Genomic Analysis of Hearing Loss in Childhood Cancer Survivors

by

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Abstract

The development of efficacious anticancer therapies has significantly improved the prognosis of childhood cancer patients at the price of predisposing survivors to extensive chronic health problems. Although not fatal, reducing treatment-related hearing loss (ototoxicity) among childhood cancer survivors (CCSs) is a priority since it is the most common toxicity of mainstay cisplatin chemotherapy regimens for pediatric solid tumors and a frequent sequela of the cranial radiation therapy often used to treat pediatric brain tumors. Disconcertingly, it is now becoming apparent that ototoxic effects are long-lasting, with impairment adversely impacting language development, neurocognitive functioning, psychosocial skills, and school performance. It is therefore critical to identify high-risk patients for ototoxicity to provide, if possible, alternative cancer therapies, targeted risk-based interventions, and follow-up care. Interindividual ototoxic variability, however, is not adequately described by demographic and clinical variables in CCSs and has led to the hypothesis that genetic susceptibility underlies these diverse responses. To address this knowledge gap, we have conducted the first genome-wide association study (GWAS) among 5-year CCSs of European genetic ancestry to identify single nucleotide polymorphisms (SNPs) associated with ototoxicity.

Genome-wide SNP genotypes and clinically-ascertained ototoxicity data were obtained from the St. Jude Lifetime Cohort Study (SJLIFE), which follows 5-year CCSs for their lifetime. To allow for possible detection of both general and treatment-specific genetic variants associated with ototoxicity, four quasi-stratified analyses were performed. Specifically, the four analyses targeted: 1) those doubly unexposed to cisplatin and cochlear radiation ≥ 20 Gy in the worst ear, 2) those exposed to cisplatin but not cochlear radiation, 3) those exposed to cochlear radiation but not cisplatin, and 4) the union of these three treatment subgroups. Adjusting for genetic ancestry

principal components and the non-genetic risk factors pertinent to each analysis group, four separate logistic regression GWASes were performed. For each GWAS, 709,023 autosomal SNPs passing quality control were iteratively tested for an additive association with ototoxicity, which was defined as having/not having a score ≥ 2 on the International Society of Pediatric Oncology Ototoxicity Scale.

While no SNP attained genome-wide statistical significance (Wald p-value < 5×10^{-8}) for an association with ototoxicity, our methodology allowed us to screen for and identify biologically-supported SNPs with suggestive significance. Our most significant hits localized to a 114 kb region on chromosome one (p-value range = 1.9×10^{-7} – 9.6×10^{-6}, odds ratio range = 2.8 – 4.8). Most of these SNPs were broadly relevant across all treatment profiles, which further modulated the strength of the genetic effect, whereas others were only relevant in the absence of cisplatin or cochlear radiation exposure. The plausibility of these nine SNPs was compelling, with molecular support from regulatory, epigenomic, and transcriptional perspectives and phenotypic support from the causal linkage of mutations in this region to progressive hearing loss in mice. Additionally, several distinct SNP signals known to reside in or influence the expression of genes implicated in sound transduction were identified.

These findings, which warrant further study, suggest these loci may have clinical utility in identifying high-risk ototoxicity patients for the provision of personalized cancer treatments and follow-up care. A whole-genome sequencing analysis is underway and a replication analysis is planned.

Preface

This thesis is an original work by Jessica Baedke, and is part of a larger research project led by Dr. Yutaka Yasui that received research ethics approval from the University of Alberta Health Research Ethics Board under project name "Statistical analyses – Genome Wide Association Study", No. Pro00042122, on August 30, 2013. The data used for analysis was provided by the St. Jude Lifetime Cohort Study (SJLIFE; funded by the National Cancer Institute, project number U01CA195547), and was collected with informed consent with the approval of the St. Jude Children's Research Hospital (SJCRH) Institutional Review Board.

The current work was collaborative, with contributions made by SJCRH faculty and staff. Drs. Yutaka Yasui, Kiri Ness, Carmen Wilson, and Johnnie Bass provided key input for study conception and design. Ms. Kyla Shelton and Drs. Nan Li, Wonjong Moon, and Yadav Sapkota provided access to SJLIFE clinical and genetic data. The audiology records of individuals eligible for study inclusion were reviewed by Dr. Johnnie Bass, who applied audiological exclusion criteria and assigned ototoxicity scores. The principal component analyses used to identify those of European genetic ancestry and to adjust for finer-level population stratification were performed by Dr. Yadav Sapkota. Cochlear radiation dose calculation was completed by Dr. Rebecca Howell's medical physics laboratory at the MD Anderson Cancer Center (MDACC). The pipeline for the whole-genome sequencing analysis discussed in the addendum was built by Drs. Yadav Sapkota and Wonjong Moon. All methodological approaches implemented in this work were performed under the supervision of my MSc advisor, Dr. Yutaka Yasui.

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List of Abbreviations

CCSs	Childhood cancer survivors
CCSS	Childhood Cancer Survivor Study
Chisq	Chi-squared statistic
CI	Confidence interval
Cis+notrt	Those exposed to cisplatin and those doubly unexposed to cisplatin and cochlear radiation ≥ 20 Gy in the worst ear.
Cis+rad+notrt	Those exposed to cisplatin but not cochlear radiation ≥ 20 Gy in the worst ear, those exposed to cochlear radiation ≥ 20 Gy but not cisplatin, or those doubly unexposed to cisplatin and cochlear radiation ≥ 20 Gy (a.k.a. combined).
CNS	Central nervous system
Common population	Those with Affymetrix and Illumina sequencing
CSF	Cerebrospinal fluid
DOF	Degrees of freedom
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
GPCs	GWAS-specific principal components
GWAS	Genome-wide association study
GWAS-only	Those with Affymetrix but not Illumina sequencing
LD	Linkage disequilibrium
LRT	Likelihood ratio test
MAF	Minor allele frequency

MDACC	MD Anderson Cancer Center
Notrt	Those doubly unexposed to cisplatin and cochlear radiation ≥ 20 Gy in the worst ear.
OR	Odds ratio
PC	Principal component
PCA	Principal component analysis
Rad+notrt	Those exposed to cochlear radiation ≥ 20 Gy and those doubly unexposed to cisplatin and cochlear radiation ≥ 20 Gy in the worst ear.
SIOP	International Society of Pediatric Oncology
SJCRH	St. Jude Children's Research Hospital
SJLIFE	St. Jude Lifetime Cohort
SNP	Single nucleotide polymorphism
TA	Tested allele
TAF	Tested allele frequency
WGS	Whole-genome sequencing
WGSA	Whole-genome sequencing analysis
WGS-only	Those with Illumina but not Affymetrix sequencing
WPCs	WGSA-specific principal components

Chapter 1: Introduction

1.1 Morbidity Among CCSs

The development of efficacious anticancer therapies has significantly improved the prognosis of childhood cancer patients. From 1960 to 2013, the five-year childhood cancer survival rate has steadily risen from less than 30% to 85%[1,2]. With this increase in survival, survivor morbidity has become a major concern: 67% of 25-year survivors have a chronic health condition and 33% have a severe to fatal chronic condition, with the latter being eight times more likely in survivors than their siblings[3]. Treatment with platinum-based chemotherapeutics, namely cisplatin and carboplatin, and radiation therapy has significantly improved survival; however, these treatments commonly impart organ-specific toxicities, contributing to this late morbidity concern among survivors. Although not fatal, reducing the onset and progression of treatment-induced hearing loss (ototoxicity) among childhood cancer survivors (CCSs) is a major priority since it is the most common dose-limiting side effect of cisplatin, a frequent sequela of cranial radiation, and can have crippling effects on CCSs' quality of life[4-7].

1.2 Cisplatin Ototoxicity

Since the development of platinum-based chemotherapy in the 1970s, cisplatin has become a mainstay chemotherapeutic for the treatment of a variety of pediatric solid and central nervous system (CNS) malignancies, including medulloblastoma, osteosarcoma, hepatoblastoma, neuroblastoma, and germ cell tumors[8]. Although cisplatin is widely regarded as one of the most potent chemotherapeutic agents for childhood cancer, its efficacy comes at the price of an extensive toxicity profile that most commonly presents with extreme nausea and vomiting, neurotoxicity, nephrotoxicity, and ototoxicity[9]. In children, the main toxicity of cisplatin is

irreversible bilateral sensorineural hearing loss (i.e., relating to the inner ear or auditory nerve pathway), which initially manifests as high frequency deficits followed by a progression to lower ranges with continued cisplatin exposure[10]. Hearing loss typically occurs during or shortly after cisplatin exposure, although ototoxicity may be sub-clinical for months to years after therapy cessation and may continue to worsen with time[11-14].

With the widespread introduction of cisplatin into cancer treatment regimens in the 1980s, the ototoxic burden shouldered by CCSs has become much heavier. In a recent Childhood Cancer Survivor Study (CCSS) publication, the incidence of hearing loss was reported to have almost doubled among CCSs diagnosed in 1990-99 compared to those diagnosed in 1970-79[15]. Combined with the partial replacement of highly neurotoxic cranial radiotherapy with cisplatin for treatment of CNS malignancies[16-18], temporal intensification of cisplatin regimens has produced a new generation of audiologically at-risk CCSs.

Estimates of ototoxicity incidence among cisplatin-exposed individuals are highly variable, with reported values ranging from 13-96%[4,7,13,19-24]. This variability is largely due to key differences in the evaluated study population, cumulative cisplatin dose distributions, the presence of co-treatments or cranial radiation, implemented study design, and choice of hearing loss grading criteria. However, it is generally expected that half of cisplatin-exposed children will develop irreversible hearing loss, with ototoxic risk and severity increasing with cumulative dose[24-30]. In particular, children who receive cumulative cisplatin doses in excess of 400 mg/m^2 or are exposed at under the age of five are considered especially at risk for hearing loss deficits[10,13,27,31,32].

1.3 Carboplatin Ototoxicity

In response to the toxicities of cisplatin, a second-generation platinum analog, carboplatin, was developed. Compared to cisplatin, carboplatin shares similar structural and pharmacologic features and has reduced ototoxic, nephrotoxic, and gastrotoxic side effects; however, it exhibits dose-limiting myelotoxicity (bone marrow suppression)[33]. Although carboplatin demonstrates a broad spectrum of anticancer activity with improved patient tolerability, the choice of platinum drug is highly dependent on tumor type and context[34], meaning that cisplatin use, and the hearing loss it imparts, is still highly prevalent among CCSs. Additionally, substitution of cisplatin with its less ototoxic carboplatin analog does not absolutely preclude hearing loss. High frequency sensorineural ototoxicity is still a major concern for pediatric patients receiving myeloablative carboplatin doses (typically $\geq$ 1500 mg/m^2) in preparation for hematopoietic stem cell transplantation[24,35,36]. To contrast, infants appear to be especially impacted by standard non-myeloablative doses[37]. Similar to cisplatin, the incidence of carboplatin-induced ototoxicity is highly variable, with reported estimates ranging from 0-79%[30,38,39]. Additionally, late onset and progression of hearing loss have also been documented for carboplatin, although onset is usually more immediate[11,37,40]. In general, carboplatin is recognized to impart much less ototoxic risk in children compared to cisplatin[39].

1.4 Cranial Radiation Ototoxicity

Platinum exposure is not a necessary component cause of ototoxicity: the routine treatment of childhood brain, head, and neck cancers with cranial radiation delineates yet another group of CCSs at high risk for hearing loss. Radiation-induced hearing loss may be sensorineural, conductive (i.e., relating to the blockage of sound from damage to the outer or middle ear), or mixed in nature[41]. Following radiation that encompasses acoustic structures, up to 40% of patients

are expected to have acute middle ear side effects, whereas one-third of adult patients will display high-frequency sensorineural hearing loss[41]. Compared to the early onset and transience often seen for conductive hearing loss, the permanence of sensorineural hearing loss is generally considered more debilitating[42,43]. The onset of ototoxicity (synonymous with sensorineural hearing loss from this point forward) typically appears several months to years following radiotherapy completion, and continued deterioration in hearing sensitivity following onset is common[41,43-45]. Due to the delayed onset of radiation ototoxicity, mitigation of adverse outcomes with reactionary dose reductions, as is frequently done with cisplatin and carboplatin, can be especially difficult.

There are a limited number of reports documenting the ototoxic experience of pediatric CCSs solely exposed to cranial radiation in isolation from platinum. Of note, a 2016 study from St Jude Children's Research Hospital (SJCRH) sought to address this knowledge gap and found that 14% of 235 pediatric brain tumor patients treated exclusively with cranial radiation had sensorineural hearing loss, of which 85% of cases were severe enough to necessitate a hearing aid[45]. This was also the first study to report younger age at radiation initiation as a risk factor for ototoxicity[45]. Radiation-induced ototoxicity is further characterized by a dose-response relationship above a minimum threshold[43-45]. Several dose thresholds have been reported, but it is generally agreed upon that cochlear doses exceeding about 30 Gy are required to evoke an ototoxic response in the absence of platinum chemotherapy[43,46]. When combined with platinum chemotherapy, ototoxic risk and severity are especially exacerbated[46-48].

1.5 Impact of Hearing Impairment Among CCSs

Although carboplatin and cranial radiation are considered less ototoxic than cisplatin, exposure to any of these treatments remains highly relevant to the ototoxic experience of CCSs. Pediatric cancer patients are especially prone to ototoxicity, and relative to adults, have a higher resulting

burden of functional impairment[25,27-29]. Sensory input to the CNS in the first few years of life is critical for language acquisition[49], so cancer diagnoses that necessitate ototoxic exposures at a young age, such as neuroblastoma where the median age of diagnosis is 19 months[50], are especially detrimental to normal child development[51]. Even mild high-frequency hearing loss amongst young children can delay the acquisition of most phonemes and render consonants inaudible, thereby delaying and impairing language development and speech recognition[28]. For preschoolers and adolescents, the ototoxic experience differs since there is a background of substantial pre-existing language; however, emotional, cognitive, and social development can still be adversely impacted if hearing deficits are not adequately addressed[51,52]. Disconcertingly, it is now becoming apparent that the adverse effects of these hearing deficits, even when supplemented with hearing aids or other assistive technologies, can be long-lasting, with impairment adversely impacting neurocognitive and psychosocial development, school performance, socioeconomic status, social isolation, and quality of life[27,32,52].

1.6 Other Ototoxic Risk Factors and Interindividual Ototoxic Variability

To minimize the detrimental impact of ototoxicity in children, many investigations have focused on elucidating clinical and demographic risk factors for cisplatin-associated hearing loss, with less emphasis placed on carboplatin and radiation-associated hearing loss. In addition to the treatment-specific risk factors previously discussed, other patient characteristics known to exacerbate platinum and/or radiation ototoxicity include concomitant treatment with aminoglycoside antibiotics[21,53-55] or loop diuretics[56], impaired renal function that may delay platinum agent excretion[27,57], tumors or surgical resections in proximity to auditory structures[58-60], administration of cisplatin via bolus injections[61], the male gender[32,57,62], and cerebrospinal fluid (CSF) shunt placement[45,63]. However, interindividual variability in ototoxic responses to cisplatin,

and perhaps carboplatin and cranial radiation, are not adequately described by these risk factors. Even under uniform treatment regimens, some patients retain normal hearing function at high cumulative doses of cisplatin, whereas others experience high grade ototoxicity at low dosages[24,29,64-66]. As a result, it has been hypothesized that genetic susceptibility to cisplatin-induced ototoxicity underlies these diverse responses[65,67-69].

1.7 Genetic Studies of Ototoxicity Susceptibility

The vast majority of studies seeking to address this unexplained variation in cisplatin-induced ototoxicity have utilized the candidate-gene approach. Broadly, these studies have focused on the *a-priori* selection of genes or single nucleotide polymorphisms (SNPs) of interest that are demonstrated or suspected to be involved with the transport, metabolism, and cellular effect of cisplatin. While many significant SNPs have been identified by individual studies using this approach, a substantial proportion have yet to be replicated in independent populations. Disconcertingly, most cisplatin-ototoxicity replication studies have inconsistent results, which are likely due to differences in cisplatin treatment regimens implemented, hearing loss grading scales used, cancer types treated, methods of statistical analysis, and/or false positives by chance[70]. To date, rs1872328 of *ACYP2* is the only SNP to have consistently been significantly associated with cisplatin-induced ototoxicity in all three studies in which it was evaluated; furthermore, the initial discovery of this association by Xu *et al.* employed a genome-wide association study (GWAS) instead of a candidate-gene approach[71-73]. Only one other cisplatin-ototoxicity GWAS has been completed, which demonstrated the significant association of rs6228305 of the Mendelian deafness gene *WFS1* with cisplatin-induced ototoxicity and its interaction with increasing cumulative cisplatin dose in survivors of adult-onset testicular cancer[74].

In the 2015 GWAS completed by Xu *et al.,* cisplatin-induced ototoxicity was evaluated in children with newly-diagnosed embryonal brain tumors in the 9 – 24 months following chemoradiotherapy initiation[71]. As a result, this study only identified genetic variants involved in relatively rapid hearing loss and did not consider variants associated with late-onset and/or progressive hearing loss. This is problematic given that the onset of cisplatin-induced hearing loss can range from being immediate to having an 11-year delay following therapy completion[13,28,45,64,75,76]. In one study of ≥ 5-year CCSs, moderate to severe ototoxicity was only observed in 11% of patients in the two years following the end of cisplatin therapy, with this percentage rising to 44% in the following 2 – 13 year period (median = 7 years) as a result of both progressive and late-onset hearing loss[13]. Similarly, the onset of radiation-induced ototoxicity can range from 0.4 – 13 years with a median of 3.6 years[45]. Therefore, consideration of genetic variants predictive of late-onset and progressive hearing loss among CCSs previously treated with platinum-based chemotherapy and/or cranial radiation is critical.

1.8 Study Objectives and Significance

There is a paucity of information on genetic determinants of treatment-induced and general hearing loss among CCSs, who are especially vulnerable to progressive and irreversible hearing loss both during and in the many years following treatment completion. To address this knowledge gap, we have completed the first comprehensive GWAS study of hearing loss among ≥ 5-year CCSs utilizing genome-wide SNP data. Identification of genomic variants that modify CCSs' risk to treatment-specific and/or general hearing loss would allow for the development of personalized cancer treatment plans and early protective interventions with the ultimate goal of reducing ototoxicity incidence among survivors. Furthermore, this work will contribute to a greater

understanding of the biological mechanisms underpinning cisplatin and radiation-related ototoxicity and perhaps general hearing loss processes.

Chapter 2: Subjects and Methodology

2.1 Study Population

All participants were enrolled in the St. Jude Lifetime Cohort (SJLIFE) study with informed consent obtained in accordance with SJCRH Institutional Review Board approval[77]. SJLIFE is an ongoing retrospective cohort study that aims to establish a lifetime cohort of CCSs to enable the prospective investigation of long-term CCS health outcomes. To support this aim, CCSs treated for a pediatric malignancy at SJCRH are recruited to undergo periodic on-campus medical assessments. SJLIFE study eligibility, recruitment, medical record abstraction, clinical assessment, and biological specimen collection have been well-documented elsewhere[77,78]. However, it must be noted that participant eligibility has expanded since SJLIFE's inception in 2007. As of 2015, the former ≥ 10 year post-diagnosis survival requirement was broadened to include those surviving ≥ 5 years, and the ≥ 18 years of age at time of recruitment criterion has been removed. As a result, the data freeze used in this work includes a combination of those recruited under both the original and updated eligibility criteria.

2.2 Audiology Assessment

Audiology records were reviewed by a SJLIFE audiologist who assigned each patient a score on the International Society of Pediatric Oncology (SIOP) ototoxicity grading scale[68]. Clinically-significant hearing loss was defined as a SIOP score ≥ 2 in the worst ear based on a patient's latest evaluation. This threshold was chosen since it corresponds with a minimum degree of functional impairment requiring educational accommodation and/or assistive technology, whereas higher grades indicate severe hearing loss requiring a hearing aid[79]. Data from ears with noise-induced

hearing loss or hearing impairment prior to cancer treatment were excluded, as well as ears on the same side of the head as tumors or surgical interventions located near auditory structures.

Similar to SJLIFE cohort eligibility, the indication for audiology exams also changed in 2015. Exam eligibility, which was previously risk-based as defined in the COG long-term follow-up guidelines[80], was broadened to include all SJLIFE participants regardless of their treatment-indicated risk. Since on-campus medical assessments are generally only scheduled every 2-5 years[77], hearing status has only been partly ascertained for this previously ineligible subpopulation. As a result, only those with completed audiological exams have been included in this work.

2.3 Cochlear Radiation Dose Calculation

Abstracted cranial, neck, spine, and total body irradiation data were evaluated by SJCRH researchers to determine if a patient was considered likely to have received cochlear radiation to at least one ear during childhood cancer therapy. Patients considered unlikely to have received cochlear radiation were assigned a cochlear dose of 0 Gy, whereas radiation records for patients with probable cochlear exposure were sent to Dr. Rebecca Howell's medical physics laboratory at the MD Anderson Cancer Center (MDACC) for cochlear radiation dose estimation. Cochlear dose was calculated to one point for each of the cochleae, which were identified by Dr. Melissa Hudson at SJCRH on the generic MDACC phantom diagram. Dose calculation only considered exposure from radiation treatment received for primary, recurrent, or metastatic cancers in the 5 years following first primary tumor diagnosis. For some patients initially receiving whole brain radiation or a large parallel-opposed head/neck treatment followed by a boost, dose estimation was incomplete due to the inability to locate the boost relative to the cochlea. For these five patients, an average dose was calculated using the minimum known dose without the boost (i.e., from whole

brain radiation) and the maximum dose the cochlea would have received had it been in the boost field.

2.4 Principal Component Analyses (PCAs)

Two separate PCAs were completed by Dr. Yadav Sapkota of the Yasui lab to account for underlying population structure likely resulting from genetic ancestry. SNP data for all SJLIFE participants with Affymetrix 6.0 sequencing (n = 2622) was combined with that of the 26 global populations from the 1000 Genomes project and subjected to an Eigenstrat-based PCA[81]. The first two principal components (PCs) were used to identify those of European genetic ancestry, and SJLIFE participants within three standard deviations from the mean PC1 and PC2 scores of the reference 1000 Genomes European population were considered as genetically European (n = 1977). After restricting the SJLIFE population to those of genetic European descent, a second PCA was completed, from which the resulting top 10 PCs were extracted to adjust for finer-level population stratification in each GWAS.

2.5 Treatment-Based Stratification

To allow for possible detection of both general and treatment-specific genetic variants associated with ototoxicity, a main analysis combining all treatment profiles followed by three supplementary treatment-specific analyses was performed. Definition of cochlear radiation and cisplatin exposure, and therefore strata membership, was informed by exploratory data analysis, which identified 20 Gy as the minimum cochlear radiation dose required to evoke an ototoxic response; on the other hand, any cisplatin exposure was considered ototoxic (Supplementary Methods and Table S1). Based on the same exploratory analysis, participants exposed to both cisplatin and cochlear radiation ≥ 20 Gy—among which hearing loss prevalence was 92% and

almost certainly due to treatment effects that would comparatively dwarf potential genetic effects—were excluded from further analysis (Table S1).

A schematic of the combined and three treatment-based analyses is shown in Figure 1. Specifically, the four analyses targeted: 1) those exposed to cisplatin but not cochlear radiation ≥ 20 Gy in the worst ear, those exposed to cochlear radiation ≥ 20 Gy but not cisplatin, or those doubly unexposed to cisplatin and cochlear radiation ≥ 20 Gy (the combined cis+rad+notrt population, n = 592); 2) those exposed to cisplatin and those doubly unexposed to cisplatin and cochlear radiation ≥ 20 Gy (cis+notrt subgroup, n = 454); 3) those exposed to cochlear radiation ≥ 20 Gy and those doubly unexposed to cisplatin and cochlear radiation ≥ 20 Gy (rad+notrt subgroup, n = 505); and 4) just those doubly unexposed to cisplatin and cochlear radiation ≥ 20 Gy (notrt subgroup = 367). We chose to perform analyses for the cis+notrt and rad+notrt subgroups instead of for those exposed to just cisplatin (cis subgroup, n = 87) or just cochlear radiation ≥ 20 Gy (rad subgroup, n = 138) due to the latter subgroups having relatively small sample sizes that would have been insufficiently powered to test the separate associations of 100,000s of SNPs with hearing loss.

2.6 Clinical Model Construction

For the purpose of adjusting for clinically relevant treatment exposures and demographic characteristics, a clinical logistic regression model was separately constructed for each of the four analyses. Medical record abstraction of these treatment and demographic characteristics was completed as described in Hudson et al., 2011[77]. The dependent variable was defined as having/not having a SIOP score ≥ 2. Risk-informed binary, categorical, and natural cubic spline variables were created and compared with their continuous counterparts to optimize non-genetic risk adjustment for the following available covariates: cumulative cisplatin dose, cumulative

carboplatin dose, cochlear radiation dose for the worst ear, any/drug-specific aminoglycoside exposure (amikacin, gentamicin, kanamycin, streptomycin, and tobramycin), and age at most recent audiology exam. Gender and placement of a CSF shunt were included as binary variables. Binary interaction terms for age at cisplatin or cochlear radiation exposure were included separately to depict the increased ototoxic risk experienced by young childhood cancer patients[10,45].

2.7 SNP Genotyping and Quality Control

Genomic DNA was extracted from the blood samples of consenting participants with the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) and genotyped with the Affymetrix Genome-Wide Human SNP 6.0 Array (Affymetrix Incorporated, Santa Clara, CA). Genotyping quality control was performed with PLINK version 1.90b[82]. SNPs falling below the following thresholds were excluded: a minor allele frequency (MAF) < 1% in the study population (n = 92,908), < 95% call rate across samples (n = 3024), and Hardy-Weinberg equilibrium p-value < $1x10^{-6}$ (n = 2). The threshold for genotype missingness per individual was set to > 5% of the SNPs passing the previous quality control step, above which participants were excluded (n = 0). Following quality control, 709,023 autosomal SNPs and 592 individuals were retained for analysis.

2.8 GWASes for the Overall and Treatment-Based Strata

A single-SNP GWAS was conducted for each of the four analysis populations using PLINK version 1.90b[82]. Adjusting for the pertinent clinical model and the top 10 PCs generated from the European PCA, each of the single SNPs passing quality control were iteratively tested for an additive association with ototoxicity, which was defined as having/not having a SIOP score ≥ 2. Each SNP variable was coded as having 0, 1, or 2 copies of the tested allele (TA). In line with

current practice[83], a two-sided Wald p-value was considered to have genome-wide significance at $\leq 5 \times 10^{-8}$. For completeness, identical analyses were repeated assuming recessive (0 or 1 vs 2 TA copies) and dominant (0 vs 1 or 2 TA copies) SNP effects.

2.9 Exploration of Treatment-Specific SNP Effect Sizes

To clarify the treatment-specific effect sizes of GWAS-identified SNPs of interest, an interaction analysis was performed separately for the cis+notrt, rad+notrt, and notrt populations with the expectation that all or most interaction terms would not be statistically significant. Adjusting for the pertinent clinical model and top 10 PCs, an additively-coded main SNP effect and interaction term were added to the logistic regression model for each SNP of interest. This interaction term was coded as 1 or 2 to indicate heterozygosity or homozygosity of the TA among individuals doubly unexposed to cisplatin and radiation and was 0 otherwise. By default, the main SNP effect described the cisplatin-/radiation-specific effect. To estimate carboplatin specificity, the same analysis was performed in the notrt population. However, instead of using a three-level categorical variable for carboplatin like in all other clinical models, a binary variable for any/no carboplatin exposure was implemented since there were only 23 carboplatin-exposed individuals in the notrt population.

Chapter 3: Results

3.1 Population Characteristics and Hearing Loss Prevalence

Among the 1235 SJLIFE participants with an audiology exam, there were 1162 CCSs with at least one ear passing audiology exclusion criteria. As can be seen in the consort diagram in Figure 2, 33 of these participants lacked cisplatin, carboplatin, and/or cochlear radiation dose information and were excluded. Those doubly exposed to cisplatin and cochlear radiation ≥ 20 Gy (n=75), among which the prevalence of hearing loss was >90%, were also excluded to enhance the detection of potential genetic signals that would otherwise be diluted with the inclusion of this nearly deterministic treatment profile. Removal of 262 people lacking Affymetrix SNP 6.0 sequencing and 200 people of non-European genetic ancestry yielded a final sample size of 592 participants.

Of the 592 participants included in this work, 87 were exposed to cisplatin (median dose [IQR] = 402 [170] mg/m^2), 138 had cochlear radiation ≥ 20 Gy (median dose [IQR] = 37.9 [27.6] Gy), and 367 experienced neither treatment (Table 1). The cisplatin stratum had the highest hearing loss prevalence at 69% and displayed a dose-response trend. A threshold dose-response relationship was observed for those exposed to cochlear radiation, with similar hearing loss prevalences for those with a dose of 0 Gy (12%) or 1-19 Gy (13%) in the notrt group and elevated risk for those with a ≥ 20 Gy insult (30-70%).

As can be seen in Table 1, demographic characteristics and their co-occurrence with hearing loss varied between the three treatment groups. While case status was evenly distributed between genders in the notrt group, males had a higher risk of hearing loss compared to females in the cisplatin (81% vs 54%) and radiation (68% vs 49%) groups and tended to form the bulk of cases.

All three groups had a similar median age of most recent audiology exam in the low to mid-thirties. Although the prevalence of hearing loss increased with exam age across all treatment strata, the baseline prevalence for those aged 19-30 years was dramatically elevated in the cisplatin (65%) and radiation (42%) strata compared to the notrt stratum (9%). Strikingly, the prevalence among the oldest notrt participants (33%) never surpassed the hearing loss burden experienced by the youngest cisplatin- and radiation-exposed participants.

Half of the CCSs exposed to cisplatin were exposed under the age of five years, whereas 32% of radiation-exposed and 41% of notrt CCSs were less than five years of age at cancer diagnosis. In line with the literature[84], the prevalence of hearing loss in the cisplatin group was heightened for those exposed to cisplatin at <5 years of age relative to those aged 5-9 years. A similar young age at radiation exposure effect was not readily apparent from the crude data. Surprisingly, older age at cisplatin exposure among those ≥ 10 years heightened ototoxic risk relative to those aged 5-9 years—however, this was explained by a positive correlation between age of diagnosis and most recent exam age (Figure S1). In particular, enforcement of the 10-year survival eligibility criterion until 2015 prevented those aged ≥ 10 years at diagnosis from having a SJLIFE audiology exam until well into adulthood. Since aging is a major risk factor for hearing loss in the general population with existing hearing loss becoming more pronounced with each passing decade, the correlation between older diagnosis and exam age portrays older exposure ages as being most detrimental for treatment-mediated ototoxicity. This same phenomenon can also be observed in the radiation stratum, and even in the notrt stratum where there is no major ototoxic treatment exposure.

In addition to the major ototoxic treatments, cisplatin and cochlear radiation, the weaker carboplatin, CSF shunt installation, and aminoglycoside ototoxic exposures were considered of

interest. Ototoxicity prevalence among carboplatin-exposed survivors did not consistently vary in a dose-response manner across treatment strata; however, it may be noted that there were few carboplatin-exposed counts within each non-zero level. Crude elevation of hearing loss risk with CSF shunt installation was evident in the radiation and notrt strata but not cisplatin stratum, whereas risk elevation by aminoglycoside exposure was the greatest among the cisplatin stratum, marginal for the radiation group, and non-existent for the notrt stratum. About a third of each treatment group was exposed to aminoglycosides.

Lastly, each treatment stratum had its own unique cancer diagnosis profile. Bone, germ cell tumor, and neuroblastoma CCSs primarily comprised the cisplatin stratum whereas CNS and leukemia CCSs formed the majority of the radiation stratum. Within the notrt stratum, about 25% of notrt participants were leukemia survivors with the other 75% being more or less evenly distributed across all other categories, with the exception of germ cell tumor CCSs (of which there were only 6). Ototoxicity prevalence varied by cancer diagnosis group both within and across treatment strata.

3.2 Clinical Model Construction

Clinical models were constructed to adjust for demographic and treatment-related characteristics to allow for better detection of ototoxic variants. The clinical model for the combined population is shown in Table 2, whereas the clinical models for the three stratified analyses are shown in Table S2. Across all analyses, age at most recent audiology exam was the most important demographic risk factor for hearing loss. For example, every ten year increase in exam age increased the adjusted odds of hearing loss 2.8-fold (p-value = 3.1×10^{-12}, likelihood ratio test (LRT) chi-squared statistic (chisq) = 48.3) in the combined population. Among analyses

with cochlear radiation or cisplatin exposure, these two treatments were the most detrimental determinants of ototoxic risk.

In the combined analysis, the cochlear radiation variables taken as a whole were strongly associated with ototoxicity with an adjusted p-value of 5.18×10^{-23} (LRT chisq = 114.0). As can be seen in Table 2, a dose-response trend was operative with odds ratios (ORs; range = 1.7 – 25.5) and significance levels (range = 5.4×10^{-1} – 2.0×10^{-17}) increasing with higher dose categories. Although the radiation dose category for 2000-2439 cGy (radcat2000) was not statistically significant (OR = 1.7, p-value = 5.4×10^{-1}) when adjusting for higher dose categories, the variable was kept rather than combined with an adjacent category because both its crude and adjusted ototoxic probabilities were distinct in magnitude from the < 2000 cGy (without cisplatin exposure) and 2440-2500 cGy (radcat2440) groups (Table S3). The young age at radiation variable was also statistically insignificant (OR = 1.9, p-value = 1.7×10^{-1}) but was retained due to its known clinical relevance[45], its point estimate's moderate effect size, and its 95% confidence interval (CI) predominantly residing above the null OR of 1.0.

Taken together, the three cisplatin variables were also significantly associated with ototoxicity in the combined population with an adjusted p-value of 5.7×10^{-31} (LRT chisq = 143.8). Two separate intercept terms were fit to distinguish those exposed to cisplatin at < or ≥ 5 years of age from those unexposed to cisplatin. Compared to those unexposed to cisplatin, the adjusted odds of ototoxicity was a colossal 14.4 times greater among those aged < 5 years (p-value = 8.8×10^{-4}) and 6.0 times greater in those aged ≥ 5 years at the time of exposure (p-value = 1.9×10^{-2}). Among both age groups, the odds of ototoxicity significantly increased by 34% with every 100 mg/m^2 dose increase (p-value = 3.7×10^{-2}).

Unlike cisplatin, the carboplatin chemotherapy variables failed to attain statistical significance when considered either as a whole (p-value = 2.1×10^{-1}, LRT chisq (2) = 3.17) or individually (low-dose p-value = 8.1×10^{-2} and high-dose p-value = 6.3×10^{-1}). With ORs of 2.4 and 1.5 for the low-dose and high-dose carboplatin terms, higher doses appeared to be less ototoxic. This observation tended to be true regardless of what dose threshold was used (Table S4). It is plausible that this counterintuitive trend resulted from high carboplatin doses tending to be prescribed in the absence of cisplatin exposure or radiation doses ≥ 20 Gy, and there being low counts of carboplatin exposure in each treatment stratum. This can be seen in Table 1, where only one person in the cisplatin group and two people in the radiation group had a carboplatin dose ≥ 3000 mg/m^2 compared to the 9 people in the notrt group. Despite this, we kept carboplatin in the clinical model due to its postulated clinical significance[16,30,35,80]. Furthermore, we modelled carboplatin as a three- and not two-level categorical variable (see Table S4 for binary model) to better adjust for the distinct risk profiles experienced by high and low dose individuals. Although the 4000 mg/m^2 category was statistically a better choice compared to the 3000 mg/m^2, the latter was enforced due to the clustering of participants below and above the 3000 mg/m^2 threshold.

The remaining variables adjusted for in the combined clinical model were gender (OR = 0.5, p-value = 8.3×10^{-3}), tobramycin (OR = 3.7, p-value = 3.5×10^{-3}), and CSF shunt placement (OR = 2.6, p-value = 6.5×10^{-2}). These variables appeared to be relevant risk factors with moderate OR sizes and convincing 95% CIs that were either significant or asymmetrically weighted away from the null. We chose to not include individual or combined terms for other aminoglycoside antibiotics due to their adjusted null effect on ototoxic risk (data not shown).

Compared to the main cis+rad+notrt clinical model, the supplementary stratified clinical models shown in Table S2 provided similar effect size estimates with acceptable significance

levels for cisplatin- and radiation-related variables as well as for continuous age at most recent audiology examination. To contrast, estimated effect sizes varied sizably between analyses for the tobramycin, CSF shunt, and carboplatin variables. The OR for gender remained relatively constant across the cis+notrt (OR = 0.6, p-value = 6.8 x 10^{-2}), rad+notrt (OR = 0.6, p-value = 5.0 x 10^{-2}), and notrt analyses (OR = 0.7, p-value = 0.38), but significance noticeably declined in the notrt analysis.

3.3 GWAS Results

Adjusting for the pertinent clinical model and the top 10 genetic ancestry PCs, separate additive, recessive, and dominant GWASes were run for the main cis+rad+notrt population as well as each of the three supplementary strata. The top 20 SNPs for each of the four populations under additive, recessive, and dominance assumptions are shown in Tables S5-S7. Since there were no obvious departures from additivity within the recessive and dominant analyses, only the additive analyses are discussed in this work.

To contextualize adjusted SNP p-values against a null distribution, QQ plots were constructed for each of the four analyses (Figure 3). Consistent with a clean QQ plot, the vast majority of observed -$\log_{10}$ p-values in each analysis fell on the X=Y line with several SNPs trailing upwards with smaller than expected p-values at the end of the null distribution. This deviation was most prominent for the combined and cis+notrt analyses, whereas the curve failed to escape the null 95% CI in the rad+notrt analysis. Given the absence of genome-wide differences in expected and observed -$\log_{10}$ p-values (i.e., genomic inflation), this ~20 SNP deviation was considered consistent with the detection of SNP-ototoxicity associations.

The analysis-specific spatial distributions of all tested variants are shown in the Manhattan plots in Figure 4. While no SNP reached genome-wide significance under the additive model (Wald p-value < 5×10^{-8}), application of the GWAS p-value threshold suggestive of significance (< 1×10^{-5}) yielded 22 unique SNPs originating from nine distinct loci. Analysis-specific ORs and p-values for these SNPs are detailed in Table 3, whereas tested allele frequencies (TAFs) are shown in Table 4 by case status.

All analyses detected both distinct and overlapping sets of SNPs with p-values < 1×10^{-5}. In the combined analysis, one distinct SNP residing in *USP28* (an ubiquitin specific peptidase) was identified with an OR of 0.2 and a p-value of 9.6×10^{-6} (Table 3). The cis+notrt analysis uniquely detected a SNP flanking the cytosolic phospholipase *PLA2G4D* promoter (OR = 3.1, p-value = 9.6×10^{-6}) and an intergenic SNP on chromosome three (OR = 3.5, p-value = 4.3×10^{-6}). The rad+notrt analysis also identified two unique SNPs—one in a promoter-flanking region 193,937 bp downstream from its closest annotated gene, the *DRD1* dopamine receptor (OR = 3.7, p-value = 9.2×10^{-6}), and a CTCF-binding intronic SNP in *ODF1*, which encodes the outer dense fiber of sperm tails (OR = 9.1, p-value = 3.5×10^{-6}). An intron variant in *NELL1* (neural epidermal growth factor-like 1; OR = 4.1, p-value = 7.6×10^{-6}) and five intron variants in the overlapping *AC020611*.2 antisense RNA and *SRGAP1* (SLIT-ROBO Rho GTPase activating protein 1) genes were uniquely identified by the notrt analysis (OR = 5.9, p-values = 8.5×10^{-6} – 9.5×10^{-6}).

SNPs were jointly identified by the combined and rad+notrt analyses near two ion channel genes: *HTR3B*, an ionotropic serotonin receptor, and *ANO4*, which is a calcium-dependent transmembrane protein that is predicted to scramble phosphatidylserine, phosphatidylcholine, and galactosylceramide. The intergenic variant near *HTR3B* had double the effect size strength but slightly weaker significance in the rad+notrt analysis vs the combined analysis (OR = 0.1 and p-

value = 6.3 x 10^{-6} vs OR = 0.2 p-value = 4.1 x 10^{-6}). The same trend of the effect size being stronger but the significance weaker in the rad+notrt analysis vs the combined analysis was also true for the *ANO4* variant (rad+notrt OR = 6.4 and p-value = 5.0 x 10^{-6} vs combined OR = 5.8 and p-value = 3.0 x 10^{-6}).

As can be seen in Table 3, a 114 kb region on chromosome 1p12 consistently produced the most significant SNP signals in each analysis, with minimum p-values for the four strata ranging from 1.9 x 10^{-7} – 1.3 x 10^{-6}. All SNPs were distributed within and between three adjacent genes: 1) *TBX15*, a phylogenetically conserved T-box 15 transcription factor involved in a myriad of developmental processes, 2) *AL139420*.1, an uncharacterized lincRNA, and 3) *WARS2*, a mitochondrial tryptophanyl-tRNA synthetase. A common core of six SNPs (rs4501872, rs4659138, rs10923726, rs12021830, rs10494218, and rs12027986—henceforth referred to as the core 1p12 signal) was detected in all four analyses, whereas another three SNPs (rs973500, rs7553422, and rs10923748) only met the p-value threshold in a subset of analyses. As can be seen in Table 5, the core 1p12 signal and rs973500 are in high linkage disequilibrium (LD; r^2 = 0.89 – 1.0), whereas rs7553422 (r^2 = 0.16 – 0.25) and rs10923748 (r^2 = 0.25 – 0.45) are not.

P-values and effect sizes varied for the nine 1p12 SNPs across the four analysis groups. Using the most significant SNP, rs4501872, as a representative example for the core 1p12 signal, it can be seen that the SNP OR imparted the largest effect in the absence of radiation-exposed participants (Table 6). Specifically, the largest OR of 4.8 was observed in the notrt GWAS, which decreased to 4.2 in the cis+notrt GWAS, 3.3 in the rad+notrt GWAS, and 3.2 in the combined GWAS. The p-value reached a minimum of 1.9 x 10^{-7} in the cis+notrt GWAS and then increased to 2.4 x 10^{-7} in the combined GWAS with the inclusion of 138 radiation participants, 8.7 x 10^{-7} in the notrt GWAS with the dual exclusion of 87 cisplatin and 138 radiation participants, and 1.3 x

10^{-6} in the rad+notrt GWAS with the exclusion of 87 cisplatin participants and inclusion of 138 radiation participants. These relative OR and p-value trends were true for all core 1p12 SNPs. Similarly, the rs973500 signal, which was detected with p-values < 1 x 10^{-5} in the combined and cis+notrt analyses, had a larger effect size with the exclusion of the radiation subgroup (Table 3). Unlike the core 1p12 signal and rs973500, the cis+notrt and notrt effect sizes were not of comparable magnitude for promoter flanking/downstream lincRNA variant rs7553422 or intron variant rs10923748, which both only met the p-value threshold in the notrt analysis. Rather, the cis+notrt ORs were closer in size to those estimated for the rad+notrt and combined populations.

3.4 Treatment-Specific Effect Sizes for 1p12 SNPs

To garner a better appreciation for treatment-specific SNP effects, an interaction analysis targeting the nine 1p12 SNPs was separately performed for the cis+notrt, rad+notrt, and notrt analyses with the addition of a treatment-specific TA copy number term to each clinically/ancestry-adjusted SNP main effects model. As can be seen in Table 7, nominally significant interactions were obtained in the cis+notrt and rad+notrt populations for the two SNPs (rs7553422 and rs10923748) that originally only met the p-value threshold of 1 x 10^{-5} in the notrt GWAS. SNP rs7553422's OR of 3.7-4.8 was strongest in the notrt/carboplatin-only subgroups and hovered around the null in the cisplatin/radiation subgroups. A similar trend was observed for rs10923748's OR, which hovered around 3.0 in the notrt subgroups and the null in the cisplatin/radiation subgroups; however, the OR peaked at 11.8 in the carboplatin-only subgroup.

Although not statistically significant, we still examined the treatment-specific ORs for the other seven 1p12 SNPs to clarify which treatment profiles were most influenced by genetic effects. SNP rs973500, which only met the p-value threshold in the combined and cis+notrt GWASes, had the largest effect size of 4.0 among cisplatin-exposed individuals, which decreased to 3.1-3.5 in

the notrt/carboplatin-only subgroups and 2.0 in the radiation subgroup. For the six core 1p12 SNPs, the genetic effect was the most substantial in the notrt/carboplatin-only subgroups (ORs = 3.9 – 23.9), moderate in the cisplatin subgroup (ORs = 2.7 – 2.9), and modest in the radiation subgroup (ORs = 1.8 – 2.0). Of note, the OR of 23.9 achieved by rs4659138 in the carboplatin-only subgroup was alone in magnitude, with all other core 1p12 carboplatin-specific ORs spanning 4.2 – 4.3.

Chapter 4: Discussion

4.1 Overview of Main Findings

There is a paucity of information on the genetic determinants of hearing loss among CCSs, who are especially vulnerable to hearing loss both during and in the many years following cancer treatment completion. To address this knowledge gap, we conducted the first GWAS in long-term CCSs and identified 22 SNPs across nine loci as being associated with hearing loss with p-values smaller than those expected by chance. However, none of these SNP p-values attained genome-wide statistical significance (Wald p-value $< 1 \times 10^{-8}$). Based on an extensive review of the literature and publicly available bioinformatic resources, we believe that the hearing loss associations of the *WARS2*/*TBX15* (chromosome 1p12), *USP28*/*HTR3B* (chromosome 11q23.2), and *ANO4* (chromosome 12q23.1) loci are strongly supported from regulatory, cellular, physiological, and phenotypic perspectives as discussed in the following sections. While the treatment-specificities of the latter two loci still need to be characterized with treatment-SNP interaction analyses, we believe we have identified a combination of SNPs in the 1p12 region that are either only relevant in the absence of strong ototoxic treatments or are broadly relevant to all treatment profiles, which modulate the strength of the genetic effect.

In the sections that follow, we first relate the construction of our clinical model to known non-genetic ototoxic risk factors described in the literature. This is followed by a thorough review and synthesis of biological and bioinformatic information supporting the associations between the 1p12, 11q23.2, and 12q23.1 loci with hearing loss in CCSs, as well as an exploration of how different treatment backgrounds modulate the effects of the 1p12 locus. Study strengths and limitations are discussed, of which the latter mainly relates to the difficulties of working with a

CCS late effect that is also extremely common in the general population and not easily distinguished from age-related hearing loss. Finally, we summarize the significance of this work from biological and clinical perspectives.

4.2 Demographic and Clinical Risk Factors Associated with Ototoxicity

To improve our ability to detect genetic signals, we constructed stratum-specific clinical models for the purpose of adjusting for non-genetic risk factors of hearing loss. Across all analyses, age at most recent audiology exam was identified as the most important demographic (i.e., not treatment-related) risk factor for hearing loss, whereas the most detrimental clinical determinants of ototoxic risk were any cisplatin exposure and cochlear radiation ≥ 20 Gy. In general, the relative effect sizes and directions of our selected variables agreed with the literature.

4.2.1 Age at Audiology Exam

As expected, age at most recent audiology exam was identified as the most important demographic risk factor for hearing loss across all analyses. It is well known that age is the strongest risk factor for hearing deficits in the general population. Even after adjusting for demographic, cardiovascular, and noise-related risk factors, age-specific ORs of hearing loss remain similar to striking crude estimates in the general population[85]. For example, in a study using data from the National Health and Nutrition Examination Survey, Hoffman et al found that the unadjusted and adjusted odds of speech-frequency hearing impairment among 60-69 year olds was respectively 40.5 and 39.5 the odds experienced by 20-29 years olds in the US[85]. To contrast, our crude ORs for age were much smaller than their adjusted counterparts owing to the sheer strength of prescribed ototoxic treatments (Table S8).

Table S9 evinces the disproportionate hearing loss burden prematurely suffered by CCSs compared to the general population. Among radiation exposed CCSs, the odds of hearing loss per passing decade were about double that of cisplatin and notrt CCSs and quadruple that of the general population, suggesting that the impact of age on hearing loss is especially detrimental in the context of cochlear radiation. Using the cisplatin-specific clinical model (Table S8b), the adjusted CCS age-specific ORs were about double the magnitude reported for the general population until about 50 years of age. Although consistent with the concept of advanced and/or accelerated "ear-age" among CCSs exposed to cisplatin chemotherapy[86], this trend was, surprisingly, also true for CCSs in the notrt group who lacked major ototoxic exposures. Although not formally evaluated for hearing loss, the stressors of intensive cancer treatment regimens are thought to promote premature aging phenotypes through cellular senescence, sterile inflammation, and mitochondrial dysfunction[87,88]. With mounting evidence for inflammatory antecedents underlying the pathogenic mechanisms of hearing loss in the general population[89], which is of in itself an aging phenotype, it would not be unreasonable to postulate that notrt CCSs exposed to intensive treatment regimens lacking cisplatin or cochlear radiation may also be at increased risk for accelerated hearing loss compared to the general population.

4.2.2 Cochlear Radiation

Sensorineural hearing loss is a relatively common adverse late effect of cranial radiation and has been reported to impact a third of exposed patients[41]. Radiation impacts hearing in a dose-dependent manner[43,45,90], with suggested dose-thresholds spanning 32-54 Gy[43,46,91]. Although version five of the COG Long-Term Follow-Up Guidelines currently recommends ototoxicity screening for survivors exposed to ≥ 30 Gy of head or brain radiation[80], we found that individuals exposed to ≥ 20 Gy of cochlear radiation were at increased ototoxic risk compared to those with

doses < 20 Gy (Table S1c). As a result, we instead used this lower threshold to define audiologically-relevant cochlear radiation exposures.

The possible origins of this threshold discrepancy are many and diverse. For instance, our cohort includes CCSs treated from 1963 – 2004, and it is well known that older radiation technologies impart increased audiological sequelae to the ear compared to their modern counterparts[41]. For example, with the invention and commercialization of intensity-modulated radiotherapy in the late 1990s, clinicians have been able to minimize the dose received by the cochlea while still maintaining tumor treatment efficacy, resulting in reduced ototoxicity rates[92-94]. In the present study, the median diagnosis year of CCSs exposed to any non-zero cochlear radiation dose was 1986 (IQR = 1978 – 1992), so it is plausible that our study population required lower overall radiation doses to invoke an ototoxic response. It is also possible that the use of a phantom diagram to calculate cochlear radiation doses for average sized and positioned cochleae may have affected our capacity to accurately estimate cochlear dose for every single study participant.

With the caveat of ascertaining a different minimum cochlear dose threshold, our results generally agreed with the literature in that higher doses were associated with higher ototoxicity odds[43,45,90]. We were unable to replicate the doubling of ototoxic risk experienced by those less than 3 years of age at exposure as described in Bass et al[45]. However, we were able to demonstrate a similar adjusted OR using five years of age at exposure as a threshold, with those < 5 years of age having about twice the odds of developing ototoxicity compared to those at or over the age of five (combined analysis: OR = 1.9, p-value = 0.17; rad+notrt analysis: OR = 1.8, p-value = 0.19).

4.2.3 Cisplatin Chemotherapy

In line with previous pediatric publications, cisplatin was found to dramatically elevate ototoxic risk. While many investigations have demonstrated a threshold response at cumulative doses ≥ 400 mg/m^2 with descriptive statistics[13,26,31], we were unable to do so with our clinical logistic regression models. Possible explanations include the small number of patients observed in previous studies with non-zero doses < 400 mg/m^2 (n = 4 – 10, ototoxicity prevalence = 0 – 13%) versus our 28 patients with a 57% ototoxicity prevalence, choice of hearing loss grading scale, and study design differences. Instead of using a threshold dose term, we elected to use a continuous cisplatin dose variable in 100s of mg/m^2, for which adjusted ORs of 1.3 (p-value = 0.037) and 1.4 (p-value = 0.031) were estimated in the combined and cis+notrt analyses. These estimates were comparable to those from a recent Dutch study evaluating determinants of ototoxicity in platinum-treated CCSs, which calculated an adjusted OR of 1.3 (95% CI 1.2 – 1.5) with every 100 mg/m^2 total cumulative dose increase[30]. In agreement with previous studies[10,31,84], we also confirmed that exposure to cisplatin under the age of five years was a sizeable and significant risk factor for hearing loss. Since not all study participants were exposed to cisplatin, we also added a statistically significant binary term for exposure to cisplatin at ≥ 5 years of age to allow this group to have a distinct y-intercept from cisplatin unexposed individuals.

4.2.4 Carboplatin Chemotherapy

While it is accepted that cisplatin and carboplatin interact synergistically to worsen hearing loss[29,30,36], whether carboplatin alone can evoke ototoxicity is controversial and heavily debated. Some studies have demonstrated carboplatin-induced ototoxicity among children without cisplatin exposure, yet many others have been unable to do so for an overlapping range of carboplatin doses[37]. Therefore, we felt compelled to consider adjusting for carboplatin exposure in our models.

Although not statistically significant, the categorical carboplatin variable was retained to account for as much non-genetic ototoxic variation as possible. Neither the removal of the three-level carboplatin variable nor its replacement with a binary term changed which top SNPs were identified in each GWAS (data not shown). An indicator term depicting a cisplatin-carboplatin interaction was not fit owing to small cell counts.

4.2.5 Other Ototoxic Risk Factors

Similar to most cisplatin ototoxicity reports[21,32], we found the male gender to be a near significant ototoxic risk factor in the cis+notrt analysis. Interestingly, the same was also true of the rad+notrt and combined populations but not the notrt subgroup, suggesting that the male gender may exacerbate radiation-related ototoxicity. Except for tobramycin, we were unable to confirm the independent and cisplatin-potentiating effects historically described for aminoglycoside antibiotics[53,95]; however, this was consistent with a recent report from the Dutch Childhood Oncology Group describing the ototoxic determinants of platinum-treated CCSs[30]. Since aminoglycoside ototoxicity is correlated with aminoglycoside blood concentration and blood levels are routinely monitored in inpatient settings in most developed countries[96], it is plausible that patient monitoring at SJCRH prevented the development of more aminoglycoside ototoxicity cases. Lastly, CSF shunt placement was estimated to increase the odds of ototoxicity 2.6 – 4.6 times (depending on the treatment stratum) compared to those without shunts, which encompassed the univariate OR of 3.6 (p-value < 0.001) reported by the Swiss Childhood Cancer Survivor Study[16]. Although the same report was unable to demonstrate an association of CSF-shunt placement with hearing loss when adjusting for treatment-related variables, sex, and age[16], our adjusted ORs approached significance with the lower boundary of each interval estimate only crossing the null slightly and the upper boundary extending towards the double digits.

4.3 Plausibility of GWAS Results

No SNP attained genome-wide statistical significance (Wald p-value < 1 x 10^{-8}) under an additive model adjusting for pertinent clinical variables and genetic ancestry PCs. However, the 22 most significant SNPs spanning nine distinct loci (Table 3) had p-values that appeared much smaller than expected as shown in the QQ plots in Figure 3. Aside from the SNPs identified in *USP28*/*HTR3B* on chromosome 11q23.2, *ANO4* on chromosome 12q23.1, and *TBX15*/*WARS2* on chromosome 1p12, there was a lack of compelling biological support for all other hearing loss-associated SNPs with p-values < 1 x 10^{-5}.

4.3.1 Signals Lacking Biological Support

Although the SNPs mapping to the *ODF1*, *NELL1*, *SRGAP1*, *PLA2G4D*, and intergenic regions had smaller than expected p-values, there was not sufficient biological evidence to corroborate these genomic regions' roles in hearing loss. These SNPs likely represent false positives, although it remains possible that they are tagging biological features that modulate hearing loss susceptibility in an unknown manner. For example, the intergenic SNP rs11956125 is enriched for enhancer and promoter chromatin states in close to 100 human cell lines and tissues[97], yet there is no significant expression data to support its role in regulating gene expression[98]. At a distance of 193,937 bp, dopamine receptor *DRD1* is the closest gene to rs11956125. Although *DRD1* is implicated in the dopaminergic signaling of cochlear nerve fibers of mice and guinea-pigs[99,100], it is unclear whether the identification of rs11956125 was coincidental to this gene's proximity, or if the mechanism by which this SNP influences *DRD1* expression has yet to be elucidated.

4.3.2 Plausibility of the 11q23.2 Region

4.3.2.1 Statistical and Bioinformatic Evidence

The combined and rad+notrt GWASes identified rs17723728 and rs7945619 of chromosome 11q23.2 as being associated with hearing loss with strong OR effect sizes of 0.1 – 0.2 and p-values on the order of 10^{-6} (Table 3). However, given that ORs of 0.2 – 0.3 with p-values spanning 2.6 x 10^{-3} – 2.0 x 10^{-2} were estimated for the smaller cis+notrt (n = 454) and notrt (n = 367) analyses, it is possible that this genomic region was only identified in the combined (n = 592) and rad+notrt (n = 505) analyses with $p < 1 \times 10^{-5}$ owing to larger sample sizes. This is consistent with cases having lower TAFs (range: 3.3 – 7.2%) than controls (range: 13.4 – 14.1%) in all four GWAS analyses (Table 4)—that is, hearing loss cases tended to have much lower TAFs than controls owing to the TAs' protective effects against hearing loss. However, given that the ORs were the most extreme in the rad+notrt analysis, it is possible that these SNPs tag features protectant against general hearing loss processes that are especially beneficial in the context of cochlear radiation insult. In the future, it would be beneficial to perform a treatment by SNP interaction analysis as was done for the 1p12 region to further characterize how these SNPs' effect sizes may vary by treatment group.

Aside from having large effect sizes, the two 11q23.2 SNPs were also of particular interest to us from regulatory, epigenomic, and transcriptional perspectives. As can be seen in Table S10, rs17723728 is an intronic variant of the ubiquitin-specific peptidase gene *USP28* with diverse variant consequences, whereas rs7945619 is an intergenic variant near the serotonin receptor subunit gene *HTR3B*. SNP rs17723728 has histone enhancer marks in stem cell lines, progenitor neuronal cells, and endoderm cells, whereas rs7945619 mainly has enhancer activity with histone enhancer and promoter marks in brain tissues and some histone enhancer marks in stem cell lines

and gastrointestinal tract tissues[97]. Additionally, SNPs in high LD with rs17723728 and rs7945619 ($r^2 \geq 0.80$) are highly enriched for promoter and enhancer activities, DNAse sensitivity sites, protein-binding sites, and dozens of motif changes[101], lending to the possibility that these two SNPs of interest are tagging other regulatory features related to hearing loss. Both SNPs were highly correlated with the expression of *MCOLN3* in human peripheral blood monocytes (p-value = 2.3 – 4.2 x 10^{-6}, Table S10)[102], which is an extensively characterized gene thought to underlie key audiological processes. Given the enrichment of these two SNPs for histone enhancer marks and their correlation with *MCOLN3* expression, it is not unreasonable to speculate that the association of these SNPs with hearing loss in CCSs is mediated by regulatory chromatin states that may be indirectly implicated in *MCOLN3* expression.

4.3.2.2 Phenotypic, Physiologic, and Cellular Impact of *MCOLN3* in Hearing Loss

MCOLN3 (a.k.a. *TRPML3*) is a pH-regulated Ca^{2+} permeable non-selective cation channel that dynamically localizes to the intracellular membranes of the endolysosomal system and the plasma membrane[103-106]. Via its pH-dependent Ca^{2+} channel activity, *MCOLN3* is thought to mediate endosome maturation and regulate protein trafficking along the endolysosomal pathway[105]. The following pathway has been proposed by Martina et al[105]: Following internalization, endocytosed vesicles possess high Ca^{2+} concentrations from the surrounding extracellular medium. At the 6.0-6.5 pH characteristic of these vesicles, MCOLN3's open pore conformation allows a rapid efflux of Ca^{2+} into the cytosol, coupled with the progressive acidification of the early endosomal lumen. As the lumen acidifies, the pH drops to the 4.5-5.0 range characteristic of lysosomes and inhibits Ca^{2+} flux by triggering the closure of *MCOLN3*'s pore. Interestingly, both overexpression and depletion of *MCOLN3* dramatically alter the endosomal pathway and impact delivery of epidermal growth factor (EGF) and epidermal growth factor receptor (EGFR) from the plasma membrane to

lysosomes for degradation[105], further supporting *MCOLN3's* role in endosome maturation and cargo trafficking along the endolysosomal pathway.

The *MCOLN3* gene is most well-known for its varitint-waddler phenotype, which is named after the variable coat colour tint and vestibular defects (waddling, head-bobbing, and circling behaviours) characteristic of mice with the Va or Va^J alleles of *MCOLN3*. Although not in the name, early-onset profound hearing loss or deafness is also a salient feature of the varitint-waddler phenotype. Both the Va and Va^J alleles possess the MCOLN3-A419P substitution responsible for the phenotype[106], but the Va^J allele also possesses a second in-cis I362T mutation[107]. Via an unknown mechanism, the I362T substitution partially rescues the A419P defect by reducing surface expression of MCOLN3-I362T/A419P relative to MCOLN3-A419P[107]. As a result, the severity of the varitint-waddler phenotype appears to depend on the specific mutations observed and their copy number. While embryonic lethality or sterility is imparted by the Va/Va genotype, the Va/+ and Va^J/Va^J genotypes impart deafness and coat colour dilution, with the former resulting in vestibular defects, whereas Va^J/+ heterozygotes retain partial hearing and only exhibit slight coat colour dilution[108].

The varitint-waddler phenotype arises from the gain-of-function A419P mutation in the pore region of *MCOLN3*[106]. Specifically, this mutation locks the *MCOLN3* channel in an open confirmation, thereby mediating a mass influx of Ca^{2+} thought to trigger cell death by apoptosis[107-110]. In the human epithelial cell line HEK293, expression of constitutively active mutant *MCOLN3* channels and subsequent Ca^{2+} overload have been shown to culminate in the translocation of phosphatidylserine from the cytoplasmic leaflet of the plasma membrane to the cell surface, which is an early marker of apoptosis[111]. In mouse melanocyte lines, which are highly specialized for melanin production, this constitutive *MCOLN3* activity has been shown to result in high resting

Ca^{2+} levels and, over time, melanocyte death[109]. Interestingly, loss of melanin-producing melanocytes seems to underlie both the pigmentation and hearing defects of varitint-waddler mice. In mutant mice, the loss of melanocytes from hair follicles results in the lightening of skin and fur colour[109], whereas the absence of melanocytes in the stria vascularis of the cochlear duct is thought to lead to hearing impairment[112].

Normally, strial melanocytes function to create the endocochlear potential required for auditory hair cell function by maintaining the ionic composition of the endolymph, which bathes the stereocilia of hair cells[113]. It is thought that melanocytes fulfill this role by acting as biological Ca^{2+} and K^{+} reservoirs. As a high capacity Ca^{2+} chelator[114,115], the melanin produced within melanocytes is thought to contribute to the calcium homeostasis of both endolymph and melanocytes[116-118]. In addition to Ca^{2+} buffering, the proposed heavy metal scavenging and antioxidant activities of melanin[117,119,120] and its precursors are thought to protect the inner ear from age-related hearing loss[121-123], noise-induced hearing loss[123,124], and ototoxicity[125-127] in rat, mouse, and guinea pig models.

Melanin is synthesized, stored, and transported in melanosomes, which are lysosome-related organelles that form from early endosomal intermediates within melanocytes[128,129]. Of relevance to this work, endosomal *MCOLN3* is thought to mediate cation efflux from melanosomes, and it has been proposed that this protein might also function in melanosomal membranes[130]. In varitint-waddler mice, hearing loss is thought to specifically arise from the Ca^{2+} overload and death of melanocytes expressing constitutively open *MCOLN3* channels within melanosomes[131]. Additionally, the export of melanosomes from melanocytes to the intrastrial space, marginal, and basal cells[132] is known to be markedly influenced by noise and ototoxic exposures[133-135]. Interestingly, the varitint-waddler cell phenotype can be rescued with various strategies[131] that

target *MCOLN3* pore opening/closing[108], stopper *MCOLN3*-mediated Ca^{2+} entry[107,109], prevent *MCOLN3* mis-localization to the plasma membrane[110], and reduce mutant surface expression[107]. However, further *in vivo* pathway experiments are needed to corroborate the relevance of these molecular rescue strategies to an actual hearing loss phenotype in mice.

Taken as a whole, it is not unreasonable to speculate that the Ca^{2+} overload and apoptosis of strial melanocytes, and the resulting reduction of the endocochlear potential, inherent of varitint-waddler mice with constitutively active endosomal *MCOLN3* channels is related to trafficking of Ca^{2+}-sequestering melanosomes. Under this conceptual framework, one could envision a scenario where subtle variations in *MCOLN3* functionality or expression may gradually affect the ability of strial melanocytes to buffer intracellular/endolymph Ca^{2+} via melanosome trafficking during the aging process. In the context of extreme oxidative stress, such as that imparted by cochlear radiation or cisplatin chemotherapy in CCSs, this hypothesized baseline sensitivity of strial melanocytes to variations in *MCOLN3* expression and suboptimal Ca^{2+} buffering could become especially pronounced and predispose individuals to ototoxicity.

In addition to the melanocytes of the stria vascularis, *MCOLN3* is also strongly expressed in the inner and to a lesser extent outer hair cells[136,137] of the inner ear, which in varitint-waddler mice display stereocilia disorganization and undergo eventual cell death[106,112]. Wildtype *MCOLN3*-containing endosomes, which are known to inwardly rectify Ca^{2+} [107,109,138], are thought to protect the subcellular organelles of inner hair cells from the large fluctuations in intracellular Ca^{2+} near the plasma membrane that are integral to the sound transduction process[137]. During sound transduction, deflection of stereocilia on the apical side of inner hair cells opens mechanically gated ion channels, allowing an influx of positive ions from the surrounding endolymph to travel into and depolarize the inner hair cell. This depolarization activates voltage gated Ca^{2+} channels

in the hair cell plasma membrane, through which Ca^{2+} floods through to trigger the release of neurotransmitters from the basal end of the hair cell that then diffuse towards and activate auditory nerve terminals. However, other signal transduction pathways, such as apoptosis, are regulated and triggered by subtle changes in Ca^{2+} concentration, so it is essential that the rapid influx of Ca^{2+} that characterizes sound transduction is mitigated to prevent both apoptotic Ca^{2+} overload (which is a known mechanism of noise-induced hearing loss[139]) and to reset the hair cell to its anticipatory state.

While outer hair cells maintain a concentrated proteinaceous buffer in their cytosol to moderate changes in Ca^{2+} concentration, inner hair cells only maintain this buffer at one tenth of the concentration seen in outer hair cells[140]. Given that *MCOLN3* is much more abundant in inner than outer hair cells and that inner hair cells lack the proteinaceous buffering capacity of outer hair cells, it has been hypothesized that *MCOLN3* functions to sequester Ca^{2+} within the inner hair cell endosomes it lines[137]. Overexpression and depletion of *MCOLN3* dramatically alters the functionality of the endosomal pathway[105], so it is conceivable that subtleties in *MCOLN3* expression, which are strongly associated with the rs17723728 and rs7945619 hearing loss SNPs identified in this work, could manifest in the predisposition of CCSs, who are especially prone to treatment-induced hearing loss and aging processes in general, to hearing loss from inadequate Ca^{2+} sequestration.

In order to evaluate whether subtle variations in *MCOLN3* expression patterns predispose human cells to Ca^{2+} overload and cell death in the context of ototoxic treatments, formal experimentation is required. Experiments evaluating the impact of our two GWAS-identified 11q23.2 SNPs on *MCOLN3* expression in human melanocyte/epithelial cell lines exposed to cisplatin, radiation, or neither treatment would be beneficial. Monitoring of intracellular Ca^{2+}

levels, endosomal Ca^{2+} sequestration, melanosome trafficking (in melanocytes only), and apoptotic marks in the context of different *MCOLN3* expression levels and treatment backgrounds would follow. In order to verify the joint relevance of *MCOLN3* expression and ototoxic treatment exposure with respect to an actual hearing loss phenotype, similar experiments would need to be performed *in vivo* with mice, wherein audiological responses and physiological changes to hair cells and strial melanocytes would need to be monitored.

4.3.3 Plausibility of the 12q23.1 Region

4.3.3.1 Statistical and Bioinformatic Evidence

Interestingly, the present study identified another SNP on a different chromosome whose resident gene is implicated in intracellular Ca^{2+} control. SNP rs11110501 is an intron variant in both the protein-coding and nonsense mediated decay transcripts of *ANO4*. Similar to the SNPs implicated in *MCOLN3* expression, rs11110501 was also detected in the combined and rad+notrt GWASes with a p-value < 1×10^{-5} (ORs = 5.8 and 6.4, p-values = 3.0×10^{-6} and 5.0×10^{-6}). Although rs11110501 and the two SNPs with which it is in high LD ($r^2 \geq 0.80$) are not particularly associated with regulatory chromatin states, rs11110501 does alter the regulatory binding motif bound by the Dmbx1 and Pax-4_5 transcription factors[101,141], the former of which contains a homeodomain and is thought to play a role in brain and sensory organ development.

4.3.3.2 Biological Plausibility

ANO4 is a Ca^{2+}-activated non-selective cation channel[142]. *ANO4* is thought to affect compartmentalized Ca^{2+} intracellular signals by emptying Ca^{2+} stores in the endoplasmic reticulum to facilitate capacitive Ca^{2+} entry across the plasma membrane[143]. Based on inferences from sequence similarity, *ANO4* is also thought to exhibit Ca^{2+}-dependent phospholipid scramblase

activity and translocate phosphatidylserine, phosphatidylcholine, and galactosylceramide between the inner and outer leaflets of the plasma membrane. Of note, Ca^{2+}-dependent scrambling of phosphatidylserine to the external side of the plasma membrane is a hallmark of apoptotic cells[144], although more transient and localized fluctuations in phosphatidylserine scrambling can mediate other cellular processes[145]. Compared to other members of the anoctamin family, exceedingly little is known about *ANO4* function. However, family-member *ANO1* has been extensively linked to diverse audiological processes in the cochlea, auditory neurons, and auditory brainstem[146-150]. Similarly, *ANO4* is known to be predominantly expressed in both mouse and human brain and nervous tissues[98,151]. Although the physiological impact of *ANO4* has yet to be elucidated, the identification of a SNP in another gene implicated in the intracellular control of Ca^{2+} levels and whose family members are intricately involved in many audiological processes is tantalizing. However, further investigation is needed to validate whether this SNP is tagging a biological signal relevant to the ototoxic experience of CCSs.

4.3.4 Plausibility of the 1p12 Region

4.3.4.1 Statistical and Bioinformatic Evidence

The evidence for the nine hearing loss associated SNPs identified in the 1p12 region was compelling with support from statistical, regulatory, epigenomic, transcriptional, and phenotypic perspectives. Of all the SNPs identified in the present work, the nine 1p12 SNPs consistently produced the smallest p-values in each of the four GWASes. The smallest p-value was 1.9×10^{-7}, which belonged to rs4501872 in the cis+notrt analysis (Table 3). All SNPs were clustered over a 114 kb region spanning the adjacent *TBX15* and *WARS2* genes and the uncharacterized *AL139420*.1 lincRNA gene. As can be seen in Table S10, query of these SNPs into Ensembl's Variant Effect Predictor returned many functional variant annotations, including: upstream and

downstream gene variants of protein-coding regions, promoter-flanking variants, downstream lincRNA gene variants, intergenic variants, downstream gene variants of processed transcripts, and intronic variants of protein-coding and processed non-coding transcripts[152].

Exploration of noncoding variant annotations with the online HaploReg portal further evinced the regulatory potential of the 1p12 signal[101]. As can be seen in Table S11, transcription factor binding site motifs were altered for many of the 1p12 variants[141], with two SNPs demonstrating DNA-protein interactions via chromatin immunoprecipitation. Specifically, rs973500 co-precipitated with transcriptional repressor protein YY1 in H1-hESC (a human embryonic stem cell line) and rs4659138 co-precipitated with transcription factor protein MAFK in HepG2 (a human liver cancer cell line used for study of polarized hepatocytes)[153]. Enrichment of promoter and/or enhancer histone marks was also observed for 1p12 SNPs in a wide variety of tissues[97], further supporting the transcriptional importance of this regulatory region. All nine variants were strongly associated with expression of *WARS2*, the nuclear-encoded mitochondrial tryptophanyl-tRNA synthetase gene, in a myriad of human cell lines and tissues with p-values ranging from 7.9×10^{-80} in peripheral blood monocytes to 6.1×10^{-4} in normal prepouch ileum[98,154,155] (Table S10). It is likely that the aforementioned regulatory motif alterations and chromatin state enrichments that characterize these SNPs modulate the association of these SNPs with observed changes in *WARS2* expression.

4.3.4.2 Linking *WARS2* Expression to Hearing Loss Susceptibility

Given the many strong correlations between our identified 1p12 SNPs and *WARS2* expression, it can be postulated that these SNPs were identified as hearing loss risk factors because they tag *WARS2* expression profiles that predispose CCSs to hearing loss. *WARS2* is a nuclear-encoded mitochondrial tryptophanyl-tRNA synthetase gene that is essential for the translation of

mitochondrial genes. Interestingly, mutations in other mitochondrial aminoacyl-tRNA synthetases, such as *LARS2*, *HARS2*, and *NARS2*, have been shown to cause sensorineural hearing loss, which is common symptom of human mitochondrial disease[156-159].

WARS2 was first linked with progressive/late-onset hearing loss in 2016 by a large-scale genetic screen in mutagenized mice for age-related disesase[160] and is known to be expressed in mouse cochleae[161]. More recently, extensive characterization of the mouse *WARS2* V117L mutant in a December 2018 study has further bolstered the plausibility of our GWAS-detected associations between hearing loss and the nine 1p12 variants. In this 2018 work, Agnew et al characterized mice harboring the *WARS2* V117L mutation, which was found to cause progressive tissue-specific pathologies[156]. These pathologies included progressive hearing loss, reduced adiposity, adipose tissue dysfunction, and hypertrophic cardiomyopathy, and were causally linked to tissue-specific mitochondrial respiratory chain deficiencies resulting from reduced *WARS2* expression levels[156]. Of particular relevance to our work, mice homozygous for the *WARS2* mutation displayed age-related hearing loss with the progressive loss of outer hair cell stereocilia bundles and a reduction in the number of spiral ganglions at the cochlear apex[156]. Unfortunately, the impact of reduced *WARS2* expression on respiratory chain deficiencies and potential compensatory mechanisms (such as mitochondrial biogenesis upregulation) remains unknown since it was not evaluated in cochlear tissues as it was for heart, kidney, liver, skeletal muscle, and adipose tissues.

Further research is needed to determine if and how reduced *WARS2* levels in mouse cochlear tissues may result in respiratory chain deficiencies culminating in hearing loss. If alterations in *WARS2* expression levels do indeed impact mitochondrial metabolism and biogenesis in the cochlea as they do in other tissues displaying progressive pathologies, it is plausible that the strong association of our GWAS-identified 1p12 SNPs with *WARS2* expression underlies human

variations in cochlear mitochondrial metabolism that may predispose CCSs to treatment- and age-related hearing loss.

4.4 Treatment-Specific Modulation of 1p12 SNP Effect Sizes

In order to further gauge the effect size and therefore relevance of the 1p12 signals to CCSs with and without major ototoxic treatments, an interaction analysis was performed with the expectation that SNPs identified in more than one stratum-specific GWAS would not demonstrate statistically significant interaction terms. In general, effect sizes tended to be the largest for the notrt and carboplatin subgroups, followed by the cisplatin and then radiation subgroups (Table 7). The attenuation of the genetic effect with ototoxic treatment severity led us to hypothesize that the 1p12 region is a general CCS hearing loss risk factor whose relative effect size is contingent on the baseline probability of CCSs lacking 1p12 genetic variants. In other words, the 1p12 effect size may appear largest for those unexposed to cisplatin and radiation since the baseline probability of hearing loss is low, whereas 1p12 effect size may be diminished in the cisplatin and radiation subgroups since the baseline probability of hearing loss is high.

One exception to this trend was observed for rs973500, which was originally detected in the combined and cis+notrt analyses and displayed cisplatin- and notrt/carbo-specific ORs of 4.0 and 3.1-3.5 in the interaction analyses. Additionally, rs973500's OR of 4.0 was much larger than any of the other cisplatin-specific ORs observed for the 1p12 signal, which ranged from 0.8 – 2.9. Given that rs973500 immunoprecipitates with transcriptional repressor YY1[153], whose protein expression is upregulated with cisplatin exposure and whose knockdown enhances the anticancer effects of cisplatin[162], it is possible that cisplatin modulates rs973500's baseline ototoxic effect via YY1.

Another interesting trend depicted by Table 7 is the null vs non-null effects of certain SNP sets in the cisplatin and radiation subgroups. Specifically, the cisplatin- and radiation-specific ORs appreciably departed from the null value of 1.0 for SNPs identified by multiple GWASes (rs973500 and the core 1p12 SNPs), whereas the cisplatin- and radiation-specific ORs for SNPs identified only in the notrt analysis did not (rs7553422 and rs10923748). This separation of SNPs with non-null vs null cisplatin/radiation effects was clearly delineated by the LD structure of the 1p12 region (Table 5), with high-LD SNPs (r^2 range = 89% - 100%) having non-null cisplatin/radiation effects and low-LD SNPs (r^2 range = 16% - 45%) having null cisplatin/radiation effects (Table 7). Inclusion of a treatment-specific SNP interaction term for the two exclusively notrt GWAS-identified/low-LD SNPs was statistically significant (Table 7). Taken together, these observations are consistent with the detection of SNP-treatment interactions, whereby rs7553422 and rs10923748 only have a hearing loss effect in the absence of major ototoxic treatment effects. It is plausible that this interaction arises from the dwarfing of genetic effects with major treatment effects.

Lastly, the astoundingly high carboplatin-specific ORs of some 1p12 SNPs must be acknowledged. Whereas all other 1p12 SNPs had carboplatin-specific effects sizes comparable to their notrt counterparts, rs4659138 and rs10923748 had carboplatin-specific ORs of 23.9 and 11.8 and notrt-specific ORs ranging from 2.9 to 4.3. Transcription factor MAFK is known to bind to rs4659138, but how this physical interaction might potentially modulate carboplatin ototoxicity is unclear; furthermore, rs10923748 is not bound by a transcription factor but still maintains a high carboplatin-specific OR. Given that there were only 23 people with carboplatin but not cisplatin/radiation exposure, the possibility that these large ORs are due to chance from small cell counts needs to be evaluated. If there are compelling differences in TAFs between carboplatin-

exposed cases and controls vs carboplatin-unexposed cases and controls in the notrt population, then these two SNPs may interact with carboplatin to dramatically elevate ototoxic risk.

4.5 Study Strengths and Limitations

A major strength of this work was our access to the exceptional genetic as well as clinical characterizations of long-term CCSs by SJLIFE, which to date remains globally unrivalled in its extensiveness. Although SJLIFE's sample size of about 3000 CCSs is much smaller than those of other major CCS cohort studies (e.g. the CCSS has upwards of 35,000 CCSs), SJLIFE is unique in that long-term survivor health outcomes are ascertained with on-campus clinical assessments instead of patient self-reporting. For ototoxicity, clinical ascertainment is especially important given the high prevalence of hearing loss in the general population, for which there are varying presentations and etiologies. Most CCSs would be unable to convey any information beyond having/not having hearing loss or a hearing aid, and inclusion of CCSs with heterogeneous hearing deficits almost certainly not related to ototoxic treatments (e.g. major noise exposures or surgery/tumors near auditory structures) would impair our ability to detect genetic risk factors. Therefore, the assessment of CCSs by on-site audiologists with expertise in the presentations of different audiological traumas is extremely valuable.

Unfortunately, there is no clear cut criteria for distinguishing between age-related and treatment-related sensorineural hearing loss. Age-related hearing loss typically begins around 40 years of age and behaves similarly to ototoxicity with respect to high frequencies being affected first with an eventual spread into lower frequencies. While other CCS ototoxicity studies have claimed to avoid this problem by evaluating survivor populations that are mainly under the age of 40 years[163], we cannot do the same since about a third of our population was ≥ 40 years at the time of their most recent audiology examination, which was used for analysis. Furthermore, it may not

be appropriate to try and separate the effects of ototoxicity and age-related hearing loss in aging CCSs if we accept the concept of accelerated ear-age, where ototoxic exposures are theorized to advance the age of the ear such that expected declines in hearing from normative aging processes occur sooner than would be expected in chronological time[86].

If we accept the concept of ear-age, it may be useful to repeat this research work as a time to event analysis, where the event is defined as the first exam in which a SIOP score ≥ 2 is observed. However, since on-campus SJLIFE medical assessments are generally only scheduled every 2-5 years[77], the proxying of hearing loss onset with the time at which a SIOP score ≥ 2 is recorded would be quite crude. Additionally, this would introduce bias in that CCSs diagnosed long before SJLIFE inception may not have had their first audiology exam as a survivor until well after the age of 40 years, when in reality hearing loss onset may have occurred at a much earlier age. Our current use of the most recent audiology exam still has the same problem in that survivors may have had hearing loss at an earlier time, but this problem is more evenly applied to all participants rather than differentially to older CCSs if we used a time to event methodology.

Alternatively, premature hearing loss could be defined as the onset of a SIOP score ≥ 2 prior to the age of 40 years with onsets following this age being classified as age-related hearing loss (non-case status) and not ototoxicity. However, this could be problematic in that this threshold is only a general guideline that could result in the misclassification of ototoxicity cases. Furthermore, it is unclear whether it would be appropriate to classify CCSs with a SIOP score of 1 at < 40 years old that then progressed to a score ≥ 2 after this age threshold as having age-related hearing loss and not ototoxicity. Both radiation and cisplatin ototoxicity can have a late onset in the years following exposure, and mild sensorineural hearing loss is known to frequently progress to more

severe forms over time[13,45]. Therefore, enforcement of an age threshold for classification of hearing loss etiology would likely result in case status misclassification.

Compared to the differential timing of outcome ascertainment in a time to event analysis and the possibility of case status misclassification with the enforcement of a 40-year threshold for case definition, we felt that the use of the most recent audiology exam and corresponding exam age was preferable. However, it must be acknowledged that the use of the most recent exam rather than the first exam with an ototoxic presentation is likely distorting the perceived effect of age on hearing loss. For example, if a substantial proportion of cisplatin-exposed CCSs had their most recent exam at the age of 35 but first demonstrated a SIOP score ≥ 2 at the age of 25, then setting the adjusted age covariate to 35 years for these individuals could distort the relationship between age and hearing loss among cisplatin-exposed CCSs. However, it must be remembered that the goal of our study is to identify genetic variants associated with hearing loss. Therefore, while suboptimal adjustment for age is not ideal, it would not confound the relationship between genetic risk factors and hearing loss since the presence/absence of SNPs within an individual's genomic profile are not influenced by age. However, it would still be beneficial to request the data for and characterize the age distribution of ototoxicity onset and the time from exposure to ototoxicity onset for each treatment group to better understand the contexts in which different genetic variants were identified.

Lastly, the impact of including notrt CCSs in this work must be discussed. Owing to the relatively small number of participants in the cisplatin-only and radiation-only groups, we were unable to perform truly stratified analyses in which treatment-specific genetic risk factors could be identified. As a compensatory measure, notrt CCSs with audiology exams (n = 367) were combined with the cisplatin-only (n = 87) and ≥ 20 Gy radiation-only groups (n = 138) to form the

quasi-stratified cis+notrt, rad+notrt, notrt, and combined analyses. This strategy was implemented to increase the sample size and thus power of each GWAS at the price of increasing subject heterogeneity, ultimately reducing our ability to identify genetic risk factors that specifically modulate sensitivity to cisplatin or radiation (depending on the analysis). Instead, we believe that we have identified genetic variants that are broadly relevant to all CCS treatment profiles whose effect sizes are modulated by the baseline risk imparted by cisplatin or radiation exposure (e.g. rs973500 and the core 1p12 signal), as well as SNPs that appear to only have non-null effect sizes in the absence of strong ototoxic exposures (e.g., rs7553422 and rs10923748 of the 1p12 locus). The identification of signals that appear to be partially or completely driven by notrt CCSs is not surprising since they comprise 62% of our study participants.

Given the importance of notrt CCSs to our analytic strategy, it is important to understand why these individuals received audiology exams in the absence of cisplatin or ≥ 20 Gy radiation. Among the 367 notrt CCSs included in this work, only 44% had carboplatin, CSF shunt, aminoglycoside, and/or non-zero cochlear radiation doses < 20 Gy to justify their audiological examination. For the other 66% of notrt CCSs with audiology exams, it is not readily apparent why these individuals had their hearing tested. The COG guidelines, which first became available in the early 2000s and are updated every few years, are used to inform audiological follow-up decisions at SJLIFE, but the final decision is ultimately made by the clinician. As a result, exposures to major or minor ototoxic treatments are not necessarily a prerequisite for audiology examination.

While not formally quantified in this work, audiologist review of a sample of notrt CCSs with no apparent ototoxic exposures provided some insight. For example, some exams were performed because a patient specifically requested it (perhaps due to a perceived decrease in hearing sensitivity resulting from age), whereas others were necessary to determine auditory function for

patients with co-morbidities such as significant vision impairment. Additionally, some patients received exams because of cranial or neck radiation exposures, which were later characterized as unlikely to impact the cochleae. In the future, it may be desirable to collaborate with an on-site audiologist and go over each of these 204 records to specifically ascertain why apparently unexposed individuals received exams. This would follow with the assessment of whether these 204 people strongly influenced GWAS results. For example, inclusion of these individuals may have biased each GWAS towards the identification of age-related hearing loss genetic risk factors that don't sensitize CCSs to adverse outcomes from major or minor ototoxic exposures.

Finally, as can be seen in the consort diagram in Figure 2, 2041 CCSs (including those of non-European genetic ancestry) did not receive audiology exams. Unexpectedly, 1020 of these survivors had at least one of the following ototoxic exposures, among which cochlear radiation dosages and CSF shunt exposures were not available: cisplatin (n = 9), carboplatin (n = 54), radiation potentially impacting the ear (n = 550), and aminoglycosides (n = 785). It is unclear to what extent audiology exams weren't performed due to the inability of these survivors to complete on-site visits versus the decision of the clinician that an exam wasn't necessary. Further investigation is required as to why these CCSs don't have audiology exams and whether there are distinct differences (e.g., decade of diagnosis, cancer type, socioeconomic status) between those with and without exams.

4.6 Conclusions and Clinical Implications

All too often, a genetic signal's ability to pass a genome-wide significance threshold is inappropriately dichotomized as the discovery, or lack thereof, of a "real" and relevant genotype-phenotype association. This dichotomization of evidence is an overly simplistic and incorrect form of thinking as it fails to consider the biological plausibility and practical importance of identified

variant-phenotype associations and the quality of the study design in which they were unearthed[164]. Instead, GWAS methodologies should be implemented as a tool to screen for top but not necessarily significant variant-phenotype associations, whose relevance should be further contextualized with available biological information. In this thesis work, we applied the latter construct by using several GWASes, adjusting for clinically relevant risk factors and genetic ancestry principal components, to screen for SNPs whose associations with hearing loss in ≥ 5-year CCSs of European genetic ancestry were supported by biological evidence. While we were unable to detect any genetic signals passing genome-wide significance, we were able to identify SNPs within three loci that displayed smaller than expected p-values and whose associations with hearing loss were supported by a wealth of published bioinformatic, biological, and phenotypic evidence sufficient to justify further investigation.

In the future, we will seek to replicate these loci's associations with hearing loss in other CCS cohorts and consider experimental constructs in mouse models and human cell lines that will better allow us to explore if and how these loci modulate hearing loss susceptibility against different treatment backgrounds. If the purported biological relevance of these loci is corroborated by these experimental constructs, we will need to evaluate whether the generated knowledge is of sufficient value and practicality to inform pediatric cancer treatment planning and hearing loss screening. Even if these loci do not modulate hearing loss sensitivities in a treatment specific manner, they may broadly and independently elevate audiological risk to the extent that their combined influence with ototoxic treatments on hearing loss may strongly predict CCS hearing deficits. Ultimately, the joint consideration of high-risk genetic and treatment-related profiles would allow for the development of personalized cancer treatment plans and early protective interventions with the goal of reducing ototoxicity incidence among survivors.

Tables and Figures

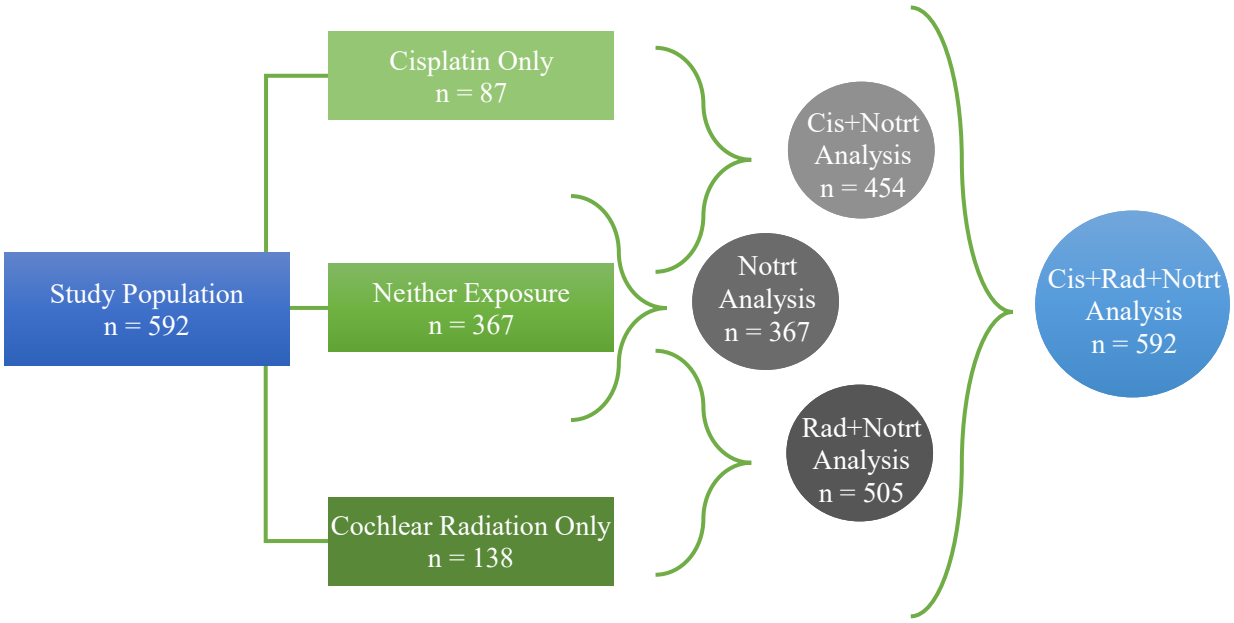
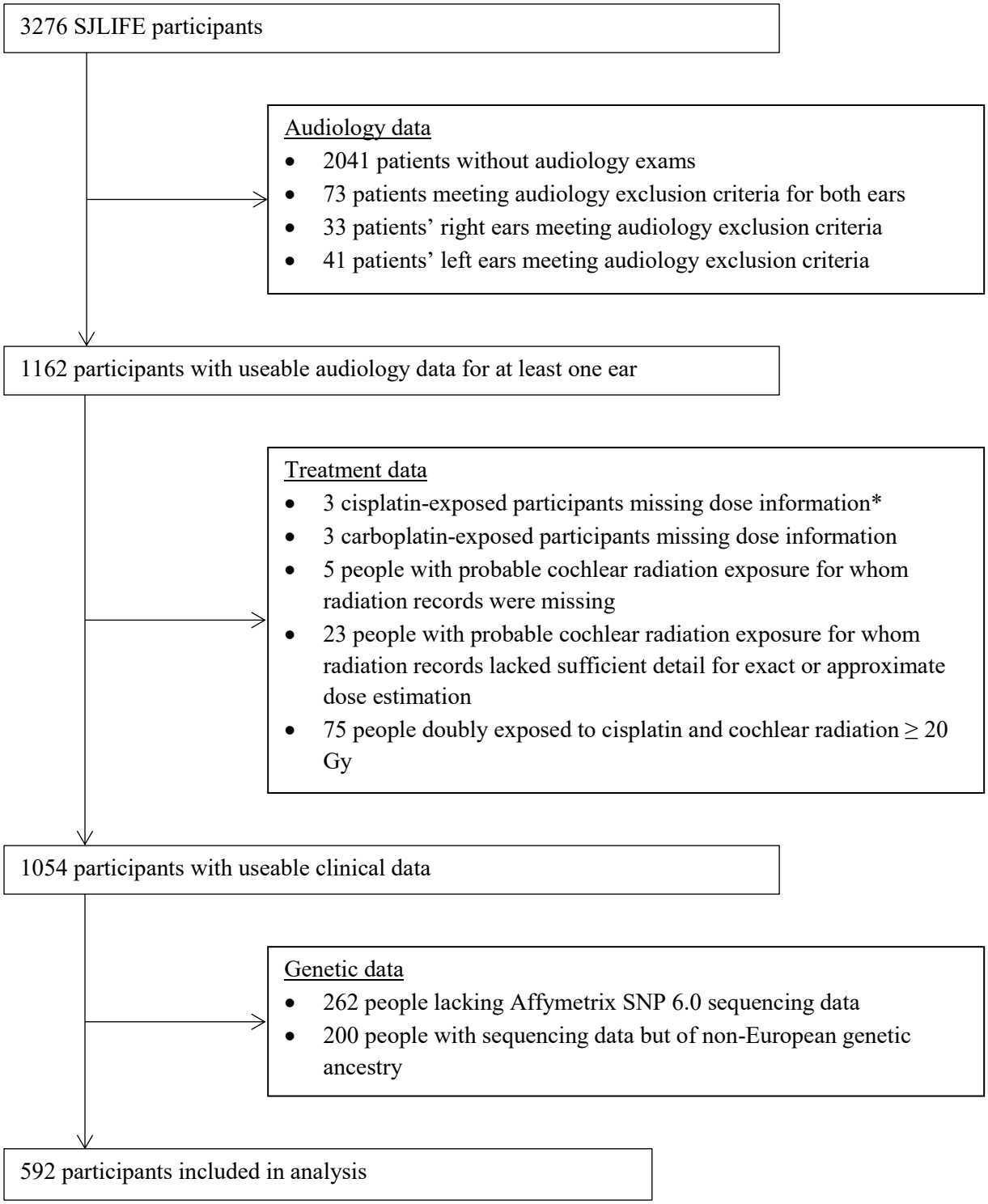


Figure 1. Participant composition for the four treatment-based GWAS analyses.



* One person excluded for missing cisplatin dose information was also counted with the 23 people lacking sufficiently detailed radiation records for cochlear dose calculation.

Figure 2. Consort diagram.

Table 1. Demographic and treatment characteristics of those exposed to cisplatin but not cochlear radiation ≥ 20 Gy, those exposed to cochlear radiation ≥ 20 Gy but not cisplatin, and those exposed to neither cisplatin nor cochlear radiation ≥ 20 Gy.

	Cisplatin Exposed					Radiation ≥ 20Gy					Neither Exposed				
Case status	Cases		Controls		Prevalence	Cases		Controls		Prevalence	Cases		Controls		Prevalence
Sample size	60	69 %	27	31 %	69 %	82	59 %	56	41 %	59 %	46	13 %	321	88 %	13 %
Gender															
Female	21	35 %	18	67 %	54 %	31	38 %	32	57 %	49 %	20	44 %	160	50 %	11 %
Male	39	65 %	9	33 %	81 %	51	62 %	24	43 %	68 %	26	57 %	161	50 %	14 %
Age at most recent audiology exam															
19≤age<30	24	40 %	13	48 %	65 %	15	18 %	21	38 %	42 %	9	20 %	91	28 %	9.0 %
30≤age<40	24	40 %	10	37 %	71 %	30	37 %	17	30 %	64 %	17	37 %	150	47 %	10 %
40≤age<50	11	18 %	4	15 %	73 %	22	27 %	16	29 %	58 %	13	28 %	66	21 %	16 %
50≤age≤65	1	1.7 %	0	0 %	100 %	15	18 %	2	3.6 %	88 %	7	15 %	14	4.4 %	33 %
Median [IQR]	31 [13]					35 [16]					36 [12]				
Age at cancer diagnosis															
age<1	15	25 %	5	19 %	75 %	0	0 %	2	3.6 %	0 %	2	4.3 %	30	9.3 %	6.3 %
1≤age<5	17	28 %	7	26 %	71 %	23	28 %	19	34 %	55 %	11	24 %	108	34 %	9.2 %
5≤age<10	2	3.3 %	4	15 %	33 %	26	32 %	18	32 %	59 %	10	22 %	71	22 %	12 %
10≤age<15	11	18 %	8	30 %	58 %	21	26 %	15	27 %	58 %	13	28 %	75	23 %	15 %
15≤age≤24	15	25 %	3	11 %	83 %	12	15 %	2	3.6 %	86 %	10	22 %	37	12 %	21 %
Median [IQR]	4.0 [13]					7.4 [7.4]					6.1 [9.5]				
Cumulative cisplatin dose (mg/m^2)															
0	0	0 %	0	0 %	--	82	100 %	56	100 %	59 %	46	100 %	321	100 %	13 %
1≤dose<300	8	13 %	8	30 %	50 %	0	0 %	0	0 %	--	0	0 %	0	0 %	--
300≤dose<450	27	45 %	14	52 %	66 %	0	0 %	0	0 %	--	0	0 %	0	0 %	--
450≤dose<600	14	23 %	3	11 %	82 %	0	0 %	0	0 %	--	0	0 %	0	0 %	--
600≤dose≤1028	11	18 %	2	7.4 %	85 %	0	0 %	0	0 %	--	0	0 %	0	0 %	--
Median [IQR]	402 [170]					0 [0]					0 [0]				

Cumulative carboplatin dose (mg/m^2)															
0	54	90 %	25	93 %	68 %	75	92 %	50	89 %	60 %	40	87 %	304	95 %	12 %
0<dose<3000	6	10 %	1	3.7 %	86 %	5	6.1 %	6	11 %	45 %	5	11 %	9	2.8 %	36 %
3000≤dose≤11,059	0	0 %	1	3.7 %	0 %	2	2.4 %	0	0 %	100 %	1	2.2 %	8	2.5 %	11 %
Median [IQR]	0 [0]					0 [0]					0 [0]				
Cochlear radiation dose* (cGy)															
0	58	97 %	25	93 %	70 %	0	0 %	0	0 %	--	38	83 %	268	84 %	12 %
1≤dose<2000	2	3.3 %	2	7.4 %	50 %	0	0 %	0	0 %	--	8	17 %	53	17 %	13 %
2000≤dose<2440	0	0 %	0	0 %	--	3	3.7 %	7	13 %	30 %	0	0 %	0	0 %	--
2440≤dose<2501	0	0 %	0	0 %	--	13	16 %	15	27 %	46 %	0	0 %	0	0 %	--
2501≤dose<4501	0	0 %	0	0 %	--	29	35 %	18	32 %	62 %	0	0 %	0	0 %	--
4501≤dose≤7280	0	0 %	0	0 %	--	37	45 %	16	29 %	70 %	0	0 %	0	0 %	--
Median [IQR]	0 [0]					3790 [2760]					0 [0]				
CSF shunt															
No	58	97 %	26	96 %	69 %	72	88 %	52	93 %	58 %	43	94 %	313	98 %	12 %
Yes	2	3.3 %	1	3.7 %	67 %	10	12 %	4	7.1 %	71 %	3	6.5 %	8	2.5 %	27 %
Aminoglycoside antibiotics															
Any**	26	43 %	5	19 %	84 %	29	35 %	16	29 %	64 %	7	15 %	97	30 %	6.7 %
Amikacin	13	22 %	2	7.4 %	87 %	7	8.5 %	7	13 %	50 %	1	2.2 %	52	16 %	1.9 %
Gentamicin	9	15 %	2	7.4 %	82 %	17	21 %	9	16 %	65 %	2	4.3 %	33	10 %	5.7 %
Kanamycin	0	0 %	0	0 %	--	4	4.9 %	0	0 %	100 %	0	0 %	2	0.6 %	0 %
Tobramycin	6	10 %	1	3.7 %	86 %	3	3.7 %	0	0 %	100 %	5	11 %	31	9.7 %	14 %
None	34	57 %	22	82 %	61 %	53	65 %	40	71 %	57 %	39	85 %	224	70 %	15 %

Cancer diagnosis category															
Bone	23	38 %	3	11 %	88 %	0	0 %	0	0 %	--	3	6.5 %	28	8.7 %	9.7 %
CNS	2	3.3 %	1	3.7 %	67 %	31	38 %	22	39 %	58 %	3	6.5 %	32	10 %	8.6 %
Germ cell tumor	6	10 %	12	44 %	33 %	0	0 %	0	0 %	--	1	2.2 %	5	1.6 %	17 %
Hodgkin lymphoma	0	0.0 %	0	0 %	--	0	0 %	3	5.4 %	0 %	9	20 %	28	8.7 %	24 %
Leukemia	0	0.0 %	0	0 %	--	31	38 %	24	43 %	56 %	4	8.7 %	91	28 %	4.2 %
Neuroblastoma	21	35 %	4	15 %	84 %	0	0 %	0	0 %	--	2	4.3 %	23	7.2 %	8.0 %
Non-Hodgkin lymphoma	0	0 %	1	3.7 %	0 %	8	9.8 %	3	5.4 %	73 %	6	13 %	35	11 %	15 %
Other	7	12 %	5	19 %	58 %	4	4.9 %	2	3.6 %	67 %	4	8.7 %	20	6.2 %	17 %
Soft tissue & extraosseous sarcomas	0	0 %	0	0 %	50 %	8	9.8 %	2	3.6 %	80 %	7	15 %	26	8.1 %	21 %
Wilms tumor	1	1.7 %	1	3.7 %	--	0	0.0 %	0	0 %	--	7	15 %	33	10 %	18 %

* Cochlear radiation dose in cGy for the ear with the highest SIOP score that also passed auditory exclusion criteria.
** A single individual may be exposed to more than one type of aminoglycoside antibiotic.

Table 2a. Clinical logistic regression model for having a SIOP score ≥ 2 in the combined (cis+rad+notrt) population.

Variable	DOF	OR	95% CI	LRT Chisq	LRT P
Carbo.low	1	2.4	0.9, 6.5	3.0	8.1E-02
Carbo.high	1	1.5	0.2, 8.0	0.2	6.3E-01
Sex	1	0.5	0.3, 0.9	7.0	8.3E-03
Tobramycin	1	3.7	1.6, 8.6	8.5	3.5E-03
Ageaudio (decades)	1	2.8	2.1, 3.8	48.3	3.7E-12
CSF shunt	1	2.6	0.9, 7.6	3.4	6.5E-02
Cisplatin dose (100s mg/m^2)	1	1.3	1.0, 1.9	4.3	3.7E-02
Youngcis	1	14.4	3.1, 64.0	11.1	8.8E-04
Oldcis	1	6.0	1.4, 23.8	5.5	1.9E-02
Youngrad	1	1.9	0.8, 4.6	1.9	1.7E-01
Radiation dose:					
radcat2000	1	1.7	0.3, 7.7	0.4	5.4E-01
radcat2440	1	3.2	1.1, 8.7	4.8	2.8E-02
radcat2501	1	9.6	4.5, 20.9	34.8	3.6E-09
radcat4501	1	25.5	11.7, 58.0	72.1	2.0E-17

Table 2b. Independent variable definitions for the combined clinical logistic regression model.

Variable	Type	Description
Carboplatin • Carbo.low • Carbo.high	Categorical with three levels and two dummy variables	• if carboplatin dose=0 mg/m^2, then carbocat.low=carbocat.high=0 • if 0 mg/m^2<carboplatin dose<3000 mg/m^2, then carbocat.low=1 and carbocat.high=0 • if carboplatin dose≥3000mg/m^2, then carbocat.low=0 and carbocat.high=1
Sex	Binary	• sex=0 if male • sex=1 if female
Tobramycin	Binary	• tobramycin=0 if unexposed • tobramycin=1 if exposed to any non-zero dose
Ageaudio	Continuous	• continuous age at most recent audiology examination in decades
CSF shunt	Binary	• shunt=0 if never had a CSF shunt placement • shunt=1 if ever had a CSF shunt placement
Cisplatin dose	Continuous	• continuous cisplatin dose in 100s of mg/m^2
Youngcis	Binary	• youngcis=1 if exposed to any non-zero cisplatin dose AND age of cancer diagnosis <5 years • youngcis=0 otherwise
Oldcis	Binary	• oldcis=1 if exposed to any non-zero cisplatin dose AND age of cancer diagnosis ≥ 5 years • oldcis=0 otherwise
Youngrad	Binary	• youngrad=1 if exposed to a radiation dose of ≥ 20 Gy in the ear with the worst SIOP score AND age of cancer diagnosis < 5 years • youngrad=0 otherwise

Radiation dose: - radcat2000 - radcat2440 - radcat2501 - radcat4501	Categorical with five levels and four dummy variables	• if cochlear radiation for the worst ear < 2000 cGy (reference group), then radcat2000=radcat2440=radcat2501=radcat4501=0 • if 2000cGy<=cochlear radiation for the worst ear<2440cGy, then radcat2000=1 and radcat2440=radcat2501=radcat4501=0 • if 2440cGy<=cochlear radiation for the worst ear<2501cGy, then radcat2440=1 and radcat2000=radcat2501=radcat4501=0 • if 2501cGy<=cochlear radiation for the worst ear<4501cGy, then radcat2501=1 and radcat2000=radcat2440=radcat4501=0 • if cochlear radiation for the worst ear>=4501cGy, then radcat4501=1 and radcat2000=radcat2501=radcat2501=0

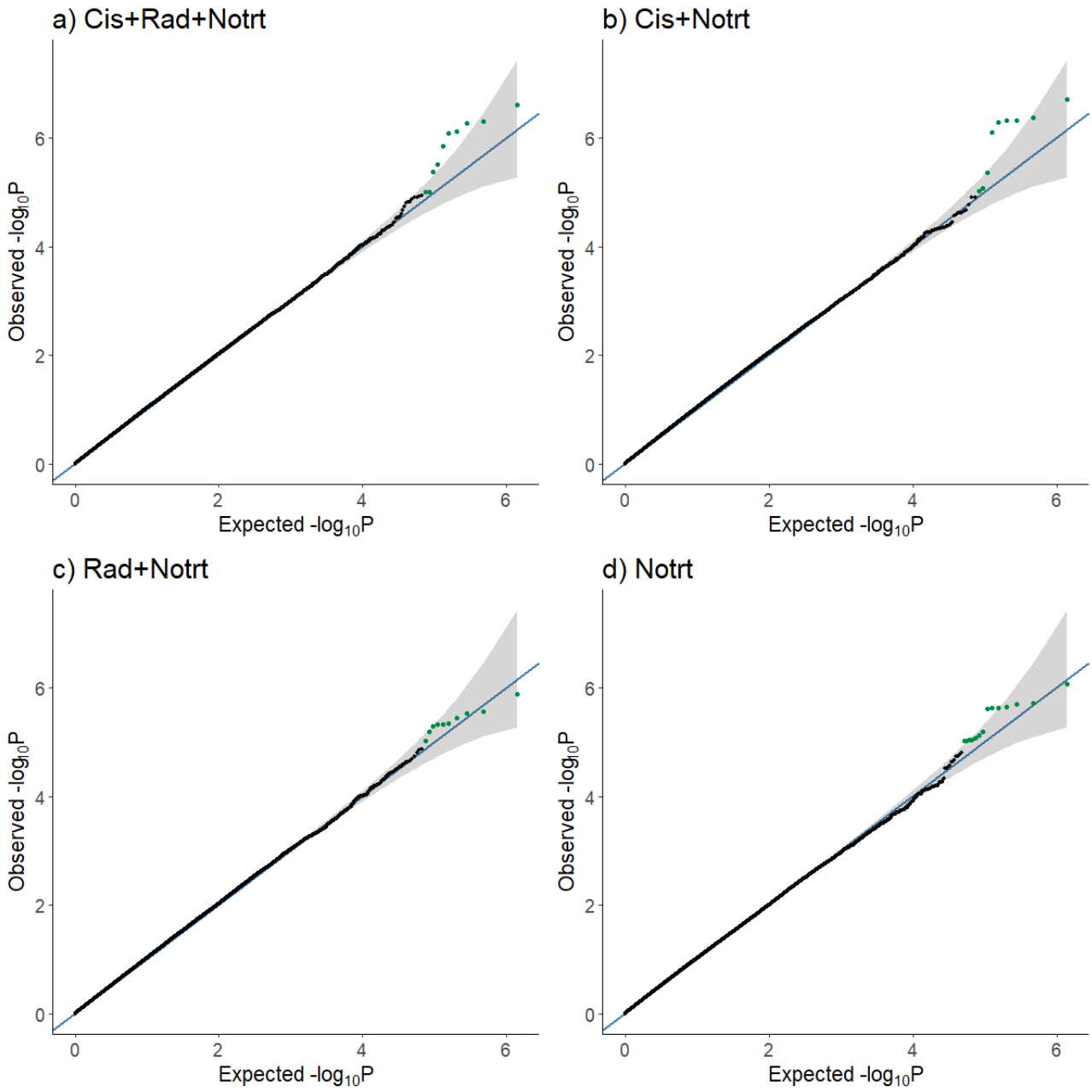


Figure 3. QQ plots of observed vs expected distributions of -$\log_{10}$ Wald p-values for the 709,023 SNPs tested in the a) combined (cis+rad+notrt), b) cis+notrt, c) rad+notrt, and d) notrt additive analyses. SNPs with a Wald p-value less than 1 x 10^{-5} are highlighted in green and 95% CIs are shown with grey shading.

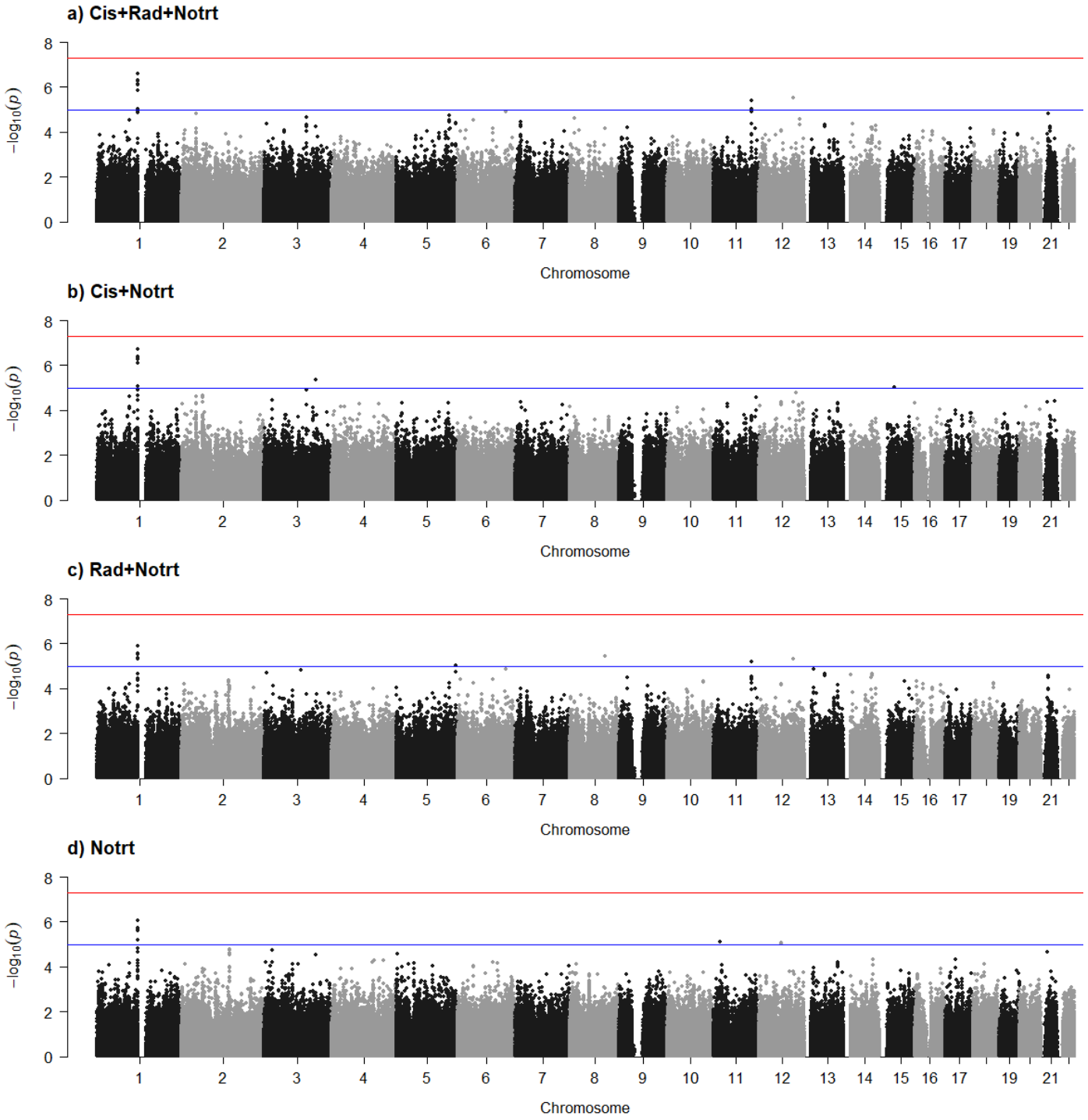


Figure 4. Manhattan plots for the a) combined (cis+rad+notrt), b) cis+notrt, c) rad+notrt, and d) notrt additive GWASes. The blue and red lines respectively indicate suggestive (p-value = 1 x 10^{-5}) and genome-wide (p-value = 5 x 10^{-8}) significance thresholds.

Table 3. SNPs with adjusted p-values < 1 x 10^{-5} in at least one of the four additive GWAS analyses. Bolded cells indicate that a SNP met the p-value threshold for a particular analysis.

rsid	Closest Transcripts (Distance)	Chr	Position	TA	Combined		Cis+Notrt		Rad+Notrt		Notrt	
					OR	P-Value	OR	P-Value	OR	P-Value	OR	P-Value
rs973500	*TBX15* (2112 bp)	1	118,991,668	G	**2.8**	**9.6E-06**	**3.5**	**8.3E-06**	2.7	7.6E-05	3.6	6.8E-05
rs7553422	*AL139420*.1 (2247 bp) *TBX15* (8539 bp)	1	118,998,096	C	1.8	1.2E-03	2.4	1.0E-04	2.2	1.4E-04	**4.8**	**2.2E-06**
rs4501872	*AL139420*.1 (8255 bp) *TBX15* (20,104 bp)	1	119,009,661	A	**3.2**	**2.4E-07**	**4.2**	**1.9E-07**	**3.3**	**1.3E-06**	**4.8**	**8.7E-07**
rs4659138	*AL139420*.1 (10,604 bp) *WARS2* (19,204 bp)	1	119,012,010	C	**3.0**	**1.4E-06**	**3.9**	**7.8E-07**	**3.1**	**4.6E-06**	**4.6**	**1.9E-06**
rs10923726	*AL139420*.1 (13,122 bp) *WARS2* (16,686 bp)	1	119,014,528	G	**3.0**	**8.0E-07**	**4.0**	**5.2E-07**	**3.1**	**4.7E-06**	**4.5**	**2.4E-06**
rs12021830	*WARS2* (11,455 bp)	1	119,019,759	C	**3.1**	**7.4E-07**	**4.0**	**4.8E-07**	**3.1**	**4.5E-06**	**4.5**	**2.3E-06**
rs10494218	*WARS2* (11,280 bp)	1	119,019,934	T	**3.1**	**5.3E-07**	**4.0**	**4.8E-07**	**3.2**	**2.9E-06**	**4.5**	**2.3E-06**
rs12027986	*WARS2* (2572 bp)	1	119,028,642	A	**3.1**	**4.9E-07**	**4.1**	**4.2E-07**	**3.2**	**2.7E-06**	**4.7**	**2.0E-06**
rs10923748	*WARS2* (0 bp)	1	119,105,323	G	1.5	1.1E-02	2.2	2.4E-04	1.7	4.3E-03	**3.4**	**6.4E-06**
rs12637439	*RP11-292E2.1* (33,648 bp) *RAP2B* (154,983 bp)	3	153,323,458	C	2.6	5.8E-05	**3.5**	**4.3E-06**	2.4	3.8E-04	3.5	3.0E-05
rs11956125	*DRD1* (193,937 bp)	5	175,246,734	A	3.1	4.4E-05	2.4	6.2E-03	**3.7**	**9.2E-06**	2.9	2.2E-03
rs4537278	*ODF1* (0 bp)	8	102,555,002	C	5.6	6.8E-05	3.6	4.1E-03	**9.1**	**3.5E-06**	5.3	9.0E-04
rs1945412	*NELL1* (0 bp)	11	21,337,924	G	1.6	1.8E-02	2.3	7.6E-04	2.0	2.3E-03	**4.1**	**7.6E-06**
rs17723728	*USP28* (0 bp) *AP003170.3* (1971 bp)	11	113,816,106	C	**0.2**	**9.6E-06**	0.3	4.3E-03	0.2	2.8E-05	0.3	2.0E-02
rs7945619	*HTR3B* (5376 bp)	11	113,899,300	A	**0.2**	**4.1E-06**	0.3	2.6E-03	**0.1**	**6.3E-06**	0.2	8.0E-03
rs11175255	*SRGAP1* (0 bp) *AC020611.2* (0 bp)	12	64,082,870	C	3.3	8.7E-05	4.1	4.9E-05	3.8	6.1E-05	**5.9**	**8.5E-06**
rs12309038	*SRGAP1* (0 bp) *AC020611*.2 (0 bp)	12	64,069,350	T	3.3	8.1E-05	4.1	4.5E-05	3.8	6.4E-05	**5.9**	**9.0E-06**
rs9652019	*SRGAP1* (0 bp) *AC020611*.2 (0 bp)	12	64,071,529	A	3.3	9.1E-05	4.1	4.9E-05	3.8	6.4E-05	**5.9**	**9.0E-06**
rs11175257	*SRGAP1* (0 bp) *AC020611*.2 (0 bp)	12	64,090,052	C	3.3	9.5E-05	4.1	5.5E-05	3.7	6.7E-05	**5.9**	**9.5E-06**
rs12302132	*SRGAP1* (0 bp) *AC020611*.2 (0 bp)	12	64,090,510	A	3.3	8.4E-05	4.1	4.6E-05	3.7	6.7E-05	**5.9**	**9.5E-06**
rs11110501	*ANO4* (0 bp)	12	100,732,070	T	**5.8**	**3.0E-06**	5.5	4.3E-05	**6.4**	**5.0E-06**	5.9	1.6E-04
rs1008782	*PLA2G4D* (9856 bp)	15	42,104,411	T	1.9	1.7E-03	**3.1**	**9.6E-06**	1.6	6.2E-02	2.6	1.2E-03

* Closest Transcripts (Distance): the annotated transcripts closest to a given variant and their distance away in base pairs, Chr: chromosome, Position: chromosomal position in base pairs, OR: odds ratio of the TA for hearing loss, P-Value: Wald p-value.

Table 4. TAFs for SNPs with adjusted p-values < 1 x 10^{-5} in at least one of the four additive GWAS analyses. Bolded cells indicate that a SNP met the p-value threshold for a particular analysis.

rsid	TA	TAFs							
		Combined		Cis+Notrt		Rad+Notrt		Notrt	
		Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
rs973500	G	**19.7%**	**10.0%**	**21.2%**	**10.2%**	20.7%	10.2%	26.1%	10.4%
rs7553422	C	65.4%	55.0%	69.3%	54.5%	68.0%	54.3%	**81.5%**	**53.7%**
rs4501872	A	**24.2%**	**11.8%**	**27.4%**	**11.9%**	**25.4%**	**11.8%**	**34.8%**	**12.0%**
rs4659138	C	**24.2%**	**12.2%**	**26.9%**	**12.3%**	**25.4%**	**12.2%**	**33.7%**	**12.3%**
rs10923726	G	**23.9%**	**11.9%**	**26.9%**	**12.0%**	**25.0%**	**12.0%**	**33.7%**	**12.0%**
rs12021830	C	**23.9%**	**11.8%**	**26.9%**	**11.9%**	**25.0%**	**11.8%**	**33.7%**	**12.0%**
rs10494218	T	**23.9%**	**11.8%**	**26.9%**	**11.9%**	**25.0%**	**11.8%**	**33.7%**	**12.0%**
rs12027986	A	**23.9%**	**11.8%**	**26.9%**	**11.9%**	**25.0%**	**11.8%**	**33.7%**	**12.0%**
rs10923748	G	36.4%	28.5%	40.6%	27.9%	38.7%	28.0%	**52.2%**	**27.3%**
rs12637439	C	17.6%	12.8%	**21.2%**	**12.6%**	18.8%	13.1%	29.4%	13.1%
rs11956125	A	10.9%	6.4%	11.3%	7.0%	**13.3%**	**6.4%**	18.5%	7.0%
rs4537278	C	6.4%	2.8%	7.5%	3.3%	**7.4%**	**2.7%**	12.0%	3.1%
rs1945412	G	24.5%	18.7%	28.3%	18.3%	26.6%	18.4%	**39.1%**	**17.9%**
rs17723728	C	**6.1%**	**14.1%**	7.1%	13.7%	4.7%	13.9%	4.3%	13.4%
rs7945619	A	**6.2%**	**14.1%**	7.2%	13.7%	**4.3%**	**14.0%**	3.3%	13.4%
rs11175255	C	11.4%	7.9%	12.7%	7.9%	12.5%	7.8%	**17.4%**	**7.8%**
rs12309038	T	11.7%	8.0%	13.2%	8.0%	12.5%	8.0%	**17.4%**	**7.9%**
rs9652019	A	11.4%	8.0%	12.7%	8.0%	12.5%	8.0%	**17.4%**	**7.9%**
rs11175257	C	11.4%	8.0%	12.7%	8.0%	12.5%	8.0%	**17.4%**	**7.9%**
rs12302132	A	11.7%	8.0%	13.2%	8.0%	12.5%	8.0%	**17.4%**	**7.9%**
rs11110501	T	**9.4%**	**3.2%**	10.6%	3.3%	**10.2%**	**3.3%**	14.4%	3.4%
rs1008782	T	23.1%	17.0%	**29.7%**	**17.0%**	19.9%	17.8%	29.4%	18.0%

Table 5. LD matrix of r^2 values for the nine 1p12 SNPs.

SNP	rs973500	rs7553422	rs4501872	rs4659138	rs10923726	rs12021830	rs10494218	rs12027986	rs10923748
rs973500	100%	16%	93%	91%	90%	90%	90%	89%	39%
rs7553422	16%	100%	17%	17%	17%	17%	17%	16%	25%
rs4501872	93%	17%	100%	98%	98%	98%	98%	97%	43%
rs4659138	91%	17%	98%	100%	99%	99%	99%	99%	44%
rs10923726	90%	17%	98%	99%	100%	100%	100%	99%	45%
rs12021830	90%	17%	98%	99%	100%	100%	100%	99%	45%
rs10494218	90%	17%	98%	99%	100%	100%	100%	99%	45%
rs12027986	89%	16%	97%	99%	99%	99%	99%	100%	45%
rs10923748	39%	25%	43%	44%	45%	45%	45%	45%	100%

Table 6. Effect size and significance of rs4501872 across treatment-based analyses, adjusting for clinical variables and genetic ancestry principal components.

Analysis	Sample Size	N lost	Ncase0	Ncontrol0	Ncase1	Ncontrol1	Ncase2	Ncontrol2	OR	P
Cis+notrt	454	138	58	269	38	75	10	4	4.2	1.9E-07
Rad+notrt	505	87	75	293	41	79	12	5	3.3	1.3E-06
Notrt	367	225	21	248	18	69	7	4	4.8	8.7E-07
Combined	592	0	112	314	61	85	15	5	3.2	2.4E-07

Ncase#: number of hearing loss cases with 0/1/2 copies of the TA.
Ncontrol#: number of controls with 0/1/2 copies of the TA.

Table 7. Treatment-specific effects estimated with interaction terms, adjusting for pertinent clinical variables, the top 10 PCs, and main SNP effects. The core 1p12 SNPs are enclosed in a bolded box and interaction terms with p-values < 0.05 are highlighted in pink. ORs are colour coded to visualize effect size differences. Carboplatin-specific ORs were only estimated for those lacking cisplatin and cochlear radiation exposures.

SNP	Cis+Notrt Population				Rad+Notrt Population				Notrt Population			
	N	Cis.OR	Notrt.OR	P-Value	N	Rad.OR	Notrt.OR	P-Value	N	Carbo.OR	Nocarbo.OR	P-Value
rs973500	454	4.0	3.4	8.1E-01	505	2.0	3.1	3.9E-01	367	3.3	3.5	9.6E-01
rs7553422	448	0.8	4.6	2.5E-04	498	1.0	4.4	4.7E-04	361	3.7	4.8	8.0E-01
rs4501872	454	2.8	4.6	4.6E-01	505	2.0	4.3	1.4E-01	367	4.3	4.7	9.2E-01
rs4659138	444	2.7	4.3	4.6E-01	495	1.8	4.2	8.7E-02	359	23.9	4.1	2.0E-01
rs10923726	453	2.8	4.3	5.3E-01	504	1.9	4.0	1.4E-01	366	4.2	4.3	9.7E-01
rs12021830	454	2.9	4.3	5.3E-01	504	1.9	3.9	1.6E-01	367	4.2	4.3	9.8E-01
rs10494218	454	2.9	4.3	5.3E-01	505	2.0	4.0	1.7E-01	367	4.2	4.3	9.8E-01
rs12027986	454	2.9	4.6	4.8E-01	505	2.0	4.1	1.5E-01	367	4.3	4.6	9.4E-01
rs10923748	454	0.9	3.1	1.2E-02	505	0.8	2.9	1.4E-03	367	11.8	3.0	1.9E-01

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Supplementary Methods

Defining Cisplatin and Radiation Exposure

To better model the crucial ototoxic effects of cisplatin and cochlear radiation, exploratory data analysis was undertaken to evaluate whether dose threshold effects were operative. This aim was achieved with the construction of cisplatin by cochlear radiation dose matrices for the ear with the worst SIOP score to visualize dose and hearing loss distributions. Dose categories were informed by the clustering of participants across dose axes as well as apparent changes in ototoxic risk.

As can be seen in Table S1a, most individuals were unexposed to neither treatment (n = 306), whereas 83 were singly-exposed to cisplatin, 199 were singly-exposed to cochlear radiation, and 40 were doubly exposed to both cisplatin and radiation. Table S1b shows the distribution of cases with a SIOP score ≥ 2 by cisplatin and radiation dose, whereas Table S1c shows the prevalence. Although the doubly unexposed cell contributed the most cases of any dose category (Table S1b), it is clear that single exposure to either cisplatin or radiation elevates ototoxic risk (Table S1c). Notably, a dose threshold effect seems operative for cochlear radiation exposure: after excluding the 10-15 Gy cell for only having two observations, it can be observed that the ototoxic prevalence fluctuates at a low baseline level of 7-20% for doses spanning 0-20 Gy. For the 20-25 Gy category, the prevalence jumps to 42%, after which it generally continues to increase with higher doses. On the basis of this observation, we defined cochlear radiation exposure as exposure to ≥ 20 Gy. To contrast, any amount of cisplatin seemed to dramatically elevate ototoxic risk and a non-zero exposure threshold was not implemented.

In addition to evaluating dose-thresholds for single exposure to cisplatin or radiation, the matrices in Table S1 also allowed us to identify exposure combinations that were nearly

deterministic for ototoxicity. Strikingly, 33 of the 36 participants doubly exposed to cisplatin and cochlear radiation ≥ 20 Gy were cases (prevalence = 92%). To avoid dilution of potential genetic signals, we excluded these 36 people for whom ototoxicity was almost certainly due to treatment effects alone.

Supplementary Tables and Figures

Table S1a. Cross-tabulation of cisplatin and cochlear radiation dose exposures among the 628 participants of European genetic ancestry with GWAS data. Lower dose bounds are inclusive and upper bounds are exclusive.

	Radiation dose (Gy)													
Cisplatin dose (mg/m2)	0	0.3-5	5-10	10-15	15-20	20-25	25-30	30-35	35-40	40-45	45-50	50-55	55-60	60-90
0	306	17	15	2	27	36	19	10	9	10	14	19	16	5
70-100	0	0	1	0	0	0	0	0	0	0	0	1	1	0
100-200	9	0	0	0	0	0	0	0	0	1	0	1	1	0
200-300	5	0	0	1	0	0	0	0	1	1	4	4	5	0
300-400	12	0	0	0	0	0	0	2	2	1	0	1	4	3
400-500	34	1	0	0	0	0	0	1	0	0	0	0	0	0
500-600	11	0	0	0	0	0	0	0	0	0	0	0	0	1
600-800	5	0	1	0	0	0	0	0	0	0	0	0	0	1
800-1100	7	0	0	0	0	0	0	0	0	0	0	0	0	0

Table S1b. Cisplatin and cochlear radiation dose distributions for the 221 cases of European genetic ancestry with GWAS data. Zeros are only shown in cells with observations. Lower dose bounds are inclusive and upper bounds are exclusive.

	Radiation dose (Gy)													
Cisplatin dose (mg/m2)	0	0.3-5	5-10	10-15	15-20	20-25	25-30	30-35	35-40	40-45	45-50	50-55	55-60	60-90
0	38	2	3	1	2	15	14	7	5	4	10	10	13	4
70-100			0									1	1	
100-200	4									1		1	1	
200-300	4			0					1	1	3	4	5	
300-400	8							2	2	1		1	3	3
400-500	24	1						0						
500-600	8													1
600-800	4		1											1
800-1100	6													

Table S1c. Prevalence of hearing loss by cisplatin and cochlear radiation dose distribution for the 628 participants of European genetic ancestry with GWAS data. Zeros are only shown in cells with observations. Lower dose bounds are inclusive and upper bounds are exclusive.

	Radiation dose (Gy)													
Cisplatin dose (mg/m2)	0	0.3-5	5-10	10-15	15-20	20-25	25-30	30-35	35-40	40-45	45-50	50-55	55-60	60-90
0	12%	12%	20%	50%	7%	42%	74%	70%	56%	40%	71%	53%	81%	80%
70-100			0%									100%	100%	
100-200	44%									100%		100%	100%	
200-300	80%			0%					100%	100%	75%	100%	100%	
300-400	67%							100%	100%	100%		100%	75%	100%
400-500	71%	100%						0%						
500-600	73%													100%
600-800	80%		100%											100%
800-1100	86%													

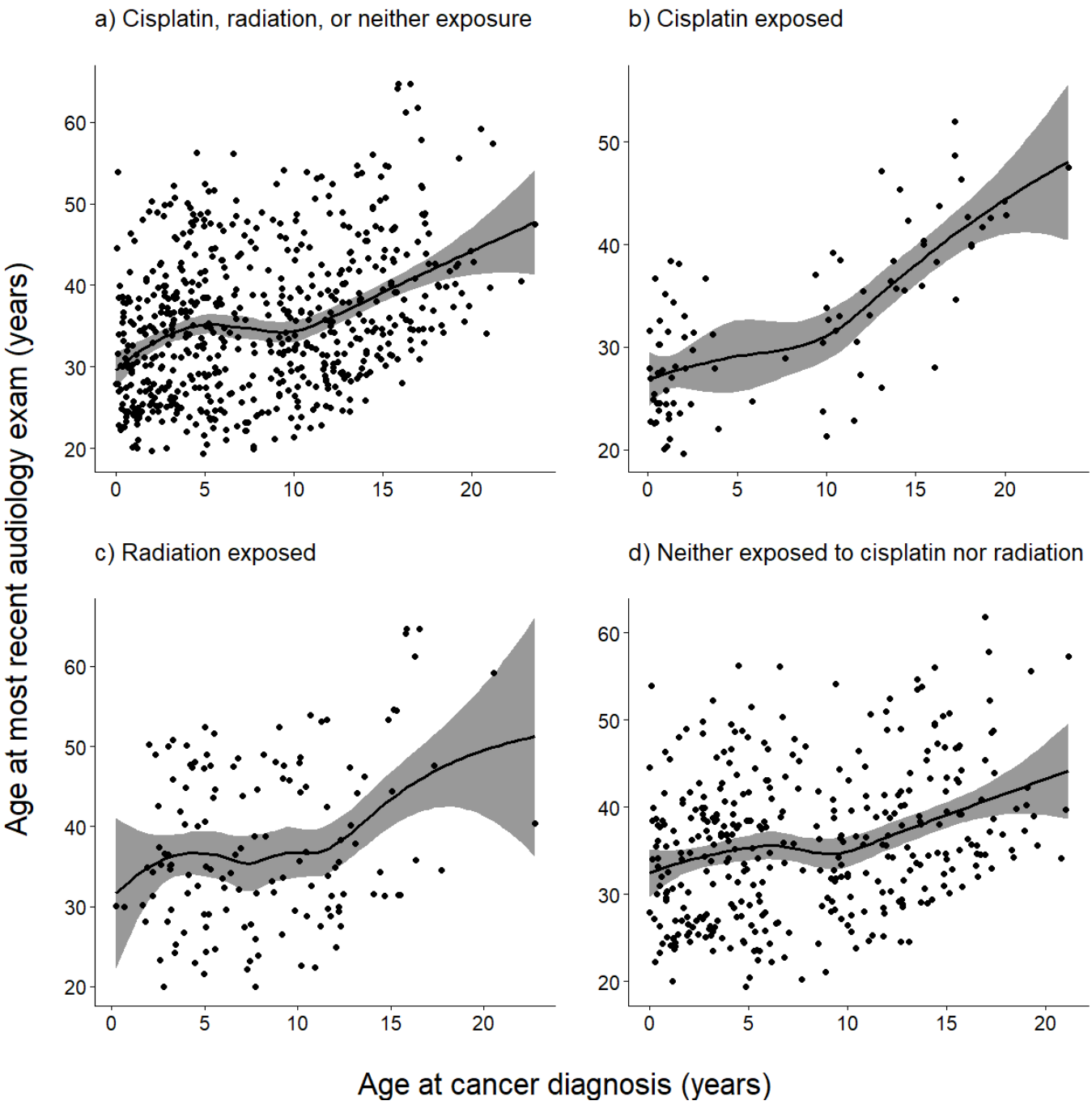

Figure S1. Lowess curves and 95% CIs for age at most recent audiology exam by age at cancer diagnosis for a) the combined cisplatin, radiation, or neither exposure population, b) the cisplatin-only exposed stratum, c) the radiation-only exposed stratum, and d) the doubly unexposed to cisplatin and radiation stratum.

Table S2a. Separate clinical logistic regression models for having a SIOP score ≥ 2 for the cis+notrt, rad+notrt, and notrt strata.

Variable	DOF	OR			95% CI			LRT Chisq			LRT P		
		Cis + Notrt	Rad + Notrt	Notrt	Cis + Notrt	Rad + Notrt	Notrt	Cis + Notrt	Rad + Notrt	Notrt	Cis + Notrt	Rad + Notrt	Notrt
Carbo.low	1	6.9	2.1	7.4	2.2, 21.6	0.7, 6.2	2.0, 25.7	10.3	1.6	8.4	1.3E-03	2.1E-01	3.7E-03
Carbo.high	1	0.7	2.9	2.1	0.0, 6.0	0.4, 14.6	0.1, 13.4	0.1	1.4	0.4	7.8E-01	2.4E-01	5.4E-01
Sex	1	0.6	0.6	0.7	0.3, 1.0	0.4, 1.0	0.4, 1.4	3.3	3.8	0.8	6.8E-02	5.0E-02	3.8E-01
Tobramycin	1	2.4	3.4	1.9	0.9, 6.0	1.3, 8.4	0.6, 5.3	3.1	5.7	1.1	7.7E-02	1.7E-02	2.9E-01
Ageaudio (decades)	1	2.4	2.8	2.4	1.7, 3.5	2.0, 3.9	1.6, 3.6	22.7	43.4	17.5	1.9E-06	4.5E-11	2.9E-05
CSF shunt	1	3.5	2.9	4.6	0.8, 13.6	1.0, 9.1	0.9, 19.1	2.8	3.6	3.4	9.6E-02	5.8E-02	6.7E-02
Cisplatin dose (100s mg/m^2)	1	1.4			1.0, 1.9			4.7			3.1E-02		
Youngcis	1	10.9			2.3, 49.6			8.8			3.0E-03		
Oldcis	1	5.2			1.2, 20.9			4.7			3.0E-02		
Youngrad	1		1.8			0.7, 4.5			1.7			1.9E-01	
Radiation dose:													
									0.4			5.1E-01	
radcat2000	1		1.7			0.3, 8.0							
radcat2440	1		3.1			1.1, 8.7			4.8			2.9E-02	
radcat2501	1		9.6			4.5, 20.9			34.8			3.7E-09	
radcat4501	1		25.3			11.5, 58.1			70.9			3.8E-17	

Table S2b. Independent variable definitions for the clinical logistic regression models.

Variable	Type	Description
Carboplatin • Carbo.low • Carbo.high	Categorical with three levels and two dummy variables	• if carboplatin dose=0 mg/m^2, then carbocat.low=carbocat.high=0 • if 0 mg/m^2<carboplatin dose<3000 mg/m^2, then carbocat.low=1 and carbocat.high=0 • if carboplatin dose≥3000mg/m^2, then carbocat.low=0 and carbocat.high=1
Sex	Binary	• sex=0 if male • sex=1 if female
Tobramycin	Binary	• tobramycin=0 if unexposed • tobramycin=1 if exposed to any non-zero amount
Ageaudio	Continuous	• continuous age at most recent audiology examination in decades
CSF shunt	Binary	• shunt=1 if ever had a CSF shunt placement • shunt=0 if never had a CSF shunt placement
Cisplatin dose	Continuous	• continuous cisplatin dose in 100s of mg/m^2
Youngcis	Binary	• youngcis=1 if exposed to any non-zero cisplatin dose AND age of cancer diagnosis <5 years • youngcis=0 otherwise
Oldcis	Binary	• oldcis=1 if exposed to any non-zero cisplatin dose AND age of cancer diagnosis ≥ 5 years • oldcis=0 otherwise
Youngrad	Binary	• youngrad=1 if exposed a radiation dose of ≥ 20 Gy in the ear with the worst SIOP score AND age of cancer diagnosis < 5 years • youngrad=0 otherwise

Radiation dose: - radcat2000 - radcat2440 - radcat2501 - radcat4501	Categorical with five levels and four dummy variables	• if cochlear radiation for the worst ear < 2000 cGy (reference group), then radcat2000=radcat2440 =radcat2501=radcat4501=0 • if 2000cGy<=cochlear radiation for the worst ear<2440cGy, then radcat2000=1 and radcat2440=radcat2501=radcat4501=0 • if 2440cGy<=cochlear radiation for the worst ear<2501cGy, then radcat2440=1 and radcat2000=radcat2501=radcat4501=0 • if 2501cGy<=cochlear radiation for the worst ear<4501cGy, then radcat2501=1 and radcat2000=radcat2440=radcat4501=0 • if cochlear radiation for the worst ear>=4501cGy, then radcat4501=1 and radcat2000=radcat2501=radcat2501=0

Table S3. Observed and predicted ototoxic probabilities by cochlear radiation dose category. The predicted probabilities were calculated for a hypothetical 35 year old female unexposed to platinum, tobramycin, or a CSF shunt with an age of cancer diagnosis ≥ 5 years.

Cochlear Radiation Dose Category	Observed Hearing Loss Probability (%)	Predicted Hearing Loss Probability (%)
0≤dose<2000 cGy	14.3	9.8
2000≤dose<2440 cGy	30.0	15.3
2440≤dose<2501 cGy	46.4	25.5
2501≤dose<4501 cGy	61.7	51.0
4501≤dose≤7280 cGy	69.8	73.4

Table S4. Adjusted ORs and p-values for three-level carboplatin variables defined with different dose thresholds for the combined analysis. Colour gradients are provided to better visualize changes in OR and p-value magnitudes.

Threshold (mg/m^2)	Low Dose Category			High Dose Category			Nocarbo*		Binary**	
	OR	Wald P-Value	N	OR	Wald P-Value	N	Chisq	LRT P-Value	Chisq	LRT P-Value
1000	4.4	2.4E-01	4	2.0	1.4E-01	40	3.3	0.19	0.36	0.55
2000	2.8	1.7E-01	13	1.9	2.1E-01	31	3.1	0.21	0.19	0.67
3000	2.4	7.9E-02	32	1.5	6.2E-01	12	3.2	0.21	0.21	0.65
4000	2.4	7.0E-02	34	1.3	8.0E-01	10	3.3	0.20	0.30	0.58
5000	2.2	9.3E-02	40	2.4	6.0E-01	4	3.0	0.23	0.004	0.95
Binary	2.2	8.2E-02	44	--	--	--	3.0	0.09	--	--

* LRT chisq and p-value compared to a model without any carboplatin variables.
** LRT chisq and p-value compared to a model with a binary carboplatin variable for zero and non-zero doses.

Table S5. The top 20 SNPs identified in each of the four additive GWAS analyses adjusting for the top 10 genetic ancestry PCs and pertinent clinical models. SNPs are colour-coded to identify spatially clustered signals, with white indicating that only one SNP was detected for a particular locus.

Additive Combined GWAS													
rsid	Chr	Position	TA	0 TA		1 TA		2 TA		NA TA		OR	P-Value
				Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls		
rs4501872	1	119,009,661	A	112	314	61	85	15	5	0	0	3.2	2.4E-07
rs12027986	1	119,028,642	A	113	313	60	87	15	4	0	0	3.1	4.9E-07
rs10494218	1	119,019,934	T	113	314	60	85	15	5	0	0	3.1	5.3E-07
rs12021830	1	119,019,759	C	113	313	60	85	15	5	0	1	3.1	7.4E-07
rs10923726	1	119,014,528	G	113	312	60	86	15	5	0	1	3.0	8.0E-07
rs4659138	1	119,012,010	C	111	302	60	88	15	4	2	10	3.0	1.4E-06
rs11110501	12	100,732,070	T	152	376	33	24	1	1	2	3	5.8	3.0E-06
rs7945619	11	113,899,300	A	164	294	21	104	1	5	2	1	0.2	4.1E-06
rs973500	1	118,991,668	G	126	327	50	73	12	4	0	0	2.8	9.6E-06
rs17723728	11	113,816,106	C	166	296	21	102	1	6	0	0	0.2	9.6E-06
rs10923704	1	118,950,308	T	119	308	56	89	12	4	1	3	2.7	1.1E-05
rs17802314	11	113,827,280	T	166	297	21	100	1	6	0	1	0.2	1.2E-05
rs10458419	1	118,972,297	T	121	313	55	84	12	7	0	0	2.6	1.2E-05
rs9484683	6	142,971,177	G	78	104	88	203	21	88	1	9	0.4	1.2E-05
rs12021544	1	118,964,609	C	122	313	54	86	12	5	0	0	2.7	1.4E-05
rs13405158	2	43,478,147	C	130	320	47	78	11	6	0	0	2.9	1.5E-05
rs13416978	2	43,478,126	G	130	320	47	78	11	6	0	0	2.9	1.5E-05
rs2825966	21	20,032,659	A	85	218	81	151	22	35	0	0	2.3	1.5E-05
rs2984649	5	158,138,960	C	100	270	74	118	14	15	0	1	2.4	1.8E-05
rs677412	3	125,153,007	C	92	132	76	208	19	60	1	4	0.4	2.2E-05

* Chr: chromosome, Position: chromosomal position in base pairs, 0/1/2/NA TA: 0, 1, 2, or unknown copies of the TA, OR: odds ratio of the TA for hearing loss, P-Value: Wald p-value.

Additive Cis+Notrt GWAS													
rsid	Chr	Position	TA	0 TA		1 TA		2 TA		NA TA		OR	P-Value
				Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls		
rs4501872	1	119,009,661	A	58	269	38	75	10	4	0	0	4.2	1.9E-07
rs12027986	1	119,028,642	A	59	268	37	77	10	3	0	0	4.1	4.2E-07
rs10494218	1	119,019,934	T	59	269	37	75	10	4	0	0	4.0	4.8E-07
rs12021830	1	119,019,759	C	59	269	37	75	10	4	0	0	4.0	4.8E-07
rs10923726	1	119,014,528	G	59	268	37	75	10	4	0	1	4.0	5.2E-07
rs4659138	1	119,012,010	C	59	258	37	77	10	3	0	10	3.9	7.8E-07
rs12637439	3	153,323,458	C	69	266	29	76	8	6	0	0	3.5	4.3E-06
rs973500	1	118,991,668	G	69	280	29	65	8	3	0	0	3.5	8.3E-06
rs1008782	15	42,104,411	T	52	240	45	96	9	11	0	1	3.1	9.6E-06
rs10923704	1	118,950,308	T	65	264	33	78	8	3	0	3	3.3	1.2E-05
rs677412	3	125,153,007	C	59	115	39	177	7	52	1	4	0.3	1.2E-05
rs11114007	12	108,599,180	A	61	253	37	83	8	12	0	0	2.9	1.7E-05
rs1665278	2	61,114,710	C	39	87	56	175	10	76	1	10	0.4	2.1E-05
rs12021544	1	118,964,609	C	67	267	31	77	8	4	0	0	3.2	2.2E-05
rs13405158	2	43,478,147	C	72	273	25	69	9	6	0	0	3.4	2.4E-05
rs13416978	2	43,478,126	G	72	273	25	69	9	6	0	0	3.4	2.4E-05
rs17398377	1	94,125,383	C	44	202	49	133	13	12	0	1	2.8	2.4E-05
rs11220301	11	125,982,378	T	55	206	42	124	9	18	0	0	2.8	2.6E-05
rs1177264	2	61,113,315	G	40	90	55	172	11	86	0	0	0.4	2.7E-05
rs10458419	1	118,972,297	T	67	267	31	75	8	6	0	0	3.0	3.6E-05

Additive Rad+Notrt GWAS													
rsid	Chr	Position	TA	0 TA		1 TA		2 TA		NA TA		OR	P-Value
				Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls		
rs4501872	1	119,009,661	A	75	293	41	79	12	5	0	0	3.3	1.3E-06
rs12027986	1	119,028,642	A	76	292	40	81	12	4	0	0	3.2	2.7E-06
rs10494218	1	119,019,934	T	76	293	40	79	12	5	0	0	3.2	2.9E-06
rs4537278	8	102,555,002	C	110	357	17	20	1	0	0	0	9.1	3.5E-06
rs12021830	1	119,019,759	C	76	292	40	79	12	5	0	1	3.1	4.5E-06
rs4659138	1	119,012,010	C	74	283	40	82	12	4	2	8	3.1	4.6E-06
rs10923726	1	119,014,528	G	76	291	40	80	12	5	0	1	3.1	4.7E-06
rs11110501	12	100,732,070	T	102	350	24	23	1	1	1	3	6.4	5.0E-06
rs7945619	11	113,899,300	A	116	275	11	97	0	4	1	1	0.1	6.3E-06
rs11956125	5	175,246,734	A	97	332	28	42	3	3	0	0	3.7	9.2E-06
rs9484683	6	142,971,177	G	56	97	58	189	13	83	1	8	0.4	1.3E-05
rs8000690	13	26,030,801	C	88	307	33	59	4	2	3	9	3.5	1.4E-05
rs11920000	3	107,948,538	C	120	367	8	10	0	0	0	0	12.6	1.6E-05
rs10059196	5	175,257,643	G	107	354	19	22	2	1	0	0	4.7	1.8E-05
rs17662322	3	7,467,601	T	72	286	52	82	4	9	0	0	2.9	2.1E-05
rs7317551	13	60,380,913	C	96	205	26	143	5	25	1	4	0.3	2.2E-05
rs1241928	14	83,535,135	C	42	86	71	200	14	91	1	0	0.4	2.3E-05
rs10923704	1	118,950,308	T	77	287	41	83	9	4	1	3	2.9	2.3E-05
rs8010599	14	20,696,675	C	102	347	26	30	0	0	0	0	5.2	2.5E-05
rs1241927	14	83,535,102	A	42	86	72	201	14	89	0	1	0.4	2.6E-05

Additive Notrt GWAS													
rsid	Chr	Position	TA	0 TA		1 TA		2 TA		NA TA		OR	P-Value
				Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls		
rs4501872	1	119,009,661	A	21	248	18	69	7	4	0	0	4.8	8.7E-07
rs4659138	1	119,012,010	C	22	239	17	71	7	3	0	8	4.6	1.9E-06
rs12027986	1	119,028,642	A	22	247	17	71	7	3	0	0	4.7	2.0E-06
rs7553422	1	118,998,096	C	31	82	13	174	2	59	0	6	4.8	2.2E-06
rs10494218	1	119,019,934	T	22	248	17	69	7	4	0	0	4.5	2.3E-06
rs12021830	1	119,019,759	C	22	248	17	69	7	4	0	0	4.5	2.3E-06
rs10923726	1	119,014,528	G	22	247	17	69	7	4	0	1	4.5	2.4E-06
rs10923748	1	119,105,323	G	11	170	22	127	13	24	0	0	3.4	6.4E-06
rs1945412	11	21,337,924	G	15	212	26	103	5	6	0	0	4.1	7.6E-06
rs11175255	12	64,082,870	C	32	272	12	48	2	1	0	0	5.9	8.5E-06
rs12309038	12	64,069,350	T	32	271	12	49	2	1	0	0	5.9	9.0E-06
rs9652019	12	64,071,529	A	32	271	12	49	2	1	0	0	5.9	9.0E-06
rs11175257	12	64,090,052	C	32	271	12	49	2	1	0	0	5.9	9.5E-06
rs12302132	12	64,090,510	A	32	271	12	49	2	1	0	0	5.9	9.5E-06
rs10923704	1	118,950,308	T	23	243	18	72	5	3	0	3	3.9	1.5E-05
rs2063802	2	139,384,878	A	30	275	16	43	0	3	0	0	5.4	1.7E-05
rs1431867	2	139,367,091	T	30	275	16	42	0	3	0	1	5.4	1.8E-05
rs9834018	3	23,074,865	G	25	251	19	67	2	2	0	1	4.9	1.8E-05
rs4659151	1	119,216,160	A	12	175	23	122	11	24	0	0	3.1	2.2E-05
rs17784886	21	19,031,847	C	36	301	9	19	1	1	0	0	8.3	2.3E-05

Table S6. The top 20 SNPs identified in each of the four recessive GWAS analyses adjusting for the top 10 genetic ancestry PCs and pertinent clinical models. SNPs are colour-coded to identify spatially clustered signals, with white indicating that only one SNP was detected for a particular locus.

Recessive Combined GWAS													
rsid	Chr	Position	TA	0 TA		1 TA		2 TA		NA TA		OR	P-Value
				Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls		
rs12027986	1	119,028,642	A	113	313	60	87	15	4	0	0	36.3	1.5E-05
rs10494218	1	119,019,934	T	113	314	60	85	15	5	0	0	23.9	1.5E-05
rs4501872	1	119,009,661	A	112	314	61	85	15	5	0	0	23.9	1.5E-05
rs4659138	1	119,012,010	C	111	302	60	88	15	4	2	10	31.5	1.6E-05
rs10923726	1	119,014,528	G	113	312	60	86	15	5	0	1	23.5	1.7E-05
rs2075624	15	40,418,524	A	101	226	62	160	25	17	0	1	6.8	1.7E-05
rs12021830	1	119,019,759	C	113	313	60	85	15	5	0	1	23.4	1.8E-05
rs12593066	15	40,421,820	T	102	227	61	159	25	18	0	0	6.6	1.9E-05
rs13289956	9	7,784,705	T	81	213	73	158	34	32	0	1	4.8	1.9E-05
rs946918	14	83,006,524	T	112	242	60	150	16	12	0	0	8.9	2.1E-05
rs7863054	9	7,780,159	T	75	205	74	161	39	38	0	0	4.3	2.6E-05
rs667874	19	23,462,392	A	90	218	69	159	29	27	0	0	5.2	2.8E-05
rs973500	1	118,991,668	G	126	327	50	73	12	4	0	0	26.6	3.0E-05
rs11085578	19	23,383,265	A	99	254	72	135	14	9	3	6	9.9	3.2E-05
rs1112898	7	1,778,361	T	129	276	49	120	9	5	1	3	16.9	3.4E-05
rs10984620	9	119,632,823	C	54	103	105	175	29	126	0	0	0.3	3.4E-05
rs12436050	14	83,036,148	G	110	234	61	152	17	18	0	0	6.7	3.5E-05
rs7099721	10	5,013,758	A	80	190	78	188	29	26	1	0	4.9	3.8E-05
rs2034254	2	53,131,815	G	110	261	63	129	15	14	0	0	8.4	4.1E-05
rs12980317	19	23,314,472	A	99	256	73	135	16	13	0	0	7.9	4.4E-05

* Chr: chromosome, Position: chromosomal position in base pairs, TA: the TA, 0/1/2/NA TA: 0, 1, 2, or unknown copies of the TA, OR: odds ratio of the TA for hearing loss, P-Value: Wald p-value.

Recessive Cis+Notrt GWAS													
rsid	Chr	Position	TA	0 TA		1 TA		2 TA		NA TA		OR	P-Value
				Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls		
rs1266444	1	19,739,640	C	29	102	38	179	39	67	0	0	4.6	5.5E-06
rs7622598	3	193,303,397	C	54	173	34	148	18	25	0	2	6.4	9.0E-06
rs2055375	5	61,235,393	G	33	93	66	174	6	81	1	0	0.1	9.6E-06
rs7644587	3	193,303,150	G	54	172	34	150	18	26	0	0	6.3	1.0E-05
rs7641152	3	193,301,978	T	53	171	35	148	18	26	0	3	6.3	1.1E-05
rs12980317	19	23,314,472	A	52	221	41	116	13	11	0	0	11.4	1.2E-05
rs7245954	19	23,315,140	G	51	220	42	117	13	11	0	0	11.4	1.2E-05
rs7249539	19	23,364,747	A	52	220	41	117	13	11	0	0	11.4	1.2E-05
rs17024368	2	100,471,135	A	47	209	43	123	15	15	1	1	8.1	1.3E-05
rs7606473	2	100,493,218	A	47	201	43	129	15	15	1	3	8.1	1.3E-05
rs10753559	1	19,371,532	G	47	150	42	167	17	30	0	1	5.8	1.5E-05
rs12977982	19	23,400,378	T	52	216	41	120	12	11	1	1	11.2	1.6E-05
rs998064	4	56,131,184	G	53	200	36	131	17	17	0	0	7.7	2.2E-05
rs11123833	2	100,474,680	C	49	206	42	127	15	15	0	0	7.5	2.2E-05
rs1519661	2	100,471,798	A	49	201	42	132	15	15	0	0	7.5	2.2E-05
rs753439	2	100,472,378	C	49	201	42	132	15	15	0	0	7.5	2.2E-05
rs1112898	7	1,778,361	T	73	234	25	106	7	5	1	3	18.6	2.3E-05
rs6723261	2	100,476,883	G	48	194	42	138	16	16	0	0	7.3	2.5E-05
rs7568283	2	100,480,141	A	48	194	42	138	16	16	0	0	7.3	2.5E-05
rs13290974	9	76,549,730	A	73	254	26	89	6	4	1	1	28.7	2.6E-05

Recessive Rad+Notrt GWAS													
rsid	Chr	Position	TA	0 TA		1 TA		2 TA		NA TA		OR	P-Value
				Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls		
rs112762	11	35,171,082	T	31	108	47	184	50	85	0	0	3.6	1.3E-05
rs806059	9	4,134,883	C	61	202	50	162	17	13	0	0	8.2	1.5E-05
rs3748900	2	134,403,184	A	54	173	50	174	24	30	0	0	5.3	1.5E-05
rs4659138	1	119,012,010	C	74	283	40	82	12	4	2	8	35.9	1.7E-05
rs686855	9	4,135,360	G	61	203	49	159	17	14	1	1	7.7	1.9E-05
rs12027986	1	119,028,642	A	76	292	40	81	12	4	0	0	38.8	2.2E-05
rs998064	4	56,131,184	G	67	215	42	144	19	18	0	0	7.5	2.4E-05
rs10494218	1	119,019,934	T	76	293	40	79	12	5	0	0	24.4	2.6E-05
rs4501872	1	119,009,661	A	75	293	41	79	12	5	0	0	24.4	2.6E-05
rs10923726	1	119,014,528	G	76	291	40	80	12	5	0	1	24.0	2.9E-05
rs17744359	14	86,370,173	T	49	153	51	185	28	39	0	0	4.5	3.0E-05
rs12021830	1	119,019,759	C	76	292	40	79	12	5	0	1	23.6	3.2E-05
rs17006750	2	61,179,303	G	80	275	41	99	7	3	0	0	28.1	3.4E-05
rs1112898	7	1,778,361	T	84	257	35	112	8	5	1	3	16.6	3.9E-05
rs7099721	10	5,013,758	A	50	177	53	174	25	26	0	0	5.1	4.5E-05
rs973500	1	118,991,668	G	85	304	33	69	10	4	0	0	27.1	4.5E-05
rs2075624	15	40,418,524	A	71	206	42	154	15	16	0	1	6.9	5.0E-05
rs574034	9	4,123,868	T	61	212	52	146	15	13	0	6	7.2	5.6E-05
rs11085578	19	23,383,265	A	71	237	44	125	11	9	2	6	9.6	5.7E-05
rs13388389	2	61,226,875	C	85	278	36	96	6	3	1	0	28.9	5.8E-05

Recessive Notrt GWAS													
rsid	Chr	Position	TA	0 TA		1 TA		2 TA		NA TA		OR	P-Value
				Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls		
rs2277365	12	48,682,028	G	24	184	12	120	10	15	0	2	10.6	1.5E-05
rs1112898	7	1,778,361	T	28	215	11	98	6	5	1	3	20.7	1.6E-05
rs443543	5	63,268,833	A	15	146	20	155	11	20	0	0	8.5	1.6E-05
rs10753558	1	19,371,421	A	19	143	13	149	14	29	0	0	6.8	1.8E-05
rs10753559	1	19,371,532	G	19	143	13	149	14	29	0	0	6.8	1.8E-05
rs10917434	1	19,380,808	A	19	144	13	148	14	29	0	0	6.8	1.8E-05
rs7667	1	19,392,330	A	19	143	13	149	14	29	0	0	6.8	1.8E-05
rs6662273	1	19,414,329	T	19	143	13	148	14	29	0	1	6.7	1.9E-05
rs7606473	2	100,493,218	A	17	189	18	115	10	14	1	3	9.2	2.0E-05
rs17744359	14	86,370,173	T	14	131	18	156	14	34	0	0	6.4	2.1E-05
rs998064	4	56,131,184	G	20	182	14	123	12	16	0	0	8.7	2.1E-05
rs17024368	2	100,471,135	A	18	197	17	109	10	14	1	1	9.1	2.1E-05
rs10923748	1	119,105,323	G	11	170	22	127	13	24	0	0	7.3	2.3E-05
rs4655414	1	215,687,891	C	10	113	16	152	20	56	0	0	5.1	2.3E-05
rs7622598	3	193,303,397	C	23	155	10	139	13	25	0	2	6.6	2.5E-05
rs7644587	3	193,303,150	G	23	155	10	140	13	26	0	0	6.5	2.7E-05
rs1566532	1	19,401,307	T	19	145	13	147	14	28	0	1	6.5	2.8E-05
rs7641152	3	193,301,978	T	22	154	11	138	13	26	0	3	6.5	3.0E-05
rs12977982	19	23,400,378	T	24	201	13	109	9	11	0	0	11.5	3.2E-05
rs12980317	19	23,314,472	A	24	203	13	107	9	11	0	0	11.5	3.2E-05

Table S7. The top 20 SNPs identified in each of the four dominant GWAS analyses adjusting for the top 10 genetic ancestry PCs and pertinent clinical models. SNPs are colour-coded to identify spatially clustered signals, with white indicating that only one SNP was detected for a particular locus.

Dominant Combined GWAS													
rsid	Chr	Position	TA	0 TA		1 TA		2 TA		NA TA		OR	P-Value
				Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls		
rs11110501	12	100,732,070	T	152	376	33	24	1	1	2	3	6.2	1.6E-06
rs7945619	11	113,899,300	A	164	294	21	104	1	5	2	1	0.2	2.4E-06
rs560906	12	9,969,605	A	121	195	57	181	10	28	0	0	0.3	3.7E-06
rs634645	12	9,964,859	A	120	191	57	182	11	28	0	3	0.3	4.6E-06
rs17662322	3	7,467,601	T	113	305	69	89	6	10	0	0	3.2	5.5E-06
rs17723728	11	113,816,106	C	166	296	21	102	1	6	0	0	0.2	5.6E-06
rs17802314	11	113,827,280	T	166	297	21	100	1	6	0	1	0.2	7.2E-06
rs677412	3	125,153,007	C	92	132	76	208	19	60	1	4	0.3	8.2E-06
rs479809	12	9,968,689	A	120	194	55	178	12	29	1	3	0.3	8.3E-06
rs673693	3	125,140,638	T	95	125	70	215	23	64	0	0	0.3	8.5E-06
rs4537278	8	102,555,002	C	165	382	22	21	1	1	0	0	7.1	8.5E-06
rs13188135	5	107,034,305	G	71	104	83	213	33	85	1	2	0.3	9.7E-06
rs521040	12	9,995,251	C	127	208	52	172	9	24	0	0	0.3	1.0E-05
rs6484459	11	29,488,678	T	84	134	76	210	24	59	4	1	0.3	1.3E-05
rs659928	12	9,996,104	A	127	210	52	170	9	24	0	0	0.3	1.4E-05
rs4501872	1	119,009,661	A	112	314	61	85	15	5	0	0	3.1	1.4E-05
rs329526	11	29,480,606	T	75	109	79	208	34	85	0	2	0.3	1.5E-05
rs990173	14	26,782,289	C	65	226	95	147	28	31	0	0	2.9	1.7E-05
rs10984618	9	119,628,305	A	29	127	104	177	54	100	1	0	3.9	2.0E-05
rs7787350	7	15,439,848	A	64	187	92	159	32	58	0	0	3.0	2.3E-05

* Chr: chromosome, Position: chromosomal position in base pairs, 0/1/2/NA TA: 0, 1, 2, or unknown copies of the TA, OR: odds ratio of the TA for hearing loss, P-Value: Wald p-value.

Dominant Cis+Notrt GWAS													
rsid	Chr	Position	TA	0 TA		1 TA		2 TA		NA TA		OR	P-Value
				Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls		
rs677412	3	125,153,007	C	59	115	39	177	7	52	1	4	0.2	7.6E-06
rs2381149	4	38,360,444	A	69	280	36	54	1	5	0	9	4.4	8.5E-06
rs4501872	1	119,009,661	A	58	269	38	75	10	4	0	0	4.0	9.9E-06
rs11110501	12	100,732,070	T	82	324	22	21	0	1	2	2	6.0	2.0E-05
rs10984618	9	119,628,305	A	14	112	57	152	34	84	1	0	6.5	2.0E-05
rs11114007	12	108,599,180	A	61	253	37	83	8	12	0	0	3.8	2.1E-05
rs1490980	5	125,623,121	C	30	156	66	144	10	48	0	0	4.5	2.3E-05
rs678197	3	125,152,898	A	59	120	39	177	8	51	0	0	0.3	2.4E-05
rs10494218	1	119,019,934	T	59	269	37	75	10	4	0	0	3.7	2.6E-05
rs12021830	1	119,019,759	C	59	269	37	75	10	4	0	0	3.7	2.6E-05
rs10923726	1	119,014,528	G	59	268	37	75	10	4	0	1	3.7	2.8E-05
rs17699211	12	3,924,325	T	75	292	29	54	2	2	0	0	4.2	2.9E-05
rs12027986	1	119,028,642	A	59	268	37	77	10	3	0	0	3.7	2.9E-05
rs751248	4	38,367,270	T	66	275	33	55	2	5	5	13	4.2	2.9E-05
rs1008782	15	42,104,411	T	52	240	45	96	9	11	0	1	3.5	3.9E-05
rs13188135	5	107,034,305	G	44	96	43	179	18	71	1	2	0.3	4.2E-05
rs6787964	3	61,679,272	G	52	121	45	177	9	49	0	1	0.3	4.8E-05
rs10984620	9	119,632,823	T	15	111	57	150	34	87	0	0	5.5	5.1E-05
rs7869670	9	119,627,534	T	15	110	58	151	33	87	0	0	5.5	5.1E-05
rs1860820	3	64,396,189	C	38	190	54	132	14	26	0	0	3.8	5.1E-05

Dominant Rad+Notrt GWAS													
rsid	Chr	Position	TA	0 TA		1 TA		2 TA		NA TA		OR	P-Value
				Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls		
rs7553422	1	118,998,096	C	66	97	42	208	20	65	0	7	4.0	9.9E-07
rs17662322	3	7,467,601	T	72	286	52	82	4	9	0	0	4.1	1.2E-06
rs4537278	8	102,555,002	C	110	357	17	20	1	0	0	0	9.5	2.1E-06
rs11110501	12	100,732,070	T	102	350	24	23	1	1	1	3	6.8	3.0E-06
rs7945619	11	113,899,300	A	116	275	11	97	0	4	1	1	0.1	5.9E-06
rs974528	2	137,113,012	T	72	149	43	176	13	52	0	0	0.3	7.2E-06
rs13188135	5	107,034,305	G	53	97	52	199	22	80	1	1	0.3	9.0E-06
rs1368061	2	137,111,199	G	71	147	44	178	13	52	0	0	0.3	9.5E-06
rs11635651	15	72,908,144	C	97	341	31	35	0	1	0	0	4.9	1.0E-05
rs7317551	13	60,380,913	C	96	205	26	143	5	25	1	4	0.3	1.2E-05
rs7787350	7	15,439,848	A	41	174	63	146	24	57	0	0	3.7	1.3E-05
rs11920000	3	107,948,538	C	120	367	8	10	0	0	0	0	12.6	1.6E-05
rs1341836	13	60,382,321	G	97	210	26	141	5	26	0	0	0.3	1.7E-05
rs2826085	21	20,209,943	C	84	297	39	74	5	6	0	0	3.8	1.9E-05
rs11956125	5	175,246,734	A	97	332	28	42	3	3	0	0	4.2	2.4E-05
rs8010599	14	20,696,675	C	102	347	26	30	0	0	0	0	5.2	2.5E-05
rs974530	2	137,112,719	A	68	141	46	180	14	56	0	0	0.3	2.6E-05
rs17723728	11	113,816,106	C	116	277	12	95	0	5	0	0	0.2	2.6E-05
rs9484683	6	142,971,177	G	56	97	58	189	13	83	1	8	0.3	2.7E-05
rs1431867	2	139,367,091	T	96	319	31	54	1	3	0	1	4.2	2.7E-05

Dominant Notrt GWAS													
rsid	Chr	Position	TA	0 TA		1 TA		2 TA		NA TA		OR	P-Value
				Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls		
rs7553422	1	118,998,096	C	31	82	13	174	2	59	0	6	7.0	5.2E-07
rs1431867	2	139,367,091	T	30	275	16	42	0	3	0	1	7.6	2.5E-06
rs2063802	2	139,384,878	A	30	275	16	43	0	3	0	0	7.6	2.7E-06
rs4954657	2	139,404,378	T	30	275	16	42	0	4	0	0	7.6	2.7E-06
rs13188135	5	107,034,305	G	26	89	12	165	7	66	1	1	0.2	1.5E-05
rs987827	11	37,405,077	C	28	104	13	156	5	59	0	2	0.2	1.9E-05
rs1945412	11	21,337,924	G	15	212	26	103	5	6	0	0	5.1	1.9E-05
rs1608463	4	124,357,734	T	27	266	19	48	0	4	0	3	5.6	2.1E-05
rs9374629	6	116,976,350	T	33	300	13	20	0	1	0	0	6.9	2.8E-05
rs10003267	4	124,045,370	T	23	69	19	178	3	73	1	1	0.2	2.9E-05
rs4501872	1	119,009,661	A	21	248	18	69	7	4	0	0	4.6	2.9E-05
rs17714360	2	139,518,691	T	37	305	9	16	0	0	0	0	9.2	3.1E-05
rs17716528	2	139,628,219	G	37	305	9	16	0	0	0	0	9.2	3.1E-05
rs2049431	2	9,679,683	G	29	279	16	39	0	1	1	2	6.1	3.2E-05
rs12645423	4	154,023,243	T	40	168	6	131	0	22	0	0	0.1	3.3E-05
rs4358107	2	66,639,635	G	10	167	30	136	6	18	0	0	5.9	3.3E-05
rs17784886	21	19,031,847	C	36	301	9	19	1	1	0	0	8.6	3.5E-05
rs6845524	4	121,225,359	T	24	71	18	177	4	73	0	0	0.2	4.5E-05
rs3198419	11	130,905,793	C	32	116	9	165	5	40	0	0	0.2	5.1E-05
rs6904594	6	117,200,208	C	35	307	11	13	0	1	0	0	8.2	5.2E-05

Table S8a. Crude and adjusted hearing loss ORs for cisplatin-exposed, radiation-exposed, and neither-exposed CCSs. Adjusted estimates were calculated using the pertinent stratum-specific clinical model shown in Table S8b. The adjusted OR for continuous age is shown for the lower bound of each age category and is per 10 year increase from the lower bound of the previous category.

Age Category (Years)	Cisplatin-Exposed					Cochlear Radiation-Exposed					Unexposed to Cisplatin and Radiation				
	Case	Control	Crude OR	Adj. OR	Prev.	Case	Control	Crude OR	Adj. OR	Prev.	Case	Control	Crude OR	Adj. OR	Prev.
19-29	24	13	1.0	1.0	65%	15	21	1.0	1.0	42%	9	91	1.0	1.0	9%
30-39	24	10	1.3	2.4	71%	30	17	2.5	4.3	64%	17	150	1.1	2.4	10%
40-49	11	4	1.5	5.6	73%	22	16	1.9	18.6	58%	13	66	2.0	5.7	16%
50-65	1	0	--	13.3	100%	15	2	10.5	80.1	88%	7	14	5.1	13.5	33%

Prev. = prevalence of hearing loss

Table S8b. Stratum-specific clinical models and sample sizes used to estimate the adjusted impact of age at most recent audiology exam among cisplatin-exposed, radiation-exposed, and neither-exposed CCSs. Aside from anycarbo (which indicates any exposure to any carboplatin dose owing to the small number of carboplatin-exposed CCSs within each stratum), all variable definitions can be found in Table S2b.

Stratum	Sample Size	Variables
Cisplatin-Exposed	87	Anycarbo, cisplatin dose, youngcis, sex, tobramycin, ageaudio, and CSF shunt
Radiation-Exposed	138	Anycarbo, youngrad, radcat2440, radcat2501, radcat4501, sex, tobramycin, ageaudio, and CSF shunt
Neither-Exposed	367	Anycarbo, sex, tobramycin, ageaudio, and CSF shunt

Table S9. Adjusted age-specific hearing loss ORs for CCSs in the present study and the general population. Adjusted continuous OR estimates for CCSs were calculated using the cis+rad+notrt (Table 2), cisplatin-specific, radiation-specific, and notrt (Table S8) clinical models with a reference of 20 years. Extracted from Table 3 of Hoffman et al 2017[85], the general population ORs are for bilateral speech-frequency impairment in US adults adjusting for demographic, cardiovascular, and noise-related risk factors.

Age	CCSs				General Population OR
	Cis+Rad+Notrt OR	Cisplatin OR	Radiation OR	Notrt OR	
20 years	1.0	1.0	1.0	1.0	1.0
30 years	2.8	2.4	4.3	2.4	1.1
40 years	7.8	5.6	18.6	5.7	3.3
50 years	22.0	13.3	80.1	13.5	13.4

Table S10. SNP annotations using public bioinformatics data from Ensembl[152] and Haploreg[101].

rsid	Gene (Distance)	Gene Description	dbSNP Functional Annotation	Ensembl Variant Consequence: Biotype	SNP eQTL Hit	eQTL P	Chromatin Regulatory State	Altered Regulatory Motifs	Proteins Bound in ChIP-Seq Experiments
rs973500	*TBX15* (2111 bp)	Transcription factor T-box 15	None	Upstream gene variant: protein coding	Serum ratio of (2-palmitoyl glycerophosphocholine) / (gamma - glutamylglutamate)	3.4E-04	Yes	Yes	YY1 in H1-hESC cells
				Regulatory region variant: promoter flanking region	WASR2 in Cells Transformed fibroblasts	2.4E-08			
					WARS2 in Whole Blood	4.5E-06			
rs7553422	*AL139420.1* (2247 bp)	LincRNA	None	Downstream gene variant: lincRNA	Gene expression of *WARS2* in peripheral blood monocytes	7.9E-80	Yes	Yes	
	TBX15 (8539 bp)	Transcription factor T-box 15		Regulatory region variant: promoter flanking region	Serum ratio of (4 - vinylphenol sulfate) / (hydroquinone sulfate)	9.0E-04			
					RP11-418J17.1 in Adipose Subcutaneous	1.5E-07			
					WARS2 in Adipose Subcutaneous	3.4E-18			
					WARS2 in Adipose Visceral Omentum	5.1E-13			
					WARS2 in Adrenal Gland	1.4E-07			
					WARS2 in Artery Aorta	3.9E-10			
					WARS2 in Artery Tibial	3.4E-17			
					WARS2 in Brain Anterior cingulate cortex BA24	2.4E-06			
					WARS2 in Brain Caudate basal ganglia	6.6E-08			
					WARS2 in Brain Cerebellar Hemisphere	6.9E-08			
					WARS2 in Brain Cerebellum	1.2E-06			
					WARS2 in Brain Frontal Cortex BA9	1.7E-06			
					WARS2 in Brain Nucleus accumbens basal ganglia	2.1E-07			
					WARS2 in Brain Putamen basal ganglia	2.1E-06			
					WARS2 in Breast Mammary Tissue	2.8E-10			
					WARS2 in Cells EBV-transformed lymphocytes	5.7E-07			

					WARS2 in Cells Transformed fibroblasts	2.0E-17			
					WARS2 in Colon Transverse	2.6E-07			
					WARS2 in Esophagus Gastroesophageal Junction	7.1E-07			
					RP11-418J17.1 in Esophagus Mucosa	6.1E-06			
					WARS2 in Esophagus Mucosa	1.3E-11			
					RP11-418J17.1 in Esophagus Muscularis	4.9E-06			
					WARS2 in Esophagus Muscularis	2.0E-11			
					WARS2 in Heart Atrial Appendage	1.2E-11			
					WARS2 in Heart Left Ventricle	2.1E-06			
					RP11-418J17.1 in Lung	3.6E-09			
					WARS2 in Lung	3.4E-16			
					WARS2 in Muscle Skeletal	1.5E-21			
					WARS2 in Nerve Tibial	1.6E-15			
					WARS2 in Pancreas	1.1E-10			
					WARS2 in Skin Not Sun Exposed Suprapubic	1.5E-14			
					RP11-418J17.1 in Skin Sun Exposed Lower leg	6.6E-06			
					WARS2 in Skin Sun Exposed Lower leg	1.7E-20			
					RP11-418J17.1 in Spleen	2.1E-06			
					WARS2 in Spleen	1.7E-09			
					RP11-418J17.1 in Stomach	2.9E-07			
					WARS2 in Stomach	1.9E-10			
					WARS2 in Thyroid	1.9E-13			
					ENSG00000116874.7 119573839 119576011 in Lymphoblastoid EUR exonlevel	7.7E-36			

rs4501872	*AL139420.1* (8255 bp) *TBX15* (20,104 bp)	LincRNA Transcription factor T-box 15	None	Intergenic variant	Serum ratio of (2-oleoylglycero phosphocholine) / (gamma – glutamyl glutamate) Gene expression of *WARS2* in normal prepouch ileum *WARS2* in Cells Transformed fibroblasts *WARS2* in whole blood	3.3E-04 6.1E-04 1.1E-09 1.2E-09	Yes	No	
rs4659138	*AL139420.1* (10,604 bp) *WARS2* (19,204 bp)	LincRNA Mitochondrial tryptophanyl tRNA synthetase 2	None	Intergenic variant	*WARS2* in artery tibial *WARS2* in Cells Transformed fibroblasts *WARS2* in whole blood	1.2E-05 1.2E-09 1.1E-09	Yes	Yes	MAFK in HepG2 cells
rs10923726	*AL139420.1* (13,122 bp) *WARS2* (16,686 bp)	LincRNA Mitochondrial tryptophanyl tRNA synthetase 2	None None	Intergenic variant	Serum ratio of (2-oleoylglycero phosphocholine) / (gamma – glutamyl glutamate) *WARS2* in Cells Transformed fibroblasts *WARS2* in whole blood	5.0E-05 4.6E-10 4.6E-10	Just 1	Yes	
rs12021830	*WARS2* (11,455 bp)	Mitochondrial tryptophanyl tRNA synthetase 2	None	Intergenic variant	Serum ratio of (2-oleoylglycero phosphocholine) / (gamma – glutamyl glutamate) *WARS2* in Cells Transformed fibroblasts *WARS2* in whole blood	9.5E-05 4.6E-10 4.3E-09	Yes	No	
rs10494218	*WARS2* (11,280 bp)	Mitochondrial tryptophanyl tRNA synthetase 2	None	Intergenic variant	Serum ratio of (1-palmitoleoyl glycerol phosphocholine) / (1-stearoyl glycerol (1-monostearin)) *WARS2* in Cells Transformed fibroblasts *WARS2* in whole blood	8.3E-05 4.6E-10 4.3E-09	Yes	No	
rs12027986	*WARS2* (2572 bp)	Mitochondrial tryptophanyl tRNA synthetase 2	None	Downstream gene variant: protein coding Downstream gene variant: processed transcript	Serum ratio of (1-palmitoleoyl glycerol phosphocholine) / (1-stearoyl glycerol (1-monostearin)) *WARS2* in Cells Transformed fibroblasts *WARS2* in whole blood	2.0E-04 3.0E-10 4.1E-09	Yes	Yes	

rs10923748	*WARS2*	Mitochondrial tryptophanyl tRNA synthetase 2	Intronic	Intron variant: protein coding Intron variant, non-coding transcript variant: processed transcript	*RP11-418J17.1* in adipose subcutaneous *RP11-418J17.1* in artery aorta *RP11-418J17.1* in artery tibial *RP11-418J17.1* in nerve tibial *RP11-418J17.1* in thyroid *WARS2* in whole blood	2.8E-06 4.5E-07 7.2E-08 3.9E-09 8.8E-07 1.9E-11	Just 1	Yes	
rs12637439	*RP11-292E2.1* (33,648 bp) *RAP2B* (154,983 bp)	LincRNA Member of RAS oncogene family	None	Intergenic variant	Gene expression of *DAPK3* in peripheral blood monocytes	8.9E-06	Yes	Yes	
rs11956125	*DRD1* (193,937 bp)	Dopamine receptor D1	None	Regulatory region variant: promoter flanking region Intergenic variant	No	No	Yes	No	
rs4537278	*ODF1*	Outer dense fiber of sperm tails 1	Intronic	Intronic variant: protein coding Regulatory region variant: CTCF binding site	No	No	Yes	Yes	
rs1945412	*NELL1*	Neural epidermal growth factor-like 1	Intronic	Intron variant: protein coding Intron variant, non-coding transcript variant: processed transcript	No	No	No	Yes	

rs17723728	*USP28*	Ubiquitin specific peptidase 28	Intronic	Intron variant: protein coding	Gene expression of *MCOLN3* in peripheral blood monocytes	4.2E-06	Yes	No	
				Downstream gene variant: nonsense mediated decay					
				Intron variant: nonsense mediated decay					
				Intron variant, nonsense mediated decay transcript variant: nonsense mediated decay					
	AP003170.3 (1971 bp)	Novel antisense RNA transcript		Upstream gene variant: antisense	ENSG00000048028.7 113745239 113745601 in lymphoblastoid EUR exonlevel tissue	6.4E-08			
				Downstream gene variant: processed pseudogene	*HTR3A* in whole blood	1.2E-03			
rs7945619	*HTR3B* (5376 bp)	Ionotropic 5-hydroxytryptamine (serotonin) receptor 3B	None	Intergenic variant	Gene expression of *MCOLN3* in peripheral blood monocytes	2.3E-06	Yes	Yes	
					ENSG00000048028.7 113745239 113745601 in lymphoblastoid EUR exonlevel tissue	3.9E-08			
					HTR3A in whole blood	2.1E-04			
rs11175255	*SRGAP1*	SLIT-ROBO Rho GTPase activating protein 1	Intronic	Intron variant: protein coding	Gene expression of *GIMAP8* in peripheral blood monocytes	8.9E-06	Yes	Yes	
				Intron variant, non-coding transcript variant: retained intron					
	AC020611.2	Novel antisense RNA transcript		Intron variant, non-coding transcript variant: antisense					
rs12309038	*SRGAP1*	SLIT-ROBO Rho GTPase activating protein 1	Intronic	Intron variant: protein coding	Gene expression of *GIMAP8* in peripheral blood monocytes	8.9E-06	Yes	Yes	
				Intron variant, non-coding transcript variant: retained intron					
	AC020611.2	Novel antisense RNA transcript		Intron variant, non-coding transcript variant: antisense					

rs9652019	*SRGAP1* *AC020611.2*	SLIT-ROBO Rho GTPase activating protein 1 Novel antisense RNA transcript	Intronic	Intron variant: protein coding Intron variant, non-coding transcript variant: retained intron Intron variant, non-coding transcript variant: antisense	Gene expression of *GIMAP8* in peripheral blood monocytes	9.3E-06	Yes	Yes	
rs11175257	*SRGAP1* *AC020611.2*	SLIT-ROBO Rho GTPase activating protein 1 Novel antisense RNA transcript	Intronic	Intron variant: protein coding Intron variant, non-coding transcript variant: antisense	No	No	Yes	No	
rs12302132	*SRGAP1* *AC020611.2*	SLIT-ROBO Rho GTPase activating protein 1 Novel antisense RNA transcript	Intronic	Intron variant: protein coding Intron variant, non-coding transcript variant: antisense	Gene expression of *GIMAP8* in peripheral blood monocytes	9.8E-06	Yes	No	
rs11110501	*ANO4*	Anoctamin 4	None	Intron variant, nonsense mediated decay transcript variant: nonsense mediated decay Intron variant: protein coding	No	No	Just 1	Yes	
rs1008782	*PLA2G4D* (9856 bp)	Cytosolic phospholipase A2, group IVD	None	Regulatory region variant: promoter flanking region Intergenic variant	*PLA2G4D* in Esophagus Mucosa	8.1E-06	Yes	Yes	

Table S11. Haploreg[101] overview of SNP annotations for the 1p12 region.

Chromosome	Hg38 Position (bp)	rsid	Reference Allele	Alternate Allele	Promoter Histone Marks	Enhancer Histone Marks	DNAse	Proteins Bound	Motifs Changed	GRASP QTL Hits	Selected eQTL Hits	GENCODE Genes
1	118991668	rs973500	A	G	IPSC	ESC, IPSC, SKIN, MUS	ESC, ESDR, ESC, IPSC, IPSC	YY1	FXR, Spz1, TATA	1 hit	2 hits	2.1kb 5' of *TBX15*
1	118998096	rs7553422	T	C		MUS			PRDM1	2 hits	38 hits	2.2kb 3' of *RP4-712E4.1*
1	119009661	rs4501872	G	A		ESC, ESDR, IPSC, FAT, STRM, BRST, MUS, SKIN, BONE				2 hits	2 hits	8.3kb 5' of *RP4-712E4.2*
1	119012010	rs4659138	A	C		ESDR, BRST, MUS	BRST	MAFK	Egr-1		3 hits	11kb 5' of *RP4-712E4.2*
1	119014528	rs10923726	A	G					Barhl1, CDP, E2A, Hoxa7, Hoxb4, Prrx2, SREBP, Sox	1 hit	2 hits	13kb 5' of *RP4-712E4.2*
1	119019759	rs12021830	T	C						1 hit	2 hits	11kb 3' of *WARS2*
1	119019934	rs10494218	A	T						1 hit	2 hits	11kb 3' of *WARS2*
1	119028642	rs12027986	G	A		ESDR, GI			Nkx2, Nkx3	1 hit	2 hits	2.6kb 3' of *WARS2*
1	119105323	rs10923748	C	G					Maf		6 hits	*WARS2*

Whole-Genome Sequencing Analysis (WGSA) Addendum

A disadvantage of GWASes using array data is that the causal mutation of interest is not necessarily genotyped. Instead, a sample of SNPs that are representative of the genome are genotyped, from which the identity of unobserved SNPs may be inferred (imputed). Of the four to five million SNPs estimated to reside within each person's genome, only about 700,000 were tested for an association with hearing loss in the present work, for which imputation was not performed. Therefore, a limitation of the present study is that our coverage of genomic variation is lacking.

However, as of 2015 SJLIFE has implemented whole genome sequencing (WGS) for participants with available biological samples. As the name indicates, WGS attempts to identify all of the nucleotides in an individual's DNA. In addition to dramatically increasing the density of called variants, WGS allows the researcher to incorporate analysis of rare variants, insertions/deletions, copy number changes, and large structural variants in addition to SNPs for the detection of loci-phenotype associations. Given the availability of WGS data for most of the participants in the present study, we opted to increase the genomic coverage of our analysis with observed WGS data rather than with unobserved imputed data as an effort to capture variants that might have been missed in our original GWASes and to refine the signals identified in the 1p12, 11q23.2, and 12q23.1 regions.

Unexpectedly, none of the top SNPs identified in our GWASes were among the top WGSA results, for which the most significant p-value was 1.9×10^{-6} (tested variants ≈ 9.7 million). In fact, only one SNP, rs1886916, was identified in the top 1000 WGSA results in the region delimited by the GWAS-identified 1p12 signal. Similarly, this SNP overlapped a chromatin region enriched for

predicted regulatory states, was significantly associated with *WARS2* expression in whole blood and transformed fibroblasts, and altered many regulatory motifs[101]. As can be seen in Table A1, the adjusted GWAS and WGSA results for the seven 1p12 SNPs identified in the cis+rad+notrt (i.e., combined) GWAS diverged greatly. Specifically, the ORs dropped from about 3.0 to 2.0 with WGSA p-values on average swelling to 184 times their corresponding GWAS value. It should also be noted that population sizes varied between the two analyses, with a total of 592 and 683 people with respective Affymetrix and Illumina sequencing. Specifically, 22 people only had Affymetrix data (i.e., the GWAS-only group), 113 people just had Illumina sequencing (i.e., the WGSA-only group), and 570 had sequencing on both platforms (i.e., the common population). Sample sizes for SNP-specific modelling varied slightly with the exclusion of participants lacking successful genotyping for a particular SNP.

Given the dramatic scale on which the WGSA and GWAS results varied, we took it upon ourselves to explore what influences may have been responsible using the seven 1p12 SNPs identified in the combined GWAS as an example. Under the assumption that the result discrepancies were predominantly the product of systematic and not random variation, we first examined the impact of GWAS vs WGSA methodologies on 1p12 SNP significance within the common population. Specifically, the influences of discrepant genotype calling and differing genetic ancestry PCs were evaluated. The following two sections ascertain the negligibility of these methodological differences. Currently, we are investigating differences in GWAS-only and WGS-only population characteristics and how they might be attenuating the ototoxic association of the 1p12 region.

Sequencing Discrepancies

To begin our GWAS vs WGSA investigation we evaluated the impact of discordant sequencing on SNP significance. To this end, we restricted our population to the 570 participants with both types of sequencing. Between-platform sequencing discrepancies for this common population are tallied for the seven GWAS-identified 1p12 SNPs in Table A2. Overall, 31 genotype discrepancies were found across 29 people. Although none of the genotypes for the seven SNPs matched perfectly between sequencing platforms for any given participant, concordance was acceptably high between 97.3% and 99.8% (Table A2).

To further quantify the degree to which sequencing differences influenced SNP effect size and significance, two series of models were run for the common population using Affymetrix and then Illumina sequencing data for the three 1p12 SNPs with discordant sequencing that wasn't solely due to missing genotypes (Table A3). These models were adjusted for the same clinical variables used in the original combined GWAS analysis. Genetic ancestry PCs, which differed between the GWAS and WGSA, weren't included as covariates to ensure that any differences in SNP significance could solely be attributed to sequencing discrepancies. As can be seen in Table A3, sequencing differences had a miniscule impact on each of the 1p12 SNPs, with all WGSA ORs falling within about ± 10% of their GWAS values and all WGSA p-values varying within their GWAS order of magnitude. Comparing these small fluctuations to the multi-order of magnitude differences observed between the original GWAS and WGSA (Table A1), it is clear that genotype discrepancies are not responsible for the WGSA-observed reduction in 1p12 SNP significance.

Principal Component Investigation

Although GWAS and WGSA PCs were both created from common variants to adjust for genetic ancestry, the analysis-specific PCs themselves were generated separately using their corresponding sequencing data. That is, GWAS PCs (GPCs) were generated using Affymetrix microarray data, which targets about one million SNPs, whereas WGSA PCs (WPCs) relied on Illumina sequencing-by-synthesis technology, which aims to interrogate the entire human genome. As such, the GPCs and WPCs both adjusted for systemic population structure, but the WPCs were informed by more genetic information. Additionally, it must be noted that the unavailability of sequencing data for all participants on both platforms meant that each set of PCs was generated with overlapping but differing populations. Therefore, we felt it necessary to confirm that the analysis-specific PCs were capturing similar aspects of population-substructure and not driving the observed differences in 1p12 SNP significance.

The degree to which the PCs captured similar information for participants with both types of sequencing was broadly evaluated with the GPC by WPC Pearson correlation matrix shown in Table A4. Of note, this matrix demonstrates a strong correlation of -0.92 between gpc1 and wpc1, a strong correlation between gpc3 and the third ($r = 0.94$) and fourth ($r = -0.67$) WPCs, and a moderate correlation of -0.34 between gpc8 and wpc10. Although all other absolute correlations ($|r|$) were small with a range of 0.001-0.26, it may be concluded that the GPC and WPC sets captured the same major features of population substructure since the first PCs, which coincide with the direction of maximum variation in the original genotype datasets, were highly correlated.

As a supplementary analysis, pairwise Pearson correlation matrices were also separately constructed between analysis-specific PCs and 1p12 SNP genotypes to ensure that a given PC was not unduly influenced by the 1p12 region in one but not the other analysis. For example, the 1p12

SNPs could hypothetically lose their significance in the WGSA if they captured information that was redundant to WPCs but not GPCs. As can been seen in Tables A5-A6, this was not the case with both matrices presenting an identical maximum SNP-PC |r| of 0.08 (median |r| = 0.03).

Quantification of PC influence on 1p12 SNP significance was achieved by re-running 1p12 genetic models with and without their corresponding GPCs and WPCs, adjusting for clinical variables. To isolate PC influence, all models were restricted to participants with concordant non-missing genotypes across both sequencing platforms. Adjusted SNP effect sizes and p-values are shown in Table A7.a for the 1p12 region while Wald p-value comparisons are made in Table A7.b. Compared to the GWAS+PCs analysis, p-values from the WGSA+PCs analysis varied within an order of magnitude of and were on average 2.2x as large as their GWAS equivalents, whereas ORs only shrunk by about 0.2. Therefore, it appears that inclusion of GPCs instead of WPCs tends to increase SNP effect size and significance on a small scale.

Removal of PCs from the GWAS+PCs and WGSA+PCs models was then performed to identify whether 1p12 SNP significance was heavily dependent on PC inclusion. Since the GWAS+PCs and WGSA+PCs models only included those with concordant non-missing genotypes, GPC and WPC removal results in one identical PC-free model (i.e., the GWAS-/WGS-PC model; Table A7). Similar to the GWAS+PCs vs WGSA+PCs comparison, removal of GPCs from the GWAS+PC model on average increased the p-value by 2.2x and reduced the OR by 0.3. To contrast, removal of the WPCs from the WGS+PCs model on average changed the p-value by 1.0x and reduced the OR by 0.1. Based on these observations, it appears that WPCs do not impact SNP significance in the common population whereas GPCs systematically enhance significance to a small extent; however, compared to the p-value differences (range = 5.5×10^{-5} to 6.9×10^{-4})

and ratios (range = 44 to 500) for the original GWAS and WGSA presented in Table A1, this influence is negligible.

As a last supplemental check, we examined the joint impact of sequencing and PC differences on 1p12 significance. As can be seen in Table A8, p-values and ORs continued to only vary on a small scale and clearly do not account for the dramatic dissimilitude observed in 1p12 SNP significance between the original GWAS and WGSA (Table A1). Given the inability of sequencing and PC differences to explain the original GWAS vs WGSA discrepancies, we concluded that the characteristics of GWAS-only and WGS-only populations are likely responsible. We are currently investigating this possibility.

Addendum Tables

Table A1. ORs and Wald p-values for the 1p12 SNPs of interest in both the original GWAS and WGS cis+rad+notrt analyses. All models are adjusted for clinical variables and their analysis-specific top 10 genetic ancestry PCs.

SNP	OR		Wald P		N		P	P
	GWAS	WGSA	GWAS	WGSA	GWAS	WGSA	Difference	Ratio
rs973500	2.8	2.0	9.60E-06	7.00E-04	592	683	6.90E-04	73
rs4501872	3.2	2.1	2.40E-07	1.20E-04	592	683	1.20E-04	500
rs4659138	3.0	2.2	1.40E-06	6.20E-05	580	683	6.10E-05	44
rs10923726	3.0	2.2	8.00E-07	7.20E-05	591	683	7.10E-05	90
rs12021830	3.1	2.2	7.40E-07	6.20E-05	591	683	6.10E-05	84
rs10494218	3.1	2.2	5.30E-07	5.60E-05	592	682	5.50E-05	106
rs12027986	3.1	2.1	4.90E-07	1.90E-04	592	682	1.90E-04	388

Table A2. Sequencing differences among the 570 people with both Affymetrix and Illumina sequencing for the seven GWAS-identified 1p12 SNPs of interest. 0 = homozygous major, 1 = heterozygous, 2 = homozygous minor, and NA = missing SNP genotype.

SNP	0 to 1 (%)	0 to 2 (%)	1 to 2 (%)	NA to 0 (%)	NA to 1 (%)	NA to 2 (%)	N Discordant (%)	N Concordant (%)
rs973500	11 (1.9%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	11 (1.9%)	559 (98.1%)
rs4501872	0 (0.0%)	0 (0.0%)	1 (0.2%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.2%)	569 (99.8%)
rs4659138	3 (0.5%)	0 (0.0%)	0 (0.0%)	11 (1.9%)	0 (0.0%)	1 (0.2%)	15 (2.6%)	555 (97.4%)
rs10923726	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.2%)	0 (0.0%)	0 (0.0%)	1 (0.2%)	569 (99.8%)
rs12021830	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.2%)	0 (0.0%)	0 (0.0%)	1 (0.2%)	569 (99.8%)
rs10494218	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.2%)	1 (0.2%)	569 (99.8%)
rs12027986	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.2%)	1 (0.2%)	569 (99.8%)

Table A3. GWAS and WGSA models for the three 1p12 SNPs that had at least one non-NA discordant genotype observation between the Affymetrix and Illumina platforms. All models were adjusted for clinical variables but not genetic ancestry PCs.

SNP	OR		Wald P		N	P Difference	P Ratio
	GWAS	WGSA	GWAS	WGSA			
rs973500	2.6	2.7	4.6E-05	2.2E-05	570	-2.4E-05	0.48
rs4501872	3.0	2.9	1.2E-06	2.3E-06	570	1.1E-06	1.97
rs4659138	2.8	2.9	5.4E-06	2.4E-06	558	-2.9E-06	0.45

Table A4. Pearson correlation matrix between WPCs and GPCs for study participants with both Affymetrix and Illumina sequencing (n = 570).

	gpc1	gpc2	gpc3	gpc4	gpc5	gpc6	gpc7	gpc8	gpc9	gpc10
wpc1	-0.92	-0.18	0.00	0.01	-0.09	-0.10	-0.07	0.02	-0.03	0.01
wpc2	-0.13	0.02	0.01	-0.04	-0.02	0.02	0.01	0.12	-0.11	0.02
wpc3	0.05	-0.11	0.94	0.08	0.12	0.02	0.03	-0.04	0.00	0.03
wpc4	0.01	0.04	-0.67	0.09	-0.03	0.10	-0.05	0.06	-0.07	-0.01
wpc5	-0.16	0.03	-0.08	-0.26	-0.02	0.02	-0.05	0.18	-0.12	0.05
wpc6	-0.02	0.11	-0.06	0.08	-0.07	0.04	0.05	0.03	-0.04	-0.01
wpc7	-0.08	0.08	-0.03	-0.06	0.08	0.01	-0.05	0.05	-0.05	0.10
wpc8	-0.03	0.01	0.02	0.05	-0.05	0.03	0.00	-0.10	-0.10	0.03
wpc9	-0.02	-0.02	-0.02	0.11	-0.12	0.08	-0.07	0.08	-0.15	-0.02
wpc10	-0.02	0.01	-0.01	0.01	0.02	-0.06	0.10	-0.34	0.26	0.05

Table A5. Pairwise Pearson correlation matrix between GPCs and 1p12 Affymetrix genotypes for study participants with both Affymetrix and Illumina sequencing (n = 570).

	gpc1	gpc2	gpc3	gpc4	gpc5	gpc6	gpc7	gpc8	gpc9	gpc10
rs973500	-0.01	0.04	0.08	0.04	-0.01	0.01	0.00	-0.07	-0.03	-0.02
rs4501872	-0.01	0.06	0.06	0.07	-0.03	0.02	-0.03	-0.06	-0.02	-0.03
rs4659138	-0.02	0.07	0.06	0.05	0.00	0.00	-0.05	-0.07	-0.03	-0.02
rs10923726	-0.02	0.07	0.06	0.07	-0.02	0.01	-0.04	-0.07	-0.02	-0.03
rs12021830	-0.01	0.06	0.06	0.07	-0.03	0.02	-0.03	-0.07	-0.02	-0.03
rs10494218	-0.01	0.06	0.06	0.07	-0.03	0.02	-0.03	-0.07	-0.02	-0.03
rs12027986	-0.01	0.06	0.06	0.06	-0.02	0.01	-0.04	-0.06	-0.02	-0.03

Table A6. Pairwise Pearson correlation matrix between WPCs and 1p12 Illumina genotypes for study participants with both Affymetrix and Illumina sequencing (n = 570).

	wpc1	wpc2	wpc3	wpc4	wpc5	wpc6	wpc7	wpc8	wpc9	wpc10
rs973500	0.01	-0.03	0.06	-0.04	-0.08	0.02	-0.03	-0.04	0.03	-0.05
rs4501872	-0.01	-0.06	0.04	-0.02	-0.08	0.00	-0.04	-0.03	0.02	-0.06
rs4659138	0.00	-0.06	0.04	-0.02	-0.07	0.01	-0.03	-0.03	0.02	-0.06
rs10923726	0.00	-0.06	0.04	-0.02	-0.08	0.01	-0.04	-0.04	0.02	-0.06
rs12021830	0.00	-0.06	0.04	-0.02	-0.07	0.01	-0.03	-0.03	0.02	-0.06
rs10494218	0.00	-0.06	0.04	-0.02	-0.07	0.00	-0.03	-0.03	0.02	-0.06
rs12027986	-0.01	-0.06	0.04	-0.02	-0.08	0.00	-0.03	-0.04	0.02	-0.07

Table A7.a. 1p12 GWAS and WGSA models with and without the top GPCs and WPCs for survivors with concordant sequencing data. All models were adjusted for clinical variables.

SNP	GWAS + PCs		WGSA + PCs		GWAS/WGSA - PCs		N
	OR	Wald P	OR	Wald P	OR	Wald P	
rs973500	3.0	9.1E-06	2.8	1.8E-05	2.7	3.0E-05	559
rs4501872	3.1	1.9E-06	2.9	4.2E-06	2.9	3.7E-06	569
rs4659138	3.1	1.8E-06	2.9	3.2E-06	2.9	3.3E-06	555
rs10923726	3.1	1.7E-06	2.9	3.7E-06	2.8	3.7E-06	569
rs12021830	3.1	1.6E-06	2.9	3.9E-06	2.8	3.5E-06	569
rs10494218	3.2	1.0E-06	2.9	2.5E-06	2.9	2.3E-06	569
rs12027986	3.0	3.9E-06	2.8	8.9E-06	2.8	7.3E-06	569

Table A7.b. GWAS and WGSA 1p12 locus p-value comparisons adjusted for clinical variables with (+) and without (-) genetic ancestry PCs.

SNP	WGSA+ vs GWAS+		GWAS-/WGSA- vs GWAS+		GWAS-/WGSA- vs WGSA+	
	P Difference	P Ratio	P Difference	P Ratio	P Difference	P Ratio
rs973500	9.0E-06	2.0	2.1E-05	3.3	1.2E-05	1.7
rs4501872	2.3E-06	2.2	1.9E-06	2.0	-4.1E-07	0.9
rs4659138	1.4E-06	1.8	1.5E-06	1.8	6.2E-08	1.0
rs10923726	2.0E-06	2.2	2.0E-06	2.2	2.5E-08	1.0
rs12021830	2.3E-06	2.4	1.9E-06	2.2	-3.5E-07	0.9
rs10494218	1.4E-06	2.4	1.2E-06	2.2	-2.0E-07	0.9
rs12027986	5.0E-06	2.3	3.4E-06	1.9	-1.6E-06	0.8

Table A8. 1p12 GWAS and WGSA models among participants with both non-NA Affymetrix and Illumina genotypes. All models were adjusted for clinical variables and analysis-specific PCs.

SNP	GWAS		WGSA		N Participants	N Non-NA Genotype Differences	P Difference	P Ratio
	OR	Wald P	OR	Wald				
rs973500	2.9	1.3E-05	2.8	1.5E-05	570	11	1.3E-06	1.1
rs4501872	3.3	5.1E-07	3.0	2.4E-06	570	1	1.9E-06	4.7
rs4659138	3.0	2.9E-06	3.0	2.5E-06	558	3	-4.1E-07	0.9
rs10923726	3.1	1.7E-06	2.9	3.7E-06	569	0	2.0E-06	2.2
rs12021830	3.1	1.6E-06	2.9	3.9E-06	569	0	2.3E-06	2.4
rs10494218	3.2	1.0E-06	2.9	2.5E-06	569	0	1.4E-06	2.4
rs12027986	3.0	3.9E-06	2.8	8.9E-06	569	0	5.0E-06	2.3