1. Study Title: GWAS of Childhood Cisplatin-induced Tinnitus and Meta-Analysis with Adult GWAS of Cisplatin-induced Tinnitus

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3. Background and rationale:

Cisplatin is one of the most commonly used chemotherapeutic agents worldwide. Cisplatin forms DNA inter- and intra-strand crosslinks that induce cell cycle arrest and apoptosis and inhibit proliferation (1). Consequently, cisplatin and its congeners carboplatin and oxaliplatin are used in the treatment of many adult-onset (cervical, colorectal, endometrial, head/neck, lung, breast, ovarian, and testicular) and pediatric malignancies (germ cell tumors, hepatoblastoma, medulloblastoma, neuroblastoma, osteosarcoma, and retinoblastoma) (2). However, cisplatin also elicits severe off-target toxicities, including nephrotoxicity, neurotoxicity, and ototoxicity that can include hearing loss and tinnitus (3, 4). Due to improved survival rates in testicular cancer (>95% 5-year survival rate) and in pediatric malignancies (partially attributed to the addition of cisplatin into treatment regimens), there is a significant number of survivors living with these severe adverse sequelae that can reduce quality of life. Cisplatin-associated ototoxicity is a notable side effect for long-term survivors because it can create functional limitations, ranging from impairment of speech development and academic achievement in children, to detrimental effects on quality of life, socialization, and cognition in adults (5).

We have previously assessed the association between genotype and susceptibility to cisplatin-induced hearing loss in a genome-wide association study (GWAS) of 511 testicular cancer survivors of European genetic ancestry (6). We identified a single nucleotide polymorphism (SNP; rs62283056) in the first intron of Mendelian deafness gene *WFS1* (wolframin ER transmembrane glycoprotein) as meeting genome-wide significance for association with cisplatin-induced hearing loss ($p = 1.4\times10^{-8}$). This SNP is an expression quantitative trait locus (eQTL) for the gene, with the risk (and minor) allele being associated with lower expression in several human tissues identified from the Genotype-Tissue Expression (GTEx) project (brain-cerebellar hemisphere ($p = 1.8\text{x}10^{-6}$), muscle-skeletal ($p = 4.9\text{x}10^{-11}$), nerve-tibial ($p = 4.1\text{x}10^{-6}$), skin (sun-exposed lower leg) ($p = 2.4\text{x}10^{-11}$), and thyroid ($p = 1.5\text{x}10^{-6}$)). Recently, we found significant association of rs62283056 with a consistent direction of effect ($p = 0.032$) in an additional 317 testicular cancer survivors treated with cisplatin. In a meta-analysis of 1,112,545 common SNPs present in both the St. Jude Children's Hospital GWAS (7) and our GWAS of cisplatin-induced hearing loss, rs62283056 remained the top signal ($p = 5.4 \text{ x } 10^{-8}$). There was a consistent direction of effect as the risk allele was the same in both investigations, but the association was primarily driven by our study (one-tailed $P_{St.Jude} = 0.09$). In addition, our results indicated that cisplatin-induced hearing loss is a polygenic trait, and that SNPs in and near 84 known Mendelian deafness genes were significantly enriched for low p-values in the GWAS ($p = 0.048$). Finally, we identified an association between decreased *WFS1* expression and general hearing loss in Vanderbilt's biobank BioVU (n = 18,620 patients, Bonferroni adjusted $p < 0.05$), revealing that Mendelian, cisplatin-induced, and general hearing loss share loss-of-function mechanisms in *WFS1*.

Another notable symptom of cisplatin is tinnitus, a perceived ringing or buzzing in the ears that can be subjective (perceived by the individual) or objective (heard by an observer) (8, 9). Although tinnitus is relatively common (affecting approximately 10-15% of individuals in the general population), the pathogenic mechanisms of this disorder are poorly understood, and there are currently no agents approved to prevent or treat associated symptoms (10). Despite its etiological ambiguity, several risk factors have been shown to contribute to the development of tinnitus, including age, hearing loss, and the administration of ototoxic drugs such as cisplatin (11, 12). Nevertheless, only half of patients with tinnitus have recognized exposures to environmental risk factors, raising the hypothesis that tinnitus is a heritable trait and its development is genetically predisposed (8). A study recently conducted using >10,000 twin pairs from the Swedish Twin Registry estimated the heritability of bilateral tinnitus to be 56% (13). Prior studies have failed to identify Mendelian patterns of inheritance (14, 15), suggesting that a more complex genetic architecture underlies tinnitus (8). A recent cross-sectional pilot tinnitus GWAS was conducted in ethnically homogeneous individuals from Belgium consisting of 167 tinnitus patients and 749 non-tinnitus controls (16). Although the study had a relatively small sample size and none of the SNPs reached genome-wide significance ($p < 5\text{x}10^{-8}$), gene-set enrichment analysis (GSEA) implicated several metabolic pathways involved in oxidative stress, endoplasmic reticulum (ER) stress, and serotonin receptor mediated signaling. The link between ER stress and the development of tinnitus is intriguing because cisplatin induces ER stress and the resulting signaling cascade is one of the agent's apoptotic mechanisms (17, 18).

A previous study of tinnitus in 528 Norwegian testicular cancer patients treated with cisplatin (19) noted that 19% reported having "quite a bit/very much" tinnitus after 1-4 cycles of chemotherapy compared to 12% of patients given no chemotherapy ($p = 0.001$). Notably, tinnitus was reported as a major symptom by 42% of patients in the dose-intensive cisplatin chemotherapy group (OR = 7.1; 95% CI: 4.1 – 12.4 compared to the no chemotherapy group). In contrast, severe tinnitus occurs among only 4.4% of men in the Norwegian general population, with results of epidemiological studies showing similar prevalence in other European countries, the United States, Japan, and in low and middle-income countries in Africa and Asia (10). We observed similar findings in our North American cohort of cisplatin treated testicular cancer survivors (20).

We evaluated cisplatin-induced tinnitus in 835 testicular cancer survivors (median age at assessment: 38 ± 10 (SD) years; median cumulative dose of cisplatin: 356 mg/m^2). Since there are no external quantitative means to assess tinnitus clinically, we assessed it as a response to the question derived from the validated SCIN (21). "Have you suffered in the last 4 weeks from: ringing in your ears?" with "not at all" (n = 614) respondents as controls, excluding "a little bit" respondents (n = 265) and combining those who answered "quite a bit" or "very much" (n = 156; 15.1% of total) as cases (**Figure 1**). In **Table** 1, we illustrate significant associations between tinnitus and cisplatin dose by 100mg/m^2 [OR: 1.36, p =0.01], age by decade [OR: 1.28, p = 0.003], reduced hearing [OR: 6.55, $p = 2x10^{-16}$], vertigo [OR: 6.37, $p = 1.4x10^{-7}$], hypertension [OR: 2.44, $p = 1.25x10^{-4}$] and use of psychotropic medication (anxiolytics or antidepressants) [OR: 2.37, p = 0.0045]. Patients who developed tinnitus (self-reported as "Quite a bit/Very much") were more likely to have a poorer self-reported health (**Figure 2A**), as well as significantly reduced hearing when compared to cisplatin-treated patients that did not develop tinnitus (**Figure 2**B), indicating that these symptoms affected quality of life. There are similar questions in the St. Jude Lifetime Cohort Study (SJLIFE) and Childhood Cancer Survivor Study (CCSS) to allow us to further determine the association of age, cumulative dose of cisplatin, clinical outcomes (i.e. dizziness, vertigo) and the effects of co-morbidities (i.e. hypertension), and self-reported health with cisplatin-induced tinnitus (**Table 1**). The numbers of individuals are approximately are 436 (54+382) cases and 6957 (254+6703) controls, after excluding survivors overlapping with the SJLIFE study. These numbers are for CCSS-only combining Fu2007 and Fu5 questionnaire (includes all patients with and without genotyping). In SJLIFE, there are 116 cases (tinnitus; yes and still present) and 773 controls (no tinnitus) – these are independent of the CCSS study and include survivors with and without GWAS. **Total Tinnitus: Cases: 552 [436 CCSS + 116 SJLIFE]; Controls: 7730 [6957 CCSS + 773 SJLIFE]**

In our cisplatin-induced tinnitus GWAS (**Figure 3**), we observed intronic SNPs ($p < 1.0x10^{-5}$) in several genes: *MIR3659, LOC105378653, DPYSL2, PPFIBP1, PIP4K2A, ADRA1A, FOXP2* including two genes that are also associated with hearing loss: *ADRA1A*, *FOXP2* (22, 23), as well as genes associated with neurodegenerative and anxiety disorders *FOXP2, DPYSL2, PIP4K2A, PPFIBP1, MACF1* (via eQTL rs60104061), and *UTP11L* (via multiple eQTL) (24, 25). The top SNP, rs6671895 ($p = 4.2x10^{-7}$), is intronic to the transcript RP5-884C9.2, and is within 25 base pairs of the micro RNA *MIR3659*. The fifth most significant SNP (rs7606353, $p = 1.5x10^{-6}$) is ~14kb upstream of *OTOS*; a gene coding for otospiralin, involved in cochlear development and implicated in vestibular disease (26, 27). In a study conducted in South Africa (22), patients treated with cisplatin-based therapy that also had baseline and follow-up audiometric data were screened for variation in 29 exonic SNPs of *OTOS* using direct cycle sequencing. In sum, rs77124181 and rs2291767 were found significantly associated with hearing loss. Furthermore, *OTOS* up-regulation significantly decreased apoptosis of spiral ligament fibrocytes induced by cisplatin, suggesting its protective role in the cochlea (23). *PPFIBP1* is also of potential interest because the gene encodes a protein that is a member of the LAR protein-tyrosine phosphatase-interacting protein (liprin) family known to interact with transmembrane protein tyrosine phosphatases functioning in axon guidance (28, 29). The genotype-based predicted expression of this gene is associated with tinnitus in Vanderbilt's BioVU ($p = 0.002$; 105 cases, 7,899 controls). GSEA revealed that the ten most enriched biological processes (FDR $q < 0.15$) are neurological in origin, including central nervous system axonogenesis ($q = 0.04$).

Although these genotype-phenotype correlations are encouraging, the SNPs of interest do not exceed the threshold of genome-wide significance, thereby warranting a meta-analysis of an independent sample set of patients with a prior history of cisplatin exposure and tinnitus. As such, we request to perform GWAS of tinnitus to extend our analysis beyond testicular cancer survivors, and characterize the genetic architecture of tinnitus in cisplatin-treated pediatric patients and de novo tinnitus (no cisplatin). **We will combine patients in the SJLIFE and CCSS cohorts. For the remainder of the proposal, overlapping samples will be included in the SJLIFE cohort and excluded from the CCSS cohort.** Dr. Les Robison, on behalf of the SJLIFE committee, has agreed to share SJLIFE data. If we are able to validate or discover novel SNPs associated with cisplatin-induced tinnitus, we will examine the underlying molecular mechanisms through which genetic variation in the genes of interest affects susceptibility to tinnitus. We will utilize gene-based association methods and *in vitro* investigation of cisplatin sensitivity in relevant cell models of ototoxicity following alterations in gene expression. We will also perform a GWAS of tinnitus in non-cisplatin treated patients to determine the specificity of their effects. We will interrogate the shared genetic architecture between cisplatin-induced and non-cisplatin-induced tinnitus to reveal mechanistic insights into this elusive condition. Our findings would then become the initial rationale for the preclinical development of otoprotectants that could be co-administered with cisplatin to prevent or mitigate tinnitus, and potentially other toxicities.

Our study marks the first time to our knowledge that data from SJLIFE and CCSS will be used to determine potential genetic predisposition to developing tinnitus as a consequence of cisplatin exposure, which is in accordance with the CCSS initiative of investigating the role of genetic susceptibility in the development of non-malignant treatment-related outcomes. Further, our research will provide a better understanding of clinical correlates and mechanisms of cisplatin-induced tinnitus that will be critical in developing effective otoprotectants that reduce toxicity without compromising therapeutic efficacy.

4. Specific Aims/Objectives/Research Hypothesis: Cisplatin acts by forming DNA adducts that inhibit replication, but also cause severe off-target toxicities such as nephrotoxicity, neurotoxicity, and ototoxicity, including tinnitus defined as ringing/buzzing sound with no external sound present (3, 4). Importantly, there still remains a paucity of research on cisplatin-induced tinnitus, and there are currently no agents approved to prevent or treat associated symptoms (10). Tinnitus is a relatively common disorder affecting 10-15% of the general population (8); however a much lower percent have severe tinnitus. For testicular cancer patients treated with cisplatin, we found significant associations between tinnitus and cisplatin dose, age, reduced hearing, vertigo, hypertension, and use of psychotropic medication (anxiolytics or antidepressants) as illustrated in **Table 1** and plan to replicate those observations in the SJLIFE and CCSS cohort. We performed to our knowledge the first GWAS of cisplatin-induced tinnitus in 770 well-characterized testicular cancer survivors (**Figure 3**); however no SNPs exceed the threshold of genome-wide significance, thereby warranting a separate analysis of these associations in independent cisplatin-treated cohorts. As such, we are requesting access to the CCSS database and collaboration to include SJLIFE in order to extend our analysis beyond testicular cancer survivors and determine potential associations between genetic variation and the development of tinnitus in pediatric patients who receive cisplatin as standard-of-care chemotherapy. We will combine the SJLIFE and CCSS participants in the analysis of cisplatin-induced tinnitus: n_{cases} = 80 (48 SJLIFE + 32 CCSS, **tinnitus; yes, still present**); $n_{controls}$ = 264 (115 SJLIFE + 149 CCSS, **no tinnitus**), and in the analysis of *de novo* tinnitus in non-platinum-treated survivors: n_{cases}= 464 (241 SJLIFE + 223 CCSS, **tinnitus; yes, still present**), $n_{controls}$ = 5726 (1889 SJLIFE + 3837 CCSS, **no tinnitus**) (**Tables 2A and 2B**). We will incorporate PrediXcan analyses to evaluate the directionality of effect and potential molecular mechanisms through which genetic variation in the genes of interest affects susceptibility to tinnitus by quantifying the association between genetically regulated levels of expression and the associated phenotype. In addition, we aim to interrogate the shared genetic architecture of cisplatin-induced and *de novo* tinnitus in the general population. We will use individuals unexposed to cisplatin in SJLIFE and CCSS and perform meta-analysis, enrichment analysis, PrediXcan, and other polygenic analyses to reveal cisplatin-dependent and independent effects. We will additionally incoporate BioVU, a large, de-identified DNA biobank linked to Vanderbilt's clinical data warehouse for phenotypic information for this purpose. We will address the specific aims described below.

Our hypothesis is that cisplatin-induced tinnitus is associated with dizziness, vertigo, hypertension, use of psychotropic medication (anxiolytics or antidepressants), and poorer self-reported health.

Aim 1: To investigate associations of cisplatin-induced and de novo tinnitus with demographic characteristics and treatment variable (like regimen and dose) and replicate phenotypic associations.

*Our **hypothesis** is that cisplatin-induced tinnitus is a heritable trait with a polygenic architecture that can be interrogated by meta-analysis GWAS to gain insights into its observed inter-individual variability.*

Aim 2: To perform: 1) a GWAS of tinnitus in cisplatin-treated pediatric cancer survivors in SJLIFE and CCSS and 2) a genome-wide meta-analysis of cisplatin-induced tinnitus in the testicular cancer survivor cohort and the combined cohort of SJLIFE and CCSS.

*Our **hypothesis** is that the interrogation of regulatory regions identified in reference transcriptome datasets (GTEx) using methods like PrediXcan would reduce multiple hypothesis testing correction and reveal possible biological mechanisms for experimental validation.*

Aim 3: To expand genome-wide analyses to include polygenic, gene-based tools like PrediXcan to reveal relevant genes and raise a hypothesized directionality of effect for future mechanistic validation studies.

*Our **hypothesis** is that there is significant overlap between genetic variants and/or genes associated with cisplatin-induced tinnitus and de novo tinnitus.*

Aim 4: To evaluate genetic variants conferring tinnitus risk in non-cisplatin exposed patients and compare genetic architecture with cisplatin-induced tinnitus using CCSS and SJLIFE and Vanderbilt's DNA-linked biobank using an array of genomic analysis tools.

5. Analysis Framework

- **Outcome of interest: Response to questions related other outcomes (dizziness/vertigo, hearing loss), medications (hypertension, antidepressants) and self-reported health. Genotypes associated with cisplatin-induced tinnitus and de novo tinnitus**
- **Subject population:** All patients who responded on either or both of the FU2007 or FU5 CCSS surveys to the question: Tinnitus or ringing in the ears? Response to these surveys is required since these ask for the first time whether the condition is still present, allowing us to eliminate those who were simply reporting a transient condition.

We propose to extend our investigation by performing GWAS of cisplatin-induced tinnitus in SJLIFE and CCSS participants to examine SNPs associated with tinnitus in cisplatin-treated pediatric patients (5, 30). Given identical phenotype definitions in both cohorts, we aim to combine them. **Figure 4** illustrates substantial increase in the percentage of patients who develop tinnitus in the cisplatin-treated group compared to carboplatin or aminoglycosides and is in accord with acquired data from SJLIFE. Our primary analysis will define cases as follows (**definition 1; Table 3**): all cisplatin-treated patients in SJLIFE and CCSS who responded "Yes and condition is still present" in both questionnaires (FU5 and FU2007) or yes on one and have a missing response on the other. A less conservative definition will be used in the secondary analysis **(definition 2; Table 4)**: "Yes and condition is still present" using participants in FU5 questionnaire only. Controls will be defined as the cohort responding "No" in SJLIFE, and "No" in both FU5 and F2007 in CCSS. Given the increased risk of cisplatin-induced tinnitus in CRT-treated patients ($p = 0.02$), we will use CRT treatment (dummy variable) and/or CRT dose as a covariate in the analysis of cases. We will also take into account patients receiving aminoglycosides and vincristine when this treatment information is available due to their potentially confounding ototoxic effects (31-33).

Exploratory clinical correlates (whether they had GWAS or not, no platinum):

- **Tinnitus (based on definition 1): Cases: 552 [436 CCSS + 116 SJLIFE]; Controls: 7730 [6957 CCSS + 773 SJLIFE]**

GWAS cohort:

- **Cisplatin-induced Tinnitus: 80 Cases, 264 Controls**
- **De novo Tinnitus (no cisplatin, no carboplatin): 464 Cases; 5726 Controls**

- **Exploratory variables**

Validation of our previously identified associations of cisplatin induced tinnitus with other variables (listed in **Table 1**) will require answers from the following demographic and clinical questions on the CCSS questionnaires: In addition, we will require genotypes to perform GWAS of all patients with and without tinnitus. **Note:** Question numbers from the Original Cohort Baseline Questionnaire, FU2007 or FU5 are in bold, with survey time indicated if from FU2007 or FU5, while question numbers from the Expanded Cohort Baseline Cohort Questionnaire are italicized.

- **FU2007 and FU5, Baseline Expansion Calculated age at surveys from dates.**
- **A.2**/*A2*. What is your sex?
- **A.4**/*A5*. To which one of the following groups do you belong (race)?
- **A.4a**/*A5a*. Are you Hispanic (ethnicity)?
  Have you ever been told by a doctor or other health care professional that you have, or have had:
  - **FU2007, FU5 D.1** /*C.1.* Hearing loss requiring a hearing aid?
  - **FU2007, FU5 D.2** *C.2.* Deafness in both ears not completely corrected by hearing aid?
  - **FU2007 , FU5 D.3***C.3.* Deafness in only one ear not completely corrected by hearing aid?
  - **FU2007 , FU5 D.4**/*C.4*. Tinnitus or ringing in the ears?
  - **FU2007 , FU5 D.5**/*C.5*. Persistent dizziness or vertigo?
  - **FU2007 , FU5 D.6/** *C.6.* Hearing loss, not requiring a hearing aid?
  - **FU2007 , FU5 D.7**/*C7***.** Any other hearing problems? If yes, describe this problem.
- **N.15** Would you say that your health is? **FU2007 L19, FU5 O1 /** *O21*. In general, would you say your health is?
- **O.8** What kind of business or industry was this job in? **FU2007 A5a, FU5 A6a /** *S3a.* Main job title? **FU2007 A5b, FU5 A6b /** *S3b.* Please briefly describe your primary job tasks.
- **P.1** Yourself. Medical history of cancer, birth defect, hereditary condition (provide specific type).
- **12.** Drugs for high blood pressure or for your heart? **FU2007 C8.5, FU5 C2.5 /** *B8.5.* Medications for high blood pressure or hypertension?
- **15.** Antidepressants or other prescribed drugs for depression or other mood disorders? **FU2007 C8.9, FU5 C2.9 /** *B8.9.* Medications for depression?

Data from the *Medical Records Abstraction Forms or Radiation dosimetry from the Radiation Dosimetry Center*

- Year of cancer diagnosis
- Ages at beginning and end of cisplatin therapy
- Cumulative dose of cisplatin
- Ages at beginning and end of vincristine treatment
- Cumulative dose of vincristine
- Ages at beginning and end of cranial radiation treatment
- Maximum Cranial Radiation dose

We will investigate the associations between tinnitus and other clinically relevant variables mentioned above: age, sex, race, other symptoms of ototoxicity, overall self-reported health, hypertension, use of antidepressants/anxiolytics, use of vincristine, cranial radiation, and aminoglycosides. We will contrast the associations of these variables with tinnitus in the cisplatin-exposed cohort with the non-cisplatin-exposed cohort. We will **not** limit this analysis to genotyped patients but rather all patients with questionnaire responses, therefore based on definition 1: **Tinnitus: Cases: 552 [436 CCSS + 116 SJLIFE]; Controls: 7730 [6957 CCSS + 773 SJLIFE]** (**Table 3**). We will additionally interrogate the time of onset of symptoms to address the chronology of associations. The estimated numbers are based on FU5 and F2007 assessed in the original cohort; however could be higher with additional questionnaires and the potential to include subjects from the expansion cohort (diagnosed 1987-1999) who responded to the baseline and/or FU5 survey. We plan to

evaluate clinical correlates and characteristics between those in cohorts of all CCSS or SJLIFE subjects, including those who did not respond to the survey's relevant for this analysis, those within the *GWAS cohort* and those used for the *Exploratory clinical correlates study* to determine whether any characteristics are significantly different between cohorts (cisplatin induced Tinnitus and de novo tinnitus). If there are meaningful differences, we will consider weighting analyses to account for participation bias.

We will utilize our sample of 770 cisplatin-treated testicular cancer survivors as a discovery cohort and combine the samples in SJLIFE and CCSS participants meeting inclusion criteria to create an independent cohort to validate associations through meta-analysis. **Figure 5A** illustrates the flowchart for our Testicular Cancer Survivor Study cohort. **Figure 5B** illustrates the flowchart for cisplatin treated pediatric patients from SJLIFE and CCSS. Both the CCSS and SJLIFE cohorts utilize the same definition of the tinnitus phenotype. The demographics and clinical characteristics of patients included in the statistical analysis can be found in **Table 3 and** 4. Quality control will be performed as previously described by the SJLIFE and CCSS working group (34), including ancestry quantification with principal component analysis (PCA) and the exclusion of non-European and admixed samples (in comparison to HapMap CEU, YRI, and ASN), related/duplicated samples, samples with excess heterozygosity, and samples with missing phenotypes, as well as SNP level quality-control in PLINK 1.9 (minor allele frequency, call rate, Hardy-Weinberg disequilibrium etc.). We will perform a GWAS on the SJLIFE and CCSS cohort followed by fixed-effects meta-analysis with our testicular cancer survivors discovery cohort using METAL. We quantified the power of estimating the total genetic variance using genome-wide SNPs given our meta-analysis sample size and assuming a point estimate of 0.56 from an early study (13) (which we recapitulated in our initial testicular cancer survivor dataset, albeit with large standard error [$h^2 = 0.57$, SE = 0.48]). At $\alpha = 0.05$, we have 42.5% power to detect the SNP heritability. We have, however, developed a more powerful strategy for identifying relevant genes using common variants. In our approach, we will utilize those variants which have previously been shown to underlie a range of pharmacogenomic (13, 35) and other complex traits (36). Using a method that aggregates the effects of regulatory SNP on gene expression, we will extrapolate SNP-level summary statistics to the gene-level by predicting gene expression, allowing us to test an endophenotype that is known to account for the majority of common variant heritability to common complex traits and reducing the multiple testing burden by at least 100-fold. We will use PrediXcan's elastic net models ($\alpha = 0.5$) trained on GTEx reference transcriptome data to predict gene expression in tissues of choice (brain, tibial nerve, whole blood) from GWAS summary statistics and perform logistic regression with cisplatin-induced tinnitus.

We will additionally perform a GWAS on tinnitus in patients with no prior exposure to cisplatin. We will consider the effects of carboplatin, cranial radiation (CRT), vincristine, and aminoglycoside use on tinnitus and exclude samples/adjust for covariates accordingly. We will investigate the shared genetic architecture between cisplatin-induced and non-platinum-induced tinnitus using several orthogonal approaches. Firstly, we will conduct a meta-analysis and identify potential genome-wide significant variants across the phenotypes. Next, we will perform permutation-based enrichment analyses to determine the extent to which top SNPs in the base GWAS are enriched in the top results of the target GWAS. We will also construct polygenic risk scores from the summary statistics in the base GWAS and evaluate their association in the target GWAS. We will also perform gene-level association using PrediXcan. We will assess the functions of SNPs associated with both cisplatin-induced and general tinnitus using ENCODE and Roadmap Epigenomics to elucidate biologically plausible mechanisms of association. We will attempt to replicate these findings independently in Vanderbilt's BioVU for further validation.

Special Considerations: PrediXcan is a gene-based method that uses reference transcriptome (genotype-gene expression) data to generate models used to 'impute' gene expression levels from genotype data and associate the predicted gene expression with phenotypes of interest (37). Specifically, we will use PrediXcan to identify genes of interest that are associated with tinnitus, both in cancer patients with no cisplatin treatment and in cancer patients who were treated with cisplatin. In addition, we will use Vanderbilt's BioVU, to determine the relationship between identified genes and tinnitus by comparing males who have a history of reported tinnitus to other patients who are in the database in an attempt to identify shared genetic architecture that

corresponds with any genes of interest from the cisplatin GWAS. Importantly, we have identified a significant overlap between GWAS of tinnitus (16) and GWAS of cisplatin-induced tinnitus (**Figure 6**). Thus, some of our findings of cisplatin-induced tinnitus may be replicated using BioVU to evaluate tinnitus in the general population.

Tables and Figures

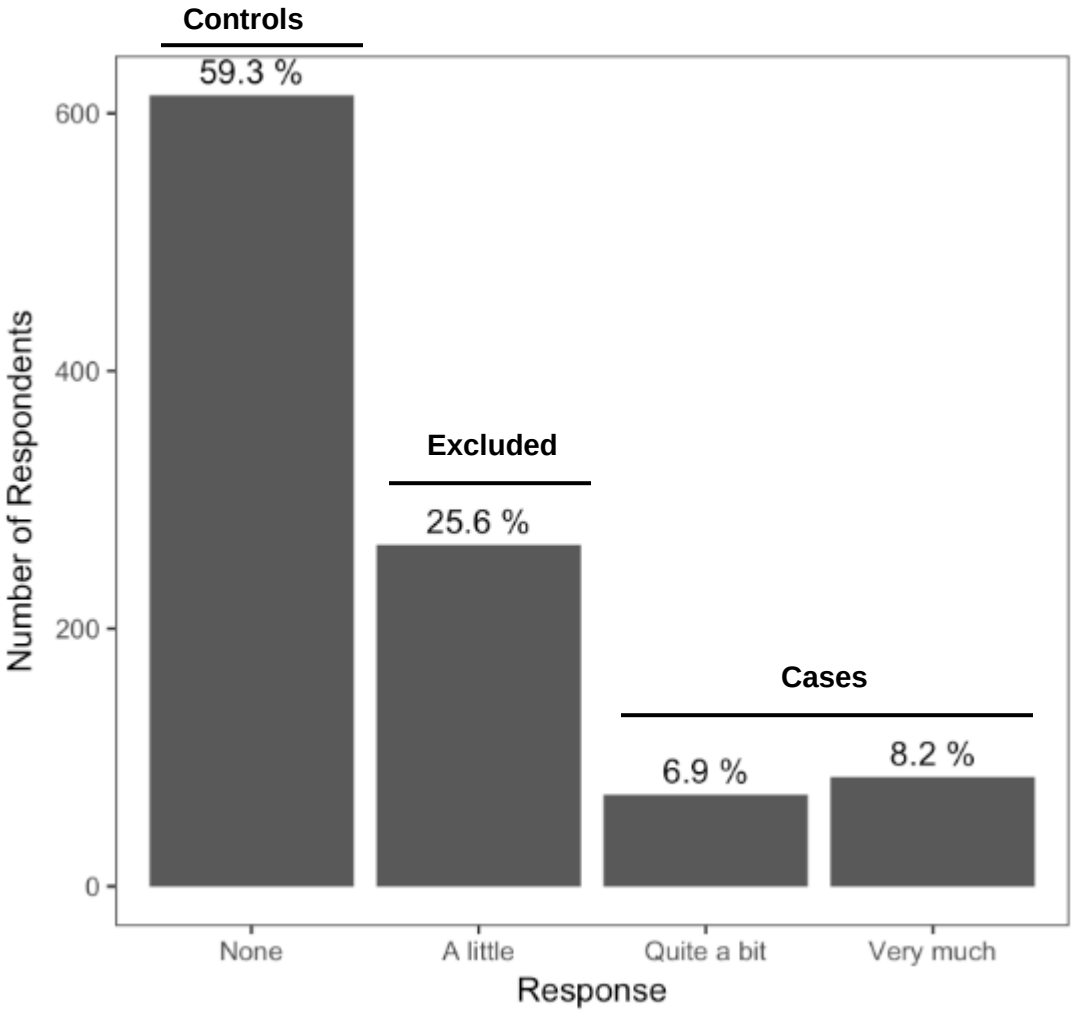


Figure 1: Percentage of cisplatin-treated testicular cancer patients who reported symptoms of tinnitus. Patients who reported symptoms as occurring “Quite a bit” or “Very much” were designated as cases, while those who responded “None” were designated as controls. Patients who reported symptoms as occurring “A little” were excluded from further analysis.

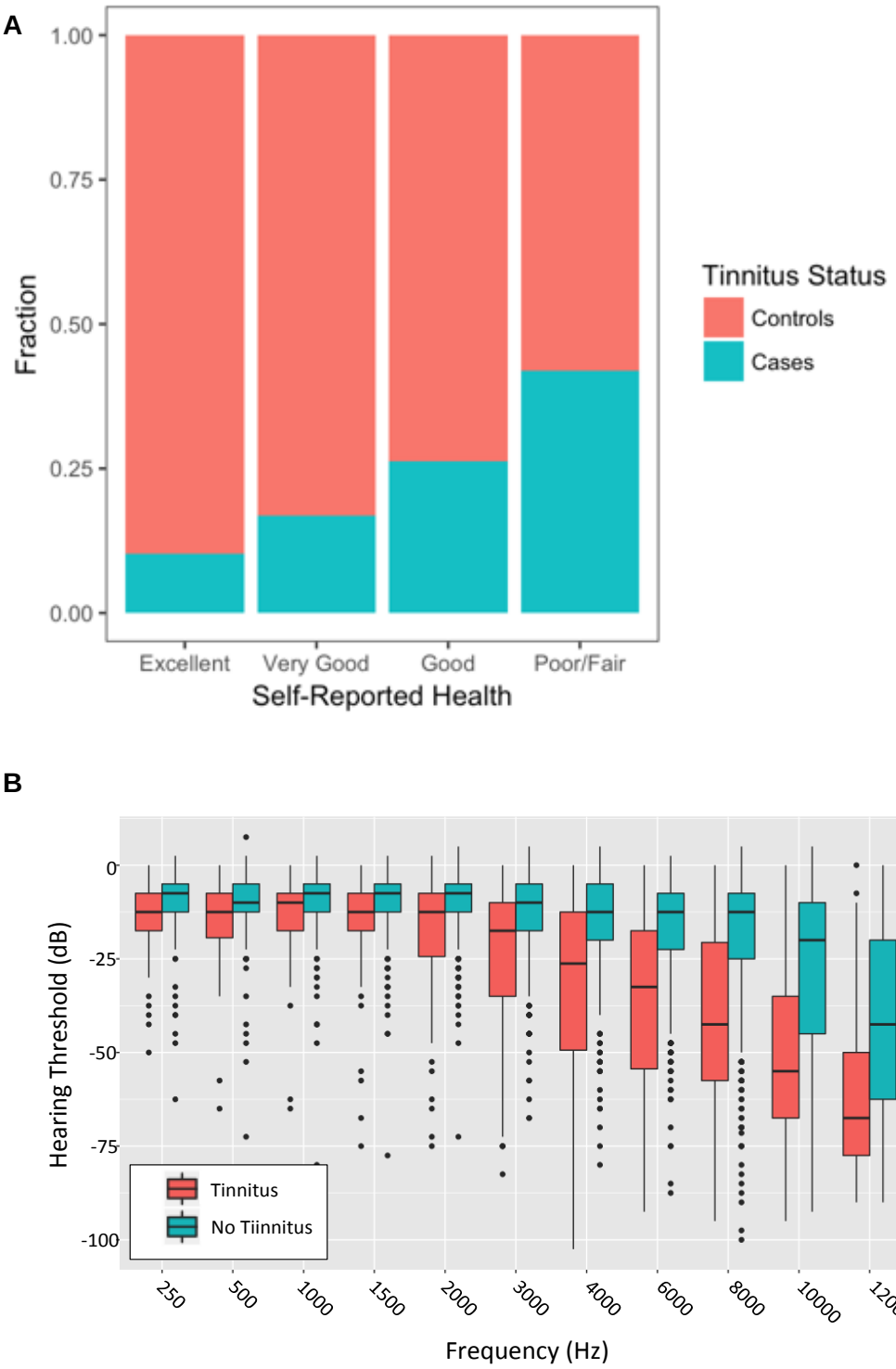


Figure 2: Comparison of self-reported health and hearing loss between cisplatin-treated testicular cancer patients who did or did not develop tinnitus. Patients answered a questionnaire that included questions on self-reported health (A) and hearing was measured using audiometry at each frequency (B) following treatment with cisplatin. Cases (n = 156) refer to cisplatin-treated patients who developed tinnitus (quite a bit/very much) following treatment, while controls (n = 614) refer to cisplatin-treated patients who did not develop tinnitus following treatment.

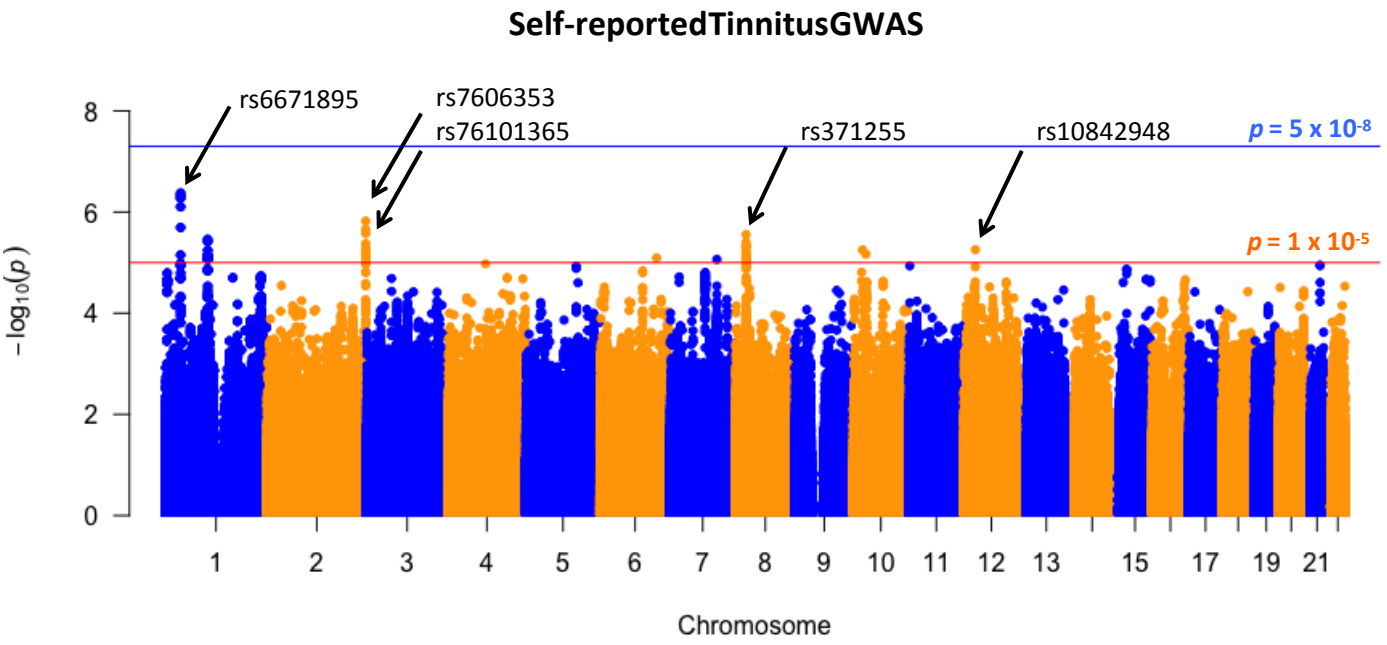


Figure 3: Manhattan plot of SNPs examined in the GWAS of cisplatin-induced tinnitus. The association of SNP genotypes and cisplatin-induced tinnitus in 770 testicular cancer survivors was tested for significance via linear regression. $-\log_{10}$ p values are plotted against the respective chromosomal position of each SNP. The red line indicates p values below $1x10^{-5}$.

Figure 4: The frequency of tinnitus by treatment (cisplatin, carboplatin, and aminoglycosides).

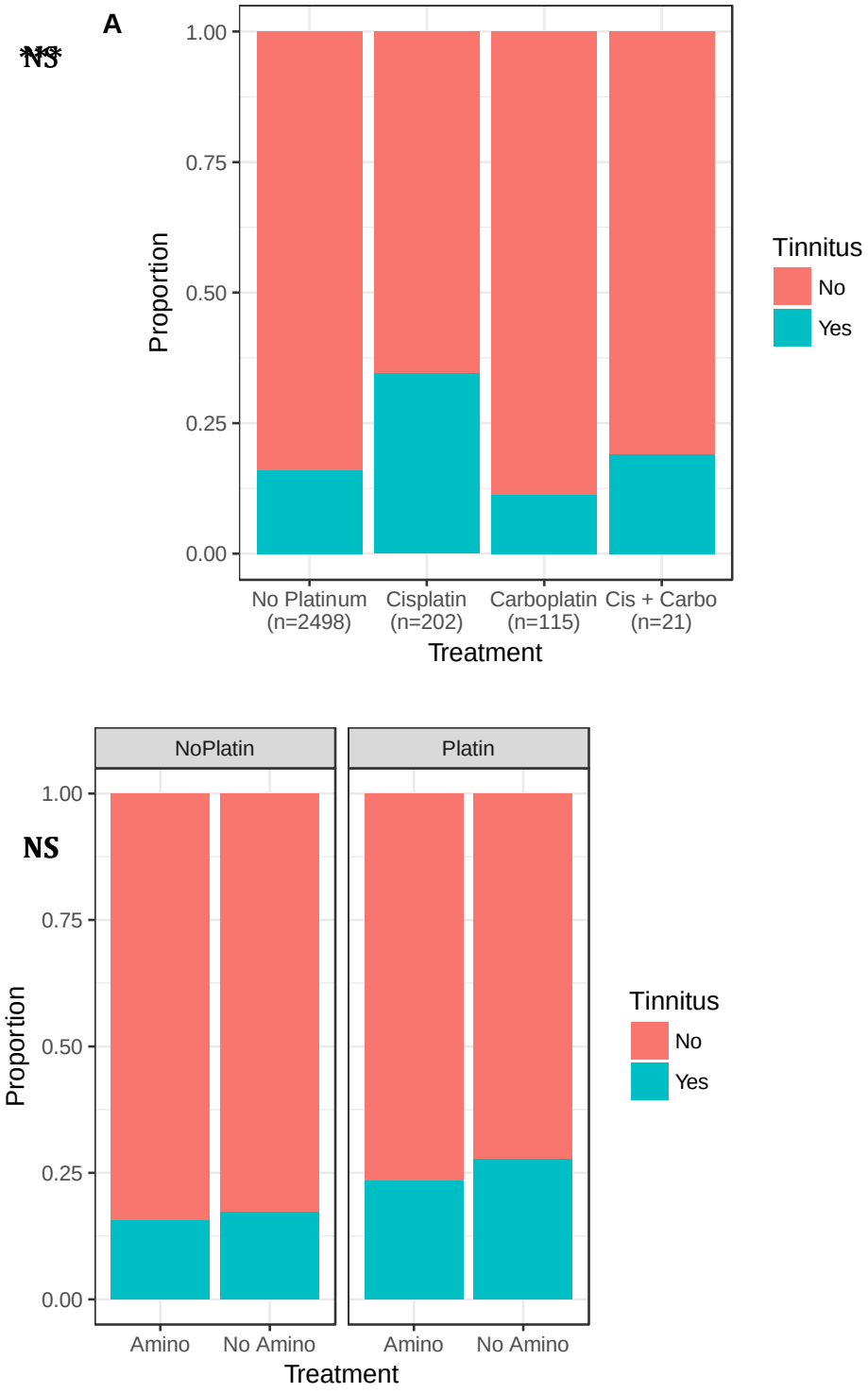
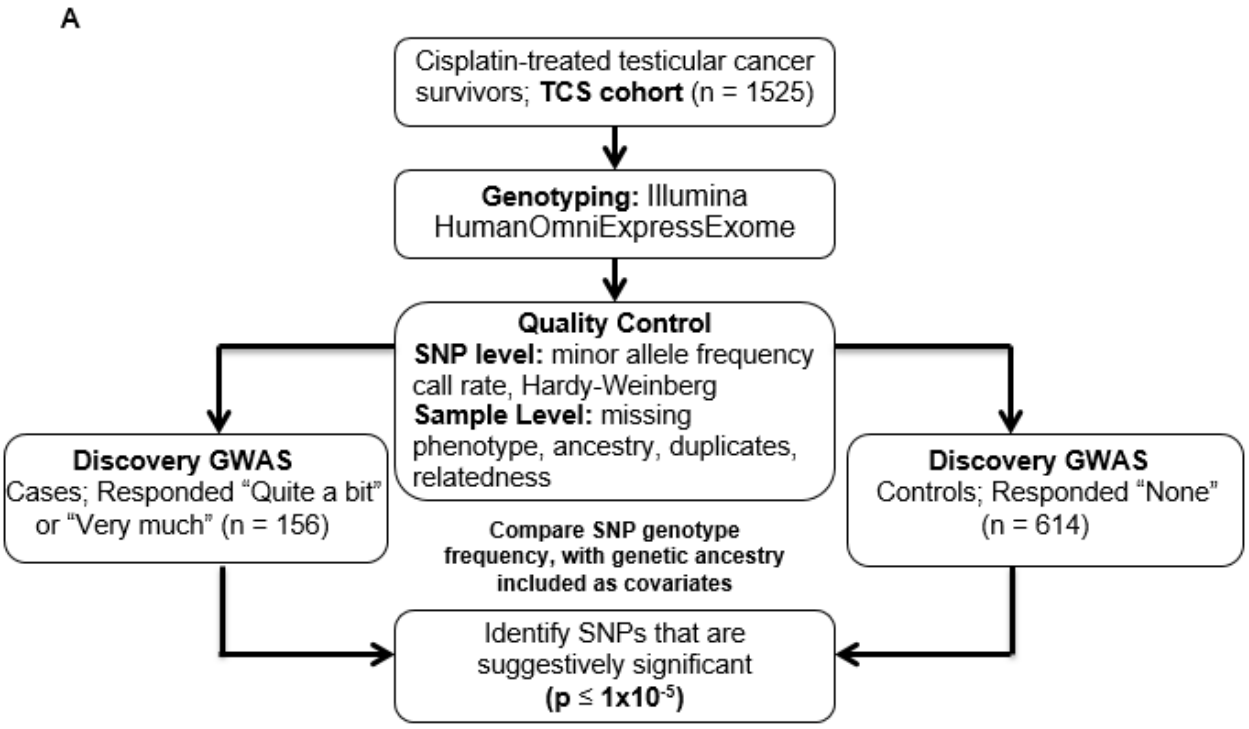


Figure 4: Bar plots showing the frequency of tinnitus by treatment group. The frequency of tinnitus is statistically significantly higher in cisplatin-treated patients than any other group ($p < 0.001$). Carboplatin, combination cisplatin and carboplatin, and aminoglycosides (Amino; either in platinum-treated or non-platinum-treated patients) did not significantly increase risk of tinnitus ($p > 0.05$).

NS = not significant, *** = $p < 0.001$

Figure 5: Data Analysis Pipeline



B

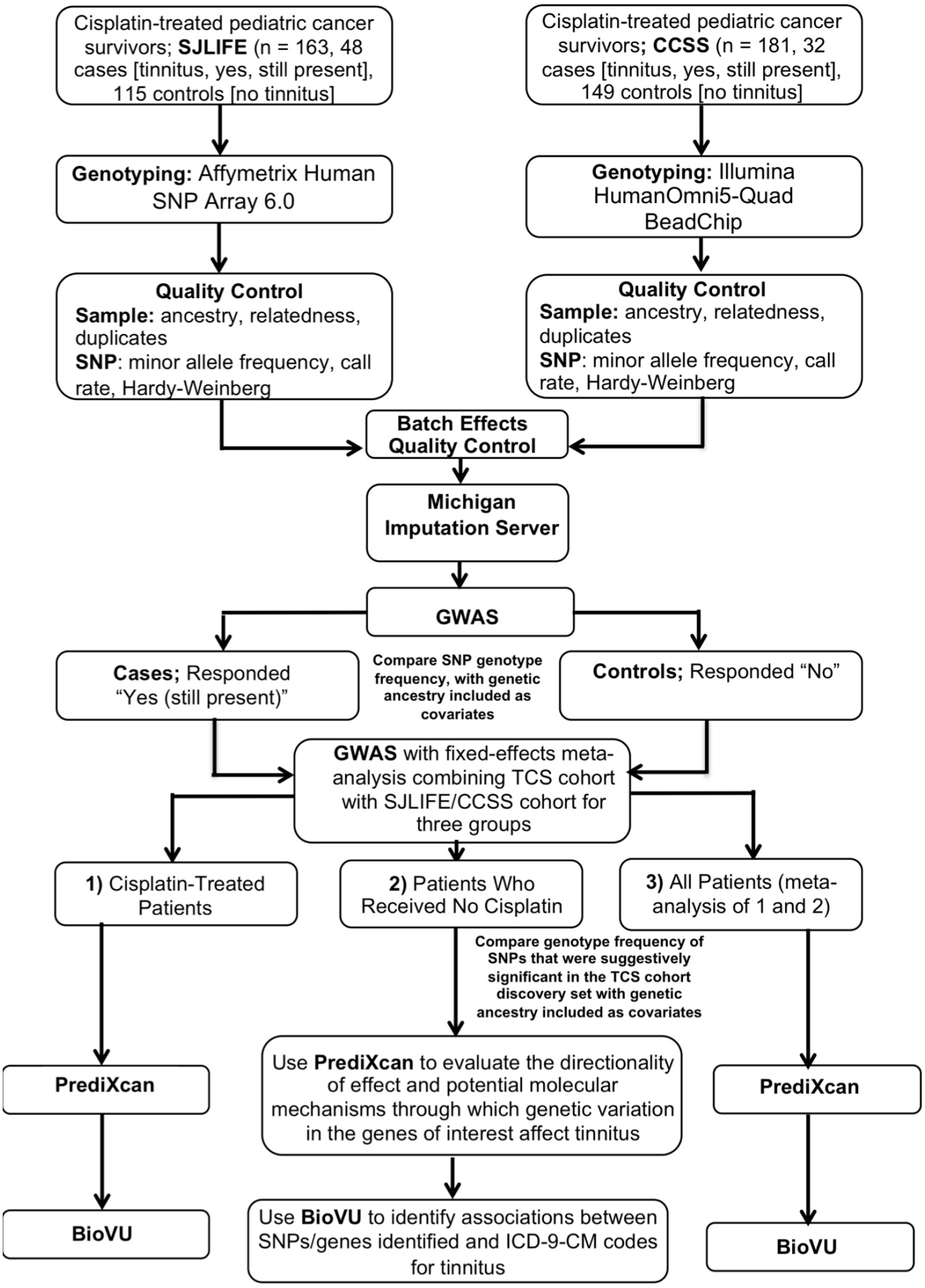

Cisplatin-treated pediatric cancer survivors; SJLIFE (n = 163, 48 cases [tinnitus, yes, still present], 115 controls [no tinnitus]
Cisplatin-treated pediatric cancer survivors; CCSS (n = 181, 32 cases [tinnitus, yes, still present], 149 controls [no tinnitus]
Genotyping: Affymetrix Human SNP Array 6.0
Genotyping: Illumina HumanOmni5-Quad BeadChip
Quality Control
Sample: ancestry, relatedness, duplicates
SNP: minor allele frequency, call rate, Hardy-Weinberg
Quality Control
Sample: ancestry, relatedness, duplicates
SNP: minor allele frequency, call rate, Hardy-Weinberg
Batch Effects Quality Control
Michigan Imputation Server
GWAS
Cases; Responded "Yes (still present)"
Compare SNP genotype frequency, with genetic ancestry included as covariates
Controls; Responded "No"
GWAS with fixed-effects meta-analysis combining TCS cohort with SJLIFE/CCSS cohort for three groups
1) Cisplatin-Treated Patients
2) Patients Who Received No Cisplatin
3) All Patients (meta-analysis of 1 and 2)
Compare genotype frequency of SNPs that were suggestively significant in the TCS cohort discovery set with genetic ancestry included as covariates
PrediXcan
Use PrediXcan to evaluate the directionality of effect and potential molecular mechanisms through which genetic variation in the genes of interest affect tinnitus
PrediXcan
BioVU
Use BioVU to identify associations between SNPs/genes identified and ICD-9-CM codes for tinnitus
BioVU

Figure 5: Flowchart of tinnitus GWAS using TCS cohort as a discovery set and SJLIFE/CCSS as a replication set. A) In the TCS cohort discovery set, cases refer to patients who responded “Quite a bit” or “Very much” when asked about having symptoms of tinnitus. Controls refer to those who responded “None.” All patients that responded “A little” were excluded from the GWAS. B) In the SJLIFE/CCSS discovery and replication set, cases refer to patients who responded “Yes (still present)” when asked about having symptoms of tinnitus. Controls refer to those who responded “No.” All patients that responded “Not sure” were excluded from the GWAS.

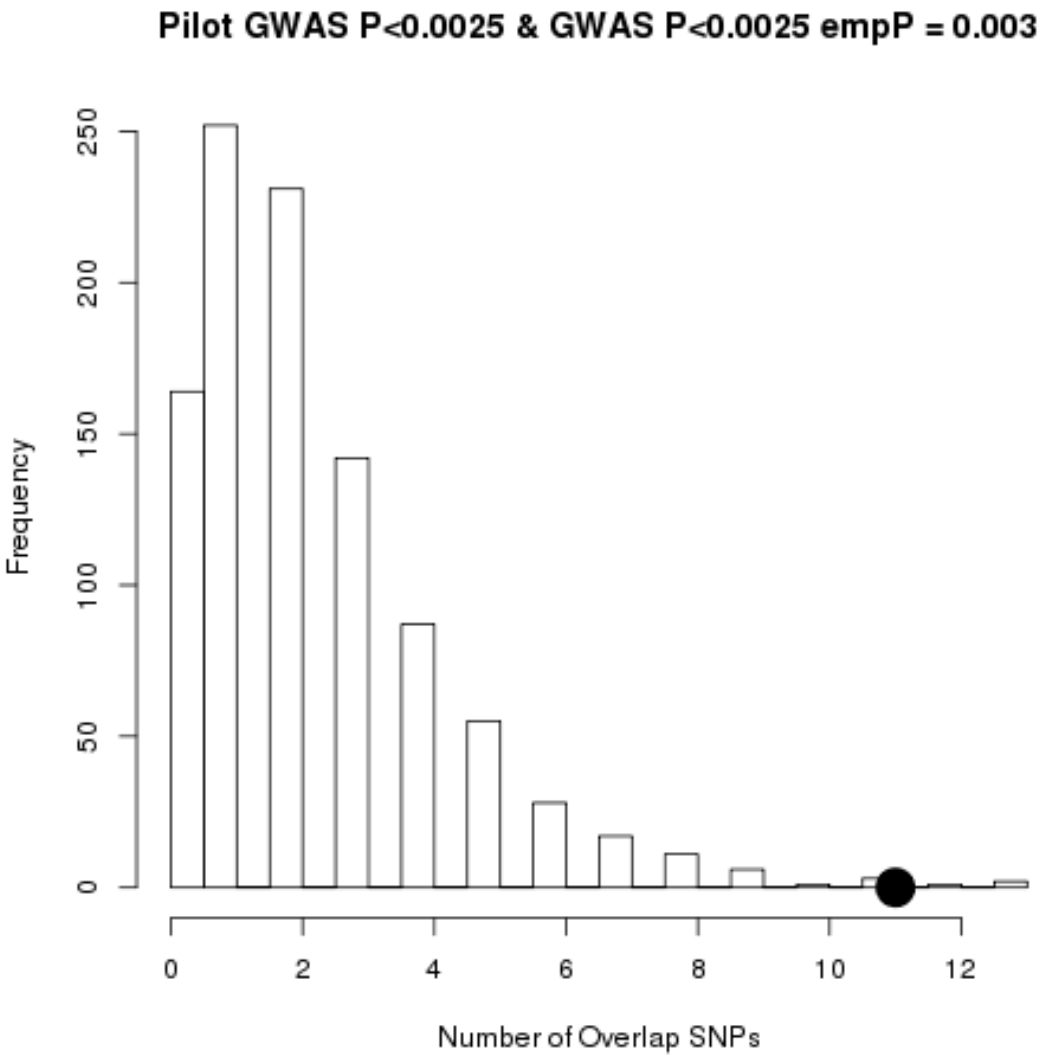


Figure 6: Overlap between GWAS of Tinnitus (p<0.0025 and GWAS of Cisplatin induced Tinnitus (p<0.0025).

Table 1: Associations of cisplatin induced tinnitus with demographic characteristics in Testicular Cancer Survivors study with drug dose and other outcomes and corresponding questions from SJLIFE and/or CCSS questionnaire.

Response	OR	p-value	SJLIFE/CCSS Question
Self Reported Health	**0.55**	**2.4E-07**	N.15 Would you say your health is?
			O21. In general, would. You say your health is?
Age of Evaluation*	**1.28**	**3.0E-03**	What is the age of the patient at his/her last treatment follow-up
Age of Diagnosis	**1.03**	**1.1E-02**	What was the year of cancer diagnosis for the patient
Vertigo	**6.37**	**1.4E-07**	C.5 Persistent dizziness or vertigo
Dizziness	**2.31**	**1.2E-12**	C.5 Persistent dizziness or vertigo
Difficulty Hearing	**4.85**	**< 2E-16**	C.7 Any other hearing problems
Problems Hearing in a Crowd	**7.86**	**< 2E-16**	C.6 Problems hearing sounds, words, or language in crowds?
Reduced Hearing	**6.55**	**< 2E-16**	C.1 Hearing loss requiring a hearing aid
			C.2 Deafness in one or both ears no completely corrected by hearing aid
			C.3 Complete deafness in either ear?
Cumulative Cisplatin Dose	**1.36**	**9.6E-03**	When did cisplatin therapy begin and end, what was cumulative cisplatin dose?
Hypertension (Dx + Rx)	**2.44**	**1.3E-04**	12. Drugs for high blood pressure or for your heart?
			B8.5 Medications for high blood pressure or hypertension?
Antianxiety/Antipsychotics (Rx)	**2.37**	**4.6E-03**	15. Antidepressants or other prescribed drugs for depression or other mood disorder?
			B8.9 Medications for depression?

*Age by decade, **Cumulative dose by 100mg/m^2

Table 2: Case/Control distribution in CCSS and SJLIFE in both the cisplatin-treated and the non-platinum-treated cohorts that have been genotyped.

Table 2A | CCSS-only GWA dataset for Tinnitus analysis

	Cisplatin		No cisplatin or carboplatin	
Tinnitus	**GWA**	**Europeans*[1]**	**GWA**	**Europeans[2]**
Yes, and still present	32	32	223	215
No	149	138	3837	3594
Total	181	170	4060	3809

Table 2B | SJLIFE GWA dataset for Tinnitus analysis**

	Cisplatin		No cisplatin or carboplatin	
Tinnitus	**GWA**	**Europeans**	**GWA**	**Europeans**
Yes, and still present	48	35	241	209
No	115	79	1889	1472
Total	163	114	2130	1681

*These numbers are based on more permissive definition of ancestry, using STRUCTURE and HapMap populations.

[1]Using PCA and 1kGP, there are 32 cases and 135 controls.

[2]Based on PCA, there are 211 cases and 3463 controls.

** Ancestry was determined based on PCA.

Table 3. Demographics and clinical characteristics of patients treated with cisplatin, or not treated with cisplatin or carboplatin in CCSS (no overlap with SJLIFE), according to definition 1 of tinnitus.

	Cisplatin		No Cisplatin or Carboplatin	
Characteristics	**Cases (Tinnitus) (N = 54)**	**Controls (No Tinnitus) (N=254)**	**Cases (Tinnitus) (N = 382)**	**Controls (No Tinnitus) (N=6703)**
Sex of patient				
Male	32 (59%)	124 (49%)	227 (59%)	3307 (49%)
Female	22 (41%)	130 (51%)	155 (41%)	3396 (51%)
Race				
NH White	51 (94%)	219 (87%)	351 (92%)	6034 (90%)
NH Black	0 (0%)	13 (5%)	4 (1%)	165 (2%)
Hispanic	3 (6%)	10 (4%)	20 (5%)	305 (5%)
Other	0 (0%)	11 (4%)	7 (2%)	170 (3%)
Diagnosis				
Leukemia	0 (0%)	4 (2%)	103 (27%)	2348 (35%)
CNS	16 (30%)	43 (17%)	97 (25%)	790 (12%)
HD			63 (16%)	893 (13%)
NHL	2 (4%)	2 (1%)	28 (7%)	511 (8%)
Kidney (Wilms)	1 (2%)	4 (2%)	14 (4%)	696 (10%)
Neuroblastoma	6 (11%)	40 (16%)	10 (3%)	444 (7%)
Soft tissue sarcoma	8 (15%)	43 (17%)	39 (10%)	586 (9%)
Bone cancer	21 (39%)	118 (46%)	28 (7%)	435 (6%)
Age at cancer diagnosis				
<1	3 (6%)	21 (8%)	11 (3%)	519 (8%)
1-4	10 (19%)	61 (24%)	93 (24%)	2248 (34%)
5-9	10 (19%)	41 (16%)	87 (23%)	1465 (22%)
15-19	31 (57%)	127 (50%)	179 (47%)	2330 (35%)
20-<21	0 (0%)	4 (2%)	12 (3%)	141 (2%)
Year of diagnosis				
1970-79	7 (13%)	25 (10%)	217 (57%)	3097 (46%)
1980-89	47 (87%)	229 (90%)	165 (43%)	3606 (54%)
Age at questionnaire				
20-29	6 (11%)	30 (12%)	19 (5%)	526 (8%)
30-39	16 (30%)	103 (41%)	100 (26%)	2534 (38%)
40-49	27 (50%)	97 (38%)	163 (43%)	2553 (38%)
50-59	5 (9%)	24 (9%)	90 (24%)	1027 (15%)
60-69			10 (3%)	63 (1%)
Cisplatin by CRT				
Cisplain only	31 (58%)	184 (74%)		
CRT only			186 (100%)	2269 (100%)
Both	22 (42%)	63 (26%)		
Cisplatin dose (mg/m^2)				
None			382 (100%)	6703 (100%)
<300	15 (28%)	73 (29%)		
300-349	8 (15%)	27 (11%)		
350-399	5 (9%)	16 (6%)		
400+	26 (48%)	138 (54%)		
Number of Cisplatin-Based Chemotherapy Cycles				
0			382 (100%)	6703 (100%)
1	51 (94%)	247 (97%)		
2	3 (6%)	7 (3%)		
CRT dose (Gy)				
None	31 (58%)	184 (74%)	189 (50%)	4247 (65%)
<20	0 (0%)	12 (5%)	45 (12%)	914 (14%)
20-29	1 (2%)	3 (1%)	44 (12%)	734 (11%)

30-49	5 (9%)	9 (4%)	17 (5%)	201 (3%)
50+	16 (30%)	39 (16%)	80 (21%)	420 (6%)
Years Between Cancer Diagnosis and Tinnitus				
0-<5	36 (67%)	0 (.%)	73 (19%)	0 (.%)
5-<10	2 (4%)	0 (.%)	36 (9%)	0 (.%)
10-<20	12 (22%)	0 (.%)	109 (29%)	0 (.%)
20-<30	4 (7%)	0 (.%)	122 (32%)	0 (.%)
30-<40			42 (11%)	0 (.%)
Fu2007: D4 Tinnitus[#]				
Yes, and condition is still present	45 (100%)	10 (4%)	339 (100%)	90 (1%)
No	0 (0%)	212 (92%)	0 (0%)	5893 (96%)
Yes, but condition is no longer present	0 (0%)	7 (3%)	0 (0%)	115 (2%)
Not sure	0 (0%)	2 (1%)	0 (0%)	53 (1%)
Yes, unknown whether still present			0 (0%)	6 (0%)
Fu5: D4 Tinnitus				
Yes, and condition is still present	37 (100%)	12 (6%)	228 (100%)	264 (6%)
No	0 (0%)	161 (85%)	0 (0%)	4169 (90%)
Yes, but condition is no longer present	0 (0%)	5 (3%)	0 (0%)	109 (2%)
Not sure	0 (0%)	11 (6%)	0 (0%)	73 (2%)
Fu2007: D1 Hearing loss requiring a hearing aid				
Yes, and condition is still present	24 (53%)	25 (11%)	60 (18%)	161 (3%)
No	20 (44%)	197 (86%)	262 (78%)	5975 (97%)
Yes, but condition is no longer present	0 (0%)	5 (2%)	0 (0%)	12 (0%)
Not sure	1 (2%)	2 (1%)	8 (2%)	10 (0%)
Yes, unknown whether still present			5 (1%)	7 (0%)
Fu5: D1 Hearing loss requiring a hearing aid				
Yes, and condition is still present	19 (51%)	24 (13%)	62 (27%)	199 (4%)
No	17 (46%)	160 (84%)	162 (71%)	4400 (95%)
Yes, but condition is no longer present	1 (3%)	3 (2%)	2 (1%)	9 (0%)
Not sure	0 (0%)	3 (2%)	2 (1%)	23 (0%)
Fu2007: D2 Deafness in both ears				
Yes, and condition is still present	6 (14%)	6 (3%)	18 (5%)	49 (1%)
No	37 (84%)	219 (95%)	313 (94%)	6097 (99%)
Yes, but condition is no longer present	0 (0%)	2 (1%)	2 (1%)	1 (0%)
Not sure	1 (2%)	3 (1%)	1 (0%)	9 (0%)
Yes, unknown whether still present			0 (0%)	4 (0%)
Fu5: D2 Deafness in both ears				
Yes, and condition is still present	8 (22%)	5 (3%)	10 (4%)	44 (1%)
No	28 (76%)	179 (94%)	212 (93%)	4547 (98%)
Yes, but condition is no longer present	0 (0%)	1 (1%)	0 (0%)	1 (0%)
Not sure	1 (3%)	5 (3%)	5 (2%)	31 (1%)
Fu2007: D3 Deafness in one ear				
Yes, and condition is still present	5 (12%)	12 (5%)	50 (15%)	91 (1%)
No	37 (86%)	215 (94%)	271 (82%)	6040 (98%)
Yes, but condition is no longer present			0 (0%)	8 (0%)
Not sure	1 (2%)	1 (0%)	3 (1%)	8 (0%)
Yes, unknown whether still present			5 (2%)	4 (0%)
Fu5: D3 Deafness in one ear				
Yes, and condition is still present	4 (11%)	5 (3%)	32 (14%)	80 (2%)
No	32 (86%)	177 (93%)	189 (84%)	4502 (98%)
Yes, but condition is no longer present	0 (0%)	1 (1%)	0 (0%)	6 (0%)
Not sure	1 (3%)	7 (4%)	4 (2%)	29 (1%)
Fu2007: D6 Hearing loss				
Yes, and condition is still present	20 (45%)	45 (20%)	135 (40%)	324 (5%)
No	24 (55%)	175 (76%)	190 (56%)	5741 (93%)
Yes, but condition is no longer present	0 (0%)	6 (3%)	3 (1%)	36 (1%)
Not sure	0 (0%)	4 (2%)	8 (2%)	35 (1%)

Yes, unknown whether still present			2 (1%)	8 (0%)
Fu5: D6 Hearing loss				
Yes, and condition is still present	17 (47%)	47 (25%)	88 (39%)	303 (7%)
No	19 (53%)	139 (73%)	127 (56%)	4230 (92%)
Yes, but condition is no longer present	0 (0%)	2 (1%)	1 (0%)	26 (1%)
Not sure	0 (0%)	3 (2%)	9 (4%)	55 (1%)
Fu2007: D7 Any other hearing problems				
Yes, and condition is still present	1 (2%)	7 (3%)	16 (5%)	78 (1%)
No	41 (95%)	207 (93%)	293 (92%)	5958 (98%)
Yes, but condition is no longer present	0 (0%)	1 (0%)	0 (0%)	27 (0%)
Not sure	1 (2%)	7 (3%)	9 (3%)	32 (1%)
Yes, unknown whether still present	0 (0%)	1 (0%)	0 (0%)	6 (0%)
Fu5: D7 Any other hearing problems				
Yes, and condition is still present	2 (6%)	8 (4%)	23 (11%)	82 (2%)
No	28 (78%)	170 (91%)	173 (81%)	4378 (96%)
Yes, but condition is no longer present			1 (0%)	15 (0%)
Not sure	6 (17%)	9 (5%)	17 (8%)	107 (2%)
Hypertension up to Fu2007, CTCAE grades^				
0	45 (83%)	207 (81%)	299 (78%)	5731 (86%)
1	2 (4%)	9 (4%)	17 (4%)	239 (4%)
2	7 (13%)	38 (15%)	66 (17%)	727 (11%)
3			0 (0%)	5 (0%)
Fu5: F5 ever had Hypertension requiring				
Yes, and condition is still present	13 (33%)	44 (23%)	54 (24%)	801 (17%)
No	26 (67%)	132 (70%)	157 (69%)	3641 (79%)
Yes, but condition is no longer present	0 (0%)	10 (5%)	12 (5%)	137 (3%)
Not sure	0 (0%)	3 (2%)	6 (3%)	27 (1%)
Fu2007: 2-year period, Drug for depression				
Yes	8 (18%)	36 (16%)	81 (24%)	987 (16%)
No	37 (82%)	195 (84%)	259 (76%)	5205 (84%)
Fu5: 2-year period, Drug for depression				
Yes	8 (21%)	38 (20%)	54 (23%)	811 (17%)
No	31 (79%)	154 (80%)	179 (77%)	3880 (83%)

#In this table, Tinnitus definition 1 is used which incorporated both Fu2007 and Fu5. For other variables that asked in both questionnaire, Qi Liu is not sure how to combined so the response from Fu2007 and Fu5 are shown separately

^ For chronic conditions by Fu2007, the CTCAE grades are available. For conditions in Fu5, the grading is not done yet so the next row shows hypertension requiring medication in Fu5.

Table 4. Demographics and clinical characteristics of patients treated with cisplatin, or not treated with cisplatin or carboplatin in CCSS (no overlap with SJLIFE), according to definition 2 of tinnitus.

	Cisplatin		No Cisplatin or Carboplatin	
Characteristics	**Cases (Tinnitus)**	**Controls**	**Cases (Tinnitus)**	**Controls**
Sex of patient				
Male	29 (59%)	82 (46%)	274 (56%)	2003 (46%)
Female	20 (41%)	95 (54%)	218 (44%)	2348 (54%)
Race				
NH White	48 (98%)	153 (86%)	464 (95%)	3930 (91%)
NH Black	0 (0%)	8 (5%)	5 (1%)	92 (2%)
Hispanic	1 (2%)	6 (3%)	14 (3%)	200 (5%)
Other	0 (0%)	10 (6%)	8 (2%)	111 (3%)
Diagnosis				
Leukemia	0 (0%)	2 (1%)	137 (28%)	1530 (35%)
CNS	14 (29%)	22 (12%)	105 (21%)	477 (11%)
HD			80 (16%)	544 (13%)
NHL	2 (4%)	2 (1%)	36 (7%)	327 (8%)
Kidney (Wilms)	0 (0%)	4 (2%)	22 (4%)	485 (11%)
Neuroblastoma	6 (12%)	26 (15%)	23 (5%)	303 (7%)
Soft tissue sarcoma	5 (10%)	31 (18%)	54 (11%)	381 (9%)
Bone cancer	22 (45%)	90 (51%)	35 (7%)	304 (7%)
Age at cancer diagnosis				
<1	5 (10%)	13 (7%)	18 (4%)	335 (8%)
1-4	5 (10%)	45 (25%)	131 (27%)	1466 (34%)
5-9	11 (22%)	18 (10%)	97 (20%)	933 (21%)
15-19	28 (57%)	98 (55%)	232 (47%)	1529 (35%)
20-<21	0 (0%)	3 (2%)	14 (3%)	88 (2%)
Year of diagnosis				
1970-79	8 (16%)	18 (10%)	271 (55%)	1995 (46%)
1980-89	41 (84%)	159 (90%)	221 (45%)	2356 (54%)
Age at questionnaire				
20-29	3 (6%)	4 (2%)	3 (1%)	35 (1%)
30-39	16 (33%)	69 (39%)	112 (23%)	1574 (36%)
40-49	24 (49%)	81 (46%)	217 (44%)	1883 (43%)
50-59	6 (12%)	23 (13%)	148 (30%)	807 (19%)
60-69			12 (2%)	52 (1%)
Cisplatin by CRT				
Cisplain only	30 (63%)	135 (79%)		
CRT only			213 (100%)	1394 (100%)
Both	18 (38%)	36 (21%)		
Cisplatin dose (mg/m^2)				
None			492 (100%)	4351 (100%)
<300	14 (29%)	51 (29%)		
300-349	5 (10%)	19 (11%)		
350-399	2 (4%)	11 (6%)		
400+	28 (57%)	96 (54%)		
Number of Chemotherapy Cycles				
0			492 (100%)	4351 (100%)
1	46 (94%)	171 (97%)		
2	3 (6%)	6 (3%)		
CRT dose (Gy)				
None	30 (63%)	135 (79%)	267 (56%)	2845 (67%)
<20	0 (0%)	9 (5%)	53 (11%)	558 (13%)
20-29	1 (2%)	2 (1%)	63 (13%)	485 (11%)

30-49	3 (6%)	7 (4%)	19 (4%)	120 (3%)
50+	14 (29%)	18 (11%)	78 (16%)	231 (5%)
Years Between Cancer Diagnosis				
0-<5	25 (68%)	0 (.%)	46 (20%)	0 (.%)
5-<10	1 (3%)	0 (.%)	24 (11%)	0 (.%)
10-<20	8 (22%)	0 (.%)	59 (26%)	0 (.%)
20-<30	3 (8%)	0 (.%)	69 (30%)	0 (.%)
30-<40			30 (13%)	0 (.%)
Fu5: D4 Tinnitus				
Yes, and condition is still present	49 (100%)	0 (0%)	492 (100%)	0 (0%)
No	0 (0%)	161 (91%)	0 (0%)	4169 (96%)
Yes, but condition is no longer	0 (0%)	5 (3%)	0 (0%)	109 (3%)
Not sure	0 (0%)	11 (6%)	0 (0%)	73 (2%)
Fu5: D1 Hearing loss requiring a				
Yes, and condition is still present	21 (44%)	20 (11%)	99 (20%)	159 (4%)
No	25 (52%)	151 (86%)	383 (78%)	4162 (96%)
Yes, but condition is no longer	1 (2%)	3 (2%)	2 (0%)	9 (0%)
Not sure	1 (2%)	2 (1%)	6 (1%)	18 (0%)
Fu5: D2 Deafness in both ears				
Yes, and condition is still present	8 (16%)	5 (3%)	20 (4%)	34 (1%)
No	38 (78%)	167 (95%)	455 (93%)	4286 (99%)
Yes, but condition is no longer	1 (2%)	0 (0%)	1 (0%)	0 (0%)
Not sure	2 (4%)	4 (2%)	13 (3%)	22 (1%)
Fu5: D3 Deafness in one ear				
Yes, and condition is still present	4 (8%)	5 (3%)	50 (10%)	60 (1%)
No	41 (84%)	166 (94%)	421 (87%)	4255 (98%)
Yes, but condition is no longer	0 (0%)	1 (1%)	0 (0%)	6 (0%)
Not sure	4 (8%)	4 (2%)	11 (2%)	20 (0%)
Fu5: D6 Hearing loss				
Yes, and condition is still present	22 (46%)	41 (23%)	164 (34%)	225 (5%)
No	26 (54%)	131 (74%)	295 (61%)	4048 (93%)
Yes, but condition is no longer	0 (0%)	2 (1%)	3 (1%)	23 (1%)
Not sure	0 (0%)	3 (2%)	20 (4%)	43 (1%)
Fu5: D7 Any other hearing				
Yes, and condition is still present	3 (6%)	7 (4%)	40 (9%)	62 (1%)
No	39 (81%)	158 (91%)	390 (84%)	4147 (96%)
Yes, but condition is no longer			2 (0%)	14 (0%)
Not sure	6 (13%)	9 (5%)	35 (7%)	87 (2%)
Fu5: F5 Hypertension requiring				
Yes, and condition is still present	14 (29%)	42 (24%)	114 (23%)	734 (17%)
No	34 (69%)	121 (69%)	346 (71%)	3433 (79%)
Yes, but condition is no longer	0 (0%)	10 (6%)	22 (4%)	127 (3%)
Not sure	1 (2%)	2 (1%)	7 (1%)	25 (1%)
Fu5: Drug for depression				
Yes	11 (22%)	34 (19%)	114 (23%)	745 (17%)
No	38 (78%)	143 (81%)	378 (77%)	3606 (83%)

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