THE UNIVERSITY OF CHICAGO

IDENTIFICATION OF RISK FACTORS AND COMORBIDITIES ASSOCIATED WITH TREATMENT-RELATED HEARING LOSS AND TINNITUS AND COMPARISON OF THEIR GENETIC ARCHITECTURE WITH DE NOVO ETIOLOGIES

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BY

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Whenever a potential graduate student or employee asks about my experience working in Dr. Dolan's lab, I always mention the collegiality shared among the lab

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ABBREVIATIONS

Acute Lymphoblastic Leukemia (ALL)

Acute Myeloid Leukemia (AML)

Area Under the Concentration-Time Curve (AUC)

Bleomycin, Etoposide, and Cisplatin (Platinol) (BEP)

Body Mass Index (BMI)

Central Nervous System (CNS)

Childhood Cancer Survivor Study (CCSS)

Cisplatin-Associated Ototoxicity (CAO)

Combined Annotation Dependent Depletion (CADD)

Cumulative Burden of Morbidity (CBM)

Electronic Health Records (EHRs)

Electronic Medical Record and Genomics (eMERGE)

Etoposide and Cisplatin (Platinol) (EP)

Expression Quantitative Trait Loci (eQTL)

Genome-Wide Association Study (GWAS)

Genotype-Tissue Expression Project (GTEx)

Germ Cell Tumor (GCT)

Hardy-Weinberg Equilibrium (HWE)

Human Leukocyte Antigen (HLA)

Identity by Descent (IBD)

Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Linkage Disequilibrium (LD)

Low-Density Lipoprotein Cholesterol (LDL)

Lower Limit of Quantification (LLOQ)

Luteinizing Hormone (LH)

Major Depressive Disorder (MDD)

Minor Allele Frequency (MAF)

National Health and Nutritional Examination Survey (NHANES)

Peripheral Motor Neuropathy (PMN)

Peripheral Sensory Neuropathy (PSN)

Polygenic Risk Score (PRS)

Quality Control (QC)

Reactive Oxygen Species (ROS)

Reactive Nitrogen Species (RNS)

Scale for Chemotherapy-Induced Long-Term Neurotoxicity (SCIN)

Single Nucleotide Polymorphism (SNP)

Single Nucleotide Variant (SNV)

Sodium Thiosulfate (STS)

Splicing Quantitative Trait Loci (sQTL)

St. Jude Lifetime Cohort (SJLIFE)

Standard Deviation (SD)

VePesid (Etoposide), Ifosfamide, and Cisplatin (Platinol) (VIP)

Vinblastine, Ifosfamide, and Cisplatin (Platinol) (VeIP)

Whole Exome Sequencing (WES)

ABSTRACT

Although cisplatin has been in clinical use for over 40 years, it remains difficult to identify the subset of patients who may develop ototoxicity following therapy completion. Therefore, we sought to identify non-genetic and genetic risk factors for cisplatin-associated ototoxicity, and then evaluate whether these associations were shared with other etiologies.

Through the use of pharmacokinetic modeling, we demonstrated that high levels of serum platinum were associated with multiple persistent cisplatin-induced toxicities, including tinnitus, and that patients with genetic variants in *MYH14*, a gene that affects kidney function, may be predisposed to retaining high levels of platinum for decades following completion of therapy. In addition, testicular cancer survivors who developed ototoxicity were more susceptible to persistent dizziness or vertigo, hypertension, and hypercholesterolemia, psychotropic drug use, and report poorer overall health. GWAS of cisplatin-induced hearing loss in testicular cancer survivors validated a previous association with rs62283056 (intronic to Mendelian deafness gene *WFS1*). Gene-based association analysis of cisplatin-induced hearing loss and tinnitus identified novel associations with *TXNRD1* and *WNT8A*, respectively, genes relevant to renal and/or neuronal maintenance. We then evaluated an overall score of neurotoxicity burden in cisplatin-treated patients that was associated with numerous risk factors and comorbidities, including age at diagnosis or clinical examination, cumulative cisplatin dose, high serum platinum levels, tobacco use (ever smoker or current smoker), hypertension, persistent dizziness/vertigo, Raynaud Phenomenon, symptoms consistent with peripheral motor neuropathy, psychotropic drug use, and poorer overall health.

Although GWAS identified no genome-wide significant SNPs, gene-based association analysis identified *FAM20C* in a gene-based association analysis, a gene that mediates cisplatin sensitivity in neurons, kidney, and bone, tissues vital for regulating cisplatin-induced toxicities.

We also demonstrated that cisplatin-associated ototoxicity is associated with the same non-genetic risk factors and comorbidities as cranial radiation therapy (CRT)-associated ototoxicity and age-related hearing loss and tinnitus. GWAS of CRT-induced hearing loss and tinnitus implicated genetic variants involved in nervous system maintenance, particularly, *ATXN1*, a gene associated with the neurodegenerative disorder spinocerebellar ataxia type 1 that had its association with hearing loss validated in an independent replication cohort of pediatric cancer survivors. Interestingly, GWAS of age-related hearing loss and tinnitus also identified numerous genes vital to inner ear/nervous system function, as well as immune system regulation. Despite exhibiting similar phenotypic correlations, we were only able to detect shared genetic architecture between CRT-associated ototoxicity and age-related hearing loss in SNP-based enrichment analysis of GWAS results and polygenic risk score analysis.

Taken together, these data indicate that elevated serum platinum levels may be of particular importance in identifying patients susceptible to developing multiple persistent toxicities following cisplatin-based chemotherapy, and that availability of circulating platinum is likely mediated by genetic variation in renal clearance and bone secretion. Further, we highlight the complexity of genetic predisposition to auditory disorders, indicating that similar phenotypes are not necessarily indicative of shared

genetic architecture. Therefore, mechanistically based treatments for treatment-related ototoxicity will need to be validated across different etiologies.

CHAPTER 1. BACKGROUND AND SIGNIFICANCE

Hearing Loss and Tinnitus

Auditory disorders are among the most frequent perturbations to human communication that either prevent sound from being appropriately amplified or interfere with mechanotransduction signaling to the brain. As the third most prevalent chronic health condition facing older adults (1), approximately one in three people in the United States between the ages of 65 and 74 has some degree of hearing loss, and nearly half of those older than 75 are hearing impaired. Tinnitus (a persistent buzzing or ringing in the ears in the absence of an external stimulus) is another frequently encountered auditory disorder, with prior epidemiological studies reporting its prevalence to range from 8 to 25.3% in the US population, and from 4.6 to 30% in other nations (2). Susceptibility to tinnitus also appears to increase with age, peaking at 14.3% in people 60-69 years of age (3). The long-term effects of hearing loss and tinnitus in the adult population are numerous, including social isolation, sleeping difficulties, and concentration problems that promote increased anxiety, depression, and insomnia (4-6). Further, it has recently been demonstrated that subclinical levels of hearing loss are highly associated with cognitive decline (7), indicating that even subtle declines in auditory processing can have substantial consequences on overall quality of life.

Treatment-Related Ototoxicity

Although hearing loss and tinnitus naturally develop in adults over time, they are also prevalent in pediatric and young adult settings due to the ototoxicity elicited by several agents. This clinical dilemma is exemplified by cisplatin, part of a class of platinating agents, that are one of the most commonly used group of chemotherapeutic

agents worldwide. Notably, cisplatin-based therapy has contributed to high five-year relative survival rates for a number of tumors (8), including testicular cancer (95%), hepatoblastoma (> 80%), medulloblastoma (70-80%), and osteosarcoma (60-80%). However, cisplatin elicits numerous adverse toxicities, including ototoxicity, neurotoxicity, nephrotoxicity, cardiometabolic toxicities, and secondary malignancies (9, 10), some of which affect patients acutely and resolve after treatment, and others that typically remain irreversible years after completion of therapy (11, 12).

Cisplatin-associated ototoxicity is a common adverse event following completion of therapy, with 75-80% developing hearing loss (13, 14) and ~40% developing subjective tinnitus (13). Mechanistic investigation has led to the rationale of mitigating cisplatin-associated ototoxicity by reducing increased reactive oxygen species (ROS levels) within cochlear tissue via the administration of protective agents such as antioxidants and potent heavy metal chelators that can act as free radical scavengers (15). However, cisplatin elicits its antineoplastic activity by forming adducts with DNA and other nucleophiles within the cell, and agents examined in clinical and animal studies that suppress ROS formation by neutralizing the potent electrophile with additional nucleophiles have failed to elicit therapeutic benefit due to observed inhibitory effects on its antineoplastic efficacy. Although sodium thiosulfate was recently approved to protect against cisplatin-induced hearing loss in children with localized hepatoblastoma (16) and other non-metastatic solid tumors, administration of this compound has been associated with reduced overall survival in pediatric patients with metastatic cancer, which may greatly hamper its clinical applicability (17). Consequently, there remains no FDA approved agent to reduce ototoxicity for the vast

majority of patients who receive cisplatin. Cisplatin-associated ototoxicity is a particularly notable side effect for long-term survivors because it can create functional limitations, ranging from speech development and impairment of academic achievement in children, to quality of life, socialization, and cognitive impairments in adults (18).

Radiation-associated ototoxicity is also a particularly notable adverse event, particularly in the pediatric setting where cranial radiation is an essential component of therapy for central nervous system (CNS) malignancies (30% of childhood tumors) and historically for prophylactic treatment of metastases from acute lymphoblastic leukemia (18-20). Despite improvements in treatment approaches, cranial radiation is highly ototoxic, directly damaging the brain and/or inner ear through inflammation, angiogenesis, and cell death (20). When evaluated in a cohort of 235 pediatric cancer survivors treated with cranial radiation, sensorineural hearing loss was prevalent in 14% of patients (2.1% had mild and 11.9% had significant hearing loss requiring hearing aids), and among 29 patients with follow-up evaluations, 65.5% experienced continued decline in hearing sensitivity in either ear (21). In addition, the risk of developing tinnitus in pediatric cancer survivors as compared to siblings is 17.2-fold elevated during therapy and 3.7-fold elevated among survivors who have survived 5 or more years after cancer diagnosis (22-25). Radiation-associated ototoxicity can also have serious ramifications in the pediatric setting due to its effects on speech, language, and cognitive development (18, 26, 27). These adverse events are further exacerbated depending on the path of the radiation beam, which can interfere with several neurocognitive domains, including executive functioning, processing speed, working memory, and ability to learn (28).

Modifiable Risk Factors Associated with Treatment-Related Ototoxicity

The non-uniformity of treatment-related ototoxicity in patient populations has been the subject of much research in efforts to circumvent its occurrence. Figure 1 provides an overview of non-genetic risk factors contributing to cisplatin-associated ototoxicity and other common toxicities. Of particular interest are the associations between older age and an increased susceptibility to ototoxicity and several other cisplatin-induced toxicities. Although the exact mechanisms of this association have not been explicitly studied, it is known that drug clearance can decrease with age, particularly when elimination is mediated by renal clearance (29). Since cisplatin is eliminated predominantly through the kidney, and is also known to be highly nephrotoxic, the agent ultimately reduces the ability for platinum to be excreted from the body (30), thereby increasing the likelihood of developing cisplatin-induced toxicities. Further, it is not surprising that older adults are associated with cisplatin-associated ototoxicity and neurotoxicity because these individuals often experience age-related hearing loss/tinnitus (2, 31) and paresthesias/neuropathies (32, 33), and the addition of cisplatin will likely exacerbate symptoms. There have been conflicting results with regard to the importance of noise exposure and cumulative cisplatin dose on hearing loss (13, 34, 35). However, in pediatric cancer patients, males appear to be more susceptible to cisplatin-induced hearing loss than females ($p = 0.005$) (35). Hypertension has also been identified as a potential risk factor for hearing loss in testicular cancer patients, with the association remaining significant when controlling for age and cumulative cisplatin dose ($p = 0.0066$) (13). Associations of non-genetic risk factors and comorbidities for cisplatin-induced tinnitus have been far less studied, but

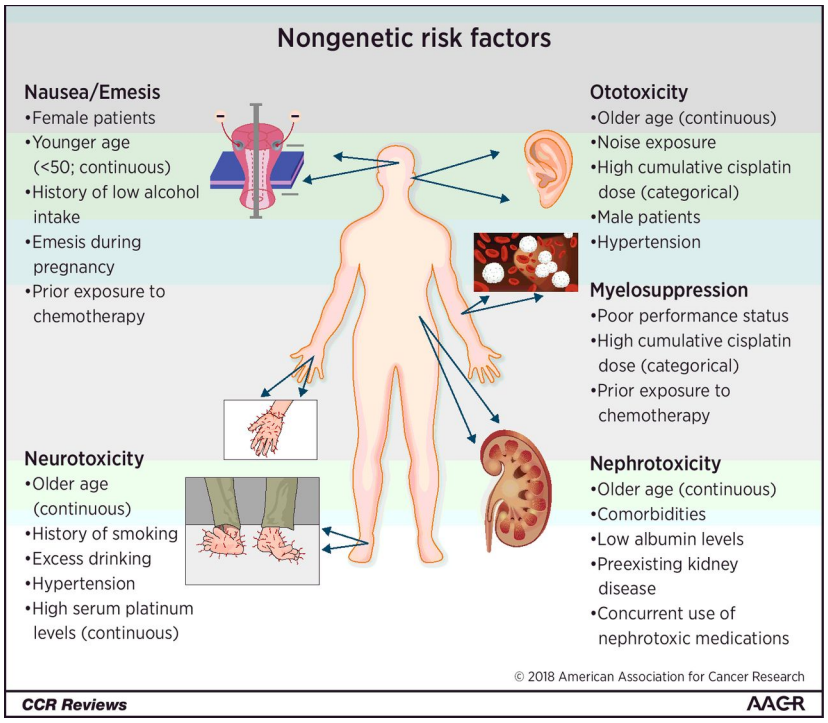

Figure 1. Non-Genetic Risk Factors that May Predispose Patients to Developing Adverse Events following Cisplatin-Based Therapy. Where relevant, risk factors are denoted as being either continuous or categorical variables based on how they were examined for association with the given toxicity. Trendowski et al. Clin Cancer Res 2019;25(4):1147-1155.

we recently identified cumulative cisplatin dose, hearing loss, persistent dizziness/vertigo, hypertension, and self-reported health to be significantly associated with cases in a cross-sectional analysis (36).

Although the evaluation of risk factors for cranial radiation-associated ototoxicity has not been as thorough, several notable associations have been observed. As with cisplatin, older age and high doses of cranial radiation (> 30 Gy) have been confirmed as independent risk factors for hearing loss and tinnitus development (19, 37-39). The mean total dose to the cochlea is particularly indicative for assessing the likelihood of ototoxicity development (39, 40). The presence of otitis media, a group of inflammatory diseases of the middle ear, has also been associated with an increased risk of sensorineural hearing loss (39).

Use of Pharmacogenomics to Identify Genetic Risk Factors

Variability in patient response can be explained in part by pharmacogenomics, which aims to provide the foundation for genetically guided treatment regimens that maximize efficacy and minimize toxicity. Initially developed from candidate gene approaches, advances in genomic sequencing technologies over the past decade have enabled agnostic genome-wide analyses of patient populations characterized for specific drug response phenotypes. Thus, pharmacogenomics has elucidated genetic variability as a key determinant in both therapeutic benefit and potential toxicities likely to be experienced during cisplatin-based chemotherapy. One of the challenges in pharmacogenomics is that most cancers are treated with a multi-drug regimen making it difficult to ascertain the genetic variants associated with a specific chemotherapeutic toxicity. Although this is typically the case for cisplatin, some toxicities (i.e. ototoxicity

and nephrotoxicity) are primarily due to cisplatin. Therefore, the genetic variants identified are most likely associated with cisplatin exposure.

Without an effective therapeutic measure by which to successfully mitigate the symptoms of cisplatin-associated ototoxicity that many patients experience, there has been an attempt to identify which individuals are more susceptible to developing this off-target toxicity through the use of pharmacogenomics. A number of pharmacogenetic studies have been conducted to identify cisplatin-associated ototoxicity risk-conferring genotypes (Appendix Table 1). In a study (41) that utilized a platform containing primarily single nucleotide polymorphisms (SNPs) in metabolizing genes, genetic variants in *TPMT* (rs12201199) and *COMT* (rs9332377) were identified that prompted the FDA to revise their label recommendations in 2012 for pediatric patients given cisplatin. However, the modification was rescinded in 2015 due to conflicting evidence of association between *TPMT* genetic variants and cisplatin-induced hearing loss provided by two replication studies and a meta-analysis (42-44). The lack of reproducibility in pharmacogenomic studies related to cisplatin is likely due to genetic heterogeneity as well as heterogeneity in treatment protocols and definition of the examined phenotype, small sample sizes, and the use of cranial radiation in combination with cisplatin which could substantially increase the likelihood of cisplatin-associated ototoxicity due to its ototoxic effects (45). This points to the importance of replication of these pharmacogenomic studies.

Technological advances have enabled agnostic, genome-wide study designs to identify contributing SNPs associated with a selected trait. Contrary to candidate gene studies, such alleles are not limited by *a priori* hypotheses of loci that generally reside in

exonic regions. In fact, the majority (90%) of disease-associated variants identified from genome-wide association studies (GWAS) reside in intergenic regions associated with transcriptional regulatory mechanisms including expression quantitative trait loci (eQTL) known to influence gene expression (46, 47). Chemotherapeutic drug susceptibility-associated SNPs, including those for cisplatin-induced cytotoxicity, are more likely to be eQTLs and be associated with the expression levels of multiple genes (48).

The first GWAS of cisplatin-induced hearing loss in 238 pediatric brain tumor patients identified an association with a SNP in *ACYP2* (rs1872328, HR = 4.5, 95% CI 2.63-7.69, $p = 3.9x10^{-8}$), and results were replicated in a second cohort of 68 pediatric patients (49). Further, increased *ACYP2* expression highly correlated with cisplatin sensitivity in lymphoblastoid cell lines *in vitro* ($p = 6.5x10^{-5}$), but the genotype at the SNP rs1872328 position was not associated with cisplatin sensitivity *in vitro*, nor was it related to expression of *ACYP2* and other genes 300 kb within this index SNP (49). Nevertheless, three studies have replicated this association with cisplatin-induced hearing loss in 156 osteosarcoma patients (50), 149 pediatric cancer patients (51), and 229 testicular cancer patients (52). *ACYP2* encodes for an enzyme that catalyzes phosphate hydrolysis in membrane pumps, most notably the Ca^{2+}/Mg^{2+} ATPase from the sarcoplasmic reticulum of skeletal muscle (53). Importantly, *ACYP2* is expressed in the cochlea for ATP-dependent Ca^{2+} signaling that is critical for hair cell development and has been directly implicated in hair cell damage (54, 55), providing a rationale for its association with cisplatin-associated ototoxicity.

In addition, Wheeler et al. performed GWAS of cisplatin-induced hearing loss in testicular cancer survivors enrolled in the Platinum Study (an NCI funded initiative that

examines the long-term effects of cisplatin treatment in testicular cancer survivors who have been cured of their disease) (56). In a GWAS of 511 testicular cancer survivors (57), a genome-wide significant SNP (rs62283056; $p = 1.4x10^{-8}$) in the first intron of Mendelian deafness gene *WFS1* (wolframin ER transmembrane glycoprotein) was identified and replicated in an independent Canadian study of 229 testicular cancer patients ($p = 5.67x10^{-3}$) (52). The SNP is an eQTL for *WFS1*, with the minor allele being associated with lower gene expression in several human tissues (57). *WFS1* encodes for wolframin, a transmembrane protein in the endoplasmic reticulum that regulates the amount of Ca^{2+} in cells, as well as facilitates protein folding by regulating these levels (58-60). As with ACYP2, wolframin is rationally associated with cisplatin-associated ototoxicity by its ability to maintain proper levels of Ca^{2+} and other charged particles (particularly K^{+} (61), the main component of the endolymph responsible for depolarizing hair cells during the mechanotransduction of mechanical to electrical energy (62).

In addition to hearing loss, we have performed a GWAS of cisplatin-induced tinnitus in 762 testicular cancer survivors enrolled in the Platinum Study, and identified rs7606353 ($p = 1.90x10^{-6}$), a SNP ~14 kb upstream of *OTOS* (36). Not only did the minor allele (G, 4% frequency) increase the risk of developing cisplatin-induced tinnitus, but the variant is in near perfect linkage disequilibrium with rs74002277 in Europeans, a SNP in the 3'-UTR region of *OTOS* that alters several transcription factor binding motifs. We also found that one *OTOS* eQTL (rs10190781, 1.5% frequency) was associated with a higher risk of developing cisplatin-induced tinnitus and lower *OTOS* expression, suggesting that higher *OTOS* expression is protective. Accordingly, tinnitus risk increased with the number of risk alleles at rs7606353 and rs10190781. We also found

a significant positive correlation between increased *OTOS* expression and decreased cellular sensitivity to cisplatin in CNS cancer cell lines *in silico* that was not observed for other chemotherapeutic agents (5-fluorouracil, bleomycin, bortezomib, cytarabine, docetaxel, etoposide, and vinblastine) and cancer cell line types (aerodigestive, breast, digestive system, lung, skin, and urogenital system). We further validated the protective effects of *OTOS* by demonstrating that its overexpression markedly reduces cisplatin sensitivity in HEI-OC1, a mouse auditory cell line routinely used for *in vitro* drug ototoxicity screening (63). *OTOS* is a recently described gene that is expressed primarily in spiral ligament fibrocytes of the cochlea. It encodes for otospiralin, a protein essential for the maintenance of normal hearing, as evidenced by *in vivo* studies reporting moderate to irreversible deafness in mice and guinea pigs following knockdown and knockout of *Otos* (64-66).

Although these GWAS revealed potential genetic susceptibilities of common SNPs to cisplatin-associated ototoxicity, the analyses were performed in cohorts of relatively small sample sizes, and may not be indicative of the overall patient population. Notably, smaller sample sizes can markedly alter the minor allele frequencies of certain SNPs, increasing the likelihood of identifying spurious associations (67). Therefore, the identified genetic variants need to be validated in additional patient cohorts before being evaluated as predictive biomarkers of cisplatin sensitivity to avoid the mistakes that were made from the findings of the *TPMT* study. There also exists the possibility that rare genetic variants in *ACYP2*, *WFS1*, *OTOS*, or other genes contribute to genetic susceptibility, as 30-40% of functional variability in previously identified pharmacogenes has been attributed to rare variants (68). As demonstrated by a recent study of

clopidogrel response in patients at risk of thromboembolism (69), whole exome sequencing has the ability to identify risk alleles for drug efficacy not previously identified by GWAS in which the majority of influential genetic variants remain unidentified based on current heritability estimates. Finally, despite the overt association between cranial radiation and auditory disorders in the pediatric setting, there has yet to be a radiogenomic study to identify genetic variants that increase susceptibility to radiation-associated ototoxicity. Due to the profound effects ototoxicity elicits on childhood development and the prevalence at which cranial radiation is administered for numerous pediatric cancers, identification of genetic risk factors that predict susceptibility would be of considerable clinical utility.

Potential for Shared Genetic Architecture Between Treatment-Related and Age-Related Hearing Loss and Tinnitus

Previously, Dolan and colleagues examined the narrow sense heritability of common genetic variants for three cisplatin-induced toxicities, and determined that cisplatin-induced hearing loss ($h^2 = 0.92 \pm 0.62$, $p = 0.04$) (57), tinnitus ($h^2 = 0.81 \pm 0.42$, $p = 0.006$) (36), and peripheral neuropathy ($h^2 = 0.74 \pm 0.48$, $p = 0.03$) (70) appear to be under appreciable genetic influence. Further, Wheeler et al. identified a SNP in Mendelian deafness gene *WFS1* (rs62283056) that increased patient susceptibility to developing cisplatin-induced hearing loss, and showed that lower PrediXcan-imputed *WFS1* expression was significantly associated with the ICD9-derived codes for hearing loss and sensorineural hearing loss in several tissues following Bonferroni correction (57). This association appeared to extend beyond *WFS1*, as permutation resampling analysis of GWAS results indicated SNPs within 50 kb of 84 genes known to cause

Mendelian nonsyndromic deafness were significantly enriched for association with cisplatin-induced hearing loss.

These results indicate cisplatin-associated ototoxicity shares genetic underpinnings with other forms of hearing loss, which is particularly important due to the increasing prevalence of age-related auditory disorders. Since susceptibility to cisplatin-induced toxicities are heritable traits and *WFS1* expression has been previously identified to influence susceptibility to hearing loss in the presence or absence of cisplatin treatment, there may exist shared genetic architecture between cisplatin-associated ototoxicity and other forms of hearing loss and tinnitus. Such findings could be used as the initial rationale for the preclinical development of otoprotective agents that could be co-administered with cisplatin to prevent or reduce symptoms of cisplatin-associated ototoxicity, and potentially improve symptoms in patients who have developed hearing loss and/or tinnitus from other etiologies.

Summary

Hearing loss and tinnitus are common auditory disorders that originate from numerous etiologies, including cancer therapy. Although curative in many settings, cisplatin is also one of the most ototoxic drugs in clinical use that causes hearing loss in 75-80% of treated patients (13) and subjective tinnitus in ~40% of patients (13). Cranial radiation is also known to induce hearing loss and tinnitus, and is frequently used in the pediatric setting because it is an essential component of therapy for CNS malignancies (30% of childhood tumors) and historically for prophylactic treatment of metastases from acute lymphoblastic leukemia (18-20). Long-term survivors who develop ototoxicity are left with debilitating functional limitations, ranging from speech development and

impairment of academic achievement in children, to quality of life, socialization, and cognitive impairments in adults (18). Although the mechanisms of treatment-related ototoxicity have been partially characterized, the development of an effective otoprotective agent that can either prevent or reduce the severity of this adverse reaction remains elusive.

There has been an attempt to identify which individuals are more susceptible to developing treatment-related ototoxicity through the use of pharmacogenomics. GWAS have elucidated the importance of genetic variation in *ACYP2* in children (49, 51) and *WFS1* in adults (52, 57) contributing to cisplatin-induced hearing loss. Further, GWAS of cisplatin-induced tinnitus in testicular cancer survivors identified a SNP upstream of *OTOS* (36). Despite its prevalence in the pediatric setting, no radiogenomic studies have been performed for radiation-associated ototoxicity.

Although these GWAS reveal potential non-genetic and genetic susceptibilities of common genetic variants to cisplatin-associated ototoxicity, there exists the possibility that rare exonic genetic variants in *ACYP2*, *WFS1*, *OTOS*, or other genes contribute as well. Further, due to the heritability of cisplatin-induced toxicities (36, 57, 70), and the fact that *WFS1* loss-of-function mutations cause a Mendelian disorder of hearing loss (Wolfram syndrome), we believe cisplatin-associated ototoxicity may share genetic architecture with age-related hearing loss and tinnitus. Cisplatin-associated ototoxicity may also share genetic architecture with other treatment-related forms of ototoxicity, including cranial radiation, which induces hearing loss and tinnitus at high frequencies in the pediatric setting (19), and is often administered in combination with cisplatin. Therefore, we intend to examine whether both common and rare genetic variants

increase patient susceptibility to developing treatment-related hearing loss and tinnitus, as well as whether these toxicities share common non-genetic risk factors and genetic architecture with age-related forms of the hearing disorders.

Significance

Advances in cancer treatment have markedly improved overall cancer survivorship, enabling more patients to live decades after their initial diagnosis. However, the efficacy of chemotherapy and radiotherapy is inherently limited by perturbations to normal tissue due to off-target toxicities, many of which are capable of inflicting permanent damage that compromises patients' overall quality of life. Consequently, as the number of long-term cancer survivors has continued to increase, the health and financial burdens associated with treatment-induced adverse sequelae has become increasingly prevalent (71, 72). Adverse reactions to chemotherapy also inherently limit the amount of drug that can be administered to the patient, and identifying drug targets capable of selectively reducing toxicity to normal tissue would reduce instances of sub-optimal dosing schedules or selection of alternative agents that are insufficient to elicit a durable antineoplastic response.

We are examining whether there exists shared genetic architecture between treatment-related hearing loss and tinnitus and age-related forms of the hearing disorders. If such a shared genetic architecture exists, it could not only lead to the development of agents that can reduce the severity and prevalence of *de novo* forms of hearing loss/tinnitus, but could also become a model for identifying genes that contribute to the development of drug-induced and disease/syndrome-induced disorders that share a similar phenotype.

Cisplatin-associated ototoxicity is a common adverse event experienced in cancer therapy, as more than 5.8 million patients are diagnosed with cancers for which first-line therapy potentially includes platinating agents (colon, rectum, cervix, endometrium, bladder, stomach, head and neck, lung, esophagus, pancreas, osteosarcoma, ovary, testis, and pediatric cancers) (56) each year. The development of mechanistic-based otoprotective drug targets would be very beneficial to the increasing population of long-term cancer survivors treated with cisplatin, as it would reduce the lifetime costs/patient associated with cisplatin-induced hearing loss ($300,000 for adults and over $1,000,000 for a child). If there is shared genetic architecture between cisplatin-induced and age-related forms of hearing loss and tinnitus, these strategies may also be applied to the much larger patient cohort of *de novo* hearing loss and tinnitus to potentially reduce the $30 billion/year spent on hearing disorders in the US alone. It may also be possible to use the identified genetic associations as predictive biomarkers of ototoxicity, enabling clinicians to determine patients who are at an increased risk of developing cisplatin- or radiation-associated ototoxicity.

Disclaimer

Figure 1 was previously published as Figure 1 in the following paper:

Trendowski MR, El Charif O, Dinh PC Jr, Travis LB, Dolan ME. Genetic and Modifiable Risk Factors Contributing to Cisplatin-induced Toxicities. Clin Cancer Res. 2019;25(4):1147-1155.

CHAPTER 2. METHODS

The Platinum Study: Patients and Data Collection

All patients were enrolled in the Platinum Study, a cross-sectional study including eight cancer centers in the United States, Canada, and the United Kingdom (9). Eligibility criteria were previously described (57, 70). Briefly, during routine follow-up, eligible testicular cancer survivors underwent physical examination/phlebotomy and completed questionnaires as well as blood collection for serum platinum levels. Data relating to germ cell tumor diagnosis and treatment were abstracted from medical records using standardized forms described previously (73). All abstractors participated in centralized, in-person training (73). Study procedures were approved by the Human Subjects Review Board at each institution, and all patients provided written consent for participation in study procedures, including genetic analyses. The studies were conducted in accordance with recognized ethical guidelines (U.S. Common Rule).

Serum Platinum Quantification

The concentration of serum platinum was quantified in two batches. For the first batch, 50 µL of serum at 10x dilution were aliquoted into 96-well plates, and a low-volume autosampler (Teledyne Technologies, Thousand Oaks, CA) introduced aliquots into an Agilent 7900 for inductively coupled plasma mass spectrometry (ICP-MS) (Agilent Technologies, Santa Clara, CA). For the second set, samples were diluted 10x and measured on the NexION 2000C for ICP-MS. Both batches utilized an iridium internal standard for calibration. Seven non-zero calibration standards ranging from 0.01 ng/L to 100 ng/L of Pt, spiked with 20 ng/L iridium for internal standardization, were analyzed every 10 samples with weighted linear regression (1/standard deviation of

triplicate sample readings as weight-factor). Linearity of calibrations was 0.9999 ± 0.0001. Method detection limit for platinum (NexIon 2000C) was 0.006 ng/L following Long and Winefordner (74). Lower limit of quantification (LLOQ) was 0.010 ng/L translating to an absolute LLOQ of 1 pg Pt. Carry-over was measured from blanks analyzed post-analysis of 100 ng/L standard (Greater Limit of Quantification) and a 35-s rinse with measured blanks below method detection limit. All samples were analyzed in a single day so no inter-day variation is noted. Within day analytical accuracy was determined by comparison of measured replicates of human serum (NIST 909c) spiked with a known amount platinum (5 ng/L) resulting in % error of 0.02. Precision, based on standard deviation of triplicate analyses of samples and standards, was better than 0.025 (relative standard deviation, 0.17%).

To evaluate inter-batch consistency, 50 samples were measured using both methods and produced a correlation coefficient of 0.94. Batches were therefore combined for analysis and the mean value of duplicates was taken following data normalization. The greater limit of quantification of both methods was used (5 ng/L), and the nonparametric reference interval for serum platinum that was previously established based on 147 non-platinum treated patient samples was used (75). This was determined as the 2.5th-97.5th percentile of 147 non-platinum treated serum samples.

Pharmacokinetic Modeling

Given the cross-sectional study design, we constructed a bi-exponential model accounting for time since treatment completion and cumulative cisplatin dose. Most patients received a cumulative cisplatin dose of 300 or 400 mg/m^2. Adding a 15 mg/m^2 margin enabled the inclusion of an additional 56 patients (5.5%). Therefore, we treated

dose as a dichotomous variable of 300±15 or 400±15 mg/m^2 and excluded remaining patients (n = 55). Prior to model fitting, cumulative cisplatin dose was taken into account by multiplying the serum platinum levels of 400±15 mg/m^2 patients by 0.75, enabling normalization to those who received 300±15 mg/m^2. We fit the following bi-exponential model to serum platinum levels:

$$\text{serum platinum} = Ae^{-\alpha t} + Be^{-\beta t}$$

where A, α, B, and ß are parameters to be estimated in the bi-exponential model, and t is years since treatment completion. Multiplicative residuals were calculated by dividing the observed serum platinum values by values expected from the fitted bi-exponential model, which were then log-transformed for normalization. These residuals are referred to as the residual platinum value. We also generated an ordinal version of residual platinum values by stratifying values based on their deviation from the mean to create three levels: “medium” (regression residuals = 0 ± 1 standard deviation (SD)), “low” (residuals < -1 SD), and “high” (residuals > 1 SD).

Cisplatin-Induced Hearing Loss and Tinnitus Phenotypes

We have previously performed audiometric testing on patients enrolled in the Platinum Study, allowing us to model hearing loss as a quantitative phenotype. Briefly, pure-tone air conduction thresholds were obtained bilaterally for each patient at frequencies of 0.25 to 12 kHz as in prior studies (76-78), covering the speech frequency range. It is important to include the frequencies of 10 and 12 kHz due to their importance in the early diagnosis of cisplatin-induced hearing loss, as high frequency

hearing is typically affected first following cisplatin treatment (79-81). We then used the geometric mean of air conduction thresholds measured at each frequency (4, 6, 8, 10, and 12 kHz) that have previously demonstrated a statistically significant relationship between cumulative cisplatin dose and hearing loss after age adjustment (13). In order to determine the geometric mean (Y_i) for each patient (i), the arithmetic mean of the natural log-transformed hearing threshold (d_i) from n frequencies was calculated and then exponentiated to return the computation to the original dB scale (i.e., the log-average):

$$Y_i = \exp\left(\frac{1}{n}\sum_{i=1}^{n}\ln d_i\right)$$

The geometric means for all patients from 4 to 12 kHz were then rank normalized to ensure a normal distribution for linear regression. This process generated a continuous variable referred to as rnGM412 that we used as the hearing loss phenotype.

By contrast, tinnitus was modeled as a binary phenotype in which cases and controls are defined based on the previously validated scale for chemotherapy-induced long-term neurotoxicity (SCIN) (82), as we did in our GWAS of cisplatin-induced tinnitus (36). Briefly, testicular cancer survivors were dichotomized to tinnitus cases/controls based on responses to the question: “Have you had in the last 4 weeks: Ringing or buzzing in the ears?” Cases are defined by those who responded “Quite a bit/Very much”, and controls are defined by those who responded “Not at all”. Those answering “A little” were excluded to establish a more rigorous phenotype. Testicular cancer

survivors were also asked: “Do you have: ringing and buzzing in the ears?” Tinnitus cases responding “No” to this question were excluded from the analysis.

Identifying Patients with Severe Hearing Loss, Tinnitus, or Peripheral Sensory Neuropathy

Hearing loss severity was assessed based on criteria of the American Speech-Language-Hearing Association (ASHA). As with the rnGM412 phenotype, audiometry data from 4-12 kHz were used. Patients who were determined to have moderate (41-55 dB), moderately severe (56-70 dB), severe (71-90 dB), or profound (> 90 dB) hearing loss at frequencies between 4-12 kHz were designated as cases, while patients with no (< 20 dB) or mild (21-40 dB) hearing loss were designated as controls. Using SCIN (82), testicular cancer survivors were dichotomized to tinnitus cases/controls as previously described (36). Peripheral sensory neuropathy was evaluated as previously described (70) using nine items in the EORTC-CIPN20 (83). Briefly, an ordinal (0-3) scale was constructed after taking the mean of symptom severity: 0 for “None”, 1 for “A little”, 2 for “Quite a bit”, and 3 for “Very much.” Groups 2 and 3 were combined to denote cases, while group 1 was eliminated to establish a more rigorous phenotype.

Establishment of the Multiple Severe Cisplatin-Induced Neurotoxicities Phenotype

Evaluation of audiometrically-defined hearing loss, tinnitus, and peripheral sensory neuropathy revealed that all toxicities were highly associated (Figure 2). Since these three symptoms typically persist for decades after completion of cisplatin-based chemotherapy, the phenotypes were combined into a single score denoting overall severe neurotoxicity burden. Patients without information for hearing loss, tinnitus, or

peripheral sensory neuropathy were eliminated from analyses to prevent non-responses from influencing the case/control definition for the phenotype (multiple severe cisplatin-induced neurotoxicities). Since the three phenotypes were grouped as cases (severe neurotoxicity: present) and controls (severe neurotoxicity: absent), their scores were combined to create an ordinal scale ranging from none (0) to all three severe toxicities being present (3). Individuals with a score of 0 were designated as controls while individuals with 2 or 3 severe toxicities were designated as cases. Patients with only one severe toxicity were eliminated from further consideration. Application of the above criteria resulted in 104 cases and 196 controls. Of 104 cases, 38 had all three severe neurotoxicities (Table 1).

Additional Patient-Reported Outcomes and Medical Records Data Abstraction

As previously described (13, 73), patients completed questionnaires ascertaining neurotoxic and other symptoms, lifestyle habits, comorbidities, and medication use. The cumulative burden of morbidity (CBM) score for cisplatin-induced toxicities was discerned by using adverse health outcomes previously related to cisplatin exposure (i.e., peripheral sensory neuropathy, hearing damage, tinnitus, and kidney disease) using a modified version described by Kerns et al. (73) that removed autonomic neuropathy. Peripheral motor neuropathy was evaluated using a summary score similar to peripheral sensory neuropathy as previously described (70) and Raynaud phenomenon was evaluated using SCIN as previously described (82). For self-reported health, patients were asked the following question: “Would you rate your health as: 1) Excellent; 2) Very good; 3) Good; 4) Fair; 5) Poor?” Responses of “Fair” and “Poor”

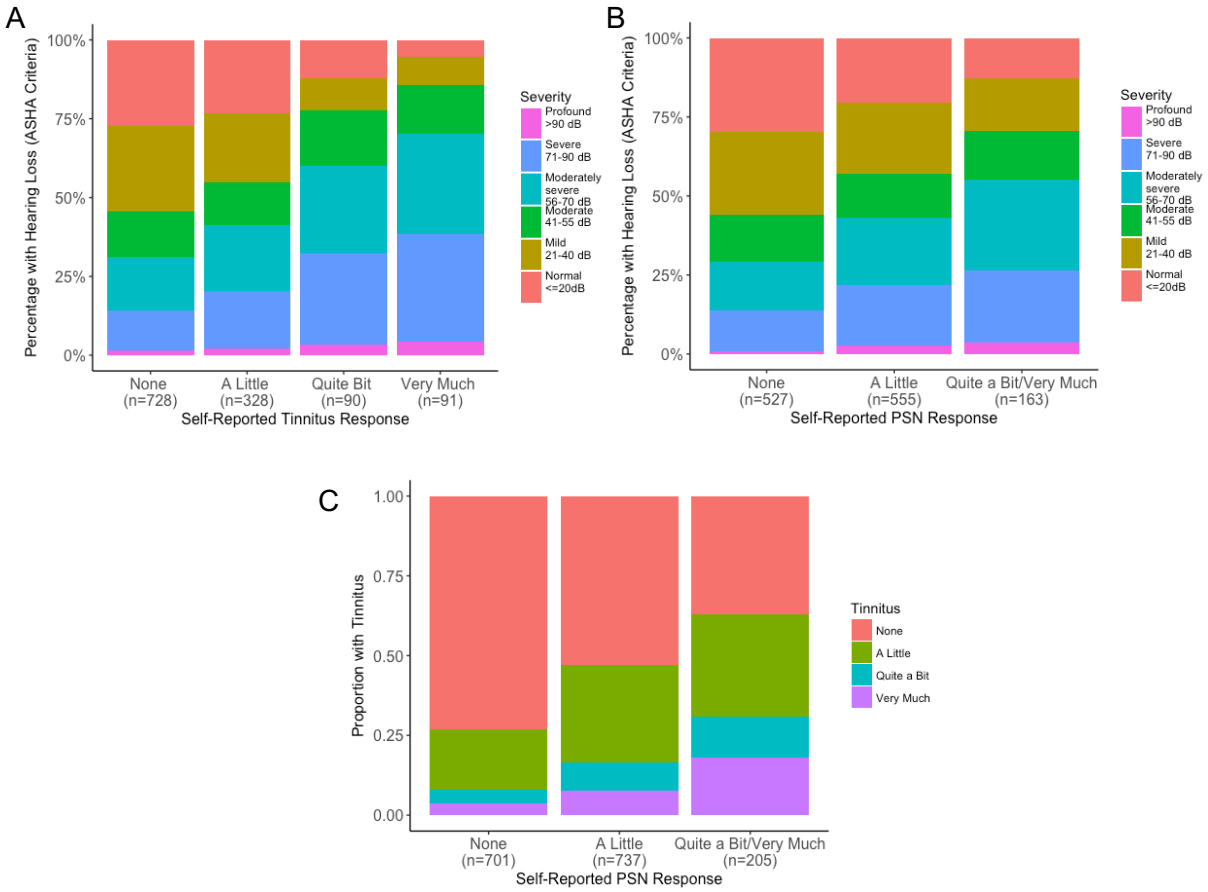

Figure 2. Relationships Between Cisplatin-Induced Hearing Loss, Tinnitus, and Peripheral Sensory Neuropathy (PSN) in Testicular Cancer Survivors. Associations between **A)** hearing loss and tinnitus ($p < 2x10^{-16}$), **B)** hearing loss and peripheral sensory neuropathy ($p = 3.94x10^{-12}$), and **C)** peripheral sensory neuropathy and tinnitus ($p < 2x10^{-16}$) are highly significant, forming the basis for combining the most severe forms of all three toxicities into a single phenotype. Statistical significance was assessed through ordinal logistic regression.

Table 1. Distribution of Severe Toxicities for Cases of the Multiple Severe-Neurotoxicities Phenotype.

Neurotoxicity	Present in Patients with Two Severe Neurotoxicities	Absent in Patients with Two Severe Neurotoxicites	Present in Patients with Three Severe Neurotoxicites
Hearing Loss	62	4	38
Tinnitus	33	33	38
Peripheral Sensory Neuropathy	37	29	38

were combined. Hypogonadism was defined as testosterone levels ≤ 3 ng/mL based on crude measurement or whether the patient was on testosterone therapy. All patients with testosterone levels > 3 ng/mL and were not on testosterone therapy were labeled as normal or high, and were grouped together as controls for linear or logistic regression analysis, as previously described (84).

We defined lifestyle habits based on patient responses to the Platinum Study questionnaire, as previously described (70). Briefly, alcohol consumption was assessed as the response to the question “During the past year, how many drinks of alcoholic beverage have you consumed on average? (1 drink = 12 oz. beer [1 can or bottle], 4 oz. glass of wine, 1 mixed drink or shot of liquor)” with the following options: Rarely/never (0), 1-3/month (1), 1/week (2), 2-4/week (3), 5-6/week (4), 1/day (5), 2-3/day (6), 4-5/day (7), and 6+/day (8). Tobacco use was assessed as the response to the following two questions: “Have you ever smoked cigarettes?” and “Do you currently smoke cigarettes?” with “Yes” (1) and “No” (0) options.

Phenotype Association Analyses in the Platinum Study

The residual platinum value was treated both continuously and ordinally (low, medium, and high, based on the deviation from the mean). Simple and multiple linear regressions were used to evaluate associations between the continuous residual

platinum values (dependent variable) and comorbidities and risk factors. Simple and age-adjusted multinomial logistic regressions were used when residual platinum values were specified ordinally, with low residual platinum values designated as the reference group. When conceptually appropriate, age at diagnosis was added as a covariate.

We previously identified several non-genetic risk factors and comorbidities associated with cisplatin-induced hearing loss (n = 488) (13) and tinnitus (n = 762) (36). In order to validate these findings, we used an expanded cohort of patients from the Platinum Study that included individuals who were not previously assessed (n = 1,680). Since not all of the patients had appropriate phenotype data, our replication analysis of cisplatin-induced hearing loss included 1,258 patients, while our replication analysis of cisplatin-induced tinnitus included 1,217 patients. Due to their different variable classifications, phenotypic correlations for cisplatin-induced hearing loss and tinnitus were evaluated with linear and logistic regression, respectively. Where indicated, associations were adjusted for age at clinical examination and cumulative cisplatin dose.

To investigate important phenotypic correlations between multiple severe cisplatin-induced neurotoxicities and responses from the Platinum Study questionnaire, univariate and multivariable logistic regressions were used to evaluate statistical significance. Where indicated, associations were adjusted for age at clinical examination and cumulative cisplatin dose. All statistical models were fit using R version 3.3.2 (http://www.R-project.org/). Statistical significance was set at $p < 0.05$.

Patient Genotyping for Common SNPs

Patient DNA was extracted from peripheral blood at the time of clinical evaluation. For the serum platinum analysis, genotyping was performed on the HumanOmniExpressExome chip (Set 1 was genotyped on the HumanOmniExpressExome-8v1-2_A chip; set 2 was genotyped on the InfiniumOmniExpressExome-8v1-3_A chip; Illumina, San Diego, CA) at the RIKEN Center (Yokohama, Japan) as previously described (57, 70). For all other genomic analyses in the Platinum Study, genotyping was performed on the Infinium Global Screening Array-24 chip (GSA-24v1-0_A1; Illumina, San Diego, CA) at Regeneron Pharmaceuticals (Tarrytown, NY).

It is important to note that genotyping for the original GWAS of cisplatin-induced hearing loss and tinnitus was performed at the RIKEN center, while the validation analysis had patient samples genotyped at Regeneron Pharmaceuticals. Genotyping all patients at Regeneron Pharmaceuticals enabled us to perform a mega-analysis of the entire expanded cohort, which has been shown to provide similar effect sizes and p-values as a meta-analysis of two separate patient populations (85).

Whole Exome Sequencing

Exome capture was completed on 1,680 patients using a high-throughput, fully automated approach developed at Regeneron Pharmaceuticals, as previously described (86). Upon completion of whole exome sequencing (WES), raw data from each Illumina NovaSeq run was gathered in local buffer storage and uploaded to the DNAnexus platform (87) for automated analysis. Sample-specific FASTQ files, representing all the reads generated for that sample, were aligned to the GRCh38 reference genome with BWA-mem (88). Aligned reads in the resultant BAM files were

then evaluated to identify and flag duplicate reads with the Picard (89) MarkDuplicates tool, and converted to GVCFs to identify single nucleotide variants (SNVs) and INDELs as compared to the reference genome. Upon completion of variant calling, individual sample BAM files were converted to fully lossless CRAM files to evaluate capture, alignment, insert size, and variant calling quality, using Picard (89), bcftools (90), and FastQC (91). Following completion of sample sequencing, samples showing disagreement between genetically determined and reported sex (n = 0), high rates of heterozygosity/contamination (D-stat > 0.4) (n = 2), low sequence coverage (less than 85% of targeted bases achieving 20x coverage) (n = 1), or genetically-identified sample duplicates (n = 11), and WES variants discordant with genotyping chip (n = 0) were excluded. Three samples were also unable to be processed, resulting in 17 individuals being excluded. The remaining 1,663 samples were then used to compile a project-level VCF for downstream analysis using the GLnexus joint genotyping tool (92). The project-level VCF then underwent additional “Goldilocks” filtering (86). Specifically, variants were filtered based on depth of coverage at a particular SNP site > 7 and allele balance in which variant sites required at least one sample to carry an alternate allele balance ≥ 15%. Multi-allelic variant sites in the project-level VCF file were normalized by left-alignment and represented as bi-allelic.

Genome-Wide Association Studies in the Platinum Study

For the serum platinum analysis, sample-level quality control (QC) criteria included: sample call rate > 0.99, pairwise identity by descent < 0.125, coefficient of inbreeding F < 6 standard deviations from the mean, and genetically European as determined by principal component analysis (performed using SMARTPCA). SNP-level

QC included: call rate > 0.99 and Hardy-Weinberg equilibrium (HWE) Chi-squared p < $1x10^{-6}$. Imputation was done on the University of Michigan Imputation Server. SNPs and samples passing QC criteria comprised the input set for imputation with EAGLE phasing, performed on the Michigan Imputation Server using the Haplotype Reference Consortium (93-95). GWAS assumed linear additive SNP effects and was adjusted for age at diagnosis and the first 10 genetic principal components. Significance was set to p ≤ $5x10^{-8}$. SNPs with imputation R^2 < 0.8, MAF < 0.01, and INFO scores > 1.05 or < 0.3 were excluded. Only subjects who passed QC were included in the GWAS (Appendix Figure 1). Narrow-sense heritability was estimated with a genetic relationship matrix variance component model as implemented by GCTA with the same covariates as GWAS. The GWAS included 909 subjects with 7,305,641 SNPs.

For the validation of our GWAS results for cisplatin-associated ototoxicity, we used linear regression (hearing loss; n = 1,079) and logistic regression (tinnitus; n = 1,044) in PLINK v1.9 (96, 97), as done previously (36, 57). To ensure GWAS resembled previous analyses, we used the same covariates for the two phenotypes (hearing loss: cumulative cisplatin dose, age at clinical examination, and 10 genetic principal components; tinnitus: cumulative cisplatin dose, age at diagnosis, noise exposure, and 5 genetic principal components). GWAS was performed on the expanded cohort of testicular cancer survivors to perform a mega-analysis on the expanded cohort. We also performed individual SNP-based association tests for the *WFS1* (rs62283056) and *OTOS* (rs7606353) SNPs to evaluate their statistical significance in an independent replication cohort, consisting of patients who were not included in the previous GWAS (hearing loss: n = 710; tinnitus: n = 462). Sample-level QC criteria included: sample call

rate > 0.99, pairwise identity by descent < 0.185, coefficient of inbreeding F < 6 standard deviations from the mean, and genetically European as determined by principal components analysis (performed using SMARTPCA). SNP-level QC first consisted of filtering variants based on depth of coverage at a particular SNP site > 7 and allele balance in which SNP sites required at least one sample to carry an alternate allele balance ≥ 15%. Remaining SNPs were then subjected to the following inclusion criteria: call rate > 0.99, MAF > 0.01, and HWE Chi-squared $p > 1x10^{-6}$. Imputation was done on the University of Michigan Imputation Server. SNPs and samples passing QC criteria comprised the input set for imputation with EAGLE phasing using the Haplotype Reference Consortium (93-95). SNPs with imputation $R^2 < 0.8$, MAF < 0.05, HWE $p < 1x10^{-6}$, and INFO scores > 1.05 or < 0.6 were excluded. Significance was set to $p \leq 5x10^{-8}$.

A GWAS of multiple severe cisplatin-induced neurotoxicities was also performed in PLINK v1.9 (96, 97) with logistic regression assuming additive effects. Cumulative cisplatin dose, age at clinical examination, and the first 20 genetic principal components (SMARTPCA) (98) were included as covariates. Sample-level and SNP-level QC were the same as the cisplatin-associated ototoxicity GWAS, as were the parameters used for imputation. Only subjects who passed QC were included in the GWAS (Appendix Figure 2). Significance was set to $p \leq 5x10^{-8}$. The GWAS included 300 subjects with 5,385,324 SNPs.

Rare Variant Analysis of Cisplatin-Induced Hearing Loss and Tinnitus

In order to identify whether rare SNVs or other genetic variants were associated with cisplatin-associated ototoxicity, we performed exome-wide association studies

(ExWAS) of cisplatin-induced hearing loss (n = 1,079) and tinnitus (n = 1,044) in which common SNPs with a MAF > 0.01 were excluded from the analyses. ExWAS for cisplatin-induced hearing loss and tinnitus were performed in PLINK v1.9 using two different criteria of deleteriousness based on annotations from SNPEff using selected gene models in Ensembl85:

1) loss-of-function (LOF) variants only; p = 0.05/13,067 (3.83×10^{-6})

2) LOF and predicted deleterious missense variants with at least 1/5 algorithms predicting a deleterious variant; p = 0.05/272,777 ($p = 1.80 \times 10^{-7}$)

The two different criteria enabled us to assess associations with confirmed deleterious mutations (LOF), while also examining rare variants that may not have been previously confirmed as deleterious (predicted variants).

Due to their low allele frequency, it is often difficult to identify valid associations between individual rare variants with small to modest effect sizes and phenotypes using traditional variant-based tests (99). Therefore, we also employed two gene-based tests, which are designed to increase power by aggregating association signals across multiple rare variants (100). For cisplatin-induced hearing loss, we performed BOLT-LMM, an approach for quantitative traits that increases statistical power by modeling more realistic, non-infinitesimal genetic architectures via a Bayesian mixture prior on marker effect sizes rather than assuming the effect sizes of examined variants are normally distributed as is done with other linear mixed models (101). It is important to note that the mathematical derivations underlying BOLT-LMM are based on a quantitative trait model, and while BOLT-LMM can be applied to analyze case-control traits, its test statistics can become miscalibrated for unbalanced case-control traits,

resulting in inflated type I error rates (102). Due to the unbalanced nature of our cisplatin-induced tinnitus phenotype, we performed gene-based association analysis with SAIGE (102), which uses the saddlepoint approximation to calibrate unbalanced case-control ratios in score tests based on logistic mixed models. For BOLT-LMM and SAIGE, covariates included age at clinical examination, cumulative cisplatin dose, and 10 genetic principal components. SAIGE analysis of cisplatin-induced tinnitus also included noise exposure as a covariate.

Childhood Cancer Survivor Study: Patient Selection

All patients were enrolled in the Childhood Cancer Survivor Study (CCSS), a large, multi-institutional collaboration of 31 participating centers in the U.S. and Canada coordinated through St. Jude Children's Research Hospital (103). Currently, CCSS has extensively characterized 14,361 five-year survivors of childhood/adolescent cancer (diagnosed 1970-1986) for pertinent demographic, disease, and treatment-related information based on medical record abstraction and surveys conducted after baseline data collection. Our phenotype association analyses and GWAS were performed in a subset of genotyped patients (n = 4,938) that was limited to 4,483 survivors that did not receive cisplatin or carboplatin. Dataset access was granted by CCSS through an approved protocol.

Establishment of the Radiation-Induced Tinnitus and Hearing Loss Phenotypes

Using two subsequent longitudinal follow-up CCSS surveys (Follow-Up-4 and Follow-Up-5) spaced seven years apart, survivors were dichotomized to tinnitus cases/controls based on the question: “Have you ever been told by a doctor or other health care professional that you have, or have had: Tinnitus or ringing in the ears?”

Cases responded “Yes, and condition is still present” on both questionnaires or “Yes, and condition is still present” on one with a missing response on the other. Conversely, controls responded “No” on both questionnaires or “No” on one with a missing response on the other. Patients who responded “Yes, no longer present” or “Not Sure” were excluded to ensure a well-defined phenotype.

Hearing loss status was derived from the questions: “Have you ever been told by a doctor or other health care professional that you have, or have had: 1) Problems hearing sounds not requiring a hearing aid? 2) Hearing loss that requires a hearing aid or hearing loss not completely corrected by a hearing aid? 3) Deafness in both ears not completely corrected by a hearing aid?” Cases responded “Yes, and condition is still present” on both questionnaires or “Yes, and condition is still present” on one with a missing response on the other. Controls responded “No” on both questionnaires or “No” on one, with a missing response on the other. Patients indicating different severities of hearing loss on both questionnaires were designated cases. Although these criteria may have excluded a few patients with “late-onset” hearing loss (i.e., no impairment on Follow-Up-4 and some impairment on Follow-Up-5), this was the most conservative approach to establish a clearly defined phenotype.

The cranial radiation doses were based on detailed abstraction of individuals’ radiotherapy records (104). For those that had cranial radiation, the maximum prescribed dose (maxTD) within the brain was taken as the sum of the prescribed dose from all overlapping fields within the brain. For individuals that received radiotherapy to regions other than the brain, we estimated stray dose (from scatter and leakage radiation) based on proximity to the brain; regions directly adjacent to the brain were

assigned stray high and more distant regions were assigned stray low, approximated as 2 Gy and 0.2 Gy, respectively.

Diagnoses of primary brain tumors were not excluded from radiation-induced tinnitus or hearing loss analyses. All patients in this study were of European ancestry based on principal components analysis of their genotype data, as previously described (98, 105).

Age at Last Observation and Other Patient-Reported Outcomes

Patients completed self-report surveys to ascertain age at last observation, neurotoxic and other symptoms, lifestyle habits, comorbidities, and medication use. Since the cohort included patients who did not report tinnitus or hearing loss status at Follow-Up 5, we generated an age at last observation variable that reflects the age of the patient at the last time point tinnitus or hearing loss status was reported (Follow-Up 4 or Follow-Up 5). Age at last observation was used for age-adjustment in both the phenotype association analyses and GWAS.

Persistent dizziness or vertigo was based on responses to the question: “Have you ever been told by a doctor or other health care professional that you have, or have had: persistent dizziness or vertigo?” Cases and controls were established based on the same criteria used for tinnitus and hearing loss (cases: Yes, and condition is still present; controls: No), with responses being consistent for both surveys or present on one, but absent on the other.

For self-reported health, patients were asked the following question in relationship to their experience over the preceding 4 weeks: “In general, would you say your health is: 1) Excellent; 2) Very good; 3) Good; 4) Fair; 5) Poor?” Since this

question is intended to determine patients' current self-reported health, only responses from Follow-Up 5 were considered. Medication history was discerned though a section of the questionnaire that asked patients to list all relevant medications they had taken regularly during the two-year period prior to Follow-Up 4 and Follow-Up 5. Types of medications extracted from the questionnaires included those for hypertension and depression, as both hypertension status and psychotropic drug use have been previously associated with treatment-related tinnitus and/or hearing loss (13, 36).

Analysis of Phenotypes with Patient Characteristics in the Childhood Cancer Survivor Study

To investigate phenotypic correlations between tinnitus/hearing loss and relevant patient characteristics collected from CCSS follow-up questionnaires, univariate and multivariable logistic regressions evaluated statistical significance. Except as indicated, phenotypes were defined based on patient responses from Follow-Up 4 and 5 questionnaires, with data from patients with inconsistent answers eliminated from the analysis. Analyses were performed in R 3.3.2, with statistical significance set at $p < 0.05$.

Genome-Wide Analyses in the Childhood Cancer Survivor Study

GWAS of radiation-induced tinnitus and hearing loss were performed in PLINK v1.9 (96, 97) with logistic regression assuming additive effects. Cumulative cranial radiation dose, age at last observation, and the first 20 European genetic principal components (98) were included as covariates. Imputation was performed based on 1000 Genomes Project release version 3 reference haplotypes (NCBI genome build 37 (hg19)) using IMPUTE version 2.3.0, as previously described (105, 106). Exclusion

criteria for samples included ≥ 8% missingness, per-sample heterozygosity < 0.11 or > 0.16, and sex discordance (X chromosome heterozygosity > 5.0% for males or < 20.0% for females), and survivors with cryptic relatedness (PI_HAT ≥ 0.2) and > 5% missingness across samples (tinnitus: n = 30; hearing loss: n = 33), as previously described (105) while exclusion criteria for SNPs included variants with MAF < 1%, and with Hardy-Weinberg equilibrium test with $p < 1x10^{-10}$, as previously described (105, 106). Genome-wide significance was set to $p < 5x10^{-8}$.

Replication of Significant SNPs

To validate SNP-ototoxicity associations reaching or approaching genome-wide significance in GWAS of either radiation-induced tinnitus or hearing loss, we performed a replication analysis in independent cohorts of childhood cancer survivors of European ancestry from the St. Jude Lifetime Cohort (SJLIFE), a clinically assessed retrospective cohort study with prospective longitudinal follow-up to characterize health outcomes of adult survivors of pediatric cancer (107-109). As with the CCSS discovery cohort, patients who received any form of cranial radiation, including stray low or stray high cranial radiation, were included in the analysis, while patients who received cisplatin or carboplatin were excluded. Patients who were present in both the CCSS discovery cohort and the SJLIFE replication cohort were removed from the analysis to ensure the validity of the replication analysis. In order to establish a comparable radiation-induced tinnitus phenotype, tinnitus status in the SJLIFE cohort was based on the same question used for the CCSS cohort: “Have you ever been told by a doctor or other health care professional that you have, or have had: Tinnitus or ringing in the ears?” Cases were defined as those who responded “Yes, and condition is still present” and

controls were those who responded “No”, producing a cohort of 952 patients (cases: 106; controls: 846). Only consistent responses were taken across surveys for the case/control definition, allowing for missing responses. For hearing loss, SJLIFE cases and controls were based on the Chang ototoxicity scale, as previously described (110). Specifically, patients with hearing loss defined as a Chang grade of 1a-4 in their worst ear were designated as cases, while patients with a Chang grade of 0 in both ears were designated as controls, producing a cohort of 331 patients (cases: 156; controls: 175). Patients who developed hearing loss due to other etiologies (congenital, Ménière's Disease, or noise exposure) were excluded prior to analysis. SNPs were evaluated for statistical significance using logistic regression. Covariates included the maximum radiation dose received to any one of the four brain segments, age at last observation, and 20 European genetic principal components accounting for population substructure.

eMERGE: Patient Selection

All patients were registered in eMERGE, a cross-sectional study including 12 medical centers in the United States (111). To ensure we examined phenotypes that accurately represented age-related hearing loss or tinnitus, patients needed to meet several inclusion criteria. All cases were diagnosed with either sensorineural hearing loss or tinnitus between the ages of 50-90 according to assigned ICD-9-CM diagnosis codes, while controls were between the ages of 50-90 according to their date of birth in eMERGE. All ICD-9-CM diagnosis codes for sensorineural hearing loss were aggregated together to form the hearing loss phenotype, while patients diagnosed with tinnitus, subjective tinnitus, or unspecified tinnitus were selected for the tinnitus phenotype (Appendix Table 2). Sensorineural hearing loss was used as the hearing loss

phenotype because patients typically develop this form after treatment with either cisplatin or cranial radiation (14, 112), enabling us to compare different etiologies of the same phenotype. Further, we excluded patients who were potentially treated with cisplatin based on ICD-9-CM diagnosis codes of cancers for which a platinating agent would be considered (Appendix Table 3). We also excluded patients with ICD-9-CM diagnosis codes that denote benign and malignant brain tumors that can lead to hearing loss or tinnitus through surgery or cranial radiation (Appendix Table 3), as well as other diseases or disorders that may cause hearing loss or tinnitus (Appendix Table 4). These criteria produced a cohort of 5,011 cases and 33,089 controls for age-related hearing loss, as well as 1,656 cases and 36,783 controls for age-related tinnitus.

Phenotypic Correlations

To validate previous phenotypic correlations for hearing loss and tinnitus (race, sex, dizziness/vertigo, depression, hypertension, and hypercholesterolemia), we extracted appropriate phenotype data from eMERGE using appropriate patient identifiers or ICD-9-CM diagnosis codes (Appendix Table 5). We investigated the correlations between age-related hearing loss or tinnitus and relevant clinical characteristics using univariate and multivariable logistic regressions. Where indicated, associations were adjusted for current age. Analyses were performed in R 3.3.2, with statistical significance being set at $p < 0.05$.

Genome-Wide Analyses in eMERGE

GWAS of age-related hearing loss and tinnitus were performed in PLINK v1.9 (96, 97) with logistic regression assuming additive effects. Current age and the first 10 European or African-specific genetic principal components were included as covariates.

Sample-level QC criteria included: sample call rate > 0.99, pairwise identity by descent < 0.185, coefficient of inbreeding F < 6 standard deviations from the mean, and genetically European or African as determined by the plink2–pca approx fast pca method, as previously described (111). Only patients who identified as white or black according to their electronic health records (EHRs) and were confirmed to be genetically European or African through principal components analysis were included in these analyses. SNP-level QC included: removing duplicated SNPs, call rate > 0.99, MAF > 0.01, and HWE Chi-squared p < $1x10^{-6}$. Imputation was done on the University of Michigan Imputation Server using the HRC1.1 variation reference in genome build 37 (hg19, hs37d5.fa) coordinates, as previously described (111). Significance was set to p ≤ $5x10^{-8}$. Due to the high proportion of white patients in eMERGE, the sample sizes for GWAS of age-related hearing loss (cases: 4,604; controls: 26,497) and tinnitus (cases: 1,459: controls: 29,841) in patients of European ancestry were larger than GWAS of age-related hearing loss (cases: 208; controls: 4,255) and tinnitus (cases: 119; controls: 4,471) in patients of African ancestry. Although we obtained age-related hearing loss and tinnitus data for patients of Asian/Pacific Islander or American Indian/Alaska Native ancestry, the sample sizes were insufficient for GWAS.

Multi-Cohort Analyses: Characterization of Identified SNPs and Gene-Based Association Analysis

Summary statistics for the GWAS from the Platinum Study, CCSS, and eMERGE were uploaded to FUMA (113) to characterize the SNPs most highly associated with each phenotype, and to run a gene-based association analysis. Identified SNPs were evaluated for functional significance through eQTL analysis based on 48 tissues in

GTEx v6-8 (114) and PsychENCODE (115), or whether they affected transcription factor binding based on RegulomeDB (116). GTEx v6-8 was used separately to identify splicing quantitative trait loci (sQTLs). The overall deleteriousness of identified SNPs was evaluated in FUMA through Combined Annotation Dependent Depletion (CADD), a tool that evaluates the deleteriousness of single nucleotide variants, as well as insertion and deletion variants in the human genome through the integration of annotations from more than 60 different databases into one metric (117, 118). CADD scores are evaluated based on a scaled metric in which single nucleotide variants within the top 10% of CADD scores are assigned to CADD-10, top 1% to CADD-20, top 0.1% to CADD-30, etc. Chromatin and eQTL interactions between identified risk loci and distal genes were mapped in FUMA using circos plotting (119). For the gene-based association analysis, SNPs from GWAS ($p < 0.05$) were mapped to protein coding genes in FUMA. The aggregated effect of all SNPs within a gene was then analyzed simultaneously using MAGMA based on a multiple linear principal components regression (120). A Bonferroni-corrected significance threshold ($p = 0.05$/number of genes) was used to define statistical significance in each analysis.

Functional Enrichment Analysis

Functional enrichment analysis was performed for radiation-associated ototoxicity and age-related hearing loss and tinnitus using Gene Ontology (121, 122) and KEGG pathways (123), along with Human Phenotype Ontology (124) and Reactome (125), using the g:Profiler package (126) in R 3.3.2 at a significance threshold of $p < 0.05$ after attributing genes the p-value of the most significantly associated SNP in or within 25 kb of gene start/end sites (GENCODE GRCh37.p12).

Evaluation of Sensitivity to Cisplatin and Radiation Based on Gene Expression *In Silico*

Gene expression data in central nervous system and other tumor cell lines was obtained from the Cancer Cell Line Encyclopedia (127). Cisplatin sensitivity, measured as the area under the dose-response curve, was obtained from the Genomics of Drug Sensitivity in Cancer Project (128). Radiosensitivity data, measured as the area under the survival curve derived from a linear-quadratic model to fit 9-day viability assay data, were obtained from the RadioGx package in R (129). Spearman correlation and linear regression were performed between gene expression and sensitivity of cancer cell lines to cisplatin or radiation with non-missing expression data in R 3.3.2.

Polygenic Risk Score Analysis

To identify whether age-related hearing loss or tinnitus shares common genetic architecture with cisplatin- or radiation-associated ototoxicity, we performed polygenic risk score (PRS) analysis using three separate patient populations of European ancestry. We have previously performed GWAS of cisplatin-induced hearing loss (57) and tinnitus (36) in testicular cancer survivors enrolled in the Platinum Study (56), but we have since acquired additional genotyped patients. Therefore, we included these patients in our cisplatin-associated ototoxicity cohort, increasing the sample size to 1,079 patients for hearing loss and 1,044 patients for tinnitus. The phenotype definition for cisplatin-induced hearing loss was defined by measured hearing thresholds for frequencies that showed statistically significant dose-response relationships with cumulative cisplatin dose (4-12 kHz) that were geometrically averaged and rank normalized, as previously described (57), while the phenotype definition for cisplatin-

induced tinnitus was discerned from responses to an extended questionnaire that were used to develop a case-control phenotype, as previously described (36). Both the radiation-induced hearing loss and tinnitus phenotypes were derived from responses from pediatric cancer survivors enrolled in CCSS (103) who responded to two questionnaires administered seven years apart to ensure patients had persistent ototoxicity, creating case-control phenotypes consisting of 2,198 (hearing loss) and 1,991 (tinnitus) patients (Manuscript Under Review).

Using the C+T method in PLINK (96, 97, 130), we trained a predictive model to assess the aggregated effects of common genetic variants on age-related hearing loss and tinnitus in patients of European ancestry, which was then evaluated in the cisplatin-associated ototoxicity and radiation-associated ototoxicity cohorts. Prior to the analysis, standard quality control was performed on the base data (age-related hearing loss and tinnitus), including the removal of ambiguous or duplicated SNPs, as well as those with MAF < 1% and INFO < 0.8. For the target data (cisplatin- and radiation-induced hearing loss and tinnitus), inclusion criteria included call rate > 0.99, and HWE Chi-squared p > $1x10^{-6}$. Target data were evaluated at two separate MAF thresholds (MAF = 0.01 or MAF = 0.05) to ensure the validity of results. PRS analysis for cisplatin-associated ototoxicity included 7,657,611 SNPs (MAF = 0.01) or 5,414,083 SNPs (MAF = 0.05). PRS analysis for radiation-associated ototoxicity included 7,257,653 SNPs (MAF = 0.01) or 5,188,521 SNPs (MAF = 0.05).

In addition, we evaluated whether radiation-associated ototoxicity (base data) shares common genetic architecture with cisplatin-associated ototoxicity (target data) using the same subjects and SNPs for the hearing loss and tinnitus evaluation. Age-

related hearing loss and tinnitus in patients of European ancestry (base data) were also compared to patients of African ancestry (target data) using 4,463 subjects (hearing loss) and 4,590 subjects (tinnitus) with 9,362,060 SNPs (MAF = 0.01).

For PRS analysis, we performed clumping to remove SNPs in high linkage disequilibrium with index SNPs (R^2 = 0.1) based on maximum likelihood haplotype frequency estimates, thereby retaining SNPs most associated with the phenotype of the base population. SNPs within 250 kb of index variants were considered for clumping, and were assigned to only one clump. The default formula in PLINK for PRS calculation (130) was used for determining the fit of the polygenic risk model:

$$PRS_j = \frac{\sum_i^N S_i * G_{ij}}{P * M_j}$$

where effect size of SNP i is S_i; the number of effect alleles observed in sample j is G_{ij}; the ploidy of the sample is P (generally 2 for humans); the total number of SNPs included in the PRS is N; and the number of non-missing SNPs observed in the sample j is M_j. If the sample has a missing genotype for SNP i, G_{ij} is replaced with the population MAF multiplied by the ploidy (MAF*P).

The p-value threshold that provided the best-fit PRS (explained the highest phenotypic variance) under the C+T method was ascertained by performing a regression between PRS calculated at a range of p-value thresholds (0.001, 0.01, 0.05, 0.20, and 0.50) in R 3.3.2. Population substructure was accounted for by incorporating the first 10 European or African principal components of the target population as covariates in the model fitting. Statistical significance of Nagelkerke R^2 values derived from the fit of PRS models was set at $p < 0.05$.

CHAPTER 3. SERUM PLATINUM ANALYSIS

Introduction

Advances in chemotherapy have markedly improved overall cancer survival, enabling more patients to live decades after completion of chemotherapeutic treatments. However, survivors often suffer from severe off-target toxicities due to chemotherapy. These toxicities can limit clinical use, compromise efficacy, and many can permanently impact survivors' quality of life. As the number of long-term cancer survivors has continued to increase, the health and financial burdens associated with chemotherapy-induced adverse sequelae have become increasingly prevalent (71, 72, 131).

This clinical problem is exemplified by cisplatin, a widely used platinating agent that is effective against several adult-onset and pediatric malignancies. High five-year relative survival rates follow cisplatin-based therapy for a number of tumors (8), including testicular cancer (95%), hepatoblastoma (> 80%), medulloblastoma (70-80%), and osteosarcoma (60-80%). Unfortunately, cisplatin also elicits a number of debilitating side effects, including ototoxicity, neurotoxicity, nephrotoxicity, cardiometabolic toxicities, and secondary malignancies (9, 10). These toxicities can be progressive and irreversible, leading to chronic health conditions in young cancer survivors. For example, approximately 18% of adults (13) and 7-22% of children (132) are left with severe to profound hearing loss. In addition, 56.2% of testicular cancer survivors given a median dose of 400 mg/m^2 report symptoms of sensory neuropathy at a median of 5 years after treatment, with 12.5% reporting severe symptoms (70). Reduced renal function is also detected in 25-35% of patients after one cisplatin dose (133). While the

majority of patients fully recover from the initial onset of nephrotoxicity, both progressive and irreversible nephrotoxicity have been reported (134, 135).

Several studies have shown detectable tissue platinum (136) and platinum-DNA adducts (137) years after cisplatin therapy. A 13-28 year follow-up study demonstrated that circulating platinum persisted for decades after treatment (138). Serum platinum levels have been shown to remain up to 1,000 times higher than normal for 20 years after completion of therapy (138-141). One study demonstrated that about 10% of circulating serum platinum remains reactive (142).

Circulating platinum has been evaluated for its contribution to the severity and persistence of cisplatin-induced neurotoxicity (paresthesias, neuropathy, and Raynaud phenomenon), nephrotoxicity, and ototoxicity (hearing loss and tinnitus). Inconsistent associations have been found between serum platinum levels and cisplatin-induced toxicities. Sprauten et al. (143) identified significant associations between crude serum platinum levels (defined as total platinum concentration) and symptoms of paresthesias, Raynaud phenomenon, and tinnitus in 169 testicular cancer survivors. Hjelle et al. (140) found crude serum platinum levels to be significantly associated with tinnitus and elevated concentrations of luteinizing hormone (LH), but not with paresthesias and Raynaud phenomenon in 292 testicular cancer survivors after adjusting for cisplatin dose. A study that longitudinally assessed 77 testicular cancer survivors regarding declines in crude serum platinum levels and toxicities counter-intuitively showed larger declines in platinum levels related to worsening of tinnitus and hand paresthesias (138), perhaps because larger declines are correlated with less time since treatment. Importantly, none of these studies accounted for the kinetics of metabolism and

clearance by accounting for variance in time since treatment. A longitudinal pharmacokinetic study serially measuring serum and urine platinum found associations of area-under-the-platinum-exposure-curve with neuropathy, but not tinnitus or Raynaud phenomenon, and noted new associations with hypertension, hypercholesterolemia, and hypogonadism (144). This study, however, had a relatively small sample size (n = 99 testicular cancer survivors) and did not compare platinum exposure to an unexposed control group.

In this study, we evaluate clinical correlates with serum platinum levels after constructing a dose- and follow-up time-adjusted pharmacokinetic model in a cohort of 1,010 testicular cancer survivors characterized for variables on diagnosis, treatment, medical history, lifestyle and behavioral factors, and comorbidities over a wide range of follow-up periods (1-35 years). We then use the model to examine the association between serum platinum levels and late adverse events associated with cisplatin toxicity. In addition, we perform a genome-wide association study (GWAS) to assess genetic contributions to our phenotype derived from serum platinum levels.

Results

Cohort Characteristics

Demographic and clinical characteristics for the entire cohort of testicular cancer survivors, as well as for each residual platinum value category, are provided in Tables 2 and 3. The median age at diagnosis and evaluation was 31 (range: 15-54) years and 37 (range: 18-75) years, respectively. Patients were treated with the following regimens: BEP (bleomycin, etoposide, and cisplatin; 54.0%), EP (etoposide and cisplatin; 30%), VIP (etoposide, ifosfamide, and cisplatin; 2.5%), VeIP (vinblastine, ifosfamide, and

cisplatin; 0.1%), and other (unspecified cisplatin-based chemotherapy; 13.4%). Of the 1,010 testicular cancer survivors, 436 and 574 (43.2% and 56.8%, respectively) were treated with 300±15 and 400±15 mg/m^2 cisplatin, respectively. Median follow-up time was 4.5 years (range 1-35 years).

Pharmacokinetic Analysis

Median serum platinum concentration was 309 ng/L (range 7.2-8,252 ng/L; 259 ng/L 6.7-8,252 ng/L after normalization). Only 62 survivors (6.1%) had serum platinum concentrations below 47 ng/L, the upper limit of the normal range determined from non-platinum-treated patient samples. The median follow-up time for this group of survivors was 19.5 years (range 6-31 years, 19.1 years for 300±15 mg/m^2 and 20.3 years for 400±15 mg/m^2, respectively). Using bi-exponential regression, we estimated C_{max} (concentration at time 0) to be 4,720 ng/L. We fit a bi-exponential model to the normalized serum platinum data (Figure 3A). Attempts to use a tri-exponential model resulted in a singular gradient error. When simulating the progression of the tri-exponential model fitting, the estimates for the tri-exponential parameters became unreasonably high, indicating that addition of the tri-exponential parameters produced an overfitted model (data not shown). Consequently, it appeared that the two-compartment model was sufficient to model the decay of serum platinum over time. The estimated time to reach the upper limit of the reference range was strikingly high (31 years), and took nearly seven half-lives. Interestingly, the bi-exponential model never reached the seventh half-life of decay (serum platinum = 36.9 ng/L) over the course of 35 years (the length of the longest follow-up period), indicating that patients are likely

Table 2. Clinical and Sociodemographic Characteristics for 1,010 Testicular Cancer Survivors According to Residual Platinum Values.

Characteristic	All Patients (n=1,010)	Residual Platinum Value		
		Low (n=118)	Medium (n=785)	High (n=107)
Age at GCT diagnosis (years)				
Median (range)	31 (15-54)	30 (15-53)	31 (15-54)	35 (16-50)
<20	70 (6.9%)	16 (13.6%)	49 (6.2%)	5 (4.7%)
20-29	390 (38.6%)	52 (44.1%)	311 (39.6%)	27 (25.2%)
30-39	351 (34.8%)	32 (27.1%)	273 (34.8%)	46 (43.0%)
40-55	199 (19.7%)	18 (15.3%)	152 (19.4%)	29 (27.1%)
Time from diagnosis to clinical evaluation (years)				
Median (range)	4.5 (0.6-35.3)	12.2 (0.6-30.3)	4.3 (0.8-30.6)	3.4 (1.1-35.3)
≤1	4 (0.3%)	2 (1.7%)	2 (0.3%)	0 (0%)
>1 and ≤5	539 (53.4%)	35 (29.7%)	437 (55.7%)	67 (62.6%)
>5 and ≤10	243 (24.1%)	14 (11.9%)	206 (26.2%)	23 (21.5%)
>10 and ≤20	181 (17.9%)	48 (40.7%)	122 (15.5%)	11 (10.3%)
>20	43 (4.3%)	19 (16.1%)	18 (2.3%)	6 (5.6%)
Treatment regimen[a,b]				
BEP	545 (54.0%)	72 (61.0%)	429 (54.7%)	44 (41.1%)
EP	303 (30.0%)	29 (24.6%)	232 (29.6%)	42 (39.3%)
VIP	25 (2.5%)	6 (5.1%)	15 (1.9%)	4 (3.7%)
VeIP	1 (0.1%)	0 (0%)	1 (0.1%)	0 (0%)
Other (includes cisplatin)	135 (13.4%)	11 (9.3%)	107 (13.6%)	17 (15.9%)
Cumulative cisplatin dose (mg/m^2)				
Median (range)	400 (286-414)	400 (292-406)	400 (286-414)	400 (297-400)
<300	20 (2.0%)	1 (0.9%)	18 (2.3%)	1 (0.9%)
300	406 (40.2%)	47 (39.8%)	317 (40.4%)	42 (39.3%)
>300 and <400	28 (2.8%)	5 (4.2%)	21 (2.7%)	2 (1.9%)
400	548 (54.3%)	64 (54.2%)	422 (53.8%)	62 (57.9%)
>400	8 (0.8%)	1 (0.9%)	7 (0.9%)	0 (0%)
Audiometrically assessed hearing loss[c,d]				
None	125 (17.1%)	15 (16.0%)	98 (17.6%)	12 (15.4%)
Mild	162 (22.2%)	17 (18.1%)	127 (22.8%)	18 (23.1%)
Moderate	111 (15.2%)	18 (19.1%)	81 (14.5%)	12 (15.4%)
Moderately severe	154 (21.1%)	17 (18.1%)	122 (21.9%)	15 (19.2%)
Severe/profound	178 (24.4%)	27 (28.7%)	130 (23.3%)	21 (26.9%)
Tinnitus[e]				
Yes	355 (37.2%)	38 (33.0%)	271 (36.7%)	46 (45.5%)
No	599 (62.8%)	77 (67.0%)	467 (63.3%)	55 (54.5%)
Peripheral sensory neuropathy[f,g]				
None	429 (43.1%)	52 (44.1%)	343 (45.3%)	9 (9.5%)
A little	472 (47.4%)	57 (48.3%)	361 (47.6%)	69 (72.6%)
Quite a bit/very much	95 (9.5%)	9 (7.6%)	54 (7.1%)	17 (17.9%)
Raynaud phenomenon[g,h]				
None	634 (64.0%)	80 (67.8%)	497 (64.5%)	57 (55.3%)
A little	205 (20.7%)	27 (22.9%)	154 (20.0%)	24 (23.3%)
Quite a bit/very much	152 (15.3%)	11 (9.3%)	119 (15.5%)	22 (21.4%)

Abbreviations: BEP: bleomycin, etoposide, and cisplatin; EP: etoposide and cisplatin; VIP: etoposide, ifosfamide, and cisplatin; VeIP: vinblastine, ifosfamide, and cisplatin

Table 2 Continued.

[a] *BEP* category includes patients who received only bleomycin, etoposide, and cisplatin; *EP* includes patients who received only etoposide and cisplatin; *VIP* includes patients who received only etoposide, ifosfamide, and cisplatin; *VeIP* includes patients who received only vinblastine, ifosfamide, and cisplatin. The *other* category includes patients who received an unspecified cisplatin-based treatment regimen.

[b] 1 participant was missing data for treatment regimen.

[c] 280 participants did not have hearing assessed audiometrically.

[d] ASHA criteria defined hearing loss severity as the following: mild: 21 to 40 dB; moderate: 41 to 55 dB; moderately severe: 56 to 70 dB; severe: 71 to 90 dB; and profound: > 90 dB; for at least one tested frequency for either ear (https://www.asha.org/public/hearing/Degree-of-Hearing-Loss).

[e] 56 participants did not report tinnitus status.

[f] 14 participants did not report peripheral sensory neuropathy status.

[g] Following conversion of the Likert scale: “none, a little, quite a bit, very much” to a 0-3 numeric scale, each individual was attributed a summary statistic for the sensory subscale (Cronbach $\alpha = 0.88$) and the motor subscale ($\alpha = 0.78$) by taking the mean of the response in the subscale: none (mean = 0), mild ($0 < \text{mean} \leq 1$), severe (mean > 1), as in Dolan et al (70).

[h] 19 participants did not report Raynaud phenomenon status.

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Table 3. Additional Clinical and Sociodemographic Characteristics for 1,010 Testicular Cancer Survivors According to Residual Platinum Values.

		Residual Platinum Value		
Characteristic	**All Patients (n=1,010)**	**Low (n=118)**	**Medium (n=785)**	**High (n=107)**
Clinical characteristics				
Age at GCT diagnosis (years)				
Median (range)	31 (15-54)	30 (15-53)	31 (15-54)	35 (16-50)
<20	70 (6.9%)	16 (13.6%)	49 (6.2%)	5 (4.7%)
20-29	390 (38.6%)	52 (44.1%)	311 (39.6%)	27 (25.2%)
30-39	351 (34.8%)	32 (27.1%)	273 (34.8%)	46 (43.0%)
40-55	199 (19.7%)	18 (15.3%)	152 (19.4%)	29 (27.1%)
BMI[a]				
Median (range)	27.4 (18.1-53.9)	29.1 (21.3-53.9)	27.2 (18.1-52.8)	27.2(19.5-46)
<25	223 (25.1%)	15 (14.0%)	178 (25.8%)	30 (32.6%)
25-29	396 (44.5%)	45 (42.1%)	315 (45.6%)	36 (39.1%)
30-34	185 (20.8%)	27 (25.2%)	137 (19.8%)	21 (22.8%)
35-39	57 (6.4%)	11 (10.3%)	43 (6.2%)	3 (3.3%)
≥40	29 (3.3%)	9 (8.4%)	18 (2.6%)	2 (2.2%)
Number of cycles, platinum-based chemotherapy				
3	436 (43.2%)	50 (42.4%)	342 (43.6%)	44 (41.1%)
4	572 (56.6%)	68 (57.6%)	443 (56.4%)	61 (57.0%)
>4	2 (0.2%)	0 (0%)	0 (0%)	2 (1.9%)
Hypogonadism[b,c]				
Yes	163 (38.4%)	23 (39.0%)	125 (37.5%)	15 (45.5%)
No	262 (61.6%)	36 (61.0%)	208 (62.5%)	18 (54.5%)
CBM score for cisplatin-induced toxicities[d,e]				
None	271 (27.1%)	38 (32.2%)	218 (27.9%)	15 (15.0%)
Very low	359 (35.9%)	36 (30.5%)	280 (35.8%)	43 (43.0%)
Low	184 (18.4%)	19 (16.1%)	148 (18.8%)	17 (17.0%)
Medium	158 (15.8%)	22 (18.6%)	118 (15.1%)	18 (18.0%)

		Residual Platinum Value		
Characteristic	**All Patients (n=1,010)**	**Low (n=118)**	**Medium (n=785)**	**High (n=107)**
High	28 (2.8%)	3 (2.5%)	18 (2.3%)	7 (7.0%)
Sociodemographic characteristics				
Race				
White	866 (85.7%)	103 (87.3%)	674 (85.9%)	89 (83.2%)
Nonwhite	17 (1.7%)	3 (2.5%)	11 (1.4%)	3 (2.8%)
Not stated	127 (12.6%)	12 (10.2%)	100 (12.7%)	15 (14.0%)
Marital status[f]				
Not married	342 (35.9%)	34 (31.8%)	279 (39.7%)	29 (31.2%)
Married/ living as married	560 (62.1%)	73 (68.2%)	423 (60.3%)	64 (68.8%)
Education				
High school or less	35 (3.5%)	9 (7.6%)	23 (2.9%)	3 (2.8%)
Some college/ college graduate	541 (53.6%)	66 (55.9%)	423 (53.9%)	52 (48.6%)
Postgraduate level	196 (19.4%)	19 (16.1%)	151 (19.2%)	26 (24.3%)
Other or unknown	238 (23.6%)	24 (20.3%)	188 (23.9%)	26 (24.3%)

Table 3 Continued.

Abbreviations: BMI: body mass index; CBM: cumulative burden of morbidity; GCT: germ cell tumor
[a]120 participants were missing BMI measurement.
[b]585 participants were not evaluated with laboratory measurements for hypogonadism.
[c]Defined as testosterone levels ≤ 3 ng/mL based on laboratory measurement or whether the patient was on testosterone therapy. All patients who had testosterone levels > 3 ng/mL and were not on testosterone therapy were labeled as normal or high, and were grouped together as controls for the multinomial regression analysis, as in Abu Zaid et al (84).
[d]10 participants did not have CBM score determined.
[e]Was calculated by using adverse health outcomes previously related to cisplatin exposure (i.e, peripheral sensory neuropathy, hearing damage, tinnitus, and kidney disease), as in Kerns et al (73).
[f]108 participants did not have marital status stated.
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still at the upper limits of the reference interval nearly four decades after completion of cisplatin-based chemotherapy. Because we could not compute concentration-time AUC with cross-sectional data, we used the log-transformed multiplicative residuals from the fit of the bi-exponential model to the normalized serum platinum data as the exposure phenotype and categorized survivors into low (n = 118, 11.7%), medium (n = 785, 77.7%), and high (n = 107, 10.6%) residual platinum values (Figure 3B and C). Importantly, residual platinum values were highly associated with normalized serum platinum levels obtained from the 1,010 patients ($p < 2x10^{-16}$), which was comparable to years since treatment completion ($p < 2x10^{-16}$), and more statistically significant than cumulative cisplatin dose (p = 0.09), weight (p = 0.23), BMI (p = 0.01), age at diagnosis (p = 0.05), age at clinical examination ($p = 6.92x10^{-15}$), or creatinine clearance (p = 0.04). Therefore, residual platinum values appear to be an accurate predictor of cisplatin clearance.

Predictors, Risk Factors, and Comorbidities

We found a significant positive association between residual platinum values and age at diagnosis ($p = 3.13x10^{-9}$), and a strong negative correlation between residual platinum values and creatinine clearance at follow-up ($p = 1.99x10^{-5}$) (Figure 4A and B, Table 4). This association appears to persist years after cisplatin-based chemotherapy has been completed, as the association between residual platinum values and creatinine clearance remains statistically significant when only evaluating patients who last received cisplatin more than 15 years prior to clinical examination (n = 100; p = 0.01). We also found a significant positive association with LH levels ($p = 6.07x10^{-3}$;

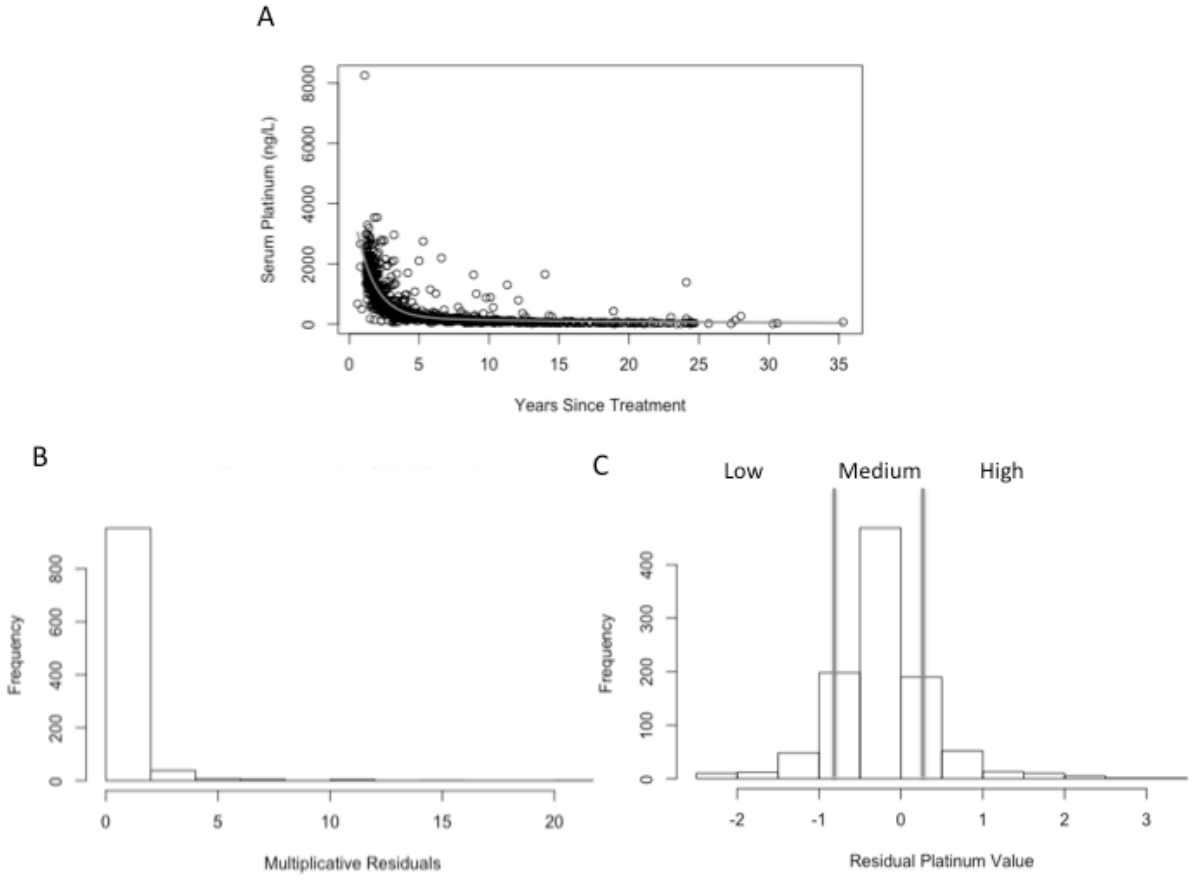

Figure 3. Population Pharmacokinetic Modeling of Long-Term Serum Platinum Levels. A) Serum platinum levels from 1,010 testicular cancer survivors were fitted to a bi-exponential model in which years since treatment completion was taken into account. Cumulative cisplatin dose was taken into account by multiplying the serum platinum levels of 400 ± 15 mg/m^2 patients by 0.75, enabling normalization to 300 ± 15 mg/m^2 patients prior to model fitting. **B)** Histogram of multiplicative residuals from the bi-exponential model. **C)** Residuals were log-transformed to fit a near normal distribution in order to examine the extent to which residual platinum values associate with cisplatin-induced toxicities. The bars denote groups of low, medium and high. Trendowski et al. Clin Cancer Res 2019;25(19):5913-5924.

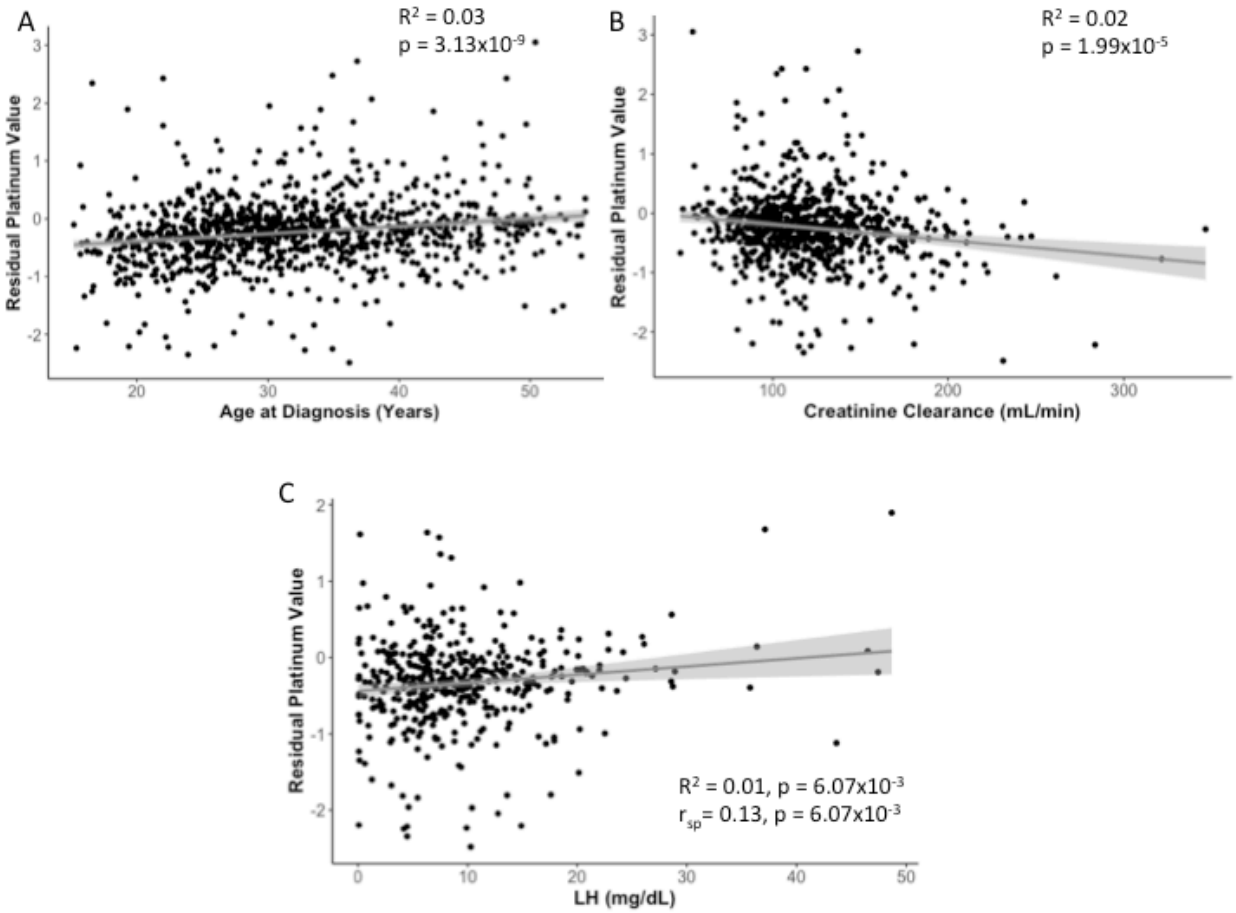

Figure 4. Linear Regression of Residual Platinum Value and Continuous Variables. Simple linear regression results are presented for **A)** age at diagnosis, **B)** creatinine clearance, and **C)** LH levels. R^2 and p-values for linear regression are reported for all phenotypes. A Spearman rank correlation test (r_{sp}) was also performed on LH levels due to its positive skew distribution, and results are shown in panel C. Fitted linear regression lines are highlighted in gray and 95% confidence intervals are indicated by the light gray shaded regions. Trendowski et al. Clin Cancer Res 2019;25(19):5913-5924.

Figure 4C, Table 4). Creatinine clearance and LH levels remained statistically significant after incorporating age at diagnosis as a covariate (Table 4). Hypogonadism was not significantly associated with residual platinum values (β = 0.07, p = 0.22, age-adjusted p = 0.75; Table 4). Hypertension, smoking, excess alcohol, and extent of physical activity were also not significantly associated with residual platinum values (p > 0.05, data not shown).

We found significant positive associations between residual platinum values and several examined drug-induced toxicities, including the cumulative burden of morbidity (CBM) score for cisplatin-induced toxicities (β = 0.04, p = 0.01), peripheral sensory neuropathy (β = 0.08, p = 7.52×10^{-3}), and Raynaud phenomenon (β = 0.07, p = 9.84×10^{-3}; Table 4). The association with Raynaud phenomenon remained statistically significant after adjusting for age at diagnosis (β = 0.05, p = 0.03), while the associations with CBM score (β = 0.02, p = 0.28) and peripheral sensory neuropathy (β = 0.04, p = 0.18) were no longer statistically significant. The association with Raynaud phenomenon also remained statistically significant after adjusting for both age and cumulative cisplatin dose (β = 0.05, p = 0.04). Tinnitus demonstrated a marginally significant association with residual platinum values (β = 0.07, p = 0.07, age-adjusted p = 0.07, age and cisplatin dose-adjusted p = 0.07), but audiometric hearing thresholds were not statistically significant (β = 0.03, p = 0.21). Although audiometric hearing thresholds became statistically significant after adjusting for age at diagnosis (β = -0.05, p = 0.05), the β-value changed from positive to negative and by more than 10%, indicating that age at diagnosis is a negative confounder for the association.

Table 4. Linear Regression of the Association Between Residual Platinum Values as a Continuous Variable and Phenotypes Relevant to Cisplatin-Based Chemotherapy.

			Linear Regression		Linear Regression with Age at Diagnosis	
	Variable Type	**n**	**β (95% CI)**	**p-value**	**β (95% CI)**	**p-value**
Risk Factors/Comorbidities						
Age at Diagnosis	continuous	1,010	0.01 ($8.68x10^{-3}$, 0.02)	**$3.13x10^{-9}$**	N/A	N/A
Creatinine clearance[a]	continuous	769	$-2.67x10^{-3}$ ($-3.89x10^{-3}$, $-1.45x10^{-3}$)	**$1.99x10^{-5}$**	$-1.95x10^{-3}$ ($-3.20x10^{-3}$, $-7.09x10^{-4}$)	**$2.13x10^{-3}$**
LDL (mg/dL)	continuous	580	$9.12x10^{-3}$($-3.61x10^{-4}$, $2.18x10^{-3}$)	0.16	$7.52x10^{-4}$ ($-5.00x10^{-4}$, $2.00x10^{-3}$)	0.24
LH (mg/dL)	continuous	437	0.01 ($3.7x10^{-3}$, 0.02)	**$6.07x10^{-3}$**	0.01 (2.91x10-3, 0.02)	**$6.58x10^{-3}$**
Hypogonadism[b,c]	categorical	428	0.07 (-0.04, 0.19)	0.22	0.02 (-0.10, 0.13)	0.75
Testosterone (ng/mL)	continuous	441	$-2.28x10^{-3}$($-7.66x10^{-3}$, $3.10x10^{-3}$)	0.41	$-1.68x10^{-3}$ ($-6.97x10^{-3}$, $3.60x10^{-3}$)	0.53
Cisplatin-Induced Toxicities						
CBM score for cisplatin-induced toxicities[d]	categorical	1003	0.04 ($8.80x10^{-3}$, 0.08)	**0.01**	0.02 (-0.02, 0.05)	0.28
Peripheral sensory neuropathy[e]	categorical	999	0.08 (0.02, 0.14)	**$7.52x10^{-3}$**	0.04 (-0.02, 0.10)	0.18
Raynaud phenomenon[e]	categorical	994	0.07 (0.02, 0.12)	**$9.84x10^{-3}$**	0.05 (4.53x10-3, 0.10)	**0.03**
Hearing Loss (rnGM412)[f]	continuous	751	0.03 (-0.02, 0.08)	0.21	-0.05 (-0.11, $6.78x10^{-5}$)	**0.05**
Tinnitus	categorical	957	0.07 (5.44x10-3, 0.15)	*0.07*	0.07 ($-7.19x10^{-3}$, 0.15)	*0.07*

Abbreviations: CBM: cumulative burden of morbidity; LDL: low-density lipoprotein cholesterol; LH: luteinizing hormone

For the linear regression model, the continuous version of residual platinum values was the dependent variable. The other phenotypes were classified as independent variables, and age at diagnosis was included as a covariate where indicated. Bold indicates $p \leq 0.05$; italics indicates $0.05 < p < 0.10$.

[a]Was calculated by using the following formula: creatinine clearance = (140-age at clinical examination)*weight (kg)/(72*serum creatinine (mg/dL).

[b]Excluded 54 patients who had measured LH values, but received testosterone therapy.

[c]Defined as testosterone levels ≤ 3 ng/mL based on crude measurement or whether the patient was on testosterone therapy. All patients who had testosterone levels > 3 ng/mL and were not on testosterone therapy were labeled as normal or high, and were grouped together as controls for the linear regression analysis, as in Abu Zaid et al (84).

[d]Calculated by using selected adverse health outcomes previously related to cisplatin exposure (i.e., peripheral sensory neuropathy, hearing damage, tinnitus, and kidney disease), using a modified version of Kerns et al. (73) by removing autonomic neuropathy.

[e]Following conversion of the Likert scale: "none, a little, quite a bit, very much" to a 0-3 numeric scale, each individual was attributed a summary statistic for the sensory subscale (Cronbach α = 0.88) and the motor subscale (α = 0.78) by taking the mean of the response in the subscale: none (mean = 0), mild (0 < mean ≤ 1), severe (mean > 1), as in Dolan et al (70).

[f]Defined by the rank normalized geometric mean of air conduction thresholds measured at 4, 6, 8, 10, and 12 kHz, as in Frisina et al (13).

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We categorized patients into low, medium, or high residual platinum values to assess whether high levels of serum platinum were more associated with cisplatin-induced toxicities than medium or low levels (Tables 5 and 6). In considering the severity of the toxicity, a higher proportion of patients who experienced more severe toxicity was demonstrated in the high residual platinum value group when compared to the low or medium groups for CBM score (Figure 5A), peripheral sensory neuropathy (Figure 5B) and Raynaud phenomenon (Figure 5C). In addition, patients with high residual platinum values were significantly more likely to have a higher CBM score ($OR_{high/low}$ = 1.26, p = 0.05), and peripheral sensory neuropathy ($OR_{high/low}$ = 1.61; p = 0.02, Table 5), but not after adjusting for age ($OR_{high/low}$ = 1.13, p = 0.32 and $OR_{high/low}$ = 1.34; p = 0.17, respectively, Table 6). Patients with high residual platinum values were significantly more likely to have Raynaud phenomenon (age-adjusted $OR_{high/low}$ = 1.46; p = 0.04, age and cisplatin dose-adjusted $OR_{high/low}$ = 1.45; p = 0.04). In regards to ototoxicity, patients with high residual platinum values had a higher likelihood of developing tinnitus ($OR_{high/low}$ = 1.69, p = 0.06), which remained marginally significant after adjusting for age at diagnosis ($OR_{high/low}$ = 1.68, p = 0.07), as well as age at diagnosis and cumulative cisplatin dose ($OR_{high/low}$ = 1.69, p = 0.07). Audiometric hearing thresholds were not significantly associated with high residual platinum values ($OR_{high/low}$ = 1.03, p = 0.86).

Genome-Wide Association Study

GWAS of residual platinum values as a continuous variable identified one SNP that met genome-wide significance: rs1377817 ($p = 4.6 \times 10^{-8}$; Figure 6A). This SNP is intronic to *MYH14*, which encodes for a heavy chain of nonmuscle myosin 2. The SNP

Table 5. Univariate Multinomial Regression of the Association Between Residual Platinum Values and Clinical Phenotypes Relevant to Cisplatin-Based Chemotherapy.

			Residual Platinum Value: Medium (n = 785)		Residual Platinum Value: High (n = 107)	
	Variable Type	**n (All Patients, Low, Medium, High)**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
Risk Factors/Comorbidities						
Age at diagnosis	continuous	1,010 (118, 785, 107)	1.03 (1.01, 1.06)	**$5.40x10^{-3}$**	1.06 (1.03, 1.10)	**$3.72x10^{-5}$**
Creatinine clearance[a]	continuous	769 (94, 597, 78)	0.99 (0.98, 0.99)	**$5.38x10^{-4}$**	0.98 (0.96, 0.99)	**$1.35x10^{-3}$**
LDL (mg/dL)	continuous	579 (73, 455, 51)	1.00 (1.00, 1.01)	*0.07*	1.00 (0.99, 1.01)	0.70
LH (mg/dL)	continuous	436 (60, 334, 32)	1.02 (0.97, 1.06)	0.46	1.02 (0.96, 1.08)	0.52
Hypogonadism[b]	categorical	425 (59, 333, 33)	0.96 (0.54, 1.69)	0.88	1.30 (0.55, 3.09)	0.55
Testosterone (ng/mL)	continuous	440 (60, 346, 34)	1.02 (0.94, 1.11)	0.58	0.97 (0.81, 1.17)	0.79
Cisplatin-Induced Toxicities						
CBM score for cisplatin-induced toxicities[c]	categorical	1,000 (118, 782, 100)	0.99 (0.83, 1.18)	0.94	1.26 (0.99, 1.60)	**0.05**
Peripheral sensory neuropathy[d]	categorical	996 (118, 773, 105)	1.02 (0.76, 1.39)	0.87	1.61 (1.07, 2.41)	**0.02**
Raynaud phenomenon[d]	categorical	991 (118, 770, 103)	1.20 (0.91, 1.59)	0.20	1.54 (1.08, 2.20)	**0.02**
Hearing Loss (rnGM412)[e]	continuous	749 (96, 570, 83)	0.87 (0.69, 1.09)	0.22	1.03 (0.76, 1.39)	0.86
Tinnitus	categorical	954 (115, 738, 101)	1.18 (0.78, 1.78)	0.44	1.69 (0.98, 2.94)	*0.06*

Abbreviations: CBM: cumulative burden of morbidity; LDL: low-density lipoprotein cholesterol; LH: luteinizing hormone

For the multinomial regression model, the ordinal version of residual platinum values (low, medium, and high) was the dependent variable, with low residual platinum values being designated as the reference group. The other phenotypes were classified as independent variables. Bold indicates $p \leq 0.05$; italics indicates $0.05 < p < 0.10$.

[a]Was calculated by using the following formula: creatinine clearance = (140-age at clinical examination)*weight (kg)/(72*serum creatinine (mg/dL).

[b]Defined as testosterone levels ≤ 3 ng/mL based on crude measurement or whether the patient was on testosterone therapy. All patients who had testosterone levels > 3 ng/mL and were not on testosterone therapy were labeled as normal or high, and were grouped together as controls for the multinomial regression analysis, as in Abu Zaid et al (84).

[c]Calculated by using selected adverse health outcomes previously related to cisplatin exposure (i.e., peripheral sensory neuropathy, hearing damage, tinnitus, and kidney disease), using a modified version of Kerns et al. (73) by removing autonomic neuropathy.

[d]Following conversion of the Likert scale: "none, a little, quite a bit, very much" to a 0-3 numeric scale, each individual was attributed a summary statistic for the sensory subscale (Cronbach ($\alpha = 0.88$) and the motor subscale ($\alpha = 0.78$) by taking the mean of the response in the subscale: none (mean = 0), mild ($0 < \text{mean} \leq 1$), severe (mean > 1), as in Dolan et al (70).

[e]Defined by the rank normalized geometric mean of air conduction thresholds measured at 4, 6, 8, 10, and 12 kHz, as in Frisina et al (13).

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Table 6. Multinomial Regression of the Association Between Residual Platinum Values and Phenotypes Relevant to Cisplatin-Based Chemotherapy with Age at Diagnosis as a Covariate.

			Residual Platinum Value: Medium (n = 785)		Residual Platinum Value: High (n = 107)	
	Variable Type	**n (All Patients, Low, Medium, High)**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
Risk Factors/Comorbidities						
Age at diagnosis	continuous	1,010 (118, 785, 107)	N/A	N/A	N/A	N/A
Creatinine clearance[a]	continuous	769 (94, 597, 78)	0.99 (0.98, 1.00)	**4.83×10^{-3}**	0.98 (0.96, 0.99)	**6.06×10^{-3}**
LDL (mg/dL)	continuous	579 (73, 455, 51)	1.00 (0.99, 1.01)	*0.08*	1.00 (0.99, 1.01)	0.79
LH (mg/dL)	continuous	436 (60, 334, 32)	1.02 (0.97, 1.06)	0.46	1.02 (0.96, 1.08)	0.55
Hypogonadism[b]	categorical	425 (59, 333, 33)	0.80 (0.45, 1.44)	0.46	1.05 (0.41, 2.44)	0.99
Testosterone (ng/mL)	continuous	440 (60, 346, 34)	1.04 (0.92, 1.18)	0.50	1.01 (0.85, 1.21)	0.87
Cisplatin-Induced Toxicities						
CBM score for cisplatin-induced toxicities[c]	categorical	1,000 (118, 782, 100)	0.93 (0.78, 1,12)	0.45	1.13 (0.89, 1.44)	0.32
Peripheral sensory neuropathy[d]	categorical	996 (118, 773, 105)	0.91 (0.67, 1.25)	0.59	1.34 (0.88, 2.03)	0.17
Raynaud phenomenon[d]	categorical	991 (118, 770, 103)	1.17 (0.88, 1.54)	0.28	1.46 (1.02, 2.09)	**0.04**
Hearing Loss (rnGM412)[e]	continuous	749 (96, 570, 83)	0.67 (0.52, 0.86)	**2.43×10^{-3}**	0.67 (0.47, 0.97)	**0.03**
Tinnitus	categorical	954 (115, 738, 101)	1.17 (0.77, 1.78)	0.45	1.68 (0.97, 2.94)	*0.07*

Abbreviations: CBM: cumulative burden of morbidity; LDL: low-density lipoprotein cholesterol; LH: luteinizing hormone

For the multinomial regression model, the ordinal version of residual platinum values (low, medium, and high) was the dependent variable, with low residual platinum values being designated as the reference group. The other phenotypes were classified as independent variables, and age at diagnosis was included as a covariate. Bold indicates $p \leq 0.05$; italics indicates $0.05 < p < 0.10$.

[a]Was calculated by using the following formula: creatinine clearance = (140-age at clinical examination)*weight (kg)/(72*serum creatinine (mg/dL).

[b]Defined as testosterone levels ≤ 3 ng/mL based on crude measurement or whether the patient was on testosterone therapy. All patients who had testosterone levels > 3 ng/mL and were not on testosterone therapy were labeled as normal or high, and were grouped together as controls for the multinomial regression analysis, as in Abu Zaid et al (84).

[c]Calculated by using selected adverse health outcomes previously related to cisplatin exposure (i.e., peripheral sensory neuropathy, hearing damage, tinnitus, and kidney disease), using a modified version of Kerns et al. (73) by removing autonomic neuropathy.

[d]Following conversion of the Likert scale: "none, a little, quite a bit, very much" to a 0-3 numeric scale, each individual was attributed a summary statistic for the sensory subscale (Cronbach ($\alpha = 0.88$) and the motor subscale ($\alpha = 0.78$) by taking the mean of the response in the subscale: none (mean = 0), mild ($0 < \text{mean} \leq 1$), severe (mean > 1), as in Dolan et al (70).

[e]Defined by the rank normalized geometric mean of air conduction thresholds measured at 4, 6, 8, 10, and 12 kHz, as in Frisina et al (13).

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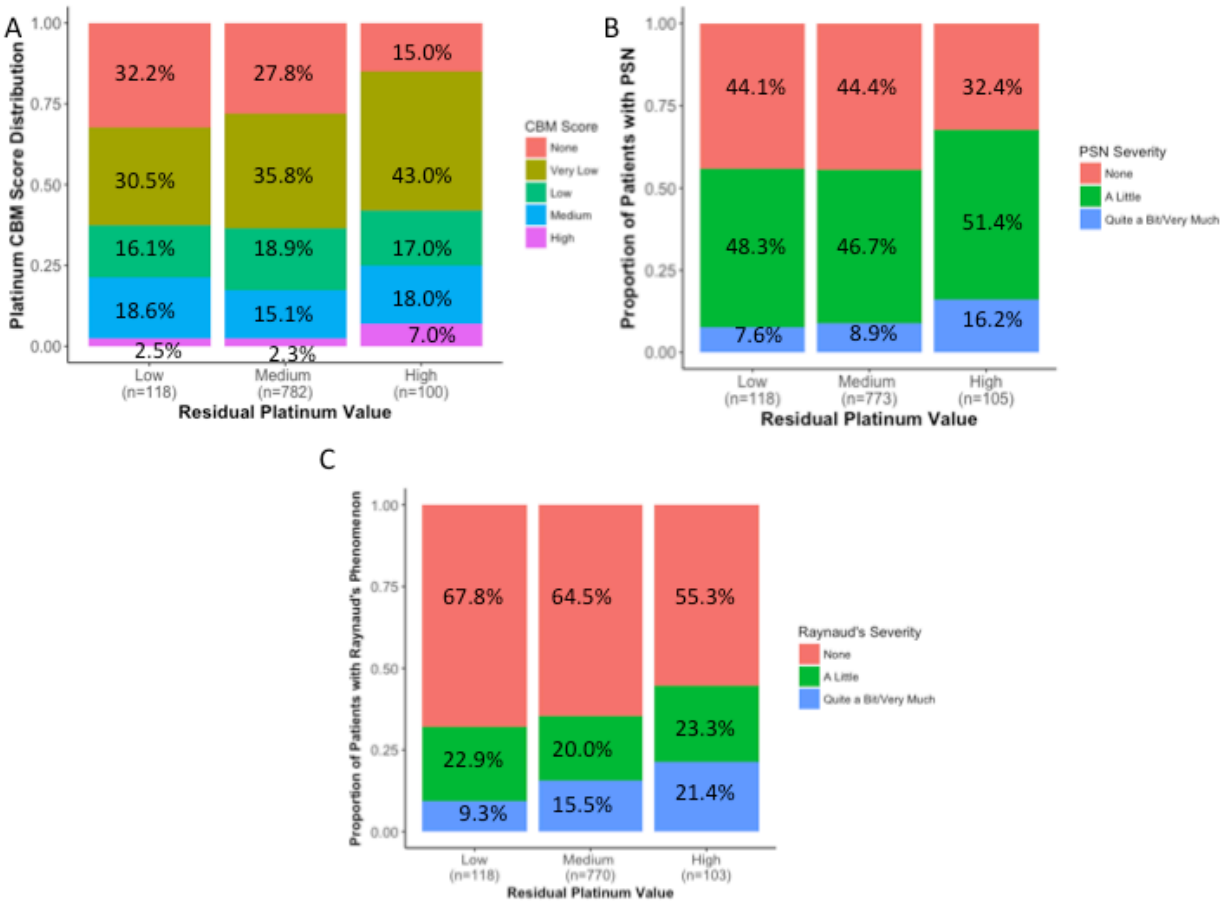

Figure 5. Distributions of Cisplatin-Induced Toxicities in Testicular Cancer Survivors Based on Residual Platinum Value. The overall distribution of **A)** CBM score for cisplatin-induced toxicities (p = 0.06), **B)** peripheral sensory neuropathy (PSN; p = 0.02), and **C)** Raynaud phenomenon (p = 0.02) in testicular cancer survivors based on having low, medium, and high residual platinum values is provided. Low, medium, and high groups reflect ordinal stratifications of residual platinum values based on their deviation from the mean: “medium” (regression residuals = 0 ± 1 standard deviation [SD]), “low” (residuals < -1 SD), and “high” (residuals > 1 SD). All three toxicities are divided into different degrees of severity, as indicated in the legend, with associated percentages provided in each panel. Sample sizes for each group are indicated within

Figure 5. Distributions of Cisplatin-Induced Toxicities in Testicular Cancer Survivors Based on Residual Platinum Value.

each panel on the x-axis. Differences between the proportions of toxicity severity observed for the low, medium, and high residual platinum value groups were evaluated for statistical significance through the Cochran-Armitage-Mantel 1df chi-squared trend test (145). Trendowski et al. Clin Cancer Res 2019;25(19):5913-5924.

is in linkage disequilibrium (LD) with rs58754699 and rs113890379 (R^2 = 1.0, p <0.0001), the next two most statistically significant genetic variants (p = $5.0x10^{-8}$ and $1.6x10^{-7}$, respectively). The SNP, rs1377817, explained 3.2% of the phenotype's variance (p = $7.11x10^{-8}$). The Q-Q plot in Figure 6B indicates that the observed p-values of associated SNPs deviate significantly from the expected distribution (null hypothesis of no association) of p-values, suggesting that multiple SNPs are associated with the residual platinum value phenotype. Appendix Table 6 lists all SNPs associated with residual platinum values with p<0.0001. Using GCTA's linear mixed model approach (146), we found that additive SNP effects did not explain phenotypic variance (h^2 = 0.04 ± 0.38, p = 0.45).

Discussion

In this study, we uniquely interrogate exposure of serum platinum levels in a cohort of 1,010 testicular cancer survivors following near uniform treatment with cisplatin-based chemotherapy and compare this with a platinum-unexposed control group. After normalizing serum platinum concentration, we fit a bi-exponential model with follow-up time as a variable. We define our measure of inter-patient variability in serum platinum as the observed serum platinum value divided by the expected value at the observed follow-up time derived from this model for each patient, followed by log-transformation. We postulate that this phenotype is correlated to the area under the concentration-time curve (AUC), which cannot be directly computed due to the cross-sectional study design. Our analytical method allows the interrogation of time-dependent data without serial sampling. Although the AUC is not directly estimated, the

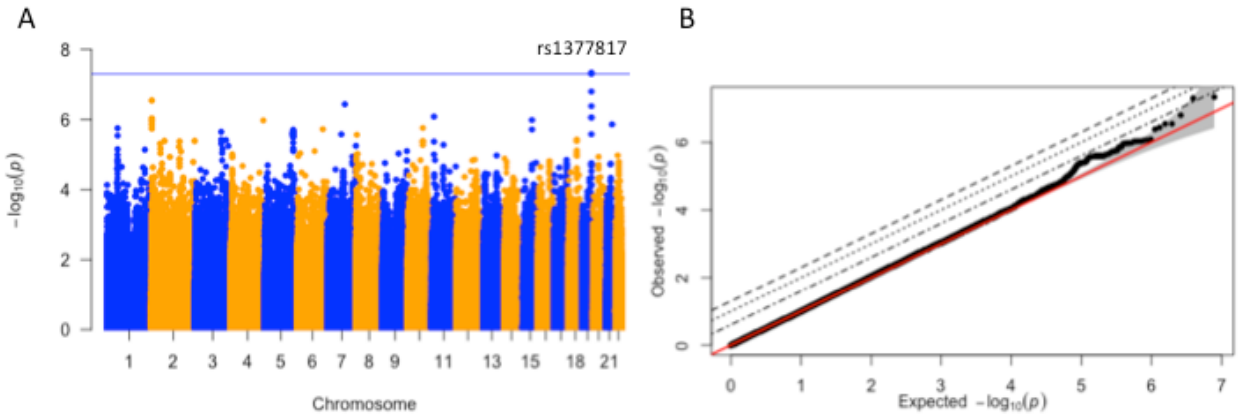

Figure 6. Genome-Wide Association Study of Residual Platinum Value as a Continuous Variable. A) Manhattan plot of GWAS results reveals one locus meeting genome-wide significance ($p \leq 5x10^{-8}$): rs1377817 ($p = 4.6x10^{-8}$). Covariates in the analysis include age at diagnosis and 10 genetic principal components accounting for population substructure. **B)** Quantile-Quantile plot of GWAS results. Trendowski et al. Clin Cancer Res 2019;25(19):5913-5924.

multiplicative residual is theoretically proportional to dose-adjusted AUC, and thus is a plausible marker of cisplatin exposure. From this, we find that serum platinum levels exceed the reference range for approximately 31 years. This is consistent with previous findings indicating long-term detection of serum platinum, but to our knowledge this is the first study to provide an estimated time-to-reference range. We interrogate the association of platinum with toxicities beyond crude serum concentration measurements, the predominant analytical strategy previously used in studies evaluating the relationship of serum platinum levels and toxicities (138, 140, 143). We do so because associations between serum platinum levels and toxicological traits are likely confounded by follow-up time, the strongest predictor of serum platinum concentration. Our data suggest that patients with high serum platinum levels are more susceptible to developing tinnitus and Raynaud phenomenon than those with medium or low serum platinum levels, but does not provide robust associations with other toxicities, including cumulative burden of morbidity scores, hearing loss, and peripheral sensory neuropathy. We additionally perform a GWAS to determine the genetic variants associated with residual platinum values.

As expected, we found a strong negative association between chronic serum platinum exposure and creatinine clearance. Cisplatin is excreted through the renal route by glomerular filtration with some tubular secretion, and is also nephrotoxic (30). Therefore, we expect that lower pre-treatment creatinine clearance would contribute to higher chronic serum concentrations of platinum, as found by Boer et al. (144). In addition, we expect that ongoing exposure to higher chronic serum platinum levels could contribute to additional nephrotoxicity. Our significant association with follow-up

creatinine clearance values may therefore be due to low renal function (possibly due to the SNP identified in our GWAS intronic to *MYH14*) raising serum platinum concentrations as well as high serum platinum concentrations lowering renal function. One limitation of our study is the lack of baseline renal function assessments.

In univariate analysis, the serum platinum phenotype was found to be significantly associated with the cumulative morbidity profile and certain toxicities, but robust associations were not detected. Boer et al. (144) identified a significant association with neuropathy; however, our dataset indicates that when age is considered, the association with neuropathy is no longer statistically significant. Taking age into account, Sprauten et al. (143) reported that both paresthesias and Raynaud phenomenon were increased two to four-fold in the highest quartile of crude serum platinum concentrations. In evaluating categorized patients (low, medium, or high residual platinum values), patients with high residual platinum values were significantly more likely to have Raynaud phenomenon even after adjusting for age.

Interestingly, hearing loss and tinnitus appeared to have contrasting levels of association with residual platinum values. Although the extent of hearing loss did not appear to be associated with residual platinum values, tinnitus did demonstrate a marginal association that was independent of age at diagnosis. Further, multinomial regression indicated that patients with high levels of serum platinum were more likely to develop tinnitus, suggesting these individuals are at an increased risk of developing this off-target toxicity. These data are in accord with Hjelle et al. (140) and Sprauten et al. (143) who both found crude serum platinum levels to be significantly associated with tinnitus after adjusting for cumulative cisplatin dose. Nevertheless, the notable

difference in statistical association for hearing loss and tinnitus is surprising in light of the fact that concentrations of platinum in the cochlea decline much slower than in serum, as cisplatin binding to the cochlea is largely irreversible (147). It is plausible that there are differences in the pathophysiology underlying cisplatin-induced hearing loss and tinnitus, as recent studies have indicated the development of tinnitus is more dependent on disruptions in the balance of excitatory and inhibitory nerve transmission within central auditory structures than pathology in the cochlea (148). However, most investigations of cisplatin-associated ototoxicity have focused on hair cell damage in the organ of corti that induces hearing loss (15), and the mechanisms underlying cisplatin-induced tinnitus are still poorly understood. Therefore, further investigation will be needed to discern potential differences regarding the importance of platinum clearance in cisplatin-induced hearing loss and tinnitus.

Our study relied in large part on patient-reported outcomes in the quantification of platinum toxicities. Patient-reported outcomes are increasingly recognized as valid and important and enable a broad interrogation of symptoms across conditions in long-term cancer survivors (149). Additionally, adverse effects like peripheral neuropathy may not be fully captured with objective assessments (150) and may be confounded by physician interpretation on physician-graded scales (151). An NCI Clinical Trials Planning Meeting (152) recently agreed that self-report for neuropathy is superior to exam and recommended EORTC-CIPN20. Self-reported data may also more adequately quantify the perceived impact on quality of life.

Residual platinum values were also associated with increased LH levels, a finding that has been previously noted when assessing either crude serum platinum

levels (140) or cumulative cisplatin dose (153). Interestingly, residual platinum values were not associated with decreased testosterone levels or hypogonadism as in Boer et al. (144), which are often observed in testicular cancer survivors due to disturbed endocrine gonadal function that impairs testosterone production (140, 153). However, disturbance of endocrine gonadal function in testicular cancer survivors is also mediated by other factors such as orchiectomy and testicular dysgenesis syndrome (153). Further, the study that initially reported an association between crude serum platinum and LH levels reported no association with testosterone levels (140). Therefore, it is plausible that unaccounted variables we have not considered and different phenotype definitions explain this discrepancy.

In GWAS, we identified a prominent signal in chromosome 19 in which rs1377817 ($p=4.6x10^{-8}$), a SNP intronic to *MYH14*, met criteria for genome-wide significance, and had several other top SNPs in LD. Importantly, *MYH14* encodes for a heavy chain that is an integral component of nonmuscle myosin 2, a protein essential for kidney development and function (153, 154). When evaluated in mice, *Myh14* was expressed throughout most segments of the renal tubules, and was implicated in the regulation of the renal epithelial transport process (155). Since cisplatin is excreted from the body predominantly through renal clearance, the importance of genetic architecture surrounding kidney development and function is apparent, and could markedly influence long-term platinum kinetics and circulation in serum. Interestingly, a mutation in *MYH14* has previously been associated with an autosomal dominant disorder of peripheral neuropathy, myopathy, hoarseness, and hearing loss (156), indicating that the gene

could also potentially influence two prominent cisplatin-induced toxicities (peripheral neuropathy and hearing loss).

It is important to note that interpretation of genetic results is especially difficult for serum platinum, as it is a highly complex phenotype. Contributions of DNA damage-related apoptotic pathways, organ development and regeneration, renal function, protein-binding, as well as the pharmacokinetic pathways of cisplatin (absorption, distribution, metabolism, and excretion) are likely all contributing to serum platinum concentrations, but are difficult to measure to enable interpretation of biomolecular mechanisms. A strategy of multiple-tissue sampling and measurement of platinum concentration has been evaluated in rodent models and has generated important insights (147), but this would be difficult in humans.

To our knowledge, at this point, there is no candidate agent that could significantly reduce toxicity without impacting antitumor activity for all cancers treated with cisplatin. Plausible detoxifying agents include antioxidants (16, 157) although potential risks must always be balanced against benefits. In addition, transporter-mediated uptake can be of importance in decreasing off-target toxicities of platinum derivatives and therefore can provide protective intervention, without compromising anticancer efficacy (158-160). Sodium thiosulfate was recently approved to protect against cisplatin-induced hearing loss in children with localized hepatoblastoma (16) and did not hamper therapeutic efficacy in localized hepatoblastoma. However, administration of this compound has been shown to reduce overall survival in pediatric patients with metastatic cancer, which may greatly hamper its clinical applicability (17). Reducing the severity of toxicities and comorbidities associated with cisplatin treatment

is an important goal, particularly for children and young adults with many decades of subsequent life.

Conclusion

Our study demonstrates the need to adjust for time since treatment and confirms previous observations that platinum follows a slow elimination and remains in circulation at exceptionally high levels for years after cisplatin treatment. Importantly, residual platinum values were associated with several cisplatin-induced toxicities and associated comorbidities. In addition, our GWAS identified rs1377817 ($p=4.6x10^{-8}$), a SNP intronic to *MYH14*, to be associated with residual platinum values, suggesting that genetic variation may predispose certain patients to high residual platinum values for years after treatment has been completed. The genetic association we report requires replication and validation before mechanistic insights can be surmised.

Summary

Serum platinum is measurable for years after completion of cisplatin-based chemotherapy. We report the largest investigation of serum platinum levels to date of 1,010 testicular cancer survivors assessed 1-35 years after cisplatin-based chemotherapy and evaluate genetic contributions to these levels. Eligible testicular cancer survivors given 300 or 400 (±15) mg/m^2 cisplatin underwent extensive audiometric testing, clinical examination, completed questionnaires and had crude serum platinum levels measured. Associations between serum platinum and various risk factors and toxicities were assessed after fitting a bi-exponential model adjusted for follow-up time and cumulative cisplatin dose. A GWAS was performed using the serum platinum residuals of the dose and time-adjusted model. Serum platinum levels

exceeded the reference range for approximately 31 years, with a strong inverse relationship with creatinine clearance at follow-up (age-adjusted $p = 2.13x10^{-3}$). We observed a significant, positive association between residual platinum values and luteinizing hormone (age-adjusted $p=6.58x10^{-3}$). Patients with high residual platinum levels experienced greater Raynaud phenomenon than those with medium or low levels (age-adjusted $OR_{high/low} = 1.46$; $p = 0.04$), as well as a higher likelihood of developing tinnitus (age-adjusted $OR_{high/low} = 1.68$, $p = 0.07$). GWAS identified one SNP meeting genome-wide significance rs1377817 ($p=4.6x10^{-8}$, a SNP intronic to *MYH14)*. This study indicates that residual platinum values are correlated with several cisplatin-related toxicities. One genetic variant is associated with these levels.

Disclaimer

Chapter 3 has been previously published as the following paper:

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Accordingly, all figures and tables presented in this chapter were derived from previously published data.

CHAPTER 4. VALIDATION OF PREVIOUS CLINICAL CHARACTERISTICS AND GENETIC PREDISPOSITIONS TO CISPLATIN-ASSOCIATED OTOTOXICITY

Introduction

Cisplatin-associated ototoxicity is a common adverse event experienced in testicular cancer survivors, with 75-80% patients developing hearing loss and 40% developing tinnitus (13). Although cisplatin has been in clinical use for over 40 years, it remains difficult to identify the subset of patients who may develop ototoxicity following therapy completion. Consequently, the identification of non-genetic risk factors, comorbidities, and genetic biomarkers to determine which patients are predisposed to developing cisplatin-induced hearing loss or tinnitus would be of tremendous benefit, enabling clinicians to more accurately counsel patients of potential risks and probable adverse toxicities prior to treatment initiation.

Previously, we determined from the Platinum Study that cumulative cisplatin dose and hypertension were associated with hearing loss (n = 488) (13), while cumulative cisplatin dose, hearing loss, persistent dizziness/vertigo, hypertension, psychotropic drug use, and self-reported health were associated with tinnitus (n = 762) (36). Although this underscores certain non-genetic risk factors and comorbidities associated with cisplatin-associated ototoxicity, it does not indicate whether genetic variation influences ototoxicity risk. Through agnostic genome-wide analyses, Dolan and colleagues have previously identified SNPs in *WFS1* (rs62283056; $p = 1.4x10^{-8}$) (57) and *OTOS* (rs7606353; $p = 1.90x10^{-6}$) (36) to be associated with increased susceptibility to developing hearing loss and tinnitus, respectively, following completion of cisplatin-based chemotherapy. However, previous candidate gene studies have

identified other SNPs to be associated with cisplatin-associated ototoxicity that have later failed to replicate in subsequent analyses (161). Although the *WFS1* SNP (rs62283056) was replicated in an independent Canadian study of 229 testicular cancer patients ($p = 5.67x10^{-3}$) (52), the *OTOS* SNP (rs7606353) has yet to be evaluated in an independent replication cohort. Further, the non-genetic risk factors and comorbidities we found for cisplatin-associated ototoxicity need to be confirmed using a larger sample size of patients. In this study, we aim to replicate previously identified non-genetic and genetic associations with cisplatin-induced hearing loss and tinnitus through the use of an uncharacterized cohort of testicular cancer survivors from the Platinum Study and use the cohort to identify novel associations. Specifically, we evaluate associations independently in the replication cohort, and then combine these individuals with those previously included in the analyses of cisplatin-induced hearing loss and/or tinnitus to form an expanded cohort for a mega-analysis of both ototoxicity phenotypes.

Results

Associations with Risk Factors and Comorbidities in the Expanded Cohort

Both age at diagnosis (hearing loss: β/10 years = 0.54, 95% CI: 0.49-0.59, $p < 2x10^{-16}$; tinnitus: OR/10 years = 1.31, 95% CI: 1.12-1.54, p = 0.001) and age at clinical examination (hearing loss: β/10 years = 0.56, 95% CI: 0.52-0.61, $p < 2x10^{-16}$; tinnitus: OR/10 years = 1.32, 95% CI: 1.15-1.52, $p = 1.16x10^{-4}$) were associated with cisplatin-induced hearing loss and tinnitus (Table 7). Cumulative cisplatin dose was also significantly associated with hearing loss and tinnitus incidence (hearing loss: β/100 mg/m^2 = 0.17, 95% CI: 0.09-0.26, $p = 7.48x10^{-5}$; tinnitus: OR/100 mg/m^2 = 1.41, 95% CI: 1.14-1.75, p = 0.001). Patients who exceeded 400 mg/m^2 cisplatin had a notably

increased likelihood of developing clinical levels of hearing loss (> 20 dB increase from 4-12 kHz: 65.5% vs. 46.3%, p = 0.01) or tinnitus (42.9% vs. 19.3%, p = 3.44×10^{-5}) when compared to patients who received 400 mg/m^2 (Figure 7), indicative of a dosing threshold.

Smoking status (ever smoking: age and dose-adjusted β = 0.04, 95% CI: -0.06-0.13, p = 0.45; chronic (> 15 years) smoking: age and dose-adjusted β = 0.16, 95% CI: -0.04-0.35, p = 0.11; currently smoking: age and dose-adjusted β = -0.02, 95% CI: -0.20-0.15, p = 0.76) did not appear to be associated with cisplatin-induced hearing loss (Table 7). Although ever smoking was not associated with cisplatin-induced tinnitus (age and dose-adjusted OR = 1.25, 95% CI: 0.94-1.67, p = 0.13), chronic smoking (age and dose-adjusted OR = 2.01, 95% CI: 1.19-3.38, p = 0.007) and current smoking status (age and dose-adjusted OR = 1.65, 95% CI: 1.02-2.60, p = 0.04) were statistically significant. Excessive drinking was not significantly associated with either phenotype (hearing loss: age and dose-adjusted β = 0.09, 95% CI: -0.04-0.23, p = 0.19; tinnitus: age and dose-adjusted OR = 0.76, 95% CI: 0.47-1.17, p = 0.23). In contrast, hearing loss and tinnitus were significantly associated with hypertension (hearing loss: age and dose-adjusted β = 0.25, 95% CI: 0.10-0.39, p = 8.50×10^{-4}; tinnitus: age and dose-adjusted OR = 2.62, 95% CI: 1.72-3.97, p = 6.83×10^{-6}). Cisplatin-induced hearing tinnitus, not hearing loss was associated with hypercholesterolemia (hearing loss: age and dose-adjusted β = 0.03, 95% CI: -0.12-0.19, p = 0.67; tinnitus: age and dose-adjusted OR =1.78, 95% CI: 1.13-2.75, p = 0.01) (Table 7).

Cisplatin-induced tinnitus was associated with hearing loss (age and dose-adjusted OR = 3.88, 95% CI: 3.00-5.11, p < 2×10^{-16}). Patients with hearing loss or

tinnitus were more likely to experience peripheral sensory neuropathy (hearing loss: age-adjusted $\beta = 0.11$, 95% CI: 0.06-0.16, $p = 5.88 \times 10^{-5}$; tinnitus: age-adjusted OR = 2.65, 95% CI: 2.13-3.31, $p < 2 \times 10^{-16}$) (Table 7). Cisplatin-induced hearing loss (age and dose-adjusted $\beta = 0.15$, 95% CI: -0.07-0.37, $p = 0.11$) was not significantly associated with persistent dizziness or vertigo, while cisplatin-induced tinnitus (age and dose-adjusted OR = 7.18, 95% CI: 4.03-12.98, $p = 3.93 \times 10^{-11}$) demonstrated a highly significant association. Similarly, only tinnitus was associated with higher psychotropic drug use (hearing loss: age-adjusted $\beta = 0.12$, 95% CI: -0.05-0.30, $p = 0.17$; tinnitus: age-adjusted OR = 2.53, 95% CI: 1.37-4.53, $p = 0.002$). Self-reported health was significantly associated with hearing loss and tinnitus (hearing loss: age-adjusted $\beta = 0.11$, 95% CI: 0.06-0.16, $p = 5.88 \times 10^{-5}$; tinnitus: age-adjusted OR = 1.83, 95% CI: 1.53-2.19, $p = 9.40 \times 10^{-11}$). When compared to the previous studies of cisplatin-induced hearing loss and tinnitus, it appears that previously identified associations remained statistically significant (Table 7), thereby verifying their statistical significance in an expanded cohort of patients. The only exception was the association between cisplatin-induced tinnitus and hypercholesterolemia, which was not previously statistically significant (expanded $p = 0.01$ vs. original $p = 0.09$) (Table 7).

Due to the high association between hearing loss and tinnitus, we included both phenotypes as covariates to determine whether the previously identified associations were confounded by either hearing disorder. After the inclusion of tinnitus as a covariate in the hearing loss analysis, only the associations with age at diagnosis, age at clinical examination, cumulative cisplatin dose, and self-reported health remained statistically

Table 7. Regression Analysis of Previously Identified Associations Between Cisplatin-Induced Hearing Loss or Tinnitus and Relevant Clinical Characteristics.

Hearing Loss[1]					
Clinical Characteristic	**n**	**Age and Dose-Adjusted β (95% CI)**	**Age and Dose-Adjusted p**	**Original Study β (95% CI)**	**Original Study p**
Age at Cancer Diagnosis*	1,258	0.54 (0.49, 0.59)	**$2x10^{-16}$**	N/A	N/A
Age at Clinical Examination*	1,258	0.56 (0.52, 0.61)	**$2x10^{-16}$**	N/A	N/A
Cumulative Cisplatin Dose*	1,258	0.17 (0.09, 0.26)	**$7.48x10^{-5}$**	N/A	**0.001**
Excessive Drinking	1,239	0.09 (-0.04, 0.23)	0.19	N/A	N/A
Ever Smoker	1,242	0.04 (-0.06, 0.13)	0.45	N/A	0.10
Current Smoker	1,204	-0.02 (-0.20, 2.60)	0.76	N/A	0.08
Hypertension	1,191	0.25 (0.10, 0.39)	**$8.50x10^{-4}$**	N/A	**0.0066**
Hypercholesterolemia	1,195	0.03 (-0.12, 0.19)	0.67	N/A	N/A
Psychotropic Drug Use	729	0.12 (-0.05, 0.30)	0.17	N/A	N/A
Peripheral Sensory Neuropathy	1,237	0.15 (0.08, 0.22)	**$7.55x10^{-6}$**	N/A	N/A
Persistent Dizziness or Vertigo	1,170	0.15 (-0.07, 0.37)	0.11	N/A	N/A
Self-Reported Health	1,235	0.11 (0.06, 0.16)	**$5.88x10^{-5}$**	N/A	N/A
Tinnitus[2]					

Clinical Characteristic	n	Age and Dose-Adjusted OR (95% CI)	Age and Dose-Adjusted p	Original Study OR (95% CI)	Original Study p
Age at Cancer Diagnosis*	1,217	1.31 (1.12, 1.54)	**0.001**	1.31 (1.1, 1.6)	**0.006**
Age at Clinical Examination*	1,217	1.32 (1.15, 1.52)	**1.16×10^{-4}**	1.30 (1.1,1.5)	**0.002**
Cumulative Cisplatin Dose*	1,217	1.41 (1.14, 1.75)	**0.001**	1.38 (1.1, 1.7)	**0.007**
Excessive Drinking	1,205	0.76 (0.47, 1.17)	0.23	0.92 (0.5, 1.5)	0.12
Ever Smoker	1,209	1.25 (0.94, 1.67)	0.13	1.26 (0.9, 1.7)	0.20
Chronic Smoker (> 15 years)	454	2.01 (1.19, 3.38)	**0.01**	2.07 (1.2, 3.8)	**0.005**
Hypertension	1,148	2.62 (1.72, 3.97)	**6.83×10^{-6}**	1.77 (1.02, 3.0)	**0.039**
Hypercholesterolemia	1,160	1.78 (1.13, 2.75)	**0.01**	1.6 (0.9, 2.8)	0.09
Psychotropic Drug Use	626	2.53 (1.37, 4.53)	**0.002**	2.40 (1.3, 4.4)	**0.003**
Peripheral Sensory Neuropathy	1,206	2.65 (2.13, 3.31)	**< 2×10^{-16}**	Range: 1.66-2.72	**< 0.0001**
Persistent Dizziness or Vertigo	1,146	7.18 (4.03, 12.98)	**3.93×10^{-11}**	6.40 (3.2, 12.9)	**< 0.0001**
Self-Reported Health	1,201	1.83 (1.53, 2.19)	**9.40×10^{-11}**	0.54 (0.4-0.7), reported in opposite direction	**< 0.0001**

Table 7 Continued.

*Clinical characteristics were only analyzed using univariate analysis.

[1]Original study p-values for cisplatin-induced hearing loss are derived from Frisina et al. (13).

[2]Original study p-values for cisplatin-induced tinnitus are derived from El Charif et al (36).

Bold indicates $p \leq 0.05$.

Table 8. Regression Analysis of Cisplatin-Induced Hearing Loss or Tinnitus Adjusted for Age at Clinical Examination, Cumulative Cisplatin Dose, and the Other Hearing Disorder.

Hearing Loss			
Clinical Characteristic	**n**	**β (95% CI)**	**p**
Age at Cancer Diagnosis*	1,258	0.49 (0.43, 0.55)	**$< 2x10^{-16}$**
Age at Clinical Examination*	1,258	0.53 (0.48, 0.57)	**$< 2x10^{-16}$**
Cumulative Cisplatin Dose*	1,258	0.13 (0.04, 0.23)	**0.005**
Current Smoker	1,204	-0.03 (-0.23, 0.17)	0.76
Chronic Smoker (> 15 years)	432	0.02 (-0.17, 0.22)	0.80
Hypertension	1,191	0.09 (-0.06, 0.25)	0.30
Hypercholesterolemia	1,195	-0.05 (-0.21, 0.11)	0.53
Psychotropic Drug Use	729	-0.10 (-0.30, 0.09)	0.31
Peripheral Sensory Neuropathy	1,237	0.06 (-0.01, 0.13)	0.11
Persistent Dizziness or Vertigo	1,170	0.05 (-0.20, 0.30)	0.70
Self-Reported Health	1,235	0.06 (0.02, 0.12)	**0.04**
Tinnitus			
Clinical Characteristic	**n**	**OR (95% CI)**	**p**
Age at Cancer Diagnosis*	1,217	0.76 (0.61, 0.95)	**0.02**

Age at Clinical Examination*	1,217	0.71 (0.58, 0.88)	**0.002**
Cumulative Cisplatin Dose*	1,217	1.15 (0.87, 1.51)	0.33
Current Smoker	1,175	2.70 (1.39, 5.13)	**0.002**
Chronic Smoker (> 15 years)	454	1.79 (0.86, 3.70)	0.12
Hypertension	1,148	2.33 (1.39, 3.89)	**0.001**
Hypercholesterolemia	1,160	1.76 (1.02, 3.02)	**0.04**
Psychotropic Drug Use	626	2.47 (1.19, 5.00)	**0.01**
Peripheral Sensory Neuropathy	1,206	2.47 (1.89, 3.24)	**4.38×10^{-11}**
Persistent Dizziness or Vertigo	1,146	6.37 (2.87, 14.46)	**6.45×10^{-6}**
Self-Reported Health	1,201	1.63 (1.31, 2.03)	**0.02**

Table 8 Continued.
*Clinical characteristics were only analyzed using the other hearing disorder as a covariate.
Bold indicates $p \leq 0.05$.

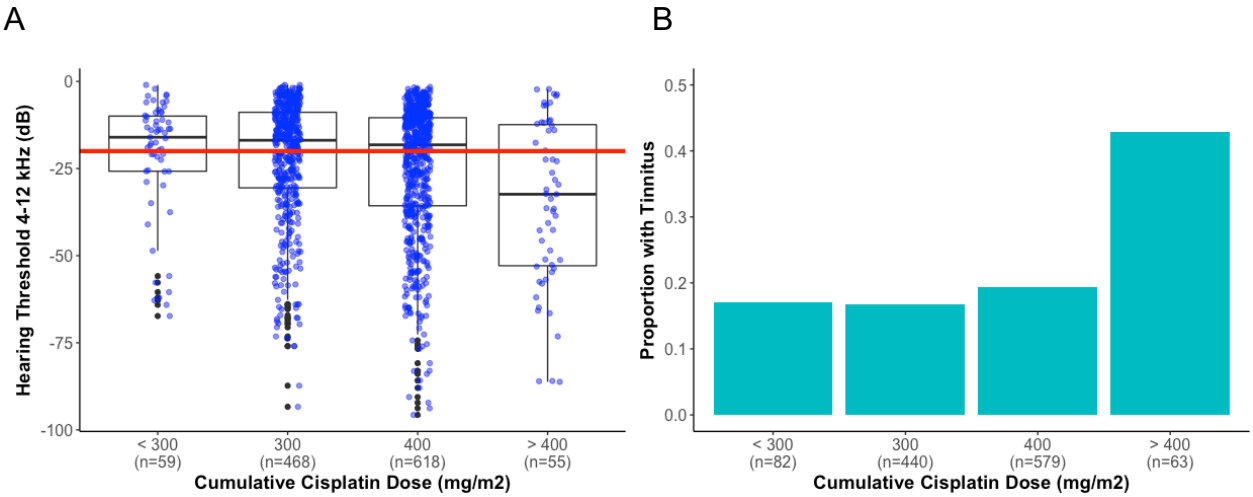


Figure 7. Effects of Cumulative Cisplatin Dose on Proportion of Patients with Cisplatin-Associated Ototoxicity. The overall proportion of testicular cancer survivors with **A)** hearing loss or **B)** tinnitus based on cumulative cisplatin dose is provided. Clinical hearing loss is defined as hearing thresholds that increase by ≥ 20 dB, and is denoted by the red line in panel A. Sample sizes for each group are indicated within each panel on the x-axis. Patients who exceeded 400 mg/m^2 cisplatin had a notably increased likelihood of developing clinical levels of hearing loss ($p = 0.01$) or tinnitus ($p = 3.44 \times 10^{-5}$) when compared to patients who received 400 mg/m^2 as determined by the two-proportions z-test.

significant (Table 8). By contrast, all of the identified non-genetic risk factors and comorbidities for tinnitus remained statistically significant when hearing loss was included as a covariate, with the exception of cumulative cisplatin dose and chronic smoking (Table 8).

Genome-Wide Association Studies

GWAS of cisplatin-induced hearing loss in the expanded cohort (n = 1,079) identified two near genome-wide significant SNPs in chromosome 1 (rs1391812, $p = 6.20 \times 10^{-8}$; rs1004517, $p = 7.08 \times 10^{-8}$) (Figure 8A and B, Appendix Table 7) that were in high linkage disequilibrium (LD) ($R^2 = 0.98$, $p < 0.0001$). Neither SNP appeared to influence gene expression or transcription factor binding, nor did any of the other SNPs in LD with rs1391812 (Figure 9). In addition, the previously identified *WFS1* SNP (rs62283056) remained marginally associated with cisplatin-induced hearing loss ($p = 4.76 \times 10^{-6}$), and was the tenth most significant SNP in the mega-analysis. The individual SNP test in previously unexamined testicular cancer survivors (n = 710) also identified rs62283056 as statistically significant (p = 0.03). Gene-based association analysis of cisplatin-induced hearing loss identified *TXNRD1* ($p = 5.78 \times 10^{-7}$) as genome-wide significant ($p = 2.70 \times 10^{-6}$) (Figure 10).

GWAS of cisplatin-induced tinnitus in the expanded cohort (n = 1,044) identified no genome-wide significant SNPs (Figure 8C and D, Appendix Table 8). The most significant SNP was rs4752423 ($p = 3.34 \times 10^{-7}$), which was in perfect LD with the next three most significant SNPs (rs4752422, $p = 3.74 \times 10^{-7}$; rs1475373, $p = 3.74 \times 10^{-7}$; rs4237520, $p = 3.74 \times 10^{-7}$) ($R^2 = 1.0$, $p < 0.0001$). All three SNPs are eQTLs for *PPAPDC1A*, as are six other SNPs in LD with rs4752423 (Figure 11). The previously

identified *OTOS* SNP (rs7606353) had a p-value of $1.45x10^{-4}$ in the mega-analysis, and a p-value of 0.70 in the individual SNP test of previously unexamined patients, indicating that the SNP no longer remained associated with cisplatin-induced tinnitus. Gene-based association analysis of cisplatin-induced tinnitus identified *WNT8A* ($p = 2.01x10^{-7}$) as genome-wide significant ($p = 2.66x10^{-6}$) (Figure 10).

Discussion

The current study is important for the clinical evaluation of testicular cancer survivors because it replicates previously identified non-genetic and genetic associations with cisplatin-associated ototoxicity in an expanded cohort of survivors, and identifies novel associations that further characterize toxicity risk. Our data indicate that patients who develop cisplatin-induced hearing loss are more likely to experience hypertension, while those with cisplatin-induced tinnitus are more likely to experience persistent dizziness/vertigo, hypertension, poorer self-reported health, and psychotropic drug use, consistent with our previous studies (13, 36). However, the current study indicates that these associations are more consistent with tinnitus incidence than hearing loss incidence. Further, non-genetic risk factors and comorbidities that were associated with hearing loss no longer remained statistically significant after controlling for tinnitus, with the exception of self-reported health. Therefore, the development of tinnitus following cisplatin-based chemotherapy may be more indicative of poorer quality of life than hearing loss.

It is also interesting to note that incidence of hearing loss and tinnitus remain fairly consistent until patients receive a cumulative cisplatin dose exceeding 400 mg/m^2.

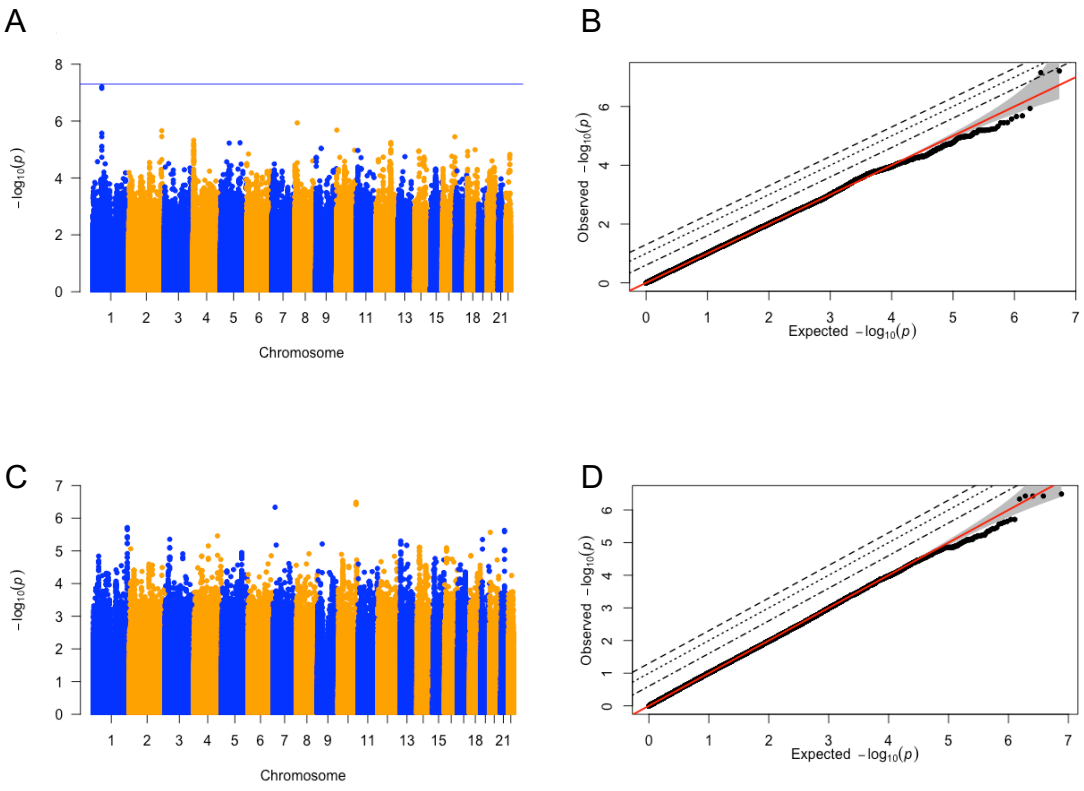


Figure 8. Genome-Wide Association Studies of Cisplatin-Associated Ototoxicity in an Expanded Cohort of Testicular Cancer Survivors. A) Manhattan plot of GWAS results for cisplatin-induced hearing loss reveals two near genome-wide significant SNPs in chromosome 1 (rs1391812, p = 6.20×10^{-8}; rs1004517, p = 7.08×10^{-8}). **B)** Quantile-Quantile plot of GWAS results for cisplatin-induced hearing loss. **C)** Manhattan plot of GWAS results for cisplatin-induced tinnitus reveals no genome-wide significant SNPs. **D)** Quantile-Quantile plot of GWAS results for cisplatin-induced tinnitus. Covariates for the cisplatin-induced hearing loss GWAS include cumulative cisplatin dose, age at clinical examination, and 10 genetic principal components accounting for population substructure. Covariates for the cisplatin-induced tinnitus GWAS include cumulative cisplatin dose, age at diagnosis, noise exposure and 5 genetic principal components accounting for population substructure.

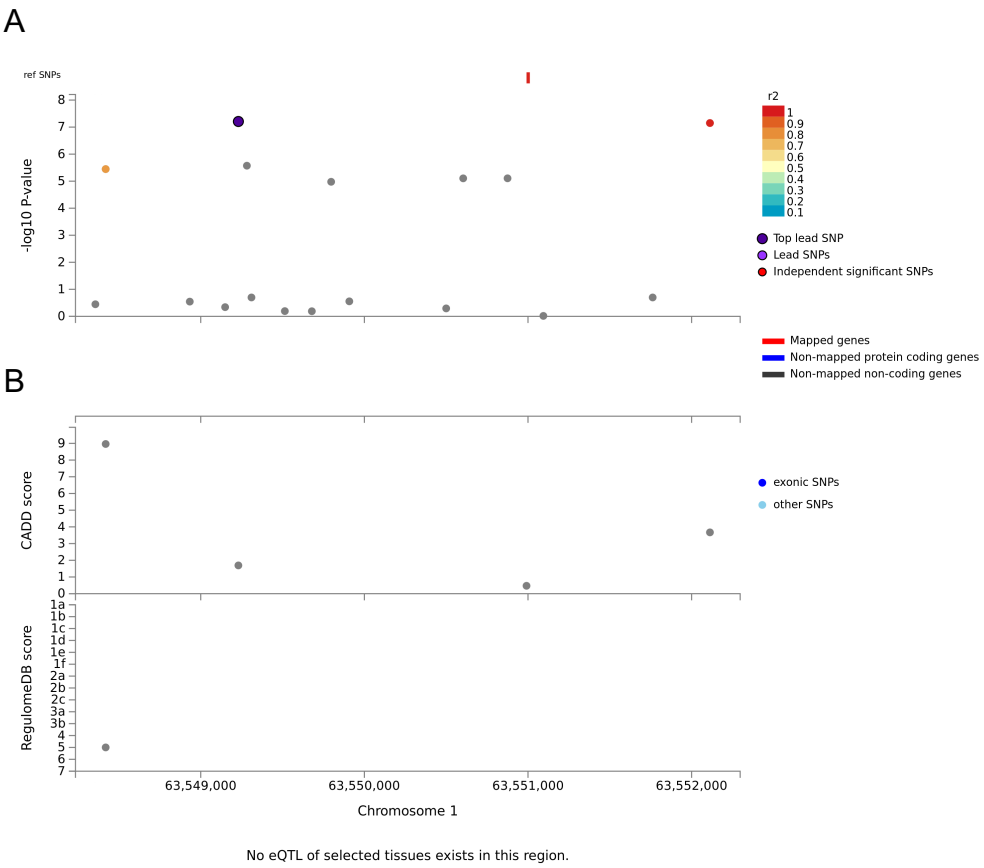


Figure 9. Regional Plot of rs1391812 for GWAS of Cisplatin-Induced Hearing Loss. **A)** A regional plot of the most significant GWAS signal (rs1391812) was generated in FUMA. Each point represents a SNP. The x-axis indicates chromosomal position, while the y-axis shows $-\log_{10}$(p-value) of association with cisplatin-induced hearing loss. The LD (R^2) of each SNP with the top signal in the region, rs1391812 (purple), is denoted by color in the legend, along with whether the gene has been mapped. **B)** SNPs that were in LD with rs1391812 were evaluated for CADD and Regulome DB scores and underwent eQTL analysis. After evaluation, SNPs were plotted in accordance with their chromosomal position. rs1391812 did not appear to influence gene expression or transcription factor binding, nor did any of the SNPs in LD.

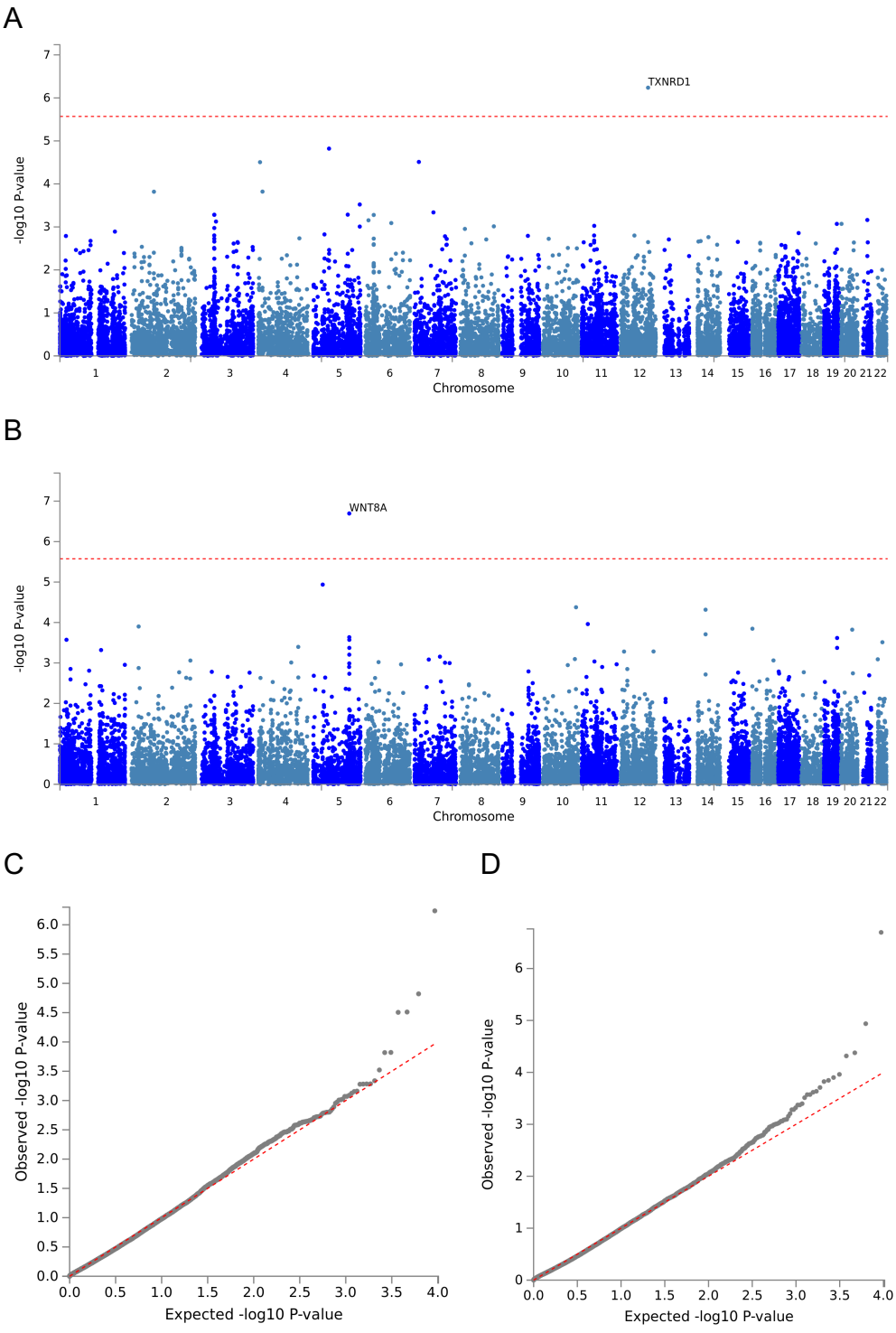


Figure 10. Gene-Based Genome-Wide Association Analyses of Cisplatin-Associated Ototoxicity. Summary statistics for the SNP-based GWAS were uploaded to FUMA to run a gene-based association analysis based on a multiple linear principal

Figure 10. Gene-Based Genome-Wide Association Analyses of Cisplatin-Associated Ototoxicity.

components regression to determine the aggregated effect of all SNPs within a gene. Inputted SNPs were mapped to 18,544 and 18,819 protein coding genes for hearing loss and tinnitus, respectively, producing a significance threshold of $p = 0.05/18,544$ (2.70×10^{-6}) or $p = 0.05/18,819$ (2.66×10^{-6}). **A)** Manhattan plot of the gene-based association analysis for cisplatin-induced hearing loss identified *TXNRD1* ($p = 5.78 \times 10^{-7}$) as genome-wide significant. **B)** Manhattan plot of the gene-based association analysis for cisplatin-induced tinnitus identified *WNT8A* ($p = 2.01 \times 10^{-7}$) as genome-wide significant. Quantile-Quantile plots of results from the gene-based association analysis for **C)** cisplatin-induced hearing loss and **D)** cisplatin-induced tinnitus are also provided.

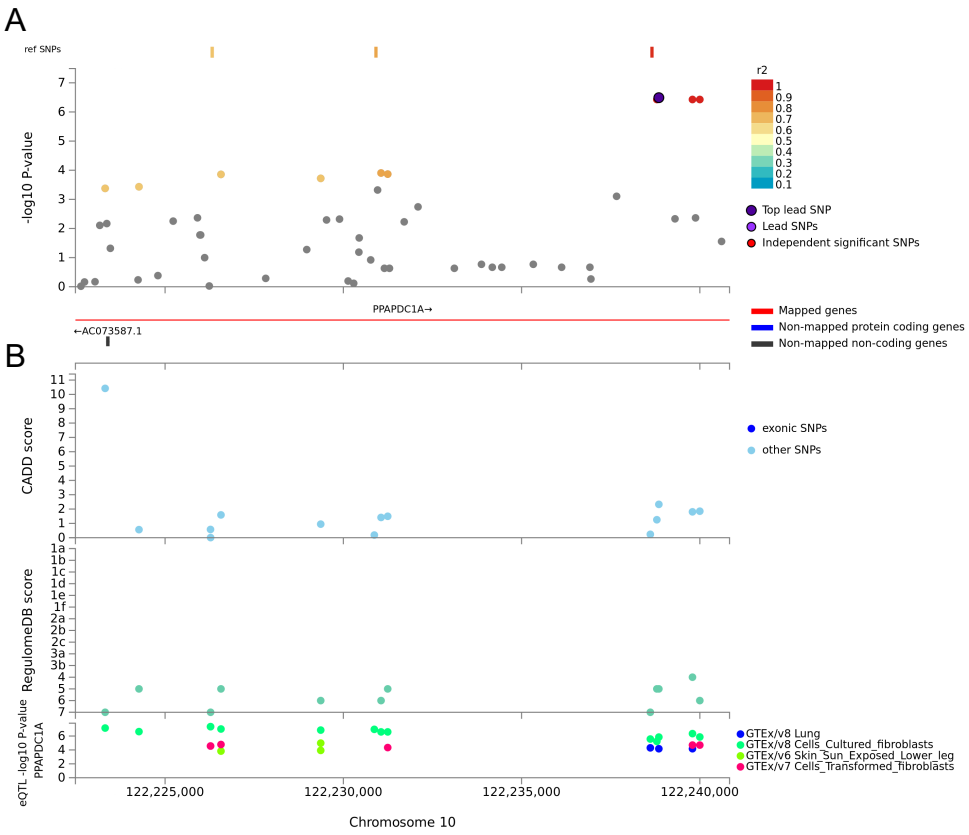


Figure 11. Regional Plot of rs4752423 for GWAS of Cisplatin-Induced Tinnitus. A) A regional plot of the most significant GWAS signal (rs4752423) was generated in FUMA. Each point represents a SNP. The x-axis indicates chromosomal position, while the y-axis shows $-\log_{10}$(p-value) of association with cisplatin-induced tinnitus. The LD (R^2) of each SNP with the top signal in the region, rs4752423 (purple), is denoted by color in the legend, along with whether the gene has been mapped. **B)** SNPs that were in LD with rs4752423 were evaluated for CADD and Regulome DB scores and underwent eQTL analysis. After evaluation, SNPs were plotted in accordance with their chromosomal position. rs4752423 is in perfect LD with the next three most significant SNPs (rs4752422, rs1475373, and rs4237520). All three SNPs are eQTLs for *PPAPDC1A*, as are six other SNPs in LD with rs4752423.

This threshold was previously identified for cisplatin-induced tinnitus (36), but was not evaluated for cisplatin-induced hearing loss (13). Due to the large sample sizes used in the hearing loss and tinnitus cohorts, the existence of a dosing threshold in which ototoxicity incidence markedly increases is probable, and is in accord with a threshold for cisplatin-induced peripheral neuropathy in which most cases do not occur until a threshold cumulative dose of 300 mg/m^2 is reached (162), and almost all patients receiving a cumulative dose of 500-600 mg/m^2 have objective evidence of nerve damage (163). The high probability for ototoxicity and other toxicities for patients who exceed 400 mg/m^2 cisplatin indicate that these patients should be made aware of their likelihood of developing multiple persistent toxicities following the completion of therapy, and should be monitored for indicative symptoms.

The GWAS of cisplatin-induced hearing loss in the expanded cohort identified two near genome-wide significant SNPs in chromosome 1 (rs1391812, $p = 6.20 \times 10^{-8}$; rs1004517, $p = 7.08 \times 10^{-8}$), but neither SNP appeared to be located in a gene or had notable biological significance. However, gene-based association analysis identified *TXNRD1* ($p = 5.78 \times 10^{-7}$) as genome-wide significant. *TXNRD1* encodes for thioredoxin reductase 1, which is used in the protection against oxidative stress, a mechanism by which cisplatin induces ototoxicity (164). Accordingly, it has been recently demonstrated that cisplatin inhibits the function of thioredoxin reductase 1, inducing profound nephrotoxicity *in vivo* 72 hours after administration (165). In addition, upregulated levels of *TXNRD1* are detected in rat primary hepatocytes following cisplatin treatment (166). Although these studies do not directly link thioredoxin reductase 1 activity directly to ototoxicity, we have previously demonstrated that high levels of serum platinum reduce

renal function in testicular cancer survivors (167). Since cisplatin is eliminated predominantly through renal clearance (30), reduced kidney function would inevitably increase the levels of platinum in circulation, ultimately potentiating the persistence of other toxicities, including ototoxicity.

The GWAS of cisplatin-induced tinnitus in the expanded cohort identified no genome-wide significant SNPs, but the most significant SNP, rs4752423 ($p = 3.34x10^{-7}$), was in LD with eight SNPs that were all eQTLs for *PPAPDC1A*. Nevertheless, the association between *PPAPDC1A* and cisplatin-induced tinnitus is difficult to surmise as the gene encodes for a phosphatidate phosphatase that has not previously been associated with cisplatin sensitivity or inner ear/nervous system development and maintenance. By contrast, gene-based association analysis of cisplatin-induced tinnitus identified *WNT8A* ($p = 2.01x10^{-7}$) as genome-wide significant. *WNT8A* encodes for a glycoprotein that is involved in several major signaling pathways, including the canonical Wnt/β-catenin signaling pathway. As with thioredoxin reductase 1, Wnt has been associated with kidney damage, as tenascin-C recruits Wnt ligands at sites of kidney injury to create a favorable microenvironment for tubular repair and regeneration (168). Further, it is known that Wnt/β-catenin signaling modulates inner ear/brain development and function. Notably, Wnt8A interacts with LRP6 to promote synaptogenesis, particularly in the formation of excitatory synapses (169). Wnt8A also appears to influence development of the inner ear, as its inactivation prevents proper development of the otic vesicle (170). Therefore, genetic variation in Wnt8a could influence Wnt signaling in several key tissues relevant to cisplatin toxicity.

The GWAS results for cisplatin-induced hearing loss and tinnitus highlight the potential utility of performing multiple genome-wide analytical approaches, as gene-based association analysis identified plausible candidate genes of cisplatin-associated ototoxicity that warrant further investigation. In our previous GWAS of hearing loss and tinnitus, we employed a traditional SNP-based approach. Such analyses are limited to the effect size of common genetic variants, which are often modest for complex traits (171). Through our gene-based approach, we were able to increase the overall power of our genetic analyses by examining the aggregated effect of SNPs in an individual gene on its association with the phenotype, and identified *TXNRD1* and *WNT8A* as genome-wide significant. Further, gene-based approaches reduce the threshold for statistical significance due to the fewer number of tests being performed. Consequently, by using an additional genome-wide analytical technique in the expanded cohort of testicular cancer survivors, we were able identify two novel genome-wide associations that have plausible biological significance to influence ototoxicity incidence following cisplatin-based chemotherapy.

Nevertheless, our SNP-based approach still had considerable utility in evaluating our prior associations of SNP variation in *WFS1* and *OTOS* and cisplatin-associated ototoxicity. The mega-analysis of the expanded cohort indicated that the *WFS1* SNP (rs62283056) was highly associated with cisplatin-induced hearing loss ($p = 4.76x10^{-6}$), which was confirmed in the individual SNP test of previously unexamined patients ($p = 0.03$). Since rs62283056 has previously been replicated in an independent Canadian study, it is highly probable that this SNP is a valid predictor of hearing loss susceptibility for testicular cancer survivors treated with cisplatin. By contrast, the *OTOS* SNP

(rs7606353) was far less associated with cisplatin-induced tinnitus in patients who were not previously examined ($p = 0.74$) than the original cohort ($p = 1.90 \times 10^{-6}$) (36), as was the case in the expanded cohort mega-analysis ($p = 1.45 \times 10^{-4}$). Therefore, the use of rs7606353 as a predictive biomarker for cisplatin-induced tinnitus is not supported by the expanded cohort study.

Major strengths of our study include the comprehensiveness of the Platinum Study questionnaire that enables us to develop robust phenotypes for hearing loss and tinnitus in an expanded cohort of testicular cancer survivors who all received cisplatin-based chemotherapy. Since all patients were enrolled in the Platinum Study, we were able to evaluate the replicability of previous associations with cisplatin-induced hearing loss and tinnitus in a larger group of patients using the same definitions for clinical characteristics. Further, the comprehensiveness of our phenotypic correlation analysis enabled us to identify novel non-genetic risk factors and comorbidities associated with cisplatin-induced hearing loss and tinnitus. An inherent limitation of our study is the fact that the previous GWASs of cisplatin-induced hearing loss and tinnitus and the mega-analyses in the current study were genotyped at different facilities with different genotyping chips (previous GWAS: HumnaOmniExpressExome chip at the RIKEN Center; expanded cohort GWAS: Infinium Global Screening Array-24 chip at Regeneron Pharmaceuticals). The use of different genotyping chips and sample sizes for genotyping indicate that the alleles included and their corresponding frequencies may have been slightly different for individuals included in both the original and expanded cohorts, which would influence GWAS results. Ideally, patients who were not previously included in the GWAS would have also been genotyped by RIKEN, which would have

enabled us to perform a mega-analysis with the original genotyping data. Nevertheless, we used the same quality control criteria and covariates that were used in the original analyses. Further, rs62283056 was replicated using the different genotyping method, which is important for its potential utility as a biomarker of cisplatin-induced hearing loss because patients will inevitably be genotyped using different methods based on the institution where they receive care. However, due to the nature of testicular cancer, and its propensity to affect men of European ancestry (172), we are unable to determine whether race or sex influences susceptibility to cisplatin-associated ototoxicity. Further, the genetic risk factors identified in the Platinum Study are limited to patients of European ancestry due to the variation in allele frequencies among different races (173). These limitations ultimately highlight the importance of using different cohorts to identify potential risk factors and comorbidities of drug-induced toxicities.

Conclusion

In summary, previous non-genetic and genetic risk factors for cisplatin-induced hearing loss and tinnitus were validated in an expanded cohort of testicular cancer survivors from the Platinum Study. The majority of these clinical characteristics (hypertension, hypercholesterolemia, psychotropic drug use, peripheral sensory neuropathy, and persistent dizziness or vertigo) are more associated with tinnitus incidence than hearing loss incidence. In addition, susceptibility to hearing loss and tinnitus markedly increases in patients who received cumulative cisplatin doses > 400 mg/m^2. Gene-based association analysis identified genetic variation in *TXNRD1* and *WNT8A* to be associated with hearing loss and tinnitus predisposition following cisplatin-based chemotherapy, respectively. Mega-analysis validated the association

between rs62283056 and cisplatin-induced hearing loss, but not the association between rs7606353 and cisplatin-induced tinnitus. In view of these findings, health care providers can improve management of cancer survivors by monitoring patients who develop tinnitus following cisplatin-based chemotherapy for overall health, as well as those who exceeded a cumulative cisplatin dose of 400 mg/m^2. In addition, investigation into the mechanisms by which *TXNRD1* and *WNT8A* genetic variation could influence susceptibility to cisplatin-associated ototoxicity is warranted. Due to its replication in two separate analyses, evaluation of rs62283056 as a predictive biomarker of cisplatin-induced hearing loss in testicular cancer survivors in a clinical trial is also warranted, and may better inform patients of their risk to adverse toxicities prior to the initiation of treatment.

Summary

Cisplatin-associated ototoxicity has previously been associated with several non-genetic and genetic risk factors that have yet to be validated in a replication analysis. We aimed to validate these associations with through the use of an uncharacterized cohort of testicular cancer survivors from the Platinum Study, as well as use the cohort to identify novel associations. Linear and logistic regression evaluated associations of cisplatin-induced hearing loss (n = 1,258) or tinnitus (n = 1,217) with non-genetic risk factors and comorbidities among an expanded cohort of survivors from the Platinum Study. GWAS of cisplatin-induced hearing loss and tinnitus were performed based on prior analyses in the Platinum Study (hearing loss: linear regression using cumulative cisplatin dose, age at clinical examination, and 10 genetic principal components as covariates; tinnitus: logistic regression using cumulative cisplatin dose, age at

diagnosis, noise exposure, and 5 genetic principal components as covariates). Both age at diagnosis (hearing loss: $p < 2x10^{-16}$; tinnitus: $p = 0.001$) and age at clinical examination (hearing loss: $p < 2x10^{-16}$; tinnitus: $p = 1.16x10^{-4}$) were associated with cisplatin-induced hearing loss and tinnitus, as was cumulative cisplatin dose (hearing loss: $p = 7.48x10^{-5}$; tinnitus: $p = 0.001$). Patients who exceeded 400 mg/m^2 cisplatin had a notably increased likelihood of developing clinical levels of hearing loss and tinnitus, indicative of a dosing threshold. In addition, cisplatin-induced hearing loss and tinnitus were significantly associated with hypertension (hearing loss: $p = 8.50x10^{-4}$; tinnitus: $p = 6.83x10^{-6}$), peripheral sensory neuropathy (hearing loss: $p = 5.88x10^{-5}$; tinnitus: $p < 2x10^{-16}$), and poorer self-reported health (hearing loss: $p = 5.88x10^{-5}$; tinnitus: $p = 9.40x10^{-11}$) after age- and dose-adjustment. Only tinnitus was associated with hypercholesterolemia ($p = 0.01$), persistent dizziness or vertigo ($p = 3.93x10^{-11}$), and higher psychotropic drug use ($p = 0.002$). The majority of these non-genetic risk factors and comorbidities are more associated with tinnitus than hearing loss incidence, as indicated by the inclusion of the hearing disorders as covariates. GWAS of the expanded cohort indicated that the previously identified *WFS1* SNP (rs62283056) was associated with cisplatin-induced hearing loss ($p = 4.76x10^{-6}$), which was validated in an individual SNP test using a cohort of previously unexamined testicular cancer survivors ($p = 0.03$). The previously identified *OTOS* SNP (rs7606353) was not replicated based on the results of the GWAS ($p = 1.45x10^{-4}$) and individual SNP test ($p = 0.70$) for cisplatin-induced tinnitus. Gene-based association analysis of cisplatin-induced hearing loss and tinnitus identified *TXNRD1* ($p = 5.78x10^{-7}$) and *WNT8A* ($p = 2.01x10^{-7}$), respectively as genome-wide significant. Taken together, the non-genetic associations

for cisplatin-induced hearing loss and tinnitus were in accord with previous studies of cisplatin-associated ototoxicity, as was the association between rs62283056 and cisplatin-induced hearing loss. Genetic variation in *TXNRD1* and *WNT8A* may also be important for predicting genetic predisposition to cisplatin-associated ototoxicity.

CHAPTER 5. RARE VARIANT ANALYSIS OF CISPLATIN-ASSOCIATED OTOTOXICITY

Introduction

Previous genome-wide association studies (GWAS) have identified potential genetic susceptibilities of common single nucleotide polymorphisms (SNPs) to cisplatin-associated ototoxicity (36, 49, 57). However, there exists the possibility that rare genetic variants (MAF < 0.01) also contribute to genetic susceptibility, as narrow sense heritability estimates of cisplatin-induced hearing loss and tinnitus indicate that phenotypic variance is not completely explained by common genetic variants (36, 57). This issue of "missing heritability" is prevalent among complex traits because GWAS focus on the identification of common variants, and do not ascertain whether rare variants explain additional disease risk or trait variability (100). Rare variants are known to influence predisposition to human diseases and other complex traits. Specifically, 30-40% of functional variability in previously identified pharmacogenes has been attributed to rare variants (68). Consequently, the identification of rare variants through whole exome sequencing (WES), which is better suited for identifying rare genetic variants in exons that are often not captured on conventional common-variant GWAS chips (174), may be a useful strategy to examine the genetic contributions of variants that are not common SNPs. Importantly, WES has the ability to detect less common mutations while capturing larger-scale information, including copy number variants and structural variants. As demonstrated by a recent study of clopidogrel response in patients at risk of thromboembolism (69), WES has the ability to identify risk alleles for drug efficacy not

previously identified by GWAS in which the majority of influential genetic variants remain unidentified based on current heritability estimates.

In the current study, we examine the genetic contribution of rare variants to cisplatin-associated ototoxicity through single variant and gene-based tests. Specifically, we perform exome-wide association studies (ExWAS) of cisplatin-induced hearing loss and tinnitus using loss-of-function (LOF) and/or predicted deleterious missense variants located in exonic regions. We also assess the aggregated effect of all rare variants within a given gene to determine whether any genes are significantly associated with either cisplatin-induced hearing loss or tinnitus.

Results

Exome-Wide Association Studies

ExWAS of cisplatin-induced hearing loss (n = 1,079) using LOF variants identified six SNVs that nearly met the exome-wide significance threshold of $p = 3.83 \times 10^{-6}$ (Figure 12A, Appendix Table 9). However, despite the fact that most of these variants reside on different chromosomes, they have the exact same level of statistical significance ($p = 3.93 \times 10^{-6}$), suggesting that the results are unreliable. These exonic variants are located in the following genes: *REN*, *LHCGR*, *WRN*, *TUBGCP5*, *TRPM1*, and *FAM69C*). No other variant approaches exome-wide significance. When examining LOF variants and those predicted to be a deleterious mutation, only one SNV had a higher level of statistical significance (2:241048707, $p = 3.45 \times 10^{-6}$) (Figure 12B Appendix Table 10). However, due to the increased significance threshold for the inclusion of predicted deleterious variants ($p = 1.80 \times 10^{-7}$), the SNV did not approach exome-wide significance. ExWAS of cisplatin-induced tinnitus (n = 1,044) using either

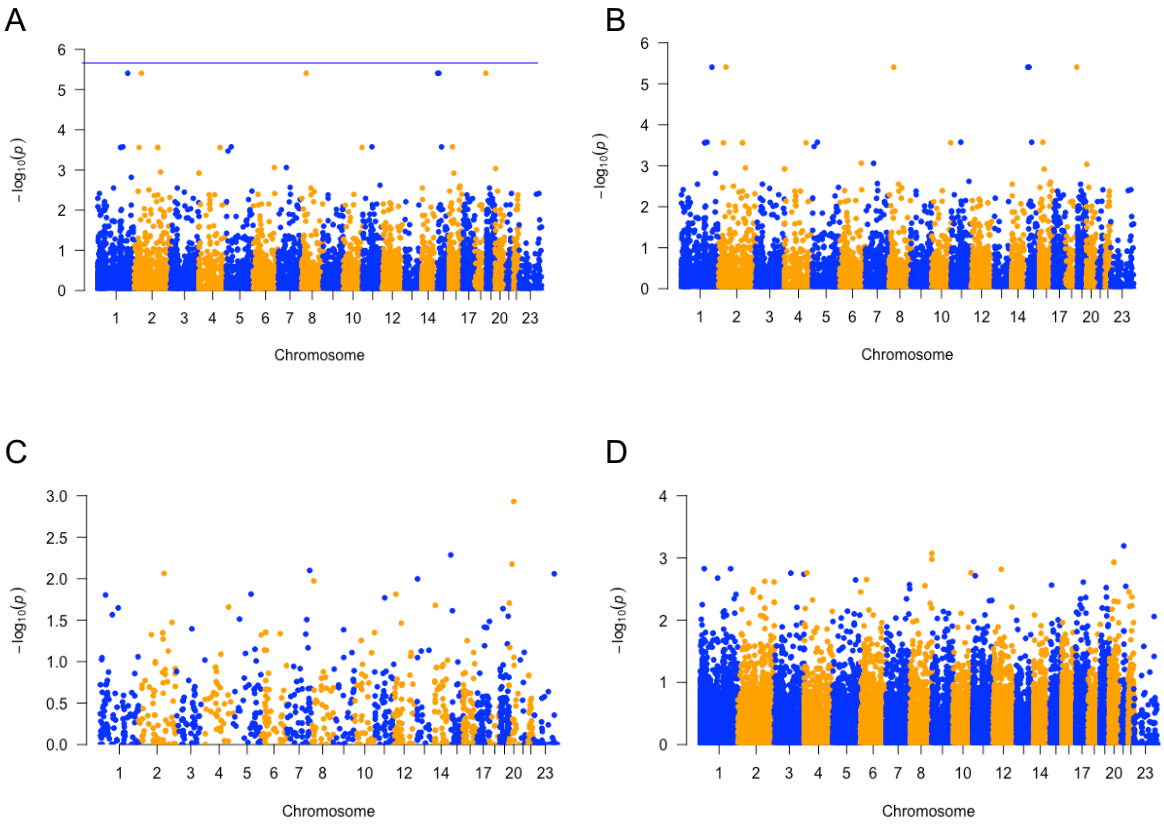


Figure 12. Exome-Wide Association Studies of Cisplatin-Associated Ototoxicity. **A)** ExWAS of cisplatin-induced hearing loss using LOF variants identified six SNVs as near exome-wide significant with the same level of statistical significance ($p = 3.93 \times 10^{-6}$). **B)** ExWAS of cisplatin-induced hearing loss using LOF and predicted deleterious variants identified no exome-wide significant variants. ExWAS of cisplatin-induced tinnitus using **C)** LOF or **D)** LOF and predicted deleterious variants identified no exome-wide significant variants.

variant method identified no exome-wide significant variants (Figure 12C and D, Appendix Table 11 and 12). It is important to note the ExWAS for cisplatin-induced hearing loss and tinnitus were substantially underpowered. For ExWAS of quantitative traits (cisplatin-induced hearing loss), 80% power for rare variants (MAF = 0.004) at p = $1x10^{-7}$ is achieved with 10,000 patients for a per allele effect of 0.69 standard deviations of the trait. Similarly, ExWAS of binary traits (cisplatin-induced tinnitus) achieves 80% power for rare variants (MAF = 0.004) with an OR = 2.0 at p = $1x10^{-7}$ with 7,450 cases, assuming a 1:2 case/control ratio.

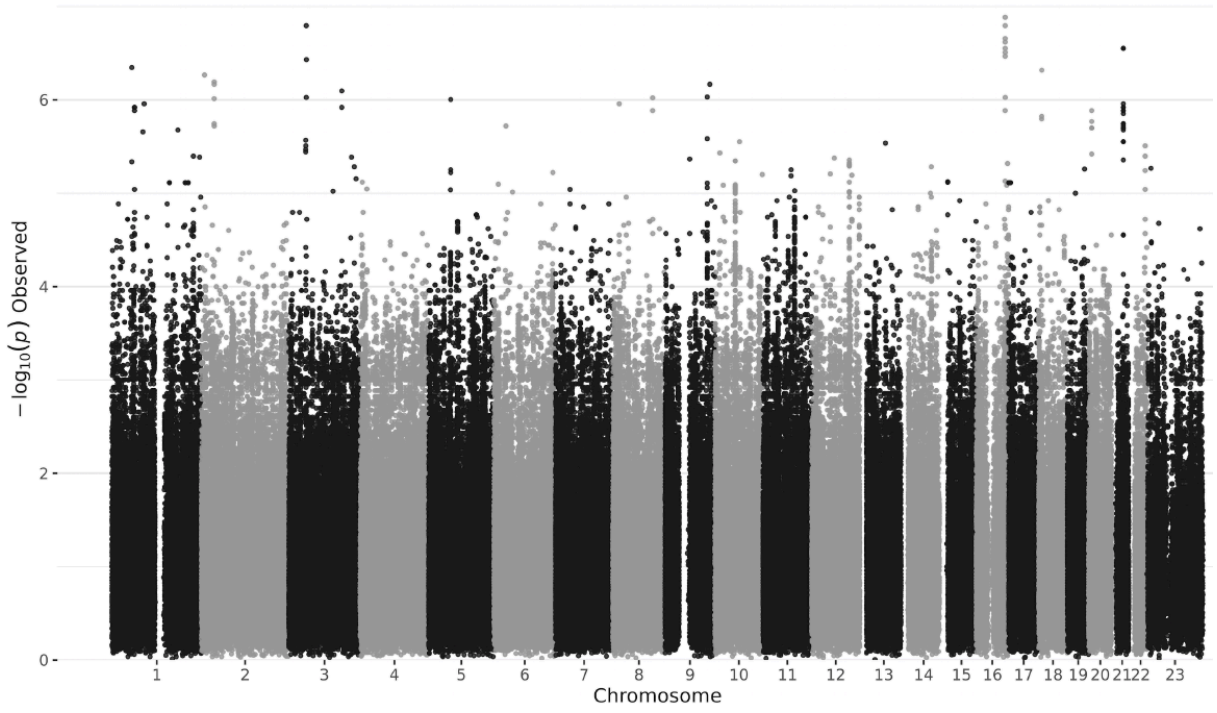
Gene-Based Association Analysis

Using BOLT-LMM, we were unable to identify any genes that met genome-wide significance for cisplatin-induced hearing loss (Figure 13A) (p = $5x10^{-8}$). Similarly, SAIGE did not identify any genes approaching genome-wide significance in the analysis of cisplatin-induced tinnitus (Figure 13B).

Discussion

Using both single variant and gene-based association analyses, we were unable to identify any rare exonic variants associated with cisplatin-associated ototoxicity. Although the ExWAS of cisplatin-induced hearing loss using LOF variants identified six near exome-wide significant SNVs, the variants all had the same p-value. Since these variants were located on different chromosomes, this anomaly is not related to linkage disequilibrium. Therefore, the most probable explanation is that the analysis was considerably underpowered, resulting in spurious associations. Single-variant tests are less powerful for rare variants than for common variants with identical effect sizes, and it has previously been noted that single variant tests have inflated type I error and very

A



B

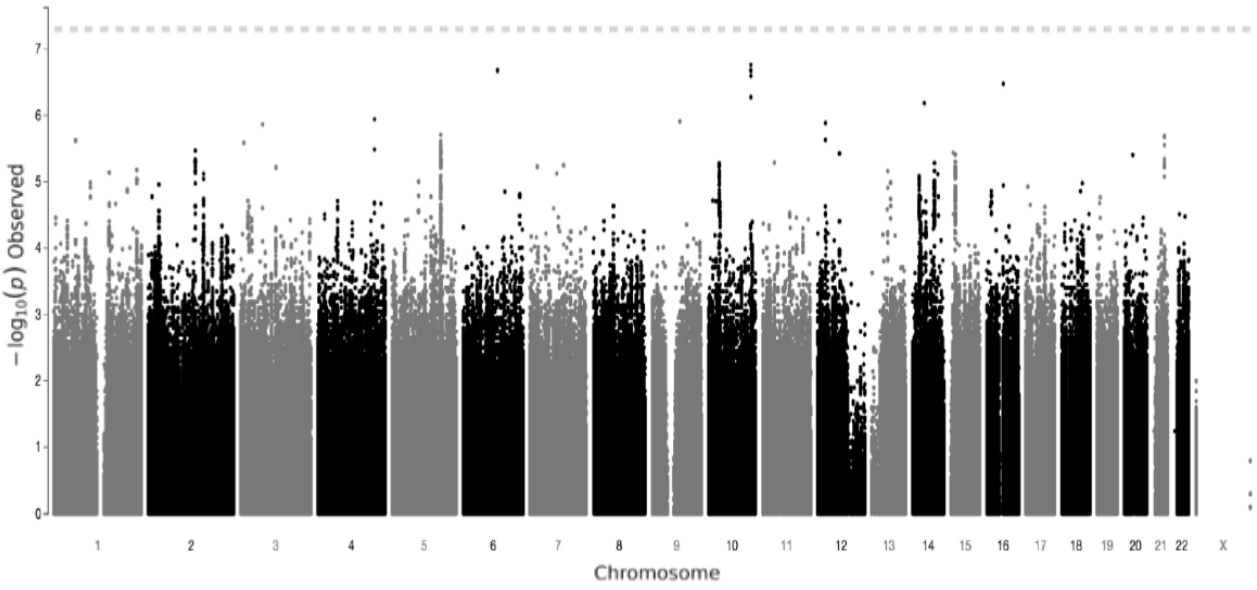


Figure 13. Gene-Based Rare Variant Association Analysis of Cisplatin-Associated Ototoxicity. A) BOLT-LMM of cisplatin-induced hearing loss identified no statistically significant genes. **B)** SAIGE of cisplatin-induced tinnitus also identified no statistically significant genes.

low power when testing rare SNVs with small to modest effect sizes, indicating that statistical inferences may not be valid (175). Further, the sample sizes of our cisplatin-induced hearing loss (n = 1,079) and tinnitus (n = 1,044) cohorts are notably small for rare variant analyses, as it can require thousands to tens of thousands of individuals to achieve 80% power for variants with a MAF < 0.01, depending on the disease prevalence and level of statistical significance (100). The small sample sizes of the examined cohorts make it difficult to ascertain reliable MAFs for rare SNVs, thereby exacerbating the already low statistical power of single rare variant association analyses.

Although the statistical power of our rare variant analyses were improved by aggregating the effect of variants within a gene through BOLT-LMM and SAIGE, neither method was able to identify any genes of statistical significance. The lack of statistical association in the gene-based association analyses does not preclude the possibility of rare variants from influencing susceptibility to cisplatin-associated ototoxicity, as these approaches may also have been underpowered to detect rare variants with small to moderate effect sizes. Notably, BOLT-LMM typically requires more than 5,000 individuals because the algorithms used rely on approximations that hold only at large sample sizes (101). The validity of SAIGE is also sample size dependent, with the original study of the software using over 408,961 individuals of European ancestry from the UK Biobank (102).

Conclusion

Due to the fact that cisplatin-treated testicular cancer survivors comprise a very specific patient demographic, it would be extremely difficult to enroll a sufficient number

of patients to resolve the current sample size limitation. One potential solution would be to broaden the scope of the study, and include all cancer survivors who were treated with ≥ 300 mg/m^2 cisplatin, as testicular cancer survivors typically receive 300 or 400 mg/m^2 (10). If necessary, the study can be further broadened to include all cancer patients who received cisplatin during the course of their treatment. Ultimately, it may prove to be unfeasible to acquire genetic data for enough cisplatin-treated patients to perform rare variant association analyses with sufficient statistical power. Therefore, it may be more productive to disregard the etiology of hearing loss or tinnitus, and attempt to identify rare variants associated with any form of the auditory disorders. In addition, it would be practical to perform rare variant analysis for age-related hearing loss or tinnitus because these auditory disorders are highly common in the elderly population, and large biobanks such as eMERGE or UK Biobank should be able to produce sufficient sample sizes.

Summary

Although GWAS have identified potential genetic susceptibilities of common genetic variants to cisplatin-associated ototoxicity, there exists the possibility that rare genetic variants (MAF < 0.01) also contribute to genetic susceptibility. Therefore, we sought to examine the genetic contribution of rare variants to cisplatin-associated ototoxicity by performing ExWAS of cisplatin-induced hearing loss and tinnitus using loss-of-function (LOF) and/or predicted deleterious variants located in exonic regions. We also assessed the aggregated effect of all rare variants within a given gene through BOLT-LMM or SAIGE to determine whether any genes are significantly associated with cisplatin-induced hearing loss or tinnitus. ExWAS of cisplatin-induced hearing loss using

LOF variants identified six near exome-wide significant SNVs, but they had the exact same level of statistical significance ($p = 3.93x10^{-6}$). The inclusion of predicted deleterious variants in the analysis provided no exome-wide significant SNVs or other variants. Neither ExWAS of cisplatin-induced tinnitus nor gene-based association analysis of cisplatin-induced hearing loss or tinnitus identified any significant associations. Due to the low statistical power from the small sample sizes of these analyses, it is possible that rare variants of small to modest effect sizes may contribute to the heritability of cisplatin-associated ototoxicity, but remained undetected using the methods of the current study. Therefore, it is necessary to increase the number of patients included in rare-variant analyses of cisplatin-associated ototoxicity to identify valid associations.

CHAPTER 6. ASSESSMENT OF MULTIPLE SEVERE NEUROTOXICITIES FOLLOWING CISPLATIN-BASED CHEMOTHERAPY

Introduction

Cisplatin is a widely used anticancer drug implemented as standard-of-care therapy for multiple adult-onset and pediatric malignancies. Although cisplatin-based chemotherapy has resulted in high five-year relative survival rates for many cancers (8), including testicular cancer (95%), hepatoblastoma (> 80%), medulloblastoma (70-80%), and osteosarcoma (60-80%), it also elicits several prominent adverse health outcomes, including ototoxicity, neurotoxicity, nephrotoxicity, cardiometabolic toxicities, and secondary malignancies (9, 10). Many of these persist for years after treatment completion, leading to chronic health conditions in a relatively young survivor population. In a recent audiometric evaluation of testicular cancer survivors (median time from treatment completion to clinical evaluation was 5 years), nearly 80% of patients exhibited some degree of hearing loss, with approximately 18% falling into the category of severe to profound hearing loss (13). This study also demonstrated that approximately 40% of patients reported tinnitus (a persistent ringing or buzzing in the ears) that is associated with multiple comorbidities, including persistent dizziness or vertigo, higher antidepressant use, and poorer self-reported health (4, 5, 176). Approximately 15.8% of these patients reported to have severe tinnitus, substantially higher than the 1-2% incidence observed in the general population (177). Further, 56.2% of testicular cancer survivors given a median dose of 400 mg/m^2 report symptoms of sensory neuropathy at a median of 5 years after treatment, with 12.5% reporting severe symptoms (70). Since patients diagnosed with testicular cancer are

typically young adults, individuals who do develop persistent neurotoxicities will likely endure the long-term consequences for decades, ultimately reducing productivity and quality of life. Hearing loss and tinnitus can lead to health issues such as sleeping difficulties and concentration problems that promote increased anxiety, depression, and insomnia (4-6); whereas peripheral sensory neuropathy can lead to reduced mobility and weight gain (70).

Although cisplatin has been in clinical use for over 40 years, it remains difficult to identify the subset of patients who may develop severe, persistent neurotoxicities following therapy completion. Previously, Kerns et al. (73) developed a score to evaluate the cumulative burden of morbidity (CBM) in 1,214 testicular cancer survivors, as well as a secondary score (CBM-Pt) that examined a subset of toxicities associated with cisplatin exposure, including peripheral sensory neuropathy, hearing damage, tinnitus, and kidney disease. Approximately 76% of patients were assigned a score of very low (8.6%), low (37.7%), or medium (29.7%), suggesting that patients' experience persistent adverse health outcomes. However, risk factors and comorbidities associated with the CBM-Pt score were not examined. We have also previously demonstrated that cisplatin-induced tinnitus is associated with symptoms of sensory neuropathy (OR range 1.66-2.72; $p < 0.0001$) (36), indicating that patients who develop tinnitus are also likely to experience neuropathy.

Due to the high incidence of hearing loss, tinnitus, and peripheral sensory neuropathy in testicular cancer survivors following cisplatin-based chemotherapy and the previously noted association between tinnitus and sensory neuropathy, we propose that a subpopulation of patients may be especially susceptible to developing multiple

severe, persistent neurotoxicities. We characterize the incidence of this outcome in testicular cancer survivors enrolled in the Platinum Study, evaluating the influence of age, cisplatin-based chemotherapy, medical history, lifestyle/behavioral factors, and other risk factors. We also perform SNP-based and gene-based analyses to identify genetic predispositions to multiple severe cisplatin-induced neurotoxicities.

Results

Cohort Characteristics

Demographic and clinical characteristics for testicular cancer survivors included in the GWAS of multiple severe cisplatin-induced neurotoxicities are provided in Tables 9 and 10. Median age at diagnosis for all patients was 28 years (range: 15-54 years), while age at clinical examination was 35 years (range: 18-68 years). Patients were treated with the following regimens: BEP (bleomycin, etoposide, and cisplatin; 53.0%), EP (etoposide and cisplatin; 38.0%), VIP (etoposide, ifosfamide, and cisplatin; 1.7%), VeIP (vinblastine, ifosfamide, and cisplatin; 0.3%), and other (unspecified cisplatin-based chemotherapy; 7.0%). Multiple severe neurotoxicity controls received a median cumulative cisplatin dose of 300 mg/m^2 (range: 100-1,000 mg/m^2), while cases received a median cumulative cisplatin dose of 400 mg/m^2 (range: 100-800 mg/m^2).

Associations with Risk Factors and Comorbidities

Both age at diagnosis (OR/10 years = 3.21, 95% CI: 2.36-4.46, p = 6.53x10^{-13}) and age at clinical examination (OR/10 years = 3.86, 95% CI: 2.82-5.45, p = 6.40x10^{-16}) were associated with multiple severe cisplatin-induced neurotoxicities (Table 11; Figure 14A and B). Cumulative cisplatin dose was significantly associated with multiple severe

Table 9. Clinical and Sociodemographic Characteristics for Testicular Cancer Survivors Based on the Occurrence of Multiple Severe Neurotoxicities.

		Status: Multiple Severe Neurotoxicities	
Characteristic	**All Patients**	**Controls (No Severe Neurotoxicities)**	**Cases (2-3 Severe Neurotoxicities)**
n	300	196	104
Age at Clinical Examination (years)			
Median (range)	35 (18-68)	32 (18-58)	45 (22-68)
< 20	3 (1.0%)	3 (1.5%)	0 (0%)
20-29	80 (26.7%)	73 (37.2%)	7 (6.7%)
30-39	108 (36.0%)	84 (42.9%)	24 (23.1%)
40-49	67 (22.3%)	28 (14.2%)	39 (37.5%)
50-59	38 (12.7%)	8 (4.1%)	30 (28.8%)
≥ 60	4 (1.3%)	0 (0%)	4 (3.8%)
Treatment Regimen			
BEP	159 (53.0%)	110 (56.1%)	49 (47.1%)
EP	114 (38.0%)	71 (36.2%)	43 (41.3%)
VIP	5 (1.7%)	2 (1.0%)	3 (2.9%)
VeIP	1 (0.3%)	1 (0.5%)	0 (0%)
Other (includes cisplatin)	21 (7.0%)	12 (6.1%)	9 (8.7%)
Cumulative Cisplatin Dose (mg/m^2)			
Median (range)	400 (100-1,000)	300 (100-1,000)	400 (100-800)
< 300	18 (6.0%)	14 (7.1%)	4 (3.8%)
300	122 (40.7%)	92 (47.0%)	30 (28.8%)
> 300 and < 400	9 (3.0%)	5 (2.6%)	4 (3.8%)
400	136 (45.3%)	80 (40.8%)	56 (53.8%)
> 400	15 (5.0%)	5 (2.6%)	10 (9.6%)
Peripheral Motor Neuropathy			
None	192 (64.0%)	167 (85.2%)	25 (24.0%)
A Little	86 (28.7%)	29 (14.8%)	57 (54.8%)

Quite a Bit/Very Much	22 (7.3%)	0 (0%)	22 (21.2%)
Raynaud Phenomenon[a]			
None	217 (72.6%)	172 (87.8%)	45 (43.7%)
A Little	33 (11.0%)	16 (8.1%)	17 (16.5%)
Quite a Bit/Very Much	49 (16.4%)	8 (4.1%)	41 (39.8%)
Self-Reported Health[b]			
Excellent	55 (18.4%)	43 (22.1%)	12 (11.7%)
Very Good	131 (44.0%)	96 (49.2%)	35 (34.0%)
Good	91 (30.5%)	53 (27.2%)	38 (36.9%)
Fair/Poor	21 (7.0%)	3 (1.5%)	18 (17.5%)

Table 9 Continued.

[a]1 patient did not report Raynaud phenomenon status.
[b]2 patients did not report their overall health.

Table 10. Additional Clinical and Sociodemographic Characteristics for Testicular Cancer Survivors Based on the Occurrence of Multiple Severe Neurotoxicities.

		Multiple Severe Neurotoxicities Status	
Characteristic	**All Patients**	**Controls (No Severe Neurotoxicities)**	**Cases (2-3 Severe Neurotoxicities)**
n	300	196	104
Age at GCT Diagnosis (years)			
Median (range)	29 (10-55)	26 (10-47)	38 (18-55)
< 20	26 (8.7%)	21 (10.7%)	5 (4.8%)
20-29	139 (46.3%)	117 (59.7%)	22 (21.2%)
30-39	74 (24.7%)	42 (21.4%)	32 (30.8%)
40-55	61 (20.3%)	16 (8.1%)	45 (43.3%)
Residual Platinum Value[a]			
Low	24 (12.4%)	20 (15.2%)	4 (6.5%)
Medium	153 (78.9%)	105 (79.5%)	48 (77.4%)
High	17 (8.8%)	7 (5.3%)	10 (16.1%)
Persistent Dizziness or Vertigo[b]			
No	276 (95.5%)	189 (97.9%)	87 (90.6%)
Yes	13 (4.5%)	4 (2.1%)	9 (9.4%)
Excessive Drinking[c]			
No	267 (89.6%)	175 (89.7%)	92 (89.3%)
Yes	31 (10.4%)	20 (10.3%)	11 (10.7%)
Ever Smoked Cigarettes[d]			
No	206 (68.9%)	145 (74.4%)	61 (58.7%)
Yes	93 (31.1%)	50 (25.6%)	43 (41.3%)
Currently Smoking Cigarettes[e]			
No	272 (93.8%)	182 (95.8%)	90 (90.0%)
Yes	18 (6.2%)	8 (4.2%)	10 (10.0%)
Prescribed Antihypertensive Medication[f]			
No	255 (88.3%)	185 (96.4%)	70 (72.2%)
Yes	34 (11.7%)	7 (3.6%)	27 (27.8%)
Prescribed High Cholesterol Medication[g]			
No	257 (88.4%)	183 (93.4%)	74 (77.9%)
Yes	34 (11.6%)	13 (6.6%)	21 (22.1%)
Prescribed Psychotropic Drugs[h]			
No	156 (90.7%)	107 (93.0%)	49 (86.0%)
Yes	16 (9.3%)	8 (7.0%)	8 (14.0%)

[a]106 patients were not evaluated for residual platinum value.
[b]11 patients did not report persistent dizziness or vertigo status.
[c]2 patients did not report excessive drinking status.
[d]1 patient did not report whether they ever smoked cigarettes.
[e]10 patients did not report whether they are currently smoking cigarettes.
[f]11 patients did not report whether they were prescribed antihypertensive medication.
[g]9 patients did not report whether they were prescribed high cholesterol medication.
[h]128 patients did not report whether they were prescribed psychotropic drugs.

neurotoxicities (OR/100 mg/m^2 = 1.97, 95% CI: 1.35-2.93, p = 5.44x10^{-4}; Table 11). Patients who received 400 mg/m^2 cisplatin had a notably increased likelihood of developing multiple severe neurotoxicities when compared to patients who received 300 mg/m^2 (41.2% vs. 24.6%, p = 0.007; Figure 14C). Serum platinum levels were also significantly associated with multiple severe neurotoxicities (age and dose-adjusted OR = 3.04, 95% CI: 1.29-7.66, p = 0.01; Table 11). Accordingly, patients with high residual platinum values had a significantly greater incidence of multiple severe neurotoxicities (58.8%) than those with medium (31.3%) or low (16.7%) serum platinum levels (p = 0.02; Figure 14D).

Ever smoking (age and dose-adjusted OR = 2.79, 95% CI: 1.52-5.23, p = 0.001) and current smoking (age and dose-adjusted OR = 5.97, 95% CI: 1.89-19.26, p = 0.002) were associated with multiple severe neurotoxicities (Table 11). Excessive drinking was not significantly associated with the multiple neurotoxicity phenotype (age and dose-adjusted OR = 1.12, 95% CI: 0.42-2.84, p = 0.82). Patients with multiple severe neurotoxicities were also more likely to have been prescribed antihypertensive medication (age and dose-adjusted OR = 3.43, 95% CI: 1.31-9.84, p = 0.02), while an association with high cholesterol medication was not statistically significant (age and dose-adjusted OR = 1.11, 95% CI: 0.46-2.70, p = 0.81; Table 11).

Patients with multiple severe neurotoxicities were also more likely to experience both Raynaud phenomenon (age and dose-adjusted OR = 3.54, 95% CI: 2.36-5.49, p = 3.71x10^{-9}) and symptoms of peripheral motor neuropathy (age and dose-adjusted OR = 14.31, 95% CI: 7.43-29.04, p = 4.30x10^{-14}; Table 11). Notably, there was a much higher proportion of patients with severe Raynaud phenomenon (Figure 15A) or peripheral

Table 11. Risk of Multiple Severe Neurotoxicities According to Socio-Demographic Features, Clinical Characteristics, Lifestyle Factors, Medication Use, and Other Variables.

Clinical Characteristic	n	OR (95% CI)	p-value	Age and Dose-Adjusted OR (95% CI)	Age and Dose-Adjusted p-value
Age at Cancer Diagnosis	300	3.21 (2.36, 4.46)	**6.53×10^{-13}**	N/A	N/A
Age at Clinical Examination	300	3.86 (2.82,5.45)	**6.40×10^{-16}**	N/A	N/A
Cumulative Cisplatin Dose	300	1.97 (1.35, 2.93)	**5.44×10^{-4}**	N/A	N/A
Residual Platinum Value	194	2.72 (1.35, 5.90)	**0.007**	3.04 (1.29, 7.66)	**0.01**
Excessive Drinking	298	1.05 (0.47, 2.24)	0.91	1.12 (0.42, 2.84)	0.82
Ever Smokers	299	2.04 (1.23, 3.40)	**0.006**	2.79 (1.52, 5.23)	**0.001**
Current Smokers	290	2.52 (0.96, 6.83)	0.06	5.97 (1.89, 19.26)	**0.002**
Prescription Antihypertensive Medication	289	10.19 (4.47, 26.38)	**2.07×10^{-7}**	3.43 (1.31, 9.84)	**0.02**
Prescription High Cholesterol Medication	291	3.99 (1.92, 8.59)	**2.56×10^{-4}**	1.11 (0.46, 2.70)	0.81
Prescription Psychotropic Drugs	172	2.45 (1.08, 5.71)	**0.03**	2.68 (0.97, 7.60)	0.06
Raynaud Phenomenon	299	4.36 (3.02, 6.52)	**5.10×10^{-14}**	3.54 (2.36, 5.49)	**3.71×10^{-9}**
Peripheral Motor Neuropathy	300	14.59 (9.38, 26.63)	**$< 2 \times 10^{-16}$**	14.31 (7.43, 29.04)	**4.30×10^{-14}**
Persistent Dizziness or Vertigo	289	4.89 (1.55, 18.44)	**0.01**	6.07 (1.54, 27.06)	**0.01**
Self-Reported Health	298	2.19 (1.61, 3.04)	**1.02×10^{-6}**	2.22 (1.54, 3.26)	**2.74×10^{-5}**

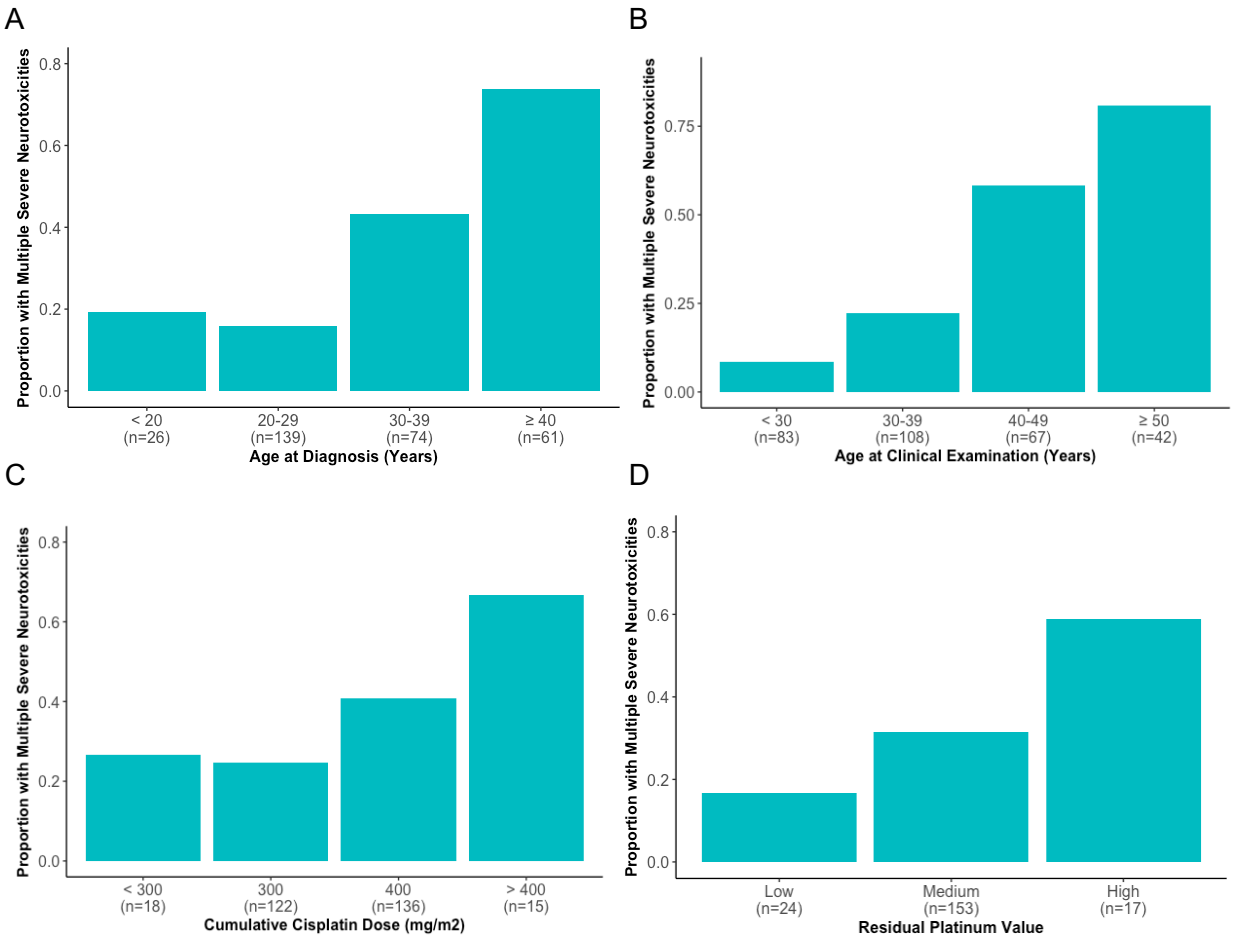


Figure 14. Effects of Age and Cisplatin Dose/Platinum Levels on Proportion of Patients with Multiple Severe Cisplatin-Induced Neurotoxicities. The overall proportion of testicular cancer survivors with multiple severe cisplatin-induced neurotoxicities is shown based on **A)** age at diagnosis ($p=5.03x10^{-5}$), **B)** age at clinical examination ($p=2.90x10^{-8}$), **C)** cumulative cisplatin dose ($p=0.04$), and **D)** residual platinum value ($p=0.03$). Statistical significance is based on logistic regression, and sample sizes for each group are indicated within each panel on the x-axis.

motor neuropathy (Figure 15B) in cases when compared to controls who had no severe neurotoxicities. The association between multiple severe neurotoxicities and Raynaud phenomenon remained statistically significant after adjusting for cumulative bleomycin dose (bleomycin dose-adjusted OR = 3.52, 95% CI: 2.23-5.80, $p = 1.88 \times 10^{-7}$).

Persistent dizziness or vertigo was also significantly associated with multiple severe cisplatin-induced neurotoxicities (age and dose-adjusted OR = 6.07, 95% CI: 1.54-27.06, p = 0.01; Table 11). Cases also reported higher psychotropic drug use that was marginally significant (age and dose-adjusted OR = 2.68, 95% CI: 0.97-7.60, p = 0.06; Table 11). Self-reported health was significantly lower in patients with multiple severe neurotoxicities than in controls (age and dose-adjusted OR = 2.22, 95% CI: 1.54-3.26, $p = 2.74 \times 10^{-5}$; Table 11). Notably, 17.5% of cases reported their overall health as fair/poor, compared to only 1.5% of controls (Figure 15C).

Genome-Wide Association Study

GWAS of multiple severe cisplatin-induced neurotoxicities identified no genome-wide significant SNPs (Appendix Table 13; Figure 16). Gene-based association analysis identified *RGS17* ($p = 3.86 \times 10^{-5}$) and *FAM20C* ($p = 5.48 \times 10^{-5}$) as near genome-wide significant ($p = 2.72 \times 10^{-6}$) (Figure 17). In addition, the normalized expression of *FAM20C* correlated with cisplatin sensitivity in CNS tumor cell lines *in silico* (Spearman Rho = 0.29, p = 0.06; $R^2 = 0.04$, p = 0.20); Figure 18A), indicative of a protective function against cisplatin-induced damage. This positive correlation between *FAM20C* expression and cisplatin sensitivity is also present in bone (Spearman Rho = 0.33, p = 0.39; $R^2 = 0.20$, p = 0.23) and kidney (Spearman Rho = 0.48, p = 0.12; $R^2 = 0.24$, p =

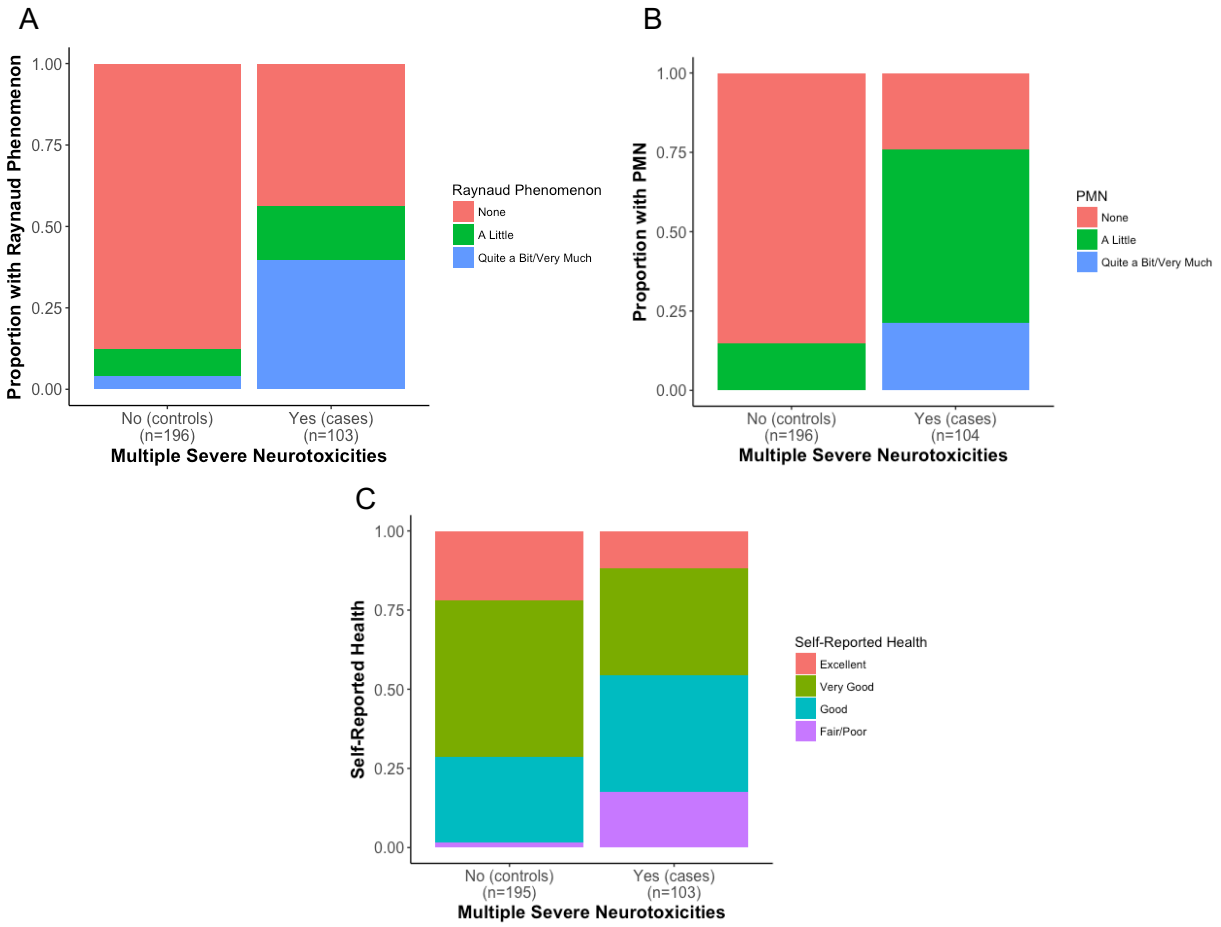
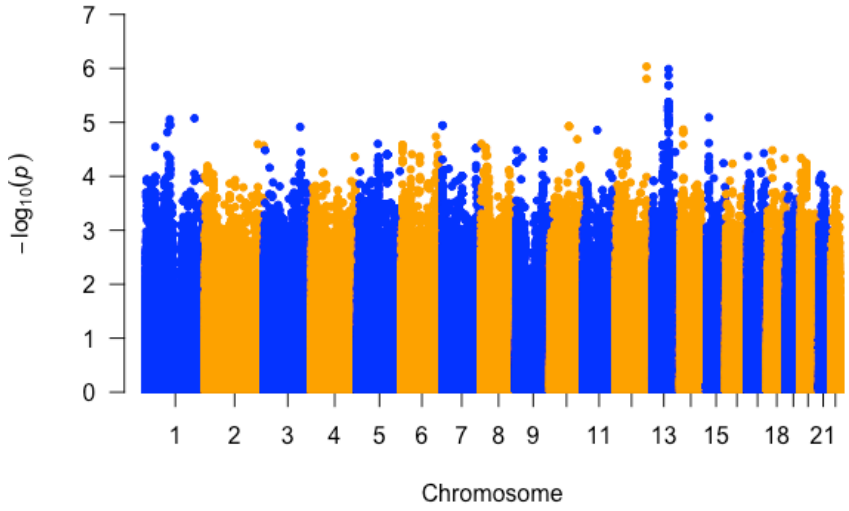


Figure 15. Distributions of Comorbidities in Testicular Cancer Survivors Based on Multiple Severe Cisplatin-Induced Neurotoxicities. The overall distribution of **A)** Raynaud phenomenon ($p < 2x10^{-16}$), **B)** peripheral motor neuropathy (PMN) ($p < 2x10^{-16}$), and **C)** self-reported health ($p = 2.92x10^{-7}$) in testicular cancer survivors based on the occurrence of multiple severe neurotoxicities is provided. All three comorbidities are divided into different degrees of severity, as indicated in the legend. Sample sizes for each group are indicated within each panel on the x-axis. Differences between the proportions of toxicity severity observed for cases and controls were evaluated for statistical significance through the Cochran-Armitage-Mantel 1df chi-squared trend test (145).

A



B

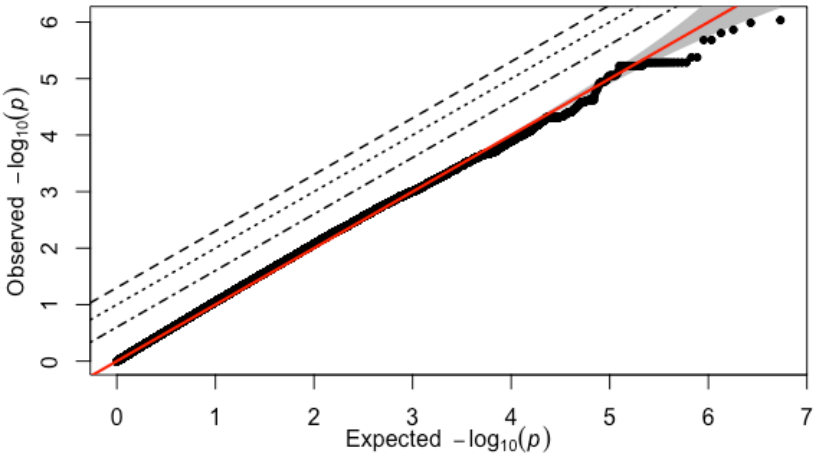


Figure 16. Genome-Wide Association Study of Multiple Severe Cisplatin-Induced Neurotoxicities in Testicular Cancer Survivors. A) Manhattan plot of GWAS results reveals no genome-wide significant SNPs. **B)** Quantile-Quantile plot of GWAS results. Covariates in the GWAS include cumulative cisplatin dose, age at clinical examination, and 20 genetic principal components accounting for population substructure.

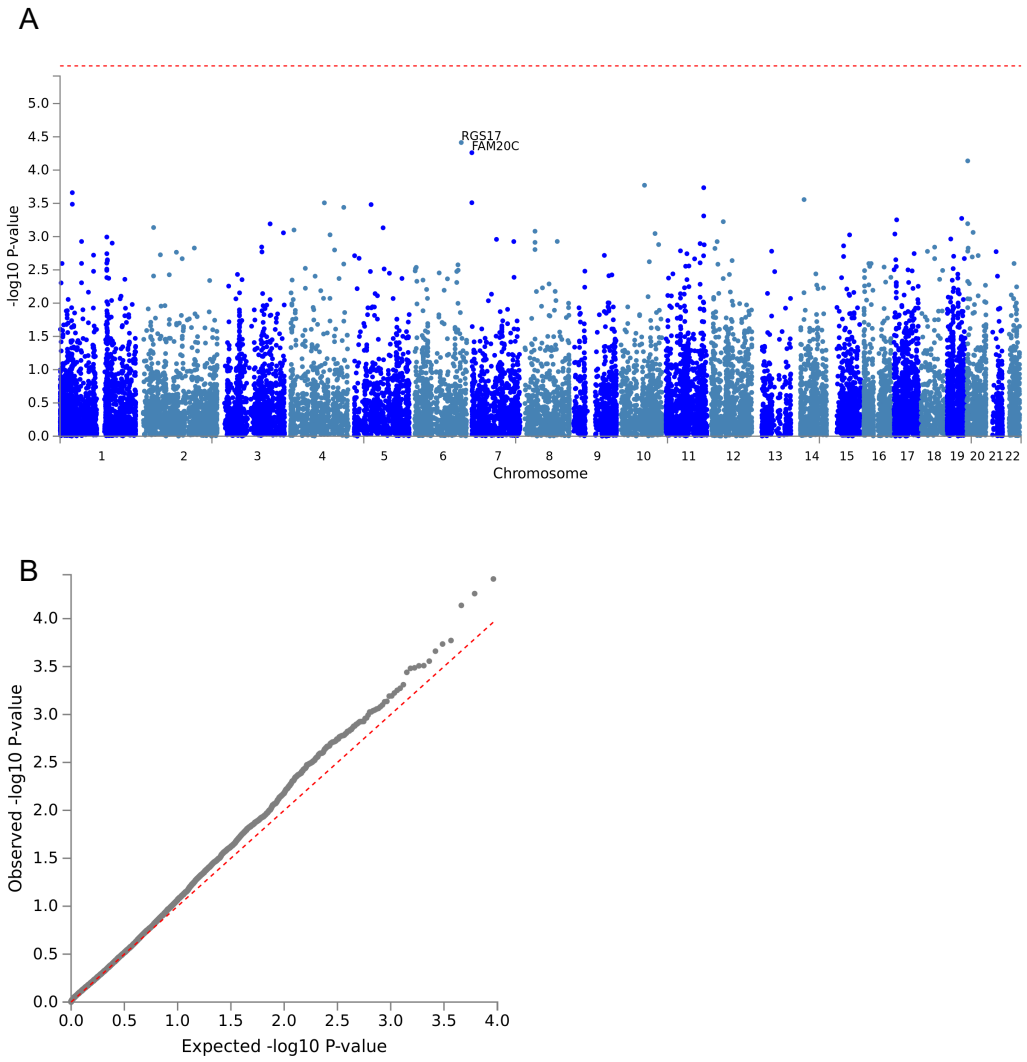


Figure 17. Gene-Based Genome-Wide Association Analysis of Multiple Severe Cisplatin-Induced Neurotoxicities. Summary statistics for SNP-based GWAS were uploaded to FUMA to run a gene-based association analysis based on a multiple linear principal components regression to determine the aggregated effect of all SNPs within a gene. Inputted SNPs were mapped to 18,404 protein coding genes, producing a significance threshold of p = 0.05/18,404 (2.72×10^{-6}). **A)** Manhattan plot of the gene-based association analysis identified *RGS17* (p = 3.86×10^{-5}) and *FAM20C* (p = 5.48×10^{-5}) as near genome-wide significant. **B)** Quantile-Quantile plots of results from the gene-based association analysis.

0.11) tumor cell lines (Figure 18B and C), two tissue types previously implicated in the persistence of cisplatin-induced toxicities (147, 167). The high correlation, but lack of statistical significance for both tissue types is likely a reflection of the small sample sizes used in the analysis (bone: n = 9; kidney: n = 12). By contrast, all other tissue types examined exhibited a negative correlation (Table 12). Normalized *RGS17* expression was not associated with cisplatin sensitivity in CNS tumor cell lines *in silico* (p = 0.99).

Discussion

The current study marks an important advance in understanding non-genetic and genetic contributions to persistent severe neurotoxicities in testicular cancer survivors. Our analyses revealed that older patients at diagnosis (and clinical examination) were more susceptible to developing multiple severe neurotoxicities. Although the exact mechanisms of this association have not been explicitly studied, renal clearance typically decreases with age (29), which not only makes cisplatin more difficult to eliminate, but also exacerbates its nephrotoxicity. Therefore, older patients likely have a reduced ability to excrete platinum from the body, increasing their likelihood of developing cisplatin-induced toxicities. Accordingly, high serum platinum levels have been associated with reduced kidney function and an increased susceptibility to developing multiple severe neurotoxicities (167). Further, older adults often experience age-related hearing loss, tinnitus, and neuropathies (161). In addition to age, it appears that patients who receive cumulative cisplatin doses ≥ 400 mg/m^2 are much more likely to develop multiple severe neurotoxicities. This observation suggests patients who receive 4 cycles of cisplatin are more susceptible to developing at least 2 neurotoxicities

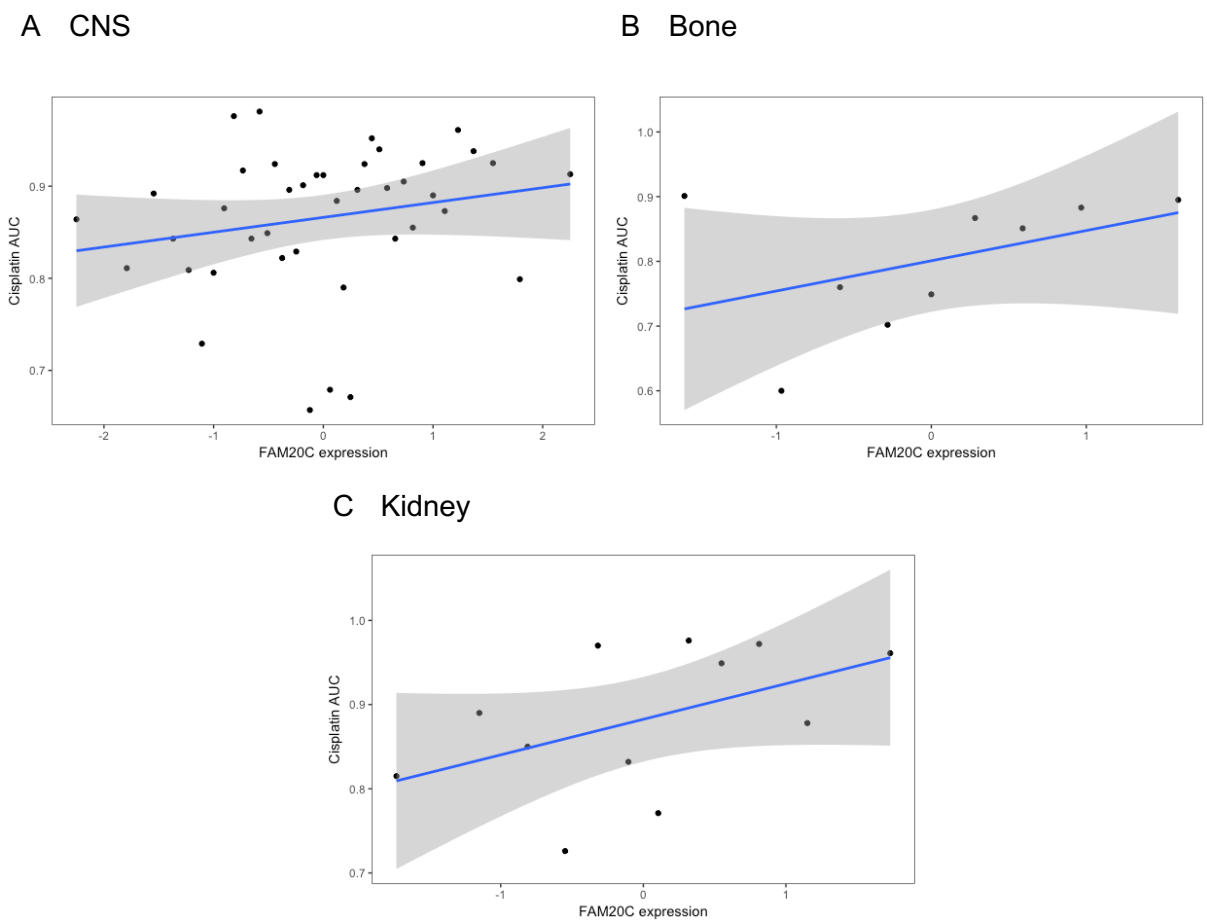


Figure 18. Scatter Plots of Cisplatin Sensitivity as a Function of Normalized *FAM20C* Expression. Scatter plots of cisplatin sensitivity as a function of normalized *FAM20C* expression are provided for **A)** CNS (ρ=0.29, p=0.06; R^2=0.04, p=0.20), **B)** bone (ρ=0.33, p=0.39; R^2=0.20, p=0.23), and **C)** kidney (ρ=0.48, p=0.12; R^2=0.24, p=0.11) tumor cell lines. Cisplatin sensitivity, measured as the area under the cisplatin dose-response curve, for all CNS tumor cell lines was extracted from CancerRX, and gene expression data were downloaded from the Cancer Cell Line Encyclopedia. Expression data were ranked normalized to fit a normal distribution prior to analysis. Correlation was assessed nonparametrically using the Spearman rank method, as well as by linear regression.

Table 12. Correlations Between *FAM20C* Expression and Cisplatin AUC Values in Different Cancer Cell Line Types.

Cancer Cell Line Type	ρ	p	R^2	p	n
Aerodigestive Tract	-0.02	0.90	0.02	0.45	34
Blood	-0.06	0.56	0.003	0.62	92
Bone	0.33	0.39	0.20	0.23	9
Breast	-0.23	0.15	0.03	0.28	38
CNS	0.29	0.06	0.04	0.20	41
Digestive System	-0.27	0.03	0.06	0.06	61
Kidney	0.48	0.12	0.24	0.11	12
Lung	-0.11	0.26	3.05×10^{-4}	0.85	116
Pancreas	-0.36	0.16	0.11	0.20	17
Skin	-0.20	0.33	0.05	0.29	25
Urogenital System	-0.27	0.06	0.05	0.14	51

Expression data were ranked normalized to fit a normal distribution prior to analysis. Significance values were calculated using the Spearman's rank-order correlation and linear regression. The number of cell lines for each analysis is provided in the n column.

(hearing loss, tinnitus, and/or peripheral sensory neuropathy) than those who receive 3 cycles.

Modifiable lifestyle factors also appeared to influence susceptibility to multiple severe cisplatin-induced neurotoxicities. Although excessive drinking did not appear to influence prevalence, smoking was significantly associated with multiple severe neurotoxicities, particularly if patients were current smokers. We previously found no association between smoking and hearing loss (13), and only an association with long-term smoking and tinnitus (36), while peripheral sensory neuropathy was associated with ever smoking and long-term smoking (70). However, our previous studies looked at each toxicity independently and did not evaluate the association between smoking and single or multiple severe forms of the toxicity. We also identified a positive association between patients who were prescribed antihypertensive medication and the development of multiple severe neurotoxicities. Hypertension has previously been associated with cisplatin-induced hearing loss (13), tinnitus (36), and peripheral sensory neuropathy in testicular cancer survivors (70), and our analysis is in accord with these data.

Testicular cancer survivors with multiple severe cisplatin-induced neurotoxicities also had an increased propensity to report numerous comorbidities. The association with persistent dizziness or vertigo is in accord with previous studies that indicate both hearing loss and tinnitus are associated with the disorder (177, 178), and can likely be attributed to the intimate relationship between the auditory and vestibular systems of the inner ear. There was also a highly significant association between multiple severe neurotoxicities and Raynaud phenomenon, as well as peripheral motor neuropathy.

Importantly, the association between multiple severe neurotoxicities and Raynaud phenomenon remained statistically significant after adjusting for cumulative bleomycin dose, an agent testicular cancer survivors receive in the bleomycin/etoposide/cisplatin regimen that is known to induce this toxicity (179, 180). The association between multiple severe neurotoxicities and peripheral motor neuropathy is particularly intriguing because cisplatin typically only induces peripheral sensory neuropathy (181). Due to the fact that peripheral sensory neuropathy and peripheral motor neuropathy were identified through responses from the Platinum Study questionnaire, it is probable that these patients have sensory neuropathy so severe that it perturbs their motor functions, subsequently influencing responses to motor neuropathy-specific questions such as problems holding a pen, which made writing difficult or difficulty manipulating small objects with your fingers. In support of this notion, patients without severe cisplatin-induced neurotoxicities had no signs of moderate to severe motor neuropathy. These data suggest that the neurotoxicity of cisplatin is highly potent in certain individuals, markedly increasing their likelihood of developing numerous neuro-otological symptoms that affect quality of life. As expected, individuals with multiple severe neurotoxicities reported higher use of antidepressants and poorer overall health. Therefore, this cohort is ideally suited to evaluate modifiable risk factors and genetics in efforts to advise patients and for potential drug development to prevent severe toxicities.

Gene-based association analysis of multiple severe cisplatin-induced neurotoxicities identified *FAM20C* as near genome-wide significant ($p = 5.48 \times 10^{-5}$), which was also positively correlated with cisplatin sensitivity in CNS tumor cell lines *in silico*. *FAM20C* encodes for a secreted protein kinase that binds calcium and

phosphorylates proteins involved in bone mineralization (182, 183). It has been recently demonstrated that high levels of platinum are stored in long bones (147), which can be slowly released over time due to its binding interaction with collagen (184). We have previously estimated that serum platinum levels remain elevated in cisplatin-treated testicular cancer survivors for an excess of 30 years, and are associated with multiple cisplatin-induced toxicities, including kidney damage, tinnitus, and Raynaud phenomenon (167). Consequently, the genetic architecture of bone modeling may play a significant role in platinum retention, which ultimately increases susceptibility to persistent toxicities. Further, the observation that *FAM20C* expression is positively correlated with cisplatin sensitivity in CNS, bone, and kidney cancer cell lines suggests that the gene exerts a protective effect in tissues affected by the long-term retention of platinum. If lower *FAM20C* expression influences the ability of bone to secrete platinum into circulation and/or subsequent renal clearance, elevated platinum levels would likely persist in long-term survivors, potentiating the neurotoxicity of cisplatin.

Major strengths of our study include the comprehensiveness of the Platinum Study questionnaire and clinical evaluation that enabled us to evaluate whether patients had multiple severe neurotoxicities following cisplatin-based chemotherapy. Our study marks the first evaluation of non-genetic and genetic risk factors contributing to multiple severe cisplatin-induced neurotoxicities, and provides a framework by which to examine other antineoplastic agents that elicit multiple persistent adverse reactions. An inherent limitation of our study is the small sample size used to evaluate genetic predispositions to multiple severe neurotoxicities (cases: 104; controls: 196) as a consequence of our strict criteria used to define cases and controls, which may have not been sufficiently

powered to identify individual genetic variants. However, we were able to increase the overall power of our genetic analysis by examining the aggregated effect of SNPs in an individual gene for their association with the phenotype, and identified *FAM20C* as near genome-wide significant. Further, *FAM20C* expression was associated with cisplatin sensitivity *in silico*. Therefore, the incorporation of gene-based analyses in pharmacogenomic studies can be highly advantageous, particularly when using a relatively small patient cohort.

Conclusion

Our study demonstrates the utility of examining patients with severe hearing loss, tinnitus, and/or peripheral sensory neuropathy following cisplatin-based chemotherapy, three of the most common and persistent toxicities experienced by testicular cancer survivors. Individuals who do develop multiple severe neurotoxicities are likely to experience concurrent dizziness or vertigo, Raynaud Phenomenon, and/or symptoms of peripheral motor neuropathy. Accordingly, these patients report higher use of antidepressants, and have a poorer quality of life when compared to patients who do not develop multiple severe neurotoxicities. In view of our results, health care providers can improve management of cancer survivors by informing patients of their risk to multiple severe cisplatin-induced neurotoxicities and associated comorbidities that may persist years after completion of therapy, particularly older patients, and those who received cumulative doses of cisplatin equivalent to or exceeding 400 mg/m^2, have a history of smoking, and are taking antihypertensive medication. In addition, our GWAS identified *FAM20C* to be associated with multiple severe cisplatin-induced

neurotoxicities, which warrants further examination to identify how perturbation of its function or expression can potentiate cisplatin-induced neurotoxicity.

Summary

Cisplatin is an essential component of first-line chemotherapy for many cancers, but causes neurotoxicity in the form of hearing loss, tinnitus, and peripheral sensory neuropathy. However, no study has comprehensively characterized risk factors for developing multiple (> 1) severe neurotoxicities. The relationship between multiple severe neurotoxicities and age, cumulative cisplatin dose, medical history, and lifestyle/behavioral factors was ascertained in 300 cisplatin-treated testicular cancer survivors using logistic regression. Case-control GWAS (cases: 104; controls: 196) was also performed. Age at diagnosis ($p = 6.53x10^{-13}$) or clinical examination ($p = 6.40x10^{-16}$) and cumulative cisplatin dose ($p = 5.44x10^{-4}$) were associated with risk of multiple severe neurotoxicities, as were high serum platinum levels (OR = 1.16, 95% CI: 1.02-1.31, p = 0.02), tobacco use (ever smoker: OR = 1.17, 95% CI: 1.07-1.30, p = 0.001, current smoker: OR = 1.35, 95% CI: 1.12-1.65, p = 0.002), and hypertension (OR = 3.04, 95% CI: 1.29-7.66, p = 0.01) after adjustment for age at clinical examination and cumulative cisplatin dose. Individuals with multiple severe neurotoxicities were more likely to experience dizziness/vertigo (OR = 6.07, 95% CI: 1.54-27.06, p = 0.01), Raynaud Phenomenon (OR = 3.54, 95% CI: 2.36-5.49, $p = 3.71x10^{-9}$), and symptoms consistent with peripheral motor neuropathy (OR = 14.31, 95% CI: 7.43-29.04, $p = 4.30x10^{-14}$) after age- and dose-adjustment. These patients also reported poorer overall health (OR = 2.22, 95% CI: 1.54-3.26, $p = 2.74x10^{-5}$) and a greater use of psychotropic drugs (OR = 2.68, 95% CI: 0.97-7.60, p = 0.06). GWAS identified no genome-wide

significant SNPs. Gene-based association analysis identified *RGS17* ($p = 3.9x10^{-5}$) and *FAM20C* ($p = 5.5x10^{-5}$) as near genome-wide significant. *FAM20C* was associated with cisplatin sensitivity *in silico*. These data indicate certain survivors are more susceptible to cisplatin-induced neurotoxicity, markedly increasing their likelihood of developing numerous neuro-otological symptoms that affect quality of life. Genome-wide analysis identified genetic variation in *FAM20C* as a potentially important risk factor.

CHAPTER 7. CLINICAL AND GENETIC RISK FACTORS ASSOCIATED WITH CRANIAL RADIATION-ASSOCIATED OTOTOXICITY

Introduction

Due to advances in chemotherapy and radiotherapy, over 85% of children diagnosed with cancer will become five-year survivors (185). However, survivors are at risk for multimorbidity and premature mortality (107, 186, 187). In terms of treatment-related ototoxicity, a Childhood Cancer Survivor Study (CCSS) investigation observed that five-year survivors compared to a sibling control group, have an excess risk of problems hearing sounds (2.3 times), hearing loss requiring an aid (4.4 times), and hearing loss in one or both ears not corrected by a hearing aid (5.2 times) (19). In a meta-analysis, the risk of tinnitus was 17.2-fold during therapy and 3.7-fold among pediatric cancer survivors compared to siblings (22). Importantly, hearing loss after pediatric cancer treatment can be permanent if inner ear damage results in sensorineural hearing loss (112). Tinnitus is also likely to be irreversible if symptoms persist for > 2 years (188). In the pediatric setting, ototoxicity has significant implications on speech, language, and cognitive development, potentially contributing to serious learning and behavioral difficulties observed in this population (18, 26, 27).

Cancer location and some treatments have well-established risks for ototoxicity. Central nervous system (CNS) tumors may perturb the central auditory nervous system, increasing susceptibility to hearing loss and tinnitus (25, 38). Platinum-based chemotherapy, supportive agents (i.e. aminoglycosides) and high dose cranial radiation therapy (> 30 Gy) are risk factors for hearing loss and tinnitus (18, 19, 37, 38). Therefore, the etiology of hearing loss and tinnitus may be multifactorial. However,

mechanisms remain poorly understood, resulting in a lack of effective prophylactic and therapeutic options.

Several GWAS of cisplatin-induced hearing loss/tinnitus have been reported (36, 49, 57), but to our knowledge, there have been no GWAS of radiation-associated ototoxicity. In this study, we aim to identify non-genetic and genetic risk factors associated with radiation-associated ototoxicity within CCSS, which provides longitudinal examination of long-term treatment-related toxicities.

Results

Cohort Characteristics

Demographic and clinical characteristics for survivors in GWAS of radiation-induced tinnitus and hearing loss are provided in Tables 13 and 14. Median age at primary cancer diagnosis for both cohorts was 8 years (range: 0-20 years), while age at last observation was 43 years (range: 23-63 years). The most common primary cancer diagnosis was acute lymphoblastic leukemia (ALL; tinnitus: n = 632; 28.9%; hearing loss: n = 692; 28.5%). Median cumulative cranial radiation dose for all cancer survivors was 2 Gy (range: 0.2-72 Gy). Survivors given cranial radiation, but without tinnitus or hearing loss received a median cumulative dose of 0.2 Gy (range: 0.2-72 Gy), while those with tinnitus or hearing loss received a median cumulative dose of 24 Gy each (tinnitus range: 0.2-62 Gy; hearing loss range: 0.2-72 Gy).

Associations with Radiation Dose, Risk Factors, and Comorbidities

Cumulative cranial radiation dose was significantly associated with tinnitus (age-adjusted OR per 15 Gy = 1.70, 95% CI: 1.48-1.95, $p = 4.82 \times 10^{-14}$) and hearing loss

Table 13. Clinical and Sociodemographic Characteristics for GWAS of Radiation-Associated Ototoxicity in Childhood Cancer Survivors.

	Self-Reported Tinnitus			Self-Reported Hearing Loss		
Characteristic	**All Patients**	**Tinnitus: No (Controls)**	**Tinnitus: Yes (Cases)**	**All Patients**	**Hearing Loss: No (Controls)**	**Hearing Loss Yes (Cases)**
n	1,991	1,845	146	2,198	1,928	270
Sex[a]						
Male	956 (48.0%)	866 (46.9%)	90 (61.6%)	1,059 (48.2%)	911 (47.3%)	148 (54.8%)
Female	1,035 (52.0%)	979 (53.1%)	56 (38.4%)	1,136 (51.8%)	1014 (52.7%)	122 (45.2%)
Age at Last Observation (Years)[b]						
Median (range)	43 (23-63)	42 (23-63)	44 (23-63)	43 (23-63)	43 (23-63)	44 (23-63)
20-29	82 (4.1%)	77 (4.2%)	5 (3.4%)	82 (3.7%)	73 (3.8%)	9 (3.3%)
30-39	618 (31.0%)	578 (31.3%)	40 (27.4%)	665 (30.3%)	587 (30.5%)	78 (28.9%)
40-49	906 (45.5%)	842 (45.6%)	64 (43.8%)	1,007 (45.8%)	885 (45.9%)	122 (45.2%)
> 50	385 (19.3%)	348 (18.9%)	37 (25.3%)	443 (20.2%)	382 (19.8%)	61 (22.6%)
Type of Cancer						
ALL	573 (28.8%)	539 (29.2%)	34 (23.3%)	627 (28.5%)	580 (30.1%)	47 (17.4%)
AML	22 (1.1%)	21 (1.1%)	1 (0.7%)	26 (1.2%)	23 (1.2%)	3 (1.1%)
Other Leukemia	8 (0.4%)	8 (0.4%)	0 (0%)	9 (0.4%)	8 (0.4%)	1 (0.4%)
Astrocytoma	136 (6.8%)	115 (6.2%)	21 (14.4%)	158 (7.2%)	104 (5.4%)	54 (20.0%)
Medulloblastoma	75 (3.8%)	58 (3.1%)	17 (11.6%)	81 (3.7%)	37 (1.9%)	44 (16.3%)
Other CNS Tumor	40 (2.0%)	35 (1.9%)	5 (3.4%)	46 (2.1%)	33 (1.7%)	13 (4.8%)
Hodgkin Lymphoma	413 (20.7%)	381 (20.7%)	32 (21.9%)	459 (20.9%)	415 (21.5%)	44 (16.3%)
Non-Hodgkin Lymphoma	165 (8.3%)	152 (8.2%)	13 (8.9%)	178 (8.1%)	165 (8.6%)	13 (4.8%)
Kidney Tumors	212 (10.6%)	206 (11.1%)	6 (4.1%)	223 (10.1%)	212 (11.0%)	11 (4.1%)
Neuroblastoma	105 (5.3%)	102 (5.5%)	3 (2.1%)	115 (5.2%)	108 (5.6%)	7 (2.6%)
Soft Tissue Sarcoma	158 (7.9%)	148 (8.0%)	10 (6.8%)	181 (8.2%)	153 (7.9%)	28 (10.4%)
Ewing Sarcoma	64 (3.2%)	61 (3.3%)	3 (2.1%)	72 (3.3%)	69 (3.6%)	3 (1.1%)
Osteosarcoma	19 (1.0%)	18 (1.0%)	1 (0.7%)	22 (1.0%)	20 (1.0%)	2 (0.7%)
Other Bone	1 (0.05%)	1 (0.05%)	0 (0%)	1 (0.5%)	1 (0.05%)	0 (0%)
Cumulative Cranial Radiation Dose (Gy)						
Median (range)	2 (0.2-72)	0.2 (0.2-72)	24 (0.2-62)	2 (0.2-72)	0.2 (0.2-72)	24 (0.2-72)
0.2-9.9	1,063 (53.4%)	1,008 (54.6%)	55 (37.7%)	1,174 (53.4%)	1,085 (56.3%)	89 (32.0%)

10-19.9	301 (15.1%)	287 (15.6%)	14 (9.6%)	324 (14.7%)	307 (15.9%)	17 (6.3%)
20-29.9	325 (16.3%)	301 (16.3%)	24 (16.4%)	356 (16.2%)	322 (16.7%)	34 (12.6%)
30-39.9	28 (1.4%)	27 (1.4%)	1 (0.6%)	34 (1.5%)	28 (1.5%)	6 (2.2%)
40-49.9	52 (2.6%)	45 (2.4%)	7 (4.8%)	57 (2.6%)	43 (2.2%)	14 (5.2%)
50-59.9	203 (10.2%)	161 (8.7%)	42 (28.8%)	231 (10.5%)	130 (6.7%)	101 (37.4%)
≥ 60	19 (1.0%)	16 (0.9%)	3 (2.1%)	22 (1.0%)	13 (0.7%)	9 (3.3%)

Table 13 Continued.

[a]3 patients did not report sex for the radiation-induced hearing loss cohort.
[b]1 patient did not report age at last observation for the radiation-induced hearing loss cohort.

All patients were of European ancestry, as determined by principal components analysis.
Abbreviations: ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; CNS: central nervous system

Table 14. Additional Clinical and Sociodemographic Characteristics for GWAS of Radiation-Associated Ototoxicity in Childhood Cancer Survivors.

	Tinnitus Status			Hearing Loss Status		
Characteristic	**All Patients**	**Tinnitus: No (Controls)**	**Tinnitus: Yes (Cases)**	**All Patients**	**Hearing Loss: No (Controls)**	**Hearing Loss: Yes (Cases)**
n	1,991	1,845	146	2,198	1,928	270
Year of Diagnosis						
Median (range)	1979 (1970-1986)	1979 (1970-1986)	1978 (1970-1986)	1979 (1970-1986)	1979 (1970-1986)	1978 (1970-1986)
1970-1974	433 (21.7%)	393 (21.3%)	40 (27.4%)	482 (21.9%)	416 (21.6%)	66 (24.4%)
1975-1979	664 (33.4%)	615 (33.3%)	49 (33.6%)	741 (33.7%)	647 (33.6%)	94 (34.8%)
1980-1984	670 (33.7%)	627 (34.0%)	43 (29.5%)	735 (33.4%)	657 (34.1%)	78 (28.9%)
1985-1986	224 (11.3%)	210 (11.4%)	14 (9.6%)	240 (10.9%)	208 (10.8%)	32 (11.9%)
Age at Diagnosis (Years)						
Median (range)	8 (0-20)	8 (0-20)	9 (0-20)	8 (0-20)	8 (0-20)	8 (0-20)
0-4	669 (33.6%)	634 (34.4%)	35 (24.0%)	742 (33.8%)	659 (34.2%)	83 (30.7%)
5-9	463 (23.3%)	423 (22.9%)	40 (27.4%)	507 (23.1%)	442 (22.9%)	65 (24.1%)
10-14	463 (23.3%)	426 (23.1%)	37 (25.3%)	501 (22.8%)	435 (22.6%)	66 (24.4%)
≥ 15	396 (19.9%)	362 (19.6%)	34 (23.3%)	448 (20.4%)	392 (20.3%)	56 (20.7%)
Age at Follow-Up 4 (2007)[a]						
Median (range)	37 (22-58)	37 (22-58)	40 (23-56)	37 (22-58)	37 (22-57)	38 (23-58)
21-29	274 (14.4%)	263 (14.9%)	11 (8.0%)	303 (14.4%)	271 (14.6%)	32 (12.5%)
30-39	862 (45.3%)	805 (45.6%)	57 (41.3%)	944 (44.7%)	828 (44.7%)	116 (45.1%)
40-49	656 (34.5%)	600 (34.0%)	56 (40.6%)	738 (35.0%)	653 (35.2%)	85 (33.1%)
50-59	111 (5.8%)	97 (5.5%)	14 (10.1%)	126 (6.0%)	102 (5.5%)	24 (9.3%)
Age at Follow-Up 5 (2014)[b]						
Median (range)	44 (29-63)	44 (29-63)	47 (33-63)	44 (29-63)	44 (29-63)	45 (30-63)
29-39	396 (27.2%)	378 (27.8%)	18 (18.9%)	452 (27.1%)	401 (27.3%)	51 (25.8%)
40-49	713 (49.0%)	667 (49.0%)	46 (48.4%)	811 (48.7%)	716 (48.8%)	95 (48.0%)
50-59	323 (22.2%)	297 (21.9%)	26 (27.4%)	376 (22.6%)	331 (22.6%)	45 (22.7%)
≥ 60	22 (1.5%)	17 (1.3%)	5 (5.3%)	26 (1.6%)	19 (1.3%)	7 (3.5%)

[a]88 participants did not complete the follow-up 4 survey for the radiation-induced tinnitus cohort, and 87 participants did not complete the follow-up 4 survey for the radiation-induced hearing loss cohort.
[b]537 participants did not complete the follow-up 5 survey for the radiation-induced tinnitus cohort, and 533 participants did not complete the follow-up 5 survey for the radiation-induced hearing loss cohort.

(age-adjusted OR per 15 Gy = 2.15, 95% CI: 1.93-2.40, $p < 2x10^{-16}$). The percentage of patients with tinnitus and hearing loss was significantly higher among survivors treated with higher cumulative cranial radiation doses (45-59.9 Gy vs. 30-44.9 Gy; tinnitus: 20.3% vs. 4.3%, p = 0.02; hearing loss: 41.6% vs. 18.2%, p = 0.002; Figure 19). Although males (median: 2 Gy; range 0.2-72 Gy) received a higher cumulative cranial radiation dose than females (median: 0.2 Gy: range 0.2-72 Gy), statistically significant differences for radiation-induced tinnitus and hearing loss between sexes were independent of both age at last observation and cumulative cranial radiation dose (tinnitus: 9.4% vs. 5.4%; age and dose-adjusted OR = 1.85, 95% CI: 1.31-2.63, $p = 5.05x10^{-4}$; hearing loss: 14.0% vs. 10.7%; age and dose-adjusted OR = 1.36, 95% CI: 1.05-1.76, p = 0.02). Radiation-induced tinnitus was significantly associated with use of antihypertensive medication (age-adjusted OR = 1.66, 95% CI: 1.14-2.41, p = 0.008), but not hearing loss (age-adjusted OR = 1.27, 95% CI: 0.94-1.71, p = 0.11; Table 15). Radiation-induced tinnitus was associated with hearing loss (age-adjusted OR = 17.99, 95% CI: 12.29-26.49, $p < 2x10^{-16}$; Figure 20A). Both radiation-induced tinnitus and hearing loss were associated with persistent dizziness or vertigo (tinnitus: age-adjusted OR = 7.91, 95% CI: 4.81-12.78, $p < 2x10^{-16}$; hearing loss: age-adjusted OR = 3.22, 95% CI: 2.10-4.85, $p = 3.71x10^{-8}$; Table 15). Tinnitus and hearing loss cases also reported higher antidepressant use (tinnitus: age-adjusted OR = 2.05, 95% CI: 1.06-3.68, p = 0.02; hearing loss: age-adjusted OR = 1.82, 95% CI: 1.10-2.91, p = 0.02; Table 15). Self-reported health was significantly lower in tinnitus and hearing loss cases than controls (tinnitus: age-adjusted OR = 1.70, 95% CI: 1.37-2.11, $p = 1.45x10^{-6}$;

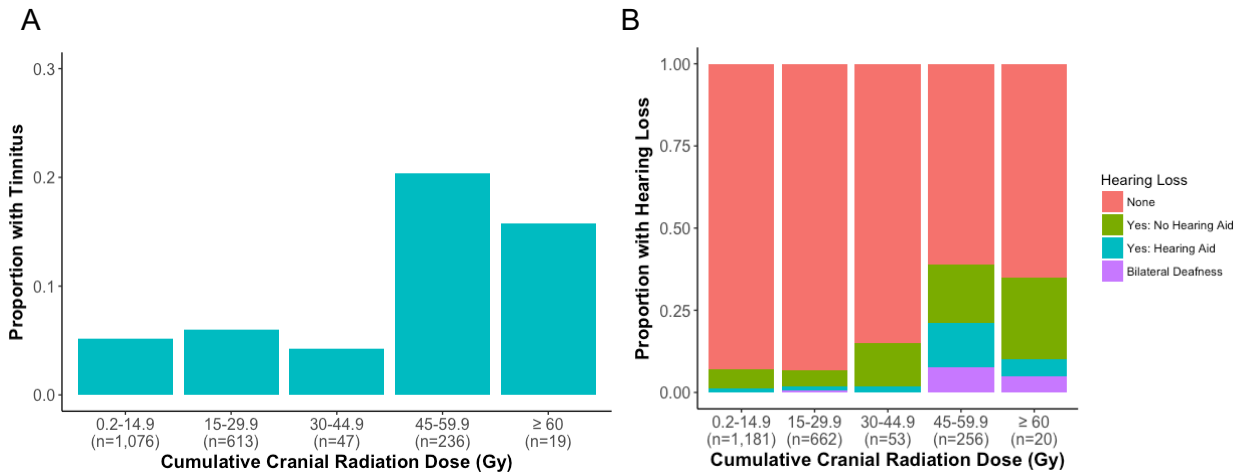


Figure 19. Effects of Cumulative Cranial Radiation Dose on Proportion of Patients with Radiation-Associated Ototoxicity. The overall proportion of pediatric cancer survivors with **A)** tinnitus ($p = 2.61 \times 10^{-15}$) or **B)** hearing loss ($p < 2 \times 10^{-16}$) based on cumulative cranial radiation dose is provided. Statistical significance is based on logistic regression, and sample sizes for each group are indicated within each panel on the x-axis.

hearing loss: age-adjusted OR = 1.45, 95% CI: 1.25-1.69, $p = 1.72 \times 10^{-6}$; Figure 20B and C).

Genome-Wide Association Studies

GWAS of radiation-induced tinnitus identified a prominent signal in chromosome 1 in which 33 SNPs met genome-wide significance ($p < 5 \times 10^{-8}$; Appendix Table 14). The most significant SNP was rs203248 (OR = 8.67, 95% CI: 1.52-47.94, $p = 1.50 \times 10^{-9}$; Figure 21A and B). This SNP is intronic to *DCAF6*, which encodes for a ligand-dependent coactivator of nuclear receptors. rs203248 is in perfect linkage disequilibrium (LD) with nearly all SNPs meeting genome-wide significance ($R^2 = 1.0$, $p < 0.0001$; Figure 22A). One SNP in perfect LD is rs73024126 (OR = 7.81, 95% CI: 1.43-43.38, $p = 1.59 \times 10^{-8}$) that has a relatively high CADD score (12.07) that places it within the top 10% of deleterious mutations (Chapter 2), and may regulate the binding of transcription factors to *DCAF6* (RegulomeDB score: 2b - likely to affect binding; Figure 22B). In addition, rs430565 (OR = 7.04, 95% CI: 1.40-35.16, $p = 1.72 \times 10^{-8}$) and rs433173 (OR = 4.87, 95% CI: 1.16-20.29, $p = 3.34 \times 10^{-6}$) are eQTLs for *DCAF6*, while rs369914 (OR = 7.03, 95% CI: 1.38-35.87, $p = 4.55 \times 10^{-8}$) and rs370952 (OR = 5.15, 95% CI: 1.19-22.42, $p = 1.78 \times 10^{-6}$) are eQTLs for *DCAF6*, *TBX19*, *MPC2*, and *GPR161* (Figure 22B). SNPs in LD with rs203248 ($R^2 > 0.6$) also encompass three other genes (*TIPRL*, *GPR161*, and *MPC2*), forming a relatively large genetic risk locus that has chromatin interactions with 13 other genes (Appendix Figure 3). Gene-based association analysis identified *DCAF6* and *NAV2* as nearly genome-wide significant ($p = 8.58 \times 10^{-6}$ and $p = 1.60 \times 10^{-5}$; Figure 23, Appendix Table 15). This is in accord with the GWAS, in which rs7106624 (OR = 1.86, 95% CI: 1.07-3.22, $p = 1.99 \times 10^{-6}$), an eQTL for *NAV2*, was one of the most

Table 15. Association Between Radiation-Induced Tinnitus or Hearing Loss and Selected Characteristics of Childhood Cancer Survivors.

Characteristic	Phenotype	n	Prevalence Among Controls	Prevalence Among Cases	OR (95% CI)	p	Age-Adjusted OR (95% CI)	Age-Adjusted p
Persistent Dizziness or Vertigo	Tinnitus	1,953	3.2%	21.0%	8.06 (4.91, 13.01)	**$< 2x10^{-16}$**	7.91 (4.81,12.78)	**$< 2x10^{-16}$**
	Hearing Loss	2,156	1.8%	13.4%	3.24 (2.11, 4.88)	**$3.13x10^{-8}$**	3.22 (2.10, 4.85)	**$3.71x10^{-8}$**
Prescribed Antidepressant	Tinnitus	1,875	4.8%	9.7%	2.15 (1.11, 3.83)	**0.01**	2.05 (1.06, 3.68)	**0.02**
	Hearing Loss	2,064	4.9%	8.7%	1.84 (1.11, 2.94)	**0.01**	1.82 (1.10, 2.91)	**0.02**
Prescribed Antihypertensive Medication	Tinnitus	1,959	22.8%	34.8%	1.80 (1.24, 2.58)	**0.002**	1.66 (1.14, 2.41)	**0.008**
	Hearing Loss	2,163	23.3%	28.4%	1.31 (0.97, 1.74)	0.07	1.27 (0.94, 1.71)	0.11

Age-adjustment reflects the use of age at last observation as a covariate in the logistic regression model. Bold indicates $p \leq 0.05$.
Patients who received cranial radiation, but did not receive cisplatin or carboplatin were included in the radiation-induced tinnitus and hearing loss cohorts.

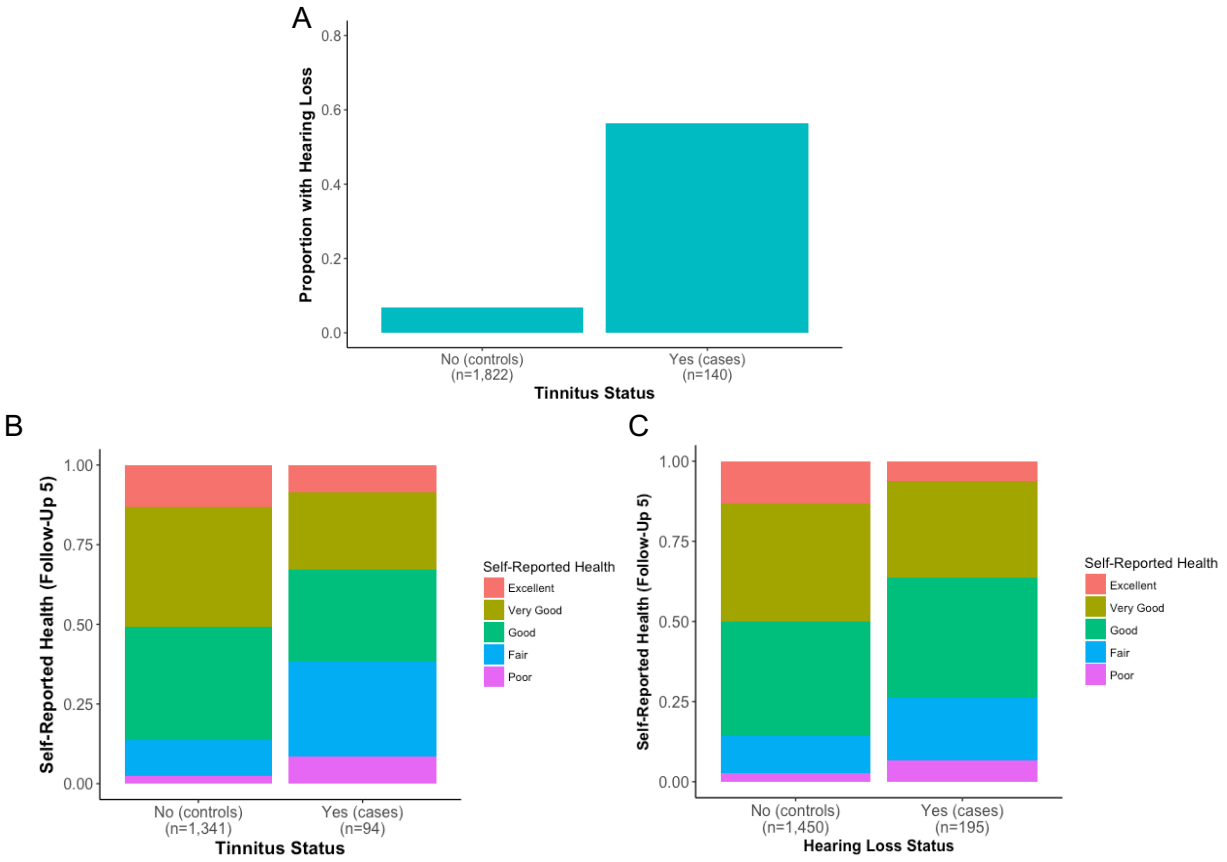


Figure 20. Co-occurrence of Hearing Loss and Tinnitus and Effects of Ototoxicity Status on Self-Reported Health for Patients Treated with Cranial Radiation. The overall distribution of **A)** hearing loss ($p < 2x10^{-16}$) and **B)** self-reported health ($p = 9.40x10^{-7}$) based on radiation-induced tinnitus status is provided. The overall distribution of **C)** self-reported health ($p = 1.30x10^{-6}$) based on radiation-induced hearing loss status is also provided. Both hearing loss and self-reported health are divided into different degrees of severity, as indicated in the legend. Statistical significance is based on logistic regression, and sample sizes for each group are indicated within each panel on the x-axis.

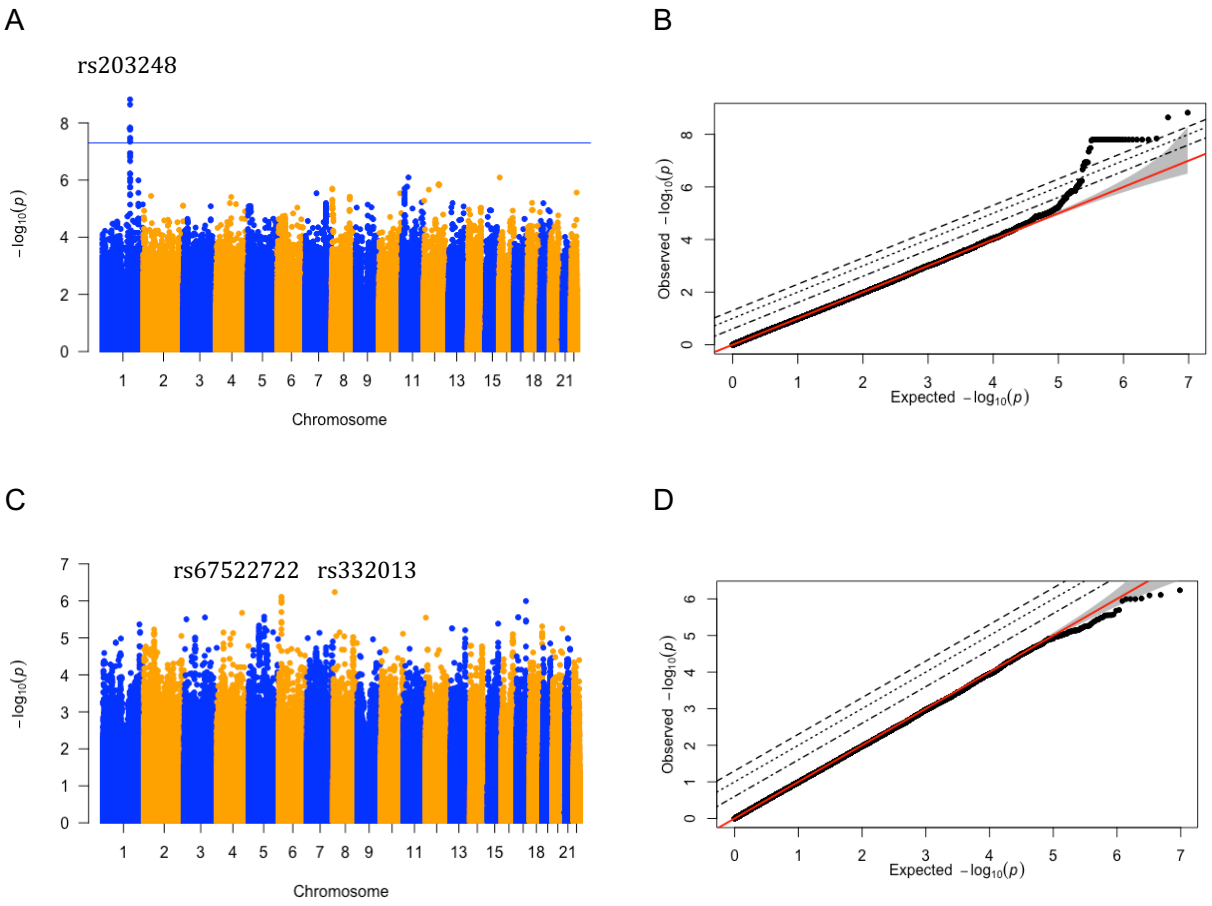


Figure 21. Genome-Wide Association Studies of Radiation-Associated Ototoxicity in Pediatric Cancer Survivors. A) Manhattan plot of GWAS results for radiation-induced tinnitus reveals a prominent signal in chromosome 1 exceeding genome-wide significance ($p < 5x10^{-8}$), with the most significant SNP being rs203248 ($p = 1.50x10^{-9}$). **B)** Quantile-Quantile plot of GWAS results for radiation-induced tinnitus. **C)** Manhattan plot of GWAS results for radiation-induced hearing loss reveals rs332013 in chromosome 8 ($p = 5.79x10^{-7}$) and rs67522722 ($p = 7.78x10^{-7}$) in chromosome 6 as nearly genome-wide significant. **D)** Quantile-Quantile plot of GWAS results for radiation-induced hearing loss. Covariates in both GWAS include cumulative cranial radiation dose, age at last observation, and 20 European genetic principal components accounting for population substructure.

significantly associated SNPs (Appendix Table 14). *NAV2* encodes for a member of the neuron navigator gene family.

GWAS of radiation-induced hearing loss identified rs332013 in chromosome 8 as near genome-wide significant (OR = 0.57, 95% CI: 0.35-0.93, $p = 5.79 \times 10^{-7}$; Figure 21C and D; Appendix Table 16). This SNP is intronic to *ERI1*, encoding for an RNA exonuclease. rs332013 has a high CADD score (17.49), indicative of a deleterious mutation, and is an eQTL for multiple genes, including *ERI1*, *MFHAS1*, and *SGK223* (Figure 24A and B). Three SNPs in LD with rs332013 (rs6991294, rs1077951, and rs1077950; $R^2 = 0.67$, $p < 0.0001$; Figure 24A) are also eQTLs for the same genes (Figure 24B and Appendix Figure 4A). The next most significant SNP is rs67522722 (OR = 2.47, 95% CI: 1.13-5.37, $p = 7.78 \times 10^{-7}$) in chromosome 6, which is intronic to *ATXN1*, a gene that encodes for ataxin-1 that regulates various aspects of protein production. rs67522722 is in perfect LD with four SNPs ($R^2 = 1.0$, $p < 0.0001$; Figure 24C), including rs34675197 (OR = 2.45, 95% CI: 1.11-5.31, $p = 1.00 \times 10^{-6}$), which has a relatively high CADD score (11.47), and appears to regulate binding of transcription factors to *ATXN1* (RegulomeDB score: 2b - likely to affect binding; Figure 24D). SNPs in LD with rs67522722 ($R^2 > 0.6$) have chromatin interactions with 5 other genes (Appendix Figure 4B). Gene-based association analysis identified no genome-wide significant genes (Figure 23; Appendix Table 17).

We further evaluated the biological significance of these findings by determining whether the expression level of genes associated with top SNPs from both GWAS correlated with radiosensitivity in CNS tumor cell lines *in silico*. Both *NAV2* and *MPC2* expression were significantly correlated with radiosensitivity (*NAV2*: Spearman Rho =

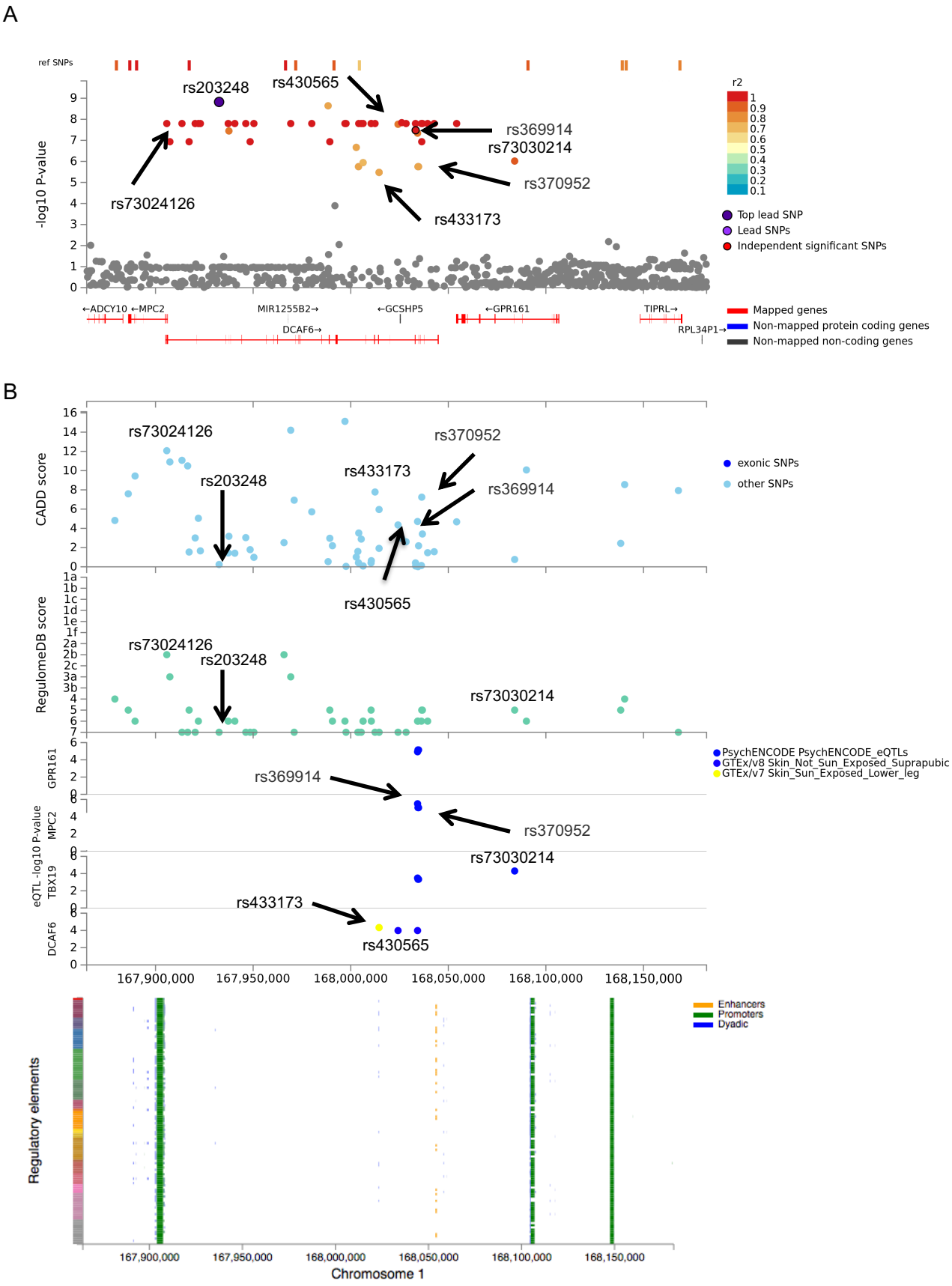


Figure 22. Regional Plot of rs203248 for GWAS of Radiation-Induced Tinnitus.

Figure 22. Regional Plot of rs203248 for GWAS of Radiation-Induced Tinnitus. A) A regional plot of the most significant GWAS signal (rs203248) was generated in FUMA. Each point represents a SNP. The x-axis indicates chromosomal position, while the y-axis shows $-\log_{10}$(p-value) of association with radiation-induced tinnitus. The LD (R^2) of each SNP with the top signal in the region, rs203248 (purple), is denoted by color in the legend, along with whether the gene has been mapped. **B)** SNPs that were in LD with rs203248 were evaluated for CADD and Regulome DB scores and underwent eQTL analysis. After evaluation, SNPs were plotted in accordance with their chromosomal position. rs73024126 has both a high CADD (12.07) and RegulomeDB score (2b) and resides within the promoter region of *DCAF6*. In addition, rs433173 and rs430565 are eQTLs for *DCAF6*, rs369914 and rs370952 are eQTLs for *DCAF6*, *TBX19*, *MPC2*, and *GPR161*, and rs73030214 is an eQTL for *TBX19* and is in LD with rs203248 ($R^2 = 0.91$; $p < 0.0001$).

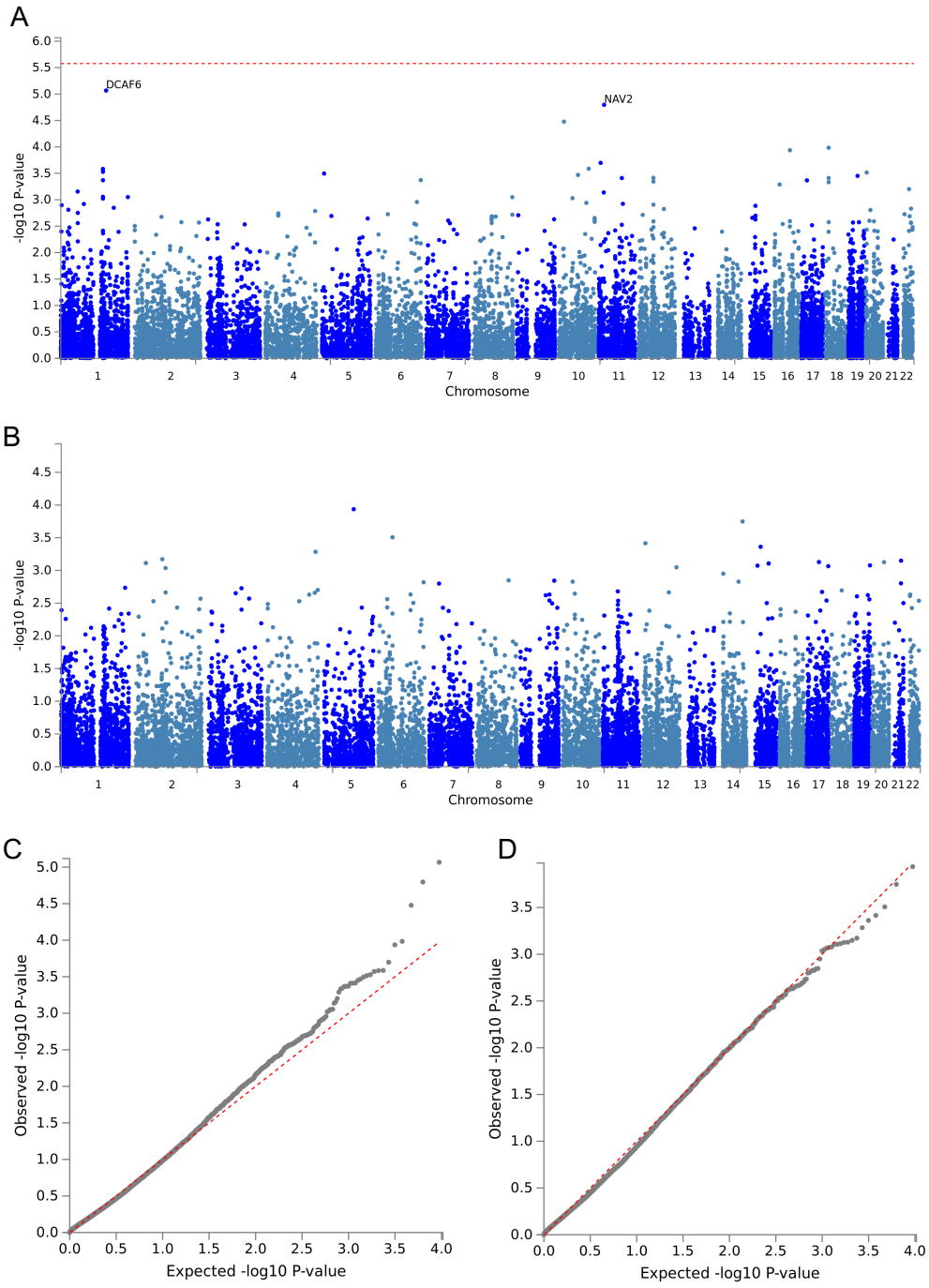


Figure 23. Gene-Based Genome-Wide Association Analyses of Radiation-Associated Ototoxicity. Summary statistics for the SNP-based GWAS were uploaded to FUMA to run a gene-based association analysis based on a multiple linear principal components regression to determine the aggregated effect of all SNPs within a gene.

Figure 23. Gene-Based Genome-Wide Association Analyses of Radiation-Associated Ototoxicity.

Inputted SNPs were mapped to 18,992 and 18,897 protein coding genes for tinnitus and hearing loss, respectively, producing a significance threshold of p = 0.05/18,992 ($2.63x10^{-6}$) or p = 0.05/18,897 ($2.65x10^{-6}$). **A)** Manhattan plot of the gene-based association analysis for radiation-induced tinnitus identified *DCAF6* (p = $8.58x10^{-6}$) and *NAV2* (p = $1.60x10^{-5}$) as nearly genome-wide significant. **B)** Manhattan plot of the gene-based association analysis for radiation-induced hearing loss revealed no genes reach genome-wide significance (p = $2.65x10^{-6}$). Quantile-Quantile plots of results from the gene-based association analysis for **C)** radiation-induced tinnitus and **D)** radiation-induced hearing loss are also provided.

0.48, p = 0.003; R^2 = 0.18, p = 0.01; *MPC2*: Spearman Rho = 0.35, p = 0.04; R^2 = 0.08, p = 0.10; Figure 25), indicative of a protective function against radiation-induced damage. No other examined gene showed a significant association with radiosensitivity in these cell lines (Table 16). The positive association between *NAV2* or *MPC2* expression and radiosensitivity also appeared to be specific to cancer cell lines of CNS origin (Table 17).

To examine whether any top genetic variants associated with radiation-induced tinnitus or hearing loss were confounded by primary brain cancer diagnosis, we performed a separate GWAS of brain cancer in CCSS patients. Among 4,435 pediatric cancer survivors (cases: 614; controls: 3,821), we performed a logistic regression-based GWAS using the same 20 principal components of European ancestry as covariates. None of the top ototoxicity SNPs had a statistically significant association with childhood brain cancer (Table 18), validating that the SNPs were associated with radiation-induced tinnitus or hearing loss independent of brain cancer.

Functional Enrichment Analysis

We mapped SNPs to genes by proximity and performed pathway enrichment with Gene Ontology terms. We identified 1,045 coding genes with $p < 5x10^{-4}$ for radiation-induced tinnitus, and 795 coding genes with $p < 5x10^{-4}$ for radiation-induced hearing loss. Multiple pathways related to nervous system development and/or maintenance were significantly over-represented in tinnitus and hearing loss using Gene Ontology (Appendix Table 18). In addition, pathway analysis through KEGG identified Rap1 signaling pathway for tinnitus (p = 0.04) and hearing loss (p = 0.01), as well as EGFR tyrosine kinase inhibitor resistance for radiation-induced tinnitus (p = 0.04). Analysis of associated human disease phenotypes through Human Phenotype

Ontology identified abnormality of the middle ear as statistically significant for tinnitus (p = 0.04) and lacrimal duct aplasia (p = 0.03) for hearing loss.

Replication Analysis in SJLIFE

We examined whether the above SNPs reaching genome-wide or near genome-wide significance were significant in an independent cohort of cancer survivors (tinnitus analysis: n = 952; hearing loss analysis: n = 331) (Table 19). None of the top GWAS-identified SNPs in *DCAF6*, *GPR161*, or *NAV2* were significantly associated with radiation-induced tinnitus. However, SNPs intronic to *ATXN1* (rs67522722 and rs34675197; p = 0.03) were significantly associated with hearing loss, and had the same direction of effect as the discovery cohort. Three SNPs intronic to *ERI1* were marginally associated with hearing loss (rs6991294, rs1077951, and rs1077950; p = 0.08), while one SNP was not significant (rs332013; p = 0.23).

Discussion

The current study marks an advance in understanding genetic and clinical risk factors to radiation-induced hearing loss/tinnitus in children. Our data indicate that pediatric cancer survivors with radiation-induced tinnitus are more likely to experience hearing loss, persistent dizziness/vertigo, poorer self-reported health, and greater use of antidepressants, consistent with studies in cisplatin-treated young adult cancer survivors (36). This indicates deleterious effects of tinnitus on quality of life regardless of etiology. Males are at greater risk for tinnitus and hearing loss than females after exposure to cranial radiation, consistent with previous observations of hearing loss and tinnitus in the general population (2, 3, 189). This association may be explained by differences in environmental factors such as noise exposure, different hypertension

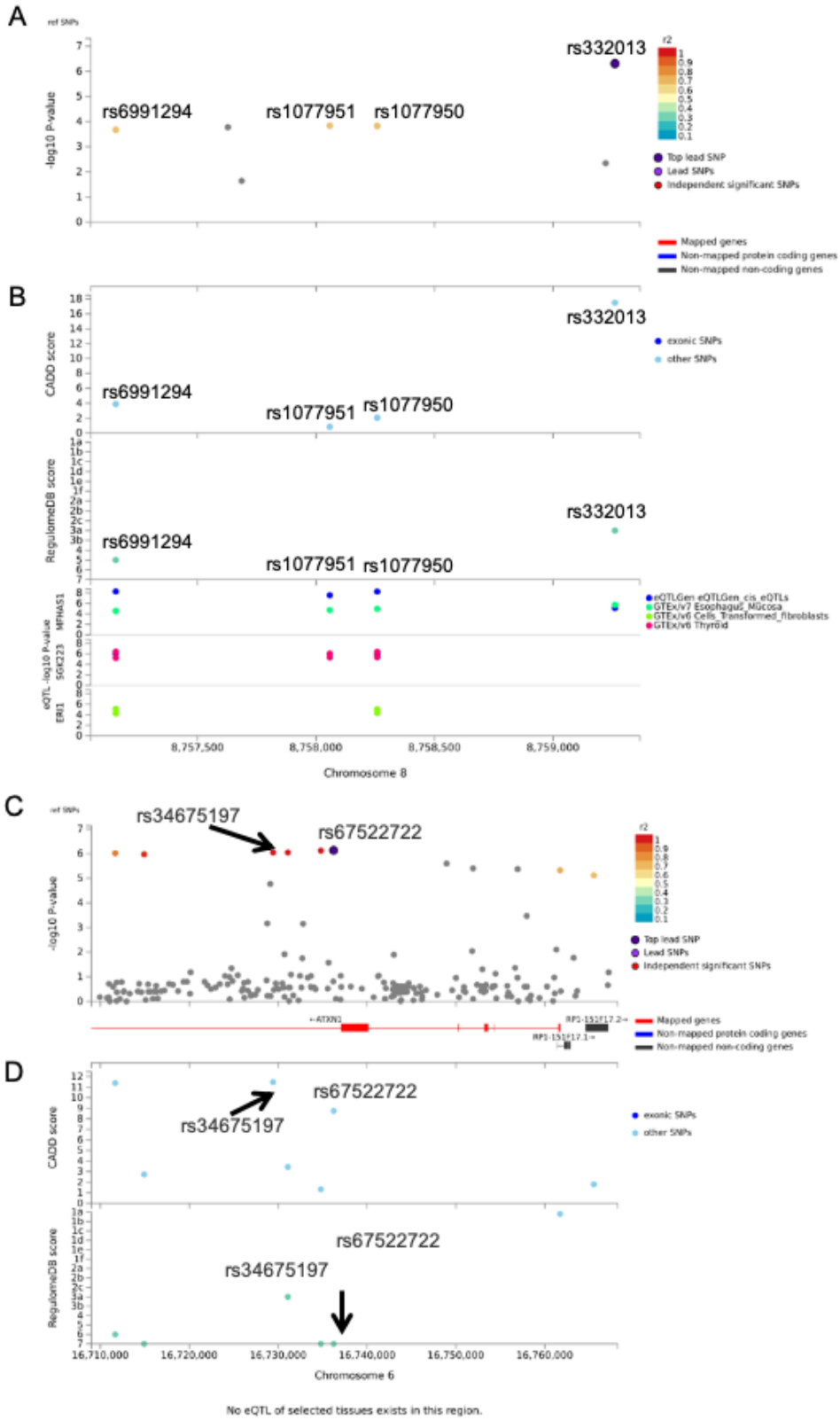


Figure 24. Regional Plot of rs332013 and rs67522722 for GWAS of Radiation-Induced Hearing Loss.

Figure 24. Regional Plot of rs332013 and rs67522722 for GWAS of Radiation-Induced Hearing Loss. A) A regional plot of the most significant GWAS signal (rs332013) was generated in FUMA. Each point represents a SNP. The x-axis indicates chromosomal position, while the y-axis shows $-\log_{10}$(p-value) of association with radiation-induced hearing loss. The LD (R^2) of each SNP with the top signal in the region, rs332013 (purple), is denoted by color in the legend, along with whether the gene has been mapped. **B)** SNPs that were in LD with rs332013 were evaluated for CADD and Regulome DB scores and underwent eQTL analysis. After evaluation, SNPs were plotted in accordance with their chromosomal position. rs332013 has a high CADD score (17.49), and appears to be an eQTL, as do three SNPs that are in LD (rs6991294, rs1077951, and rs1077950). **C)** A regional plot of the second most significant GWAS signal (rs67522722) was generated in FUMA. **D)** rs34675197 is in LD with rs67522722 and has both a high CADD (11.47) and RegulomeDB score (2b).

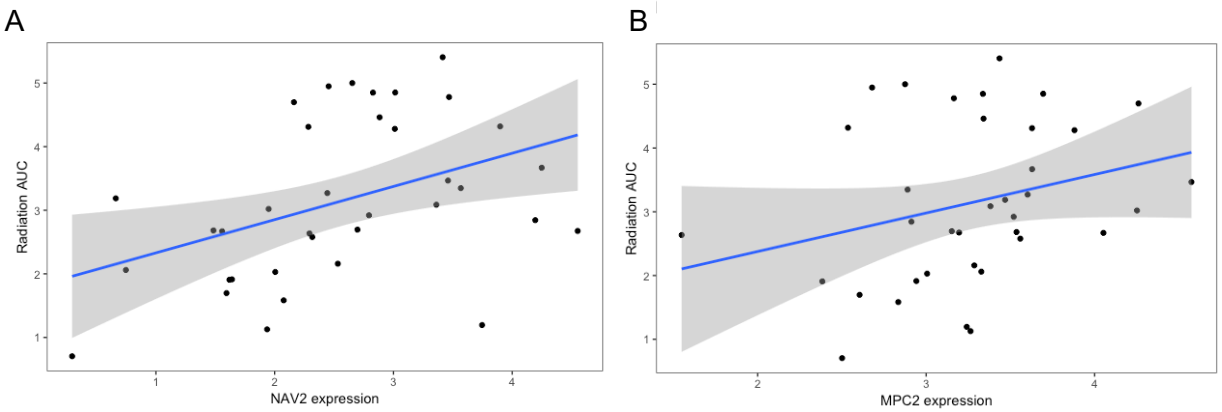


Figure 25. Scatter Plots of Radiosensitivity as a Function of Normalized *NAV2* or *MPC2* Expression. Scatter plots of radiosensitivity as a function of normalized gene expression are provided for **A)** *NAV2* ($\rho = 0.48$, $p = 0.003$; $R^2 = 0.18$, $p = 0.01$) and **B)** *MPC2* ($\rho = 0.35$, $p = 0.04$; $R^2 = 0.08$, $p = 0.10$). Radiosensitivity, measured as the area under the survival curve derived from a linear-quadratic model to fit 9-day viability assay data, for all 36 CNS tumor cell lines, was obtained from the RadioGx package in R, and normalized gene expression data were downloaded from the Cancer Cell Line Encyclopedia. Correlation was assessed nonparametrically using the Spearman rank method, as well as by linear regression.

Table 16. Evaluation of Radiosensitivity as a Function of Normalized Expression of Genes Identified in GWAS of Radiation-Associated Ototoxicity.

	Radiation AUC			
Gene	**ρ**	**p**	**R^2**	**p**
Tinnitus				
DCAF6	0.08	0.65	8.30×10^{-5}	0.96
TBX19	-0.12	0.43	0.004	0.72
GPR161	0.06	0.72	0.005	0.67
MPC2	0.35	**0.04**	0.08	0.10
NAV2	0.48	**0.003**	0.18	**0.01**
Hearing Loss				
ERI1	-0.15	0.38	0.02	0.37
MFHAS1	0.11	0.52	3.08×10^{-6}	0.99
SGK223	0.03	0.87	0.004	0.70
ATXN1	-0.07	0.67	0.009	0.58

Significance values were calculated using Spearman's rank-order correlation and linear regression. P-values < 0.05 are highlighted in bold.
Radiosensitivity, measured as the area under the survival curve derived from a linear-quadratic model to fit 9-day viability assay data, for all 36 CNS tumor cell lines, was obtained from the RadioGx package in R, and normalized gene expression data were downloaded from the Cancer Cell Line Encyclopedia.

Table 17. Correlations Between *NAV2* or *MPC2* Expression and Radiosensitivity in Different Cancer Cell Line Types.

Cancer Cell Line Type	ρ	p	R^2	p	n
NAV2					
Aerodigestive Tract	0.17	0.39	0.03	0.39	26
Bone	0.64	0.14	0.38	0.14	7
Breast	-0.74	**7.63×10^{-6}**	0.34	**0.001**	28
CNS	0.48	**0.003**	0.18	**0.01**	36
Digestive System	0.15	0.33	5.47×10^{-4}	0.88	42
Kidney	-0.08	0.84	0.01	0.92	9
Lung	-0.01	0.91	3.79×10^{-6}	0.87	72
Pancreas	0.21	0.56	0.07	0.45	10
Skin	0.11	0.68	0.01	0.71	16
Urogenital System	0.09	0.60	0.03	0.39	38
MPC2					
Aerodigestive Tract	0.32	0.10	0.06	0.22	26
Bone	-0.46	0.30	0.02	0.77	7
Breast	0.18	0.36	0.05	0.26	28
CNS	0.35	**0.04**	0.08	0.10	36
Digestive System	-0.17	0.28	0.02	0.39	42
Kidney	0.37	0.33	0.07	0.51	9
Lung	-0.05	0.65	2.06×10^{-5}	0.97	72
Pancreas	-0.33	0.35	0.13	0.31	10
Skin	0.06	0.82	0.03	0.50	16
Urogenital System	0.05	0.77	0.002	0.78	38

Significance values were calculated using Spearman's rank-order correlation and linear regression. P-values ≤ 0.05 are highlighted in bold. The number of cell lines for each analysis is provided in the n column. Radiosensitivity, measured as the area under the survival curve derived from a linear-quadratic model to fit 9-day viability assay data, was obtained from the RadioGx package in R, and normalized gene expression data were downloaded from the Cancer Cell Line Encyclopedia.

Table 18. Comparison of P-Values Between Top SNPs of Radiation-Induced Tinnitus and Hearing Loss GWAS for their Association with Childhood Brain Cancer.

SNP	Gene	Tinnitus P	Hearing Loss P	Brain Cancer P
Radiation-Induced Tinnitus				
rs203248	*DCAF6*	1.50×10^{-9}	0.04	0.71
rs73024126	*DCAF6*	1.59×10^{-8}	0.04	0.64
rs430565	*DCAF6*	1.72×10^{-8}	0.02	0.26
rs369914	*DCAF6*	4.55×10^{-8}	0.03	0.44
rs370952	*DCAF6*	1.78×10^{-6}	0.07	0.39
rs433173	*DCAF6*	3.34×10^{-6}	0.10	0.49
rs73030214	*GPR161*	9.65×10^{-7}	0.09	0.39
rs7106624	*NAV2*	1.99×10^{-6}	0.003	0.41
Radiation-Induced Hearing Loss				
rs332013	*ERI1*	0.02	5.79×10^{-7}	0.93
rs6991294	*ERI1*	0.09	2.11×10^{-4}	0.61
rs1077951	*ERI1*	0.08	1.47×10^{-4}	0.48
rs1077950	*ERI1*	0.09	1.51×10^{-4}	0.52
rs67522722	*ATXN1*	0.01	7.78×10^{-7}	0.66
rs34675197	*ATXN1*	0.01	1.01×10^{-6}	0.67

Table 19. Evaluation of Top SNPs in Radiation-Induced Tinnitus and Hearing Loss GWAS in SJLIFE.

SNP	Gene	MAF (Cases)	MAF (Controls)	GWAS OR	GWAS p	SJLIFE OR (95% CI)	SJLIFE p
Radiation-Induced Tinnitus							
rs203248	*DCAF6*	0.05	0.008	8.67 (1.52, 47.94)	1.50×10^{-9}	0.77 (0.09, 6.50)	0.81
rs73024126	*DCAF6*	0.05	0.008	7.81 (1.43, 43.38)	1.59×10^{-8}	0.77 (0.09, 6.50)	0.81
rs430565	*DCAF6*	0.05	0.01	7.04 (1.40, 35.16)	1.72×10^{-8}	1.38 (0.29, 6.65)	0.69
rs369914	*DCAF6*	0.05	0.009	7.03 (1.38, 35.87)	4.55×10^{-8}	1.38 (0.29, 6.65)	0.69
rs370952	*DCAF6*	0.05	0.01	5.15 (1.19, 22.42)	1.78×10^{-6}	1.20 (0.33, 4.33)	0.78
rs433173	*DCAF6*	0.05	0.01	4.87 (1.16, 20.29)	3.34×10^{-6}	1.20 (0.33, 4.33)	0.78
rs7106624	*NAV2*	0.45	0.31	1.86 (1.07, 3.22)	1.99×10^{-6}	1.15 (0.83, 1.58)	0.40
Radiation-Induced Hearing Loss							
rs332013	*ERI1*	0.28	0.38	0.57 (0.35, 0.93)	5.79×10^{-7}	1.26 (0.87, 1.82)	0.23

rs6991294	*ERI1*	0.23	0.30	0.63 (0.39, 1.01)	$2.12x10^{-4}$	1.42 (0.95, 2.12)	0.08
rs1077951	*ERI1*	0.23	0.30	0.62 (0.38, 1.01)	$1.47x10^{-4}$	1.42 (0.95, 2.12)	0.08
rs1077950	*ERI1*	0.23	0.30	0.62 (0.38, 1.01)	$1.51x10^{-4}$	1.42 (0.95, 2.12)	0.08
rs67522722	*ATXN1*	0.09	0.04	2.47 (1.13, 5.37)	$7.78 x10^{-7}$	2.64 (1.12, 6.20)	**0.03**
rs34675197	*ATXN1*	0.09	0.04	2.45 (1.11, 5.31)	$1.01x10^{-6}$	2.64 (1.12, 6.20)	**0.03**

Table 19 Continued.

Significance values were calculated using logistic regression with the same covariates from GWAS. P-values ≤ 0.05 are highlighted in bold.
The sample sizes for the discovery cohorts of radiation-induced tinnitus and hearing loss (CCSS) were 1,991 and 2,198 patients, while the sample sizes for the replication cohorts of radiation-induced tinnitus and hearing loss (SJLIFE) were 952 and 331 patients.

rates, smoking rates, and biological sex differences (189). Sex differences exist in both peripheral and central auditory processing, and higher levels of circulating estrogen have been associated with better hearing thresholds (190).

The GWAS of radiation-induced tinnitus identified a prominent signal in chromosome 1 led by rs203248 ($p = 1.50x10^{-9}$), a SNP intronic to *DCAF6*, a gene that encodes for a ligand-dependent coactivator of nuclear receptors, including glucocorticoid receptor and androgen receptor. Recent studies have confirmed the importance of glucocorticoid receptor in the maintenance of normal hearing, as hyporeactivity of the HPA axis has been documented in patients suffering from chronic tinnitus and hearing loss, while individuals with tinnitus and hearing loss secrete significantly less cortisol than control subjects without tinnitus (191, 192). A recent *in vivo* study has also noted that auditory trauma markedly increases glucocorticoid receptor expression in rat brains, which is known to suppress neurogenesis, and could provide a potential mechanism for how hearing loss and tinnitus may be modulated by stress (193). Further, androgen receptor has been identified in the inner ear of vertebrates (194, 195), indicating that multiple nuclear receptors could influence auditory processes. It should be noted that SNPs in LD with rs203248 encompassed several other genes on chromosome 1 (*TIPRL*, *GPR161*, and *MPC2*), forming a relatively large genetic risk locus that had chromatin interactions with 13 other genes. Consequently, the genetic susceptibilities underlying radiation-induced tinnitus are complex and likely polygenic in nature. In addition, SNPs intronic to *DCAF6* were not replicated in SJLIFE. Due to the much smaller sample size of the replication analysis, it would be worthwhile to assess *DCAF6* SNPs identified in the GWAS in additional

cohorts of pediatric cancer survivors. Importantly, CCSS does have an expanded cohort of non-overlapping patients diagnosed with pediatric cancer between 1987-1999 that should have whole genome sequencing data available by the end of 2020. Based on estimates from the original cohort, this group would include approximately 1,335 patients for hearing loss (164 cases and 1,171 controls) and 1,210 patients for tinnitus (89 cases and 1,121 controls for tinnitus).

In addition to *DCAF6*, *NAV2* and *MPC2* were positively correlated with radiosensitivity in CNS tumor cell lines *in silico*, with *NAV2* also being identified as near genome-wide significant in the gene-based analysis ($p = 1.60x10^{-5}$). It has previously been demonstrated that Nav2 (Neuron navigator 2) mutant mice embryos have an overall reduction in nerve fiber density, as well as specific defects in cranial nerves, suggesting that Nav2 is vital for mammalian nervous system development (196). Interestingly, Nav2 hypomorphic mutant adult mice also displayed a blunted baroreceptor response compared to wild-type controls (196), implicating the gene in blood pressure regulation. This observation could provide insight into the association between radiation-induced tinnitus and hypertension, as perturbed Nav2 function could exert pleotropic effects on both phenotypes. Further, Nav2 has been shown to be essential for all-trans retinoic acid induction of neurite outgrowth, as well as direct localization with the microtubule cytoskeleton, implicating the protein in axonal elongation and interactions between microtubules and other proteins that facilitate the formation and stability of growing neurites (197). *MPC2* encodes for mitochondrial pyruvate carrier 2, a protein vital for neuron survival due their reliance on glucose and pyruvate metabolism to generate ATP (198). Importantly, MPC function has also been

linked to radiosensitivity, as inhibiting the carrier increases oxygen availability, markedly sensitizing SiHa xenografts in nude mice to radiation (199). Our *in silico* analysis in CNS tumor cell lines is in accord with these *in vivo* data, as lower *MPC2* expression was associated with increased radiosensitivity.

The GWAS of radiation-induced hearing loss identified rs332013 as near genome-wide significant ($p = 5.79x10^{-7}$), a SNP intronic to *ERI1*, which encodes for an RNA exonuclease. Interestingly, rs332013 and three SNPs in LD (rs6991294, rs1077951, and rs1077950) appear to be eQTLs for *ERI1*, as well as *MFHAS1* and *SGK223*. However, these three SNPs were only marginally associated with radiation-induced hearing loss in the SJLIFE cohort. MFHAS1 appears to influence recovery in the CNS following injury, as knock-down by Mfhas1 siRNA in rats improved cognitive impairment induced by cecal ligation and puncture (200). In addition, a recently described microdeletion in *ERI1* and *MFHAS1* has been associated with a developmental disorder that includes intellectual disability (201), suggesting that both genes could influence nervous system development. *SGK223* (*PRAG1*) has also been implicated in nervous system development, as it has been demonstrated that RND2 regulates neurite outgrowth by functioning as a RhoA activator through PRAG1 (202). Further, PRAG1 is known to co-activate NOTCH1 (203), a protein required for both neuron and glial formation, as well as modulating the onset of neurogenesis within the cerebellar neuroepithelium (204).

The next most significant SNP in the radiation-induced hearing loss GWAS, rs67522722 ($p = 7.78x10^{-7}$) is intronic to *ATXN1*, and is in perfect LD with rs34675197 that appears to regulate the binding of transcription factors to *ATXN1*. Further,

rs67522722 was significantly associated with radiation-induced hearing loss in SJLIFE ($p = 0.03$), and had the same direction of effect. Spinocerebellar ataxia type 1 is an inherited neurodegenerative disease associated with a gain of function mutation in ataxin-1 that contributes to cerebellar and brain stem degeneration. Recently, mechanistic studies in iPSC-derived neurons from affected patients and relevant mouse models have validated the importance of ATXN1-CIC complexes in the pathophysiology of the disease (205, 206), highlighting the importance of ataxin-1 in neuronal maintenance.

These data suggest that SNPs associated with radiation-induced tinnitus and hearing loss appear to be near or intronic to genes involved in neuronal development and maintenance. Accordingly, pathway analysis via GO and KEGG identified multiple pathways associated with nervous system development and maintenance for both radiation-induced tinnitus and hearing loss, including Rap1 signaling (tinnitus: $p = 0.04$; hearing loss: $p = 0.01$). During the development of the mammalian neocortex, the generation and migration of neurons are closely coordinated by Rap1 GTPases that promote both cortical organization and cell polarity (207, 208). Consequently, loss of Rap1 GTPases in developing neurons prevents the formation of axons and leading processes, thereby interfering with radial migration (208). In addition, Rap1 stimulates multiple downstream pathways necessary for neuronal cell maintenance, including ERK1/2 signaling (209), which also appears to be vital for regulating inner ear cell survival, particularly after auditory or ototoxic insult (210, 211). The downstream activation of ERK via Rap1 also provides insight regarding the association between EGFR tyrosine kinase inhibitor resistance and radiation-induced tinnitus identified

through KEGG (p = 0.04), as EGFR inhibitor resistance is often mediated by aberrant activation of ERK signaling (212).

Analysis of human disease phenotypes associated with SNPs enriched for statistical significance in the two GWAS identified abnormality of the middle ear as statistically significant for tinnitus (p = 0.04) and lacrimal duct aplasia (p = 0.03) for hearing loss. The association between middle ear pathologies and tinnitus is evident, as patients with tinnitus often complain about a sensation of fullness or blockage in the middle ear, indicative of issues with middle ear pressure or increased impedance of the ossicular chain (188). Although not as inherent, associations between lacrimal duct aplasia and hearing loss have been documented in multiple hereditary disorders, including LADD syndrome. Importantly, families with genetic predispositions to LADD syndrome often have heterozygous mutations in the tyrosine kinase domains of *FGFR2* and *FGFR3*, as well as in *FGF10* (213), which appear to be integral to its pathophysiology (214, 215). As with Rap1, ERK1/2 is an important mediator of FGF signaling in many biological processes due to the ability of ERK to transduce the FGF signal to the nucleus and other cellular compartments, and is an essential interaction for mammalian ear development (216, 217). Due to the importance of Rap1 and ERK signaling in influencing the development and maintenance of the auditory system, further investigation into their association with radiation-associated ototoxicity is warranted.

Major strengths of our study include the longitudinal nature of the CCSS data collection that enabled the first genome-wide analysis of radiation-associated ototoxicity, our rigorous definition of phenotypes, and replication of *ATXN1* variants

approaching genome-wide significance in an independent cohort. Due to the dearth of information regarding genetic susceptibility to ototoxicity and its associated pathophysiology, our study provides novel information on a treatment-related toxicity that is frequently encountered in the clinical setting among pediatric and adult-onset cancer survivors. Inherent limitations of our study include the lack of collection of several factors associated with ototoxicity, including the usage of aminoglycosides or other ototoxic supportive care agents, the specific location of brain tumors, and brain surgery status. Furthermore, the radiation exposure variable used in the analysis was not based upon direct estimation of radiation dose to the cochlea. In addition, the hearing loss phenotype differed in the CCSS discovery set (self-report) and SJLIFE cohort (Chang score based on audiometry). Although the presence of brain tumors could be a confounder, we demonstrate that SNPs associated with tinnitus/hearing loss are not associated with brain tumors.

It is important to note that approximately 29% of patients in the CCSS hearing loss and tinnitus cohorts were given prophylactic cranial radiation for ALL; yet recent evidence indicates that prophylactic cranial radiation is unnecessary in ALL, as only 2 of ~1,100 children not given cranial radiation died due to CNS relapse (218, 219). Consequently, the overall proportion of pediatric cancer survivors exposed to cranial radiation will decrease in the near future, reducing the incidence of radiation-associated ototoxicity.

Conclusion

Untreated hearing loss has been linked to many health conditions including cognitive decline and dementia (7, 220). Importantly, midlife hearing loss is the single

largest modifiable risk factor for dementia (220). Given the detrimental effects of tinnitus/hearing loss on neurological and behavioral development, patients and their families should be educated with regard to potential non-genetic risk factors and comorbidities associated with treatment-related ototoxicity prior to therapy initiation and during long-term follow-up. Further validation of *ATXN1*, *NAV2*, and *MPC2* regarding their association with radiation-associated ototoxicity *in vitro* and *in vivo* is warranted.

Summary

Cranial radiation therapy is associated with ototoxicity that manifests as hearing loss and tinnitus. We sought to identify clinical determinants and genetic risk factors for ototoxicity among adult survivors of pediatric cancer treated with cranial radiation. Logistic regression evaluated associations of tinnitus (n = 1,991) and hearing loss (n = 2,198) with non-genetic risk factors and comorbidities among cranial radiation-treated survivors of European ancestry in the Childhood Cancer Survivor Study who did not receive platinum-based chemotherapy. Genome-wide association studies (GWAS) of radiation-induced tinnitus and hearing loss were performed using cumulative cranial radiation dose, age at last observation, and 20 genetic principal components as covariates. Males were more likely to report radiation-induced tinnitus (9.4% vs. 5.4%; $p = 5.81 \times 10^{-4}$) and hearing loss (14.0% vs. 10.7%; $p = 0.02$) than females after adjusting for cumulative cranial radiation dose and age at last observation. Survivors with tinnitus or hearing loss were more likely to experience persistent dizziness or vertigo (tinnitus: $p < 2 \times 10^{-16}$; hearing loss: $p = 6.35 \times 10^{-9}$), take antidepressants (tinnitus: $p = 0.02$; hearing loss: $p = 0.01$) and report poorer overall health (tinnitus: $p = 9.40 \times 10^{-7}$; hearing loss: $p = 1.30 \times 10^{-6}$) compared to controls after age-adjustment. GWAS of radiation-induced

tinnitus revealed a genome-wide significant signal in chromosome 1 led by rs203248 ($p = 1.50x10^{-9}$), while GWAS of radiation-induced hearing loss identified rs332013 ($p = 5.79x10^{-7}$) in chromosome 8 and rs67522722 ($p = 7.78x10^{-7}$) in chromosome 6 as near genome-wide significant. Additional analysis in the St. Jude Lifetime Cohort identified rs67522722, intronic to *ATXN1*, a gene associated with spinocerebellar ataxia type 1, to be significantly associated with radiation-induced hearing loss ($p = 0.03$). Taken together, radiation-associated ototoxicity was associated with sex, several neuro-otological symptoms, increased antidepressant use, and poorer self-reported health. GWAS of radiation-induced hearing loss identified rs67522722, which was further supported in an independent cohort of pediatric cancer survivors.

CHAPTER 8. COMPARISON OF GENETIC ARCHITECTURE BETWEEN AGE-RELATED HEARING LOSS AND TINNITUS AND TREATMENT-RELATED OTOTOXICITY

Introduction

Age-related disorders are becoming increasingly prevalent as the median age of global populations continues to increase (221). This is epitomized by hearing loss and tinnitus, two common auditory disorders that are particularly prevalent among elderly populations. As the third most prevalent chronic health condition facing older adults (1), approximately one in three people in the United States between the ages of 65 and 74 has some degree of hearing loss, and nearly half of those older than 75 are hearing impaired. Susceptibility to tinnitus also appears to increase with age, peaking at 14.3% in people 60-69 years of age (3). Interestingly, there appears to be differences in hearing loss and tinnitus prevalence based on race, as blacks have a lower incidence of both hearing disorders than whites (3, 189). Further, it has been reported that males have a 5.5-fold higher risk of developing age-related hearing loss than females (189). Evaluating tinnitus susceptibility among men and women has proven to be far less definitive (3, 222), partially due to differences in perceived stress following symptoms of tinnitus (223). Nevertheless, men generally exhibit a slightly higher risk of developing tinnitus (222). The long-term effects of tinnitus are numerous, including sleeping difficulties and concentration problems that promote increased anxiety, depression, and insomnia (4, 5); similarly hearing loss is an established risk factor for depression (6, 224) but also dementia (225). Further, it has recently been demonstrated that subclinical levels of hearing loss are highly associated with cognitive decline (7),

indicating that even subtle declines in auditory processing can have substantial consequences on overall quality of life.

Several studies have suggested a significant heritable component to *de novo* hearing loss and tinnitus (177, 226), resulting in an increased interest in identifying genetic variants that are associated with hearing loss/tinnitus susceptibility. Although heritability estimates for age-related hearing loss range from 19% to 65% (227, 228) variability in phenotyping methods, small sample sizes and differing demographics have led to inconsistent GWAS results. Although pure-tone audiometry is the gold standard for measuring hearing loss, most large population based studies use self-report or electronic health records because of the requirement for specialized equipment and audiology expertise. A GWAS of self-reported hearing loss and speech reception threshold data from electronic health records in 6,527 cases and 45,882 controls identified 2 novel SNP associations (in genes *ISG20* and *TRIOBP*) in non-Hispanic whites that were replicated in two subsequent analysis (229). A more recent GWAS of 250,000 UK Biobank volunteers aged between 40 and 69 years using participant responses to questionnaires identified 44 independent genome-wide significant loci ($p < 5x10^{-8}$), with 34 loci being novel associations with hearing loss of any form (230). These studies underscore the polygenicity of age-related hearing loss, and provide evidence that the phenotype is a complex trait in need of further study. In contrast to hearing loss, GWAS of *de novo* tinnitus has only been performed in a small cohort of 167 cases and 749 controls of European descent that identified no genome-wide significant SNPs (227) indicative of the importance of evaluating GWAS of tinnitus in a large cohort.

Similar to many GWAS of complex traits (231), there is a paucity of data on genetics of hearing loss and tinnitus in individuals of African ancestry. In the current study, we use the Electronic MEdical Record and GEnomics (eMERGE) network, a large consortium of over 85,000 genotyped patients who are linked to phenotype information through electronic health records (EHRs), to determine genetic variants associated with hearing loss in individuals of European and African descent. We further characterized non-genetic and genetic risk factors to age-related hearing loss and tinnitus, but also to determine whether these disorders share common genetic architecture with treatment-related ototoxicity. Specifically, we interrogate variables on age, race, sex, treatment, medical history, and lifestyle and behavioral factors to identify potential non-genetic risk factors and comorbidities. We then perform SNP-based and gene-based genome-wide analyses to identify genetic predispositions to age-related hearing loss and tinnitus among patients of European and African ancestry. Finally, we perform enrichment and polygenic risk score (PRS) analysis of age-related hearing loss and tinnitus in our European and African cohorts to determine whether these phenotypes share common genetic architecture in different races, and whether age-related hearing loss and tinnitus share genetic architecture with ototoxicity following treatment with cisplatin or cranial radiation.

Results

Cohort Characteristics

Demographic and clinical characteristics for patients diagnosed with age-related hearing loss and tinnitus are provided in Table 20. Median age at diagnosis was 69 years (range: 50-89 years) and 65 (range: 50-89) for hearing loss and tinnitus,

respectively. Median current age of patients with age-related hearing loss was 72 (range: 54-99), while the median current age of patients with age-related tinnitus was 73 (range: 53-99).

Associations with Risk Factors and Comorbidities

The distribution of patients with age-related hearing loss or tinnitus based on race is provided in Table 21. Both white (14.8%; age-adjusted OR = 2.48, 95% CI: 2.15-2.88, $p < 2x10^{-16}$) and American Indian/Alaska Native (27.1%; age-adjusted OR = 6.95, 95% CI: 3.42-13.33, $p = 1.82x10^{-8}$) patients were much more likely to report hearing loss than black patients (4.7%). Similarly, both patient populations had a higher incidence of tinnitus (white: 4.7%; age-adjusted OR = 1.49, 95% CI: 1.24 -1.83, $p = 3.52x10^{-5}$; American Indian/Alaska Native: 11.1%; age-adjusted OR = 4.21, 95% CI: 1.43-9.97, p = 0.003) when compared to black patients (2.6%). Asian patients did not have a significant difference in hearing loss (9.8%; age-adjusted OR = 1.91, 95% CI: 0.71-4.31, p = 0.15) or tinnitus (0%; age-adjusted OR = $1.71x10^{-5}$, 95% CI: $3.96x10^{-22}$-$9.12x10^{-4}$, p = 0.91) incidence compared to black patients. In addition, males were much more likely than females to report age-related hearing loss (15.0% vs. 11.3%; age-adjusted OR = 1.31, 95% CI: 1.23-1.39, $p < 2x10^{-16}$), but there was no statistical difference in tinnitus incidence (4.4% vs. 4.2%; age-adjusted OR = 1.09, 95% CI: 0.99-1.21, p = 0.08; Table 22).

Both age-related hearing loss and tinnitus were associated with dizziness (hearing loss: age-adjusted OR = 2.88, 95% CI: 2.70-3.07, $p < 2x10^{-16}$; tinnitus: age-adjusted OR = 3.32, 95% CI: 3.00-3.68, $p < 2x10^{-16}$) and vertigo (hearing loss: age-adjusted OR = 3.94, 95% CI: 3.54-4.38, $p < 2x10^{-16}$; tinnitus: age-adjusted OR = 3.46,

95% CI: 2.98-4.01, $p < 2x10^{-16}$; Table 23). Hearing loss and tinnitus cases also reported higher incidences of major depressive disorder single episode (hearing loss: age-adjusted OR = 2.26, 95% CI: 2.00-2.55, $p < 2x10^{-16}$; tinnitus: age-adjusted OR = 2.18, 95% CI: 1.83-2.59, $p < 2x10^{-16}$) and recurrent episode (hearing loss: age-adjusted OR = 1.88, 95% CI: 1.66-2.12, $p < 2x10^{-16}$; tinnitus: age-adjusted OR = 2.06, 95% CI: 1.73-2.44, $p < 2x10^{-16}$). Patients with age-related hearing loss and tinnitus were also more likely to be diagnosed with more severe forms of major depressive disorder single episode (hearing loss: $p = 2.84x10^{-17}$; tinnitus: $p = 3.91x10^{-4}$) or recurrent episode (hearing loss: p = 0.11; tinnitus: $p = 6.20x10^{-4}$) when compared to age-matched controls (Figure 26). In addition, patients with age-related hearing loss and tinnitus experienced higher rates of hypercholesteremia (hearing loss: age-adjusted OR = 1.50, 95% CI: 1.41-1.59, $p < 2x10^{-16}$; tinnitus: age-adjusted OR = 1.82, 95% CI: 1.65-2.01, $p < 2x10^{-16}$) and hypertension (hearing loss: age-adjusted OR = 1.17, 95% CI: 1.09-1.27, $p = 2.36x10^{-5}$; tinnitus: age-adjusted OR = 1.17, 95% CI: 1.04-1.32, p = 0.01). As with depression, the severity of hypertension was associated with age-related hearing loss ($p < 2x10^{-16}$) and tinnitus ($p = 1.77x10^{-11}$) (Figure 27).

Genome-Wide Association Studies

GWAS of age-related hearing loss in patients of European ancestry identified two genome-wide significant SNPs (rs9272454, $p = 5.49x10^{-11}$; rs3828840, $p = 4.36x10^{-8}$), as well as one near-genome wide significant SNP (rs68148149, $p = 5.74x10^{-8}$) in chromosome 6 (Figure 28A and B, Appendix Table 19). Both genome-wide significant signals reside in human leukocyte antigen (HLA) genes as rs9272454 is intronic to *HLA-DQA1*, while rs3828840 is intronic to *HLA-DRB4*. rs68148149 is in an intergenic region

Table 20. Clinical and Sociodemographic Characteristics of Patients in eMERGE Based on Hearing Loss and Tinnitus Status.

	Hearing Loss Status (50-90 years old)		Tinnitus Status (50-90 years old)	
Characteristic	**Hearing Loss Controls**	**Hearing Loss Cases**	**Tinnitus Controls**	**Tinnitus Cases**
n	33,089	5,011	36,783	1,656
Sex[a]				
Male	16,106 (48.7%)	2,840 (56.7%)	18,276 (49.7%)	805 (48.6%)
Female	16,982 (51.3%)	2,171 (43.3%)	18,506 (50.3%)	851 (51.4%)
Race				
White	26,497 (80.1%)	4,604 (91.9%)	29,841 (81.1%)	1,459 (88.1%)
Black	4,255 (12.9%)	208 (4.2%)	4,471 (12.2%)	119 (7.2%)
American Indian or Alaska Native	35 (0.1%)	13 (0.3%)	40 (0.1%)	5 (0.3%)
Asian or Pacific Islander	55 (0.2%)	6 (0.1%)	67 (0.2%)	0 (0%)
Unknown	2,247 (6.8%)	180 (3.6%)	2,364 (6.4%)	73 (4.4%)
Age at Diagnosis				
Median (range)	N/A	69 (50-89)	N/A	65 (50-89)
50-59	N/A	936 (18.7%)	N/A	508 (30.7%)
60-69	N/A	1,749 (34.9%)	N/A	620 (37.4%)
70-79	N/A	1,611 (32.1%)	N/A	403 (24.3%)
80-90	N/A	715 (14.3%)	N/A	125 (7.5%)
Current Age				
Median (range)	72 (54-99)	82 (54-99)	73 (53-99)	77 (53-99)
50-59	4,052 (12.2%)	61 (1.2%)	4,619 (12.6%)	29 (1.8%)
60-69	9,406 (28.4%)	633 (12.6%)	9,688 (26.3%)	323 (19.5%)
70-79	10,188 (30.8%)	1,547 (30.9%)	11,034 (30.0%)	615 (37.1%)
80-89	6,501 (19.7%)	1,509 (30.1%)	7,509 (20.4%)	431 (26.0%)
90-99	2,942 (8.9%)	1,261 (25.2%)	3,933 (10.7%)	258 (15.6%)
Dizziness	6,352 (19.2%)	2,261 (45.1%)	7,760 (21.1%)	821 (49.6%)
Vertigo	1,063 (3.2%)	682 (13.6%)	1,490 (4.1%)	238 (14.4%)
MDD (Single Episode)	1,332 (4.0%)	421 (8.4%)	1,631 (4.4%)	153 (9.2%)
MDD (Recurrent Episode)	1,759 (5.3%)	379 (7.6%)	2,019 (5.5%)	159 (9.6%)
Hyper-cholesterolemia	11,812 (35.7%)	2,449 (48.9%)	13,381 (36.4%)	881 (53.2%)
Hypertension	23,299 (70.4%)	3,983 (79.5%)	26,074 (70.9%)	1,279 (77.2%)

Abbreviation: MDD: major depressive disorder

[a]1 patient did not report sex for the age-related hearing loss cohort.

Table 21. Distribution of Hearing Loss and Tinnitus Based on Race.

Type	Race	Total Number of Patients (Percent with Phenotype)	OR (95% CI)	p	Age-Adjusted OR (95% CI)	Age-Adjusted p
Age-Related Sensorineural Hearing Loss	Black	4,463 (4.7%)	N/A	N/A	N/A	N/A
	White	31,101 (14.8%)	3.55 (3.09, 4.11)	**$< 2x10^{-16}$**	2.48 (2.15, 2.88)	**$< 2x10^{-16}$**
	Asian or Pacific Islander	61 (9.8%)	2.23 (0.85, 4.84)	0.07	1.91 (0.71, 4.31)	0.15
	American Indian or Alaska Native	48 (27.1%)	7.60 (3.82, 14.23)	**$1.06x10^{-9}$**	6.95 (3.42, 13.33)	**$1.82x10^{-8}$**
Age-Related Tinnitus	Black	4,590 (2.6%)	N/A	N/A	N/A	N/A
	White	31,300 (4.7%)	1.84 (1.53, 2.23)	**$3.17x10^{-10}$**	1.49 (1.24, 1.83)	**$3.52x10^{-5}$**
	Asian or Pacific Islander	67 (0%)	$1.77x10^{-5}$ ($2.63x10^{-22}$, $9.89x10^{-4}$)	0.91	$1.71x10^{-5}$ ($3.96x10^{-22}$, $9.12x10^{-4}$)	0.91
	American Indian or Alaska Native	45 (11.1%)	4.70 (1.60, 11.07)	**0.001**	4.21 (1.43, 9.97)	**0.003**

P-value is calculated based on using blacks as a reference group. Bold indicates $p \leq 0.05$.

Table 22. Distribution of Tinnitus and Hearing Loss Based on Sex.

Type	Sex	Total Number of Patients (Percent Affected)	OR (95% CI)	p	Age-Adjusted OR (95% CI)	Age-Adjusted p
Age-Related Sensorineural Hearing Loss	Female	19,153 (11.3%)	N/A	N/A	N/A	N/A
	Male	18,946 (15.0%)	1.37 (1.30, 1.46)	**$< 2x10^{-16}$**	1.31 (1.23, 1.39)	**$< 2x10^{-16}$**
Age-Related Tinnitus	Female	19,081 (4.2%)	N/A	N/A	N/A	N/A
	Male	19,357 (4.4%)	1.04 (0.95, 1.15)	0.39	1.09 (0.99, 1.21)	0.08

P-value is calculated based on using females as a reference group. Bold indicates $p \leq 0.05$.

Table 23. Logistic Regression Analyses of the Association Between Hearing Loss or Tinnitus and Relevant Clinical Characteristics.

Characteristic	Phenotype (Age-Related)	OR (95% CI)	p	Age-Adjusted OR (95% CI)	Age-Adjusted p
Dizziness	Sensorineural Hearing Loss	3.46 (3.25, 3.68)	**$< 2x10^{-16}$**	2.88 (2.70, 3.07)	**$< 2x10^{-16}$**
	Tinnitus	3.67 (3.33, 4.06)	**$< 2x10^{-16}$**	3.32 (3.00, 3.68)	**$< 2x10^{-16}$**
Vertigo	Sensorineural Hearing Loss	4.75 (4.29, 5.25)	**$< 2x10^{-16}$**	3.94 (3.54, 4.38)	**$< 2x10^{-16}$**
	Tinnitus	3.98 (3.43, 4.60)	**$< 2x10^{-16}$**	3.46 (2.98, 4.01)	**$< 2x10^{-16}$**
Major Depressive Disorder Single Episode	Sensorineural Hearing Loss	2.19 (1.95, 2.45)	**$< 2x10^{-16}$**	2.26 (2.00, 2.55)	**$< 2x10^{-16}$**
	Tinnitus	2.19 (1.84, 2.60)	**$< 2x10^{-16}$**	2.18 (1.83, 2.59)	**$< 2x10^{-16}$**
Major Depressive Disorder Recurrent Episode	Sensorineural Hearing Loss	1.46 (1.30,1.63)	**$1.48x10^{-10}$**	1.88 (1.66, 2.12)	**$< 2x10^{-16}$**
	Tinnitus	1.83 (1.54, 2.16)	**$2.97x10^{-12}$**	2.06 (1.73, 2.44)	**$< 2x10^{-16}$**
Hyper-cholesterolemia	Sensorineural Hearing Loss	1.72 (1.62,1.83)	**$< 2x10^{-16}$**	1.50 (1.41, 1.59)	**$< 2x10^{-16}$**
	Tinnitus	1.99 (1.80, 2.19)	**$< 2x10^{-16}$**	1.82 (1.65, 2.01)	**$< 2x10^{-16}$**
Hypertension	Sensorineural Hearing Loss	1.63 (1.51, 1.75)	**$< 2x10^{-16}$**	1.17 (1.09, 1.27)	**$2.36x10^{-5}$**
	Age-Related Tinnitus	1.39 (1.24, 1.57)	**$2.77x10^{-8}$**	1.17 (1.04, 1.32)	**0.01**

Age-adjustment reflects the use of current age as a covariate in the logistic regression model. Bold indicates $p \leq 0.05$.

on chromosome 6. Notably, rs3828840 is an eQTL and sQTL for multiple HLA genes (Table 24). Gene-based association analysis identified *MAD2L1* ($p = 1.47x10^{-5}$) and *CSN1S1* ($p = 1.76x10^{-5}$) in chromosome 4 as nearly genome-wide significant ($p = 2.67x10^{-6}$) (Figure 29).

GWAS of age-related tinnitus in patients of European ancestry identified 15 independent signals consisting of 290 genome-wide significant SNPs (Figure 28C and D, Appendix Table 20). The most significant SNP was rs9273081 ($p = 5.02x10^{-20}$), which is intronic to *HLA-DQA1*. rs9273081 is also an eQTL and sQTL for multiple HLA genes (Table 25). Gene-based association analysis identified seven genes to be genome-wide significant (*EXD3*, $p = 2.79x10^{-9}$; *PRKAR1B*, $p = 6.01x10^{-8}$; *SRSF10*, $p = 3.10x10^{-7}$; *NRC2*, $p = 4.36x10^{-7}$; *MIB2*, $p = 5.77x10^{-7}$; *EGLN3*, $p = 8.16x10^{-7}$; and *PLEKHN1*, $p = 1.61x10^{-6}$), four of which are located in chromosome 1 (*SRSF10*, *NRC2*, *MIB2*, and *PLEKHN1*) (Figure 29).

GWAS of age-related hearing loss in patients of African ancestry identified two independent genome-wide significant signals in chromosomes 4 and 19 (Figure 30A and B, Appendix Table 21). The most significant SNP was rs77750421 ($p = 5.94x10^{-9}$), which is intronic to *FSTL5*. rs77750421 does not appear to influence gene expression or transcription factor binding, nor does it have SNPs in linkage disequilibrium (LD) (Figure 31). The next most significant SNP was rs144555968 ($p = 1.56x10^{-8}$), which is intronic to *SMARCA4*. Multiple SNPs are in high LD with rs144555968 (Figure 32A), including rs550869012 ($R^2 = 0.75$, $p < 0.0001$) that is located in an exonic region of *CARM1* and has a relatively high CADD score (13.97), placing it within the top 10% of deleterious

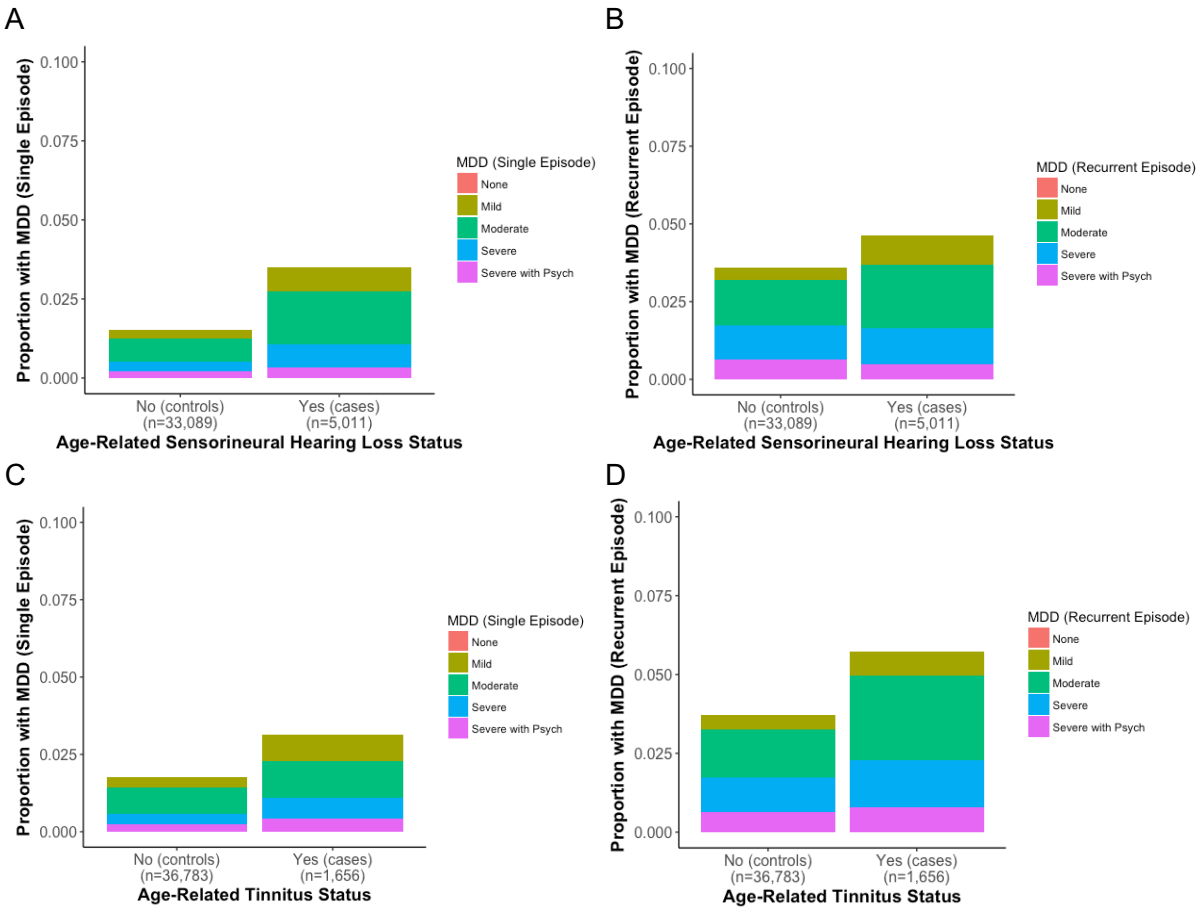


Figure 26. Association of Age-Related Sensorineural Hearing Loss and Tinnitus with the Severity of Major Depressive Disorder. The overall distribution of **A)** major depressive disorder (MDD) single episode ($p = 2.84 \times 10^{-17}$) and **B)** MDD recurrent episode ($p = 0.11$) based on age-related sensorineural hearing loss status is provided. The overall distribution of **C)** MDD single episode ($p = 3.91 \times 10^{-4}$) and **D)** MDD recurrent episode ($p = 6.20 \times 10^{-4}$) based on age-related tinnitus status is also provided. MDD single and recurrent episode are divided into different degrees of severity, as indicated in the legend. Sample sizes for each group are indicated within each panel on the x-axis. Differences between the proportions of MDD severity observed for cases and controls were evaluated for statistical significance through the Cochran-Armitage-Mantel 1df chi-squared trend test (145).

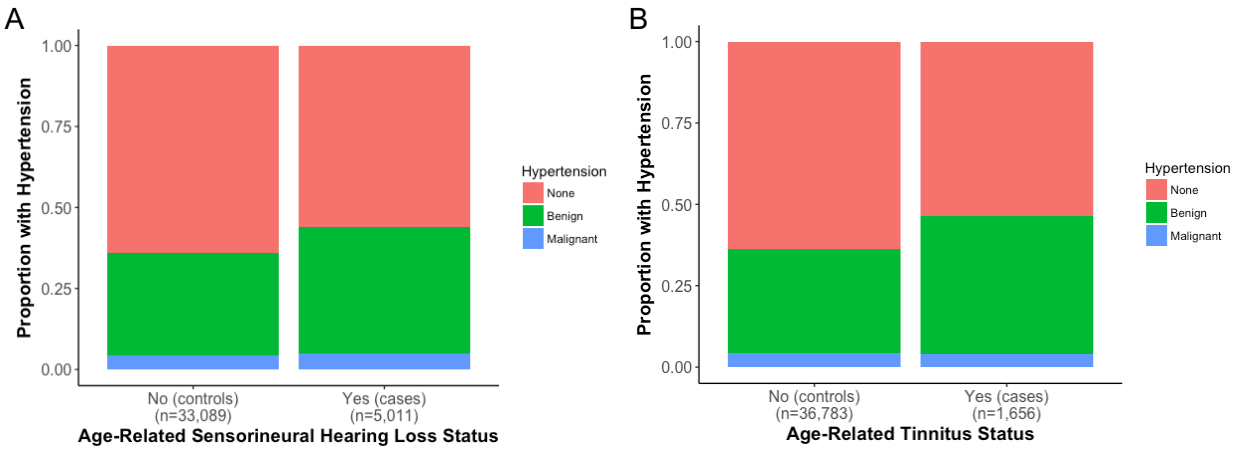


Figure 27. Association of Age-Related Sensorineural Hearing Loss and Tinnitus with the Severity of Hypertension. The overall distribution of hypertension ($p < 2x10^{-16}$) based on **A)** age-related sensorineural hearing loss status is provided. The overall distribution of hypertension ($p = 1.77x10^{-11}$) based on **B)** age-related tinnitus status is also provided. Hypertension is divided into degrees of severity, as indicated in the legend. Sample sizes for each group are indicated within each panel on the x-axis. Differences between the proportions of hypertension severity observed for cases and controls were evaluated for statistical significance through the Cochran-Armitage-Mantel 1df chi-squared trend test (145).

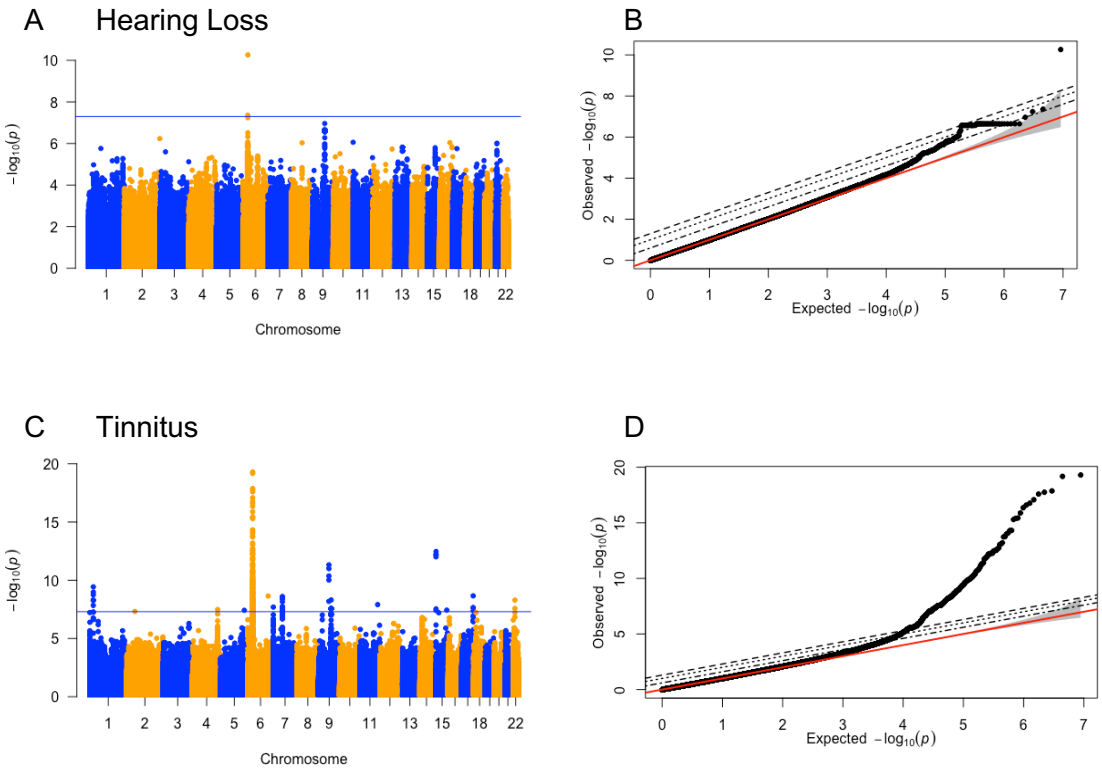


Figure 28. Genome-Wide Association Studies of Age-Related Hearing Loss and Tinnitus in Patients of European Ancestry. A) Manhattan plot of GWAS results for age-related hearing loss in patients of European ancestry reveals two genome-wide significant SNPs (rs9272454, p = 5.49×10^{-11}; rs3828840, p = 4.36×10^{-8}), as well as one near-genome wide significant SNP (rs68148149, p = 5.74×10^{-8}) in chromosome 6. **B)** Quantile-Quantile plots of GWAS results for age-related hearing loss. **C)** Manhattan plot of GWAS results for age-related tinnitus in patients of European ancestry reveals 15 independent signals consisting of 290 genome-wide significant SNPs, with the most significant SNP being rs9273081 (p = 5.02×10^{-20}) in chromosome 6. **D)** Quantile-Quantile plots of GWAS results for age-related tinnitus. Covariates in both GWAS include current age and 10 European genetic principal components accounting for population substructure.

mutations, as well as rs139420375 that may regulate the binding of transcription factors to *C19orf38* (RegulomeDB score: 2b - likely to affect binding) (Figure 32B). In addition, 28 eQTLs are in high LD with rs144555968 (Figure 32B), and regulate the expression of 6 genes on chromosome 19 (*DNM2*, *AP1M2*, *COL5A3*, *ZNF627*, *KEAP1*, and *PDE4A*). In total, SNPs in LD with rs144555968 ($R^2 > 0.6$) encompass 7 genes (*SMARCA4*, *C19orf52*, *YIPF2*, *CARM1*, *C19orf38*, *TMED1*, and *DNM2*) forming a relatively large genetic risk locus on chromosome 19 that has chromatin interactions with 17 other genes and eQTL interactions with 5 other genes (Appendix Figure 5). Gene-based association analysis identified no genome-wide significant genes (Figure 33), with the most significant being *SLC27A2* ($p = 5.13 \times 10^{-5}$) in chromosome 15 and *KCNIP3* ($p = 1.09 \times 10^{-4}$) in chromosome 2.

GWAS of age-related tinnitus in patients of African ancestry identified one genome-wide significant SNP (rs8127374, $p = 4.75 \times 10^{-8}$) in chromosome 5, and one near genome-wide significant SNP (rs62349633, $p = 6.65 \times 10^{-8}$) that was in near perfect LD with the lead SNP ($R^2 = 0.99$, $p < 0.0001$) (Figure 30C and D, Appendix Table 22). rs8127374 is located in an intergenic region, with the closest gene being *RPL36AP21*. Neither SNP appeared to influence gene expression or transcription factor binding, nor did any of the other SNPs in LD with rs8127374 (Figure 34). Gene-based association analysis identified no genome-wide significant genes (Figure 33), with the most significant being *SHISA7* ($p = 8.97 \times 10^{-5}$) in chromosome 19 and *CXCL3* ($p = 1.03 \times 10^{-4}$) in chromosome 4.

Table 24. Identified eQTLs and sQTLs for rs3828840.

eQTL							
Gencode Id	**Gene Symbol**	**Variant Id**	**SNP Id**	**P-Value**	**NES**	**Tissue**	
ENSG00000198502.5	HLA-DRB5	chr6_32553130_T_C_b38	rs3828840	2.20E-66	-0.8	Muscle - Skeletal	
ENSG00000198502.5	HLA-DRB5	chr6_32553130_T_C_b38	rs3828840	8.30E-61	-0.87	Thyroid	
ENSG00000229391.7	HLA-DRB6	chr6_32553130_T_C_b38	rs3828840	2.10E-59	0.86	Lung	
ENSG00000198502.5	HLA-DRB5	chr6_32553130_T_C_b38	rs3828840	4.90E-58	-0.74	Artery - Tibial	
ENSG00000198502.5	HLA-DRB5	chr6_32553130_T_C_b38	rs3828840	2.40E-55	-0.79	Adipose - Subcutaneous	
ENSG00000229391.7	HLA-DRB6	chr6_32553130_T_C_b38	rs3828840	5.30E-55	0.85	Skin - Sun Exposed (Lower leg)	
ENSG00000229391.7	HLA-DRB6	chr6_32553130_T_C_b38	rs3828840	7.70E-55	0.82	Nerve - Tibial	
ENSG00000229391.7	HLA-DRB6	chr6_32553130_T_C_b38	rs3828840	4.70E-54	0.79	Thyroid	
ENSG00000196301.3	HLA-DRB9	chr6_32553130_T_C_b38	rs3828840	1.30E-53	0.79	Lung	
ENSG00000179344.16	HLA-DQB1	chr6_32553130_T_C_b38	rs3828840	1.00E-52	-0.81	Skin - Sun Exposed (Lower leg)	
sQTL							
Gencode Id	**Gene Symbol**	**Variant Id**	**SNP Id**	**P-Value**	**NES**	**Phenotype Id**	**Intron Id**
ENSG00000179344.16	HLA-DQB1	chr6_32553130_T_C_b38	rs3828840	8.30E-70	1.1	chr6:32660249:32757773:clu_29048:ENSG00000179344.16	32660249:32757773:clu_29048
ENSG00000232629.8	HLA-DQB2	chr6_32553130_T_C_b38	rs3828840	8.30E-70	1.1	chr6:32660249:32757773:clu_29048:ENSG00000232629.8	32660249:32757773:clu_29048
ENSG00000179344.16	HLA-DQB1	chr6_32553130_T_C_b38	rs3828840	1.10E-65	-1.1	chr6:32660249:32661347:clu_36992:ENSG00000179344.16	32660249:32661347:clu_36992
ENSG00000232629.8	HLA-DQB2	chr6_32553130_T_C_b38	rs3828840	1.10E-65	-1.1	chr6:32660249:32661347:clu_36992:ENSG00000232629.8	32660249:32661347:clu_36992

ENSG00000179344.16	HLA-DQB1	chr6_32553130_T_C_b38	rs3828840	7.20E-64	1.1	chr6:32660249:32757773:clu_36072:ENSG00000179344.16	32660249:32757773:clu_36072
ENSG00000232629.8	HLA-DQB2	chr6_32553130_T_C_b38	rs3828840	7.20E-64	1.1	chr6:32660249:32757773:clu_36072:ENSG00000232629.8	32660249:32757773:clu_36072
ENSG00000196126.11	HLA-DRB1	chr6_32553130_T_C_b38	rs3828840	2.60E-63	-0.98	chr6:32517752:32518054:clu_29044:ENSG00000196126.11	32517752:32518054:clu_29044
ENSG00000198502.5	HLA-DRB5	chr6_32553130_T_C_b38	rs3828840	2.60E-63	-0.98	chr6:32517752:32518054:clu_29044:ENSG00000198502.5	32517752:32518054:clu_29044
ENSG00000229391.7	HLA-DRB6	chr6_32553130_T_C_b38	rs3828840	2.60E-63	-0.98	chr6:32517752:32518054:clu_29044:ENSG00000229391.7	32517752:32518054:clu_29044
ENSG00000179344.16	HLA-DQB1	chr6_32553130_T_C_b38	rs3828840	5.80E-63	1.1	chr6:32660249:32757773:clu_38507:ENSG00000179344.16	32660249:32757773:clu_38507

Table 24 Continued.

Only the 10 most significant eQTLs and sQTLs are presented. A complete list of the 406 eQTLs and 294 sQTLs associated with rs3828840 can be found on the GTEx portal.

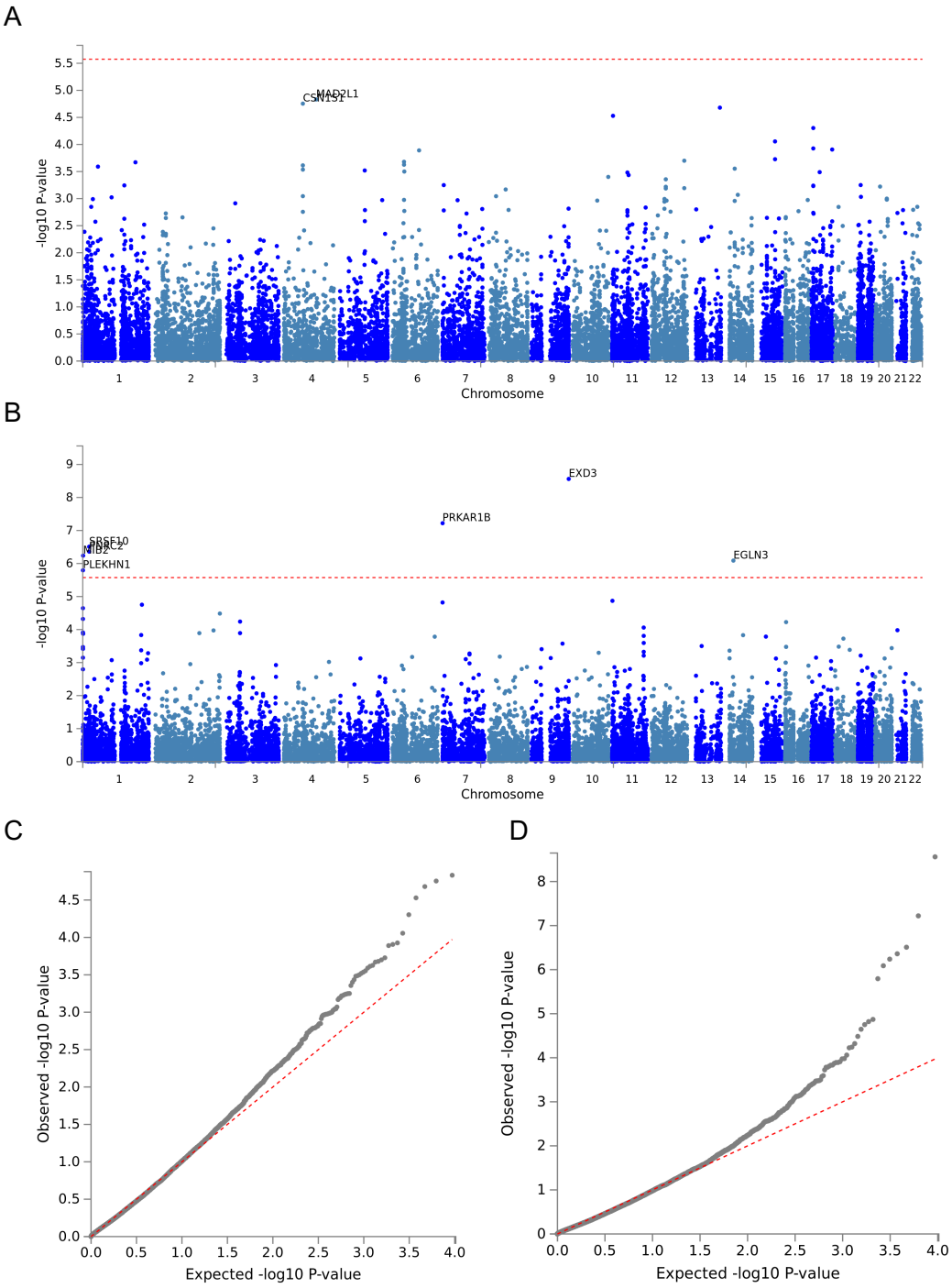


Figure 29. Gene-Based Genome-Wide Association Analyses of Age-Related Hearing Loss or Tinnitus in Patients of European Ancestry. Summary statistics for the SNP-based GWAS were uploaded to FUMA to run a gene-based association analysis based on a multiple linear principal components regression to determine the

Figure 29. Gene-Based Genome-Wide Association Analyses of Age-Related Hearing Loss or Tinnitus in Patients of European Ancestry.

aggregated effect of all SNPs within a gene. Inputted SNPs were mapped to 18,756 protein coding genes for age-related hearing loss and tinnitus, producing a significance threshold of p = 0.05/18,756 (2.67×10^{-6}). **A)** Manhattan plot of the gene-based association analysis for age-related hearing loss identified *MAD2L1* (p = 1.47×10^{-5}) and *CSN1S1* (p = 1.76×10^{-5}) in chromosome 4 as nearly genome-wide significant. **B)** Manhattan plot of the gene-based association analysis for age-related tinnitus identified seven genes to be genome-wide significant (*EXD3*, p = 2.79×10^{-9}; *PRKAR1B*, p = 6.01×10^{-8}; *SRSF10*, p = 3.10×10^{-7}; *NRC2*, p = 4.36×10^{-7}; *MIB2*, p = 5.77×10^{-7}; *EGLN3*, p = 8.16×10^{-7}; and *PLEKHN1*, p = 1.61×10^{-6}). Quantile-Quantile plots of results from the gene-based association analysis for age-related **C)** hearing loss and **D)** tinnitus are also provided.

Table 25. Identified eQTLs and sQTLs for rs9273081.

eQTL							
Gencode Id	**Gene Symbol**	**Variant Id**	**SNP Id**	**P-Value**	**NES**	**Tissue**	
ENSG00000179344.16	HLA-DQB1	chr6_32644559_G_A_b38	rs9273081	6.90E-186	-1.1	Skin - Sun Exposed (Lower leg)	
ENSG00000179344.16	HLA-DQB1	chr6_32644559_G_A_b38	rs9273081	5.60E-184	-0.77	Whole Blood	
ENSG00000179344.16	HLA-DQB1	chr6_32644559_G_A_b38	rs9273081	5.50E-177	-1	Muscle - Skeletal	
ENSG00000232629.8	HLA-DQB2	chr6_32644559_G_A_b38	rs9273081	3.20E-168	0.97	Whole Blood	
ENSG00000179344.16	HLA-DQB1	chr6_32644559_G_A_b38	rs9273081	6.10E-154	-1	Adipose - Subcutaneous	
ENSG00000179344.16	HLA-DQB1	chr6_32644559_G_A_b38	rs9273081	5.40E-144	-0.96	Thyroid	
ENSG00000179344.16	HLA-DQB1	chr6_32644559_G_A_b38	rs9273081	7.70E-142	-0.96	Skin - Not Sun Exposed (Suprapubic)	
ENSG00000232629.8	HLA-DQB2	chr6_32644559_G_A_b38	rs9273081	4.80E-134	1.1	Nerve - Tibial	
ENSG00000179344.16	HLA-DQB1	chr6_32644559_G_A_b38	rs9273081	8.40E-129	-0.95	Nerve - Tibial	
ENSG00000232629.8	HLA-DQB2	chr6_32644559_G_A_b38	rs9273081	5.70E-128	0.92	Artery - Tibial	
sQTL							
Gencode Id	**Gene Symbol**	**Variant Id**	**SNP Id**	**P-Value**	**NES**	**Phenotype Id**	**Intron Id**
ENSG00000179344.16	HLA-DQB1	chr6_32644559_G_A_b38	rs9273081	2.00E-224	1.5	chr6:32660249:32757773:clu_36072:ENSG00000179344.16	32660249:32757773:clu_36072
ENSG00000232629.8	HLA-DQB2	chr6_32644559_G_A_b38	rs9273081	2.00E-224	1.5	chr6:32660249:32757773:clu_36072:ENSG00000232629.8	32660249:32757773:clu_36072
ENSG00000179344.16	HLA-DQB1	chr6_32644559_G_A_b38	rs9273081	4.10E-214	1.4	chr6:32660249:32757773:clu_29048:ENSG00000179344.16	32660249:32757773:clu_29048
ENSG00000232629.8	HLA-DQB2	chr6_32644559_G_A_b38	rs9273081	4.10E-214	1.4	chr6:32660249:32757773:clu_29048:ENSG00000232629.8	32660249:32757773:clu_29048
ENSG00000179344.16	HLA-DQB1	chr6_32644559_G_A_b38	rs9273081	4.40E-209	1.5	chr6:32660249:32757773:clu_3	32660249:32757773:clu_38507

						8507:ENSG00000179344.16	
ENSG00000232629.8	HLA-DQB2	chr6_32644559_G_A_b38	rs9273081	4.40E-209	1.5	chr6:32660249:32757773:clu_38507:ENSG00000232629.8	32660249:32757773:clu_38507
ENSG00000179344.16	HLA-DQB1	chr6_32644559_G_A_b38	rs9273081	1.00E-206	1.5	chr6:32660249:32757773:clu_37171:ENSG00000179344.16	32660249:32757773:clu_37171
ENSG00000232629.8	HLA-DQB2	chr6_32644559_G_A_b38	rs9273081	1.00E-206	1.5	chr6:32660249:32757773:clu_37171:ENSG00000232629.8	32660249:32757773:clu_37171
ENSG00000179344.16	HLA-DQB1	chr6_32644559_G_A_b38	rs9273081	2.60E-197	1.5	chr6:32660249:32757773:clu_37871:ENSG00000179344.16	32660249:32757773:clu_37871
ENSG00000232629.8	HLA-DQB2	chr6_32644559_G_A_b38	rs9273081	2.60E-197	1.5	chr6:32660249:32757773:clu_37871:ENSG00000232629.8	32660249:32757773:clu_37871

Table 25 Continued.
Only the 10 most significant eQTLs and sQTLs are presented. A complete list of the 381 eQTLs and 268 sQTLs associated with rs9273081 can be found on the GTEx portal.

Functional Enrichment Analysis

We mapped SNPs to genes by proximity and performed pathway enrichment with Gene Ontology terms. SNPs with GWAS $p < 5x10^{-5}$ mapped to 154 coding genes for age-related hearing loss in patients of European ancestry, 273 coding genes with $p < 5x10^{-5}$ for age-related tinnitus in patients of European ancestry, 248 coding genes with $p < 5x10^{-5}$ for age-related hearing loss in patients of African ancestry, and 557 coding genes with $p < 1x10^{-4}$ for age-related tinnitus in patients of African ancestry. A significance threshold of $p < 1x10^{-4}$ was used for age-related tinnitus in patients of African ancestry because there was no significant findings using a threshold of $p < 5x10^{-5}$.

Numerous pathways related to immune system maintenance or diseases associated with immune system deficiencies were significantly over-represented in age-related hearing loss in patients of European ancestry using Gene Ontology or KEGG (Appendix Table 23). Accordingly, analysis of associated human disease phenotypes through Human Phenotype Ontology identified C8 deficiency as statistically significant ($p = 0.04$), and analysis of associated biochemical reactions through Reactome identified phosphorylation of CD3 and TCR zeta chains ($p = 1.73x10^{-4}$), generation of second messenger molecules ($p = 0.001$), translocation of ZAP-70 to immunological synapse ($p = 0.004$), and PD-1 signaling ($p = 0.008$). Similarly, pathway analysis of age-related tinnitus in patients of European ancestry using GO and KEGG identified term names highly associated with the immune response (Appendix Table 24), with Reactome analysis identifying translocation of ZAP-70 to Immunological synapse ($p = 6.57x10^{-5}$), phosphorylation of CD3 and TCR zeta chains ($p = 1.66x10^{-4}$), PD-1 signaling

($p = 2.18x10^{-4}$), generation of second messenger molecules ($p = 0.002$), MHC class II antigen presentation ($p = 0.03$), and interferon gamma signaling ($p = 0.01$) as statistically significant. Pathway analysis of age-related hearing loss in patients of African ancestry also identified statistically significant immune system term names (Appendix Table 25), while also identifying pathways associated with inner ear/nervous system development and maintenance, including nervous system development ($p = 0.05$), mechanoreceptor differentiation ($p = 0.05$), inner ear receptor cell differentiation ($p = 0.05$), and neuron part ($p = 0.05$). Finally, pathway analysis of age-related tinnitus in patients of African ancestry identified statistically significant term names associated with nervous system maintenance and development, along with general cellular functions (Appendix Table 26).

Comparison of GWAS Results of Age-Related Hearing Loss and Tinnitus in Patients of Different Genetic Ancestry

Using summary statistics from the GWAS of age-related hearing loss and tinnitus, we assessed whether the SNPs most highly associated with hearing loss and tinnitus in patients of African ancestry ($p < 0.01$) were comparable to those associated with patients of European ancestry. The Q-Q plots in Figure 35A and B indicate that the observed p-values of hearing loss and tinnitus SNPs at $p < 0.01$ in patients of African ancestry are higher than the expected distribution of p-values in the corresponding European ancestry GWAS, indicative of potential enrichment. This is particularly evident for age-related tinnitus, as multiple SNPs from the African ancestry GWAS are more significant than SNPs from the European ancestry GWAS. Similarly, when PRS of age-related tinnitus in patients of European ancestry were evaluated in patients of African

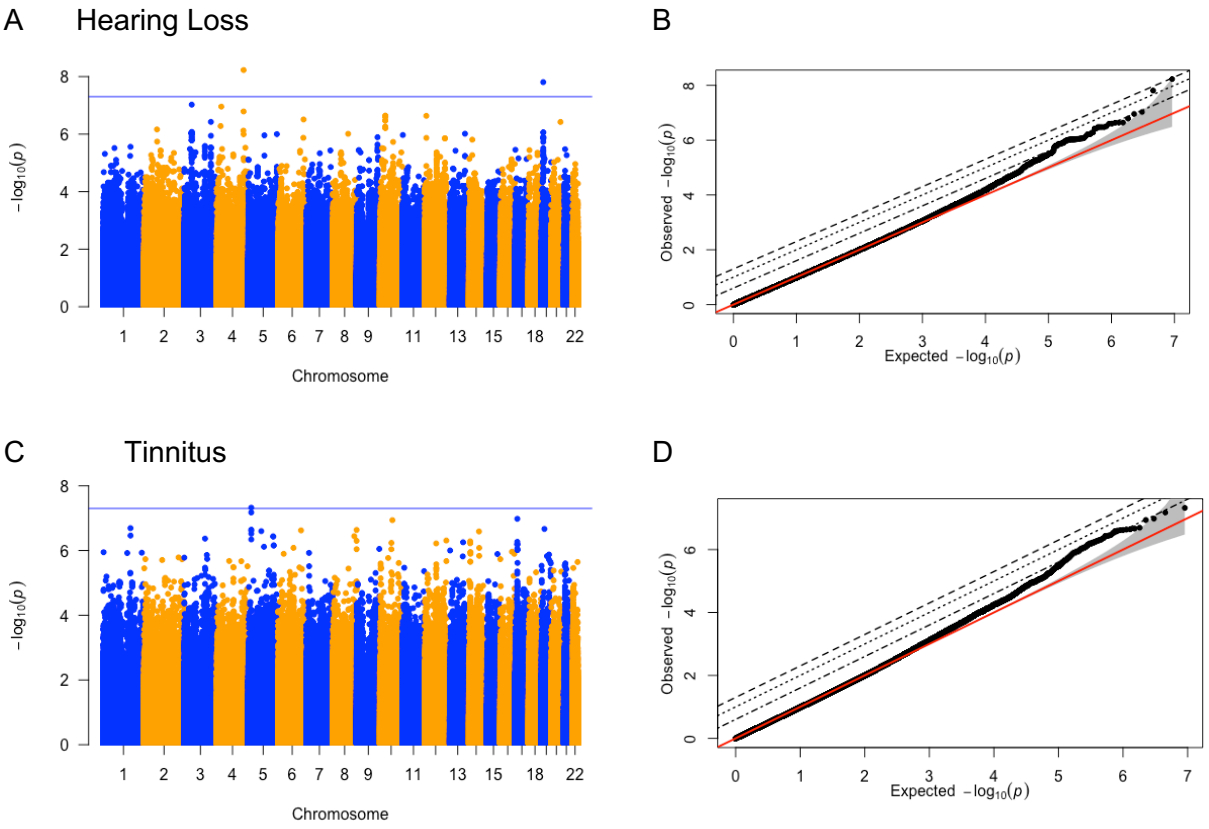
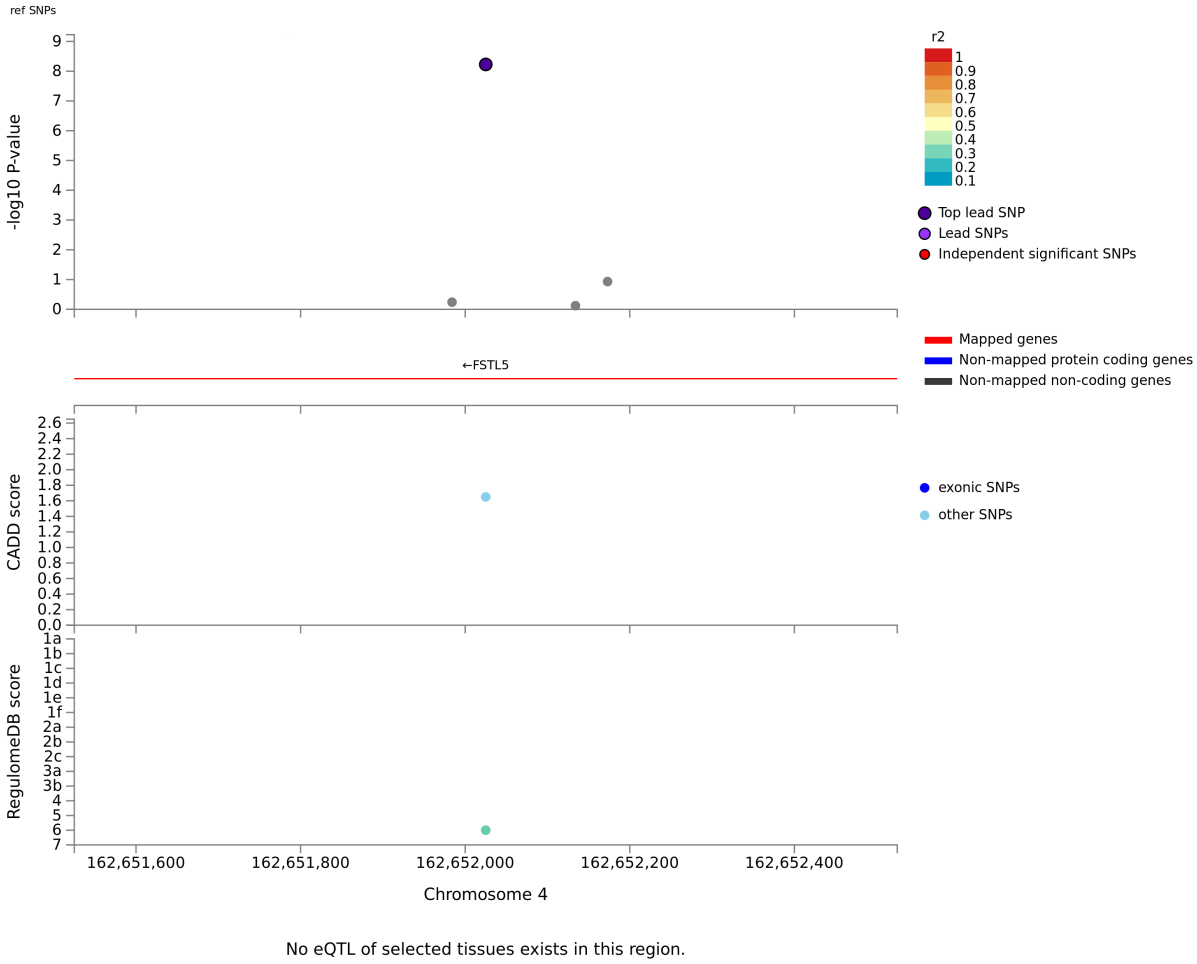


Figure 30. Genome-Wide Association Studies of Age-Related Hearing Loss and Tinnitus in Patients of African Ancestry. A) Manhattan plot of GWAS results for age-related hearing loss in patients of African ancestry reveals two independent genome-wide significant signals in chromosomes 4 and 19 (rs77750421, p = 5.94×10^{-9}; rs144555968, p = 1.56×10^{-8}). **B)** Quantile-Quantile plots of GWAS results for age-related hearing loss. **C)** Manhattan plot of GWAS results for age-related tinnitus in patients of African ancestry reveals one genome-wide significant SNP (rs8127374, p = 4.75×10^{-8}) and one near genome-wide significant SNP (rs62349633, p = 6.65×10^{-8}) in chromosome 5. **D)** Quantile-Quantile plots of GWAS results for age-related tinnitus. Covariates in both GWAS include current age and 10 African genetic principal components accounting for population substructure.

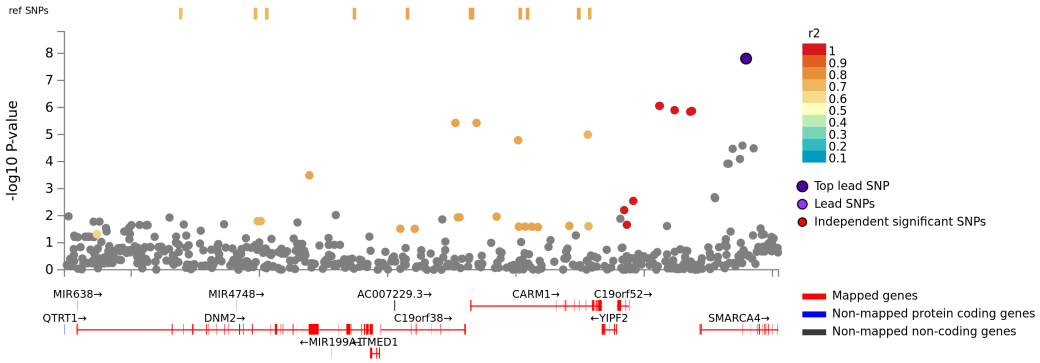
A



B

Figure 31. Regional Plot of rs77750421 for GWAS of Age-Related Hearing Loss in Patients of African Ancestry. A) A regional plot of the most significant GWAS signal (rs77750421) was generated in FUMA. Each point represents a SNP. The x-axis indicates chromosomal position, while the y-axis shows $-\log_{10}$(p-value) of association with age-related hearing loss. The LD (R^2) of each SNP with rs77750421 (purple) is denoted by color in the legend, along with whether the gene has been mapped. **B)** No SNPs were in LD with rs77750421, and the SNP had a low Regulome DB score and did not appear to be an eQTL.

A



B

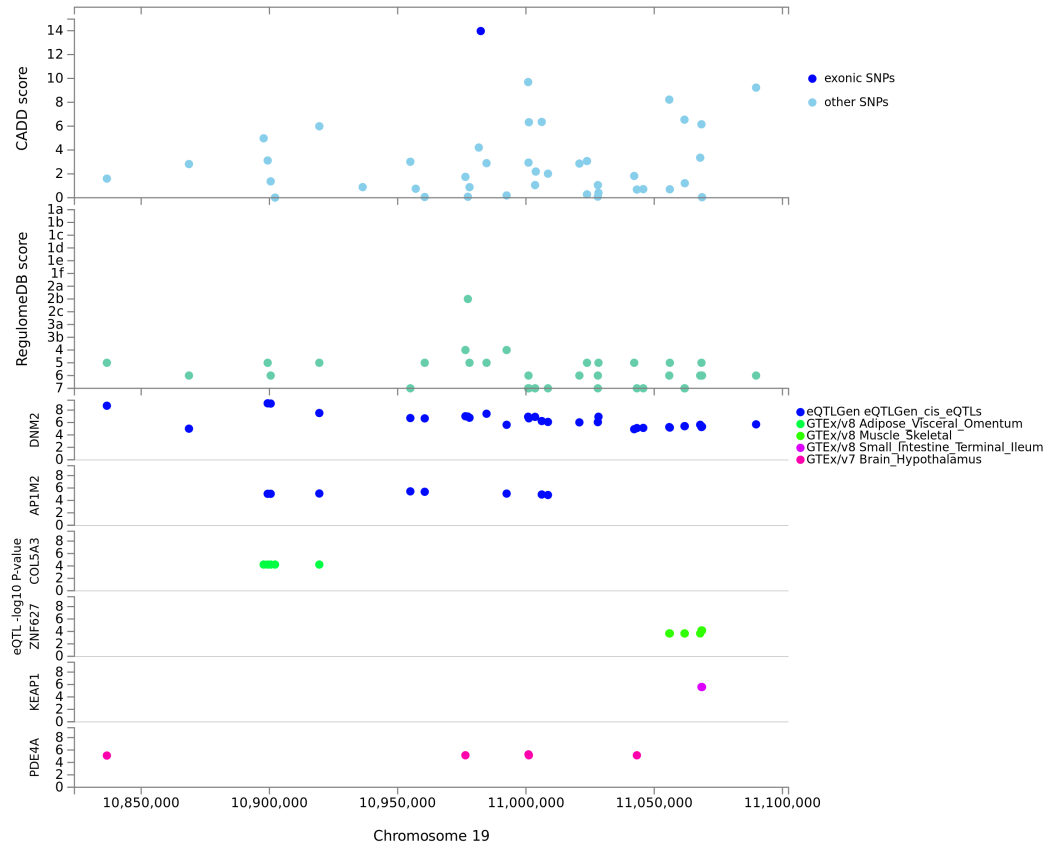


Figure 32. Regional Plot of rs144555968 for GWAS of Age-Related Hearing Loss in Patients of African Ancestry. A) A regional plot of the most significant GWAS signal (rs144555968) was generated in FUMA. Each point represents a SNP. The x-axis indicates chromosomal position, while the y-axis shows $-\log_{10}$(p-value) of association

Figure 32. Regional Plot of rs144555968 for GWAS of Age-Related Hearing Loss in Patients of African Ancestry.

with age-related hearing loss. The LD (R^2) of each SNP with rs144555968 (purple) is denoted by color in the legend, along with whether the gene has been mapped. **B)** SNPs that were in LD with rs144555968 were evaluated for CADD and Regulome DB scores and underwent eQTL analysis. After evaluation, SNPs were plotted in accordance with their chromosomal position. Multiple SNPs are in high LD with rs144555968, including rs550869012 that is located in an exonic region of *CARM1* and has a relatively high CADD score (13.97), as well as rs139420375 that may regulate the binding of transcription factors to *C19orf38* (RegulomeDB score: 2b). In addition, 28 eQTLs are in high LD with rs144555968, and regulate the expression of 6 genes on chromosome 19.

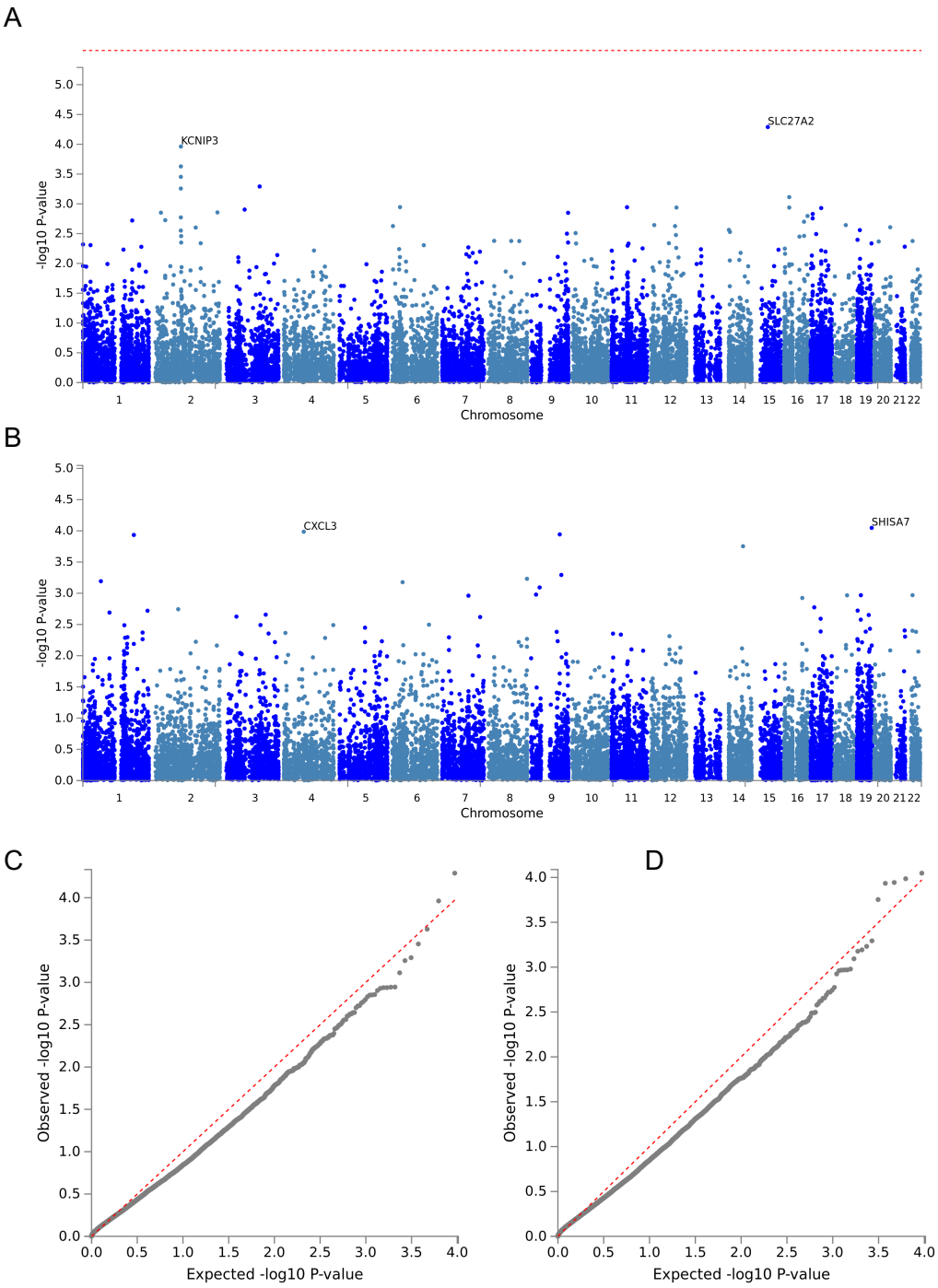


Figure 33. Gene-Based Genome-Wide Association Analyses of Age-Related Hearing Loss or Tinnitus in Patients of African Ancestry. Summary statistics for the SNP-based GWAS were uploaded to FUMA to run a gene-based association analysis based on a multiple linear principal components regression to determine the aggregated effect of all SNPs within a gene. Inputted SNPs were mapped to 18,756 protein coding

Figure 33. Gene-Based Genome-Wide Association Analyses of Age-Related Hearing Loss or Tinnitus in Patients of African Ancestry.

genes for age-related hearing loss and tinnitus, producing a significance threshold of p = 0.05/18,756 ($2.67x10^{-6}$). **A)** Manhattan plot of the gene-based association analysis for age-related hearing loss identified no genome-wide significant genes, with the most significant being *SLC27A2* (p = $5.13x10^{-5}$) in chromosome 15 and *KCNIP3* (p = $1.09x10^{-4}$) in chromosome 2. **B)** Manhattan plot of the gene-based association analysis for age-related tinnitus identified no genome-wide significant genes, with the most significant being *SHISA7* (p = $8.97x10^{-5}$) in chromosome 19 and *CXCL3* (p = $1.03x10^{-4}$) in chromosome 4. Quantile-Quantile plots of results from the gene-based association analysis for age-related **C)** hearing loss and **D)** tinnitus are also provided.

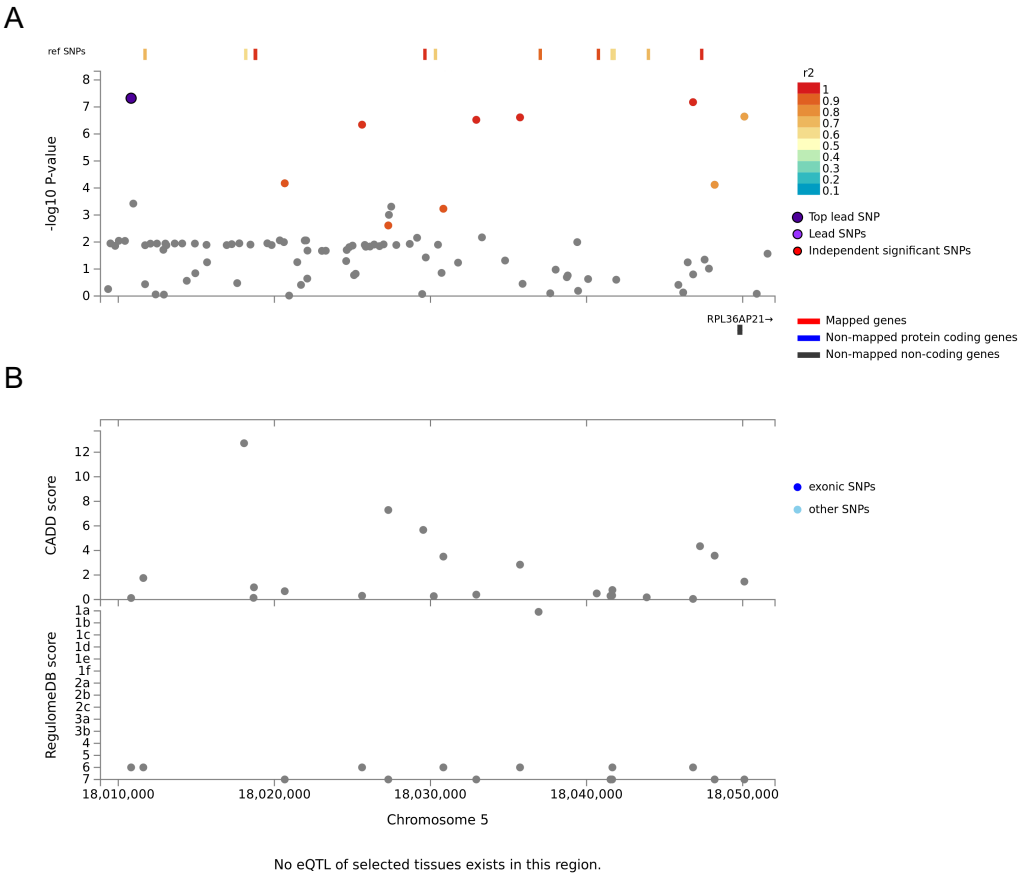


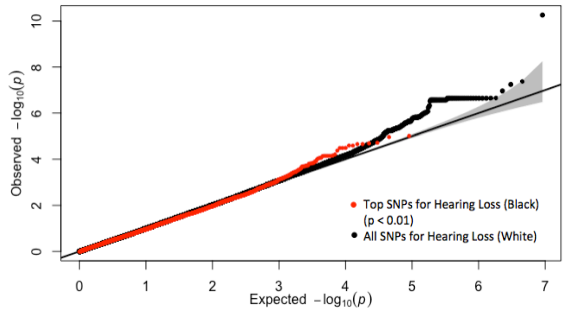
Figure 34. Regional Plot of rs8127374 for GWAS of Age-Related Tinnitus in Patients of African Ancestry. A) A regional plot of the most significant GWAS signal (rs8127374) was generated in FUMA. Each point represents a SNP. The x-axis indicates chromosomal position, while the y-axis shows $-\log_{10}$(p-value) of association with age-related tinnitus. The LD (R^2) of each SNP with rs8127374 (purple) is denoted by color in the legend, along with whether the gene has been mapped. **B)** SNPs that were in LD with rs8127374 were evaluated for CADD and Regulome DB scores and underwent eQTL analysis. After evaluation, SNPs were plotted in accordance with their chromosomal position. rs8127374 does not appear to influence gene expression or transcription factor binding, nor did any of the SNPs in LD.

ancestry, logistic regression identified the inclusion of SNPs with a p-value threshold of 0.01 to be significantly associated with tinnitus in patients of African ancestry (Nagelkerke $R^2 = 8.77x10^{-4}$, p = 0.04) (Figure 36, Table 26). By contrast, PRS of age-related hearing loss did not find any p-value thresholds that were statistically significant.

Comparison of GWAS Results of Age-Related Hearing Loss and Tinnitus and Treatment-Related Ototoxicity

Using summary statistics from our GWAS of cisplatin- and radiation-induced hearing loss and tinnitus, we assessed whether the SNPs most highly associated with treatment-related ototoxicity ($p < 0.01$) were comparable to age-related hearing loss and tinnitus in patients of European ancestry. The Q-Q plots in Figure 37A and B indicate that the observed p-values of radiation-induced hearing loss and tinnitus SNPs at $p < 0.01$ are slightly higher than the expected distribution of p-values in the corresponding age-related GWAS. There also appears to be slight enrichment for the cisplatin-induced hearing loss and tinnitus SNPs at $p < 0.01$ (Figure 37C and D). When evaluating the aggregate effects of SNPs from the age-related GWAS, age-related hearing loss SNPs at a p-value threshold of 0.50 were significantly associated with radiation-induced hearing loss (MAF = 0.01: Nagelkerke $R^2 = 0.009$, p = 0.01; MAF = 0.05: Nagelkerke $R^2 = 0.007$, p = 0.11) (Figure 38, Table 26). The quantile plot of the best-fit PRS model (MAF = 0.01) demonstrates the positive nature of this relationship as the odds ratio for radiation-induced hearing loss generally increases with a greater polygenic load for age-related hearing loss (Figure 39A). Pediatric cancer survivors with higher PRS for age-related hearing loss also exhibit an increased likelihood of developing radiation-induced hearing loss (Figure 39B). Age-related tinnitus SNPs were not significantly associated

A Hearing Loss



B Tinnitus

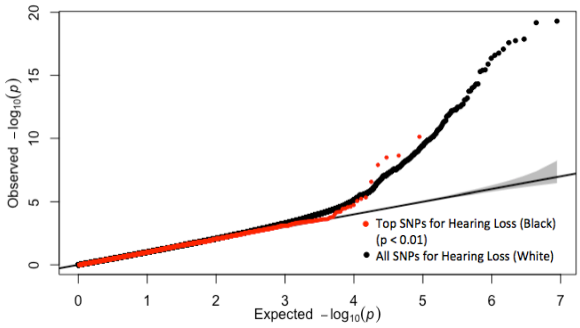


Figure 35. SNP-Based Comparison of Genetic Architecture of Age-Related Hearing Loss and Tinnitus Between Patients of European and African Ancestry. **A)** Using summary statistics from GWAS, the SNPs most highly associated with age-related hearing loss ($p < 0.01$) in the African cohort were assessed for enrichment in the GWAS of the European cohort. **B)** The same enrichment analysis was performed for age-related tinnitus SNPs ($p < 0.01$) in the African cohort and the GWAS of the European cohort. Black dots refer to all SNPs assessed for statistical significance in the age-related hearing loss or tinnitus GWAS in patients of European ancestry. Red dots refer to the most statistically significant SNPs in the age-related hearing loss or tinnitus GWAS in patients of African ancestry that were evaluated for statistical significance in the European analyses.

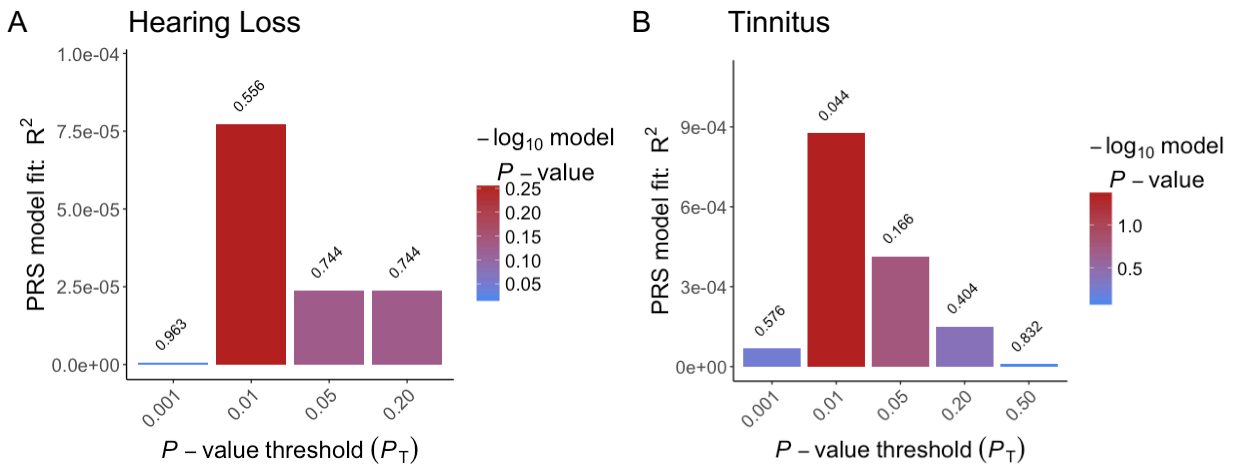


Figure 36. Polygenic Risk Score Analysis of Age-Related Hearing Loss and Tinnitus Between Patients of European and African Ancestry. A) Multiple SNP p-value thresholds from the GWAS of age-related hearing loss and tinnitus in patients of European ancestry were used to generate a PRS that was then assessed for its association for hearing loss or tinnitus in patients from the African cohort. **B)** PRS for different p-value thresholds were also generated for age-related tinnitus in patients of European ancestry to assess their association with tinnitus in the African cohort. The x-axis refers to the different p-values used from the base population to generate PRS, while the y-axis refers to the correlation (R^2) of the models to hearing loss or tinnitus in the target population. The p-value of each model is shown above the bars in the panels, along with its corresponding $-\log_{10}$ value in the legend. PRS models with $p \leq 0.05$ were designated as statistically significant.

Table 26. Comparison of Best-Fit Polygenic Risk Score Models.

Base Population	Target Population	MAF in Target Population	P-Value Threshold	Nagelkerke R^2	P
Age-Related Hearing Loss (European)	Age-Related Hearing Loss (African)	0.01	0.01	7.72×10^{-5}	0.56
		0.05			
Age-Related Tinnitus (European)	Age-Related Tinnitus (African)	0.01	0.01	8.77×10^{-4}	0.04
		0.05			
Age-Related Hearing Loss (European)	Radiation-Induced Hearing Loss (European)	0.01	0.50	0.009	0.01
		0.05	0.20	0.007	0.11
Age-Related Tinnitus (European)	Radiation-Induced Tinnitus (European)	0.01	0.20	-8.12×10^{-4}	0.48
		0.05	0.20	5.27×10^{-4}	0.09
Age-Related Hearing Loss (European)	Cisplatin-Induced Hearing Loss (European)	0.01	0.50	0.001	0.38
		0.05	0.50	0.002	0.34
Age-Related Tinnitus (European)	Cisplatin-Induced Tinnitus (European)	0.01	0.20	0.003	0.47
		0.05	0.20	0.002	0.52
Radiation-Induced Hearing Loss (European)	Cisplatin-Induced Hearing Loss (European)	0.01	0.50	0.002	0.22
		0.05	0.001	0.002	0.24
Radiation-Induced Tinnitus (European)	Cisplatin-Induced Tinnitus (European)	0.01	0.20	0.004	0.07
		0.05	0.20	0.004	0.08

with radiation-induced tinnitus at a MAF of 0.01, but appeared to be marginally associated at a MAF of 0.05 using a p-value threshold of 0.20 (Nagelkerke R^2 = $5.27x10^{-4}$, p = 0.09) (Figure 38**)**. However, PRS derived from the best-fit age-related tinnitus model did not exhibit as clear of an association with odds ratios or proportion of patients with radiation-induced tinnitus (Figure 39C and D). None of the PRS generated for age-related hearing loss or tinnitus were significantly associated with cisplatin-associated ototoxicity (Figure 40, Table 26).

Comparison of GWAS Results of Treatment-Related Ototoxicity

Using summary statistics from our GWAS of cisplatin-induced hearing loss and tinnitus in testicular cancer survivors, we assessed whether the SNPs most highly associated with cisplatin-associated ototoxicity ($p < 0.01$) were enriched in the GWAS for radiation-associated ototoxicity. The Q-Q plots in Figure 41A and B indicate that the observed p-values of cisplatin-induced hearing loss SNPs at $p < 0.01$ are higher than the expected distribution of p-values in the corresponding radiation-induced hearing loss GWAS, which is not observed for the cisplatin-induced tinnitus SNPs. However, PRS analysis indicated that the aggregated effects of SNPs associated with radiation-induced hearing loss were not associated with cisplatin-induced hearing loss at all evaluated p-value thresholds (Figure 42, Table 26). SNPs associated with radiation-induced tinnitus were marginally associated with cisplatin-induced tinnitus at a p-value threshold of 0.20 (MAF = 0.01: Nagelkerke R^2 = 0.004, p = 0.07; MAF = 0.05: Nagelkerke R^2 = 0.004, p = 0.08) (Figure 42).

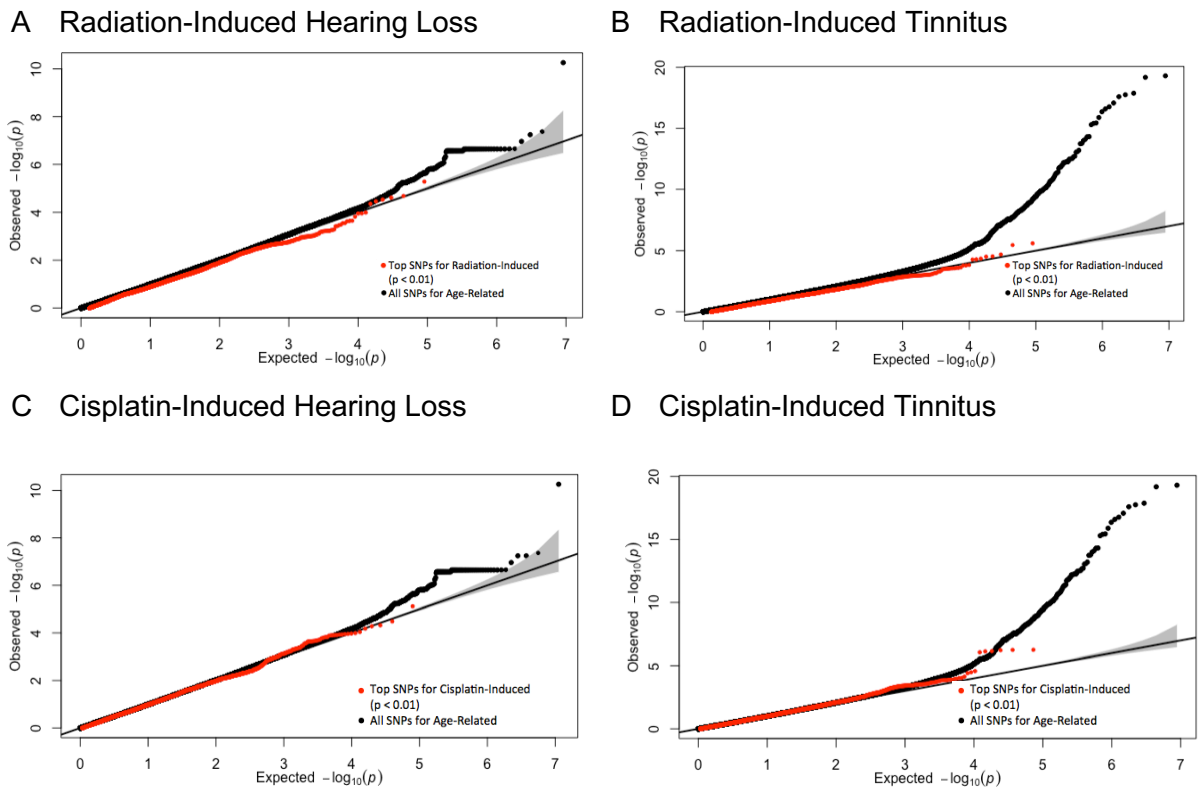


Figure 37. SNP-Based Comparison of Genetic Architecture Between Age-Related Hearing Loss and Tinnitus and Treatment-Related Ototoxicity. A) Using summary statistics from GWAS of radiation-associated ototoxicity from CCSS, the SNPs most highly associated with radiation-induced hearing loss ($p < 0.01$) were assessed for enrichment in the GWAS of age-related hearing loss. **B)** The same enrichment analysis was performed for radiation-induced tinnitus SNPs ($p < 0.01$) and the GWAS of age-related tinnitus. **C)** Using summary statistics from GWAS of cisplatin-associated ototoxicity from the Platinum Study, the SNPs most highly associated with cisplatin-induced hearing loss ($p < 0.01$) were assessed for enrichment in the GWAS of age-related hearing loss. **D)** The same enrichment analysis was performed for cisplatin-induced tinnitus SNPs ($p < 0.01$) and the GWAS of age-related tinnitus. Black dots refer to all SNPs assessed for statistical significance in the age-related hearing loss or

Figure 37. SNP-Based Comparison of Genetic Architecture Between Age-Related Hearing Loss and Tinnitus and Treatment-Related Ototoxicity.

tinnitus GWAS in patients of European ancestry. Red dots refer to the most statistically significant SNPs in GWAS of cisplatin- or radiation-induced hearing loss/tinnitus that were evaluated for statistical significance with the age-related cohorts.

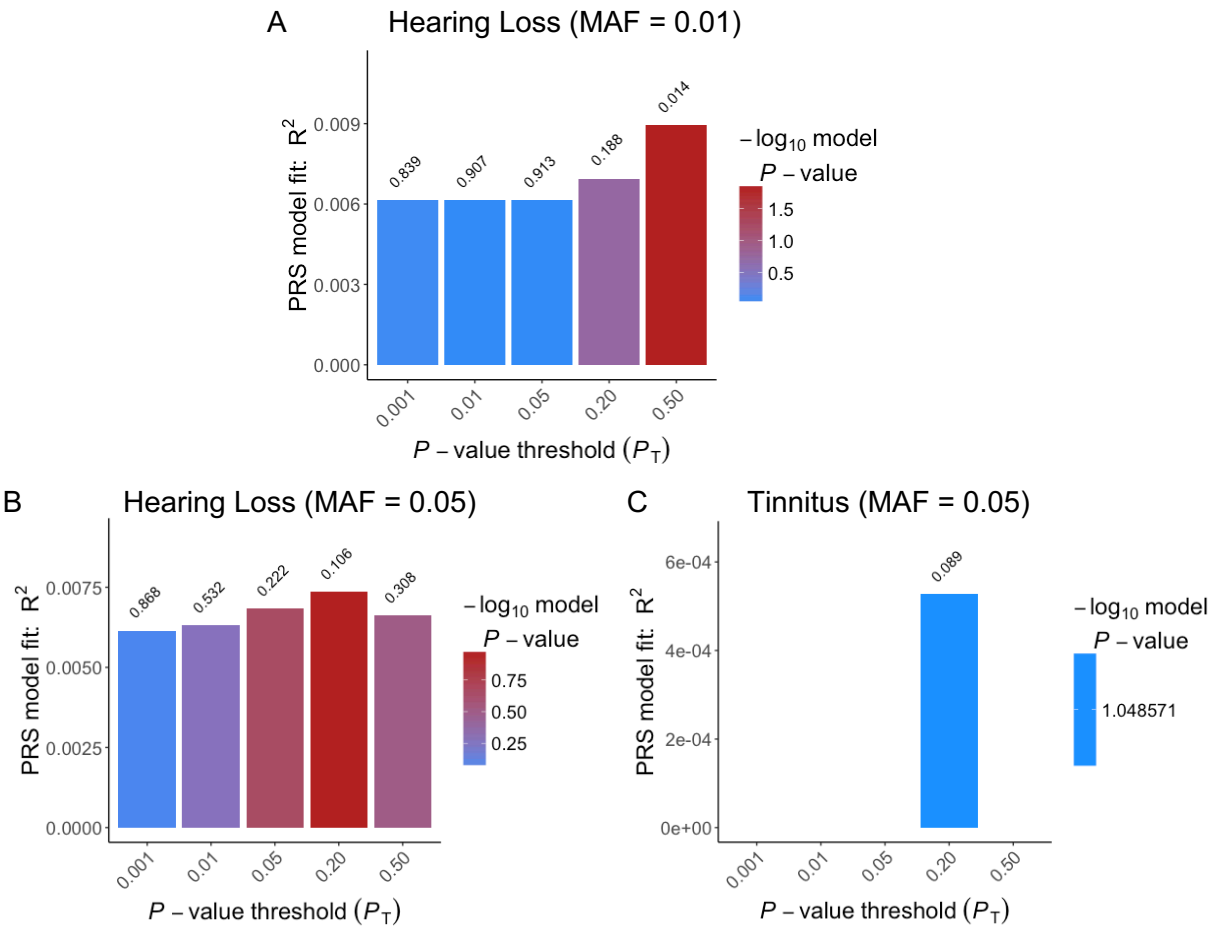


Figure 38. Polygenic Risk Score Analysis Between Age-Related Hearing Loss and Tinnitus and Radiation-Associated Ototoxicity. Multiple SNP p-value thresholds from the GWAS of age-related hearing loss and tinnitus in patients of European ancestry were used to generate a PRS that was then assessed for its association for radiation-induced hearing loss using a MAF threshold of **A)** 0.01. There is no plot for a MAF threshold of 0.05 because the R^2 values for all of the PRS models were negative. PRS for different p-value thresholds were also generated for age-related tinnitus in patients of European ancestry to assess their association with radiation-induced tinnitus using a MAF threshold of **B)** 0.01 or **C)** 0.05. The x-axis refers to the different p-values used from the base population to generate PRS, while the y-axis refers to the correlation (R^2) of the models to hearing loss or tinnitus in the target population. The p-value of each model is shown above the bars in the panels, along with its corresponding $-\log_{10}$ value in the legend. PRS models with $p \leq 0.05$ were designated as statistically significant.

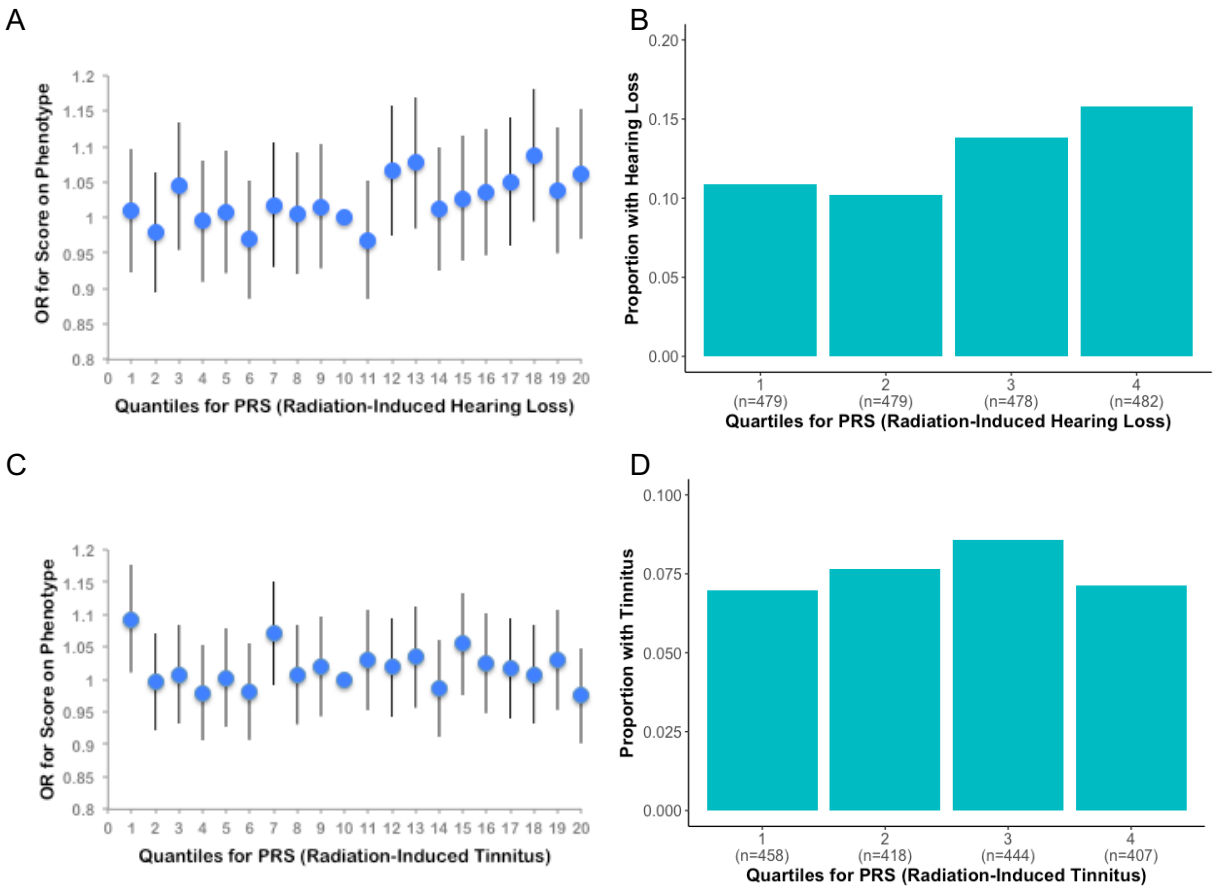


Figure 39. Evaluation of Polygenic Risk Scores Derived from Age-Related Hearing Loss and Tinnitus for Their Association with Radiation-Associated Ototoxicity in Pediatric Cancer Survivors. PRS generated from the best-fit model of age-related hearing loss (MAF = 0.01) or tinnitus (MAF = 0.05) were divided into quantiles or quartiles to assess their association with radiation-associated ototoxicity in pediatric cancer survivors from CCSS. Pediatric cancer survivors with a higher age-related hearing loss PRS have an increased **A)** odds ratio and **B)** overall likelihood of developing radiation-induced hearing loss. By contrast, the association between higher age-related tinnitus PRS scores and **C)** odds ratios and **D)** overall likelihood of developing radiation-induced tinnitus is less apparent.

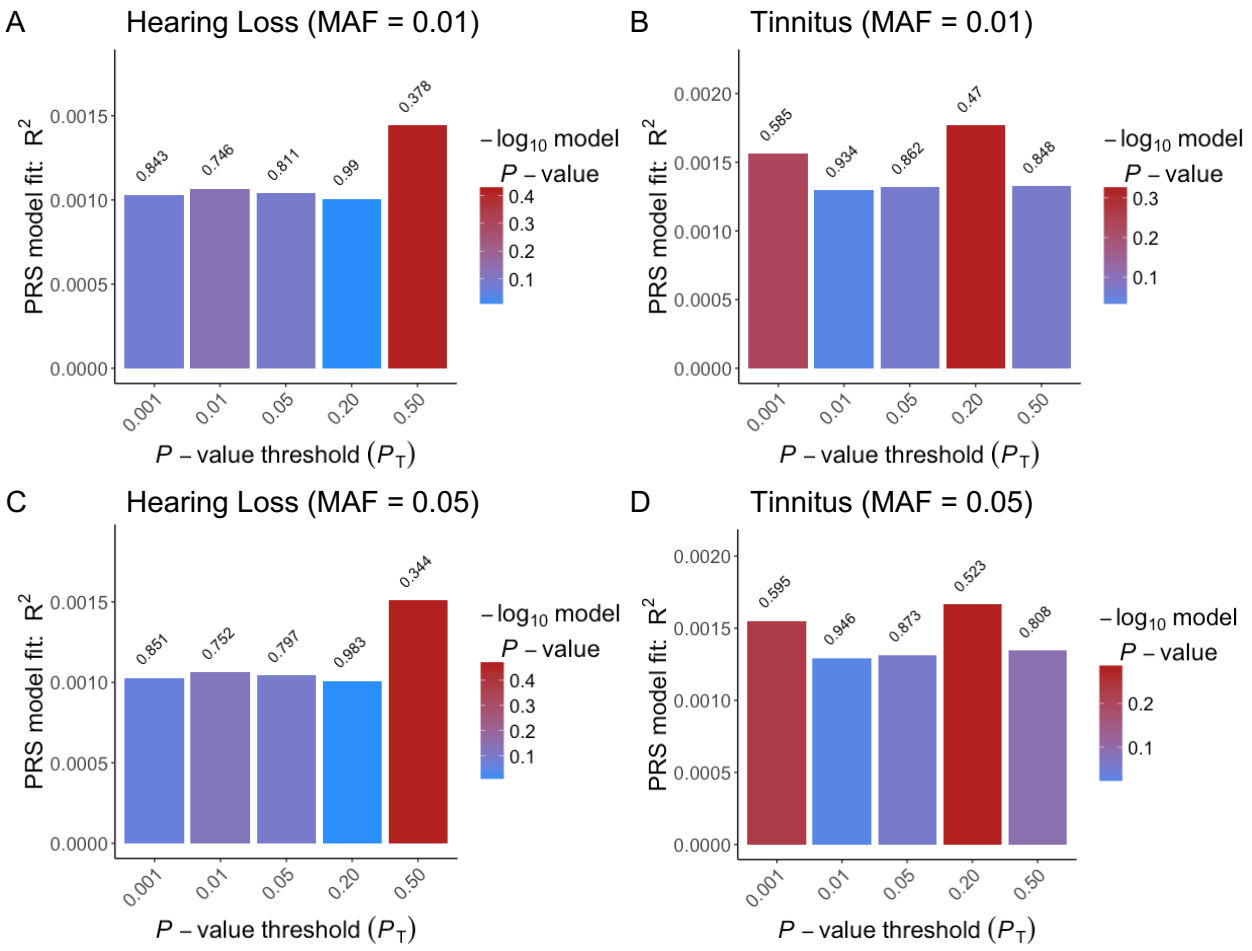


Figure 40. Polygenic Risk Score Analysis Between Age-Related Hearing Loss and Tinnitus and Cisplatin-Associated Ototoxicity. Multiple SNP p-value thresholds from the GWAS of age-related hearing loss and tinnitus in patients of European ancestry were used to generate a PRS that was then assessed for its association for cisplatin-induced hearing loss using a MAF threshold of **A)** 0.01 or **B)** 0.05. PRS for different p-value thresholds were also generated for age-related tinnitus in patients of European ancestry to assess their association with cisplatin-induced tinnitus using a MAF threshold of **C)** 0.01 or **D)** 0.05. The x-axis refers to the different p-values used from the base population to generate PRS, while the y-axis refers to the correlation (R^2) of the models to hearing loss or tinnitus in the target population. The p-value of each model is shown above the bars in the panels, along with its corresponding $-\log_{10}$ value in the legend. PRS models with $p \leq 0.05$ were designated as statistically significant.

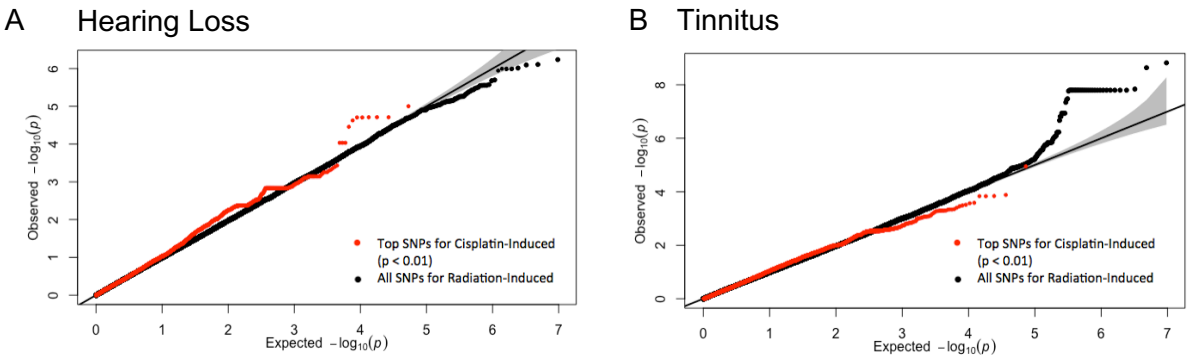


Figure 41. SNP-Based Comparison of Genetic Architecture of Radiation- and Cisplatin-Associated Ototoxicity. A) Using summary statistics from GWAS of cisplatin-associated ototoxicity, the SNPs most highly associated with cisplatin-induced hearing loss ($p < 0.01$) were assessed for enrichment in the GWAS of radiation-induced hearing loss. **B)** The same enrichment analysis was performed for cisplatin-induced tinnitus SNPs ($p < 0.01$) and the GWAS of radiation-induced tinnitus. Black dots refer to all SNPs assessed for statistical significance in the radiation-induced hearing loss or tinnitus GWAS. Red dots refer to the most statistically significant SNPs in the cisplatin-induced hearing loss or tinnitus GWAS that were evaluated for statistical significance in the radiation-associated ototoxicity analyses.

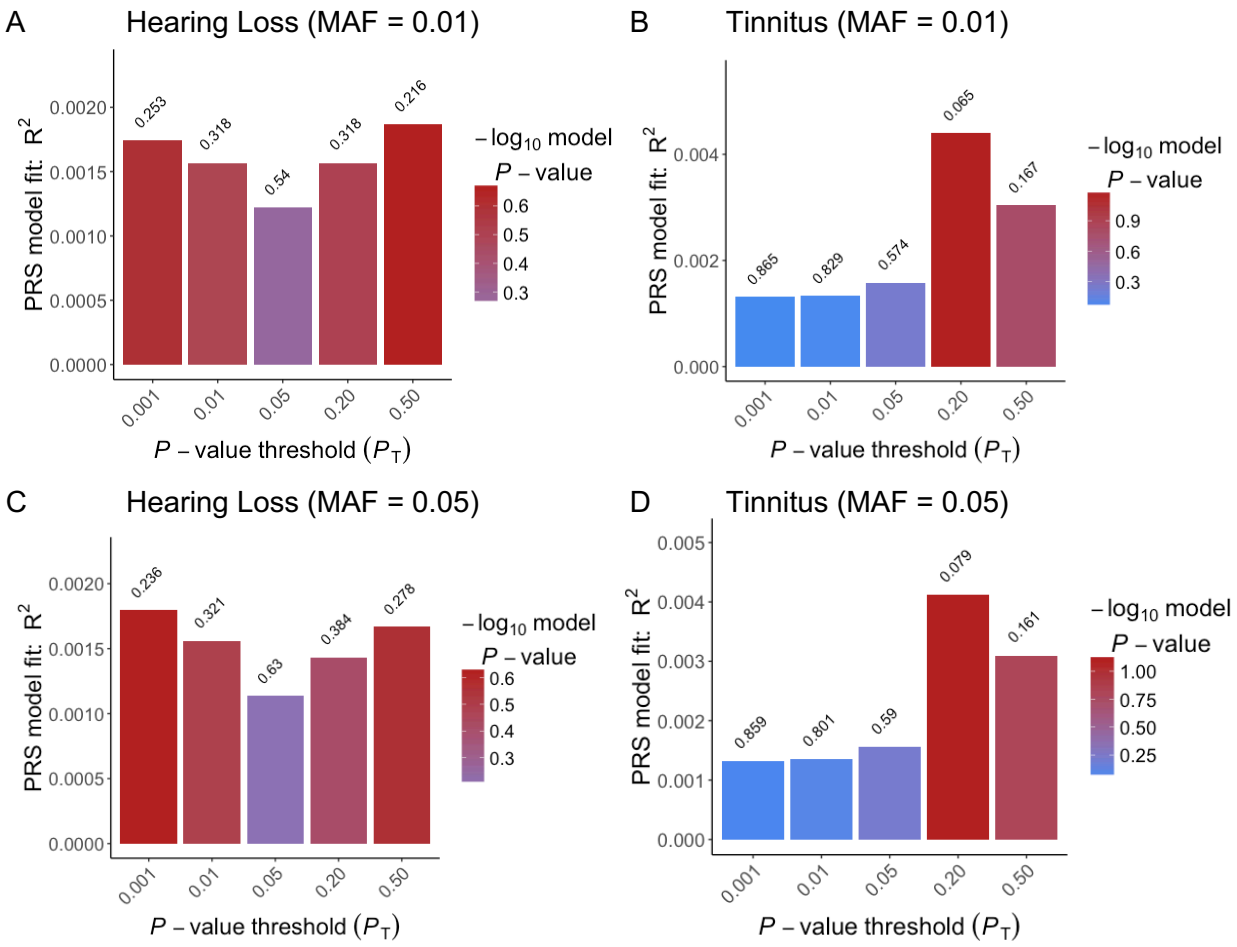


Figure 42. Polygenic Risk Score Analysis Between Radiation- and Cisplatin-Associated Ototoxicity. Multiple SNP p-value thresholds from the GWAS of radiation-induced hearing loss and tinnitus were used to generate a PRS that was then assessed for its association for cisplatin-induced hearing loss using a MAF threshold of **A)** 0.01 or **B)** 0.05. PRS for different p-value thresholds were also generated for radiation-induced tinnitus to assess their association with cisplatin-induced tinnitus using a MAF threshold of **C)** 0.01 or **D)** 0.05. The x-axis refers to the different p-values used from the base population to generate PRS, while the y-axis refers to the correlation (R^2) of the models to hearing loss or tinnitus in the target population. The p-value of each model is shown above the bars in the panels, along with its corresponding $-\log_{10}$ value in the legend. PRS models with $p \leq 0.05$ were designated as statistically significant.

Discussion

The current study marks an important advance in understanding the non-genetic and genetic risk factors for age-related hearing loss and tinnitus, as well as whether different races or etiologies of these hearing disorders share common genetic architecture. Patients who identified as white were more likely to develop age-related sensorineural hearing loss and tinnitus (hearing loss: 14.8%; tinnitus: 4.7%) than those who identified as black (hearing loss: 4.7%; tinnitus: 2.6%) for the ages of 50-90, which is in accord with previous studies (3, 189). In addition, Asian/Pacific Islander patients (hearing loss: 9.8%; tinnitus: 0%) had incidences of hearing loss and tinnitus that were non-significant when compared to black patients, while American Indian/Alaska Native patients (hearing loss: 27.1%; tinnitus: 11.1%) were the most likely to develop either hearing disorder. However, these results are far less definitive due to the small sample sizes of Asian/Pacific Islander and American Indian/Alaska Native patients. The difference in hearing loss susceptibility among white and black patients has been previously identified, with Agrawal et al. (189) reporting odds of hearing loss to be 70% lower in black subjects vs. white subjects, and Shargorodsky et al. (3) reporting blacks (OR = 0.62, 95% CI: 0.55-0.69) and Hispanics (OR = 0.70, 95% CI: 0.61-0.80) to have lower odds of developing tinnitus when compared to the white reference group in the National Health and Nutritional Examination Survey (NHANES). Further, the associations between race/ethnicity and tinnitus remained significant in participants without hearing impairment (3), suggesting a mechanism for tinnitus that is independent of hearing impairment. Although the differences of hearing loss and tinnitus incidence among whites and blacks may be indicative of potential socioeconomic and lifestyle

factors, it has been previously demonstrated that skin pigmentation is correlated with hearing thresholds. In an analysis of pure tone hearing thresholds from NHANES (232), it was not only demonstrated race/ethnicity was associated with hearing thresholds (black participants with the best hearing followed by Hispanics and then white individuals), but in race-stratified analyses, darker-skinned Hispanics as defined by Fitzpatrick skin type (233) had better hearing than lighter skinned Hispanics by an average of −2.5 dB (95% CI, −4.8 to −0.2 dB) and −3.1 dB (95% CI,−5.3 to −0.8 dB) for speech and high-frequency pure-tone averages, respectively. This ultimately suggests that skin pigmentation is independently associated with hearing loss, and may mediate the strong association observed between race/ethnicity and hearing loss. In support of this concept, melanocytes produce melanin in both the skin and cochlea, and are more abundant in the stria vascularis and Rosenthal's canal of black patients (234), indicating that increased melanin in the inner ear may help protect the cochlea against age-related cellular declines and subsequent hearing loss.

Our data also indicated that males (15.0%) had a significantly higher incidence of age-related hearing loss than females (11.3%), but differences in tinnitus incidence (males: 4.4%; females: 4.2%) were not statistically significant. This difference between hearing loss and tinnitus susceptibility has been previously observed for *de novo* hearing loss (189) and tinnitus (2, 3, 222). Differences in hearing loss prevalence is that the association could merely be a reflection of environmental and non-genetic risk factors that increase hearing loss susceptibility among men such as excessive noise exposure, working in loud conditions, increased rates of hypertension, or an increased likelihood of smoking (189). In addition, it has been established that sex differences

exist in both peripheral and central auditory processing, and that circulating levels of estrogen have been associated with hearing thresholds, indicating that higher levels of estrogen correlate with better overall hearing (190). In regards to the negligible difference in tinnitus prevalence, it has previously been observed that women exhibit more annoyance and perceive more stress than men after developing symptoms of tinnitus (223). In addition, women scored lower than men in proactive coping, sense of coherence, and personal resources, yet had lower levels of hearing loss and tinnitus loudness than men. These findings suggest that women would be more likely than men to view their tinnitus as problematic, resulting in a higher proportion being diagnosed with tinnitus (as represented by ICD-9-CM diagnosis code status). Therefore, although the incidence of tinnitus among men and women are similar in the eMERGE dataset, it is plausible that there is a much higher proportion of men who remain undiagnosed, reducing its overall prevalence.

Age-related hearing loss and tinnitus were associated with multiple comorbidities, including dizziness, vertigo, and major depressive disorder single episode or recurrent episode. The associations between hearing loss, tinnitus, dizziness, and vertigo are in accord with previous studies (177, 178), and are likely due to the intimate relationship between the auditory and vestibular systems of the inner ear. Hearing loss and tinnitus also often lead to several mental health issues such as sleeping difficulties and concentration problems that promote increased anxiety and insomnia (5, 6, 178), and can ultimately promote cognitive decline and dementia (7, 220). Accordingly, our study indicated hearing loss and tinnitus were associated with higher incidences of major depressive disorder, as well as the most severe forms of both single and recurrent

depressive episodes, with the exception of hearing loss and recurrent depressive episodes ($p = 0.11$).

In regards to modifiable risk factors, both the overall incidence and severity of hypertension were associated with hearing loss and tinnitus. In a previous study of 274 patients between the ages of 45-64, hearing thresholds between 0.25 and 8 kHz increased in patients with grade 1-3 hypertension. In addition, an evaluation of overall blood pressure variability over a 9-year period in 8,646 males indicated that pure-tone average thresholds at low, intermediate, and high frequencies grew gradually with increasing systolic blood pressures (235). Our study also indicated that patients with hearing loss and tinnitus were more likely to be diagnosed with hypercholesterolemia. Gopinath et al. (236) have previously demonstrated that the likelihood of prevalent hearing loss (pure-tone average of frequencies 0.5, 1.0, 2.0, and 4.0 kHz > 25 dB) increased from the lowest (reference) to the highest quartile of dietary cholesterol intake, and individuals who used cholesterol-lowering medication had a 48% reduced odds of prevalent hearing loss. Similarly, Sutbas et al. (237) found that hearing loss and tinnitus are particularly common in patients with hyperlipidemia, and lowering serum lipid levels through a low-cholesterol diet or antihyperlipidemic therapy for up to 24 months significantly lowered average hearing thresholds and tinnitus intensity. Importantly, previous studies have indicated that age-related sensorineural hearing loss is related to a microcirculatory insufficiency that occurs due to vascular occlusion, which could be exacerbated by hypertension or hypercholesterolemia (238, 239). One potential explanation is that cochlear hair cells are markedly sensitive to ischemic conditions due to its high reliance on active aerobic metabolism to maintain its function

(240). Since higher blood pressure and cholesterol likely lead to compromised blood supplies for the cochlea and subsequent hair cell death, lowering blood pressure and cholesterol may be novel targets for preventing age-related hearing loss. It is important to note that the clinical characteristics evaluated in the current study were all derived from *a priori* observations for hearing loss and tinnitus, and it is likely that there are other modifiable risk factors or comorbidities associated with age-related hearing loss and tinnitus. Since patients in eMERGE have phenotype status determined from detailed EHRs, it is plausible to run an EHR-WAS in which hearing loss and tinnitus are evaluated against all ICD-9-CM diagnosis codes to agnostically identify other phenotypes of statistical significance (241).

The GWAS of age-related hearing loss in patients of European ancestry identified genome-wide significant SNPs (rs9272454, $p = 5.49 \times 10^{-11}$; rs3828840, $p = 4.36 \times 10^{-8}$) that are intronic to *HLA-DQA1* and *HLA-DRB4*, respectively, with rs3828840 being an eQTL for multiple HLA genes. These findings are in accord with the GWAS of age-related hearing loss with UK Biobank patients, as one of the genome-wide significant signals was in *HLA-DQA1* (230). In addition, the GWAS of age-related tinnitus in patients of European ancestry identified 290 genome-wide significant SNPs in which the most significant SNP (rs9273081, $p = 5.02 \times 10^{-20}$), is intronic to *HLA-DQA1*, and is an eQTL and sQTL for multiple HLA genes. These findings suggest that HLA genes are associated with age-related hearing loss and tinnitus in genetically European patients. HLA genes encode for major histocompatibility complex proteins that are responsible for the regulation of the immune system in humans, and previous GWAS have identified SNPs in HLA genes to be associated with the immune response and

autoimmune diseases (242-244). Therefore, it is plausible that variations in the immune response due to SNP heterogeneity in HLA genes may influence predisposition to hearing loss and tinnitus. It is also possible that these two strong signals are a result of population substructure in our European ancestry cohorts, as the global patterns of HLA nucleotide diversity among populations are significantly correlated to geography, and can lead to spurious genome-wide significant signals (245). However, because we were evaluating hearing loss and tinnitus in two genetically distinct populations, we accounted for population substructure prior to our GWAS by ensuring that the self-reported race of individuals coincided with their genetic ancestry as determined by principal components analysis, and then performed an additional principal components analysis that was specific to genetically determined Europeans or Africans.

Several candidate gene studies have previously identified associations between HLA genes and sensorineural hearing loss (246-248), providing evidence that SNP variability within this region may predispose patients to hearing loss. In addition, autoimmunity or immunologic disorders have been suggested as possible causes of idiopathic progressive sensorineural hearing loss and tinnitus, as several genes within the major histocompatibility complex are potentially involved in the immunologic process of inner ear diseases (246, 249, 250). Functional enrichment analysis provides further evidence of the association between the immune system and age-related hearing loss and tinnitus in patients of European ancestry, as significant associations were nearly all derived from immune response terms, including C8 deficiency and PD1 signaling. Individuals with C8 deficiency are susceptible to recurrent bacterial infections, particularly by *N. meningitides* that causes meningitis, a disorder known to markedly

increase susceptibility to hearing loss (251). PD1 signaling is also of potential interest as a recent case report indicated that the PD1 inhibitor pembrolizumab could induce sudden bilateral sensorineural hearing loss (252), providing evidence that PD1 blockade can lead to an autoimmune inner ear disease.

Variation in the immune response may also be of particular importance for age-related hearing loss and tinnitus in patients of African Ancestry. Although neither African ancestry GWAS identified genome-wide significant SNPs that were associated with the immune response, functional enrichment analysis of age-related hearing loss identified multiple statistically significant immune system term names, suggesting that the SNPs most significantly associated with hearing loss in this cohort ($p < 5x10^{-5}$) are located within genes vital for the immune response. Further, gene-based association analysis of age-related tinnitus in patients of African ancestry identified *CXCL3* ($p = 1.03x10^{-4}$) as the second most significantly associated gene with the phenotype. *CXCL3* encodes for a protein that regulates migration and adhesion of monocytes and mediates its effects on its target cell, particularly during inflammatory responses (253). It has been previously demonstrated that sensorineural hearing loss can arise from acute mitochondrial dysfunction that induces secondary inflammatory responses in the cochlear lateral wall through the IL-6/CCL-2 inflammatory pathway and activation of monocytes (254). Consequently, immune-driven inflammation of the cochlea may mediate *de novo* sensorineural hearing loss, and could serve as a potential therapeutic target.

It is important to note that the genetic architecture surrounding age-related hearing loss and tinnitus susceptibility is complex, and is not mediated entirely through

immune response mechanisms. Notably, GWAS of age-related tinnitus in patients of European ancestry identified 15 separate genome-wide significant signals, while gene-based association analysis identified 7 genes that surpassed the genome-wide significance threshold. These genes encode for proteins of a vast array of cellular functions, including an exonuclease (*EXD3*), a regulatory subunit of cyclic AMP-dependent protein kinase A (*PRKAR1B*), a protein involved in constitutive and regulated RNA splicing (*SRSF10*), a serine-threonine protein kinase (*NRC2*), E3 ubiquitin-protein ligase (*MIB2*), an alpha-ketoglutarate-dependent hydroxylase (*EGLN3*), and a protein that decreases mRNA stability and promotes apoptosis through enhancing BAX-BAK hetero-oligomerization (*PLEKHN1*). Of these genes, four have previously been associated with nervous system development and maintenance (*PRKAR1B* (255), *SRSF10* (256), *EGLN3* (257), and *MIB2* (258), highlighting the importance of nerve function in auditory processing. Accordingly, functional enrichment analysis of age-related hearing loss and tinnitus in patients of African ancestry identified pathways associated with inner ear/nervous system development and maintenance.

The genetic architecture underlying age-related hearing loss in patients of African ancestry was particularly complex, as the most significant SNP (rs77750421, $p = 5.94 \times 10^{-9}$), did not appear to influence gene expression or transcription factor binding, while the next most significant SNP (rs144555968, $p = 1.56 \times 10^{-8}$), was intronic to *SMARCA4*, and was in LD with multiple SNPs of potential biological significance, including an exonic variant in *CARM1* (rs550869012), a SNP that regulates the binding of *C19orf38* (rs139420375), and 28 eQTLs that regulate the expression of 6 genes on chromosome 19 (*DNM2*, *AP1M2*, *COL5A3*, *ZNF627*, *KEAP1*, and *PDE4A*). These

SNPs are all located on chromosome 19, encompassing 7 genes that form chromatin interactions with 17 other genes and eQTL interactions with 5 other genes, indicative of a complex polygenic architecture. Of the genes that have expression influenced by the risk locus through transcription factor binding or eQTL interaction, 5 of the genes have previously been associated with nervous system development or maintenance, including *CARM1* (259), *DNM2* (260, 261), *COL5A3* (262), *KEAP1* (263), and *PDE4A* (264). Therefore, despite the polygenic nature of age-related hearing loss and tinnitus, there is a clear enrichment for genes that exert an influence on the nervous system and central auditory processing.

The enrichment of genes involved in the immune response or inner ear/nervous system development and maintenance for GWAS in the European and African cohorts suggests that there may exist shared genetic architecture between age-related hearing loss and tinnitus in these patient populations. PRS analysis provided support for this genetic correlation, as the aggregated effects of SNPs highly associated with tinnitus in the European cohort (p-value threshold of 0.01) were significantly associated with tinnitus in patients of African ancestry. Shared genetic architecture for tinnitus in patients of European and African ancestry is not immediately apparent, given that black patients are far less likely to experience both hearing disorders. Further, PRS from age-related hearing loss in the European cohort were not significantly associated with cases in the African cohort, indicative of a difference in the overall shared genetic architecture of hearing loss and tinnitus in these two patient populations. One possibility is that the mechanisms underlying tinnitus susceptibility in European and African individuals are more similar than those observed for hearing loss. Other factors, such as differences in

melanin content of the cochlea or the influence of environmental factors may be more important for hearing loss prevalence than tinnitus, suggesting that the underlying genetic architecture for hearing loss may be less important or more heterogeneous than that observed for tinnitus. The underlying mechanisms of susceptibility for tinnitus are far less defined than they are for hearing loss (177), and will require additional research to discern whether the genetic architecture surrounding hearing loss and tinnitus susceptibility has substantial differences.

Survivors of several adult-onset and pediatric malignancies are highly susceptible to developing hearing loss and tinnitus following treatment with either cisplatin-based chemotherapy or cranial radiation therapy, as described in Chapters 4 and 7, respectively. When compared to treatment-related etiologies, age-related hearing loss and tinnitus appear to share genetic architecture with radiation-associated ototoxicity, but not cisplatin-associated ototoxicity. The genetic correlation between age-related hearing loss and radiation-induced hearing loss was particularly apparent, as the higher PRS derived from the age-related cohort in the eMERGE dataset were associated with both higher odds ratios and increased prevalence of radiation-induced hearing loss in pediatric cancer survivors. We have previously demonstrated that the ototoxic phenotypes potentiated by cisplatin and cranial radiation are similar, as they are associated with comparable non-genetic risk factors and comorbidities (36, 57) (Manuscript Under Review). However, the current study indicates that the genetic predispositions by which ototoxicity is induced by the two etiologies are potentially unique, and in the case of cisplatin, is different than those observed for age-related hearing loss and tinnitus. This is not surprising because of the differences in

mechanisms of toxicity for cisplatin and radiation. Cisplatin is a systemic agent that is retained by the cochlea for months to years following treatment (147), inducing bilaterial sensorineural hearing loss or tinnitus in a dose dependent manner (15). By contrast, cranial radiation causes unilateral or bilateral hearing loss or tinnitus typically after doses exceed 30 Gy, with hearing loss being attributed to conductive (middle ear) or sensorineural (inner ear) mechanisms depending on the direction of the radiation beam. This heterogeneity in affected areas ultimately suggests that genetic variants influencing cisplatin- and radiation-associated ototoxicity may be inherently different, resulting in unique genetic predispositions.

Ototoxicity elicited by cranial radiation is caused predominantly by reactive oxygen species (ROS) and reactive nitrogen species (RNS), providing a rationale for why it shares genetic architecture with age-related hearing loss and tinnitus. It has been previously established that ROS are pivotal for regulating inflammatory signaling, as they contribute to pro-inflammatory cytokine release (265, 266). These pro-inflammatory signals are associated with macrophage activation that can damage the cochlear lateral wall, leading to hearing loss and/or tinnitus. Excessive levels of ROS are also known to be highly cytotoxic to hair cells (267, 268), which are essential for mechanotransduction within the inner ear. Importantly, aging is often attributed to accumulated damage elicited by free radicals and other reactive species that increase in production over time (269). Increased levels of ROS in aging patients would not only activate pro-inflammatory signaling within the inner ear that could lead to immune system activation, but also would directly damage hair cells. As with aging, cranial radiation could markedly increases ROS availability in the cochlea depending on the path of the

radiation beam, thereby eliciting very similar deleterious effects to the auditory system. Although it is known cisplatin induces ROS within the inner ear, it does not appreciably accumulate within hair cells, and also elicits damage by platinating mitochondrial DNA and activating ER stress (270, 271). Therefore, the mechanisms of age-related hearing loss and tinnitus are more similar to the ototoxicity elicited by cranial radiation, resulting in a higher proportion of shared genetic architecture.

Major strengths of our study include that this represents the first discovery GWAS to our knowledge of age-related hearing loss and tinnitus in patients of African ancestry, enabling a direct comparison of genetic susceptibilities to patients of European ancestry. These analyses are particularly important for age-related tinnitus, as the investigation of its genetic architecture has long lagged behind hearing loss, and the current study may provide novel inroads to studying its pathogenesis. In addition, our access to genetic data for cisplatin- and radiation-based etiologies from collaborations with the Platinum Study and CCSS enabled a direct comparison of common genetic architecture between age-related hearing loss and tinnitus and treatment-related ototoxicity. Through SNP-based enrichment and PRS analyses, we were able to determine that age-related and treatment-related hearing loss and tinnitus do not necessarily share common genetic architecture, indicating that genetically targeted approaches to prevent or mitigate hearing disorders may have to be distinct depending on its etiology. Further, the comprehensiveness of EHRs in the eMERGE dataset enabled us to confirm previous associations between hearing loss/tinnitus and multiple clinical characteristics. As such, it should be feasible to perform agnostic

association analyses to identify other non-genetic risk factors and comorbidities for these hearing disorders that have not been previously identified.

An inherent limitation of our study is the fact that hearing loss is based on EHRs instead of quantitative audiometry, indicating that the severity of hearing loss is unable to be discerned. A similar issue of ambiguity is observed for our definition of tinnitus. Although there are currently no quantitative measures to define tinnitus, we have previously used detailed questionnaires to obtain the severity of tinnitus patients experience after cisplatin-based chemotherapy (36). However, due to the large sample size of the eMERGE dataset, it is plausible that the vast majority of patients assigned ICD-9-CM diagnosis codes of sensorineural hearing loss or tinnitus can be considered as valid cases. Similarly, our data had considerably fewer age-related hearing loss and tinnitus patients of African ancestry in comparison to those of European ancestry, indicating that the genome-wide analyses for genetically African patients were not as powerful for identifying associated SNPs and genes. Further, it is plausible that the genetic architecture underlying hearing loss or tinnitus susceptibility can change with age and that the differences in genetic correlation observed for cisplatin-associated and radiation-associated ototoxicity may be attributed to the differences in age of exposure to the ototoxic agent.

Conclusion

Taken together, our results suggest that the prevalence of age-related hearing loss and tinnitus is associated with race, and those who develop the hearing disorders are more likely to experience dizziness or vertigo, experience a single or recurrent episode of major depressive disorder, and be diagnosed with either

hypercholesterolemia or hypertension. GWAS of genetically European or African patients identified genome-wide significant signals for SNPs and genes that are associated with the immune response or inner ear/nervous system development and maintenance. Along with SNP-based enrichment and PRS analysss, these data indicate that there exists shared genetic architecture between age-related hearing loss and tinnitus in these two patient populations, particularly for age-related tinnitus. PRS analysis also revealed that age-related hearing loss and tinnitus share a higher proportion of common genetic architecture with radiation-associated ototoxicity than cisplatin-associated ototoxicity. The identification of shared genetic architecture between age-related hearing loss and tinnitus and radiation-associated ototoxicity is particularly important because the development of mechanistically-based agents that can reduce the severity and prevalence of ototoxicity following cranial radiation may also be applied to the much larger patient population of age-related hearing loss and tinnitus to potentially reduce the $30 billion/year spent on hearing disorders in the United States alone. Therefore, further investigation into the influence of the immune system and ROS on cochlear degeneration and subsequent hearing loss or tinnitus susceptibility is warranted.

Summary

Hearing loss and tinnitus are common auditory disorders that increase in frequency with age. We sought to identify non-genetic risk factors and comorbidities associated with age-related hearing loss and tinnitus and then perform GWAS to identify genetic predispositions in patients of European and African ancestry. Logistic regression was used to evaluate patients diagnosed with sensorineural hearing loss or

tinnitus between the ages of 50-90, along with age-matched controls, in the eMERGE network for associations with clinical characteristics based on ICD-9-CM diagnosis code status. GWAS of age-related hearing loss and tinnitus in the European and African cohorts were performed using current age and 10 ancestry specific-genetic principal components as covariates. Both cohorts of European and American Indian/Alaska Native descent reported higher rates of age-related hearing loss and tinnitus than black patients. Males were more likely to experience hearing loss than females, but tinnitus prevalence was comparable. Individuals with hearing loss or tinnitus were more likely to experience dizziness, vertigo, major depressive disorder, hypercholesterolemia, and hypertension. GWAS of age-related hearing loss and tinnitus revealed genome-wide significant SNPs that were intronic to HLA genes. Although GWAS in patients of African ancestry identified genome-wide significant SNPs in different chromosomes, functional enrichment analysis identified the immune response and/or inner ear/nervous system maintenance and development to be highly associated with hearing loss and tinnitus in these genetically distinct populations. PRS analysis identified shared genetic architecture for age-related tinnitus in the European and African cohorts. PRS analysis also identified that cranial radiation-associated ototoxicity, but not cisplatin-associated ototoxicity, shares identifiable genetic architecture with age-related hearing loss and tinnitus.

CHAPTER 9. DISCUSSION

The analysis of hearing loss and tinnitus susceptibility for treatment-related ototoxicity and age-related hearing loss identified several shared non-genetic risk factors and comorbidities, as well as similarities regarding genetic predisposition. In the replication analysis of the expanded cohort of testicular cancer survivors from the Platinum Study, we demonstrated that cisplatin-induced hearing loss and tinnitus were significantly associated age at diagnosis/clinical examination, cumulative cisplatin dose, hypertension, peripheral sensory neuropathy, and poorer self-reported health. Interestingly, only tinnitus was associated with hypercholesterolemia, persistent dizziness or vertigo, and higher psychotropic drug use. The evaluation of radiation-associated ototoxicity in CCSS identified hearing loss and tinnitus to be associated with persistent dizziness or vertigo, antidepressant use and report poorer overall health compared to controls after age-adjustment, while only tinnitus was associated with hypertension. Finally, age-related hearing loss and tinnitus in the eMERGE dataset did not show any discrepancy, as both were associated with dizziness, vertigo, major depressive disorder single/recurrent episode, hypercholesterolemia, and hypertension. The results of these analyses are summarized in Table 27. Regardless of etiology and patient background, it appears that the hearing disorders have shared non-genetic risk factors and comorbidities, indicating that the observed phenotype for treatment-related ototoxicity and age-related hearing loss and tinnitus are very similar.

It is important to note that hearing loss and tinnitus in the treatment-related cohorts are not always associated with the same clinical characteristics, which is in

Table 27. Comparison of P-Values for Non-Genetic Risk Factors and Comorbidities Among Treatment-Related Ototoxicity and Age-Related Hearing Loss and Tinnitus.

Hearing Loss				
Clinical Characteristic	**Cisplatin-Induced p (Original) (n = 488)**	**Cisplatin-Induced p (Expanded) (n = 1,258)**	**Radiation-Induced p (n = 2,198)**	**Age-Related p (n = 38,100)**
Cumulative Dose	**0.001**	**7.48×10^{-5}**	**$p < 2 \times 10^{-16}$**	x
Current Smoker	0.08	0.76	x	x
Hypertension	**0.0066**	**8.50×10^{-4}**	0.11	**2.36×10^{-5}**
Hypercholesterolemia	Not Evaluated	0.67	x	**$< 2 \times 10^{-16}$**
Persistent Dizziness or Vertigo	Not Evaluated	0.11	**3.71×10^{-8}**	**$< 2 \times 10^{-16}$**
Psychotropic Drug Use/Depression	Not Evaluated	0.17	**0.02**	**$< 2 \times 10^{-16}$**
Self-Reported Health	Not Evaluated	**5.88×10^{-5}**	**1.72×10^{-6}**	x
Tinnitus				
Clinical Characteristic	**Cisplatin-Induced p (Original) (n = 762)**	**Cisplatin-Induced p (Expanded) (n = 1,217)**	**Radiation-Induced p (n = 1,991)**	**Age-Related p (n = 38,439)**
Cumulative Dose	**0.007**	**0.001**	**4.82×10^{-14}**	x
Chronic Smoker	**0.005**	**0.01**	x	x
Hypertension	**0.039**	**6.83×10^{-6}**	**0.008**	0.01
Hypercholesterolemia	0.09	**0.01**	x	**$< 2 \times 10^{-16}$**
Persistent Dizziness or Vertigo	**< 0.0001**	**3.93×10^{-11}**	**$< 2 \times 10^{-16}$**	**$< 2 \times 10^{-16}$**
Psychotropic Drug Use/Depression	**0.003**	**0.002**	**0.02**	**$< 2 \times 10^{-16}$**
Self-Reported Health	**< 0.0001**	**9.40×10^{-11}**	**1.45×10^{-6}**	x

Bold indicates $p \leq 0.05$. Clinical characteristics given a value of x were not evaluated because the data were unavailable for the cohort.

contrast to age-related hearing loss and tinnitus. Due to the fact that either hearing loss or tinnitus in the treatment-related cohorts were significantly associated with the same clinical characteristics evaluated in the age-related cohort, it is likely that the observed discrepancies are a consequence of much smaller sample sizes, which reduces the power to identify true statistical associations. The associations of age-related hearing loss and tinnitus were evaluated in cohorts of 38,100 and 38,439 patients, respectively. By comparison, the expanded cohorts of testicular cancer survivors for cisplatin-induced hearing loss and tinnitus included 1,258 and 1,217 patients, and the pediatric cancer cohorts of radiation-induced hearing loss and tinnitus included 2,198 and 1,991 patients. In addition, there appears to be more discrepancies among statistically significant clinical characteristics for hearing loss than tinnitus. One possibility is that the phenotype definitions for hearing loss were more heterogeneous, as hearing loss in the three datasets was based on either continuous or categorical variables, while tinnitus was only based on categorical definitions.

Although the current study indicated the risk factors and comorbidities associated with hearing loss and tinnitus are shared among all three etiologies, enrichment analysis of GWAS results and PRS analysis indicated that the genetic architecture surrounding susceptibility to age-related hearing loss and tinnitus may be more similar for radiation-associated ototoxicity than cisplatin-associated ototoxicity. A possible mechanistic explanation for this discrepancy based on ROS-induced immune activation is provided in the Discussion of Chapter 8, but may not be sufficient due to the fact that cisplatin also induces ROS within the cochlea (57, 272-275). However, because ROS accumulation occurs in different areas of the inner ear depending on the agent, it may

be the case that cranial radiation elicits damage in a pattern more similar to what is observed in aging. In addition, cisplatin is known to induce ototoxicity through ER stress and mitochondrial DNA platination (270, 271), which inevitably increases the diversity of genes that could influence hearing loss or tinnitus susceptibility. In addition, failure to detect shared genetic architecture between age-related hearing loss/tinnitus and cisplatin-associated ototoxicity through our methods of evaluation does not preclude its existence. In the gene-based association analysis of cisplatin-induced tinnitus, we identified a genome-wide significant association for *WNT8A*, a gene that is vital for proper development of the inner ear and nervous system. In the genome-wide and functional enrichment analyses of age-related hearing loss and tinnitus, we identified numerous genes also involved in inner ear/nervous system development and maintenance to be highly associated with both phenotypes. Similarly, the same analytical approaches for radiation-associated ototoxicity implicated multiple genes involved in inner ear/nervous system development and maintenance, particularly *ATXN1* and *NAV2*. Consequently, it is most probable that both forms of treatment-related ototoxicity share appreciable genetic architecture with age-related hearing loss and tinnitus, with radiation-associated ototoxicity sharing a greater overall proportion of heritability.

The fact that hearing loss and tinnitus are associated with many of the same neuro-otological characteristics indicated that patients who develop severe forms of these hearing disorders following cisplatin treatment likely also develop peripheral sensory neuropathy, and that a considerable proportion of testicular cancer survivors develop multiple severe neurotoxicities. Once we confirmed that incidence of all three

phenotypes are highly associated with each other, we developed an overall score of neurotoxicity burden for testicular cancer survivors to examine how multiple severe neurotoxicites could affect quality of life. It was apparent that patients with multiple severe neurotoxicities were likely to experience multiple persistent adverse health outcomes, ultimately leading to poorer-self reported health (17.5% reported fair/poor overall health in comparison to 1.5% of controls).

The development of an overall neurotoxicity score following cisplatin treatment is a beneficial approach that could potentially be used for other drugs known to elicit multiple related toxicities that affect the same tissue. In addition, it may be worthwhile to examine whether other ototoxic agents/etiologies increase susceptibility to additional nervous system disorders. Due to limitations from the CCSS questionnaire design, we were unable to evaluate whether radiation-induced hearing loss and tinnitus were associated with peripheral sensory neuropathy. Since cranial radiation is a locally applied agent, it is probable that nerve function in the extremities would not be correlated with hearing loss and tinnitus susceptibility, as is observed with cisplatin, a systemic agent. However, it would be of interest to examine whether patients who are predisposed to developing auditory disorders are also likely to experience nerve damage in other areas of the body. Such an analysis could be performed in the eMERGE dataset, as all ICD-9-CM diagnosis codes are provided for each patient. Consequently, an EHR-WAS of hearing loss or tinnitus could be used to identify novel associations with other adverse health events, including peripheral neuropathy and other neurological disorders. Associations between hearing loss and neurological disorders/cognitive decline have already been reported (7, 220), and it would be of

potential interest to evaluate whether hearing loss and tinnitus are associated with nerve-related disorders outside of the CNS.

The predisposition to multiple cisplatin-induced toxicities may also extend beyond neurotoxicity. In our analysis of serum platinum levels in testicular cancer survivors, it appeared that elevated levels of circulating platinum were associated with poorer kidney function. Although cisplatin is eliminated predominantly through renal clearance (30), it is also known to be highly nephrotoxic. Patients with kidneys highly sensitive to cisplatin would therefore have considerable difficulty eliminating cisplatin, ultimately increasing the levels of platinum in circulation. In addition to kidney function, bone structure is influential in cisplatin elimination, as high levels of platinum are stored in long bones (147), and are then slowly released over time due to its binding interaction with collagen (184). GWAS of multiple severe neurotoxicity status identified *FAM20C* as nearly genome-wide significant, a gene essential for proper bone development. *FAM20C* expression was also positively correlated with cisplatin sensitivity in CNS, bone, and kidney cancer cell lines, suggesting that the gene exerts a protective effect in tissues affected by the long-term retention of platinum. Similarly, GWAS of cisplatin-induced hearing loss in the expanded cohort identified *TXNRD1*, a gene that mediates cisplatin renal sensitivity (165), while GWAS of cisplatin-induced tinnitus identified *WNT8A*, a gene involved in kidney repair (168) and inner ear/nervous system function (169, 170). These analyses indicate that genetic variation in genes vital to kidney function or bone modeling may influence susceptibility to ototoxicity and other toxicities elicited by cisplatin. If patients have genetic polymorphisms that influence the ability of bone to secrete platinum into circulation and/or subsequent renal clearance, it would ultimately

elevate platinum levels in long-term survivors, thereby potentiating the numerous persistent toxicities associated with cisplatin treatment. Therefore, the overall genetic architecture relevant to cisplatin-induced hearing loss and tinnitus is complex, indicating that numerous SNPs or other mutations influencing nerve, kidney, and bone development and maintenance could increase patient risk for developing cisplatin-associated ototoxicity.

CHAPTER 10. FUTURE DIRECTIONS

Analysis of Expanded Patient Cohorts

Although we were able to use three separate cohorts to evaluate non-genetic risk factors, comorbidities, and genetic predispositions in two different forms of treatment related-otoxicity and age-related hearing loss and tinnitus, a common limitation was the number of patients enrolled in the Platinum Study and CCSS. Both cohorts had far fewer patients than eMERGE, indicating that the statistical power of the non-genetic and genetic analyses was reduced. Due to the fact that these cohorts are derived from a much smaller proportion of the population (long-term cancer survivors) than age-related hearing loss and tinnitus patients (patients diagnosed between the ages of 50-90 who meet inclusion criteria), it is unlikely that this limitation will be resolved.

However, there does exist the possibility of enrolling more patients in the Platinum Study or CCSS to improve the statistical power of these analyses. By including previously uncharacterized patients in the Platinum Study to form an expanded cohort of patients, we were not only able to validate previously identified non-genetic associations and one genetic predisposition (rs62283056 for cisplatin-induced hearing loss), we were able to identify two new genes of plausible biological significance to be associated with cisplatin-associated ototoxicity (*TXNRD1* and *WNT8A*). Consequently, it would be of particular importance to validate our phenotypic correlations and genetic risk factors for radiation-associated ototoxicity in an expanded cohort. Although we were able to replicate the association between SNPs intronic to *ATXN1* (rs67522722 and rs34675197) and radiation-induced hearing loss in the SJLIFE cohort, it was considerably underpowered, which may have ultimately precluded the validation of the

prominent signal in *DCAF6* with radiation-induced tinnitus. Importantly, CCSS does have an expanded cohort of non-overlapping patients diagnosed with pediatric cancer between 1987-1999 that should have whole genome sequencing data available by the end of 2020. Based on estimates from the original cohort, this group would include approximately 1,335 patients for hearing loss (164 cases and 1,171 controls) and 1,210 patients for tinnitus (89 cases and 1,121 controls). This expanded cohort could be used as either a replication dataset or combined with the original cohort of patients diagnosed from 1971-1985 to enable a mega-analysis of our previously identified non-genetic and genetic associations.

Use of eMERGE to Identify Novel Associations with Hearing Loss and Tinnitus

The eMERGE dataset is also particularly compelling for future analyses because of the sheer volume of phenotypes that can be analyzed in a patient population currently exceeding 85,000 individuals. Although we used eMERGE to characterize age-related hearing loss and tinnitus, there exists the possibility of evaluating hereditary hearing disorders through the use of age at diagnosis and appropriate ICD-9-CM diagnosis code criteria. This would not only enable a direct comparison of genetic predispositions to congenital hearing loss and tinnitus between patients of European and African ancestry, but also whether these auditory disorders share common genetic architecture with other etiologies. Further, our phenotype association analyses of age-related hearing loss and tinnitus merely confirmed previously identified associations with other clinical characteristics. Since patients in eMERGE have phenotype status determined from detailed EHRs, it is plausible to run an EHR-WAS in which hearing loss and tinnitus are evaluated against all ICD-9-CM diagnosis codes to agnostically

identify other phenotypes of statistical significance. As mentioned in Chapter 9, such an approach could be used to identify novel associations for hearing loss and tinnitus with other adverse health events, including neurological disorders outside the CNS. Once these associations have been identified, PRS analysis or other techniques such as SNP-based enrichment and LD score regression can be performed to determine whether hearing loss or tinnitus share common genetic architecture with these phenotypes. Further, our previous genetic analyses focused predominantly on examining whether individual genetic variants were associated with a given phenotype such as hearing loss or tinnitus. However, it is known that genes can exhibit pleiotropy in which variation in one gene can influence seemingly unrelated phenotypic traits. Since eMERGE provides detailed EHR information on all included patients, it is feasible for us to perform phenome-wide association studies (PheWAS) in which phenotypes are agnostically evaluated for their association with our identified SNPs. Through PheWAS, we would be able to determine whether patients who have genetic predispositions to hearing loss or tinnitus are susceptible to developing other adverse events prior to their manifestation.

Reducing the Current Health Disparity of Genomic Studies

In addition, we were only able to compare the genetic architecture of treatment-related ototoxicity and age-related hearing loss and tinnitus in patients of European ancestry because GWAS of cisplatin- and radiation-associated ototoxicity have only been performed in this patient population. To our knowledge, we also performed the first GWAS of age-related hearing loss and tinnitus in patients of African ancestry. This is in accord with the observation that a disproportionate number of genetic studies evaluate

cohorts of European ancestry, despite known differences in allele frequencies and effect sizes among individuals of differing ancestries (276). Although we identified shared genetic architecture of age-related tinnitus between patients of European and African ancestry, the individual genetic variants that were associated with hearing loss and tinnitus in these cohorts were different. Consequently, the identification of genetic risk factors for phenotypes in European-based studies may not be relevant in patients of other genetic ancestries, thereby promoting a health disparities gap. Although these slight genetic variations may appear to be subtle nuances among heterogeneous patient populations, finding causal associations of adverse sequelae is a hallmark paradigm of precision medicine, and may eventually enable treatment regimens and doses to be tailored specifically to the individual patient to maximize treatment efficacy while limiting toxicities. Therefore, there is an immediate need to perform genome-wide analyses of disease susceptibility, drug response, and other phenotypes relevant to public health in patients who are not of European descent, which will provide the framework for an equitable use of precision medicine in future patients.

Functional Validation of Identified Genetic Associations

Regardless of the *in silico* approach used to identify genetic variants of potential interest, it is paramount that associations are functionally validated *in vitro* and/or *in vivo* (277). Through this critical step, the biological significance of the identified genetic variants can be definitively ascertained. Moreover, physiological validation of the genetic architecture underlying different drug-induced toxicities may potentiate the discovery of novel drug targets that can mitigate the adverse effects, thereby reducing their overall morbidity. These mechanistically based therapeutic strategies may

ultimately be leveraged to identify novel drug targets for treatment-related hearing loss and tinnitus that can reduce selected toxicities without inhibiting antineoplastic efficacy. Due to the existence of shared genetic architecture between treatment-related and age-related etiologies, it is possible that identified treatment strategies for ototoxicity could be applied to the much broader population of patients who develop irreversible hearing loss and tinnitus.

CHAPTER 11. APPENDIX

LIST OF APPENDIX FIGURES

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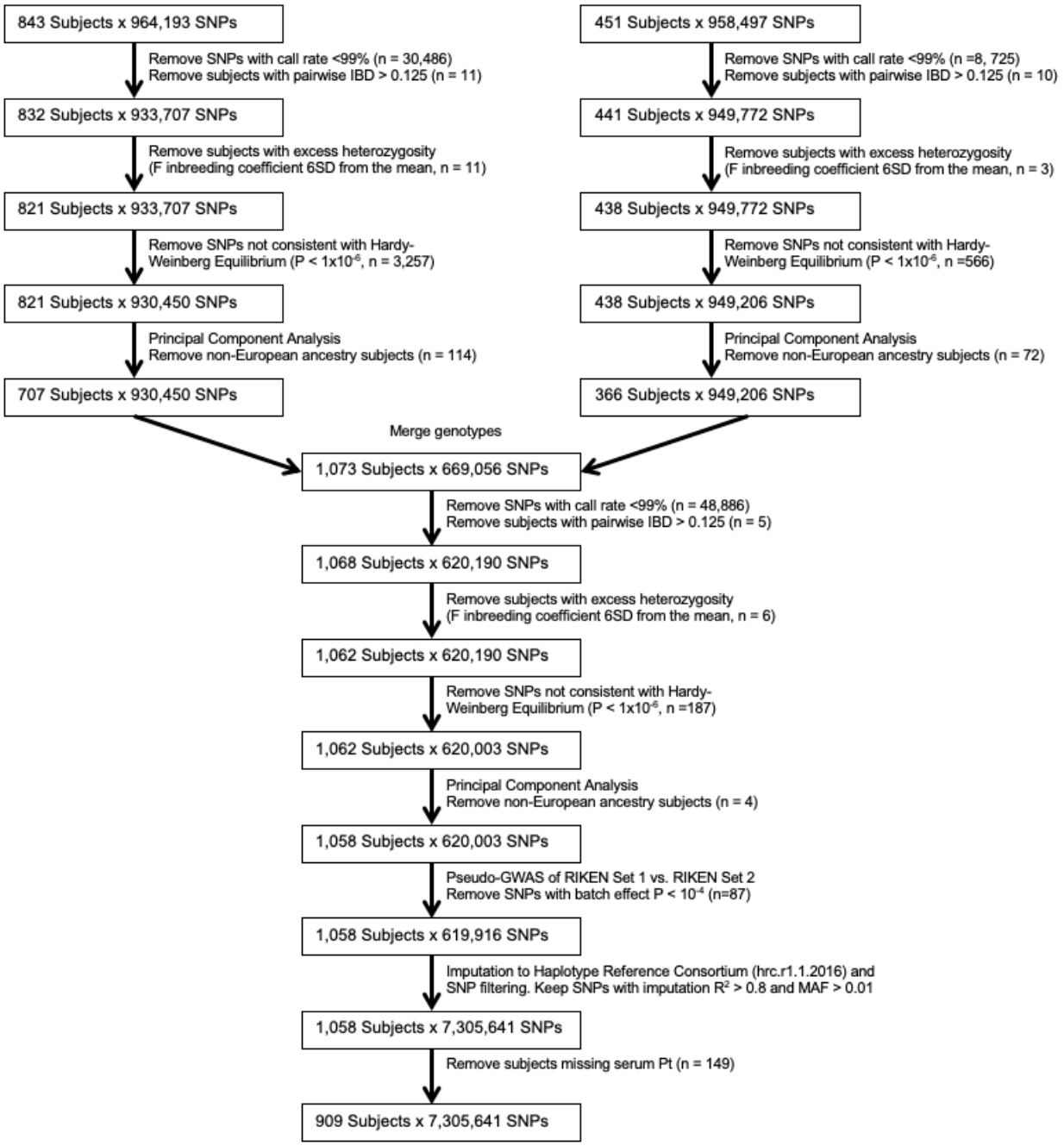
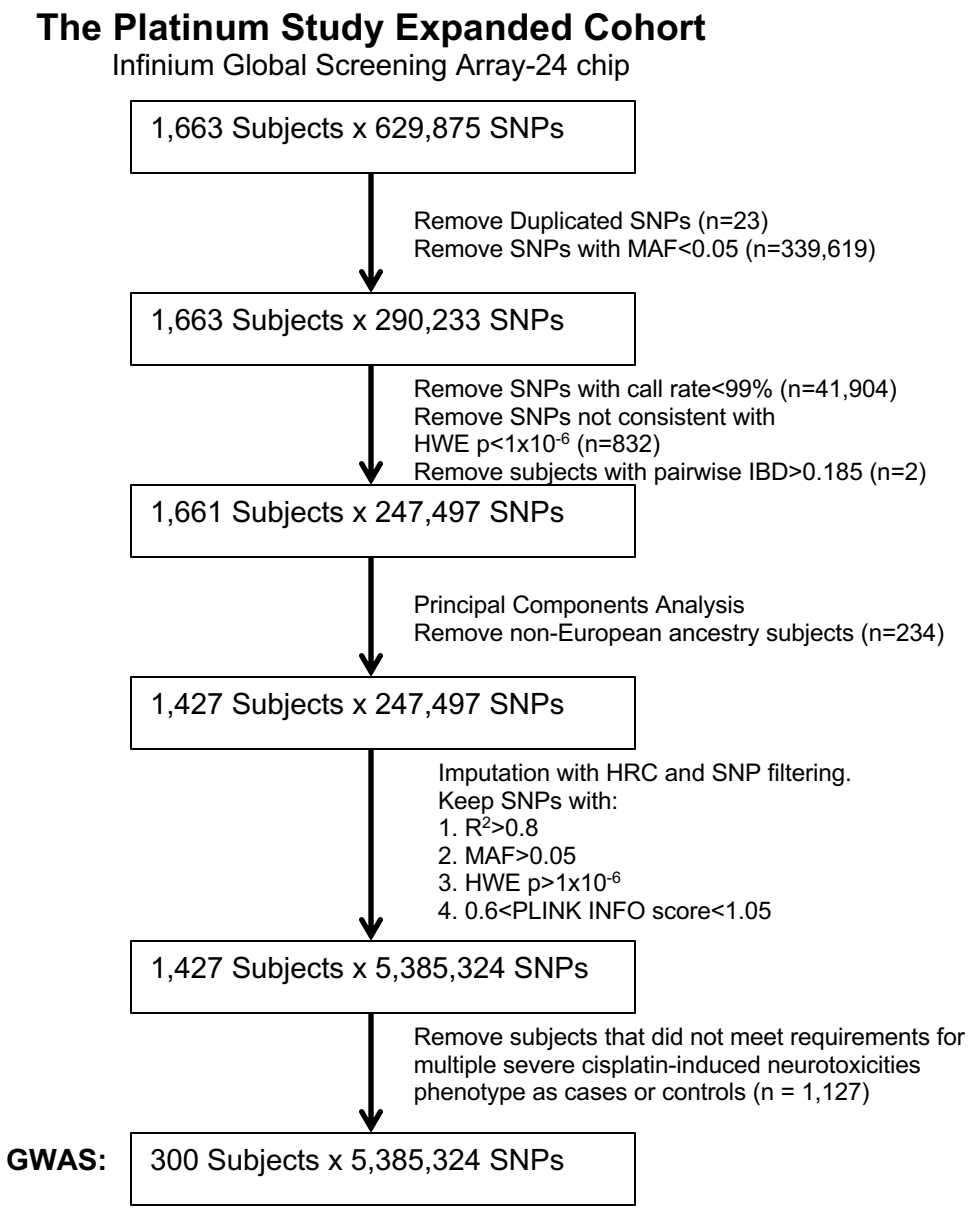
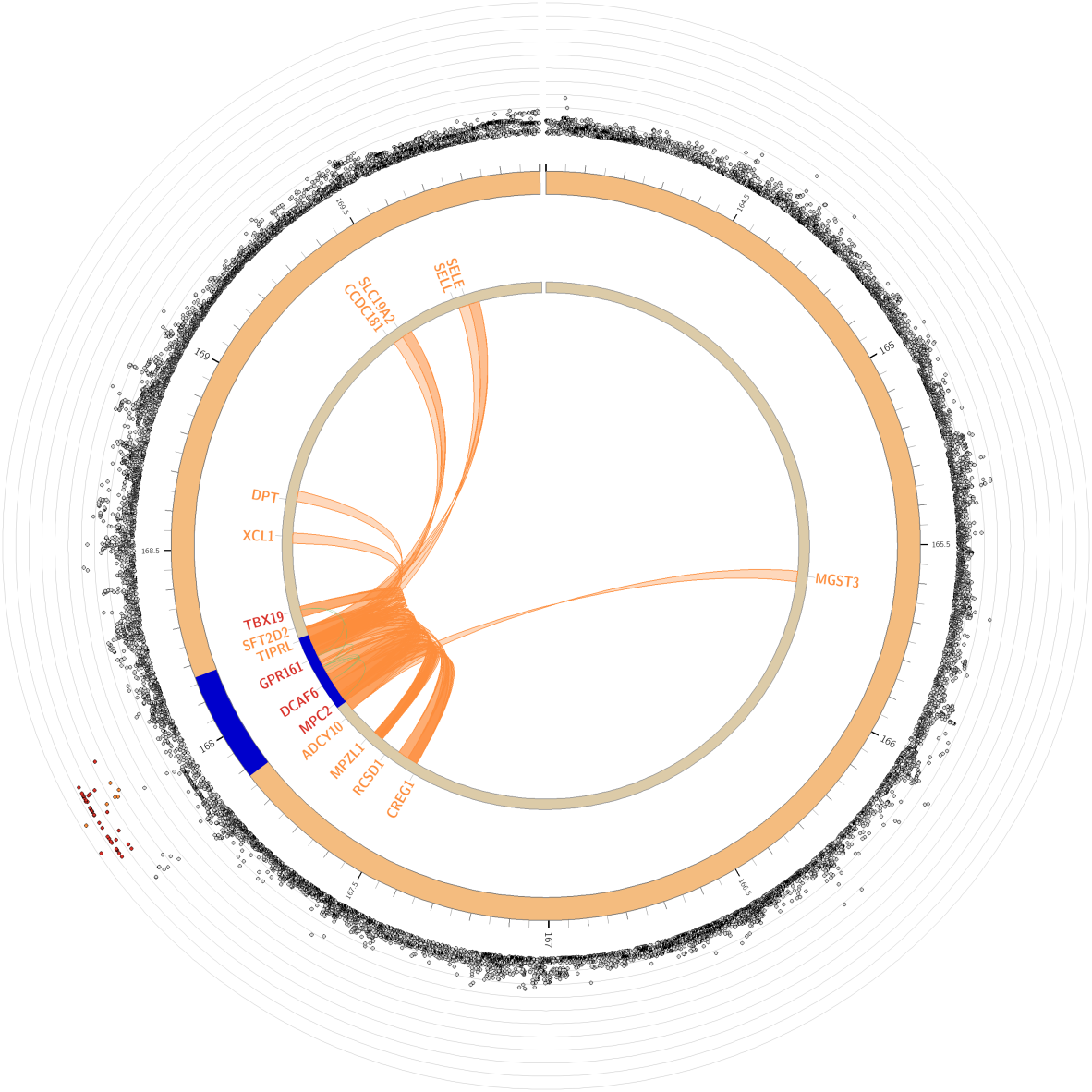


Appendix Figure 1. GWAS of Serum Platinum Levels and Quality Control Pipeline.

Abbreviations: IBD: identity by descent; SD: standard deviation; MAF: minor allele frequency. Trendowski et al. Clin Cancer Res 2019;25(19):5913-5924.



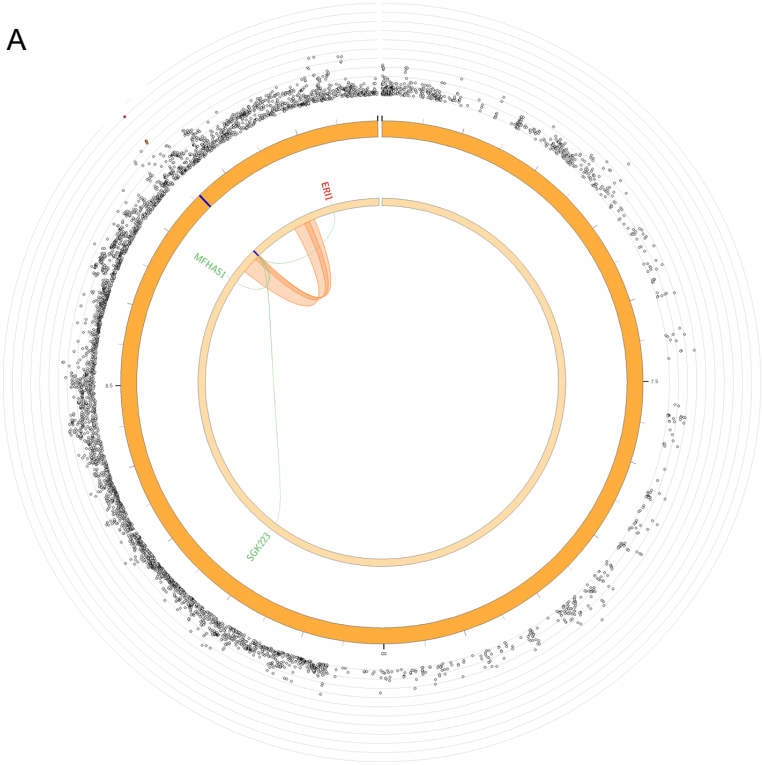
Appendix Figure 2. Study Design for Subject and Genotype Quality Control for GWAS of Multiple Severe Cisplatin-Induced Neurotoxicities. The flow chart depicts the steps used in selecting SNPs and subjects for testing in the GWAS. Abbreviations: SNP: single nucleotide polymorphism; IBD: identity by descent; MAF: minor allele frequency; HWE: Hardy-Weinberg Equilibrium; GWAS: genome-wide association study.



Appendix Figure 3. Circos Plot of eQTL and Chromatin Interactions with Genes Associated with Top SNPs in GWAS of Radiation-Induced Tinnitus. SNPs in LD (R^2 > 0.6) with rs203248 encompass four genes (*DCAF6*, *TIPRL*, *GPR161*, and *MPC2*) that together form a relatively large genetic risk locus. Chromatin interaction mapping indicated 16 other genes within chromosome 1 interacting with this region. The outer layer depicts the Manhattan plot containing the $-\log_{10}$-transformed p-value of each SNP in the GWAS. Only SNPs with $p < 0.05$ are displayed. SNPs in the genetic risk locus

(blue shaded region) are color-coded as a function of their maximum R^2 to rs203248, as follows: red ($R^2 > 0.8$) and orange ($R^2 > 0.6$). SNPs that are not in LD with rs203248 ($R^2 \leq 0.2$) are gray. Chromosome position is indicated by the circular axis between the Manhattan plot and associated genes. Links colored orange are chromatin interactions between the genetic risk locus and other genes, while links colored green are eQTL interactions.

A



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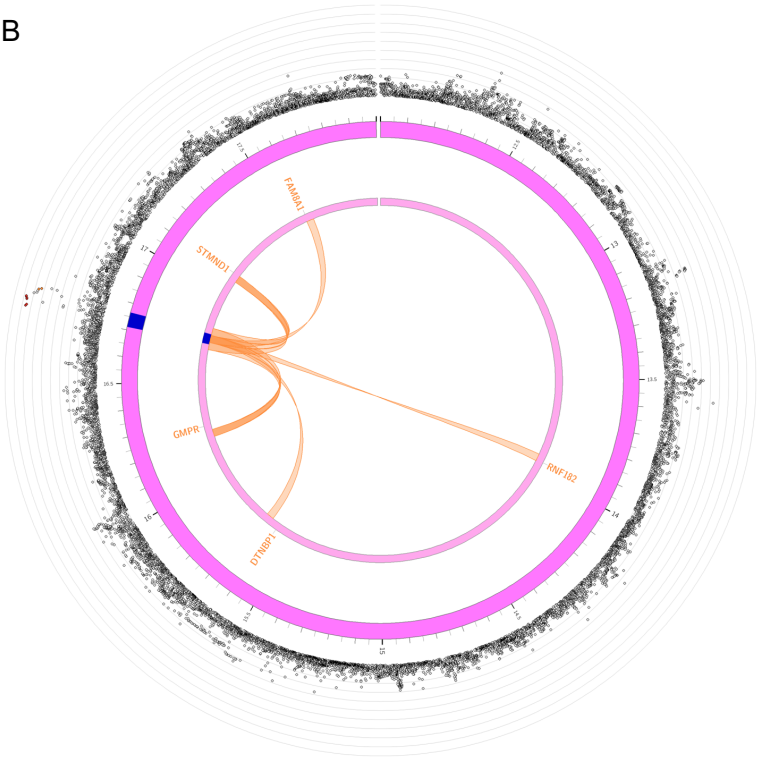
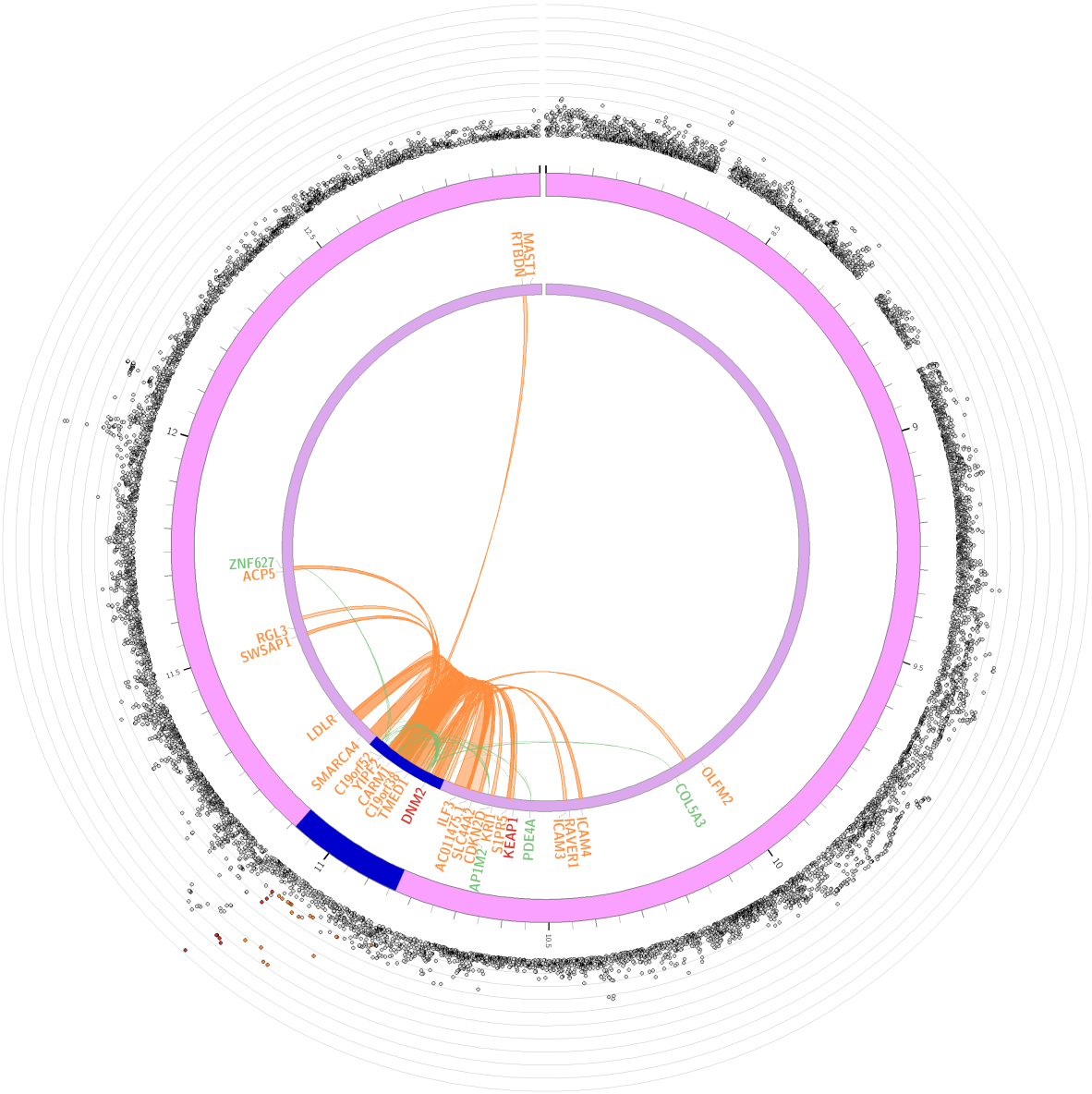


Appendix Figure 4. Circos Plots of eQTL and Chromatin Interactions with Genes Associated with Top SNPs in GWAS of Radiation-Induced Hearing Loss.

Appendix Figure 4. Circos Plots of eQTL and Chromatin Interactions with Genes Associated with Top SNPs in GWAS of Radiation-Induced Hearing Loss. A) rs332013 is an eQTL for *ERI1*, and SNPs in LD ($R^2 > 0.6$) with rs332013 are eQTLs for *MFHAS1* and *SGK223*. **B)** SNPs in LD with rs67522722 ($R^2 > 0.6$) have chromatin interactions with 5 other genes. The outer layer of the plots depict the Manhattan plots containing the $-\log_{10}$-transformed p-value of each SNP in the GWAS. Only SNPs with $p < 0.05$ are displayed. SNPs in the genetic risk locus (blue shaded region) are color-coded as a function of their maximum R^2 to rs332013 or rs67522722, as follows: red ($R^2 > 0.8$) and orange ($R^2 > 0.6$). SNPs that are not in LD with rs332013 or rs67522722 ($R^2 \leq 0.2$) are gray. Chromosome position is indicated by the circular axis between the Manhattan plot and associated genes. Links colored orange are chromatin interactions between the genetic risk locus and other genes, while links colored green are eQTL interactions.



Appendix Figure 5. Circos Plot of Chromatin and eQTL Interactions with Genes Associated with Top SNPs in GWAS of Age-Related Hearing Loss in Patients of African Ancestry. SNPs in LD (R^2 > 0.6) with rs144555968 encompass seven genes (*SMARCA4*, *C19orf52*, *YIPF2*, *CARM1*, *C19orf38*, *TMED1*, and *DNM2*) that together form a relatively large genetic risk locus on chromosome 19. Chromatin interaction and eQTL mapping indicated 22 other genes within chromosome 19 interact with this region (17 chromatin interactions and 5 eQTLs). The outer layer depicts the Manhattan plot

Appendix Figure 5. Circos Plot of Chromatin and eQTL Interactions with Genes Associated with Top SNPs in GWAS of Age-Related Hearing Loss in Patients of African Ancestry.

containing the $-\log_{10}$-transformed p-value of each SNP in the GWAS. Only SNPs with $p < 0.05$ are displayed. SNPs in the genetic risk locus (blue shaded region) are color-coded as a function of their maximum R^2 to rs144555968, as follows: red ($R^2 > 0.8$) and orange ($R^2 > 0.6$). SNPs that are not in LD with rs144555968 ($R^2 \leq 0.2$) are gray. Chromosome position is indicated by the circular axis between the Manhattan plot and associated genes. Links colored orange are chromatin interactions between the genetic risk locus and other genes, while links colored green are eQTL interactions. Genes with red text have both chromatin and eQTL interactions.

Appendix Table 1. Previous Pharmacogenomic Studies on Cisplatin-Associated Ototoxicity.

Gene/Protein	Function	Discovery Study(s)	Replication Study(s)
ABCC3/canalicular multispecific organic anion transporter 2	Transports molecules across extra and intracellular membranes. *ABCC3* is induced as a hepatoprotective response to pathologic liver conditions (278).	**1)** In 155 pediatric patients, an association was identified in *ABCC3* variant rs1051640 (p = 0.036, OR = 1.8). Predictive model combining variants in *TPMT*, *ABCC3*, and *COMT* with clinical variables (age, vincristine treatment, germ-cell tumor, and cranial irradiation) significantly improved prediction of hearing-loss compared with clinical risk factors alone (43).	**1)** GWAS of 511 cisplatin-treated testicular cancer patients did not find *ABCC3* SNP rs1051640 to be significantly associated with CAO (p = 0.703) (57).
ACYP2/ acylphosphatase-2	Catalyzes hydrolysis of carboxyl-phosphate bond of acyl phosphates in the phosphoenzyme intermediate of different membrane pumps, particularly the Ca^{2+}/Mg^{2+} ATPase in the sarcoplasmic reticulum of skeletal muscle (53).	**1)** GWAS of 238 children with brain tumors identified genetic variations in *ACYP2* associated with CAO (rs1872328, p = 3.9×10^{-8}, hazard ratio = 4.5). Validated in independent replication in 68 similarly treated children (49).	**1)** Independent cohort of 156 patients genotyped for rs1872328 variant identified significant association between carriership of A allele and CAO (p = 0.027) (50). **2)** Genotyping of 149 children (seven UK centers) using retrospective cohort study design performed on *ACYP2* SNP rs1872328: shown to be associated with ototoxicity (p =0.027; worst ear, OR: 5.91; 95% CI: 1.51-23.16) (51). **3)** GWAS of 511 cisplatin-treated testicular cancer patients did not find replicate association with CAO (p = 0.76) (57).

Appendix Table 1 Continued.

			4) Candidate gene study of 229 cisplatin-treated testicular cancer patients indicated *ACYP2* SNP rs1872328 was significantly associated with CAO [$p = 2.83 \times 10^{-3}$, OR (95% CI): 14.7 (2.6-84.2)] (52).
COMT/catechol-O -methyl transferase	Methylates compounds with a catechol structure (including catecholamine neurotransmitters; dopamine, epinephrine, and norepinephrine) to facilitate their degradation (279).	**1)** Identified a genetic variant in *COMT* (rs9332377, p = 0.00018, OR = 5.5, 95% CI 1.9-15.9) associated with cisplatin-induced hearing loss in children (41).	**1-5)** Similar findings with *TPMT* in the same studies except *COMT* variant rs4646316 remained statistically significant in the meta-analysis (OR A versus T allele: 1.52, 95% CI: 1.16-1.99, p =0.003) (44). **6)** Demonstrated an association of early onset CAO with the T allele of rs9332377 in *COMT* (p=0.001) (280).
GST/glutathione *S* -transferase	Group of isoenzymes that catalyze the conjugation of potentially damaging electrophiles with glutathione (281). Subclasses M1, T1, and P1 have been demonstrated to influence the outcome of chemotherapy with platinum compounds, and are directly involved in their detoxification (281-283).	**1)** *GSTM3**B allele appeared to have an otoprotective effect with a frequency of 0.18 in the group with normal hearing after therapy versus 0.025 in the group with hearing impairment. ($\chi^2 = 5.37$; p = 0.02) (284). **2)** Risk of having an inferior audiometric result was more than four times higher in patients with 105Ile/105Ile-*GSTP1* or 105Val/105Ile-*GSTP1* compared with 105Val/105Val-*GSTP1* (OR = 4.21; 95% CI, 1.99 to 8.88; p < 0.001) (285).	**1)** Study on medulloblastoma and *GST* polymorphisms did not find any relationship between development or time-to-development of ≥ grade 3 ototoxicity and the study variables, including the *GST* polymorphisms (288). **2)** A large group of genotyped Canadian pediatric patients failed to identify significant associations with CAO and *GSTP1* (Fisher exact p = 0.61, OR = 0.71) or GSTM1 (Fisher exact p = 0.51, OR = 0.78) (41).

Appendix Table 1 Continued.

		3) *GSTP1*-GG genotype experienced less tinnitus (p = 0.008, OR 0.33 [0.14-0.74]) and hearing impairment (p = 0.025, OR 1.81 [1.08-3.03]) (286). **4)** *GSTT1* wild type occurred with higher frequency in patients with ototoxicity (p = 0.023; OR, 10; 95% CI, 1.80-56.00) (287). **5)** Demonstrated an association of early onset of CAO with the presence of two copies of *GSTT1* (p = 0.009) (280).	**3)** GWAS of 511 cisplatin-treated testicular cancer patients did not find *GSTP1* SNP rs1695 to be significantly associated with CAO (p = 0.469) (57).
LRP2/ megalin	Multiligand binding receptor that mediates endocytosis of ligands leading to degradation in lysosomes or transcytosis (289, 290). May play a crucial role in the development of the inner ear (290).	**1)** Observed a higher frequency of the A allele of rs2075252 in the group with hearing impairment than in the group with normal hearing after cisplatin therapy (0.32 versus 0.14) (χ^2 = 5.83, p <0.02; OR: 3.45; 95% CI: 1.11-11.2) (291). **2)** C-allele of rs2228171 occurred with higher frequency in patients with ototoxicity (p =0.034; OR, 2.67; 95% CI, 1.22-5.82) (287).	**1)** A large group of genotyped Canadian pediatric patients failed to identify significant associations with CAO and the *LRP2* rs2075252 A allele (in the combined cohort, Fisher exact allelic p = 0.55, OR = 1) (41).
NFE2L2/nuclear factor (erythroid-derived 2)-like 2 (Nrf2)	Basic leucine zipper transcription factor that regulates the expression of antioxidant proteins used to protect against oxidative stress (292).	**1)** In patients who were exposed to cumulative cisplatin doses ≥ 200 mg/m^2 (n = 113), rs6721961 was associated with protection against ototoxicity, according to three different	No published independent replication studies.

Appendix Table 1 Continued.

		grading scales of hearing loss (ASHA, p = 0.005; Chang, p = 0.028; CTCAE, p = 0.004) (287).	
OTOS/otospiralin	Essential for the maintenance of normal hearing, as evidenced by *in vivo* studies reporting moderate to irreversible deafness in mice and guinea pigs following knockdown and knockout of *OTOS* (64-66). Overexpression of *OTOS* significantly decreased apoptosis of spiral ligament fibrocytes following treatment with cisplatin (293).	**1)** Candidate gene analysis of *OTOS* SNPs indicated that rs77124181 (c.-192-182C>G) and rs2291767 (c.-192-22A>G) were significantly associated with patients who experienced no hearing loss following treatment (p = 0.022) (294).	No published independent replication studies.
SLC16A5/Solute carrier family 16, member 5	Localizes to the cell membrane and acts as a proton-linked transporter of bumetanide. Murine Slc16a5 is expressed in cochlear and utricle hair cells, and mutations in genes uniquely expressed in ear hair cells have been shown to cause deafness (295).	**1)** Association and fine-mapping analyses in 188 patients identified rs4788863 in *SLC16A5*, to be associated with protection against CAO in two independent cohorts (combined cohort: OR, 0.06; 95%CI, 0.02-0.22; $p = 2.17 \times 10^{-7}$). Statistically significant ($p < 1.0 \times 10^{-4}$) differences in cell viability were observed between *SLC16A5*-silenced cells and non-targeting siRNA-treated cells. In addition, *SLC16A5* was significantly induced by cisplatin	No published independent replication studies.

Appendix Table 1 Continued.

		in a dose dependent manner ($p < 1.0 \times 10^{-4}$) (296).	
SLC22A2 (OCT2)/solute carrier family 22 member 2	Polyspecific organic cation transporters in the liver, kidney, intestine, and other organs that eliminates endogenous small organic cations, drugs, and environmental toxins (297).	**1)** *SLC22A2* variant rs316019 (c.808G>T; Ser270Ala) was significantly associated with protection from cisplatin-induced ototoxicity in the pediatric cohort (Fisher's exact p = 0.022) and the adult cohort (Fisher's exact p = 0.048) (298).	**1)** GWAS of 511 cisplatin-treated testicular cancer patients did not find *SLC22A2* SNP rs316019 to be significantly associated with CAO (p = 0.2515) (57).
SLC31A1 (*CTR1*)/ high affinity copper uptake protein 1	High-affinity copper transporter found in the cell membrane that also facilitates cisplatin uptake (299). Mouse *CTR1* (*Ctr1*) is abundantly expressed and highly localized at primary sites of cisplatin toxicity in the inner ear (300).	**1)** *CTR1* rs10981694 A>C polymorphism was associated with CAO, as C-carrier subjects presented poorer tolerance to ototoxicity (p<0.05) (301).	No published independent replication studies.
SOD2/superoxide dismutase 2	Reduces oxidative stress by converting superoxide into hydrogen peroxide and diatomic oxygen, preventing the accumulation of ROS (302).	**1)** After correcting for multiple comparisons in five *SOD* variants genotyped in 71 patients (only 42% were non-Hispanic white, and other genetic ancestries were included in the analysis), the C-allele of the rs4880 variant was significantly associated with ototoxicity (OR = 3.06, 95% CI: 1.30-7.20 (303).	No published independent replication studies.

Appendix Table 1 Continued.

TPMT/thiopurine S - methyltransferase	Carries out S-methylation on aromatic and heterocyclic sulfhydryl compounds, which is vital for the metabolism of thiopurines (304).	**1)** Identified rs12201199 in *TPMT* to be highly associated with cisplatin-induced hearing loss in children (p = 0.00022, OR = 17.0, 95% CI 2.3-125.9) (41). Association with ototoxicity was so significant that the FDA changed cisplatin label to include warnings for patients with this genotype.	**1)** Genotyping of 213 medulloblastoma patients revealed no association between *TPMT* variants rs1800462 (excluded from genotype-phenotype association analyses due to monomorphism, rs12201199 (p = 0.71), rs1800460 (p = 1.00) and rs1142345 (p = 0.69) and ototoxicity, but there was a trend for less ototoxicity in those with rs1142345 (p = 0.14). In addition, there was no significant difference of functional hearing loss or hair cell damage between *TPMT* knockout and wild type mice following cisplatin treatment, and no *TPMT* variant was associated with cisplatin cytotoxicity in lymphoblastoid cell lines (42). **2)** Using an independent cohort of 155 pediatric patients, an association was replicated in *TPMT* variant rs12201199 (p = 0.0013, OR = 6.1) (43). **3)** Meta-analysis indicated that the association between *TPMT* variants rs12201199 (p = 0.16, OR = 2.15 [0.74, 6.26]), rs1800460 (p = 0.45, OR = 1.55 [0.50, 4.87]), and rs1142345 (p = 0.23, OR = 1.93 [0.66, 5.64]), and cisplatin-induced hearing loss was not statistically significant (44).

Appendix Table 1 Continued.

			4) Genotyping of 149 children from seven UK centers using a retrospective cohort study design was performed for three *TPMT* variants (rs12201199, rs1142345 and rs1800460), and none were statistically significantly associated with CAO (51). **5)** GWAS of 511 cisplatin-treated testicular cancer patients did not find *TMPT* SNPs rs12201199 (p = 0.820), rs1142345 (p = 0.334), and rs1800460 (p = 0.070) to be significantly associated with CAO (57). **6)** Expression constructs of *TPMT* genetic variants had *TPMT**3A levels > 20-fold lower than the wild type, and the expression of wild type *TPMT* (*TPMT**1) in two murine ear cell lines, HEI-OC1 and UB/OC-1, significantly reduced cisplatin sensitivity (305).
WFS1/wolframin	Mediates endoplasmic reticulum (ER) stress response through degradation of ATF6α, a key transcription factor involved in ER stress signaling (306).	**1)** GWAS of 511 cisplatin-treated testicular cancer survivors observed that rs62283056 in the first intron of *WFS1* met genome-wide significance for association with cisplatin-induced hearing loss ($p = 1.4 \times 10^{-8}$). Higher cisplatin doses appeared to worsen hearing loss in patients with the	**1)** Candidate gene study of 229 cisplatin-treated testicular cancer patients indicated *WFS1* SNP rs62283056 was not significantly associated with CAO (p = 0.39). However, the genetic variant was associated with hearing loss attributable to any cause [$p = 5.67 \times 10^{-3}$, OR (95% CI): 3.2 (1.4-7.7)] (52).

Appendix Table 1 Continued.

		rs62283056 genotype (p = 0.035), and the association between decreased *WFS1* expression and hearing loss was replicated in an independent BioVU cohort (n = 18,620 patients, Bonferroni adjusted p < 0.05) (57).	

Abbreviations used are as follows: CAO (cisplatin-associated ototoxicity), GWAS (genome-wide association study), ROS (reactive oxygen species), and SNP (single nucleotide polymorphism). Trendowski et al. Clin Cancer Res 2019;25(4):1147-1155.

Appendix Table 2. ICD-9-CM Diagnosis Codes for Sensorineural Hearing loss and Tinnitus Phenotypes.

Diagnosis	ICD-9-CM Code
Tinnitus	**388.3**
Unspecified tinnitus	388.30
Subjective tinnitus	388.31
Sensorineural hearing loss	**389.1**
Sensorineural hearing loss, unspecified Non-specific	389.10
Sensory hearing loss, bilateral	389.11
Neural hearing loss, bilateral	389.12
Neural hearing loss, unilateral	389.13
Central hearing loss	389.14
Sensorineural hearing loss, unilateral	389.15
Sensorineural hearing loss, asymmetrical	389.16
Sensory hearing loss, unilateral	389.17
Sensorineural hearing loss, bilateral	389.18
Mixed conductive and sensorineural hearing loss	**389.2**
Mixed hearing loss, unspecified Non-specific	389.20
Mixed hearing loss, unilateral	389.21
Mixed hearing loss, bilateral	389.22

Appendix Table 3. ICD-9-CM Diagnosis Codes for Brain Tumors or Cancers Treated with Either Cisplatin or Cranial Radiation.

Primary Diagnosis	ICD-9-CM Code
Cisplatin	
Cervical Cancer	180
Endometrial Cancer	179, 182
Bladder Cancer	188
Stomach Cancer	151
Head and Neck Cancer	195.0
Lung Cancer	162
Esophageal Cancer	150
Pancreatic Cancer	157
Osteosarcoma	170
Ovarian Cancer	183
Testicular Cancer, Germ Cell Tumor	183, 186
Breast Cancer	174
Hodgkins Lymphoma	201
Hepatoblastoma	155
Medulloblastoma	191.7
Neuroblastoma	194
Cranial Radiation	
Malignant neoplasm of brain	191
Malignant neoplasm of frontal lobe	191.1
Malignant neoplasm of temporal lobe	191.2
Malignant neoplasm of parietal lobe	191.3
Malignant neoplasm of occipital lobe	191.4
Malignant neoplasm of ventricles	191.5
Malignant neoplasm of cerebellum nos	191.6
Malignant neoplasm of other parts of brain	191.8
Malignant neoplasm of brain, unspecified	191.9

Benign neoplasm of brain and other parts of nervous system	225
Benign neoplasm of brain	225.0
Benign neoplasm of cranial nerve	225.1
Benign neoplasm of cerebral meninges	225.2
Benign neoplams of nervous system	225.9
Malignant neoplasm of cranial nerve	192.0

Appendix Table 3 Continued.

Appendix Table 4. ICD-9-CM Diagnosis Codes for Diseases that Increase Susceptibility to Hearing and/or Tinnitus.

Diagnosis	ICD-9-CM Code
Meniere's disease	**386.0**
Ménière's disease NOS	386.00
Actv Ménière, cochlvestib	386.01
Active Ménière, cochlear	386.02
Actv Ménière, vestibular	386.03
Inactive Ménière's dis	386.04
Head injury, unspecified	959.01
Injury of face and neck	959.09
Temporomandibular joint disorders	524.6
Temporomandibular joint disorders, unspecified	524.60
Temporomandibular joint disorders, adhesions and ankylosis (bony or fibrous)	524.61
Temporomandibular joint disorders, arthralgia oftemporomandibular joint	524.62
Temporomandibular joint disorders, articular disc disorder (reducing or non-reducing)	524.63
Temporomandibular joint sounds on opening and/or closing the jaw	524.64
Other specified temporomandibular joint disorders	524.69

Appendix Table 5. ICD-9-CM Diagnosis Codes for Additional Phenotypes of Interest.

Diagnosis	ICD-9-CM Code
Dizziness and giddiness	**780.4**
Vertigo	**438.85**
Epidemic vertigo	078.81
Peripheral vertigo NOS	386.10
Peripheral vertigo NEC	386.19
Benign parxysmal vertigo	386.11
Central origin vertigo	386.2
Other and unspecified peripheral vertigo	386.1
Hypercholesterolemia	
Pure hypercholesterolemia	272.0
Anxiety/Depression	
Major depressive disorder, single episode	296.2
Major depressive affective disorder, single episode, unspecified	296.20
Major depressive affective disorder, single episode, mild	296.21
Major depressive affective disorder, single episode, moderate	296.22
Major depressive affective disorder, single episode, severe, without mention of psychotic behavior	296.23
Major depressive affective disorder, single episode, severe, specified as with psychotic behavior	296.24
Major depressive affective disorder, single episode, in partial or unspecified remission	296.25
Major depressive affective disorder, single episode, in full remission	296.26
Major depressive disorder, recurrent episode	296.3
Major depressive affective disorder, recurrent episode, unspecified	296.30

Major depressive affective disorder, recurrent episode, mild	296.31
Major depressive affective disorder, recurrent episode, moderate	296.32
Major depressive affective disorder, recurrent episode, severe, without mention of psychotic behavior	296.33
Major depressive affective disorder, recurrent episode, severe, specified as with psychotic behavior	296.34
Major depressive affective disorder, recurrent episode, in partial or unspecified remission	296.35
Major depressive affective disorder, recurrent episode, in full remission	296.36
Generalized anxiety disorder	300.02
Hypertension	
Essential hypertension	401
Malignant essential hypertension	401.0
Benign essential hypertension	401.1
Unspecified essential hypertension	401.9

Appendix Table 5 Continued.

Appendix Table 6. GWAS Results for Residual Platinum Value as a Continuous Variable (P < 0.0001).

CHR	BP	SNP	EFFECT	REFERENCE	FRQ	INFO	BETA	SE	P
19	50804066	rs1377817	G	T	0.1299	1.0081	-0.2303	0.0418	4.62E-08
19	50803571	rs58754699	T	C	0.1298	1.0088	-0.2299	0.0418	5.00E-08
19	50798679	rs113890379	A	G	0.1311	1.0321	-0.2178	0.0412	1.60E-07
2	2047397	rs6759709	G	A	0.2572	0.9887	0.1671	0.0323	2.86E-07
2	2047397	rs6759709	G	A	0.2572	0.9887	0.1671	0.0323	2.86E-07
7	96167810	rs150691387	G	A	0.0135	0.9847	0.63	0.123	3.68E-07
19	50787642	rs78604854	C	G	0.1337	1.0641	-0.2058	0.0403	4.14E-07
11	12807189	rs61878760	A	G	0.0896	0.9563	0.2499	0.0503	8.25E-07
19	50795001	rs7247511	A	G	0.1865	1.0918	-0.1731	0.0349	8.65E-07
19	50794970	rs7247501	A	G	0.187	1.0908	-0.1728	0.0349	8.86E-07
2	2102687	rs6755567	G	A	0.2402	1.024	0.1607	0.0325	9.37E-07
2	2102687	rs6755567	G	A	0.2402	1.024	0.1607	0.0325	9.37E-07
2	2103999	rs13019515	G	A	0.2403	1.0235	0.1607	0.0326	9.43E-07
2	2103999	rs13019515	G	A	0.2403	1.0235	0.1607	0.0326	9.43E-07
15	70810984	rs36116683	G	A	0.2011	0.9747	0.1746	0.0355	1.03E-06
4	183553033	rs138013532	T	C	0.0107	0.8981	0.7147	0.1454	1.06E-06
2	2109794	rs35314672	G	A	0.2414	1.0173	0.1602	0.0326	1.07E-06
2	2109794	rs35314672	G	A	0.2414	1.0173	0.1602	0.0326	1.07E-06
2	2110503	rs34210742	C	T	0.2414	1.0172	0.1601	0.0326	1.09E-06
2	2110503	rs34210742	C	T	0.2414	1.0172	0.1601	0.0326	1.09E-06
21	46563820	rs13047590	T	C	0.1363	0.9937	0.1998	0.0411	1.37E-06
2	2086374	rs1617213	C	T	0.2541	0.9844	0.1585	0.0326	1.39E-06
2	2086374	rs1617213	C	T	0.2541	0.9844	0.1585	0.0326	1.39E-06
10	85778375	rs11200804	G	A	0.1573	0.9777	0.1899	0.0394	1.74E-06
1	59556847	rs4543825	A	G	0.212	0.9768	-0.1678	0.0349	1.77E-06
2	2091619	rs1421614	T	C	0.2536	0.994	0.1561	0.0325	1.81E-06
2	2091619	rs1421614	T	C	0.2536	0.994	0.1561	0.0325	1.81E-06

Appendix Table 6 Continued.

6	144224312	rs9484827	G	T	0.0133	1.0704	0.5719	0.1192	1.89E-06
15	70811082	rs6494875	C	A	0.2008	0.9771	0.1701	0.0355	1.92E-06
5	159341075	rs80184476	T	C	0.0349	0.9922	0.3691	0.0771	1.96E-06
5	159342026	rs77875476	A	G	0.0349	0.9934	0.3675	0.077	2.13E-06
5	159342345	rs78007572	G	A	0.0349	0.9938	0.3669	0.077	2.19E-06
3	146147613	rs563551865	T	C	0.0126	0.9303	-0.6234	0.131	2.25E-06
5	159342843	rs74425400	T	C	0.0349	0.9943	0.3661	0.0769	2.28E-06
5	159343166	rs139748609	A	C	0.0349	0.9946	0.3657	0.0769	2.33E-06
5	159342808	rs74440770	C	T	0.0356	0.9888	0.363	0.0764	2.36E-06
5	159344083	rs2229181	T	C	0.035	0.9958	0.364	0.0768	2.52E-06
5	159354532	rs114294707	A	G	0.035	0.9966	0.3638	0.0768	2.53E-06
5	159347613	rs76661640	A	C	0.035	0.9966	0.3638	0.0768	2.53E-06
5	159348134	rs77476585	G	A	0.035	0.9966	0.3638	0.0768	2.53E-06
5	159348665	rs77392734	A	C	0.035	0.9966	0.3638	0.0768	2.53E-06
5	159346342	rs79224140	A	G	0.035	0.9964	0.3638	0.0768	2.54E-06
5	159346362	rs78464698	T	C	0.035	0.9964	0.3638	0.0768	2.54E-06
5	159344446	rs3730284	T	C	0.035	0.996	0.3639	0.0768	2.54E-06
5	159345906	rs75038956	G	T	0.035	0.9963	0.3638	0.0768	2.54E-06
5	159352459	rs75398586	A	T	0.035	0.9966	0.3638	0.0768	2.54E-06
5	159345112	rs78609450	G	A	0.035	0.9961	0.3638	0.0768	2.55E-06
5	159354433	rs76889583	G	A	0.035	0.9959	0.3637	0.0768	2.55E-06
5	159353055	rs76458976	C	T	0.035	0.996	0.3637	0.0768	2.56E-06
5	159353439	rs79686163	G	A	0.035	0.9959	0.3637	0.0768	2.56E-06
5	159352409	rs74417290	A	G	0.035	0.9951	0.3636	0.0768	2.56E-06
5	159355336	rs115188683	T	C	0.035	0.9954	0.3636	0.0768	2.58E-06
5	159355916	rs74319842	C	T	0.035	0.9942	0.3634	0.0768	2.62E-06
5	159355942	rs114663835	A	G	0.035	0.9941	0.3634	0.0768	2.62E-06
19	50795879	rs659773	G	T	0.186	1.0949	-0.1651	0.0349	2.63E-06
7	76820800	rs10252204	A	C	0.4261	0.9849	-0.1349	0.0285	2.63E-06
5	159356026	rs76011910	A	G	0.0351	0.993	0.3631	0.0768	2.67E-06

Appendix Table 6 Continued.

8	3913060	rs183748570	A	G	0.0147	0.8064	0.6129	0.1298	2.72E-06
5	159356875	rs77670862	T	C	0.0351	0.9919	0.3628	0.0769	2.73E-06
5	159356906	rs116547954	T	C	0.0351	0.9918	0.3627	0.0769	2.74E-06
5	159357298	rs78253043	A	G	0.0352	0.9905	0.3626	0.0768	2.77E-06
1	59548314	rs17466219	C	T	0.2131	0.9729	-0.1644	0.0349	2.88E-06
5	159358878	rs74641610	C	T	0.0353	0.9895	0.3613	0.0768	2.97E-06
5	159359512	rs78223549	A	G	0.0353	0.9885	0.3606	0.0768	3.07E-06
5	159360438	rs115000732	G	A	0.0353	0.9883	0.3602	0.0768	3.13E-06
5	159360770	rs116446221	T	C	0.0354	0.9884	0.3598	0.0768	3.20E-06
3	146142255	rs115704620	C	T	0.0632	0.8886	-0.2879	0.0619	3.75E-06
18	47309540	rs75276171	G	A	0.0611	0.9539	0.2797	0.0601	3.77E-06
18	47309909	rs7237253	G	A	0.0611	0.9539	0.2797	0.0601	3.77E-06
3	172462474	rs75979836	G	C	0.0547	0.8062	0.3189	0.0686	3.85E-06
18	47297521	rs143619252	A	G	0.0615	0.9523	0.2784	0.06	3.95E-06
18	47310967	rs80068204	A	T	0.0613	0.9512	0.279	0.0601	4.00E-06
18	47309884	rs7233791	C	G	0.0599	0.9477	0.2826	0.0609	4.00E-06
18	47296455	rs142158273	T	C	0.0616	0.9531	0.2779	0.0599	4.02E-06
18	47295988	rs3889856	T	G	0.0616	0.9535	0.2777	0.0599	4.06E-06
2	241177042	rs114949804	C	G	0.01	0.8619	0.7073	0.1526	4.08E-06
2	241177042	rs114949804	C	G	0.01	0.8619	0.7073	0.1526	4.08E-06
2	72156702	rs7587256	A	G	0.0396	1.0172	0.3333	0.0719	4.08E-06
2	72156702	rs7587256	A	G	0.0396	1.0172	0.3333	0.0719	4.08E-06
10	85775159	rs10788306	A	G	0.1937	0.9588	0.1698	0.0366	4.14E-06
10	85778523	rs11200805	T	A	0.1937	0.9588	0.1698	0.0366	4.15E-06
10	85778903	rs6585773	C	T	0.1937	0.9588	0.1698	0.0366	4.16E-06
2	156570190	rs7579510	C	A	0.012	0.943	0.6159	0.133	4.19E-06
2	156570190	rs7579510	C	A	0.012	0.943	0.6159	0.133	4.19E-06
11	12794946	rs3915398	G	A	0.1119	0.9101	0.2176	0.047	4.26E-06
10	85779923	rs7098745	C	T	0.1936	0.9582	0.1696	0.0367	4.29E-06
10	85780168	rs7902759	T	C	0.1936	0.9581	0.1695	0.0367	4.32E-06

Appendix Table 6 Continued.

5	159376710	rs78335203	A	T	0.0359	0.9706	0.3552	0.077	4.55E-06
2	48070044	rs7568939	T	C	0.0203	0.8401	0.5003	0.1088	4.88E-06
2	48070044	rs7568939	T	C	0.0203	0.8401	0.5003	0.1088	4.88E-06
3	146180070	rs76258070	A	G	0.0479	0.8864	-0.3231	0.0703	4.88E-06
11	65637273	rs501630	G	A	0.5545	0.9897	-0.1299	0.0284	5.25E-06
10	85786038	rs7921728	C	A	0.1935	0.9565	0.1682	0.0367	5.25E-06
10	85786610	rs7908599	A	G	0.1935	0.9565	0.1682	0.0367	5.27E-06
7	157930243	rs10949698	C	A	0.3239	0.9838	0.1383	0.0303	5.61E-06
3	185604948	rs111649035	C	T	0.0114	0.9692	0.6144	0.1345	5.65E-06
2	154366274	rs114985352	T	C	0.0153	0.7911	0.5881	0.1291	5.96E-06
2	154366274	rs114985352	T	C	0.0153	0.7911	0.5881	0.1291	5.96E-06
5	159299561	rs78989266	C	T	0.0368	0.9552	0.3472	0.0766	6.60E-06
2	50420757	rs115487787	T	C	0.0463	0.9773	0.3084	0.0681	6.71E-06
2	50420757	rs115487787	T	C	0.0463	0.9773	0.3084	0.0681	6.71E-06
3	153334750	rs17506814	G	A	0.0131	0.8316	0.6165	0.1363	6.93E-06
1	59555169	rs12404965	G	A	0.2237	0.9769	-0.1549	0.0343	7.03E-06
1	212136070	rs115440456	C	T	0.0495	0.7991	-0.3291	0.0729	7.23E-06
5	159323156	rs74747711	G	A	0.037	0.9863	0.3391	0.0753	7.58E-06
18	47292377	rs8083124	T	C	0.0611	0.9424	0.2723	0.0605	7.60E-06
6	98198517	rs144939408	T	A	0.0106	0.8459	0.6703	0.1491	7.90E-06
5	159349985	rs79416469	C	T	0.034	0.9867	0.3521	0.0784	7.92E-06
9	136613623	rs78280308	A	T	0.0325	0.8882	0.3793	0.0844	7.92E-06
2	164257937	rs10469688	G	A	0.0264	0.9492	0.4037	0.0899	8.10E-06
2	164257937	rs10469688	G	A	0.0264	0.9492	0.4037	0.0899	8.10E-06
3	151509744	rs9289856	C	T	0.0283	0.973	0.3856	0.0863	8.99E-06
18	47326426	rs183716438	T	C	0.0619	0.9489	0.2676	0.0599	9.05E-06
2	50443675	rs2222316	C	T	0.0459	0.9825	0.3045	0.0682	9.08E-06
2	50443675	rs2222316	C	T	0.0459	0.9825	0.3045	0.0682	9.08E-06
6	16951626	rs76903139	G	T	0.043	0.8413	0.3411	0.0764	9.11E-06
9	90504714	rs73653973	T	G	0.0425	0.967	0.3189	0.0715	9.31E-06

Appendix Table 6 Continued.

18	47327759	rs77983677	G	A	0.0619	0.9493	0.2671	0.0599	9.37E-06
8	8490501	rs74615832	A	G	0.0727	0.8638	0.2689	0.0604	9.56E-06
4	10198932	rs11732805	C	T	0.011	0.9276	0.6235	0.1402	9.74E-06
11	12772203	rs117301475	A	G	0.0184	0.7717	0.5304	0.1193	9.91E-06
18	47329871	rs7238567	T	C	0.0619	0.9483	0.2665	0.06	1.00E-05
1	59552704	rs9970281	G	T	0.2242	0.979	-0.152	0.0342	1.01E-05
8	1287641	rs73170474	A	C	0.0523	0.99	-0.2819	0.0635	1.01E-05
18	47330081	rs74481622	C	T	0.0619	0.9483	0.2664	0.06	1.01E-05
22	29813175	rs9625792	C	T	0.0441	0.9197	0.3173	0.0716	1.05E-05
13	93666376	rs460850	C	T	0.8516	0.9364	-0.1815	0.041	1.06E-05
18	47331659	rs78558624	C	T	0.0619	0.9489	0.2655	0.06	1.07E-05
18	47332163	rs140573335	A	G	0.0619	0.9489	0.2654	0.06	1.08E-05
6	6336740	rs116617212	T	C	0.0134	0.9042	0.5737	0.1297	1.10E-05
4	10210648	rs185700151	A	C	0.0132	0.975	0.5514	0.1249	1.14E-05
7	138728018	rs59023152	T	C	0.0196	0.9713	0.4595	0.1041	1.14E-05
20	31873286	rs6141913	A	G	0.2173	0.959	0.1541	0.0349	1.15E-05
4	10440936	rs140477370	T	A	0.0133	0.9633	0.5524	0.1253	1.16E-05
2	102731596	rs1007027	C	A	0.3294	0.9657	-0.1361	0.0309	1.16E-05
2	102731596	rs1007027	C	A	0.3294	0.9657	-0.1361	0.0309	1.16E-05
4	10194891	rs11727128	G	A	0.0133	0.9706	0.5497	0.1248	1.20E-05
8	1284211	rs73170467	A	G	0.0244	1.0009	-0.4011	0.0914	1.27E-05
14	23291551	rs117373989	A	C	0.0448	0.8485	0.3233	0.0737	1.29E-05
2	156566726	rs73965656	T	C	0.0118	0.9468	0.5867	0.1339	1.32E-05
2	156566726	rs73965656	T	C	0.0118	0.9468	0.5867	0.1339	1.32E-05
8	1284547	rs56071402	A	C	0.0244	0.9999	-0.4005	0.0914	1.32E-05
1	59534195	rs34071988	T	A	0.227	0.9847	-0.1488	0.034	1.36E-05
2	156555762	rs77079095	C	T	0.0118	0.9432	0.5872	0.1343	1.38E-05
2	156555762	rs77079095	C	T	0.0118	0.9432	0.5872	0.1343	1.38E-05
12	106302694	rs78225733	T	C	0.0162	0.8238	0.536	0.1226	1.39E-05
17	71130961	rs2344994	C	T	0.7719	1.0018	0.1464	0.0335	1.41E-05

Appendix Table 6 Continued.

11	18286807	rs750948	T	C	0.0216	0.9679	-0.4304	0.0986	1.43E-05
10	73047181	rs151211976	T	C	0.0126	0.7884	0.6199	0.1421	1.44E-05
3	151512034	rs115608419	G	T	0.0296	0.9763	0.3677	0.0843	1.45E-05
12	92342258	rs63482062	C	T	0.0132	0.8673	0.578	0.1326	1.46E-05
9	136112029	rs78433526	G	A	0.0111	0.8763	0.6308	0.1447	1.47E-05
9	136112069	rs79382109	A	G	0.011	0.8765	0.6307	0.1447	1.47E-05
1	59540105	rs4421614	C	T	0.2253	0.976	-0.1491	0.0342	1.48E-05
8	1286467	rs73170473	A	G	0.0243	0.9945	-0.3994	0.0918	1.53E-05
1	59537214	rs7536474	A	G	0.1421	0.9983	0.1753	0.0403	1.53E-05
16	65820295	rs7198565	A	C	0.0911	0.9905	0.2143	0.0493	1.53E-05
16	65818152	rs28386180	T	G	0.0906	0.9921	0.2144	0.0493	1.54E-05
14	34436723	rs8005064	C	G	0.6362	0.8796	0.1352	0.0311	1.57E-05
22	36639067	rs13054261	T	C	0.2074	0.9588	0.1541	0.0355	1.60E-05
7	138727771	rs111377784	A	C	0.0207	0.9705	0.4392	0.1013	1.61E-05
7	138727353	rs10245897	A	T	0.0207	0.97	0.4392	0.1013	1.61E-05
2	233346027	rs746379	G	C	0.2657	0.9523	0.142	0.0328	1.63E-05
2	233346027	rs746379	G	C	0.2657	0.9523	0.142	0.0328	1.63E-05
16	65813222	rs9931033	A	G	0.0917	0.9908	0.2126	0.0491	1.65E-05
16	65812859	rs28399714	T	G	0.0917	0.9909	0.2126	0.0491	1.65E-05
22	36639308	rs5750243	T	C	0.208	0.9662	0.1529	0.0353	1.68E-05
7	157906182	rs2335844	G	C	0.3272	0.9817	0.1308	0.0302	1.70E-05
22	36639421	rs4378900	A	G	0.2081	0.9673	0.1527	0.0353	1.70E-05
15	93010321	rs116928729	A	G	0.0176	0.8056	0.5182	0.1199	1.72E-05
7	157909775	rs4909241	G	T	0.3272	0.9814	0.1307	0.0302	1.72E-05
7	157917414	rs4909253	A	G	0.3274	0.9818	0.1306	0.0302	1.74E-05
2	156556800	rs11901099	G	A	0.0113	0.9412	0.5949	0.1378	1.75E-05
2	156556800	rs11901099	G	A	0.0113	0.9412	0.5949	0.1378	1.75E-05
9	14726174	rs76660602	C	G	0.0106	0.7463	0.688	0.1593	1.75E-05
19	50816020	rs116046797	A	G	0.0832	0.9607	-0.2269	0.0525	1.75E-05
7	157920125	rs13437759	A	G	0.3274	0.9819	0.1305	0.0302	1.75E-05

Appendix Table 6 Continued.

17	71127471	rs28410243	A	G	0.1862	0.9895	-0.1569	0.0363	1.77E-05
14	34674161	rs12590234	C	T	0.4919	0.9223	-0.1268	0.0294	1.78E-05
1	59535204	rs12402173	C	T	0.2258	0.9809	-0.1472	0.0341	1.79E-05
19	50816768	rs9916985	G	T	0.0837	0.9586	-0.2261	0.0524	1.81E-05
2	58523256	rs74887875	T	C	0.0226	0.9241	0.426	0.0988	1.81E-05
2	58523256	rs74887875	T	C	0.0226	0.9241	0.426	0.0988	1.81E-05
22	36636794	rs34223341	A	G	0.2114	0.9422	0.1533	0.0356	1.82E-05
21	39202275	rs117930146	G	T	0.0339	1.0513	0.3275	0.076	1.83E-05
7	157927537	rs6944055	G	A	0.323	0.9746	0.1312	0.0305	1.85E-05
7	157927166	rs6943534	T	A	0.323	0.9747	0.1312	0.0305	1.85E-05
8	87748951	rs139032104	A	G	0.0187	0.8576	0.4849	0.1126	1.85E-05
7	157927193	rs6960916	A	G	0.323	0.9747	0.1312	0.0305	1.86E-05
7	157927858	rs10231602	G	T	0.323	0.9746	0.1311	0.0305	1.86E-05
17	71126836	rs9902588	T	C	0.186	0.9836	-0.1569	0.0365	1.87E-05
8	14596257	rs138531759	C	T	0.0179	0.9113	-0.4809	0.1118	1.90E-05
21	37538063	rs9984678	A	G	0.1957	0.9433	-0.158	0.0367	1.90E-05
7	138735270	rs7782919	T	C	0.0328	0.9371	0.3525	0.082	1.91E-05
8	36673279	rs147078296	G	A	0.0271	0.9442	0.3838	0.0893	1.93E-05
16	65805462	rs72784570	A	G	0.0914	0.9907	0.2113	0.0492	1.94E-05
19	50817702	rs77696005	C	A	0.0842	0.958	-0.2245	0.0523	1.94E-05
15	70818373	rs4407020	G	T	0.2131	1.027	0.1457	0.0339	1.95E-05
20	31865697	rs7262822	A	G	0.2242	0.9789	0.147	0.0342	1.96E-05
15	70817811	rs4539552	G	A	0.2092	1.0371	0.1459	0.034	1.96E-05
22	36640533	rs5756113	A	C	0.2086	0.9659	0.1516	0.0353	1.96E-05
10	29325167	rs117756384	A	T	0.0351	0.9702	-0.3329	0.0776	1.98E-05
7	157928503	rs4349930	A	T	0.3224	0.9761	0.1306	0.0305	2.00E-05
7	157928660	rs6459841	C	G	0.3222	0.9755	0.1307	0.0305	2.00E-05
7	157928710	rs4475429	T	C	0.3219	0.9779	0.1305	0.0304	2.00E-05
7	157928642	rs4458820	A	G	0.3222	0.9755	0.1307	0.0305	2.00E-05
7	157928460	rs6966663	C	G	0.3224	0.9763	0.1306	0.0305	2.01E-05

Appendix Table 6 Continued.

7	157928610	rs6459840	G	A	0.3224	0.9763	0.1306	0.0305	2.01E-05
7	157928572	rs4349931	A	G	0.3224	0.9763	0.1305	0.0305	2.02E-05
7	157928272	rs6967448	G	C	0.3224	0.9762	0.1305	0.0305	2.02E-05
16	65800850	rs141403560	G	C	0.0921	0.99	0.2101	0.049	2.02E-05
5	16854994	rs2560858	G	A	0.2809	0.9237	0.1395	0.0325	2.02E-05
16	65802899	rs72784567	T	C	0.0915	0.9912	0.2106	0.0491	2.03E-05
20	31862091	rs6141909	T	C	0.2246	0.9826	0.1462	0.0341	2.05E-05
7	157928998	rs7803631	A	G	0.3225	0.9762	0.1304	0.0305	2.06E-05
7	157928976	rs7803621	A	G	0.3225	0.9762	0.1304	0.0305	2.06E-05
5	142433411	rs2398565	G	A	0.023	1.0387	0.3955	0.0924	2.07E-05
7	157929076	rs7787501	T	A	0.3226	0.9764	0.1303	0.0305	2.08E-05
16	65802172	rs55958810	A	G	0.0916	0.9918	0.2102	0.0491	2.09E-05
7	157929223	rs3800858	A	G	0.3226	0.9765	0.1302	0.0305	2.10E-05
19	50809274	rs144748691	A	G	0.0653	0.9342	-0.2548	0.0596	2.11E-05
4	67676396	rs114671118	A	G	0.0229	0.8345	-0.4419	0.1034	2.11E-05
7	157928246	rs6966230	A	G	0.3224	0.9762	0.1302	0.0305	2.12E-05
16	65801698	rs1966503	A	G	0.0916	0.9926	0.2099	0.0491	2.12E-05
17	71126929	rs9909102	G	A	0.1853	0.9828	-0.1561	0.0365	2.13E-05
17	71343370	rs184838697	T	A	0.011	0.9286	-0.6013	0.1408	2.14E-05
22	36640927	rs13055888	T	G	0.2087	0.9659	0.1508	0.0353	2.15E-05
7	157918094	rs4909255	T	C	0.3252	0.9833	0.1293	0.0303	2.16E-05
7	157920247	rs13437762	T	G	0.3279	0.9802	0.1292	0.0302	2.17E-05
7	157918582	rs4909094	T	C	0.3252	0.9834	0.1293	0.0303	2.17E-05
11	134019675	rs11223715	C	T	0.0748	0.9161	0.238	0.0558	2.19E-05
3	172567112	rs79520329	G	A	0.0612	0.8139	0.2769	0.0649	2.19E-05
2	156553677	rs11901342	T	G	0.0114	0.9379	0.5852	0.1372	2.20E-05
2	156553677	rs11901342	T	G	0.0114	0.9379	0.5852	0.1372	2.20E-05
11	65637076	rs570387	C	T	0.5198	1.0051	-0.1198	0.0281	2.23E-05
16	65800656	rs139685695	T	C	0.0916	0.9904	0.2095	0.0492	2.24E-05
16	15870985	rs113267683	T	C	0.0151	0.7832	-0.5577	0.1309	2.25E-05

Appendix Table 6 Continued.

1	83300093	rs11580950	A	G	0.0999	0.7669	0.2297	0.0539	2.25E-05
20	31873821	rs3746393	T	C	0.2228	0.9761	0.1463	0.0343	2.26E-05
7	157931263	rs3752371	C	G	0.3231	0.9786	0.1295	0.0304	2.27E-05
20	31868249	rs7263699	C	T	0.2262	0.9895	0.1447	0.034	2.29E-05
7	157930036	rs6977814	G	C	0.3228	0.9777	0.1295	0.0304	2.29E-05
14	34638094	rs12886379	T	C	0.4212	1.0042	-0.1208	0.0284	2.30E-05
7	157930137	rs12668329	A	T	0.3228	0.9778	0.1295	0.0304	2.30E-05
8	1287382	rs73670773	C	T	0.0578	0.9706	-0.2606	0.0613	2.32E-05
1	202424535	rs114269248	T	G	0.0127	0.8762	0.5733	0.1348	2.32E-05
2	8033212	rs11682406	C	T	0.0176	0.9606	0.4684	0.1101	2.33E-05
2	8033212	rs11682406	C	T	0.0176	0.9606	0.4684	0.1101	2.33E-05
7	157930974	rs3752366	T	C	0.3231	0.9769	0.1294	0.0304	2.34E-05
1	59488772	rs2716108	T	G	0.1991	0.8031	-0.168	0.0395	2.35E-05
7	157930619	rs10949699	A	C	0.3229	0.9793	0.1292	0.0304	2.36E-05
17	29920645	rs77144095	T	C	0.0199	0.8976	0.4512	0.1061	2.36E-05
20	31867286	rs6141911	A	T	0.2247	0.9836	0.145	0.0341	2.40E-05
8	14585192	rs186518397	A	G	0.0173	0.9109	-0.4834	0.1139	2.41E-05
11	65636509	rs659824	G	A	0.5227	1.0042	-0.1193	0.0281	2.44E-05
18	47398040	rs79374597	T	C	0.0622	0.9527	0.2536	0.0598	2.47E-05
18	47390805	rs4939898	T	C	0.0622	0.9525	0.2536	0.0598	2.47E-05
10	44307607	rs7906426	G	A	0.0932	1.0022	-0.206	0.0486	2.48E-05
18	47393044	rs4939596	A	G	0.0622	0.9526	0.2536	0.0598	2.48E-05
8	1283042	rs73170465	T	C	0.0167	1.0189	-0.4611	0.1088	2.49E-05
10	104249839	rs1977382	T	A	0.2619	0.9485	0.1396	0.0329	2.49E-05
2	57931576	rs187470710	T	C	0.0173	0.901	0.4834	0.1142	2.52E-05
2	57931576	rs187470710	T	C	0.0173	0.901	0.4834	0.1142	2.52E-05
22	36641792	rs62233823	A	G	0.2092	0.9635	0.1496	0.0353	2.53E-05
17	71153671	rs9904568	A	G	0.1964	0.9822	-0.1514	0.0358	2.54E-05
1	59533996	rs4128414	A	C	0.2253	0.9826	-0.1446	0.0341	2.54E-05
1	59533777	rs4128413	G	A	0.2253	0.9824	-0.1445	0.0341	2.55E-05

Appendix Table 6 Continued.

1	59533336	rs12402674	T	A	0.2253	0.9821	-0.1445	0.0342	2.56E-05
20	31870090	rs4911311	C	G	0.2248	0.9829	0.1445	0.0341	2.56E-05
15	70817246	rs8034299	G	T	0.2068	1.0362	0.1445	0.0341	2.56E-05
15	70817223	rs8033107	T	C	0.2068	1.0363	0.1445	0.0341	2.57E-05
1	59533223	rs17118659	A	G	0.2253	0.982	-0.1445	0.0342	2.57E-05
18	47395770	rs78814171	T	C	0.0627	0.9519	0.2521	0.0596	2.58E-05
2	58556174	rs186863356	A	C	0.0229	0.8884	0.4243	0.1003	2.58E-05
2	58556174	rs186863356	A	C	0.0229	0.8884	0.4243	0.1003	2.58E-05
22	36642018	rs34235585	A	T	0.2092	0.9634	0.1494	0.0353	2.59E-05
1	59532637	rs6689928	C	A	0.2253	0.9814	-0.1445	0.0342	2.60E-05
11	65638719	rs633800	A	G	0.5235	1.0006	-0.1191	0.0282	2.60E-05
10	44307693	rs7917583	G	A	0.0927	1.0017	-0.2061	0.0487	2.61E-05
10	44307687	rs7900485	T	G	0.0927	1.0017	-0.206	0.0487	2.61E-05
1	59531700	rs12016654	A	G	0.2254	0.981	-0.1444	0.0342	2.62E-05
4	10137711	rs16893993	A	C	0.0698	0.9905	0.2354	0.0557	2.63E-05
1	212138895	rs116415521	A	C	0.0408	0.782	-0.3414	0.0808	2.63E-05
8	1277213	rs73170459	G	A	0.0236	1.017	-0.3892	0.0921	2.64E-05
7	44221942	rs117544459	A	C	0.0166	0.8783	0.4998	0.1184	2.66E-05
10	44307717	rs7920802	A	T	0.0927	1.0018	-0.2058	0.0487	2.67E-05
15	70817307	rs8033277	T	C	0.2062	1.0309	0.1447	0.0343	2.68E-05
3	30631458	rs80138614	T	G	0.0628	0.8581	-0.2654	0.0629	2.71E-05
5	142438134	rs2190778	A	T	0.0228	1.0247	0.3937	0.0936	2.84E-05
17	71153536	rs62071603	A	G	0.1958	0.9777	-0.151	0.0359	2.84E-05
16	65840560	rs72786512	T	C	0.0577	0.9803	0.2588	0.0616	2.88E-05
5	142437071	rs2270067	G	A	0.0228	1.0256	0.393	0.0935	2.91E-05
9	135187714	rs142304305	T	C	0.017	0.7625	0.5264	0.1253	2.92E-05
16	65797622	rs9938100	T	C	0.0912	0.9844	0.2076	0.0494	2.92E-05
7	157930979	rs3752367	G	C	0.3237	0.9758	0.1279	0.0304	2.94E-05
12	83670415	rs187048210	A	C	0.0111	0.8438	0.6124	0.1459	2.96E-05
7	87071530	rs45539339	G	T	0.072	0.9872	0.2321	0.0553	2.98E-05

Appendix Table 6 Continued.

7	87071639	rs45579433	A	G	0.072	0.9872	0.2321	0.0553	2.98E-05
12	114298643	rs12231525	T	A	0.1693	0.9518	0.1621	0.0386	2.98E-05
7	157931119	rs1130500	G	A	0.3235	0.9781	0.1276	0.0304	2.98E-05
7	157931144	rs1130499	T	C	0.3235	0.9781	0.1276	0.0304	2.98E-05
8	1287425	rs189301764	T	G	0.0238	0.9864	-0.3914	0.0933	2.98E-05
7	87072226	rs17149601	T	C	0.072	0.9873	0.2321	0.0553	2.99E-05
7	87072505	rs17149606	A	C	0.072	0.9873	0.232	0.0553	2.99E-05
7	87072140	rs45503798	C	T	0.072	0.9873	0.232	0.0553	2.99E-05
12	85520152	rs73182415	T	A	0.0131	0.8348	0.5728	0.1365	3.00E-05
19	50818252	rs115192757	C	G	0.0849	0.9513	-0.2192	0.0523	3.01E-05
7	157894119	rs4909223	G	A	0.3278	0.9604	0.1282	0.0306	3.03E-05
22	36643428	rs71314972	C	G	0.2096	0.9621	0.1481	0.0353	3.04E-05
11	99038365	rs10892836	G	A	0.4914	0.9256	-0.1233	0.0294	3.04E-05
4	10145910	rs34448220	G	A	0.0705	0.9863	0.233	0.0556	3.06E-05
16	65795849	rs72784560	T	C	0.0918	0.9806	0.2067	0.0494	3.11E-05
10	104499232	rs12762176	T	A	0.3185	0.9569	0.1294	0.0309	3.14E-05
2	46179662	rs12471357	C	T	0.595	1.0231	-0.1187	0.0284	3.17E-05
2	46179662	rs12471357	C	T	0.595	1.0231	-0.1187	0.0284	3.17E-05
18	12487778	rs79993352	C	T	0.0105	0.8713	0.6169	0.1476	3.20E-05
16	63154176	rs1625555	C	T	0.7131	0.9667	0.1342	0.0321	3.21E-05
3	60011473	rs183872205	G	A	0.0525	0.9799	0.2674	0.064	3.22E-05
7	157931928	rs11763130	A	G	0.581	1.0035	-0.1188	0.0284	3.24E-05
8	80585763	rs143460322	A	C	0.0124	0.8563	-0.5765	0.138	3.26E-05
6	25466324	rs144503707	T	C	0.0123	0.8478	0.5802	0.1389	3.26E-05
4	161872848	rs10011575	T	C	0.3483	0.9845	0.1246	0.0299	3.28E-05
18	47372315	rs58349995	A	G	0.0627	0.9517	0.2488	0.0596	3.28E-05
14	34634563	rs10144484	A	C	0.4679	0.9545	-0.1206	0.0289	3.29E-05
18	47367536	rs2276169	G	C	0.0627	0.9519	0.2488	0.0596	3.29E-05
5	16632304	rs77937257	C	A	0.0756	0.8549	0.2404	0.0576	3.29E-05
18	47359787	rs870590	A	G	0.0627	0.952	0.2487	0.0596	3.29E-05

Appendix Table 6 Continued.

18	47359444	rs16951101	G	A	0.0627	0.9519	0.2487	0.0596	3.30E-05
13	93516511	rs9301840	C	G	0.2963	0.9829	0.1299	0.0312	3.35E-05
18	47311513	rs17657133	G	A	0.0573	0.9537	0.2595	0.0623	3.37E-05
1	212102560	rs115291660	G	A	0.0499	0.8249	-0.2985	0.0716	3.39E-05
4	10155484	rs9991911	G	A	0.9273	0.984	-0.2287	0.0549	3.40E-05
2	102728004	rs1861284	T	C	0.3275	0.9642	-0.1289	0.031	3.44E-05
2	102728004	rs1861284	T	C	0.3275	0.9642	-0.1289	0.031	3.44E-05
13	29328193	rs7331284	A	T	0.4617	1.0144	-0.1169	0.0281	3.47E-05
1	59535812	rs7530655	A	G	0.1477	0.9925	0.166	0.0399	3.49E-05
11	61961938	rs7926905	A	G	0.02	0.9568	0.4274	0.1027	3.50E-05
22	36643986	rs35150149	T	A	0.21	0.9612	0.1468	0.0353	3.59E-05
7	87061534	rs6977739	T	G	0.0731	0.9838	0.2283	0.055	3.60E-05
7	87056011	rs1017054	C	T	0.073	0.9848	0.2283	0.055	3.60E-05
11	14251440	rs61883840	A	G	0.2056	0.9898	-0.1465	0.0353	3.62E-05
16	65794420	rs58827016	A	C	0.092	0.9785	0.2051	0.0494	3.62E-05
5	159364062	rs78695043	C	A	0.0415	0.9764	0.2974	0.0716	3.62E-05
13	45263685	rs7331853	C	A	0.3794	1.0189	-0.1201	0.0289	3.62E-05
4	10194377	rs76850735	C	T	0.0252	0.9464	0.3847	0.0927	3.63E-05
5	159363996	rs76342362	C	A	0.0415	0.9764	0.2974	0.0716	3.63E-05
9	135209595	rs137935435	T	G	0.0171	0.7709	0.5155	0.1242	3.65E-05
5	159362973	rs80043756	C	T	0.0415	0.9768	0.2972	0.0716	3.67E-05
9	135207272	rs149838860	G	A	0.0171	0.7695	0.5161	0.1244	3.69E-05
3	151438811	rs75124309	A	C	0.0361	0.9938	0.3169	0.0764	3.70E-05
5	159362184	rs76615609	C	T	0.0415	0.977	0.297	0.0716	3.71E-05
3	150670866	rs60340247	T	C	0.0137	0.9143	0.5444	0.1313	3.71E-05
5	159361847	rs114842646	A	G	0.0415	0.9769	0.2969	0.0716	3.73E-05
12	114396307	rs73401074	C	T	0.1224	1.0136	0.1776	0.0429	3.74E-05
2	102722402	rs13388182	T	C	0.3209	0.9856	-0.1277	0.0308	3.79E-05
2	102722402	rs13388182	T	C	0.3209	0.9856	-0.1277	0.0308	3.79E-05
17	3600433	rs117973460	T	C	0.1625	0.8853	0.168	0.0406	3.79E-05

Appendix Table 6 Continued.

22	36644151	rs35345680	A	G	0.2103	0.9594	0.1464	0.0353	3.79E-05
17	71127132	rs8075806	C	A	0.1809	0.9695	-0.1539	0.0372	3.80E-05
9	23714264	rs113488840	G	A	0.015	1.0019	0.4798	0.1159	3.83E-05
18	58821028	rs117766488	A	G	0.0205	0.8271	0.4524	0.1093	3.84E-05
5	142432311	rs62374604	A	C	0.0226	1.0196	0.3897	0.0942	3.86E-05
8	1275473	rs73170458	G	C	0.0237	1.0336	-0.3775	0.0913	3.87E-05
22	36644251	rs35769663	A	G	0.2108	0.9595	0.1461	0.0353	3.87E-05
13	93652913	rs308231	T	C	0.8849	0.9413	-0.1886	0.0456	3.90E-05
10	8647358	rs72777027	T	C	0.0247	1.0013	0.3754	0.0908	3.92E-05
8	104423011	rs118081385	A	G	0.0146	0.9491	0.5024	0.1216	3.92E-05
2	102712236	rs56193008	A	G	0.3183	0.9757	-0.1283	0.031	3.93E-05
2	102712236	rs56193008	A	G	0.3183	0.9757	-0.1283	0.031	3.93E-05
5	116483701	rs35928434	T	C	0.0179	0.8694	-0.4707	0.1139	3.93E-05
13	93653818	rs308236	T	C	0.885	0.9419	-0.1884	0.0456	3.94E-05
7	87056176	rs2230028	C	T	0.0733	0.9864	0.2267	0.0549	3.94E-05
13	93654114	rs308237	T	C	0.885	0.9422	-0.1884	0.0456	3.97E-05
4	10090931	rs3775931	T	C	0.0823	0.9785	0.2146	0.052	3.97E-05
13	93654272	rs170103	G	A	0.8851	0.9425	-0.1883	0.0456	3.98E-05
13	93654351	rs308238	C	A	0.8851	0.9427	-0.1883	0.0456	3.98E-05
6	45820214	rs142310373	G	A	0.0289	0.9226	0.362	0.0877	3.99E-05
13	29326727	rs9508131	A	G	0.462	1.015	-0.1159	0.0281	4.00E-05
2	102711009	rs933494	A	G	0.318	0.979	-0.1279	0.031	4.03E-05
2	102711009	rs933494	A	G	0.318	0.979	-0.1279	0.031	4.03E-05
18	47335627	rs138571974	T	C	0.0643	0.9404	0.2443	0.0592	4.04E-05
10	104296419	rs78657278	G	T	0.11	0.9315	0.1929	0.0467	4.04E-05
10	104296418	rs79159857	G	C	0.11	0.9316	0.1929	0.0467	4.04E-05
13	93655194	rs308239	T	C	0.8852	0.9436	-0.1881	0.0456	4.04E-05
13	29326036	rs12184861	T	G	0.4621	1.0154	-0.1158	0.0281	4.04E-05
5	142431653	rs7727861	G	A	0.0226	1.0189	0.389	0.0943	4.05E-05
13	29325932	rs12184835	T	C	0.462	1.0157	-0.1158	0.0281	4.06E-05

Appendix Table 6 Continued.

13	29325914	rs12184834	T	C	0.4621	1.0158	-0.1158	0.0281	4.06E-05
13	93655620	rs308240	G	T	0.8852	0.944	-0.1881	0.0456	4.06E-05
13	93655647	rs308241	T	C	0.8852	0.9442	-0.188	0.0456	4.08E-05
13	93655675	rs308242	A	G	0.8852	0.9443	-0.188	0.0456	4.08E-05
13	93655803	rs308243	G	A	0.8853	0.9445	-0.188	0.0456	4.09E-05
13	22713611	rs4769203	T	C	0.8052	0.8349	0.1614	0.0392	4.09E-05
13	93655843	rs308244	A	C	0.8853	0.9446	-0.188	0.0456	4.10E-05
13	93656075	rs308246	T	C	0.8853	0.9453	-0.1879	0.0456	4.12E-05
17	71154910	rs2044718	A	C	0.2019	0.9764	-0.1462	0.0355	4.14E-05
8	104462801	rs117575603	C	T	0.0147	0.9253	0.5041	0.1223	4.14E-05
13	93656534	rs308247	C	T	0.8854	0.9462	-0.1877	0.0456	4.18E-05
13	93656633	rs457190	C	T	0.8854	0.9465	-0.1876	0.0456	4.20E-05
13	93656800	rs308248	T	C	0.8855	0.947	-0.1875	0.0456	4.22E-05
2	58086587	rs78703671	C	A	0.0177	0.9039	0.4636	0.1127	4.23E-05
2	58086587	rs78703671	C	A	0.0177	0.9039	0.4636	0.1127	4.23E-05
13	93657064	rs167390	A	T	0.8855	0.9472	-0.1875	0.0456	4.24E-05
13	29324426	rs9579229	T	A	0.4624	1.0177	-0.1154	0.028	4.25E-05
13	29324203	rs9579228	G	A	0.4624	1.018	-0.1153	0.028	4.27E-05
17	37196446	rs55646995	T	C	0.0288	1.0263	0.3432	0.0834	4.28E-05
13	93657160	rs160147	A	T	0.8855	0.9475	-0.1874	0.0456	4.28E-05
13	93657855	rs401043	C	G	0.8856	0.9481	-0.1873	0.0456	4.29E-05
13	93657860	rs400535	A	C	0.8856	0.9482	-0.1873	0.0456	4.29E-05
12	48775764	rs10783249	C	T	0.2633	0.9825	0.1321	0.0321	4.30E-05
13	93658331	rs160135	T	G	0.8856	0.9492	-0.1872	0.0455	4.33E-05
2	18435253	rs140060408	G	T	0.0172	1.0478	-0.4355	0.106	4.33E-05
2	18435253	rs140060408	G	T	0.0172	1.0478	-0.4355	0.106	4.33E-05
13	93658420	rs160136	G	A	0.8856	0.9493	-0.1872	0.0455	4.33E-05
13	93658505	rs160137	C	T	0.8857	0.9497	-0.1871	0.0455	4.35E-05
2	18459672	rs141660103	C	T	0.0172	1.0473	-0.4355	0.106	4.35E-05
2	18459672	rs141660103	C	T	0.0172	1.0473	-0.4355	0.106	4.35E-05

Appendix Table 6 Continued.

2	18435641	rs12467096	G	C	0.0172	1.0475	-0.4355	0.106	4.35E-05
2	18435641	rs12467096	G	C	0.0172	1.0475	-0.4355	0.106	4.35E-05
2	18459610	rs114366824	A	C	0.0172	1.0476	-0.4355	0.106	4.35E-05
2	18459610	rs114366824	A	C	0.0172	1.0476	-0.4355	0.106	4.35E-05
2	102713621	rs72817889	T	C	0.3181	0.9755	-0.1276	0.0311	4.36E-05
2	102713621	rs72817889	T	C	0.3181	0.9755	-0.1276	0.0311	4.36E-05
20	44474810	rs197664	T	C	0.0272	0.9435	0.3659	0.0891	4.38E-05
2	18442302	rs146749043	G	A	0.0172	1.0466	-0.4355	0.106	4.38E-05
2	18442302	rs146749043	G	A	0.0172	1.0466	-0.4355	0.106	4.38E-05
13	93658638	rs160138	T	C	0.8857	0.95	-0.187	0.0455	4.38E-05
13	29319116	rs7338057	T	C	0.4543	1.0193	-0.1152	0.0281	4.40E-05
5	11381876	rs188888729	G	A	0.0122	0.9248	0.5497	0.1339	4.40E-05
2	18439607	rs115539892	A	C	0.0172	1.0471	-0.4352	0.106	4.40E-05
2	18439607	rs115539892	A	C	0.0172	1.0471	-0.4352	0.106	4.40E-05
11	14251553	rs4757243	A	G	0.2054	0.9853	-0.1453	0.0354	4.40E-05
13	29319711	rs7339280	A	C	0.4543	1.0196	-0.1151	0.028	4.41E-05
8	22820753	rs113286888	C	G	0.0162	0.8492	0.5018	0.1223	4.46E-05
2	2066677	rs756282	G	T	0.3884	0.9523	0.1221	0.0298	4.49E-05
2	2066677	rs756282	G	T	0.3884	0.9523	0.1221	0.0298	4.49E-05
14	73466566	rs10138746	C	G	0.0302	1.0372	0.3336	0.0814	4.51E-05
7	157927316	rs6943714	G	A	0.326	0.9757	0.1247	0.0304	4.51E-05
2	177005519	rs79120932	T	G	0.0128	0.9857	0.5219	0.1273	4.51E-05
2	177005519	rs79120932	T	G	0.0128	0.9857	0.5219	0.1273	4.51E-05
19	50821781	rs28479615	C	T	0.0856	0.9334	-0.2152	0.0525	4.52E-05
7	87052479	rs6977539	C	T	0.0694	0.9942	0.2296	0.056	4.52E-05
7	87052605	rs6957680	G	A	0.0694	0.9942	0.2296	0.056	4.52E-05
13	29323164	rs1928502	T	C	0.4626	1.0197	-0.1148	0.028	4.52E-05
2	102714540	rs11685997	T	C	0.3178	0.9766	-0.1273	0.0311	4.54E-05
2	102714540	rs11685997	T	C	0.3178	0.9766	-0.1273	0.0311	4.54E-05
5	10109385	rs906071	C	T	0.2794	0.9657	0.1313	0.032	4.55E-05

Appendix Table 6 Continued.

20	31861671	rs6141908	G	C	0.2224	0.9811	0.1406	0.0343	4.55E-05
16	9677797	rs12444198	T	A	0.1454	0.9927	0.1645	0.0401	4.58E-05
13	93660312	rs172590	A	G	0.8859	0.9542	-0.1863	0.0455	4.60E-05
21	39223881	rs12483485	T	C	0.033	1.0158	0.321	0.0784	4.60E-05
7	87053611	rs45609336	C	G	0.0694	0.9933	0.2293	0.056	4.61E-05
4	112335436	rs114307289	A	C	0.0108	0.9627	-0.5698	0.1392	4.62E-05
13	29316654	rs9314917	G	A	0.4549	1.018	-0.1149	0.0281	4.64E-05
13	29319840	rs1928501	T	C	0.4627	1.02	-0.1146	0.028	4.70E-05
13	29319871	rs7139542	T	C	0.4627	1.0201	-0.1145	0.028	4.70E-05
13	29320287	rs7324361	A	G	0.4626	1.0201	-0.1145	0.028	4.72E-05
1	59530164	rs12016651	A	G	0.2319	0.9833	-0.1383	0.0338	4.73E-05
13	93652425	rs308230	G	A	0.8792	0.9243	-0.1843	0.0451	4.73E-05
13	29320286	rs7325403	C	T	0.4626	1.02	-0.1145	0.028	4.73E-05
20	31874257	rs3827028	G	A	0.2694	0.9314	0.1346	0.0329	4.74E-05
7	157928817	rs7799902	A	G	0.3194	0.9706	0.1252	0.0306	4.78E-05
13	29320084	rs7323963	C	G	0.4627	1.0207	-0.1144	0.028	4.78E-05
13	29314645	rs9508125	A	G	0.5381	1.014	0.1147	0.0281	4.78E-05
13	29320342	rs7324041	G	A	0.4628	1.0213	-0.1143	0.028	4.80E-05
13	93652387	rs308229	C	A	0.8792	0.924	-0.1842	0.0451	4.81E-05
13	93662149	rs160140	G	A	0.8862	0.9581	-0.1855	0.0454	4.81E-05
13	29320935	rs1539056	G	A	0.4628	1.0223	-0.1143	0.028	4.81E-05
13	29320883	rs1539057	A	G	0.4628	1.0223	-0.1143	0.028	4.82E-05
10	107638257	rs17236454	G	T	0.021	0.8569	0.437	0.107	4.82E-05
11	65635559	rs594689	A	G	0.5198	0.9932	-0.1156	0.0283	4.84E-05
13	93662235	rs160141	C	T	0.8862	0.9585	-0.1854	0.0454	4.84E-05
18	47318473	rs17713584	C	G	0.0577	0.9526	0.2536	0.0621	4.84E-05
2	64364632	rs6733160	C	T	0.5338	0.9962	-0.1159	0.0284	4.85E-05
2	64364632	rs6733160	C	T	0.5338	0.9962	-0.1159	0.0284	4.85E-05
8	22636936	rs112910828	A	T	0.0155	0.8965	0.4984	0.1221	4.85E-05
19	40038334	rs143814327	A	G	0.0142	0.9582	0.4987	0.1222	4.86E-05

Appendix Table 6 Continued.

20	31862280 rs1964852	T	C	0.2752	0.9472	0.1325	0.0325	4.86E-05
2	64367694 rs6730262	T	C	0.5338	0.9963	-0.1159	0.0284	4.88E-05
2	64367694 rs6730262	T	C	0.5338	0.9963	-0.1159	0.0284	4.88E-05
3	43808049 rs190927863	C	G	0.0104	1.0546	0.5547	0.1359	4.89E-05
13	93662748 rs466561	T	G	0.8862	0.9596	-0.1852	0.0454	4.91E-05
13	29314452 rs9508124	G	A	0.5384	1.0159	0.1145	0.0281	4.91E-05
13	93662845 rs462910	C	T	0.8862	0.9597	-0.1852	0.0454	4.92E-05
13	93663635 rs464563	A	T	0.8862	0.9597	-0.1852	0.0454	4.92E-05
13	93663970 rs460846	C	T	0.8862	0.9597	-0.1852	0.0454	4.92E-05
13	93664425 rs445462	T	C	0.8862	0.9597	-0.1852	0.0454	4.92E-05
13	93664775 rs452880	G	C	0.8862	0.9597	-0.1852	0.0454	4.92E-05
13	93664954 rs399314	G	A	0.8862	0.9597	-0.1852	0.0454	4.92E-05
2	8030276 rs17838382	C	T	0.0164	0.9597	0.4643	0.1138	4.92E-05
2	8030276 rs17838382	C	T	0.0164	0.9597	0.4643	0.1138	4.92E-05
13	93663210 rs463719	G	A	0.8862	0.9596	-0.1852	0.0454	4.92E-05
2	2031036 rs1213579	C	T	0.3539	0.9506	0.1239	0.0304	4.92E-05
2	2031036 rs1213579	C	T	0.3539	0.9506	0.1239	0.0304	4.92E-05
5	159308481 rs116749599	T	C	0.0159	0.9497	0.4722	0.1158	4.93E-05
2	2007981 rs68087472	A	G	0.3665	0.9505	0.1231	0.0302	4.93E-05
2	2007981 rs68087472	A	G	0.3665	0.9505	0.1231	0.0302	4.93E-05
13	93665488 rs374894	A	T	0.8862	0.9597	-0.1851	0.0454	4.96E-05
13	93665712 rs405994	C	T	0.8862	0.9596	-0.1851	0.0454	4.98E-05
13	93665835 rs424228	T	C	0.8862	0.9596	-0.185	0.0454	4.99E-05
2	102725013 rs17767183	T	C	0.3179	0.9759	-0.1267	0.0311	5.00E-05
2	102725013 rs17767183	T	C	0.3179	0.9759	-0.1267	0.0311	5.00E-05
13	29316836 rs9314918	A	G	0.4548	1.0187	-0.1143	0.028	5.00E-05
13	93665860 rs374048	T	C	0.8862	0.9596	-0.185	0.0454	5.00E-05
13	93665924 rs447741	G	C	0.8862	0.9596	-0.185	0.0454	5.01E-05
13	29315831 rs9506037	T	C	0.4552	1.0223	-0.1141	0.028	5.02E-05
13	93665955 rs451715	T	G	0.8862	0.9596	-0.185	0.0454	5.02E-05

Appendix Table 6 Continued.

16	68255037	rs145240988	G	T	0.0123	0.9234	0.5449	0.1337	5.02E-05
12	114396345	rs73401076	C	A	0.1198	1.021	0.1758	0.0432	5.03E-05
5	167458946	rs13158058	T	G	0.4063	0.9251	-0.1229	0.0302	5.06E-05
2	102718225	rs17818195	C	T	0.3179	0.9737	-0.1267	0.0311	5.06E-05
2	102718225	rs17818195	C	T	0.3179	0.9737	-0.1267	0.0311	5.06E-05
12	114388301	rs3816579	G	A	0.1199	1.0216	0.1757	0.0431	5.08E-05
12	114389242	rs10850253	C	T	0.1199	1.0216	0.1757	0.0431	5.08E-05
13	93666328	rs462792	T	C	0.8863	0.9595	-0.1849	0.0454	5.09E-05
13	29321378	rs9508128	G	T	0.4626	1.0204	-0.114	0.028	5.12E-05
12	106335822	rs75404309	T	A	0.0459	0.8534	0.2983	0.0733	5.12E-05
13	93666857	rs467354	G	A	0.8863	0.9595	-0.1848	0.0454	5.13E-05
1	192355216	rs10159276	C	G	0.2819	0.9868	-0.1292	0.0318	5.14E-05
13	29316991	rs9314919	C	G	0.4544	1.0188	-0.1141	0.0281	5.18E-05
13	29316941	rs9506039	C	T	0.4544	1.0187	-0.1141	0.0281	5.18E-05
2	177008484	rs79440139	A	T	0.0134	0.9784	0.5063	0.1245	5.20E-05
2	177008484	rs79440139	A	T	0.0134	0.9784	0.5063	0.1245	5.20E-05
2	84184053	rs116366566	T	C	0.0123	0.9375	0.538	0.1323	5.20E-05
2	84184053	rs116366566	T	C	0.0123	0.9375	0.538	0.1323	5.20E-05
9	26698957	rs142256843	T	C	0.012	0.9436	0.5438	0.1337	5.21E-05
9	76909652	rs11143897	G	T	0.1538	1.043	-0.1565	0.0385	5.21E-05
2	64369336	rs10496107	C	A	0.5333	0.9988	-0.1152	0.0283	5.22E-05
2	64369336	rs10496107	C	A	0.5333	0.9988	-0.1152	0.0283	5.22E-05
14	73462076	rs4903085	G	A	0.0304	1.0324	0.3306	0.0813	5.22E-05
12	106233178	rs10861522	C	T	0.3458	1.0646	0.1172	0.0288	5.23E-05
13	113207608	rs76588804	A	C	0.0164	0.8344	0.4921	0.1211	5.23E-05
3	131415723	rs75119553	C	T	0.0473	0.9271	0.28	0.0689	5.24E-05
20	31874869	rs6119362	A	C	0.2742	0.9369	0.1326	0.0326	5.28E-05
21	39223696	rs12483570	C	T	0.0332	1.0203	0.3167	0.078	5.32E-05
2	102719104	rs72996520	T	C	0.3175	0.9756	-0.1263	0.0311	5.32E-05
2	102719104	rs72996520	T	C	0.3175	0.9756	-0.1263	0.0311	5.32E-05

Appendix Table 6 Continued.

8	22818529	rs2872346	C	A	0.2849	0.9604	-0.1297	0.0319	5.32E-05
13	93668983	rs191044	T	A	0.8863	0.9593	-0.1844	0.0454	5.34E-05
13	93669289	rs160150	T	C	0.8863	0.9593	-0.1844	0.0454	5.36E-05
20	31867032	rs6059230	T	C	0.2753	0.9455	0.1319	0.0325	5.36E-05
5	16858282	rs2625206	C	T	0.3119	0.9526	-0.1269	0.0313	5.36E-05
3	116193370	rs143848409	T	C	0.0171	1.0078	0.4404	0.1085	5.39E-05
20	31867840	rs941682	G	A	0.2756	0.9465	0.1317	0.0325	5.40E-05
18	3114419	rs11081010	A	G	0.8943	0.9678	0.1898	0.0468	5.40E-05
2	102721117	rs1019296	C	T	0.3177	0.9759	-0.1261	0.0311	5.43E-05
2	102721117	rs1019296	C	T	0.3177	0.9759	-0.1261	0.0311	5.43E-05
14	81561209	rs117631391	G	A	0.024	0.849	-0.4065	0.1002	5.43E-05
4	10082515	rs737678	T	C	0.0684	0.9774	0.2302	0.0568	5.45E-05
1	192351359	rs7549244	A	T	0.2817	0.9726	-0.1297	0.032	5.49E-05
17	71132748	rs62071582	C	A	0.1836	1.021	-0.1461	0.0361	5.52E-05
15	32088895	rs117740173	T	C	0.0117	0.8738	0.5677	0.1401	5.52E-05
5	55142537	rs10737957	A	C	0.7023	0.9255	0.131	0.0323	5.55E-05
16	65830216	rs1118653	C	G	0.1057	0.9816	0.1879	0.0464	5.55E-05
17	71153739	rs9906459	T	C	0.1941	0.9743	-0.1462	0.0361	5.58E-05
20	31859395	rs1884883	C	T	0.2232	0.9798	0.1388	0.0343	5.60E-05
9	113931440	rs183439396	A	G	0.0107	0.7941	0.6248	0.1543	5.61E-05
3	153366389	rs116429691	T	C	0.0161	0.8771	0.4882	0.1206	5.61E-05
22	42949748	rs3213548	G	C	0.2455	0.8275	-0.1458	0.036	5.61E-05
2	102717337	rs10490571	T	C	0.318	0.979	-0.1256	0.031	5.62E-05
2	102717337	rs10490571	T	C	0.318	0.979	-0.1256	0.031	5.62E-05
20	31854547	rs6141379	T	C	0.2231	0.9793	0.1389	0.0343	5.63E-05
11	61964425	rs17577608	T	C	0.0208	0.9505	0.41	0.1013	5.64E-05
9	24911447	rs75221401	G	A	0.028	0.8022	-0.3872	0.0957	5.66E-05
17	71133864	rs12602530	T	A	0.1837	1.0245	-0.1456	0.036	5.67E-05
13	93673083	rs308253	C	G	0.8863	0.9591	-0.1838	0.0454	5.69E-05
21	37550509	rs2845753	C	G	0.3277	0.9854	-0.1229	0.0304	5.70E-05

Appendix Table 6 Continued.

14	73468970	rs12323834	A	G	0.0294	1.0474	0.3315	0.082	5.72E-05
2	102728716	rs1861283	T	C	0.3272	0.9706	-0.1249	0.0309	5.72E-05
2	102728716	rs1861283	T	C	0.3272	0.9706	-0.1249	0.0309	5.72E-05
10	35556466	rs79940628	G	A	0.0162	0.8953	0.4775	0.1181	5.77E-05
2	241170677	rs147288234	A	G	0.0182	0.8209	0.4706	0.1165	5.79E-05
2	241170677	rs147288234	A	G	0.0182	0.8209	0.4706	0.1165	5.79E-05
13	93673921	rs184254	G	C	0.8863	0.959	-0.1836	0.0454	5.79E-05
7	87055635	rs11768699	T	G	0.0699	0.9931	0.2255	0.0558	5.80E-05
7	87057699	rs6956661	A	G	0.0699	0.9931	0.2255	0.0558	5.80E-05
17	3556471	rs188118937	T	C	0.0135	0.886	0.5238	0.1297	5.80E-05
12	114396518	rs73401078	T	G	0.1193	1.0222	0.1744	0.0432	5.86E-05
2	223645982	rs74367692	T	A	0.0509	0.7511	-0.2986	0.074	5.86E-05
2	223645982	rs74367692	T	A	0.0509	0.7511	-0.2986	0.074	5.86E-05
1	192351321	rs12143695	C	T	0.2814	0.9707	-0.1294	0.0321	5.87E-05
6	148104634	rs12664057	G	A	0.0802	0.9385	0.2168	0.0537	5.88E-05
2	241170675	rs113694575	C	T	0.0184	0.8167	0.4699	0.1164	5.89E-05
2	241170675	rs113694575	C	T	0.0184	0.8167	0.4699	0.1164	5.89E-05
7	87058570	rs45466200	T	G	0.0699	0.9925	0.2254	0.0558	5.90E-05
6	166331331	rs12528241	A	G	0.0601	0.9896	0.2429	0.0602	5.99E-05
7	157888228	rs9654673	C	G	0.3241	0.9403	0.125	0.031	6.00E-05
7	87059700	rs45474405	A	G	0.0699	0.9919	0.2252	0.0559	6.02E-05
10	104493444	rs11191381	T	C	0.3174	0.9499	0.1253	0.0311	6.05E-05
2	177008161	rs148638470	T	C	0.0127	0.973	0.5175	0.1284	6.06E-05
2	177008161	rs148638470	T	C	0.0127	0.973	0.5175	0.1284	6.06E-05
19	40027464	rs117900938	G	A	0.0221	1.0299	0.3826	0.0949	6.06E-05
2	8022943	rs61235986	A	G	0.0145	0.9902	0.4797	0.1191	6.07E-05
2	8022943	rs61235986	A	G	0.0145	0.9902	0.4797	0.1191	6.07E-05
12	114381499	rs73399070	A	G	0.1043	0.9782	0.1887	0.0468	6.08E-05
2	8023073	rs6721147	A	C	0.0145	0.9899	0.4798	0.1191	6.09E-05
2	8023073	rs6721147	A	C	0.0145	0.9899	0.4798	0.1191	6.09E-05

Appendix Table 6 Continued.

22	26517670	rs139445921	A	G	0.0141	0.8302	0.5295	0.1314	6.09E-05
22	36644522	rs35829971	T	C	0.2103	0.961	0.1423	0.0353	6.12E-05
14	73456238	rs28609654	C	T	0.0306	1.0296	0.3269	0.0812	6.14E-05
10	104266239	rs113451617	A	G	0.2294	0.9408	0.1394	0.0346	6.21E-05
8	84195830	rs143117419	A	T	0.0133	0.8172	0.5477	0.1362	6.24E-05
7	87061753	rs45547639	T	G	0.0699	0.9909	0.2249	0.0559	6.26E-05
7	87061974	rs45473093	G	A	0.0699	0.9909	0.2249	0.0559	6.27E-05
17	71142994	rs7213907	C	T	0.7731	1.0224	0.1339	0.0333	6.27E-05
7	87062112	rs1034821	G	T	0.0699	0.9909	0.2249	0.0559	6.28E-05
1	211732947	rs6663436	T	A	0.8828	0.9624	0.1803	0.0448	6.29E-05
3	172572471	rs1553176	T	C	0.1521	0.9012	0.1661	0.0413	6.34E-05
2	88418770	rs74832257	G	A	0.0143	0.8651	0.5144	0.128	6.37E-05
2	88418770	rs74832257	G	A	0.0143	0.8651	0.5144	0.128	6.37E-05
1	54852036	rs151070537	G	C	0.0109	0.8592	0.5908	0.1471	6.38E-05
16	68316582	rs139936925	A	G	0.0127	0.9253	0.5283	0.1315	6.39E-05
17	71143527	rs10852744	G	T	0.7731	1.0213	0.1338	0.0333	6.40E-05
1	192351266	rs12142904	G	A	0.2818	0.9728	-0.1286	0.032	6.41E-05
7	87049583	rs45540936	G	C	0.0741	0.9746	0.2209	0.055	6.42E-05
17	8290362	rs112443430	T	C	0.0291	0.9514	0.3473	0.0865	6.44E-05
3	8806526	rs115356575	A	G	0.0179	0.8575	0.4599	0.1145	6.44E-05
3	153381359	rs147808876	C	T	0.011	0.8277	0.6012	0.1497	6.44E-05
7	96425603	rs118081765	C	T	0.0147	0.9952	0.4723	0.1177	6.50E-05
13	29316482	rs1792049	G	A	0.4582	1.0102	-0.113	0.0282	6.51E-05
4	10082464	rs4478188	A	C	0.0689	0.9763	0.2273	0.0567	6.51E-05
2	48012027	rs3136239	C	G	0.0285	0.8533	0.369	0.092	6.55E-05
2	48012027	rs3136239	C	G	0.0285	0.8533	0.369	0.092	6.55E-05
5	75233405	rs11958971	G	A	0.4816	0.9723	-0.1155	0.0288	6.63E-05
4	77869872	rs75272931	C	G	0.0372	0.9125	0.3134	0.0782	6.63E-05
1	238772108	rs61829290	T	G	0.0396	0.8882	0.3085	0.077	6.64E-05
1	91249095	rs78851948	T	C	0.0248	0.8743	0.3884	0.0969	6.64E-05

Appendix Table 6 Continued.

2	239463148	rs6726956	A	G	0.321	1.006	-0.122	0.0304	6.65E-05
2	239463148	rs6726956	A	G	0.321	1.006	-0.122	0.0304	6.65E-05
7	138733340	rs11981663	C	T	0.0233	1.0177	0.3744	0.0934	6.68E-05
16	23743126	rs117701014	G	T	0.0203	0.7901	0.4528	0.113	6.69E-05
14	73477629	rs143319887	C	T	0.0285	1.0086	0.3393	0.0847	6.69E-05
11	14252728	rs7951630	G	T	0.7978	0.9792	0.1434	0.0358	6.69E-05
5	75232389	rs1357997	G	A	0.4814	0.9734	-0.1154	0.0288	6.69E-05
5	159713955	rs72812242	C	A	0.1001	0.8351	-0.206	0.0514	6.71E-05
7	138733313	rs11978040	T	C	0.0233	1.0182	0.3742	0.0934	6.73E-05
17	71136248	rs9911292	G	A	0.1835	1.0319	-0.1436	0.0359	6.75E-05
17	71135860	rs55944075	G	T	0.1835	1.032	-0.1436	0.0359	6.75E-05
17	71135791	rs56053845	A	G	0.1835	1.0323	-0.1436	0.0359	6.76E-05
17	71138465	rs55739021	A	T	0.1834	1.0312	-0.1436	0.0359	6.82E-05
17	71138696	rs55832596	A	G	0.1833	1.0313	-0.1436	0.0359	6.87E-05
10	104398895	rs12764219	T	G	0.279	0.9799	0.1278	0.032	6.89E-05
9	135181695	rs118172942	A	G	0.0346	0.8772	0.3337	0.0835	6.92E-05
6	78791379	rs3904384	A	C	0.0426	0.9207	0.2929	0.0733	6.94E-05
13	93661677	rs9301847	C	T	0.1111	0.9629	0.1829	0.0458	6.99E-05
13	29311798	rs12855922	C	T	0.4589	1.0183	-0.112	0.028	7.02E-05
8	81253576	rs148737414	T	C	0.0105	0.8375	-0.6066	0.1519	7.02E-05
2	102747313	rs1024794	G	A	0.2974	0.9689	-0.127	0.0318	7.04E-05
2	102747313	rs1024794	G	A	0.2974	0.9689	-0.127	0.0318	7.04E-05
15	79601742	rs146192528	A	G	0.0136	0.8418	-0.5294	0.1326	7.04E-05
4	10436482	rs7675445	C	T	0.1476	0.9515	0.1627	0.0408	7.10E-05
2	64360433	rs7573532	A	G	0.5401	0.99	-0.1138	0.0285	7.11E-05
2	64360433	rs7573532	A	G	0.5401	0.99	-0.1138	0.0285	7.11E-05
3	72610393	rs114820373	T	C	0.0205	0.8863	0.4229	0.106	7.15E-05
10	104303748	rs67646802	T	C	0.2701	0.953	0.1301	0.0326	7.15E-05
7	87070089	rs11768036	A	G	0.0698	0.9893	0.2234	0.056	7.17E-05
13	23884476	rs2770055	T	C	0.4941	0.9738	-0.1148	0.0288	7.20E-05

Appendix Table 6 Continued.

2	84236178	rs115405840	G	A	0.0126	0.9211	0.5277	0.1324	7.24E-05
2	84236178	rs115405840	G	A	0.0126	0.9211	0.5277	0.1324	7.24E-05
13	29316948	rs1305103	C	A	0.4578	1.011	-0.1123	0.0282	7.26E-05
3	25438248	rs144594145	A	T	0.0156	0.7515	-0.5254	0.1318	7.28E-05
5	75234881	rs4235688	G	C	0.4815	0.9726	-0.1149	0.0288	7.28E-05
7	87070298	rs45533331	A	G	0.0698	0.9893	0.2232	0.056	7.29E-05
3	151517616	rs76034984	T	C	0.0332	0.9643	0.32	0.0803	7.30E-05
13	93663214	rs9516149	C	A	0.111	0.9651	0.1822	0.0457	7.30E-05
19	52493717	rs1241462	A	G	0.9144	0.8707	0.2148	0.0539	7.33E-05
1	116491488	rs114879091	T	C	0.0172	0.7889	-0.4875	0.1224	7.34E-05
1	1069535	rs113355263	A	G	0.0921	0.8583	-0.2096	0.0526	7.36E-05
20	44445880	rs113035198	C	T	0.0271	0.9353	0.3573	0.0897	7.37E-05
10	104532828	rs12776506	G	A	0.3198	0.9363	0.1244	0.0312	7.44E-05
2	162653362	rs1551051	A	T	0.7445	1.0144	-0.1281	0.0322	7.45E-05
2	233490999	rs2091250	T	C	0.6912	0.9878	-0.1234	0.031	7.45E-05
2	162653362	rs1551051	A	T	0.7445	1.0144	-0.1281	0.0322	7.45E-05
2	233490999	rs2091250	T	C	0.6912	0.9878	-0.1234	0.031	7.45E-05
4	10462231	rs183203239	T	G	0.0106	0.896	0.5772	0.145	7.45E-05
1	59515972	rs79349044	C	A	0.2033	0.9206	-0.1461	0.0367	7.48E-05
3	176857157	rs57242082	C	A	0.0235	1.0094	-0.3677	0.0924	7.50E-05
3	176858770	rs1316286	T	G	0.0235	1.0092	-0.3677	0.0924	7.50E-05
12	93406190	rs12231012	G	T	0.0232	1.0162	0.3729	0.0937	7.50E-05
5	75227767	rs4704268	G	A	0.4682	0.9799	-0.1142	0.0287	7.50E-05
7	87070096	rs11767995	T	C	0.0699	0.9888	0.2227	0.056	7.51E-05
4	10073785	rs12509082	A	G	0.081	0.9863	0.2075	0.0522	7.60E-05
11	100564126	rs17095341	A	G	0.0106	0.8416	0.6001	0.151	7.61E-05
13	29310643	rs9578058	T	A	0.4596	1.0168	-0.1115	0.0281	7.61E-05
13	29310467	rs9578057	A	T	0.4596	1.0167	-0.1115	0.0281	7.62E-05
4	17375165	rs34004560	T	C	0.1378	0.92	0.1705	0.0429	7.62E-05
2	2479617	rs116194950	T	A	0.0106	0.83	0.599	0.1507	7.63E-05

Appendix Table 6 Continued.

2	2479617	rs116194950	T	A	0.0106	0.83	0.599	0.1507	7.63E-05
11	65641033	rs10896064	C	G	0.5549	1.0257	-0.1112	0.028	7.64E-05
5	94141845	rs75081050	G	A	0.0135	0.8094	0.5387	0.1356	7.68E-05
8	102479654	rs507852	A	G	0.9689	1.0702	-0.3112	0.0783	7.71E-05
11	25382598	rs529834045	A	G	0.0112	0.8431	-0.5794	0.1459	7.72E-05
14	73470524	rs10134432	A	G	0.0316	1.0396	0.3157	0.0795	7.72E-05
4	10106798	rs12509609	T	C	0.07	0.9743	0.2229	0.0561	7.77E-05
17	71148027	rs8064740	T	C	0.1826	1.0348	-0.1425	0.0359	7.78E-05
5	11365825	rs61750663	C	A	0.0125	0.9677	0.5148	0.1297	7.78E-05
5	11364595	rs61750672	T	G	0.0125	0.9675	0.5148	0.1297	7.78E-05
9	102291854	rs10819685	C	G	0.1052	0.875	0.1957	0.0493	7.79E-05
16	65806627	rs11648644	C	T	0.1053	0.9842	0.1842	0.0464	7.79E-05
5	11363004	rs61750682	A	G	0.0125	0.9676	0.5147	0.1297	7.80E-05
2	8025686	rs11693863	T	C	0.0164	0.9621	0.4511	0.1137	7.81E-05
2	8025686	rs11693863	T	C	0.0164	0.9621	0.4511	0.1137	7.81E-05
17	71148059	rs9893457	T	C	0.1825	1.0343	-0.1425	0.0359	7.83E-05
5	11362061	rs61750690	T	C	0.0125	0.9682	0.5143	0.1296	7.83E-05
5	11369794	rs79976016	A	T	0.0125	0.9664	0.5145	0.1297	7.87E-05
14	73472776	rs4903088	T	C	0.0329	1.0296	0.3109	0.0784	7.88E-05
7	138731343	rs3187141	A	G	0.0233	1.0249	0.3697	0.0932	7.90E-05
17	71147968	rs8082088	C	G	0.1826	1.0355	-0.1423	0.0359	7.90E-05
5	79548131	rs147804141	A	G	0.0359	0.8866	0.3231	0.0815	7.90E-05
17	71147889	rs8064580	T	C	0.1826	1.0355	-0.1423	0.0359	7.92E-05
17	71147734	rs8069963	T	C	0.1826	1.0365	-0.1422	0.0359	7.92E-05
16	68027854	rs142421824	G	C	0.013	0.9362	0.5121	0.1291	7.93E-05
4	99138280	rs116515688	T	G	0.0155	0.7876	0.512	0.1292	7.94E-05
22	36646839	rs5756114	T	C	0.2109	0.9615	0.1399	0.0353	7.95E-05
14	73475755	rs4903091	T	C	0.0329	1.0302	0.3107	0.0784	7.96E-05
10	104528509	rs11191394	C	A	0.3199	0.9358	0.1239	0.0313	7.96E-05
17	71147862	rs8069173	C	T	0.1826	1.0369	-0.1421	0.0359	7.98E-05

Appendix Table 6 Continued.

10	104523634	rs12775376	A	G	0.3199	0.937	0.1238	0.0312	7.99E-05
12	106306914	rs74969942	G	A	0.0157	0.8155	0.4962	0.1252	8.01E-05
15	92444871	rs72750075	C	T	0.086	0.986	-0.201	0.0507	8.01E-05
14	73466463	rs10138468	T	C	0.0318	1.0318	0.3152	0.0795	8.02E-05
3	71990143	rs138114136	G	A	0.0182	0.9036	0.4391	0.1108	8.02E-05
10	104513049	rs11191385	T	G	0.3188	0.9447	0.1234	0.0311	8.04E-05
17	71147708	rs8070513	G	A	0.1826	1.0365	-0.1421	0.0359	8.04E-05
8	20146260	rs7012277	T	C	0.0692	0.9482	0.2281	0.0576	8.05E-05
2	125330422	rs1542918	T	A	0.9805	0.9659	-0.4122	0.1041	8.07E-05
2	125330422	rs1542918	T	A	0.9805	0.9659	-0.4122	0.1041	8.07E-05
4	10277869	rs11736814	C	T	0.0265	0.9538	0.3568	0.0901	8.12E-05
10	104288777	rs1536308	G	T	0.3232	0.9843	-0.1202	0.0304	8.13E-05
20	62120948	rs1757785	T	C	0.5234	0.9617	0.1148	0.029	8.23E-05
21	37547518	rs11702069	C	T	0.4707	1.0111	-0.1114	0.0281	8.24E-05
16	15499997	rs12927590	G	A	0.0799	0.7502	0.2378	0.0601	8.25E-05
4	10097446	rs3796820	A	G	0.0692	0.9593	0.2249	0.0569	8.26E-05
13	93672943	rs308254	A	T	0.8857	0.9553	-0.1797	0.0454	8.27E-05
5	16852734	rs7719940	C	T	0.2842	0.9447	-0.1275	0.0322	8.30E-05
21	37553209	rs7275804	C	T	0.3302	0.9877	-0.1198	0.0303	8.30E-05
21	37553248	rs2835299	T	C	0.3304	0.9895	-0.1197	0.0303	8.32E-05
16	9684775	rs12921634	G	A	0.1439	1.0025	0.1587	0.0401	8.32E-05
12	114281011	rs3782427	A	G	0.1657	0.9491	0.1545	0.0391	8.35E-05
3	9552457	rs145987757	C	A	0.0359	0.9236	-0.3121	0.079	8.39E-05
13	25562174	rs12585964	C	T	0.1431	0.9754	0.1619	0.041	8.41E-05
8	23935531	rs78669873	G	A	0.0101	0.9901	0.5633	0.1426	8.42E-05
4	10110878	rs76754641	C	T	0.0706	0.9662	0.2218	0.0562	8.50E-05
13	29328854	rs1536900	C	T	0.4653	1.0098	-0.1112	0.0282	8.51E-05
6	148103855	rs17077323	G	A	0.0789	0.9441	0.2132	0.054	8.53E-05
3	176718089	rs62298919	C	G	0.0221	0.8677	-0.4055	0.1027	8.53E-05
18	61135583	rs7233385	C	G	0.4606	0.9851	-0.1129	0.0286	8.54E-05

Appendix Table 6 Continued.

4	10115139	rs71603987	A	G	0.0708	0.9664	0.2214	0.0561	8.56E-05
5	142878977	rs73797758	A	C	0.0111	0.9872	-0.5373	0.1361	8.57E-05
5	75232842	rs34268756	C	T	0.4663	0.9815	-0.1132	0.0287	8.59E-05
19	43324908	rs147520235	T	A	0.0324	0.7984	0.3527	0.0894	8.59E-05
3	108908581	rs6797888	C	T	0.0243	1.011	0.3598	0.0912	8.60E-05
5	111015405	rs1673758	T	C	0.7924	0.9675	-0.1398	0.0354	8.61E-05
6	97766783	rs369767725	A	G	0.0109	0.8097	0.5956	0.151	8.62E-05
2	241035359	rs116099385	G	A	0.0455	0.7913	0.2997	0.076	8.64E-05
2	241035359	rs116099385	G	A	0.0455	0.7913	0.2997	0.076	8.64E-05
15	33116047	rs77312922	C	T	0.0101	0.9284	0.5773	0.1464	8.64E-05
4	10295852	rs74723388	T	C	0.0286	0.9504	0.3423	0.0868	8.67E-05
14	73453530	rs7154084	A	G	0.0301	1.0309	0.3225	0.0818	8.68E-05
4	10054337	rs12108388	A	T	0.0812	0.9789	0.2064	0.0523	8.68E-05
12	114294019	rs11066791	C	G	0.1665	0.9521	0.1537	0.039	8.68E-05
17	71146408	rs9892282	T	C	0.1825	1.0355	-0.1415	0.0359	8.70E-05
9	90500224	rs58932350	T	C	0.0419	0.9773	0.2833	0.0719	8.71E-05
5	159378582	rs6884129	C	G	0.0936	0.9792	0.1937	0.0491	8.73E-05
2	162640725	rs2892792	G	A	0.7447	1.025	-0.1262	0.032	8.74E-05
2	162640725	rs2892792	G	A	0.7447	1.025	-0.1262	0.032	8.74E-05
4	10143250	rs71603991	G	A	0.071	1.0022	0.2169	0.055	8.75E-05
1	59524507	rs17118653	A	G	0.2307	0.9774	-0.134	0.034	8.75E-05
3	172533725	rs4491935	A	G	0.1715	1.0117	0.1468	0.0372	8.76E-05
21	37547256	rs11701901	T	G	0.47	1.0133	-0.1108	0.0281	8.76E-05
12	114294570	rs1559836	T	C	0.1665	0.9522	0.1536	0.039	8.78E-05
1	59528234	rs9970411	T	C	0.2302	0.9817	-0.1338	0.034	8.78E-05
17	71145132	rs72846723	A	G	0.1826	1.0331	-0.1415	0.0359	8.80E-05
14	73401196	rs10134292	C	T	0.966	1.0124	-0.3076	0.0781	8.81E-05
4	10060984	rs11727418	T	C	0.0691	0.9739	0.223	0.0566	8.84E-05
5	142883391	rs73797766	C	G	0.0111	0.9868	-0.5354	0.136	8.86E-05
5	75236560	rs10038303	A	G	0.4703	0.9713	-0.1137	0.0289	8.88E-05

Appendix Table 6 Continued.

12	130888473	rs73150881	A	G	0.0134	1.0601	0.4722	0.1199	8.89E-05
21	37550116	rs2845751	C	T	0.3313	0.9823	-0.1195	0.0304	8.96E-05
3	97402639	rs75355167	A	G	0.0129	0.7948	0.5516	0.1402	8.98E-05
7	87047813	rs7788404	C	T	0.0738	0.9852	0.2158	0.0548	8.99E-05
7	87047039	rs35737120	T	C	0.0738	0.9852	0.2158	0.0548	8.99E-05
7	87040296	rs45521742	T	G	0.0738	0.9853	0.2158	0.0548	8.99E-05
7	87045565	rs45505301	A	G	0.0738	0.9853	0.2158	0.0548	8.99E-05
7	87046470	rs4148829	G	A	0.0738	0.9853	0.2158	0.0548	8.99E-05
11	23582746	rs16911535	C	T	0.0192	0.9356	0.4283	0.1089	9.00E-05
7	87036227	rs45543841	T	C	0.0738	0.9852	0.2158	0.0548	9.00E-05
4	31195130	rs116216646	T	C	0.03	0.8218	-0.3586	0.0912	9.05E-05
3	48531227	rs12491849	T	C	0.4079	1.0143	0.1123	0.0286	9.06E-05
2	177001962	rs79025511	T	C	0.0126	0.9768	0.5069	0.1289	9.08E-05
2	177001962	rs79025511	T	C	0.0126	0.9768	0.5069	0.1289	9.08E-05
1	234795238	rs24185	A	T	0.4066	1.01	0.113	0.0287	9.08E-05
14	73400038	rs8004641	G	A	0.966	1.0116	-0.3073	0.0782	9.09E-05
12	111356134	rs61943011	C	T	0.0113	0.9632	0.5417	0.1378	9.09E-05
6	166315261	rs117667378	T	C	0.0584	0.9881	0.2402	0.0611	9.10E-05
1	59526686	rs12409598	T	G	0.2303	0.982	-0.1335	0.034	9.10E-05
15	78247331	rs62008631	A	G	0.1464	0.8845	0.1668	0.0424	9.12E-05
8	126995981	rs148815837	C	A	0.0126	0.833	0.5446	0.1385	9.13E-05
17	71144700	rs113091587	A	G	0.1826	1.035	-0.1411	0.0359	9.13E-05
22	28178694	rs45623132	C	G	0.0125	0.8336	0.5453	0.1388	9.15E-05
3	172533919	rs4264754	C	T	0.1709	1.0117	0.1466	0.0373	9.17E-05
12	111351651	rs61940352	A	G	0.0113	0.9747	0.539	0.1372	9.17E-05
16	68069280	rs77184626	A	G	0.0128	0.9289	0.515	0.1311	9.18E-05
17	71143988	rs66497426	C	T	0.8172	1.0343	0.141	0.0359	9.18E-05
6	110620177	rs41288584	C	T	0.0158	1.0398	0.4358	0.1109	9.18E-05
2	241039187	rs114533404	G	T	0.0456	0.7908	0.2982	0.0759	9.24E-05
2	241039187	rs114533404	G	T	0.0456	0.7908	0.2982	0.0759	9.24E-05

Appendix Table 6 Continued.

10	104219908	rs76085620	C	T	0.0418	0.9152	0.2916	0.0743	9.25E-05
17	37865659	rs56114611	T	C	0.0124	0.8676	0.5366	0.1367	9.28E-05
12	114312417	rs11066798	C	T	0.1695	0.9586	0.1513	0.0385	9.29E-05
16	68068768	rs147014076	A	G	0.0128	0.928	0.5151	0.1312	9.30E-05
15	92447710	rs55772252	C	T	0.0857	0.9851	-0.1995	0.0508	9.32E-05
2	177007102	rs67435554	T	G	0.0765	0.9897	0.2089	0.0532	9.33E-05
2	177007102	rs67435554	T	G	0.0765	0.9897	0.2089	0.0532	9.33E-05
7	138726856	rs59047924	C	A	0.0155	0.9673	0.4599	0.1172	9.35E-05
5	134742235	rs7731133	T	C	0.0225	0.9389	-0.3843	0.0979	9.37E-05
4	10314188	rs727996	T	G	0.0263	0.9554	0.3553	0.0905	9.39E-05
7	79561556	rs37170	C	T	0.902	1.0394	-0.1832	0.0467	9.39E-05
7	79558403	rs9649000	C	A	0.902	1.0398	-0.1831	0.0467	9.40E-05
5	142887902	rs73301884	G	C	0.0112	0.9803	-0.533	0.1359	9.42E-05
7	87037158	rs45443210	C	G	0.0744	0.9842	0.2145	0.0547	9.42E-05
7	87038074	rs17149539	G	A	0.0744	0.9842	0.2145	0.0547	9.42E-05
7	87041856	rs11761050	A	C	0.0744	0.9842	0.2145	0.0547	9.42E-05
7	87043583	rs7807638	G	A	0.0744	0.9842	0.2145	0.0547	9.42E-05
21	37548110	rs9983855	G	A	0.453	1.0154	-0.1105	0.0282	9.45E-05
18	47318885	rs150342704	A	G	0.039	0.9733	0.2907	0.0741	9.46E-05
16	67938523	rs545838710	A	G	0.0125	0.9141	0.5232	0.1334	9.47E-05
13	29312555	rs1305095	T	C	0.4626	1.0082	-0.1105	0.0282	9.49E-05
13	29312541	rs1305096	A	C	0.4626	1.0082	-0.1105	0.0282	9.49E-05
3	151540101	rs76248492	G	A	0.0319	0.9548	0.3227	0.0823	9.49E-05
5	75231497	rs4703689	A	G	0.4639	0.9865	-0.1123	0.0286	9.52E-05
20	62119875	rs7265428	C	T	0.4187	0.9545	-0.1159	0.0296	9.54E-05
2	18427456	rs115923952	T	A	0.0211	1.031	-0.3794	0.0968	9.55E-05
2	18427456	rs115923952	T	A	0.0211	1.031	-0.3794	0.0968	9.55E-05
7	87034845	rs11773089	T	C	0.0744	0.9831	0.2143	0.0547	9.56E-05
2	18426454	rs12466959	G	A	0.0211	1.0304	-0.3794	0.0968	9.56E-05
2	18426454	rs12466959	G	A	0.0211	1.0304	-0.3794	0.0968	9.56E-05

Appendix Table 6 Continued.

11	65603252	rs7107912	A	G	0.525	0.9927	-0.1112	0.0284	9.57E-05
4	67654510	rs115002912	T	C	0.0243	0.8426	-0.3918	0.1	9.57E-05
3	30603093	rs17567548	G	A	0.0687	0.8353	-0.2399	0.0612	9.58E-05
3	151535976	rs9810654	A	C	0.0333	0.9512	0.3163	0.0807	9.59E-05
2	235505900	rs7573410	C	A	0.6826	0.9206	0.1237	0.0316	9.59E-05
2	235505900	rs7573410	C	A	0.6826	0.9206	0.1237	0.0316	9.59E-05
4	10111341	rs12498746	T	C	0.0708	0.9708	0.2195	0.056	9.61E-05
5	28620309	rs4867148	C	A	0.1322	0.974	0.1666	0.0425	9.63E-05
12	111346613	rs61940349	T	C	0.0112	0.983	0.5374	0.1372	9.63E-05
4	10313755	rs116613191	G	A	0.0263	0.9563	0.3544	0.0905	9.63E-05
5	159716449	rs73308692	G	C	0.0909	0.866	-0.2062	0.0526	9.64E-05
4	10085301	rs2241471	T	C	0.0706	0.9728	0.2195	0.056	9.64E-05
9	117992142	rs150433143	A	C	0.0153	1.0331	0.4436	0.1133	9.66E-05
3	172536130	rs71310525	T	A	0.171	1.0088	0.1463	0.0374	9.69E-05
1	234819747	rs485615	T	C	0.4705	1.0104	0.1106	0.0282	9.69E-05
4	10101104	rs2241487	C	A	0.0706	0.9729	0.2193	0.056	9.73E-05
12	4482973	rs10744644	C	T	0.9765	1.007	-0.3658	0.0934	9.75E-05
1	234819800	rs485790	A	G	0.4705	1.0108	0.1105	0.0282	9.75E-05
10	104422046	rs4522108	T	C	0.3185	0.9642	0.1215	0.031	9.77E-05
1	192354156	rs1936711	T	A	0.2705	0.9802	-0.1262	0.0322	9.77E-05
12	111350807	rs61940351	A	G	0.0111	0.9866	0.5368	0.1371	9.77E-05
4	10089121	rs2241479	C	T	0.0706	0.9726	0.2193	0.056	9.77E-05
12	111345404	rs61940348	T	C	0.0112	0.9817	0.5373	0.1373	9.78E-05
12	4483139	rs7295857	C	T	0.9765	1.0075	-0.3657	0.0934	9.79E-05
6	9436198	rs76835941	A	G	0.0193	0.8211	0.4433	0.1133	9.80E-05
10	19386040	rs10826941	T	C	0.2542	0.9988	-0.1271	0.0325	9.81E-05
6	148113931	rs55908285	A	G	0.0825	0.9258	0.209	0.0534	9.82E-05
4	10099018	rs3822238	G	T	0.0706	0.9726	0.2192	0.056	9.82E-05
5	11380024	rs78575730	A	C	0.0126	0.9523	0.5091	0.1301	9.82E-05
10	104421679	rs12570611	C	G	0.3185	0.9643	0.1214	0.031	9.82E-05

Appendix Table 6 Continued.

12	111343279	rs61940347	T	C	0.0112	0.9778	0.5375	0.1374	9.84E-05
12	111351204	rs146401175	G	T	0.0111	0.9854	0.5367	0.1372	9.85E-05
7	31996149	rs73310525	G	A	0.1768	0.9499	-0.1486	0.038	9.86E-05
7	87032251	rs45437295	T	G	0.0746	0.9818	0.2139	0.0547	9.90E-05
2	239469653	rs62194972	A	G	0.3069	1.0143	-0.1197	0.0306	9.92E-05
2	239469653	rs62194972	A	G	0.3069	1.0143	-0.1197	0.0306	9.92E-05
12	111351932	rs148639029	A	C	0.0111	0.9842	0.5366	0.1372	9.92E-05
4	10141515	rs11735463	C	T	0.0709	1.0064	0.2148	0.0549	9.92E-05
12	111339307	rs61940346	A	C	0.0112	0.9716	0.5382	0.1376	9.93E-05
10	44304600	rs78931007	C	A	0.0553	0.9974	-0.2416	0.0618	9.93E-05
12	111373510	rs61943022	A	C	0.0145	0.9295	0.4843	0.1239	9.93E-05
12	111337298	rs144597752	G	A	0.0112	0.9679	0.5386	0.1378	9.94E-05
6	148113313	rs3966656	A	G	0.0824	0.9257	0.2088	0.0534	9.94E-05
21	37548099	rs9984009	A	G	0.4671	1.0081	-0.1102	0.0282	9.95E-05
10	104241983	rs12763720	C	T	0.2314	0.9313	0.1358	0.0347	9.95E-05
2	8018940	rs73153537	G	T	0.0146	1.008	0.4612	0.118	9.96E-05
2	8018940	rs73153537	G	T	0.0146	1.008	0.4612	0.118	9.96E-05
15	92448712	rs72750081	C	G	0.0858	0.9869	-0.1984	0.0507	9.98E-05
1	192352776	rs1936712	T	C	0.2704	0.9826	-0.1259	0.0322	9.98E-05
15	31973676	rs147900146	G	A	0.0124	0.867	0.5362	0.1372	9.99E-05
21	37553132	rs11911615	G	T	0.3295	0.9838	-0.1187	0.0304	9.99E-05

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Appendix Table 7. GWAS Results for Cisplatin-Induced Hearing Loss in an Expanded Cohort of Testicular Cancer Survivors ($P < 1x10^{-5}$).

CHR	BP	SNP	EFFECT	REFERENCE	BETA	STAT	P
1	63549230	rs1391812	T	C	0.1968	5.452	6.20E-08
1	63552112	rs1004517	A	G	0.196	5.428	7.08E-08
8	17746374	rs34612399	A	G	0.3119	4.891	1.16E-06
10	3138265	rs7074733	T	C	0.1901	4.772	2.08E-06
2	227402018	rs16825777	T	C	0.2816	4.762	2.19E-06
1	63549282	rs953654	T	C	-0.1604	-4.72	2.68E-06
2	227416662	rs11676181	T	C	0.27	4.663	3.51E-06
16	86910194	rs11642990	G	C	-0.218	-4.66	3.57E-06
16	86910194	rs11642990	T	C	-0.218	-4.66	3.57E-06
1	63548419	rs10493333	G	C	0.1626	4.66	3.57E-06
1	63548419	rs10493333	T	C	0.1626	4.66	3.57E-06
4	6276630	rs62283056	C	G	0.1927	4.599	4.76E-06
12	104638773	rs4406890	G	T	0.2046	4.561	5.69E-06
12	104638773	rs11785081	T	G	0.2046	4.561	5.69E-06
5	134443908	rs72802321	C	T	-0.3614	-4.556	5.81E-06
5	60069057	rs13158665	T	C	-0.1546	-4.55	5.98E-06
4	6275735	rs17718958	A	G	0.2155	4.538	6.33E-06
4	6276805	rs62283057	T	C	0.2155	4.538	6.33E-06
12	104645363	rs7960443	A	G	0.2042	4.533	6.46E-06
12	104645669	rs6539132	A	C	0.2042	4.533	6.46E-06
12	104646770	rs4964267	A	G	0.2042	4.533	6.46E-06
12	104649733	rs4964728	A	G	0.2042	4.533	6.46E-06
12	104649733	rs4964728	C	G	0.2042	4.533	6.46E-06
12	104651110	rs10861181	C	T	0.2042	4.533	6.46E-06
12	104651482	rs7297560	T	C	0.2042	4.533	6.46E-06
4	6284965	rs4568307	G	A	0.1646	4.498	7.60E-06

Appendix Table 7 Continued.

1	63550875	rs1466834	A	G	-0.153	-4.492	7.84E-06
1	63550604	rs6662149	T	C	-0.1533	-4.491	7.86E-06
4	6283460	rs10028875	T	C	0.1647	4.489	7.93E-06
9	36309845	rs72624021	A	G	0.1918	4.457	9.19E-06
9	36311610	rs7853674	A	G	0.1918	4.457	9.19E-06

Appendix Table 8. GWAS Results for Cisplatin-Induced Tinnitus in an Expanded Cohort of Testicular Cancer Survivors ($P < 1x10^{-5}$).

CHR	BP	SNP	EFFECT	REFERENCE	BETA	STAT	P
10	122238850	rs4752423	A	G	0.4202	-5.109	3.24E-07
10	122238794	rs4752422	A	C	0.4223	-5.082	3.74E-07
10	122239793	rs1475373	G	C	0.4223	-5.082	3.74E-07
10	122239999	rs4237520	C	T	0.4223	-5.082	3.74E-07
7	14200667	rs4107135	G	A	2.97	5.041	4.62E-07
7	14200667	rs11983244	A	C	2.97	5.041	4.62E-07
7	14200667	rs11983244	T	C	2.97	5.041	4.62E-07
1	234826975	rs607708	A	T	1.753	4.759	1.95E-06
1	234827009	rs512578	C	A	1.753	4.759	1.95E-06
1	234827824	rs541021	A	T	1.746	4.731	2.23E-06
1	234827824	rs541021	G	T	1.746	4.731	2.23E-06
21	38901620	rs13051500	A	C	1.803	4.719	2.37E-06
21	38902379	rs13052357	A	C	1.797	4.701	2.58E-06
20	4711755	rs117098605	T	G	4.462	4.692	2.71E-06
4	162489247	rs76102346	T	C	7.686	4.64	3.48E-06
1	234829747	rs655506	G	T	1.72	4.627	3.72E-06
1	234830361	rs2770371	C	T	1.72	4.627	3.72E-06
1	234830590	rs498518	A	G	1.718	4.612	3.99E-06
3	33131888	rs112303980	A	G	2.657	4.591	4.41E-06
19	10020385	rs75764500	T	C	2.579	4.587	4.49E-06
13	24515624	rs2765107	A	G	1.788	4.565	5.01E-06
1	234820331	rs490559	A	G	0.5829	-4.529	5.91E-06
1	234820419	rs491427	C	T	0.5829	-4.529	5.91E-06
1	234820827	rs588965	T	G	0.5829	-4.529	5.91E-06
9	31848524	rs75341355	A	G	2.518	4.521	6.15E-06
13	24514328	rs2810669	T	C	1.778	4.52	6.19E-06

Appendix Table 8 Continued.

13	24514518	rs2765105	T	G	1.778	4.52	6.19E-06
13	24515162	rs2765106	C	A	1.778	4.52	6.19E-06
13	24515162	rs8128468	A	G	1.778	4.52	6.19E-06
7	22097807	rs139884628	C	T	4.066	4.505	6.65E-06
13	65270250	rs78597370	A	G	2.898	4.501	6.77E-06
13	65275989	rs141170839	A	G	2.898	4.501	6.77E-06
4	99557766	rs846004	T	A	5.919	4.493	7.04E-06
14	37480996	rs751202	T	A	1.818	4.47	7.82E-06
14	37481442	rs909015	G	C	1.818	4.47	7.82E-06
14	37481442	rs909015	T	C	1.818	4.47	7.82E-06
14	37481442	rs2973246	A	T	1.818	4.47	7.82E-06
14	37481812	rs17105951	A	G	1.818	4.47	7.82E-06
3	33064935	rs7610270	G	C	2.462	4.467	7.94E-06
16	13124884	rs10459770	G	C	4.236	4.459	8.22E-06
16	13125741	rs7203886	G	A	4.236	4.459	8.22E-06
16	13126708	rs76477961	G	C	4.236	4.459	8.22E-06
16	13127010	rs7190439	A	G	4.236	4.459	8.22E-06
3	33079760	rs78672100	C	A	2.457	4.459	8.24E-06
2	8549456	rs4425054	C	T	0.3083	-4.448	8.65E-06
19	9990729	rs150211563	G	A	2.625	4.445	8.81E-06
16	13133980	rs79792779	G	C	3.376	4.44	9.01E-06
14	37459015	rs2022730	C	T	1.792	4.433	9.28E-06
21	38902907	rs35682991	T	C	1.744	4.432	9.34E-06
21	38722674	rs2835700	G	T	1.728	4.425	9.63E-06
21	38892198	rs8131077	G	C	1.734	4.418	9.97E-06

Appendix Table 9. ExWAS Results for Cisplatin-Induced Hearing Loss using LOF Variants (P < 0.001).

CHR	BP	VARIANT	GENE	EFFECT	REFERENCE	BETA	STAT	P
1	204161290	1:204161290	REN	G	A	-3.635	-4.64	3.93E-06
2	48725674	2:48725674	LHCGR	G	A	-3.635	-4.64	3.93E-06
8	31081132	8:31081132	WRN	T	C	-3.635	-4.64	3.93E-06
15	23027222	15:23027222	TUBGCP5	C	A	-3.635	-4.64	3.93E-06
15	31066075	15:31066075	TRPM1	T	C	-3.635	-4.64	3.93E-06
18	74447124	18:74447124	FAM69C	A	C	-3.635	-4.64	3.93E-06
1	171319817	1:171319817	FMO4	C	G	-2.891	-3.658	0.0002667
5	32270901	5:32270901	MTMR12	C	G	-2.891	-3.658	0.0002667
11	62422322	11:62422322	SCGB1A1	T	C	-2.891	-3.658	0.0002667
15	48878338	15:48878338	EID1	A	G	-2.891	-3.658	0.0002667
16	21049684	16:21049684	DNAH3	G	T	-2.891	-3.658	0.0002667
1	156242814	1:156242814	BGLAP	G	C	2.867	3.65	0.0002754
1	156242814	1:156242814	PMF1-BGLAP	G	C	2.867	3.65	0.0002754
2	32442322	2:32442322	BIRC6	G	A	2.867	3.65	0.0002754
2	159881241	2:159881241	LY75	G	C	2.867	3.65	0.0002754
2	159881241	2:159881241	LY75-CD302	G	C	2.867	3.65	0.0002754
4	143185466	4:143185466	USP38	T	G	2.867	3.65	0.0002754
10	128116002	10:128116002	MKI67	A	T	2.867	3.65	0.0002754
5	10448263	5:10448263	ROPN1L	A	T	1.633	3.595	0.0003389
6	143863420	6:143863420	LTV1	T	C	-1.87	-3.34	0.0008687
7	55537524	7:55537524	VOPP1	A	G	1.176	3.339	0.0008716
20	3191686	20:3191686	DDRGK1	A	T	1.855	3.321	0.0009272

Appendix Table 10. ExWAS Results for Cisplatin-Induced Hearing loss using LOF and Predicted Deleterious Variants (P < 0.0001).

CHR	BP	VARIANT	GENE	EFFECT	REFERENCE	BETA	STAT	P
2	241048707	2:241048707	SNED1	C	G	-2.588	-4.667	3.45E-06
1	114856610	1:114856610	SYCP1	G	C	-3.635	-4.64	3.93E-06
1	159192691	1:159192691	CADM3	C	A	-3.635	-4.64	3.93E-06
1	204161290	1:204161290	REN	G	A	-3.635	-4.64	3.93E-06
1	212358753	1:212358753	PPP2R5A	T	G	-3.635	-4.64	3.93E-06
2	48725674	2:48725674	LHCGR	G	A	-3.635	-4.64	3.93E-06
2	60791859	2:60791859	PAPOLG	A	G	-3.635	-4.64	3.93E-06
2	72968452	2:72968452	SFXN5	T	C	-3.635	-4.64	3.93E-06
2	149575839	2:149575839	MMADHC	T	A	-3.635	-4.64	3.93E-06
2	149575839	2:149575839	MMADHC	C	A	-3.635	-4.64	3.93E-06
2	215404616	2:215404616	FN1	T	G	-3.635	-4.64	3.93E-06
2	219483098	2:219483098	SPEG	T	C	-3.635	-4.64	3.93E-06
2	231108702	2:231108702	HTR2B	C	T	-3.635	-4.64	3.93E-06
2	232407651	2:232407651	ALPPL2	A	T	-3.635	-4.64	3.93E-06
2	238166960	2:238166960	ERFE	C	G	-3.635	-4.64	3.93E-06
3	9743746	3:9743746	BRPF1	A	G	-3.635	-4.64	3.93E-06
3	9950090	3:9950090	PRRT3	C	A	-3.635	-4.64	3.93E-06
3	47910660	3:47910660	MAP4	A	G	-3.635	-4.64	3.93E-06
3	52208136	3:52208136	ALAS1	G	A	-3.635	-4.64	3.93E-06
3	52360012	3:52360012	DNAH1	A	G	-3.635	-4.64	3.93E-06
3	100178314	3:100178314	CMSS1	A	G	-3.635	-4.64	3.93E-06
3	138097820	3:138097820	DZIP1L	C	T	-3.635	-4.64	3.93E-06
3	195786640	3:195786640	MUC4	A	G	-3.635	-4.64	3.93E-06
4	137530384	4:137530384	PCDH18	T	C	-3.635	-4.64	3.93E-06
4	150844183	4:150844183	LRBA	T	C	-3.635	-4.64	3.93E-06
4	189955114	4:189955114	FRG1	C	T	-3.635	-4.64	3.93E-06

Appendix Table 10 Continued.

5	38484788	5:38484788	LIFR	A	G	-3.635	-4.64	3.93E-06
5	55110061	5:55110061	GZMA	A	G	-3.635	-4.64	3.93E-06
5	142131859	5:142131859	NDFIP1	T	C	-3.635	-4.64	3.93E-06
5	146471524	5:146471524	TCERG1	T	G	-3.635	-4.64	3.93E-06
6	18215109	6:18215109	KDM1B	T	C	-3.635	-4.64	3.93E-06
6	20402254	6:20402254	E2F3	T	G	-3.635	-4.64	3.93E-06
6	31702612	6:31702612	ABHD16A	G	C	-3.635	-4.64	3.93E-06
6	32052729	6:32052729	TNXB	G	T	-3.635	-4.64	3.93E-06
6	80171374	6:80171374	BCKDHB	G	A	-3.635	-4.64	3.93E-06
7	3301594	7:3301594	SDK1	C	G	-3.635	-4.64	3.93E-06
7	45660062	7:45660062	ADCY1	G	C	-3.635	-4.64	3.93E-06
7	108572938	7:108572938	DNAJB9	G	A	-3.635	-4.64	3.93E-06
8	3406057	8:3406057	CSMD1	T	C	-3.635	-4.64	3.93E-06
8	23028487	8:23028487	TNFRSF10B	A	C	-3.635	-4.64	3.93E-06
8	31081132	8:31081132	WRN	T	C	-3.635	-4.64	3.93E-06
8	99013848	8:99013848	VPS13B	A	C	-3.635	-4.64	3.93E-06
8	99809501	8:99809501	VPS13B	T	G	-3.635	-4.64	3.93E-06
9	32631393	9:32631393	TAF1L	T	C	-3.635	-4.64	3.93E-06
9	65283434	9:65283434	FOXD4L5	G	C	-3.635	-4.64	3.93E-06
9	114168316	9:114168316	COL27A1	G	C	-3.635	-4.64	3.93E-06
9	129177599	9:129177599	IER5L	A	C	-3.635	-4.64	3.93E-06
9	129177599	9:129177599	IER5L	T	C	-3.635	-4.64	3.93E-06
9	133407008	9:133407008	REXO4	A	G	-3.635	-4.64	3.93E-06
9	136845984	9:136845984	C9orf172	A	G	-3.635	-4.64	3.93E-06
10	27734542	10:27734542	MKX	A	G	-3.635	-4.64	3.93E-06
11	294187	11:294187	ATHL1	T	C	-3.635	-4.64	3.93E-06
11	396665	11:396665	PKP3	A	G	-3.635	-4.64	3.93E-06
11	6410810	11:6410810	APBB1	A	G	-3.635	-4.64	3.93E-06
11	6622792	11:6622792	DCHS1	A	G	-3.635	-4.64	3.93E-06
11	19170483	11:19170483	ZDHHC13	A	G	-3.635	-4.64	3.93E-06
11	64015258	11:64015258	MACROD1	T	C	-3.635	-4.64	3.93E-06

Appendix Table 10 Continued.

11	123754512	11:123754512	OR6X1	G	T	-3.635	-4.64	3.93E-06
11	130411515	11:130411515	ADAMTS8	T	C	-3.635	-4.64	3.93E-06
11	134345090	11:134345090	GLB1L2	G	C	-3.635	-4.64	3.93E-06
12	8048175	12:8048175	FOXJ2	G	C	-3.635	-4.64	3.93E-06
12	27737586	12:27737586	MRPS35	A	C	-3.635	-4.64	3.93E-06
12	31103860	12:31103860	DDX11	T	C	-3.635	-4.64	3.93E-06
12	31982020	12:31982020	KIAA1551	C	A	-3.635	-4.64	3.93E-06
12	53534607	12:53534607	ATF7	T	C	-3.635	-4.64	3.93E-06
12	53534607	12:53534607	RP11-793H13.10	T	C	-3.635	-4.64	3.93E-06
13	32535902	13:32535902	N4BP2L2	C	G	-3.635	-4.64	3.93E-06
14	24143634	14:24143634	PSME2	A	G	-3.635	-4.64	3.93E-06
14	64017613	14:64017613	SYNE2	A	G	-3.635	-4.64	3.93E-06
14	89989742	14:89989742	TDP1	A	G	-3.635	-4.64	3.93E-06
14	105411477	14:105411477	TEX22	C	G	-3.635	-4.64	3.93E-06
15	23027222	15:23027222	TUBGCP5	C	A	-3.635	-4.64	3.93E-06
15	31066075	15:31066075	TRPM1	T	C	-3.635	-4.64	3.93E-06
15	56103657	15:56103657	RFX7	C	T	-3.635	-4.64	3.93E-06
16	3657032	16:3657032	DNASE1	T	C	-3.635	-4.64	3.93E-06
17	7353505	17:7353505	KCTD11	A	G	-3.635	-4.64	3.93E-06
17	10531770	17:10531770	MYH2	T	C	-3.635	-4.64	3.93E-06
17	11598806	17:11598806	DNAH9	C	T	-3.635	-4.64	3.93E-06
17	11598806	17:11598806	DNAH9	G	T	-3.635	-4.64	3.93E-06
17	19782059	17:19782059	ULK2	C	G	-3.635	-4.64	3.93E-06
17	19958129	17:19958129	AKAP10	G	T	-3.635	-4.64	3.93E-06
17	35650689	17:35650689	AP2B1	A	C	-3.635	-4.64	3.93E-06
18	10761050	18:10761050	PIEZO2	C	T	-3.635	-4.64	3.93E-06
18	74447124	18:74447124	FAM69C	A	C	-3.635	-4.64	3.93E-06
19	49023106	19:49023106	CGB3	A	G	-3.635	-4.64	3.93E-06
19	55455646	19:55455646	ISOC2	A	G	-3.635	-4.64	3.93E-06
22	26540862	22:26540862	TPST2	T	C	-3.635	-4.64	3.93E-06

Appendix Table 10 Continued.

22	50448587	22:50448587	SBF1	A	G	-3.635	-4.64	3.93E-06
23	1348498	23:1348498	IL3RA	A	G	-3.635	-4.64	3.93E-06
23	154478308	23:154478308	LAGE3	G	T	-3.635	-4.64	3.93E-06
17	47708784	17:47708784	TBKBP1	C	T	-3.635	-4.638	3.97E-06
8	8892509	8:8892509	MFHAS1	C	G	-2.343	-4.207	2.82E-05
3	147402748	3:147402748	ZIC4	T	C	-1.893	-4.154	3.53E-05
6	52836287	6:52836287	GSTA5	C	T	0.9256	4.061	5.24E-05
20	56452603	20:56452603	CASS4	C	T	2.247	4.035	5.86E-05
17	10529472	17:10529472	MYH2	C	A	-1.822	-4.004	6.68E-05
11	105029739	11:105029739	CASP1	C	T	1.782	3.921	9.38E-05

Appendix Table 11. ExWAS Results for Cisplatin-Induced Tinnitus using LOF Variants (P < 0.01).

CHR	BP	VARIANT	GENE	EFFECT	REFERENCE	OR	STAT	P
20	31389027	20:31389027	DEFB119	C	A	44.46	3.245	0.001174
15	20534261	15:20534261	GOLGA6L6	G	A	3.302	2.796	0.005176
20	20052678	20:20052678	CRNKL1	A	G	5.309	2.712	0.006689
7	149806746	7:149806746	SSPO	T	G	6.243	2.653	0.007966
2	168907858	2:168907858	G6PC2	T	C	7.113	2.625	0.008653
23	135722986	23:135722986	CT45A1	T	C	3.575	2.622	0.008731

Appendix Table 12. ExWAS Results for Cisplatin-Induced Tinnitus using LOF and Predicted Deleterious Variants (P < 0.005).

CHR	BP	VARIANT	GENE	EFFECT	REFERENCE	OR	STAT	P
21	32325266	21:32325266	URB1	A	G	5.122	3.414	0.0006413
8	143868132	8:143868132	EPPK1	T	C	7.557	3.338	0.0008452
8	143717733	8:143717733	MAPK15	A	G	7.136	3.277	0.00105
20	31389027	20:31389027	DEFB119	C	A	44.46	3.245	0.001174
20	31389209	20:31389209	DEFB119	C	T	44.46	3.245	0.001174
1	26344165	1:26344165	AIM1L	A	C	5.979	3.177	0.001489
1	201210396	1:201210396	IGFN1	A	G	5.812	3.176	0.001493
12	52568266	12:52568266	KRT74	A	G	14.84	3.17	0.001524
10	119898888	10:119898888	SEC23IP	T	C	5.009	3.132	0.001736
4	17633777	4:17633777	FAM184B	C	T	37.23	3.131	0.00174
3	109000589	3:109000589	MORC1	G	C	8.418	3.129	0.001753
3	195783839	3:195783839	MUC4	C	T	5.07	3.117	0.00183
11	14644570	11:14644570	PDE3B	A	C	7.592	3.1	0.001936
1	114984743	1:114984743	SYCP1	T	C	9.193	3.074	0.00211
6	41161514	6:41161514	TREM2	T	C	5.94	3.058	0.002228
5	150010405	5:150010405	HMGXB3	A	G	4.841	3.054	0.00226
2	178590625	2:178590625	TTN	T	C	10.06	3.038	0.00238
2	240130020	2:240130020	COPS9	G	C	6.684	3.031	0.002438
17	49973193	17:49973193	DLX4	C	G	27.62	3.029	0.002453
7	154975767	7:154975767	PAXIP1	T	C	8.966	3.001	0.002691
15	32633101	15:32633101	ARHGAP11A	A	G	4.358	2.996	0.002734
8	98021213	8:98021213	MATN2	A	G	4.261	2.989	0.002797
21	45456090	21:45456090	COL18A1	T	C	4.738	2.983	0.002857
19	38525492	19:38525492	RYR1	A	G	12.24	2.968	0.003002
7	156963087	7:156963087	NOM1	A	G	3.44	2.953	0.003142
2	102235211	2:102235211	IL1RL2	T	C	13.84	2.945	0.003229
22	19724232	22:19724232	GP1BB	T	C	7.335	2.919	0.003516

Appendix Table 12 Continued.

6	2678307	6:2678307	MYLK4	T	C	7.245	2.917	0.003536
2	99322621	2:99322621	TXNDC9	G	A	33.5	2.914	0.003568
1	236558300	1:236558300	HEATR1	A	G	7.075	2.889	0.003866
22	39522517	22:39522517	ATF4	T	C	28.38	2.861	0.004226
19	40396869	19:40396869	PRX	G	C	3.815	2.859	0.004251
2	147958332	2:147958332	ORC4	G	A	8.78	2.856	0.004296
17	66053863	17:66053863	CEP112	C	G	9.357	2.854	0.004317
1	224305081	1:224305081	NVL	A	T	9.601	2.839	0.004528
7	135045501	7:135045501	AGBL3	T	C	3.862	2.837	0.004549
17	32021694	17:32021694	LRRC37B	T	C	6.141	2.836	0.004564
4	55105862	4:55105862	KDR	T	C	12.18	2.824	0.004739
11	124440377	11:124440377	OR8B8	T	C	9.08	2.819	0.004815
11	108161887	11:108161887	NPAT	T	C	3.922	2.815	0.004879
20	31793857	20:31793857	TPX2	A	G	4.46	2.81	0.004948

Appendix Table 13. GWAS Results for Multiple Severe Neurotoxicities ($P < 1x10^{-5}$).

CHR	BP	SNP	EFFECT	REFERENCE	OR	STAT	P
12	129184432	rs12829696	T	G	0.1076	-4.907	9.23E-07
13	86386112	rs34832729	G	A	5.554	4.886	1.03E-06
13	86453372	rs57528547	A	G	4.785	4.831	1.36E-06
13	86453372	rs57384334	A	A	4.785	4.831	1.36E-06
12	129184889	rs34721993	G	A	0.1185	-4.804	1.56E-06
13	86439593	rs9602858	G	A	5.266	4.747	2.07E-06
13	86442909	rs10162185	A	G	5.266	4.747	2.07E-06
13	86462814	rs914887077	T	A	4.301	4.601	4.20E-06
13	86462814	rs9602864	T	A	4.301	4.601	4.20E-06
13	86462814	rs72615744	T	G	4.301	4.601	4.20E-06
13	86463135	rs9602865	A	G	4.301	4.601	4.20E-06
13	86392672	rs72631091	A	G	4.998	4.557	5.19E-06
13	86394952	rs72631094	G	A	4.998	4.557	5.19E-06
13	86397630	rs56229215	G	A	4.998	4.557	5.19E-06
13	86404422	rs55694964	T	C	4.998	4.557	5.19E-06
13	86405905	rs72631097	T	G	4.998	4.557	5.19E-06
13	86406320	rs72631099	T	C	4.998	4.557	5.19E-06
13	86408756	rs55739240	A	C	4.998	4.557	5.19E-06
13	86409020	rs72632703	C	T	4.998	4.557	5.19E-06
13	86409356	rs112214436	C	G	4.998	4.557	5.19E-06
13	86412484	rs72632706	G	C	4.998	4.557	5.19E-06
13	86414490	rs72632707	T	A	4.998	4.557	5.19E-06
13	86415821	rs72632708	G	T	4.998	4.557	5.19E-06
13	86416254	rs72632710	A	G	4.998	4.557	5.19E-06
13	86416582	rs72632713	T	C	4.998	4.557	5.19E-06
13	86416735	rs72632715	G	A	4.998	4.557	5.19E-06
13	86416804	rs72632717	A	G	4.998	4.557	5.19E-06
13	86387829	rs72631086	G	C	4.851	4.53	5.89E-06

Appendix Table 13 Continued.

13	86418918	rs8002082	G	A	4.961	4.528	5.95E-06
13	86424251	rs72632726	T	A	4.961	4.528	5.95E-06
13	86426705	rs55872307	A	G	4.961	4.528	5.95E-06
13	86427744	rs72632729	T	G	4.961	4.528	5.95E-06
13	86428559	rs72632734	G	A	4.961	4.528	5.95E-06
13	86431538	rs56347335	G	A	4.961	4.528	5.95E-06
13	86432448	rs55749217	C	T	4.961	4.528	5.95E-06
13	86432658	rs55755529	G	C	4.961	4.528	5.95E-06
13	86435537	rs72632738	G	C	4.961	4.528	5.95E-06
13	86435760	rs72632739	T	C	4.961	4.528	5.95E-06
13	86442620	rs72632747	G	C	4.961	4.528	5.95E-06
13	86442876	rs72632748	C	G	4.961	4.528	5.95E-06
13	86443720	rs72632750	G	A	4.961	4.528	5.95E-06
13	86446047	rs72632751	T	C	4.961	4.528	5.95E-06
13	86446672	rs55637305	C	T	4.961	4.528	5.95E-06
13	86449236	rs56033536	G	A	4.961	4.528	5.95E-06
13	86451114	rs72632754	G	A	4.961	4.528	5.95E-06
13	86451668	rs72632756	G	A	4.961	4.528	5.95E-06
13	86386305	rs72631085	C	A	4.798	4.484	7.33E-06
15	31471472	rs1223889	G	G	0.2677	-4.463	8.09E-06
1	204359764	rs1512114	G	G	3.676	4.454	8.43E-06
1	102024990	rs1484309	A	A	5.944	4.446	8.77E-06
13	86420105	rs72632719	C	A	4.164	4.444	8.83E-06
13	86420864	rs72632722	T	C	4.164	4.444	8.83E-06
13	86422978	rs56065131	G	C	4.164	4.444	8.83E-06
13	86426611	rs55747338	T	C	4.164	4.444	8.83E-06
13	86427156	rs56169913	A	T	4.164	4.444	8.83E-06
13	86428479	rs72632732	C	T	4.164	4.444	8.83E-06
13	86442119	rs72632745	T	C	4.164	4.444	8.83E-06
13	86374910	rs72631081	A	G	4.681	4.434	9.27E-06
13	86377916	rs72631084	T	A	4.681	4.434	9.27E-06

Appendix Table 14. GWAS Results for Radiation-Induced Tinnitus ($P < 1x10^{-5}$), Adjusting for Age at Last Observation, Cumulative Cranial Radiation Dose, and 20 European Principal Components.

CHR	BP	SNP	EFFECT	REFERENCE	OR	STAT	P
1	167932562	rs203248	T	G	8.666	6.044	1.50E-09
1	167988413	rs57738266	A	AG	7.631	5.976	2.28E-09
1	168026131	rs201535	T	C	7.841	5.668	1.45E-08
1	167940616	rs202245	T	C	7.813	5.651	1.59E-08
1	168005387	rs73028104	G	A	7.813	5.651	1.59E-08
1	168012455	rs41271645	T	C	7.813	5.651	1.59E-08
1	168036735	rs399273	T	C	7.813	5.651	1.59E-08
1	168039418	rs58082401	G	A	7.813	5.651	1.59E-08
1	168054273	rs1051426	G	A	7.813	5.651	1.59E-08
1	167922957	rs202271	T	C	7.813	5.651	1.59E-08
1	168036208	rs58731723	T	G	7.813	5.651	1.59E-08
1	167920312	rs59261808	G	A	7.813	5.651	1.59E-08
1	168004122	rs60775715	A	T	7.813	5.651	1.59E-08
1	167913532	rs61238731	G	T	7.813	5.651	1.59E-08
1	168028368	rs61643149	G	T	7.813	5.651	1.59E-08
1	167905757	rs73024126	T	G	7.813	5.651	1.59E-08
1	167921945	rs73024143	T	C	7.813	5.651	1.59E-08
1	167950390	rs73026142	A	C	7.813	5.651	1.59E-08
1	167969262	rs73026155	G	T	7.813	5.651	1.59E-08
1	167997051	rs73026198	G	T	7.813	5.651	1.59E-08
1	167997590	rs73026200	T	A	7.813	5.651	1.59E-08
1	168010436	rs73028106	C	T	7.813	5.651	1.59E-08
1	168010558	rs73028108	G	A	7.813	5.651	1.59E-08
1	167946239	rs111444726	A	T	7.813	5.651	1.59E-08
1	167937237	rs111710153	A	G	7.813	5.651	1.59E-08
1	168006340	rs112837846	T	C	7.813	5.651	1.59E-08

Appendix Table 14 Continued.

1	168033199	rs66923770	T	TA	7.813	5.651	1.59E-08
1	168042770	rs150699452	C	CCT	7.813	5.651	1.59E-08
1	167980043	rs140916263	T	TTTC	7.813	5.651	1.59E-08
1	168024244	rs430565	T	C	7.044	5.638	1.72E-08
1	168033269	rs169309	T	C	7.241	5.523	3.33E-08
1	167937585	rs141859624	AC	A	7.255	5.51	3.58E-08
1	168034256	rs369914	T	A	7.031	5.468	4.55E-08
1	168036395	rs79316144	C	T	7.199	5.299	1.16E-07
1	167907340	rs73024128	G	T	7.199	5.299	1.16E-07
1	167917198	rs73024136	A	G	7.199	5.299	1.16E-07
1	167989291	rs73026187	T	G	7.199	5.299	1.16E-07
1	167948456	rs113160934	T	C	7.199	5.299	1.16E-07
1	167837724	rs203815	G	A	11.46	5.247	1.54E-07
1	167837432	rs203813	C	T	11.45	5.246	1.55E-07
1	168002844	rs201536	T	C	6.215	5.185	2.17E-07
1	167847955	rs203840	G	A	10.09	4.993	5.95E-07
1	167847984	rs203841	C	T	10.09	4.993	5.95E-07
1	167848531	rs203844	G	T	10.09	4.993	5.95E-07
1	167848671	rs203845	G	A	10.05	4.986	6.17E-07
11	43090589	rs139710329	T	C	6.338	4.934	8.07E-07
16	3260406	rs74427749	C	T	5.425	4.933	8.12E-07
1	167838804	rs129717	T	C	10.86	4.913	8.95E-07
1	167838805	rs129718	C	T	10.86	4.913	8.95E-07
1	168083997	rs73030214	A	G	6.51	4.899	9.65E-07
1	218487190	rs17557846	C	T	3.89	4.887	1.02E-06
1	168006342	rs380682	T	C	5.411	4.866	1.14E-06
12	92991145	rs7977829	A	G	0.5131	-4.825	1.40E-06
12	93000452	rs2385096	C	G	0.5137	-4.82	1.44E-06
12	92999807	rs6538339	C	T	0.5137	-4.82	1.44E-06
12	93001578	rs35642478	AG	A	0.5137	-4.82	1.44E-06
12	92991427	rs7313463	G	A	0.5138	-4.817	1.46E-06

Appendix Table 14 Continued.

12	92992792	rs10466947	G	A	0.5138	-4.817	1.46E-06
12	92994180	rs1961763	G	A	0.5138	-4.817	1.46E-06
12	92993933	rs1961764	A	T	0.5138	-4.817	1.46E-06
12	92995131	rs2082899	C	A	0.5138	-4.817	1.46E-06
12	92997562	rs7309092	A	C	0.5139	-4.815	1.47E-06
11	33964278	rs117955565	T	C	6.448	4.785	1.71E-06
11	33965972	rs117644109	G	A	6.448	4.785	1.71E-06
1	167837285	rs203812	C	A	9.767	4.779	1.77E-06
1	168003979	rs169310	G	T	5.152	4.777	1.78E-06
1	168034466	rs369851	C	G	5.152	4.777	1.78E-06
1	168034763	rs370952	A	G	5.152	4.777	1.78E-06
11	19485416	rs7106624	G	G	1.861	4.755	1.99E-06
8	3565380	rs76070030	T	G	2.345	4.754	1.99E-06
8	3569197	rs75015496	G	C	2.34	4.743	2.10E-06
12	31917041	rs73092328	G	A	4.673	4.737	2.17E-06
11	19485410	rs2632041	C	T	1.845	4.698	2.62E-06
22	51123411	rs113604661	T	C	4.991	4.69	2.74E-06
11	19485950	rs5790074	G	A	1.844	4.69	2.74E-06
11	19485259	rs2729868	G	T	1.838	4.689	2.75E-06
1	218482195	rs17485987	C	G	4.943	4.686	2.79E-06
7	66602866	rs141635338	T	C	4.092	4.678	2.89E-06
10	127847013	rs66634335	A	AC	0.4214	-4.678	2.90E-06
1	167861989	rs80124755	A	C	6.033	4.662	3.13E-06
1	168014532	rs417427	G	T	4.865	4.649	3.34E-06
1	168014533	rs433173	T	G	4.865	4.649	3.34E-06
11	19483904	rs2200568	G	A	1.829	4.647	3.38E-06
2	46072278	rs116091362	G	T	4.535	4.633	3.60E-06
8	109335760	rs111306410	G	C	6.238	4.617	3.89E-06
8	109327783	rs111920990	T	C	6.238	4.617	3.89E-06
8	109296501	rs112617707	G	T	6.238	4.617	3.89E-06
4	91511874	rs111315244	A	G	6.609	4.617	3.90E-06

Appendix Table 14 Continued.

11	19487617	rs1386450	G	A	1.819	4.6	4.22E-06
8	109317468	rs78313442	T	G	5.558	4.577	4.72E-06
11	19484855	rs2632042	A	A	1.81	4.574	4.77E-06
8	3555836	rs2251863	T	G	2.256	4.57	4.87E-06
8	3559270	rs74668325	G	T	2.259	4.563	5.04E-06
12	3250245	rs149734922	C	G	6.673	4.554	5.26E-06
11	19483360	rs4757002	G	G	1.809	4.554	5.27E-06
11	123444439	rs72645487	A	G	3.973	4.526	6.00E-06
11	19483521	rs9633840	T	T	1.805	4.526	6.01E-06
11	19483086	rs4757000	G	G	1.803	4.519	6.21E-06
18	28350663	rs140901585	A	G	5.296	4.518	6.25E-06
8	3552027	rs2624059	G	C	1.973	4.515	6.34E-06
7	123959767	rs72569177	C	T	1.796	4.515	6.34E-06
13	41895087	rs9525472	T	C	2.905	4.513	6.39E-06
11	19483304	rs4757001	G	G	1.802	4.512	6.42E-06
19	18908436	rs74346119	T	A	2.067	4.512	6.43E-06
4	83293596	rs116281082	C	T	5.269	4.512	6.44E-06
1	218546474	rs149269977	T	C	4.278	4.502	6.73E-06
11	19486772	rs2729876	G	A	1.794	4.5	6.81E-06
11	19487089	rs2702635	A	G	1.794	4.5	6.81E-06
11	19487065	rs2702636	T	G	1.794	4.5	6.81E-06
4	120476490	rs62319607	T	A	3.495	4.496	6.93E-06
1	245003778	rs41269391	T	C	6.562	4.49	7.13E-06
1	167849701	rs203850	A	G	6.381	4.488	7.20E-06
9	72526318	rs6559847	T	C	1.927	4.487	7.23E-06
7	123957875	rs7807675	T	C	1.782	4.477	7.58E-06
7	123967410	rs6962230	C	T	1.791	4.471	7.80E-06
7	123967411	rs6980369	A	G	1.791	4.471	7.80E-06
2	240394854	rs6710481	C	T	2.229	4.469	7.85E-06
2	240394611	rs6706469	T	A	2.263	4.466	7.95E-06
1	218502090	rs17558084	T	A	3.875	4.461	8.14E-06

Appendix Table 14 Continued.

5	18385650	rs201175189	CTTTAG	C	3.456	4.461	8.16E-06
3	165231082	rs77309421	A	G	5.883	4.459	8.22E-06
8	3550647	rs76583473	T	A	2.239	4.459	8.23E-06
5	6902327	rs2047325	T	T	3.293	4.459	8.24E-06
13	109065322	rs200726793	A	ATAACT	4.336	4.456	8.36E-06
8	3555970	rs2449208	T	C	2.203	4.451	8.55E-06
8	3555985	rs2449207	C	T	2.203	4.451	8.55E-06
7	123971770	rs6963923	A	G	1.782	4.444	8.83E-06
9	95211713	rs192417371	G	T	3.39	4.442	8.93E-06
7	123967319	rs6962063	T	C	1.782	4.441	8.96E-06
13	35040313	rs117805826	G	A	3.198	4.441	8.97E-06
7	123973340	rs6945501	A	G	1.78	4.439	9.03E-06
6	121143347	rs565921615	C	G	4.575	4.436	9.16E-06
9	5121726	rs111578703	G	A	3.687	4.436	9.16E-06
14	97993431	rs10142577	C	T	4.218	4.429	9.46E-06
14	97991917	rs7152926	A	G	4.218	4.429	9.46E-06
14	97992149	rs7158627	C	T	4.218	4.429	9.46E-06
14	97993274	rs10142467	C	T	4.218	4.429	9.46E-06
14	97993509	rs10142666	G	T	4.218	4.429	9.46E-06
14	97994343	14_97994343	CTATG	C	4.218	4.429	9.46E-06
11	67507419	rs12146441	C	T	2.799	4.42	9.86E-06
5	18385659	rs57661894	T	G	3.411	4.418	9.98E-06
5	18385658	rs61541330	C	A	3.411	4.418	9.98E-06

Appendix Table 15. Gene-Based Association Analysis Results for Radiation-Induced Tinnitus (P < 0.001).

GENE	SYMBOL	CHR	START	STOP	NSNPS	NPARAM	N	ZSTAT	P
ENSG00000143164	DCAF6	1	167905021	168045081	272	19	1991	4.2989	8.58E-06
ENSG00000166833	NAV2	11	19372271	20143144	2672	248	1991	4.1589	1.60E-05
ENSG00000204740	MALRD1	10	19492779	20079330	2852	94	1991	3.9881	3.33E-05
ENSG00000215527	AP005482.1	18	12658042	12663470	24	4	1991	3.7099	0.00010368
ENSG00000159579	RSPRY1	16	57220049	57274387	126	16	1991	3.682	0.00011572
ENSG00000166368	OR2D2	11	6912721	6913830	3	2	1991	3.5394	0.00020052
ENSG00000108039	XPNPEP1	10	111624524	111683311	117	16	1991	3.4711	0.00025915
ENSG00000198952	SMG5	1	156219015	156252620	97	11	1991	3.4689	0.00026128
ENSG00000163468	CCT3	1	156278759	156337664	151	12	1991	3.4619	0.00026818
ENSG00000163472	TMEM79	1	156252726	156262976	20	4	1991	3.4347	0.00029661
ENSG00000132670	PTPRA	20	2844830	3019722	333	18	1991	3.4271	0.00030499
ENSG00000215217	C5orf49	5	7830491	7851603	82	11	1991	3.4149	0.00031903
ENSG00000172731	LRRC20	10	72058729	72142382	372	35	1991	3.3982	0.00033914
ENSG00000124302	CHST8	19	34112861	34264414	597	70	1991	3.3865	0.00035397
ENSG00000197757	HOXC6	12	54384408	54424607	78	14	1991	3.363	0.00038546
ENSG00000128789	PSMG2	18	12658737	12725739	225	17	1991	3.3614	0.00038779
ENSG00000150676	CCDC83	11	85566144	85631064	238	19	1991	3.3609	0.00038851
ENSG00000120436	GPR31	6	167569759	167571817	4	2	1991	3.3356	0.00042559
ENSG00000198715	C1orf85	1	156259880	156265463	12	4	1991	3.3346	0.00042707
ENSG00000184185	KCNJ12	17	21279509	21323179	189	80	1991	3.3329	0.00042978
ENSG00000273049	RP11-834C11.12	12	54379629	54428672	95	16	1991	3.3183	0.00045276
ENSG00000101624	CEP76	18	12661832	12702776	142	13	1991	3.3107	0.00046526
ENSG00000170537	TMC7	16	18995256	19075264	274	17	1991	3.2825	0.00051448
ENSG00000128283	CDC42EP1	22	37956454	37965412	27	7	1991	3.2257	0.00062838
ENSG00000132849	INADL	1	62208149	62629592	1686	102	1991	3.1946	0.00070021
ENSG00000151116	UEVLD	11	18551156	18610294	209	23	1991	3.183	0.00072876
ENSG00000160781	PAQR6	1	156213206	156217881	7	4	1991	3.1281	0.00087983

Appendix Table 15 Continued.

ENSG00000183310	OR2T34	1	248737022	248738080	5	2	1991	3.1246	0.00089012
ENSG00000158106	RHPN1	8	144451057	144466390	54	9	1991	3.1241	0.00089188
ENSG00000138293	NCOA4	10	51565108	51590734	34	8	1991	3.1103	0.0009344
ENSG00000163467	TSACC	1	156307105	156316786	19	6	1991	3.1053	0.0009505

Appendix Table 16. GWAS Results for Radiation-Induced Hearing Loss ($P < 1x10^{-5}$), Adjusting for Age at Last Observation, Cumulative Cranial Radiation Dose, and 20 European Principal Components.

CHR	BP	SNP	EFFECT	REFERENCE	OR	STAT	P
8	8759261	rs332013	T	C	0.5664	-4.998	5.79E-07
6	16736244	rs67522722	C	G	2.469	4.941	7.78E-07
6	16734812	rs72827827	T	C	2.466	4.935	8.03E-07
6	16711676	rs34533789	G	A	2.429	4.898	9.69E-07
6	16729433	rs34675197	T	C	2.446	4.89	1.01E-06
6	16731097	rs67805820	C	T	2.446	4.89	1.01E-06
17	57378490	rs76751661	C	T	2.407	4.89	1.01E-06
6	16714933	rs13220378	T	C	2.421	4.867	1.14E-06
6	16748948	rs72827833	G	T	3.076	4.753	2.01E-06
4	150781561	rs13140340	G	A	2.316	4.743	2.10E-06
5	93213420	rs17314423	C	T	1.622	4.694	2.67E-06
17	10678422	rs141272251	A	G	3.514	4.687	2.78E-06
17	10677832	rs186509238	A	C	3.514	4.687	2.78E-06
3	124337973	rs11455473	CT	C	1.777	4.685	2.80E-06
12	1617348	rs56285428	C	T	3.878	4.683	2.83E-06
3	12500065	rs28595399	C	T	1.622	4.661	3.15E-06
5	93196537	rs111886364	C	T	1.617	4.654	3.26E-06
17	57416562	rs75559421	G	T	2.285	4.646	3.38E-06
17	57420076	rs117182204	A	C	2.261	4.63	3.65E-06
6	16751926	rs72827835	T	C	3.015	4.628	3.70E-06
6	16756944	rs16879071	A	G	3.001	4.613	3.96E-06
15	82189860	rs72745984	A	G	2.111	4.606	4.11E-06
1	220892716	rs76972700	T	C	2.61	4.595	4.32E-06
5	62164941	rs10447104	A	G	1.719	4.581	4.64E-06
18	74330301	rs17059651	G	A	1.879	4.57	4.88E-06
5	62169754	rs3107557	T	A	1.713	4.552	5.32E-06

Appendix Table 16 Continued.

5	62157949	rs2140044	T	C	1.715	4.546	5.47E-06
5	96416985	rs35858014	CATAT	C	2.063	4.546	5.47E-06
13	25909341	rs17499463	C	T	4.575	4.544	5.53E-06
13	25913193	rs75274095	G	C	4.574	4.543	5.54E-06
20	58616328	rs6071091	A	G	0.6189	-4.541	5.60E-06
6	16761715	6_16761715	TCTC	TCTCCTC	2.377	4.531	5.87E-06
2	60475820	rs359244	A	G	0.6369	-4.529	5.93E-06
13	105355049	rs773375	C	T	1.637	4.519	6.20E-06
5	62161578	rs141186166	C	T	1.707	4.515	6.34E-06
5	108248885	rs9326754	T	C	1.993	4.515	6.34E-06
5	62162549	rs7701138	G	C	1.705	4.502	6.73E-06
1	224812470	rs144997352	T	C	3.761	4.496	6.92E-06
5	146902304	rs35694396	T	C	2.007	4.493	7.01E-06
8	38320976	rs150512143	C	T	3.47	4.491	7.08E-06
4	38293599	rs201161162	AC	A	0.5808	-4.489	7.15E-06
18	74329307	rs9945989	G	A	1.859	4.486	7.27E-06
18	74329300	rs9948432	C	T	1.859	4.486	7.27E-06
7	75487187	rs118040706	C	T	3.298	4.485	7.31E-06
1	224777924	rs140603600	C	T	3.748	4.484	7.32E-06
4	96328935	rs116622294	A	G	3.977	4.481	7.43E-06
5	93257240	rs2167763	G	A	1.583	4.48	7.47E-06
2	60473094	rs359237	T	C	0.64	-4.48	7.47E-06
2	60471823	rs359235	A	G	0.6402	-4.477	7.58E-06
2	60472298	rs359236	A	G	0.6403	-4.476	7.62E-06
10	133343618	rs116875824	A	G	4.054	4.472	7.75E-06
5	62166515	rs57448893	CAAAAAAAA	C	1.69	4.471	7.78E-06
6	16765502	rs628572	T	C	2.307	4.468	7.89E-06
16	68754479	rs117777238	A	G	3.914	4.465	8.00E-06
16	68743256	rs148442070	G	C	3.914	4.465	8.00E-06
18	74330000	rs9959420	G	A	1.854	4.464	8.06E-06
5	62166979	rs1521994	T	A	1.687	4.458	8.26E-06

Appendix Table 16 Continued.

2	60466491	rs359273	A	G	0.6404	-4.457	8.33E-06
2	225045293	rs534951162	T	C	4.968	4.443	8.86E-06
18	74328029	rs9952968	A	G	1.848	4.443	8.88E-06
8	18378221	rs10099199	C	T	0.4702	-4.441	8.97E-06
5	62169769	rs3107558	A	C	1.683	4.438	9.06E-06
18	74326971	rs28637216	C	T	1.847	4.438	9.08E-06
18	74327084	rs17059643	T	C	1.847	4.438	9.08E-06
8	66680248	rs74805159	A	G	2.461	4.437	9.11E-06
5	93286329	5_93286329	CA	C	1.574	4.436	9.15E-06
5	62160942	rs151251104	A	C	1.683	4.433	9.27E-06
8	120405472	rs16892488	A	G	4.096	4.426	9.60E-06
8	120403141	rs117331970	T	C	4.096	4.426	9.60E-06
5	62157962	rs2177101	G	C	1.684	4.424	9.70E-06
5	62158290	rs3111617	A	G	1.684	4.424	9.70E-06
5	62158542	rs3111614	A	G	1.683	4.422	9.79E-06
18	74330869	rs937897	C	G	1.852	4.421	9.83E-06
3	64930453	rs73126001	C	A	2.341	4.419	9.93E-06
5	62164981	rs10447086	G	T	1.68	4.418	9.98E-06
3	64919406	rs112175640	T	C	2.324	4.418	9.98E-06

Appendix Table 17. Gene-Based Association Analysis Results for Radiation-Induced Hearing Loss (p < 0.001).

GENE	SYMBOL	CHR	START	STOP	NSNPS	NPARAM	N	ZSTAT	P
ENSG00000165929	TC2N	14	92246095	92333880	225	21	2198	3.6461	0.00013313
ENSG00000198961	PJA2	5	108670410	108745695	336	16	2198	3.6281	0.00014274
ENSG00000174156	GSTA3	6	52761437	52774483	43	8	2198	3.3899	0.00034959
ENSG00000089818	NECAP1	12	8234807	8250367	32	10	2198	3.3248	0.00044235
ENSG00000108379	WNT3	17	44839872	44910520	156	29	2198	3.2958	0.00049077
ENSG00000137821	LRRC49	15	71145578	71342414	274	22	2198	3.2534	0.0005702
ENSG00000152439	ZNF773	19	58011283	58029772	143	5	2198	3.2514	0.00057425
ENSG00000163171	CDC42EP3	2	37869032	37965611	448	46	2198	3.2141	0.00065438
ENSG00000185332	TMEM105	17	79285074	79304474	84	9	2198	3.2093	0.00066531
ENSG00000062524	LTK	15	41795836	41806085	32	8	2198	3.1919	0.00070681
ENSG00000249008	RP11-487E13.1	4	178163693	178169927	31	8	2198	3.1879	0.0007166
ENSG00000149654	CDH22	20	44802372	44937137	345	51	2198	3.1583	0.00079342
ENSG00000225362	CT62	15	71402583	71407833	12	2	2198	3.1565	0.00079842
ENSG00000075568	TMEM131	2	98372799	98612388	585	20	2198	3.1516	0.00081197
ENSG00000175727	MLXIP	12	122516628	122631894	236	8	2198	3.1362	0.0008558
ENSG00000186522	10-Sep	2	110300559	110371783	227	25	2198	3.1218	0.00089887
ENSG00000119669	IRF2BPL	14	77490888	77495034	9	4	2198	3.094	0.00098725

Appendix Table 18. Functional Enrichment Analysis of Most Significant SNPs ($p < 5x10^{-4}$) in GWAS of Radiation-Associated Ototoxicity.

Tinnitus

HP

term.id	domain	term.name	p	subgraph.number	relative.depth
HP:0000370	HP	Abnormality of the middle ear	0.04		1

KEGG

term.id	domain	term.name	p	subgraph.number	relative.depth
KEGG:04015	keg	Rap1 signaling pathway	0.04		1
KEGG:01521	keg	EGFR tyrosine kinase inhibitor resistance	0.04		1

GO

term.id	domain	term.name	p	subgraph.number	relative.depth
GO:0007155	BP	cell adhesion	0.000275	7	2
GO:0022610	BP	biological adhesion	0.000335	7	1
GO:0018149	BP	peptide cross-linking	0.000419	9	1
GO:0007275	BP	multicellular organism development	0.000563	12	3
GO:0048731	BP	system development	0.000699	12	3
GO:0032501	BP	multicellular organismal process	0.00128	12	1
GO:0007416	BP	synapse assembly	0.00466	8	1
GO:0032502	BP	developmental process	0.00466	12	1
GO:0050808	BP	synapse organization	0.0059	8	1
GO:0048856	BP	anatomical structure development	0.00677	12	2
GO:0035303	BP	regulation of dephosphorylation	0.00705	1	1
GO:0099560	BP	synaptic membrane adhesion	0.00843	8	2
GO:0009887	BP	animal organ morphogenesis	0.0108	12	3
GO:0009653	BP	anatomical structure morphogenesis	0.0141	12	2
GO:0034220	BP	ion transmembrane transport	0.0143	4	1
GO:0048513	BP	animal organ development	0.0164	12	3
GO:0009612	BP	response to mechanical stimulus	0.0221	16	1

Appendix Table 18 Continued.

GO:0050896	BP	response to stimulus	0.0375	2	1
GO:0097120	BP	receptor localization to synapse	0.0441	17	1
GO:0071709	BP	membrane assembly	0.0441	13	1
GO:0010921	BP	regulation of phosphatase activity	0.0441	1	2
GO:0055085	BP	transmembrane transport	0.0479	4	1
GO:0120025	CC	plasma membrane bounded cell projection	4.68E-06	10	2
GO:0042995	CC	cell projection	6.82E-06	10	1
GO:0005886	CC	plasma membrane	2.99E-05	3	2
GO:0071944	CC	cell periphery	4.20E-05	3	1
GO:0044459	CC	plasma membrane part	4.20E-05	3	3
GO:0030054	CC	cell junction	5.56E-05	5	1
GO:0098590	CC	plasma membrane region	0.000153	3	4
GO:0043005	CC	neuron projection	0.000153	10	3
GO:0044463	CC	cell projection part	0.000153	10	2
GO:0120038	CC	plasma membrane bounded cell projection part	0.000153	10	3
GO:0097458	CC	neuron part	0.00109	10	1
GO:0030424	CC	axon	0.00109	10	4
GO:0044297	CC	cell body	0.00336	10	1
GO:0001533	CC	cornified envelope	0.00363	3	3
GO:0045202	CC	synapse	0.00516	6	1
GO:0005887	CC	integral component of plasma membrane	0.00818	3	1
GO:0031226	CC	intrinsic component of plasma membrane	0.01	3	4
GO:0016020	CC	membrane	0.0108	3	1
GO:0036477	CC	somatodendritic compartment	0.0108	10	2
GO:0005912	CC	adherens junction	0.0119	5	3
GO:0070161	CC	anchoring junction	0.0126	5	2
GO:0030425	CC	dendrite	0.016	10	4
GO:0097447	CC	dendritic tree	0.0166	10	4
GO:0005856	CC	cytoskeleton	0.0308	11	1
GO:0033267	CC	axon part	0.0354	10	4

Appendix Table 18 Continued.

GO:0043025	CC	neuronal cell body	0.0395	10	2
GO:0044425	CC	membrane part	0.0429	3	2
GO:0008179	MF	adenylate cyclase binding	0.0445	14	1
GO:0019901	MF	protein kinase binding	0.0445	15	1
Hearing Loss					
HP					
term.id	**domain**	**term.name**	**p**	**subgraph.number**	**relative.depth**
HP:0007925	HP	Lacrimal duct aplasia	0.03	1	1
KEGG					
term.id	**domain**	**term.name**	**p**	**subgraph.number**	**relative.depth**
KEGG:04015	keg	Rap1 signaling pathway	0.01	1	1
GO					
term.id	**domain**	**term.name**	**p**	**subgraph.number**	**relative.depth**
GO:1905114	BP	cell surface receptor signaling pathway involved in cell-cell signaling	0.000116	25	4
GO:0007267	BP	cell-cell signaling	0.000481	25	2
GO:0022610	BP	biological adhesion	0.00129	16	1
GO:0007155	BP	cell adhesion	0.00129	16	2
GO:0048856	BP	anatomical structure development	0.00129	2	2
GO:0032989	BP	cellular component morphogenesis	0.00129	2	1
GO:0000902	BP	cell morphogenesis	0.00129	2	2
GO:0198738	BP	cell-cell signaling by wnt	0.0014	25	3
GO:2000147	BP	positive regulation of cell motility	0.00146	25	3
GO:0016055	BP	Wnt signaling pathway	0.00232	25	5
GO:0032502	BP	developmental process	0.00232	2	1
GO:0007154	BP	cell communication	0.00244	25	1
GO:0051272	BP	positive regulation of cellular component movement	0.00244	25	2
GO:1901699	BP	cellular response to nitrogen compound	0.00258	25	1
GO:0030111	BP	regulation of Wnt signaling pathway	0.00258	25	1
GO:0009653	BP	anatomical structure morphogenesis	0.00258	2	2
GO:1901700	BP	response to oxygen-containing compound	0.0032	25	3

Appendix Table 18 Continued.

GO:0040017	BP	positive regulation of locomotion	0.0032	25	2
GO:0023052	BP	signaling	0.0032	25	1
GO:0030335	BP	positive regulation of cell migration	0.00343	25	4
GO:0048468	BP	cell development	0.00363	2	3
GO:0048513	BP	animal organ development	0.00385	2	3
GO:0032879	BP	regulation of localization	0.0041	25	1
GO:0098916	BP	anterograde trans-synaptic signaling	0.0041	25	5
GO:0007268	BP	chemical synaptic transmission	0.0041	25	6
GO:0099536	BP	synaptic signaling	0.00419	25	3
GO:0099537	BP	trans-synaptic signaling	0.00419	25	4
GO:1901698	BP	response to nitrogen compound	0.00426	25	3
GO:0051716	BP	cellular response to stimulus	0.00426	25	2
GO:2000145	BP	regulation of cell motility	0.00426	25	2
GO:0030178	BP	negative regulation of Wnt signaling pathway	0.00426	25	2
GO:0007399	BP	nervous system development	0.00426	2	4
GO:0048869	BP	cellular developmental process	0.00426	2	2
GO:0007166	BP	cell surface receptor signaling pathway	0.00447	25	3
GO:0051270	BP	regulation of cellular component movement	0.00518	25	1
GO:0030334	BP	regulation of cell migration	0.00773	25	3
GO:0050808	BP	synapse organization	0.00782	1	1
GO:0034332	BP	adherens junction organization	0.00782	21	1
GO:0009719	BP	response to endogenous stimulus	0.009	25	2
GO:0009725	BP	response to hormone	0.0097	25	3
GO:1901701	BP	cellular response to oxygen-containing compound	0.0097	25	4
GO:0040012	BP	regulation of locomotion	0.0097	25	2
GO:0065007	BP	biological regulation	0.00991	25	1
GO:0097062	BP	dendritic spine maintenance	0.00991	1	1
GO:0048731	BP	system development	0.0101	2	3
GO:0007165	BP	signal transduction	0.0106	25	2

Appendix Table 18 Continued.

GO:0060560	BP	developmental growth involved in morphogenesis	0.0106	2	3
GO:0071417	BP	cellular response to organonitrogen compound	0.0112	25	2
GO:0001964	BP	startle response	0.0114	5	1
GO:0001101	BP	response to acid chemical	0.0116	25	3
GO:0040011	BP	locomotion	0.0122	25	1
GO:0016477	BP	cell migration	0.0122	25	3
GO:0007275	BP	multicellular organism development	0.0125	2	3
GO:0061061	BP	muscle structure development	0.0129	2	3
GO:0048699	BP	generation of neurons	0.0129	2	5
GO:0050789	BP	regulation of biological process	0.0153	25	2
GO:0048511	BP	rhythmic process	0.0153	13	1
GO:0060078	BP	regulation of postsynaptic membrane potential	0.0161	25	2
GO:0032906	BP	transforming growth factor beta2 production	0.0164	8	1
GO:0032909	BP	regulation of transforming growth factor beta2 production	0.0164	8	2
GO:0006928	BP	movement of cell or subcellular component	0.0176	25	1
GO:0010243	BP	response to organonitrogen compound	0.0176	25	4
GO:0010646	BP	regulation of cell communication	0.0176	25	2
GO:0042330	BP	taxis	0.0176	25	1
GO:0006935	BP	chemotaxis	0.0176	25	2
GO:0060079	BP	excitatory postsynaptic potential	0.0176	25	3
GO:0090090	BP	negative regulation of canonical Wnt signaling pathway	0.0176	25	2
GO:0003008	BP	system process	0.0176	2	2
GO:0051128	BP	regulation of cellular component organization	0.0176	2	1
GO:0022604	BP	regulation of cell morphogenesis	0.0176	2	2
GO:0008286	BP	insulin receptor signaling pathway	0.0176	27	1
GO:0051703	BP	intraspecies interaction between organisms	0.0178	15	1
GO:0035176	BP	social behavior	0.0178	15	2

Appendix Table 18 Continued.

GO:0023051	BP	regulation of signaling	0.018	25	2
GO:0030182	BP	neuron differentiation	0.0183	2	4
GO:0014812	BP	muscle cell migration	0.0189	25	4
GO:0046777	BP	protein autophosphorylation	0.0199	24	1
GO:0051674	BP	localization of cell	0.0199	25	1
GO:0048870	BP	cell motility	0.0199	25	2
GO:0106027	BP	neuron projection organization	0.0199	1	1
GO:1905874	BP	regulation of postsynaptic density organization	0.0199	20	1
GO:0032501	BP	multicellular organismal process	0.0202	2	1
GO:0022603	BP	regulation of anatomical structure morphogenesis	0.0211	2	3
GO:0000904	BP	cell morphogenesis involved in differentiation	0.0213	2	3
GO:0060693	BP	regulation of branching involved in salivary gland morphogenesis	0.0213	19	1
GO:0032870	BP	cellular response to hormone stimulus	0.0217	25	1
GO:0050896	BP	response to stimulus	0.0227	25	1
GO:0099565	BP	chemical synaptic transmission	0.0228	25	
GO:0050905	BP	neuromuscular process	0.0243	5	1
GO:0010648	BP	negative regulation of cell communication	0.0245	25	3
GO:0072359	BP	circulatory system development	0.0246	2	4
GO:0030154	BP	cell differentiation	0.0246	2	3
GO:0023057	BP	negative regulation of signaling	0.0258	25	1
GO:0030030	BP	cell projection organization	0.0264	2	1
GO:0097485	BP	neuron projection guidance	0.0272	25	1
GO:0044093	BP	positive regulation of molecular function	0.0275	4	1
GO:0018105	BP	peptidyl-serine phosphorylation	0.03	11	1
GO:0120039	BP	plasma membrane bounded cell projection morphogenesis	0.03	2	3
GO:0031344	BP	regulation of cell projection organization	0.03	2	2
GO:0048666	BP	neuron development	0.0303	2	4
GO:0048667	BP	cell morphogenesis involved in neuron differentiation	0.0311	2	4

Appendix Table 18 Continued.

GO:0018209	BP	peptidyl-serine modification	0.0313	11	1
GO:0050919	BP	negative chemotaxis	0.0313	25	3
GO:0032990	BP	cell part morphogenesis	0.0317	2	2
GO:0048858	BP	cell projection morphogenesis	0.0317	2	2
GO:0097061	BP	dendritic spine organization	0.0321	1	3
GO:0042221	BP	response to chemical	0.0325	25	2
GO:0001525	BP	angiogenesis	0.0342	12	1
GO:0022008	BP	neurogenesis	0.0348	2	4
GO:0098735	BP	positive regulation of the force of heart contraction	0.0353	22	1
GO:0031589	BP	cell-substrate adhesion	0.0353	16	3
GO:0010882	BP	regulation of cardiac muscle contraction by calcium ion signaling	0.0353	7	1
GO:0031175	BP	neuron projection development	0.0364	2	5
GO:0031346	BP	positive regulation of cell projection organization	0.0364	2	1
GO:0007420	BP	brain development	0.0366	2	4
GO:0120035	BP	regulation of plasma membrane bounded cell projection organization	0.0369	2	3
GO:0051593	BP	response to folic acid	0.0376	25	1
GO:0007613	BP	memory	0.0376	18	1
GO:0050918	BP	positive chemotaxis	0.0383	25	3
GO:0060322	BP	head development	0.0383	2	3
GO:0042391	BP	regulation of membrane potential	0.0395	25	1
GO:0032869	BP	cellular response to insulin stimulus	0.0395	27	1
GO:1901653	BP	cellular response to peptide	0.0417	25	3
GO:0048585	BP	negative regulation of response to stimulus	0.043	25	1
GO:0099173	BP	postsynapse organization	0.043	1	2
GO:0060828	BP	regulation of canonical Wnt signaling pathway	0.0448	25	1
GO:0007411	BP	axon guidance	0.0471	25	2
GO:0048583	BP	regulation of response to stimulus	0.0475	25	2
GO:0008360	BP	regulation of cell shape	0.0488	2	3

Appendix Table 18 Continued.

GO:0043085	BP	positive regulation of catalytic activity	0.0488	4	1
GO:0045202	CC	synapse	0.00109	3	1
GO:0071944	CC	cell periphery	0.00129	3	1
GO:0005886	CC	plasma membrane	0.00146	3	1
GO:0044459	CC	plasma membrane part	0.00244	3	2
GO:0098794	CC	postsynapse	0.0031	3	1
GO:0030054	CC	cell junction	0.0032	31	1
GO:0044425	CC	membrane part	0.00343	3	2
GO:0016020	CC	membrane	0.00394	3	1
GO:0044456	CC	synapse part	0.0041	3	1
GO:0097458	CC	neuron part	0.00419	3	1
GO:0031224	CC	intrinsic component of membrane	0.00419	3	3
GO:0098590	CC	plasma membrane region	0.00426	3	3
GO:0097060	CC	synaptic membrane	0.00426	3	4
GO:0098797	CC	plasma membrane protein complex	0.00426	3	1
GO:0045211	CC	postsynaptic membrane	0.00598	3	2
GO:0016021	CC	integral component of membrane	0.0106	3	4
GO:0031012	CC	extracellular matrix	0.0151	23	1
GO:0120025	CC	plasma membrane bounded cell projection	0.0178	3	2
GO:0098589	CC	membrane region	0.0183	3	1
GO:0042995	CC	cell projection	0.0227	3	1
GO:0043005	CC	neuron projection	0.0227	3	3
GO:0043235	CC	receptor complex	0.0245	26	1
GO:0048786	CC	presynaptic active zone	0.0246	3	1
GO:0031226	CC	intrinsic component of plasma membrane	0.03	3	3
GO:0042383	CC	sarcolemma	0.0353	3	2
GO:0098857	CC	membrane microdomain	0.0376	3	2
GO:0045121	CC	membrane raft	0.0376	3	3
GO:0043167	MF	ion binding	0.00129	6	1
GO:0019899	MF	enzyme binding	0.0032	32	1
GO:0030169	MF	low-density lipoprotein particle binding	0.0032	28	3

Appendix Table 18 Continued.

GO:0071814	MF	protein-lipid complex binding	0.0068	28	1
GO:0071813	MF	lipoprotein particle binding	0.0068	28	2
GO:0043169	MF	cation binding	0.00782	6	2
GO:0005102	MF	signaling receptor binding	0.0101	17	1
GO:0005509	MF	calcium ion binding	0.0106	6	4
GO:0008013	MF	beta-catenin binding	0.0129	14	1
GO:0004714	MF	transmembrane receptor protein tyrosine kinase activity	0.0176	10	1
GO:0046872	MF	metal ion binding	0.0178	6	3
GO:0019199	MF	transmembrane receptor protein kinase activity	0.0246	10	1
GO:0030695	MF	GTPase regulator activity	0.0292	29	1
GO:0070513	MF	death domain binding	0.0295	9	1
GO:0008144	MF	drug binding	0.0295	30	1

Appendix Table 19. GWAS Results for Age-Related Hearing Loss in Patients of European Ancestry ($p < 1x10^{-6}$).

CHR	BP	SNP	EFFECT	REFERENCE	OR	STAT	P
6	32605525	rs9272454	T	C	0.7935	-6.557	5.49E-11
6	32520907	rs3828840	C	T	0.8063	-5.476	4.36E-08
6	32511725	rs68148149	A	C	0.8078	-5.427	5.74E-08
9	83440246	rs114510757	G	A	20.93	5.311	1.09E-07
9	83413883	rs12002000	T	C	23.75	5.176	2.27E-07
9	83414951	rs78000763	A	C	23.75	5.176	2.27E-07
9	83415638	rs115181811	G	C	23.75	5.176	2.27E-07
9	83415639	rs115792541	G	A	23.75	5.176	2.27E-07
9	83416588	rs77837664	T	A	23.75	5.176	2.27E-07
9	83417077	rs112901552	T	C	23.75	5.176	2.27E-07
9	83417957	rs75369370	C	T	23.75	5.176	2.27E-07
9	83418869	rs79988151	T	C	23.75	5.176	2.27E-07
9	83419209	rs112897760	G	A	23.75	5.176	2.27E-07
9	83419971	rs112920846	C	A	23.75	5.176	2.27E-07
9	83420300	rs12002421	A	C	23.75	5.176	2.27E-07
9	83420846	rs876289	G	A	23.75	5.176	2.27E-07
9	83420846	rs112377444	G	A	23.75	5.176	2.27E-07
9	83422251	rs79336126	C	T	23.75	5.176	2.27E-07
9	83426823	rs12207727	T	C	23.75	5.176	2.27E-07
9	83426823	rs113871275	A	G	23.75	5.176	2.27E-07
9	83428608	rs7847714	A	G	23.75	5.176	2.27E-07
9	83431729	rs151272432	A	G	23.75	5.176	2.27E-07
9	83431761	rs113054643	G	A	23.75	5.176	2.27E-07
9	83434087	rs189738401	G	A	23.75	5.176	2.27E-07
9	83438293	rs11832789	G	A	23.75	5.176	2.27E-07
9	83438293	rs112576470	A	G	23.75	5.176	2.27E-07
9	83439053	rs113232445	T	C	23.75	5.176	2.27E-07

Appendix Table 19 Continued.

9	83439143	rs114977152	G	A	23.75	5.176	2.27E-07
9	83439559	rs78808397	A	G	23.75	5.176	2.27E-07
9	83439625	rs79314744	A	C	23.75	5.176	2.27E-07
9	83379877	rs77245261	C	T	26.13	5.144	2.69E-07
9	83382954	rs12003483	A	T	26.13	5.144	2.69E-07
9	83384336	rs12004061	C	T	26.13	5.144	2.69E-07
9	83404851	rs78616911	A	G	26.13	5.144	2.69E-07
9	83413607	rs9449532	A	G	26.13	5.144	2.69E-07
9	83413607	rs9449532	A	G	26.13	5.144	2.69E-07
9	83413607	rs113283869	A	G	26.13	5.144	2.69E-07
9	83417502	rs76521249	C	T	26.13	5.144	2.69E-07
9	83421342	rs75441551	T	C	26.13	5.144	2.69E-07
9	83425690	rs78438850	G	A	26.13	5.144	2.69E-07
9	83426363	rs12004927	A	T	26.13	5.144	2.69E-07
9	83426493	rs11999328	G	A	26.13	5.144	2.69E-07
9	83431698	rs143309055	A	C	26.13	5.144	2.69E-07
9	83432023	rs138706856	G	C	26.13	5.144	2.69E-07
9	83432820	rs145789584	T	C	26.13	5.144	2.69E-07
9	83434376	rs142694279	C	T	26.13	5.144	2.69E-07
9	83435303	rs11999981	T	G	26.13	5.144	2.69E-07
9	83435304	rs11999982	C	G	26.13	5.144	2.69E-07
9	83436419	rs75025786	A	G	26.13	5.144	2.69E-07
9	83436680	rs76968659	T	G	26.13	5.144	2.69E-07
9	83439821	rs74589527	C	A	26.13	5.144	2.69E-07
9	83440286	rs113537701	C	T	26.13	5.144	2.69E-07
9	83440524	rs112743269	A	G	26.13	5.144	2.69E-07
6	32584625	rs3129758	G	A	0.8349	-5.122	3.03E-07
6	32573574	rs9270970	T	C	0.8341	-5.037	4.73E-07
9	83424430	rs148448770	A	G	19.92	5.015	5.29E-07
2	242647404	rs62191342	G	C	0.8669	-4.998	5.81E-07
6	32577715	rs9271162	T	G	0.8359	-4.924	8.49E-07

Appendix Table 19 Continued.

11	1471095	rs117021012	T	C	1.294	4.918	8.73E-07
16	70187938	rs58878452	T	C	1.553	4.91	9.10E-07
8	74343932	rs11997512	A	G	1.151	4.906	9.28E-07
21	20985414	rs11679386	C	T	1.128	4.898	9.67E-07
21	20985414	rs9981304	A	G	1.128	4.898	9.67E-07
6	32587782	rs9271414	T	C	0.8471	-4.896	9.78E-07
6	32577733	rs9271163	T	C	0.8367	-4.895	9.83E-07

Appendix Table 20. GWAS Results for Age-Related Tinnitus in Patients of European Ancestry ($p < 5x10^{-8}$).

CHR	BP	SNP	EFFECT	REFERENCE	OR	STAT	P
6	32612336	rs9273081	A	G	0.6519	-9.164	5.02E-20
6	32612343	rs9273083	C	T	0.6782	-9.133	6.65E-20
6	32571876	rs9270896	G	A	0.708	-8.802	1.35E-18
6	32613746	rs9273216	A	C	0.6839	-8.77	1.79E-18
6	32571873	rs9270895	T	C	0.7148	-8.728	2.59E-18
6	32612339	rs9273082	T	C	0.6933	-8.594	8.41E-18
6	32612034	rs9273049	T	C	0.7001	-8.511	1.73E-17
6	32571403	rs543504257	C	A	0.6799	-8.464	2.58E-17
6	32613712	rs9273215	A	G	0.7016	-8.401	4.41E-17
6	32562023	rs534705706	A	G	0.6967	-8.273	1.31E-16
6	32607735	rs9272607	A	G	0.7039	-8.148	3.69E-16
6	32615578	rs17612590	A	G	0.7126	-8.139	3.99E-16
6	32613976	rs9273237	T	C	0.6629	-8.11	5.06E-16
6	32612309	rs9273078	A	T	0.6931	-7.831	4.85E-15
6	32609358	rs28383453	T	C	0.6676	-7.827	4.99E-15
6	32614749	rs17612489	A	T	0.6929	-7.767	8.03E-15
6	32607652	rs9272601	T	A	0.6961	-7.737	1.02E-14
6	32612601	rs112282948	C	T	0.7077	-7.668	1.74E-14
6	32612601	rs9273105	A	G	0.7077	-7.668	1.74E-14
6	32614552	rs9273290	T	C	0.6879	-7.66	1.85E-14
6	32536348	rs34624766	T	C	0.7215	-7.502	6.31E-14
6	32614334	rs9273268	C	T	0.6698	-7.468	8.14E-14
6	32611474	rs9272998	A	G	0.6449	-7.455	8.96E-14
6	32615652	rs17843583	C	A	0.7419	-7.355	1.91E-13
6	32531245	rs12661707	T	C	0.7218	-7.34	2.14E-13
6	32571845	rs9270893	T	C	0.7389	-7.298	2.93E-13
6	32607666	rs9272602	A	G	0.7334	-7.289	3.11E-13

Appendix Table 20 Continued.

6	32614883	rs17612510	T	C	0.7173	-7.284	3.25E-13
15	20405954	rs4114732	T	C	0.7636	-7.277	3.42E-13
6	32594916	rs9271824	G	A	0.7162	-7.215	5.41E-13
6	32612655	rs9273113	G	A	0.7128	-7.213	5.46E-13
6	32613163	rs9273167	A	C	0.7391	-7.212	5.52E-13
15	20399505	rs4114734	G	A	0.7658	-7.198	6.13E-13
6	32590463	rs9271568	A	G	0.6393	-7.196	6.20E-13
15	20393644	rs1840020	A	T	0.7666	-7.188	6.57E-13
15	20448056	rs8024950	G	A	0.7668	-7.136	9.62E-13
6	32613584	rs9273207	A	C	0.668	-7.12	1.08E-12
6	32607008	rs9272553	T	G	0.7603	-7.082	1.42E-12
6	32612627	rs9273109	G	A	0.7009	-7.076	1.48E-12
6	32607225	rs9272569	A	G	0.6888	-7.063	1.63E-12
6	32542148	rs9269458	G	A	0.7278	-7.041	1.91E-12
6	32538503	rs3819692	C	A	0.6649	-6.946	3.77E-12
6	32620469	rs17612816	G	T	0.7212	-6.929	4.24E-12
9	68335265	rs11262907	T	C	0.7628	-6.911	4.81E-12
6	32623348	rs9273335	G	A	0.7479	-6.911	4.82E-12
6	32560859	rs28366298	C	A	0.6753	-6.879	6.03E-12
6	32565465	rs2760981	A	G	0.7543	-6.848	7.48E-12
9	68328507	rs1948126	G	A	0.7652	-6.806	1.00E-11
6	32612326	rs9273079	T	C	0.6692	-6.782	1.19E-11
6	32610934	rs3667	G	A	0.6837	-6.748	1.50E-11
6	32594704	rs9271784	A	T	0.7326	-6.743	1.55E-11
6	32574736	rs618095	A	G	0.7108	-6.721	1.80E-11
6	32610994	rs1130161	A	C	0.6593	-6.711	1.94E-11
6	32556635	rs35331833	A	G	0.6877	-6.701	2.07E-11
6	32571903	rs9270898	C	T	1.293	6.69	2.23E-11
6	32613618	rs114931587	A	T	0.6825	-6.663	2.68E-11
6	32612251	rs9273071	G	T	0.6386	-6.635	3.25E-11
6	32634795	rs9274550	A	G	0.7438	-6.594	4.27E-11

Appendix Table 20 Continued.

9	68329250	rs11262902	C	T	0.7715	-6.592	4.33E-11
6	32612793	rs9273131	T	G	0.6639	-6.569	5.08E-11
6	32612733	rs9273123	A	G	0.7337	-6.563	5.27E-11
6	32627352	rs9273417	G	A	0.74	-6.546	5.91E-11
6	32561880	rs28366331	G	T	0.6799	-6.516	7.24E-11
6	32560757	rs9270562	T	G	0.7406	-6.509	7.54E-11
6	32561186	rs28366313	A	G	0.6765	-6.507	7.67E-11
6	32614037	rs34276369	T	C	0.7005	-6.502	7.91E-11
6	32615470	rs17612583	A	G	0.7278	-6.486	8.81E-11
9	68328670	rs1972894	A	G	0.7758	-6.474	9.57E-11
6	32594457	rs111477174	G	A	0.7334	-6.47	9.81E-11
6	32532385	rs72492343	T	C	0.7405	-6.452	1.11E-10
6	32561175	rs28366312	C	T	0.6604	-6.439	1.21E-10
6	32615421	rs7745002	G	A	0.7584	-6.432	1.26E-10
6	32611982	rs9273045	C	A	0.6913	-6.427	1.30E-10
6	32607660	rs28383392	G	A	0.6842	-6.421	1.36E-10
6	32594299	rs114969562	A	T	0.6889	-6.42	1.36E-10
6	32615446	rs17612562	G	T	0.7211	-6.419	1.37E-10
6	32618493	rs4952212	C	G	0.6577	-6.415	1.41E-10
6	32618493	rs545361737	A	G	0.6577	-6.415	1.41E-10
6	32561093	rs9270578	A	G	0.7395	-6.388	1.68E-10
6	32593585	rs9271727	A	G	0.6904	-6.386	1.70E-10
6	32612915	rs9273144	C	T	0.6784	-6.376	1.82E-10
6	32589282	rs9271498	A	G	0.7041	-6.369	1.90E-10
6	32593010	rs9271692	A	C	0.7664	-6.351	2.13E-10
6	32600602	rs9272129	T	C	0.7598	-6.351	2.14E-10
6	32566072	rs2284483	T	C	0.7022	-6.332	2.41E-10
6	32566072	rs9270658	C	T	0.7022	-6.332	2.41E-10
6	32594953	rs9271842	A	C	0.724	-6.328	2.48E-10
6	32549005	rs28732310	C	T	0.6496	-6.328	2.48E-10
6	32609453	rs28383456	T	C	0.7103	-6.296	3.05E-10

Appendix Table 20 Continued.

1	24294587	rs35233638	A	G	0.7812	-6.266	3.71E-10
6	32565164	rs28366358	G	A	0.6474	-6.265	3.73E-10
6	32509829	rs66521709	A	G	0.7422	-6.265	3.73E-10
6	32618822	rs375069308	T	C	0.6474	-6.257	3.92E-10
6	32569119	rs9270782	G	A	0.7164	-6.248	4.15E-10
6	32561885	rs28366332	A	C	0.6787	-6.243	4.28E-10
6	32623393	rs9273338	T	A	0.7752	-6.242	4.31E-10
6	32609673	rs10492389	C	T	0.6954	-6.231	4.64E-10
6	32609673	rs9272737	A	G	0.6954	-6.231	4.64E-10
6	32615458	rs17612576	A	G	0.7322	-6.203	5.53E-10
6	32609286	rs1048087	T	C	0.7409	-6.2	5.65E-10
6	32553125	rs113600317	T	A	0.7288	-6.198	5.73E-10
6	32612843	rs9273138	T	G	0.6357	-6.19	6.01E-10
6	32561068	rs9270576	T	C	0.7332	-6.181	6.37E-10
6	32595194	rs116603449	T	C	0.7413	-6.17	6.83E-10
6	32565056	rs28366355	G	T	0.7376	-6.15	7.73E-10
6	32620454	rs73573027	A	G	0.7654	-6.135	8.49E-10
6	32620454	rs17843608	C	A	0.7654	-6.135	8.49E-10
6	32589305	rs77947651	T	C	0.6936	-6.118	9.49E-10
6	32589305	rs9271502	A	G	0.6936	-6.118	9.49E-10
6	32593507	rs9271720	A	G	0.7669	-6.115	9.64E-10
6	32609106	rs118085305	A	G	0.7188	-6.104	1.04E-09
6	32609106	rs746358168	TAAC	T	0.7188	-6.104	1.04E-09
1	24288476	rs3211053	C	T	0.7867	-6.101	1.06E-09
6	32570400	rs2516049	C	T	0.6582	-6.092	1.12E-09
6	32509579	rs28819746	C	G	0.7498	-6.09	1.13E-09
6	32619188	rs527589011	C	T	0.7174	-6.083	1.18E-09
6	32607611	rs9272598	T	G	0.7137	-6.079	1.21E-09
6	32611971	rs455893	A	G	0.6896	-6.077	1.22E-09
6	32611971	rs9273044	C	G	0.6896	-6.077	1.22E-09
6	32509979	rs72492311	T	C	0.7506	-6.076	1.23E-09

Appendix Table 20 Continued.

6	32619099	rs574576046	T	A	0.7129	-6.072	1.26E-09
6	32565115	rs16834877	C	T	0.7396	-6.065	1.32E-09
6	32565115	rs9270614	A	C	0.7396	-6.065	1.32E-09
6	32613997	rs9273238	G	A	0.6909	-6.064	1.32E-09
6	32564699	rs28366337	G	A	0.7575	-6.057	1.39E-09
6	32612161	rs9273062	A	C	0.7019	-6.055	1.41E-09
1	24335104	rs4474201	G	T	0.7918	-6.016	1.78E-09
6	32608931	rs9272679	A	T	0.7932	-6.005	1.91E-09
6	32553816	rs200771848	T	C	0.7332	-6.004	1.92E-09
6	32570401	rs2454138	A	G	0.6849	-6.003	1.94E-09
6	32514144	rs34369284	T	C	0.754	-6	1.98E-09
6	32561201	rs28366314	G	A	0.7119	-5.997	2.01E-09
6	32613680	rs9273211	A	G	0.6433	-5.99	2.10E-09
6	32521083	rs3828832	C	T	0.7508	-5.989	2.11E-09
17	77351123	rs77938915	A	G	12.44	5.984	2.18E-09
6	136861575	rs746973	A	C	1.476	5.978	2.26E-09
7	61520749	rs36188061	C	G	0.7062	-5.962	2.50E-09
7	61520403	rs141542022	T	C	0.7062	-5.952	2.65E-09
6	32504757	rs72908914	C	A	0.7552	-5.944	2.78E-09
6	32504757	rs67529500	C	G	0.7552	-5.944	2.78E-09
7	61522670	rs141792915	C	T	0.7073	-5.941	2.83E-09
6	32574190	rs615698	G	A	0.736	-5.93	3.03E-09
6	32627310	rs9273415	T	C	0.7793	-5.924	3.14E-09
6	32620800	rs17843620	G	A	0.768	-5.922	3.18E-09
6	32623487	rs9273348	G	T	0.7374	-5.918	3.27E-09
6	32539391	rs9269285	C	T	0.6774	-5.909	3.44E-09
6	32607453	rs9272583	T	C	0.7965	-5.892	3.82E-09
6	32550819	rs28724031	A	C	0.7319	-5.888	3.90E-09
6	32572290	rs9270915	A	G	0.776	-5.887	3.93E-09
6	32523627	rs77501456	G	A	0.7432	-5.886	3.96E-09
7	61516731	rs137981357	G	A	0.709	-5.885	3.97E-09

Appendix Table 20 Continued.

6	32622103	rs17843692	G	A	0.774	-5.884	4.00E-09
6	32619455	rs572019305	C	A	0.749	-5.863	4.55E-09
6	32620852	rs17612907	A	G	0.7189	-5.852	4.86E-09
6	32503488	rs75684124	T	C	0.7541	-5.851	4.88E-09
1	24298595	rs34369430	C	T	0.7935	-5.85	4.92E-09
9	83424430	rs148448770	A	G	17.84	5.849	4.93E-09
22	28241457	rs5752647	A	T	0.6683	-5.844	5.10E-09
6	32593386	rs9271708	A	C	0.7421	-5.842	5.17E-09
6	32625209	rs941707873	T	C	0.751	-5.841	5.18E-09
1	24286649	rs35666672	A	G	0.7937	-5.837	5.33E-09
6	32576721	rs647455	A	G	0.7198	-5.836	5.35E-09
1	24300238	rs61772989	C	T	0.7904	-5.832	5.47E-09
6	32617104	rs188694433	T	C	0.6879	-5.821	5.86E-09
6	32561207	rs28366315	T	C	0.7301	-5.818	5.94E-09
6	32619089	rs534331448	C	T	0.7208	-5.818	5.96E-09
6	32605713	rs9272469	C	A	0.6821	-5.816	6.04E-09
6	32568685	rs9270761	T	C	0.7048	-5.813	6.13E-09
6	32549935	rs35095850	G	C	0.7475	-5.808	6.34E-09
9	68507869	rs1968925	T	C	0.7991	-5.803	6.51E-09
6	32627250	rs9273410	A	C	0.7779	-5.803	6.53E-09
7	61532334	rs148946498	A	C	0.7055	-5.79	7.04E-09
6	32520272	rs11757159	T	C	0.7303	-5.789	7.08E-09
6	32612111	rs9273059	A	G	0.679	-5.787	7.16E-09
6	32549356	rs117816615	A	G	0.7572	-5.781	7.44E-09
6	32549356	rs1024612	G	A	0.7572	-5.781	7.44E-09
6	32549356	rs2308818	A	T	0.7572	-5.781	7.44E-09
6	32511650	rs421653	A	C	0.727	-5.779	7.51E-09
6	32511650	rs111797109	T	C	0.727	-5.779	7.51E-09
6	32561887	rs28366333	C	G	0.6597	-5.772	7.81E-09
6	32560934	rs28366302	C	G	0.7382	-5.771	7.87E-09
6	32624660	rs112391576	A	T	0.7879	-5.77	7.91E-09

Appendix Table 20 Continued.

6	32615527	rs17843579	G	T	0.8036	-5.762	8.34E-09
6	32570979	rs9270861	A	G	0.7097	-5.754	8.71E-09
6	32560875	rs28366300	T	C	0.6797	-5.751	8.85E-09
6	32519391	rs79638048	A	C	0.7492	-5.74	9.45E-09
6	32612460	rs9282051	GAA	G	0.7064	-5.731	9.98E-09
6	32589959	rs9271535	T	C	0.716	-5.728	1.02E-08
6	33054619	rs929	A	G	0.7195	-5.725	1.03E-08
6	32593003	rs9271690	C	T	0.6735	-5.72	1.07E-08
6	32593392	rs9271709	A	G	0.7483	-5.719	1.07E-08
6	32574204	rs615719	A	G	0.7448	-5.712	1.12E-08
6	32560727	rs28366296	A	C	0.7297	-5.703	1.18E-08
11	119431826	rs386375057	GC	G	1.459	5.695	1.24E-08
6	32521022	rs2806163	C	T	0.7297	-5.681	1.34E-08
6	32521022	rs111343881	C	T	0.7297	-5.681	1.34E-08
6	32589326	rs9271503	A	C	0.7312	-5.676	1.38E-08
6	32603936	rs9272309	G	A	0.7703	-5.676	1.38E-08
6	32570184	rs2516051	T	C	0.7413	-5.676	1.38E-08
1	24286897	rs17338135	T	C	0.7839	-5.667	1.45E-08
6	32571872	rs9270894	G	A	0.7473	-5.666	1.46E-08
6	32514041	rs112587701	A	T	0.7312	-5.647	1.63E-08
6	32616512	rs553276313	A	C	0.7328	-5.647	1.64E-08
6	32619632	rs566907865	T	G	0.7231	-5.646	1.64E-08
6	32571897	rs12211942	G	A	0.7415	-5.643	1.67E-08
6	32560107	rs28366274	T	G	0.7636	-5.637	1.73E-08
6	32588630	rs9271469	C	T	0.7514	-5.634	1.77E-08
6	32605423	rs9272446	C	T	0.6563	-5.633	1.77E-08
6	32575325	rs2858867	G	A	0.7569	-5.63	1.80E-08
7	61523241	rs144589334	C	A	0.714	-5.629	1.81E-08
7	61523243	rs138769825	T	A	0.7141	-5.628	1.83E-08
6	33054675	rs9277529	G	C	0.7191	-5.625	1.85E-08
6	32624272	rs756687619	G	A	0.7191	-5.625	1.86E-08

Appendix Table 20 Continued.

7	550737	rs62433334	C	G	0.7378	-5.61	2.03E-08
7	61526312	rs147440980	T	A	0.7179	-5.608	2.04E-08
6	32552416	rs34951355	A	C	0.7015	-5.607	2.05E-08
6	32607659	rs4608850	A	G	0.6707	-5.598	2.17E-08
6	32607659	rs28383391	T	C	0.6707	-5.598	2.17E-08
7	61525545	rs145221375	A	C	0.7184	-5.596	2.20E-08
17	77346178	rs115839112	T	G	12.03	5.591	2.26E-08
17	77346462	rs76473581	A	G	12.03	5.591	2.26E-08
7	61521896	rs12219821	A	G	0.7207	-5.591	2.26E-08
7	61521896	rs143654121	G	A	0.7207	-5.591	2.26E-08
7	61515881	rs372871506	C	T	0.7185	-5.591	2.26E-08
7	61515503	rs147083677	A	G	0.7189	-5.581	2.39E-08
6	32594354	rs35366682	C	T	0.7163	-5.575	2.48E-08
22	28248840	rs1062731	A	C	0.6471	-5.563	2.65E-08
6	32610781	rs1048643	A	C	0.7063	-5.561	2.68E-08
6	32623580	rs190594352	T	C	0.7158	-5.555	2.78E-08
9	83379877	rs77245261	C	T	18.43	5.555	2.78E-08
9	83382954	rs12003483	A	T	18.43	5.555	2.78E-08
9	83384336	rs12004061	C	T	18.43	5.555	2.78E-08
9	83404851	rs78616911	A	G	18.43	5.555	2.78E-08
9	83413607	rs9449532	A	G	18.43	5.555	2.78E-08
9	83413607	rs113283869	A	G	18.43	5.555	2.78E-08
9	83417502	rs76521249	C	T	18.43	5.555	2.78E-08
9	83421342	rs75441551	T	C	18.43	5.555	2.78E-08
9	83425690	rs78438850	G	A	18.43	5.555	2.78E-08
9	83426363	rs12004927	A	T	18.43	5.555	2.78E-08
9	83426493	rs11999328	G	A	18.43	5.555	2.78E-08
9	83431698	rs143309055	A	C	18.43	5.555	2.78E-08
9	83432023	rs138706856	G	C	18.43	5.555	2.78E-08
9	83432820	rs145789584	T	C	18.43	5.555	2.78E-08
9	83434376	rs142694279	C	T	18.43	5.555	2.78E-08

Appendix Table 20 Continued.

9	83435303	rs11999981	T	G	18.43	5.555	2.78E-08
9	83435304	rs11999982	C	G	18.43	5.555	2.78E-08
9	83436419	rs75025786	A	G	18.43	5.555	2.78E-08
9	83436680	rs76968659	T	G	18.43	5.555	2.78E-08
9	83439821	rs74589527	C	A	18.43	5.555	2.78E-08
9	83440286	rs113537701	C	T	18.43	5.555	2.78E-08
9	83440524	rs112743269	A	G	18.43	5.555	2.78E-08
7	61517701	rs36151211	T	C	0.7204	-5.553	2.82E-08
15	20459374	rs4578619	C	T	0.8087	-5.548	2.89E-08
7	61065560	rs9314232	C	G	0.7276	-5.547	2.91E-08
7	61065340	rs9690089	A	G	0.7278	-5.543	2.97E-08
6	32607230	rs9272570	A	G	0.7141	-5.533	3.14E-08
6	32611964	rs9273042	T	C	0.7074	-5.532	3.16E-08
6	32595060	rs9271850	G	A	0.7597	-5.529	3.22E-08
6	32604994	rs9272414	T	A	0.7161	-5.528	3.23E-08
6	32611537	rs9273007	T	C	0.7519	-5.528	3.24E-08
4	169307112	rs2029382	A	G	6.497	5.526	3.28E-08
6	32596908	rs6929020	C	G	0.7352	-5.523	3.33E-08
6	33050089	rs9277365	A	G	0.7014	-5.523	3.34E-08
15	92489280	rs73530678	A	G	16.95	5.506	3.68E-08
7	61517563	rs12708840	C	T	0.723	-5.5	3.79E-08
7	61517563	rs36182412	T	A	0.723	-5.5	3.79E-08
7	61064518	rs3886032	C	T	0.7331	-5.5	3.80E-08
5	157554434	rs116795454	T	C	8.132	5.5	3.80E-08
6	32560385	rs34291045	T	A	0.6123	-5.498	3.84E-08
6	32608211	rs9272636	C	T	0.6988	-5.496	3.88E-08
15	20416977	rs6599858	C	T	0.7984	-5.496	3.89E-08
7	61520884	rs36150504	G	T	0.7234	-5.49	4.03E-08
6	32605371	rs9272440	C	G	0.6933	-5.49	4.03E-08
17	77330264	rs4790012	G	A	5.219	5.482	4.21E-08
6	32607124	rs9272559	C	T	0.7078	-5.48	4.26E-08

Appendix Table 20 Continued.

7	61524018	rs150668574	T	G	0.7242	-5.478	4.30E-08
6	32590428	rs113512385	G	C	0.7539	-5.472	4.44E-08
6	32590428	rs12943279	T	C	0.7539	-5.472	4.44E-08
6	32590428	rs9271563	C	T	0.7539	-5.472	4.44E-08
6	32564681	rs17191234	C	A	0.7826	-5.471	4.47E-08
6	32578278	rs2858863	T	C	0.707	-5.467	4.58E-08
6	33054656	rs930	C	A	0.7286	-5.467	4.58E-08
7	61522282	rs147919157	C	T	0.7246	-5.462	4.72E-08
6	32627386	rs12918	T	C	0.7444	-5.46	4.75E-08
2	54693490	rs6755780	G	T	0.6727	-5.46	4.76E-08
7	61059285	rs114639770	A	G	0.7319	-5.46	4.77E-08
6	33050078	rs9277362	G	A	0.7166	-5.457	4.85E-08
6	32561565	rs34553045	C	T	0.6263	-5.455	4.89E-08
6	32603583	rs9272273	G	A	0.719	-5.455	4.91E-08
6	32620661	rs17843614	G	T	0.8061	-5.454	4.92E-08

Appendix Table 21. GWAS Results for Age-Related Hearing Loss in Patients of African Ancestry ($p < 1x10^{-6}$).

CHR	BP	SNP	EFFECT	REFERENCE	OR	STAT	P
4	162652025	rs77750421	A	G	7.692	5.818	5.94E-09
19	11089718	rs144555968	C	T	8.005	5.655	1.56E-08
3	46216812	rs546897628	A	G	2.221	5.337	9.44E-08
4	27558997	rs75671871	A	G	5.158	5.308	1.11E-07
4	162577814	rs78749316	G	A	6.121	5.237	1.63E-07
10	32614096	rs148394603	C	T	15.16	5.174	2.29E-07
10	32635672	rs190786071	G	C	15.16	5.174	2.29E-07
12	10371763	rs142626101	T	C	7.365	5.172	2.32E-07
10	32302558	rs118079618	A	G	12.4	5.159	2.49E-07
10	32572631	rs143127607	C	G	14.72	5.156	2.53E-07
6	154667800	rs150164161	G	A	9.049	5.117	3.10E-07
10	32264123	rs151290517	T	C	11.81	5.103	3.35E-07
10	32312541	rs144996047	C	A	11.81	5.103	3.35E-07
10	32337032	rs141315833	T	C	11.81	5.103	3.35E-07
10	32346639	rs148744607	A	G	11.81	5.103	3.35E-07
3	162441769	rs115027738	G	A	4.581	5.081	3.76E-07
20	56269499	rs149759674	G	A	7.495	5.08	3.78E-07
4	23288496	rs144428189	A	G	8.95	5.017	5.25E-07
10	32221998	rs141895309	G	A	10.95	5.01	5.44E-07
10	32513152	rs138728206	A	G	12.88	4.988	6.09E-07
10	32528516	rs140509673	C	T	12.88	4.988	6.09E-07
10	32540708	rs145804759	A	G	12.88	4.988	6.09E-07
2	78813206	rs143400310	T	C	15.77	4.965	6.88E-07
4	161267156	rs78021844	T	A	5.624	4.946	7.59E-07
3	46199936	rs113328541	T	C	2.089	4.926	8.40E-07
3	46200281	rs113628900	A	G	2.088	4.922	8.57E-07
3	46204467	rs41366344	G	A	2.089	4.921	8.61E-07

Appendix Table 21 Continued.

19	11055872	rs142402195	G	C	7.028	4.918	8.73E-07
19	11056093	rs139973513	A	C	7.028	4.918	8.73E-07
3	46210555	rs80083138	A	T	2.085	4.912	9.01E-07
3	46211800	rs78565306	A	G	2.085	4.912	9.01E-07
3	46212302	rs113932294	A	C	2.085	4.912	9.01E-07
3	46215524	rs77952080	A	G	2.08	4.906	9.28E-07
3	46209900	rs112429202	T	C	2.083	4.906	9.28E-07
3	46221929	rs112368983	G	T	2.079	4.903	9.43E-07
3	46222767	rs113297542	T	G	2.079	4.903	9.43E-07
3	46222914	rs80046651	T	A	2.079	4.903	9.43E-07
3	46224704	rs79949848	C	T	2.079	4.903	9.43E-07
3	46225331	rs41465848	A	G	2.079	4.903	9.43E-07
3	46225507	rs12317144	G	A	2.079	4.903	9.43E-07
3	46225507	rs41480548	A	G	2.079	4.903	9.43E-07
3	46226088	rs75700111	G	C	2.079	4.903	9.43E-07
3	46213584	rs75771214	G	C	2.078	4.9	9.57E-07
3	46214470	rs41326147	C	A	2.078	4.9	9.57E-07
13	110948811	rs78555758	G	A	2.169	4.898	9.68E-07
8	94592825	rs7821191	C	T	3.167	4.897	9.74E-07

Appendix Table 22. GWAS Results for Age-Related Tinnitus in Patients of African Ancestry ($p < 1x10^{-6}$).

CHR	BP	SNP	EFFECT	REFERENCE	OR	STAT	P
5	18010823	rs8127374	C	G	2.114	5.46	4.75E-08
5	18046818	rs62349633	A	G	2.109	5.4	6.65E-08
17	12876781	rs74730836	T	A	4.062	5.318	1.05E-07
10	75366566	rs189395276	T	G	15.57	5.301	1.15E-07
1	167642121	rs566872713	T	TC	3.86	5.196	2.03E-07
19	18198923	rs150543577	T	C	5.563	5.185	2.16E-07
5	18050107	rs10941964	T	C	2.028	5.176	2.27E-07
8	145718435	rs56384511	G	A	2.331	5.172	2.31E-07
6	139036178	rs146216359	T	C	7.44	5.166	2.39E-07
5	18035736	rs1390630	A	G	2.048	5.163	2.43E-07
5	78881863	rs72766775	G	A	19.21	5.156	2.52E-07
14	80894253	rs144504181	A	T	13.04	5.152	2.58E-07
5	18032934	rs10941950	A	G	2.034	5.124	2.99E-07
1	167641379	rs72697715	G	A	3.696	5.097	3.45E-07
8	131572108	rs117495235	C	T	7.367	5.089	3.60E-07
5	151132615	rs28917192	G	A	10.83	5.085	3.68E-07
5	151141403	rs139565113	A	G	10.83	5.085	3.68E-07
3	126936902	rs1107250	T	C	3.955	5.056	4.29E-07
5	18025617	rs1496380	G	A	2.008	5.045	4.53E-07
12	131653862	rs72488117	C	T	2.63	5.03	4.90E-07
8	145715450	rs67607986	A	G	2.197	5.026	5.02E-07
14	27772450	rs17603025	G	A	7.015	5.019	5.19E-07
17	12873484	rs2058090	T	G	3.844	5.012	5.40E-07
13	99537104	rs138242012	G	T	11.72	5.006	5.56E-07
10	69313004	rs34596854	A	G	26.11	4.998	5.79E-07
14	27624926	rs144187995	G	T	8.473	4.992	5.97E-07
12	70267720	rs11177921	T	G	2.901	4.99	6.03E-07
17	12875963	rs145669577	T	C	3.805	4.978	6.43E-07

Appendix Table 22 Continued.

17	12875963	rs12651403	C	T	3.805	4.978	6.43E-07
17	12876266	rs75780741	C	T	3.805	4.978	6.43E-07
17	12876867	rs75772201	A	C	3.805	4.978	6.43E-07
5	154091050	rs76645988	G	T	6.839	4.963	6.94E-07
5	154077497	rs76458420	T	C	6.829	4.961	7.00E-07
5	95095768	rs72783474	A	G	9.087	4.944	7.66E-07
6	93206330	rs116483190	G	A	2.735	4.929	8.27E-07
14	82241629	rs77177730	T	A	5.42	4.929	8.28E-07
17	12878253	rs117451061	A	G	3.739	4.917	8.79E-07
9	136895818	rs72766607	G	T	10.43	4.914	8.92E-07
8	145706027	rs55955028	A	G	2.164	4.91	9.10E-07
10	69214201	rs71496043	C	G	21.93	4.91	9.10E-07
17	14108645	rs11656587	C	G	2.268	4.902	9.49E-07
17	14108653	rs11656522	T	C	2.267	4.899	9.66E-07
13	21043496	rs113471684	G	C	10.58	4.893	9.96E-07

Appendix Table 23. Functional Enrichment Analysis of Most Significant SNPs ($p < 5x10^{-5}$) in GWAS of Age-Related Hearing Loss in Patients of European Ancestry.

GO						
term.id	**domain**	**term.name**	**p**	**subgraph. number**	**relative. depth**	**genes**
GO:0042613	CC	MHC class II protein complex	0.0128	1	2	HLA-DQB1,HLA-DRB1,HLA-DQA1,HLA-DRB5
GO:0042611	CC	MHC protein complex	0.0357	1	1	HLA-DQB1,HLA-DRB1,HLA-DQA1,HLA-DRB5
HP						
HP:0004434	hp	C8 deficiency	0.0494	1	1	C8B,C8A
KEGG						
KEGG:05330	keg	Allograft rejection	0.011	10	1	HLA-DQB1,HLA-DRB1,HLA-DQA1,HLA-DRB5
KEGG:05310	keg	Asthma	0.00447	6	1	HLA-DQB1,HLA-DRB1,HLA-DQA1,HLA-DRB5
KEGG:05321	keg	Inflammatory bowel disease (IBD)	0.00902	2	1	IL21R,HLA-DQB1,HLA-DRB1,HLA-DQA1,HLA-DRB5
KEGG:04672	keg	Intestinal immune network for IgA production	0.0321	8	1	HLA-DQB1,HLA-DRB1,HLA-DQA1,HLA-DRB5
KEGG:05332	keg	Graft-versus-host disease	0.0137	1	1	HLA-DQB1,HLA-DRB1,HLA-DQA1,HLA-DRB5
KEGG:04940	keg	Type I diabetes mellitus	0.0205	5	1	HLA-DQB1,HLA-DRB1,HLA-DQA1,HLA-DRB5
KEGG:05323	keg	Rheumatoid arthritis	0.039	9	1	ATP6V1G3,HLA-DQB1,HLA-DRB1,HLA-DQA1,HLA-DRB5
KEGG:05320	keg	Autoimmune thyroid disease	0.0443	7	1	HLA-DQB1,HLA-DRB1,HLA-DQA1,HLA-DRB5
KEGG:05322	keg	Systemic lupus erythematosus	0.0364	3	1	C8B,C8A,HLA-DQB1,HLA-DRB1,HLA-DQA1,HLA-DRB5
KEGG:04145	keg	Phagosome	0.0102	4	1	TUBB4A,ATP6V1G3,HLA-DQB1,HLA-DRB1,HLA-DQA1,HLA-DRB5,TUBB3
Reactome						
REAC:R-HSA-202430	rea	Translocation of ZAP-70 to	0.00367	1	1	HLA-DQB1,HLA-DRB1,HLA-DQA1,HLA-DRB5

Appendix Table 23 Continued.

		Immunological synapse				
REAC:R-HSA-202427	rea	Phosphorylation of CD3 and TCR zeta chains	0.000173	2	1	PTPRC,HLA-DQB1,HLA-DRB1,HLA-DQA1,HLA-DRB5
REAC:R-HSA-389948	rea	PD-1 signaling	0.00786	4	1	HLA-DQB1,HLA-DRB1,HLA-DQA1,HLA-DRB5
REAC:R-HSA-202433	rea	Generation of second messenger molecules	0.00135	3	1	HLA-DQB1,HLA-DRB1,EVL,HLA-DQA1,HLA-DRB5

Appendix Table 24. Functional Enrichment Analysis of Most Significant SNPs ($p < 5x10^{-5}$) in GWAS of Age-Related Tinnitus in Patients of European Ancestry.

GO term.id	domain	term.name	p	subgraph. number	relative. depth	genes
GO:0002504	BP	antigen processing and presentation of peptide or polysaccharide antigen via MHC class II	0.00308	2	2	EC24B,MARCH1,HLA-DQB1,AP2A2,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1
GO:0019882	BP	antigen processing and presentation	0.00516	2	1	SEC24B,MARCH1,RAB4A,HLA-DQB1,AP2A2,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1,HLA-B
GO:0002495	BP	antigen processing and presentation of peptide antigen via MHC class II	0.00992	2	3	SEC24B,MARCH1,AP2A2,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1
GO:0048002	BP	antigen processing and presentation of peptide antigen	0.015	2	2	SEC24B,MARCH1,AP2A2,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1,HLA-B
GO:0071346	BP	cellular response to interferon-gamma	0.015	1	1	D44,GAPDH,TLR4,VPS26B,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1,HLA-B
GO:0060333	BP	interferon-gamma-mediated signaling pathway	0.0189	1	2	CD44,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1,HLA-B
GO:0002478	BP	antigen processing and presentation of exogenous peptide antigen	0.0265	2	3	SEC24B,AP2A2,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1,HLA-B
GO:0034341	BP	response to interferon-gamma	0.027	1	1	CD44,GAPDH,TLR4,VPS26B,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1,HLA-B

Appendix Table 24 Continued.

GO:0019886	BP	antigen processing and presentation of exogenous peptide antigen via MHC class II	0.0317	2	4	SEC24B,AP2A2,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1
GO:0019884	BP	antigen processing and presentation of exogenous antigen	0.0345	2	2	SEC24B,AP2A2,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1,HLA-B
GO:0042611	CC	MHC protein complex	6.32E-05	5	1	HLA-DQB1,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1,HLA-B
GO:0042613	CC	MHC class II protein complex	6.49E-05	5	2	HLA-DQB1,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1
GO:0030134	CC	COPII-coated ER to Golgi transport vesicle	0.000374	7	2	SEC31B,SEC24B,VANGL2,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1,HLA-B
GO:0012507	CC	ER to Golgi transport vesicle membrane	0.000374	7	2	SEC31B,SEC24B,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1,HLA-B
GO:0098553	CC	lumenal side of endoplasmic reticulum membrane	0.000374	6	1	HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1,HLA-B
GO:0071556	CC	integral component of lumenal side of endoplasmic reticulum membrane	0.000374	6	2	HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1,HLA-B
GO:0030662	CC	coated vesicle membrane	0.00353	7	2	SEC31B,SEC24B,COPG2,SYT9,AP2A2,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1,HLA-B
GO:0030660	CC	Golgi-associated vesicle membrane	0.00353	7	1	SEC31B,SEC24B,COPG2,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1,HLA-B
GO:0030669	CC	clathrin-coated endocytic vesicle membrane	0.0044	7	1	AP2A2,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1
GO:0030135	CC	coated vesicle	0.00957	7	1	EC31B,SEC24B,VPS33A,COPG2,VANGL2,SYT9,AP2A2,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1,HLA-B

Appendix Table 24 Continued.

GO:0030658	CC	transport vesicle membrane	0.00957	7	2	SEC31B,AMPH,SEC24B,SYNPR,SYT9,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1,HLA-B
GO:0005798	CC	Golgi-associated vesicle	0.00966	7	1	SEC31B,SEC24B,COPG2,VANGL2,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1,HLA-B
GO:0044433	CC	cytoplasmic vesicle part	0.015	7	1	RNASET2,CD44,SRI,SEC31B,AMPH,DSP,NCF4,KPNB1,GRB14,STEAP4,SLC44A2,CLIP1,TLR4,SEC24B,VPS33A,GALNS,MARCH1,DYNLT1,COLEC12,COPG2,SYNPR,RAB4A,SYT9,OSCAR,PRL,AP2A2,HLA-DRB1,HLA-DQA1,PDGFA,TOR4A,HLA-DRB5,APRT,HLA-DPB1,HLA-DPA1,HLA-B,TARM1
GO:0030133	CC	transport vesicle	0.015	7	1	SEC31B,AMPH,SEC24B,DGKI,COPG2,SYNPR,SYTL3,SYT9,HLA-DRB1,TRAPPC4,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1,HLA-B
GO:0071944	CC	cell periphery	0.015	8	1	HSD7A,PRKAR2B,ADAM22,PHLDB1,CD44,SRI,AMPH,FKBP1A,DSP,SLC5A4,NCF4,RASSF2,NALCN,PIEZO1,UPK2,SLC1A2,GAPDH,VIPR1,GRB14,SPTBN1,PCYOX1,TREH,KIF14,RAB3GAP2,PTGIS,KIR2DL1,STEAP4,SLC44A2,RHBDF2,ITGA7,MAP7,TLR4,SLC44A5,LGR5,MDGA2,CDH13,MARCH1,GABRA6,LRRC4C,AKAP6,DISP1,NCAM2,SLC28A1,CACNA2D3,DGKI,COLEC12,NRG2,CLDN14,SCUBE1,FCRL3,VANGL2,FCRL1,SYTL3,SLC13A4,DLC1,GPR176,POTED,KIR3DL1,RAB4A,GFRA2,ADAM29,SYT9,OSCAR,KSR2,DSCAM,XKR3,MC5R,SLCO3A1,DSCAML1,HLA-DQB1,TIGIT,NTM,AP2A2,GPC6,MUC6,TNFAIP2,NKAIN3,CD300E,DCC,PLEKHN1,KLHL17,PRKAR1B,NKAIN2,PARVB,SHISA6,CNR2,KIR2DL4,HLA-DRB1,HLA-DQA1,MAP3K5,HLA-DRB5,CD300LD,C9ORF135,HLA-DPB1,HLA-DPA1,HLA-B,TARM1
GO:0045334	CC	clathrin-coated endocytic vesicle	0.0189	7	1	AP2A2,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1
GO:0032588	CC	trans-Golgi network membrane	0.0189	3	1	LGR5,MARCH1,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1
GO:0005886	CC	plasma membrane	0.0189	8	1	THSD7A,PRKAR2B,ADAM22,CD44,SRI,AMPH,FKBP1A,DSP,SLC5A4,NCF4,RASSF2,NALCN,PIEZO1,UPK2,SLC1A2,GAPDH,VIPR1,GRB14,SPTBN1,PCYOX1,TREH,KIF14,RAB3GAP2,PTGIS,KIR2DL1,STEAP4,SLC44A2,RHBDF2,ITGA7,MAP7,TLR4,SLC44A5,LGR5,MDGA2,CDH13,MARCH1,GABRA6,LRRC4C,AKAP6,DISP1,NCAM2,SLC28A1,CACNA2D3,DGKI,COLEC12,NRG2,CLDN14,SCUBE1,FCRL3,VANGL2,FCRL1,SYTL3,SLC13A4,DLC1,GPR176,POTED,KIR3DL1,RAB4A,GFRA2,ADAM29,SYT9,OSCAR,KSR2,DSCAM,XKR3,MC5R,SLCO3A1,DSCAML1,HLA-DQB1,TIGIT,NTM,AP2A2,GPC6,MUC6,NKAIN3,CD300E,DCC,PLEKHN1,KLHL17,PRKAR1B,NKAIN2,PARVB,SHISA6,CNR2,KIR2DL4,HLA-DRB1,HLA-DQA1,MAP3K5,HLA-DRB5,CD300LD,C9ORF135,HLA-DPB1,HLA-DPA1,HLA-B,TARM1

Appendix Table 24 Continued.

GO:0030659	CC	cytoplasmic vesicle membrane	0.0265	7	2	CD44,SRI,SEC31B,AMPH,DSP,SLC44A2,CLIP1,SEC24B,MARCH1,COLEC12,COPG2,SYNPR,RAB4A,SYT9,AP2A2,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1,HLA-B,TARM1
GO:0012506	CC	vesicle membrane	0.0331	7	1	CD44,SRI,SEC31B,AMPH,DSP,SLC44A2,CLIP1,SEC24B,MARCH1,COLEC12,COPG2,SYNPR,RAB4A,SYT9,AP2A2,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1,HLA-B,TARM1
GO:0030665	CC	clathrin-coated vesicle membrane	0.0448	7	3	SYT9,AP2A2,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1
GO:0042605	MF	peptide antigen binding	0.00048	9	1	HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1,HLA-B
GO:0032395	MF	MHC class II receptor activity	0.027	4	1	HLA-DRB1,HLA-DQA1,HLA-DPA1
KEGG						
KEGG:05310	keg	Asthma	7.44E-05	4	1	HLA-DQB1,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1
KEGG:05332	keg	Graft-versus-host disease	3.38E-08	7	1	KIR2DL1,KIR3DL1,HLA-DQB1,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1,HLA-B
KEGG:04640	keg	Hematopoietic cell lineage	1.21E-02	5	1	CD44,HLA-DQB1,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1
KEGG:04940	keg	Type I diabetes mellitus	4.52E-05	2	1	HLA-DQB1,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1,HLA-B
KEGG:04145	keg	Phagosome	1.02E-03	15	1	NCF4,TLR4,COLEC12,HLA-DQB1,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1,HLA-B
KEGG:05145	keg	Toxoplasmosis	3.40E-02	12	1	TLR4,HLA-DQB1,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1
KEGG:04672	keg	Intestinal immune network for IgA production	1.55E-03	9	1	HLA-DQB1,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1
KEGG:05140	keg	Leishmaniasis	1.63E-04	6	1	NCF4,TLR4,HLA-DQB1,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1
KEGG:05150	keg	Staphylococcus aureus infection	3.17E-03	11	1	HLA-DQB1,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1
KEGG:04514	keg	Cell adhesion molecules (CAMs)	9.39E-05	13	1	LRRC4C,NCAM2,CLDN14,HLA-DQB1,TIGIT,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1,HLA-B
KEGG:05323	keg	Rheumatoid arthritis	6.90E-03	14	1	TLR4,HLA-DQB1,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1
KEGG:05330	keg	Allograft rejection	1.44E-05	3	1	HLA-DQB1,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1,HLA-B
KEGG:05320	keg	Autoimmune thyroid disease	1.84E-04	1	1	HLA-DQB1,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1,HLA-B

Appendix Table 24 Continued.

KEGG:04612	keg	Antigen processing and presentation	7.50E-07	18	1	KIR2DL1,KIR3DL1,HLA-DQB1,KIR2DL4,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1,HLA-B
KEGG:05321	keg	Inflammatory bowel disease (IBD)	7.15E-05	16	1	TBX21,TLR4,HLA-DQB1,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1
KEGG:05416	keg	Viral myocarditis	4.02E-04	8	1	HLA-DQB1,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1,HLA-B
KEGG:04658	keg	Th1 and Th2 cell differentiation	9.22E-03	17	1	TBX21,HLA-DQB1,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1
KEGG:04659	keg	Th17 cell differentiation	2.42E-02	10	1	TBX21,HLA-DQB1,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1
Reactome						
REAC:R-HSA-2132295	rea	MHC class II antigen presentation	3.03E-02	3	1	SEC24B,HLA-DQB1,AP2A2,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1
REAC:R-HSA-202427	rea	Phosphorylation of CD3 and TCR zeta chains	1.66E-04	1	1	HLA-DQB1,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1
REAC:R-HSA-202430	rea	Translocation of ZAP-70 to Immunological synapse	6.57E-05	2	1	HLA-DQB1,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1
REAC:R-HSA-202433	rea	Generation of second messenger molecules	1.95E-03	5	1	HLA-DQB1,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1
REAC:R-HSA-877300	rea	Interferon gamma signaling	9.98E-03	6	1	CD44,HLA-DQB1,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1,HLA-B
REAC:R-HSA-389948	rea	PD-1 signaling	2.18E-04	4	1	HLA-DQB1,HLA-DRB1,HLA-DQA1,HLA-DRB5,HLA-DPB1,HLA-DPA1

Appendix Table 25. Functional Enrichment Analysis of Most Significant SNPs ($p < 5x10^{-5}$) in GWAS of Age-Related Hearing Loss in Patients of African Ancestry.

GO term.id	domain	term.name	p	subgraph. number	relative. depth	genes
GO:0007399	BP	nervous system development	0.048	8	1	LRRC7,ARID1B,NTN1,CTNNA2,LRP6,WDR1,SEMA3A,ULK2,JAG1,SLC7A5,CDH23,SPOCK2,MAPKAP1,TNN,RUNX2,BMP4,SMARCA4,POSTN,SBF2,BZW2,FAM129B,SLC6A3,CARM1,EFNA3,GABRB2,NFIB,CRB2,PCDH15,DLG5,ASTN1,ABR,ZNF148,KLF15,TMC1,KIF27,PBX3,CTNND2,YWHAG,KIF5B,GRIK1,CNTNAP2,ERBB4,PTPN11,CXCR2,PLCB1,PARK2,COL4A1,MBP,EFNA4,SHANK3
GO:1904207	BP	regulation of chemokine (C-C motif) ligand 2 secretion	0.048	9	1	POSTN,MBP
GO:1904209	BP	positive regulation of chemokine (C-C motif) ligand 2 secretion	0.048	9	1	HIP1,HIP1R
GO:0042490	BP	mechanoreceptor differentiation	0.048	1	1	JAG1,CDH23,BMP4,GABRB2,PCDH15,TMC1
GO:0060113	BP	inner ear receptor cell differentiation	0.048	1	2	JAG1,CDH23,BMP4,GABRB2,PCDH15,TMC1
GO:0030336	BP	negative regulation of cell migration	0.048	4	2	PFN2,SEMA3A,JAG1,RGCC,MIIP,TNN,TPM1,ADAM15,DLG5,ABR,MCTP1,ERBB4,PLCB1
GO:0007267	BP	cell-cell signaling	0.048	16	1	ARID1B,LRP6,PFN2,TRHDE,RAPGEF4,SMPD3,RNF43,IL17A,KMO,TNN,TNFAIP6,BMP4,SMARCA4,EXOC4,FAM129B,SLC6A3,EFNA3,GABRB2,PCDH15,CACNA2D1,SLC24A2,CCR1,NPY5R,CTNND2,YWHAG,SYT9,KIF5B,GRIK1,MCTP1,PTPN11,PLCB1,PARK2,MPZL1,MBP,RYR2,EFNA4,SHANK3
GO:0051179	BP	localization	0.048	6	1	LRRC7,NTN1,CTNNA2,LRP6,PFN2,OSBPL3,WDR1,IPCEF1,SEMA3A,KLHL20,ADCYAP1R1,RPS5,PITPNM2,RAPGEF4,JAG1,RGCC,SMPD3,MON1B,SLC7A5,HSPB1,CDH23,SPOCK2,SLC2A9,CREBL2,IL17A,KCNIP3,IL1R2,THADA,MFN2,MIIP,SRSF11,KMO,SLC5A9,MFSD1,DENND1A,TNN,TCP1,A1BG,TBC1D15,TNFAIP6,BMP4,MSTO1,HIP1,MAU2,HIP1R,EXOC4,POSTN,RPS15A,DNAJC1,FAM129B,KIF13A,FRAS1,SLC7A1,VPS37B,TPM1,PCSK6,SLC6A3,CARM1,ADAM15,MEGF10,GABRA6,GABRB2,CRB2,ASTN2,ABCB9,RILPL2,DLG5,ADAM8,ASTN1,HAUS1,CNKSR3,CACNA2D1,PROM2,SLC24A2,SMG1,AGAP1,ABR

Appendix Table 25 Continued.

						,CXCR1,CCR1,KLF15,NPY5R,TMC1,ARHGAP12,LDB2,YWHAG,NSG2,ARL6IP1,SYT9,KIF5B,GRIK1,CNTNAP2,RSRC1,MCTP1,ERBB4,LDLRAD3,PTPN11,CXCR2,TRAPPC5,PLCB1,CTNNA3,TMPRSS2,PARK2,MBP,RYR2,CHML,SHANK3,NCOA4
GO:0006810	BP	transport	0.048	6	3	LRRC7,NTN1,LRP6,PFN2,OSBPL3,WDR1,IPCEF1,KLHL20,ADCYAP1R1,RPS5,PITPNM2,RAPGEF4,RGCC,SMPD3,MON1B,SLC7A5,HSPB1,CDH23,SLC2A9,CREBL2,IL17A,KCNIP3,IL1R2,THADA,MFN2,SRSF11,KMO,SLC5A9,MFSD1,DENND1A,TCP1,A1BG,TBC1D15,TNFAIP6,BMP4,HIP1,HIP1R,EXOC4,POSTN,RPS15A,DNAJC1,FAM129B,KIF13A,FRAS1,SLC7A1,VPS37B,PCSK6,SLC6A3,MEGF10,GABRA6,GABRB2,ASTN2,ABCB9,RILPL2,ADAM8,CNKSR3,CACNA2D1,PROM2,SLC24A2,SMG1,AGAP1,ABR,CXCR1,CCR1,KLF15,NPY5R,TMC1,ARHGAP12,YWHAG,NSG2,ARL6IP1,SYT9,KIF5B,GRIK1,RSRC1,MCTP1,ERBB4,LDLRAD3,PTPN11,CXCR2,TRAPPC5,TMPRSS2,PARK2,MBP,RYR2,CHML,SHANK3,NCOA4
GO:0032879	BP	regulation of localization	0.048	6	2	NTN1,PFN2,WDR1,SEMA3A,ADCYAP1R1,RAPGEF4,JAG1,RGCC,SMPD3,HSPB1,SPOCK2,CREBL2,IL17A,KCNIP3,IL1R2,THADA,MIIP,KMO,TNN,TCP1,TBC1D15,TNFAIP6,BMP4,HIP1,HIP1R,POSTN,DNAJC1,TPM1,ADAM15,ASTN2,DLG5,ADAM8,CNKSR3,CACNA2D1,PROM2,ABR,CCR1,KLF15,NPY5R,TMC1,LDB2,YWHAG,ARL6IP1,SYT9,KIF5B,MCTP1,ERBB4,PTPN11,CXCR2,PLCB1,CTNNA3,PARK2,MBP,RYR2,SHANK3
GO:0048729	BP	tissue morphogenesis	0.048	13	1	NTN1,LRP6,WDR1,SEMA3A,JAG1,BMP4,EXOC4,FRAS1,TPM1,CRB2,ASTN2,PCDH15,DLG5,KIF26B,ARHGAP12,ZFPM1,CXCR2,COL4A1,RYR2,SHANK3
GO:0038112	BP	interleukin-8-mediated signaling pathway	0.048	10	1	CXCR1,CXCR2
GO:2000588	BP	positive regulation of platelet-derived growth factor receptor-beta signaling pathway	0.048	12	1	HIP1,HIP1R
GO:0051234	BP	establishment of localization	0.0483	6	1	LRRC7,NTN1,LRP6,PFN2,OSBPL3,WDR1,IPCEF1,KLHL20,ADCYAP1R1,RPS5,PITPNM2,RAPGEF4,RGCC,SMPD3,MON1B,SLC7A5,HSPB1,CDH23,SLC2A9,CREBL2,IL17A,KCNIP3,IL1R2,THADA,MFN2,SRSF11,KMO,SLC5A9,MFSD1,DENND1A,TCP1,A1BG,TBC1D15,TNFAIP6,BMP4,HIP1,HIP1R,EXOC4,POSTN,RPS15A,DNAJC1,FAM129B,KIF13A,FRAS1,SLC7A1,VPS37B,PCSK6,SLC6A3,MEGF10,GABRA6,GABRB2,ASTN2,ABCB9,RILPL2,ADAM8,CNKSR3,CACNA2D1,PROM2,SLC24A2,SMG1,AGAP1,ABR,CXCR1,CCR1,KLF15,NPY5R,TMC1,ARHGA

Appendix Table 25 Continued.

						P12,YWHAG,NSG2,ARL6IP1,SYT9,KIF5B,GRIK1,RSRC1,MCTP1,ERBB4,LDLRAD3,PTPN11,CXCR2,TRAPPC5,TMPRSS2,PARK2,MBP,RYR2,CHML,SHANK3,NCOA4
GO:2000146	BP	negative regulation of cell motility	0.0495	4	1	PFN2,SEMA3A,JAG1,RGCC,MIIP,TNN,TPM1,ADAM15,DLG5,ABR,MCTP1,ERBB4,PLCB1
GO:0042995	CC	cell projection	0.048	5	1	THSD7A,LRRC7,OPN3,CTNNA2,PFN2,OSBPL3,WDR1,SEMA3A,KLHL20,ADCYAP1R1,HSPB1,ANKMY2,CDH23,FRMD4B,KCNIP3,DENND1A,TNN,HIP1R,EXOC4,GABARAPL1,TPH2,TPM1,SLC6A3,ADAM15,MEGF10,SNTG1,NFIB,PCDH15,RILPL2,DLG5,PROM2,NPY5R,TMC1,KIF27,CTNND2,NSG2,KIF5B,CNTNAP2,CTNNA3,PARK2,AGBL4,MBP,CYS1,SHANK3
GO:0097458	CC	neuron part	0.048	3	1	LRRC7,ARID1B,OPN3,CTNNA2,LRP6,PFN2,SEMA3A,KLHL20,ADCYAP1R1,WDR7,HSPB1,CDH23,KCNIP3,DENND1A,TNN,SMARCA4,HIP1,HIP1R,EXOC4,GABARAPL1,TPH2,SLC6A3,NFIB,ASTN2,PCDH15,DLG5,ASTN1,NPY5R,TMC1,CTNND2,NSG2,SYT9,KIF5B,CNTNAP2,MCTP1,PARK2,MBP,SHANK3
GO:0044459	CC	plasma membrane part	0.048	14	1	LRRC7,OPN3,CTNNA2,LRP6,TRHDE,ADCYAP1R1,JAG1,SLC7A5,FCER2,SIPA1L3,RNF43,SLC2A9,TSPAN11,IL17A,KCNIP3,PAPPA2,SLC5A9,DENND1A,HIP1,HIP1R,EXOC4,KLRD1,GABARAPL1,SLC7A1,TPM1,SLC6A3,HMCN1,EFNA3,MEGF10,GABRA6,GABRB2,SNTG1,CRB2,PCDH15,DLG5,ADAM8,ASTN1,CD96,CNKSR3,CACNA2D1,PROM2,SLC24A2,TM2D1,CCR1,NPY5R,TMC1,ARHGAP12,GRIK1,CNTNAP2,ERBB4,CXCR2,CCR3,TMPRSS2,MPZL1,CYS1,EFNA4
GO:0032051	MF	clathrin light chain binding	0.048	11	1	HIP1,HIP1R,NSG2
GO:0005515	MF	protein binding	0.048	7	1	LRRC7,MYOM2,ARID1B,NTN1,MCM10,CTNNA2,LRP6,PFN2,OSBPL3,WDR1,SEMA3A,KLHL20,MLLT10,ADCYAP1R1,RIF1,ULK2,ZNF446,RPS5,PITPNM2,WDR7,RAPGEF4,JAG1,RGCC,SMPD3,MON1B,SLC7A5,FCER2,HSPB1,ANKMY2,CDH23,RNF43,ELF2,TBC1D19,IL17A,KCNIP3,IL1R2,THADA,MFN2,MIIP,SRSF11,CTH,MAPKAP1,DENND1A,TNN,CENPL,ACAT2,TCP1,EPC1,ELF1,TBC1D15,TNFAIP6,PMEPA1,RUNX2,BMP4,SMARCA4,HIP1,CGNL1,MAU2,TUBGCP2,YIPF2,CCDC62,HIP1R,EXOC4,PRMT7,DAP3,POSTN,SBF2,RPS15A,KLRD1,COL4A2,ERCC5,BZW2,DNAJC1,FAM129B,KIF13A,ASCC1,GABARAPL1,SLC7A1,VPS37B,TPM1,PCSK6,NLRC5,SLC6A3,CARM1,ADAM15,EFNA3,MEGF10,GABRB2,WTAP,SNTG1,CRB2,SLX4IP,ABCB9,RILPL2,DLG5,ADAM8,ZNF385D,TMEM132D,HAUS1,C18ORF25,CNKSR3,L3MBTL4,SLC24A2,SMG1,MX1,HLCS,ABR,KIF26B,DCST1,YY1AP1,CXCR1,CCR1,ZNF148,KLF15,KIF27,ARHGAP12,PBX3,GFM1,FSTL5,LDB2,CTNND2,OTUD7A,YWHAG,NSG2,ARL6IP1,SYT9,KIF5B,ZNF439,ZNF440,KLF11,SH3RF3,ZBTB21,AGR3,CNTNAP2,RSRC1,ST8SIA3,ERBB4,PTPN11,ZFPM1,CCDC129,CXCR2,TRAPPC5,ARL6IP4,CLEC4G,PLCB1,CTNNA3,CCR3,TMPRSS2,RALYL,PARK2,AGBL4,COL4A1,JAK

Appendix Table 25 Continued.

						MIP3,DPYD,FHIT,ADH7,MPZL1,MBP,RYR2,RCSD1,CHML,CENPW,CYS1,KIAA0040,EFNA4,SHANK3,MSMB,NCOA4,GPR75-ASB3
GO:0004918	MF	interleukin-8 receptor activity	0.048	2	1	CXCR1,CXCR2
GO:0019956	MF	chemokine binding	0.0495	15	1	CXCR1,CCR1,CXCR2,CCR3

Appendix Table 26. Functional Enrichment Analysis of Most Significant SNPs ($p < 1x10^{-4}$) in GWAS of Age-Related Tinnitus in Patients of African Ancestry.

GO term.id	domain	term.name	p	subgraph. number	relative. depth	genes
GO:0048856	BP	anatomical structure development	0.037	3	1	ITGA3,FARP2,ARHGAP44,MARK4,MGST1,MKS1,WWTR1,NRXN3,EHD2,TNFRSF1B,MUSK,CUL3,FLT4,CUL7,PREX2,COL9A2,FOXN3,DGKG,CALCRL,ATXN3,SP100,ROCK1,RPS6KA2,AFF4,TP63,PPP2R3A,CELSR1,RBFOX1,EPB41L3,GNA11,KIF16B,COL9A3,IL12RB1,NFATC2,TCFL5,RGCC,ZNF423,CRISPLD2,FOXF1,ACSBG1,TJP1,BLOC1S6,PRKD2,AHR,NCS1,PPP3CB,LIPA,WNT3,MDM1,MAK,UST,PPP2R5D,DUSP22,COL4A3BP,PDE4D,CDX1,FOXP1,GPD2,CCDC88A,NID1,ATF6,NRP2,FGF23,DNMT3A,CD274,MTHFD1L,SERP1,HOXC13,HOXC11,HOXC12,ZNF831,MEA1,GCM2,TBC1D20,TCF15,HIVEP3,PTPRB,LRFN1,ARHGAP22,UNC13A,SPATA6,PPARG,MYH10,DCLK1,BTBD2,CSNK1G2,CNTN6,SORT1,KIDINS220,GRHL1,SOX5,EPHA7,NHSL1,CPM,IGF2BP3,RAPGEF5,BZW2,ALPK3,IL1RN,TMEM14B,TRPC6,BMPR1B,MDGA2,ABHD2,ARHGDIA,EIF4A3,NFIC,FBN3,HSPG2,SYPL2,MAEL,ILDR2,CNTN4,PTPRG,TMEM108,EAF2,ADAMTS16,PAM,LECT2,DCDC2,MFHAS1,CSGALNACT1,ASTN2,LRRC4C,KIRREL3,PIP4K2A,AKAP6,ADAMTS12,FER,PELO,BMP6,ASAP1,PTPRD,TRIP12,MSI2,TNIK,C21ORF91,CHODL,TTN,FMN2,CYLC2,C8ORF37,HTR5A,HYDIN,ERG,NCK1,MRAS,CLSTN2,RCAN1,RUNX1,SPON2,RDH13,RECQL4,FOXH1,PDPN,ACP6,DISC1,LMOD1,RYBP,ADAMTS9,ZC3H12A,EMCN,RHOBTB3,HEY1,DLC1,WRN,TTC8,TRAPPC9,SEPT2,BMP1,LDLRAD4,BTD,ROBO1,NPAS2,SGCD,ALK,GAA,FGG,FGA,FGB,DSCAM,DNAI2,MACROD2,GNG12,IQCB1,TDRD12,CNTNAP2,FOSL1,FOXG1,MYOZ1,AQP11,AMBN,ZHX2,CDH4,ZBTB18,IFNL1,NTM,GPC6,SLIT3,EFNA5,SGCZ,SMYD3,PRKG1,PBX1,NPAP1,PCLO,FOXI2,TEAD1,FOXD3,DCC,AMTN,EYS,HMGB1,PRTN3,LAMA2,PIWIL2,TXNRD1,DPPA5,CSNK1E,TENM3,PRKDC
GO:0007399	BP	nervous system development	0.0376	2	1	ITGA3,FARP2,ARHGAP44,MARK4,MKS1,NRXN3,MUSK,CUL7,PREX2,DGKG,ATXN3,ROCK1,TP63,CELSR1,RBFOX1,EPB41L3,ZNF423,ACSBG1,BLOC1S6,NCS1,PPP3CB,WNT3,UST,PPP2R5D,FOXP1,CCDC88A,NRP2,DNMT3A,MTHFD1L,GCM2,LRFN1,UNC13A,PPARG,DCLK1,BTBD2,CNTN6,KIDINS220,SOX5,EPHA7,NHSL1,RAPGEF5,BZW2,TMEM14B,TRPC6,BMPR1B,MDGA2,ARHGDIA,HSPG2,SYPL2,CNTN4,PTPRG,TMEM108,PAM,DCDC2,CSGALNACT1,LRRC4C,KIRREL3,BMP6,ASAP1,PTPRD,TNIK,C21ORF91,CHODL,C8ORF37,HTR5A,HYDIN,NCK1,CLSTN2,RCAN1,RUNX1,SPON2,RDH1

Appendix Table 26 Continued.

						3,DISC1,ZC3H12A,DLC1,WRN,TTC8,TRAPPC9,SEPT2,BTD,ROBO1,NPAS2,ALK,DSCAM,MACROD2,GNG12,CNTNAP2,FOXG1,ZHX2,CDH4,NTM,SLIT3,EFNA5,PRKG1,PBX1,PCLO,DCC,HMGB1,LAMA2,CSNK1E,TENM3,PRKDC
GO:0048666	BP	neuron development	0.0376	2	2	ITGA3,FARP2,ARHGAP44,MKS1,NRXN3,CUL7,PREX2,DGKG,ROCK1,EPB41L3,BLOC1S6,NCS1,PPP3CB,WNT3,UST,FOXP1,CCDC88A,NRP2,LRFN1,UNC13A,DCLK1,KIDINS220,EPHA7,TRPC6,BMPR1B,ARHGDIA,CNTN4,PTPRG,TMEM108,LRRC4C,KIRREL3,ASAP1,PTPRD,TNIK,C21ORF91,CHODL,C8ORF37,NCK1,RUNX1,SPON2,RDH13,TTC8,SEPT2,ROBO1,ALK,DSCAM,CNTNAP2,CDH4,NTM,SLIT3,EFNA5,PRKG1,DCC,HMGB1,LAMA2,TENM3
GO:0009653	BP	anatomical structure morphogenesis	0.0376	3	1	ITGA3,ARHGAP44,MKS1,WWTR1,NRXN3,EHD2,TNFRSF1B,CUL3,FLT4,CUL7,PREX2,FOXN3,CALCRL,SP100,ROCK1,TP63,CELSR1,EPB41L3,KIF16B,NFATC2,RGCC,CRISPLD2,FOXF1,TJP1,PRKD2,PPP3CB,LIPA,WNT3,UST,COL4A3BP,CDX1,FOXP1,NRP2,MTHFD1L,SERP1,HOXC13,HOXC11,TBC1D20,TCF15,PTPRB,LRFN1,ARHGAP22,UNC13A,PPARG,MYH10,DCLK1,CSNK1G2,KIDINS220,EPHA7,CPM,IGF2BP3,IL1RN,TRPC6,BMPR1B,ARHGDIA,EIF4A3,NFIC,FBN3,HSPG2,MAEL,CNTN4,TMEM108,ADAMTS16,PAM,CSGALNACT1,ASTN2,LRRC4C,KIRREL3,ADAMTS12,FER,BMP6,PTPRD,TNIK,TTN,C8ORF37,ERG,RUNX1,SPON2,RDH13,FOXH1,PDPN,LMOD1,ADAMTS9,ZC3H12A,EMCN,HEY1,DLC1,TTC8,BMP1,ROBO1,GAA,FGG,FGA,FGB,DSCAM,CNTNAP2,FOXG1,MYOZ1,AMBN,CDH4,GPC6,SLIT3,EFNA5,PBX1,FOXI2,FOXD3,DCC,AMTN,LAMA2,TXNRD1,CSNK1E,TENM3,PRKDC
GO:0022610	BP	biological adhesion	0.0467	4	1	ITGA3,FARP2,NRXN3,ATXN3,ROCK1,STK10,SCARB1,CELSR1,CD200,IL12RB1,RGCC,FOXF1,PRKD2,SIGLEC8,DUSP22,NID1,NRP2,CD274,CDHR3,MYH10,CNTN6,EPHA7,IL1RN,ARHGDIA,CNTN4,RSU1,LRRC4C,KIRREL3,ADAMTS12,FER,CNTNAP4,PTPRD,NCK1,CLSTN2,RUNX1,SPON2,PDPN,ADAMTS9,ZC3H12A,EMCN,DLC1,ROBO1,FGG,FGA,FGB,DSCAM,FIBP,COL6A5,CNTNAP2,AMBN,CDH4,CCR8,IFNL1,NTM,CTNNA3,EFNA5,PRKG1,AMTN,HMGB1,LAMA2,ELANE,ITGBL1,TENM3,SIGLEC12,PCDH20
GO:0030030	BP	cell projection organization	0.0467	2	1	ITGA3,ARHGAP44,MARK4,MKS1,WWTR1,NRXN3,CUL3,CUL7,PREX2,NAV3,ROCK1,CDC14A,EPB41L3,BLOC1S6,NCS1,PPP3CB,WNT3,MAK,UST,FOXP1,CCDC88A,NRP2,CEP89,RAB3IP,LRFN1,UNC13A,SPATA6,DCLK1,KIDINS220,EPHA7,TRPC6,BMPR1B,ARHGDIA,CNTN4,PTPRG,TMEM108,ADAMTS16,DCDC2,LRRC4C,KIRREL3,FER,ASAP1,PTPRD,TNIK,C21ORF91,CHODL,HYDIN,NCK1,DZIP1L,SPON2,PDPN,DISC1,TTC8,SEPT2,ROBO1,DSCAM,DNAI2,IQCB1,CNTNAP2,CDH4,SLIT3,EFNA5,PRKG1,DNAH14,DCC,HMGB1,KIF19,LAMA2,CSNK1E,TENM3
GO:0120036	BP	plasma membrane	0.0467	2	2	ITGA3,ARHGAP44,MARK4,MKS1,WWTR1,NRXN3,CUL7,PREX2,NAV3,ROCK1,CDC14A,EPB41L3,BLOC1S6,NCS1,PPP3CB,WNT3,M

Appendix Table 26 Continued.

		bounded cell projection organization				AK,UST,FOXP1,CCDC88A,NRP2,CEP89,RAB3IP,LRFN1,UNC13A,SPATA6,DCLK1,KIDINS220,EPHA7,TRPC6,BMPR1B,ARHGDIA,CNTN4,PTPRG,TMEM108,ADAMTS16,DCDC2,LRRC4C,KIRREL3,FER,ASAP1,PTPRD,TNIK,C21ORF91,CHODL,HYDIN,NCK1,DZIP1L,SPON2,PDPN,DISC1,TTC8,SEPT2,ROBO1,DSCAM,DNAI2,IQCB1,CNTNAP2,CDH4,SLIT3,EFNA5,PRKG1,DNAH14,DCC,HMGB1,KIF19,LAMA2,CSNK1E,TENM3
GO:0022008	BP	neurogenesis	0.0467	2	1	ITGA3,FARP2,ARHGAP44,MKS1,NRXN3,CUL7,PREX2,DGKG,ROCK1,CELSR1,EPB41L3,BLOC1S6,NCS1,PPP3CB,WNT3,UST,FOXP1,CCDC88A,NRP2,DNMT3A,GCM2,LRFN1,UNC13A,PPARG,DCLK1,BTBD2,CNTN6,KIDINS220,SOX5,EPHA7,NHSL1,TRPC6,BMPR1B,MDGA2,ARHGDIA,CNTN4,PTPRG,TMEM108,DCDC2,LRRC4C,KIRREL3,BMP6,ASAP1,PTPRD,TNIK,C21ORF91,CHODL,C8ORF37,NCK1,RUNX1,SPON2,RDH13,DISC1,TTC8,TRAPPC9,SEPT2,ROBO1,ALK,DSCAM,CNTNAP2,ZHX2,CDH4,NTM,SLIT3,EFNA5,PRKG1,PBX1,DCC,HMGB1,LAMA2,CSNK1E,TENM3
GO:0048699	BP	generation of neurons	0.0467	2	2	ITGA3,FARP2,ARHGAP44,MKS1,NRXN3,CUL7,PREX2,DGKG,ROCK1,CELSR1,EPB41L3,BLOC1S6,NCS1,PPP3CB,WNT3,UST,FOXP1,CCDC88A,NRP2,DNMT3A,LRFN1,UNC13A,PPARG,DCLK1,CNTN6,KIDINS220,SOX5,EPHA7,NHSL1,TRPC6,BMPR1B,MDGA2,ARHGDIA,CNTN4,PTPRG,TMEM108,DCDC2,LRRC4C,KIRREL3,BMP6,ASAP1,PTPRD,TNIK,C21ORF91,CHODL,C8ORF37,NCK1,RUNX1,SPON2,RDH13,DISC1,TTC8,TRAPPC9,SEPT2,ROBO1,ALK,DSCAM,CNTNAP2,ZHX2,CDH4,NTM,SLIT3,EFNA5,PRKG1,PBX1,DCC,HMGB1,LAMA2,CSNK1E,TENM3
GO:0030182	BP	neuron differentiation	0.0467	2	1	ITGA3,FARP2,ARHGAP44,MKS1,NRXN3,CUL7,PREX2,DGKG,ROCK1,EPB41L3,BLOC1S6,NCS1,PPP3CB,WNT3,UST,FOXP1,CCDC88A,NRP2,DNMT3A,LRFN1,UNC13A,DCLK1,CNTN6,KIDINS220,EPHA7,TRPC6,BMPR1B,MDGA2,ARHGDIA,CNTN4,PTPRG,TMEM108,LRRC4C,KIRREL3,BMP6,ASAP1,PTPRD,TNIK,C21ORF91,CHODL,C8ORF37,NCK1,RUNX1,SPON2,RDH13,TTC8,TRAPPC9,SEPT2,ROBO1,ALK,DSCAM,CNTNAP2,ZHX2,CDH4,NTM,SLIT3,EFNA5,PRKG1,PBX1,DCC,HMGB1,LAMA2,CSNK1E,TENM3
GO:0031175	BP	neuron projection development	0.0467	2	3	ITGA3,ARHGAP44,MKS1,NRXN3,CUL7,PREX2,ROCK1,EPB41L3,BLOC1S6,NCS1,PPP3CB,WNT3,UST,FOXP1,CCDC88A,NRP2,LRFN1,UNC13A,DCLK1,KIDINS220,EPHA7,TRPC6,BMPR1B,ARHGDIA,CNTN4,PTPRG,TMEM108,LRRC4C,KIRREL3,ASAP1,PTPRD,TNIK,C21ORF91,CHODL,NCK1,SPON2,TTC8,SEPT2,ROBO1,DSCAM,CNTNAP2,CDH4,SLIT3,EFNA5,PRKG1,DCC,HMGB1,LAMA2,TENM3
GO:0043062	BP	extracellular structure organization	0.0467	1	1	ITGA3,TNFRSF1B,COL9A2,SCARB1,COL9A3,RGCC,CRISPLD2,FOXF1,NID1,TEX14,TCF15,PXDN,STAM,HSPG2,CSGALNACT1,PDPN,OLFML2B,ADAMTS9,BMP1,FGG,FGA,FGB,EFEMP2,P4HB,LAMA2,ELANE

Appendix Table 26 Continued.

GO:0007275	BP	multicellular organism development	0.0467	3	2	ITGA3,FARP2,ARHGAP44,MARK4,MGST1,MKS1,WWTR1,NRXN3,TNFRSF1B,MUSK,CUL3,FLT4,CUL7,PREX2,COL9A2,FOXN3,DGKG,CALCRL,ATXN3,SP100,ROCK1,RPS6KA2,TP63,PPP2R3A,CELSR1,RBFOX1,EPB41L3,GNA11,KIF16B,COL9A3,IL12RB1,NFATC2,TCFL5,RGCC,ZNF423,CRISPLD2,FOXF1,ACSBG1,TJP1,BLOC1S6,PRKD2,AHR,NCS1,PPP3CB,LIPA,WNT3,MDM1,MAK,UST,PPP2R5D,DUSP22,COL4A3BP,CDX1,FOXP1,GPD2,CCDC88A,NID1,ATF6,NRP2,FGF23,DNMT3A,CD274,MTHFD1L,SERP1,HOXC13,HOXC11,HOXC12,ZNF831,MEA1,GCM2,TBC1D20,TCF15,HIVEP3,PTPRB,LRFN1,ARHGAP22,UNC13A,SPATA6,PPARG,DCLK1,BTBD2,CNTN6,SORT1,KIDINS220,GRHL1,SOX5,EPHA7,NHSL1,RAPGEF5,BZW2,ALPK3,IL1RN,TMEM14B,TRPC6,BMPR1B,MDGA2,ARHGDIA,EIF4A3,NFIC,HSPG2,SYPL2,MAEL,ILDR2,CNTN4,PTPRG,TMEM108,EAF2,ADAMTS16,PAM,LECT2,DCDC2,MFHAS1,CSGALNACT1,LRRC4C,KIRREL3,PIP4K2A,AKAP6,ADAMTS12,BMP6,ASAP1,PTPRD,TRIP12,TNIK,C21ORF91,CHODL,TTN,FMN2,CYLC2,C8ORF37,HTR5A,HYDIN,ERG,NCK1,MRAS,CLSTN2,RCAN1,RUNX1,SPON2,RDH13,RECQL4,FOXH1,PDPN,ACP6,DISC1,RYBP,ADAMTS9,ZC3H12A,EMCN,RHOBTB3,HEY1,DLC1,WRN,TTC8,TRAPPC9,SEPT2,BMP1,LDLRAD4,BTD,ROBO1,NPAS2,SGCD,ALK,GAA,DSCAM,DNAI2,MACROD2,GNG12,IQCB1,TDRD12,CNTNAP2,FOSL1,FOXG1,AQP11,AMBN,ZHX2,CDH4,ZBTB18,IFNL1,NTM,GPC6,SLIT3,EFNA5,SGCZ,PRKG1,PBX1,NPAP1,PCLO,TEAD1,FOXD3,DCC,AMTN,HMGB1,PRTN3,LAMA2,PIWIL2,TXNRD1,DPPA5,CSNK1E,TENM3,PRKDC
GO:0032989	BP	cellular component morphogenesis	0.0467	3	2	ARHGAP44,NRXN3,CUL3,CUL7,PREX2,ROCK1,EPB41L3,PPP3CB,LIPA,WNT3,UST,COL4A3BP,FOXP1,NRP2,TBC1D20,LRFN1,UNC13A,MYH10,DCLK1,CSNK1G2,KIDINS220,EPHA7,TRPC6,BMPR1B,ARHGDIA,MAEL,CNTN4,TMEM108,LRRC4C,KIRREL3,FER,PTPRD,TNIK,TTN,C8ORF37,SPON2,PDPN,LMOD1,DLC1,TTC8,ROBO1,FGG,FGA,FGB,DSCAM,CNTNAP2,MYOZ1,CDH4,SLIT3,EFNA5,DCC,LAMA2,CSNK1E,TENM3
GO:0000902	BP	cell morphogenesis	0.0467	3	3	ARHGAP44,NRXN3,CUL3,CUL7,PREX2,ROCK1,EPB41L3,PPP3CB,LIPA,WNT3,UST,COL4A3BP,FOXP1,NRP2,TBC1D20,LRFN1,UNC13A,MYH10,DCLK1,CSNK1G2,KIDINS220,EPHA7,TRPC6,BMPR1B,ARHGDIA,MAEL,CNTN4,TMEM108,LRRC4C,KIRREL3,FER,PTPRD,TNIK,C8ORF37,SPON2,PDPN,DLC1,TTC8,ROBO1,FGG,FGA,FGB,DSCAM,CNTNAP2,CDH4,SLIT3,EFNA5,DCC,LAMA2,CSNK1E,TENM3
GO:0044459	CC	plasma membrane part	0.0121	6	1	FPI,ITGA3,ARHGAP44,SLC6A7,SLC7A9,NRXN3,EHD2,TNFRSF1B,MUSK,FLT4,GNA15,CALCRL,TRPC7,SCARB1,CELSR1,EPB41L3,GP6,GNA11,RAB11FIP3,CD200,IL12RB1,KCNK10,SLC10A1,EEA1,TJP1,ZDHHC2,SLC39A14,TSPAN13,NCS1,CUBN,PPP3CB,PRMT8,PDE4D,ABCC5,KISS1R,KIAA1324,DLGAP3,SLC5A9,CD274,ENOX1,RAMP3,GRM4,TM9SF2,TSPAN8,PTPRB,LRFN1,UNC13A,MYH10

Appendix Table 26 Continued.

						,DCLK1,DTNA,EPHA7,KCNMB4,TRPC6,BMPR1B,SLC39A8,ARHGDIA,HSPG2,GPA33,PTH2R,PTPRG,TMEM108,LRRC4C,CACNA2D4,AKAP6,FER,CNTNAP4,ASAP1,PTPRD,TNIK,PTPRN2,GRIA1,SLC28A1,FCHO2,HTR5A,ESYT3,CLSTN2,CD1C,SCIMP,PDPN,DISC1,ANTXR2,SLC15A2,SGMS2,EMCN,GRIK2,DLC1,TTC8,MFSD3,SEPT2,SLC35G2,ROBO1,MUC17,SGCD,ALK,FGG,FGA,FGB,DSCAM,DNAI2,SNTB1,GNG12,CNTNAP2,IGDCC3,DPP10,FOSL1,SLCO3A1,CDH4,GRM8,CCR8,KCNE1,FKRP,IFNL1,GPC6,EFNA5,SGCZ,P4HB,GRK1,SHISA7,HMGB1,PRTN3,CYS1,TENM3,GPIHBP1
GO:0005577	CC	fibrinogen complex	0.0467	5		FGL2,FGG,FGA,FGB

CHAPTER 12. REFERENCES

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