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| **External Collaborator Proposal** *for* **eMERGE Network Analysis**Project/Manuscript Concept Sheet |
| **Reference Number** | NT257 |
| **Submission Date** | May 17, 2018 |
| **Tentative Lead Investigator** *(first author with contact information and affiliation)* | M. Eileen Dolan, Omar El Charif, Matthew R. Trendowski, Lois Travis representing The Platinum Study Research Group and Marylyn Ritchie representing eMERGE |
| **Tentative Senior Author** *(last author)* | The Platinum Study Research Group |
| **eMERGE Site Sponsor & Contact** | M. Eileen Dolan, PhD (Affiliate member)  |
| **Project Title** | Replication of GWAS of Cisplatin-induced Ototoxicity |
| **All Other Authors**  | Marylyn Ritchie and anyone contributing from eMERGE. We will rely on eMERGE to let us know the appropriate authors. We will add the eMERGE banner if requested. |
| **Other eMERGE Sites Involved** | We invite participation from all eMERGE sites that are interested. |
| **Background / Significance** | Cisplatin is one of the most commonly used chemotherapeutic agents worldwide. As a potent electrophile, cisplatin forms DNA inter- and intra-strand crosslinks that induce cell cycle arrest and apoptosis, as well as inhibit proliferation (1). Consequently, cisplatin and its congeners carboplatin and oxaliplatin are used in the treatment of many adult-onset (cervical, colorectal, endometrial, head and 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/or 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 are a significant number of survivors living with severe adverse sequelae that affect quality of life. Cisplatin-associated ototoxicity (CAO) is a particularly notable side effect for long-term survivors because it can create functional limitations, ranging from speech development and impairment of academic achievement in children, to quality of life, socialization, and cognitive impairments in adults (5). We have previously assessed the genetic susceptibility to CAO 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 (p = 1.4×10-8). 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 = 1.8x10-6), musculoskeletal (p = 4.9x10-11), nerve-tibial (p = 4.1x10-6), skin (sun-exposed lower leg) (p = 2.4x10-11), and thyroid (p = 1.5x10-6)). We found a significant interaction between cumulative cisplatin dose and the rs62283056 genotype (p = 0.035), indicating that higher cisplatin doses exacerbate hearing loss in patients with the risk allele. 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 CAO, rs62283056 remained the top signal (P = 5.4 x 10-8). There was a consistent direction of effect, but the association was primarily driven by our study (one-tailed PSt.Jude = 0.09). Recently, we evaluated rs62283056 in an additional 317 testicular cancer survivors, and again found a significant association with a consistent direction of effect (p = 0.032). Because the SNP lies in a Mendelian deafness gene, we hypothesized that the genetic architecture of CAO and other forms of hearing loss are partly shared. We tested this by investigating the enrichment of SNPs in other Mendelian deafness genes in our GWAS using permutation resampling and found statistically significant enrichment (p = 0.048). Using BioVU as an independent replication cohort (n = 18,620), we tested the hypothesis that *WFS1* is associated with hearing loss in the general population as well. We used PrediXcan to predict the tissue-specific genetically regulated expression of *WFS1* from patients’ genotypes. We found a significant association between decreased *WFS1* expression and hearing loss (Bonferroni adjusted p < 0.05).Another notable symptom of CAO is tinnitus, a perceived ringing, buzzing, beeping, or hissing 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), 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). A comprehensive study recently conducted using >10,000 twin pairs from the Swedish Twin Registry estimated the heritability of bilateral tinnitus to be 56% (13). Several prior studies failed to identify Mendelian patterns of inheritance, suggesting that tinnitus follows more complex patterns of polygenic inheritance (9). Therefore, GWAS may be more appropriate for the identification of variants explaining the trait’s heritability. 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 (14). Although the study had a relatively small sample size and none of the SNPs reached genome-wide significance (p < 5x10-8), 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 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 (15, 16). A previous study of tinnitus in 528 Norwegian testicular cancer patients treated with cisplatin (17) noted 22% as reporting having “quite a bit/very much” tinnitus, indicating significantly higher rates of severe tinnitus, which only occurs in about 1-2% of the general population (10). We observed similar findings in our 488 cisplatin treated testicular cancer survivors (18), which demonstrated that a high percentage (40%) experienced some degree of tinnitus years after treatment (median = 4.3 years), with a substantial fraction of survivors exhibiting symptoms of severe tinnitus (15.8%). To expand upon these findings, we analyzed a larger cohort of well-characterized testicular cancer survivors with cisplatin-induced tinnitus (n = 762) and found 41% of our cohort developed some degree of tinnitus, with 15.2% experiencing symptoms that occurred “quite a bit” or “very much” (referred to as moderate/severe). Testicular cancer survivors (n= 762) were dichotomized to cases (moderate/severe tinnitus; n=154) and controls (none; n=608) removing from further analysis those reporting a little. Logistic regression was used to evaluate associations with comorbidities and SNP dosages in GWAS following quality control and imputation (covariates: age, noise exposure, cisplatin dose, genetic principal components). Patients with tinnitus were more likely to report poorer health (OR=2.9; P=0.0005; Figure 1A) and greater use of psychotropic medications (OR=2.1; P=0.003; Figure 1B) than did those without tinnitus, indicating that these symptoms affected quality of life. The frequency of tinnitus increased significantly with increasing age at treatment (P=0.007) and cumulative cisplatin dose (P=0.007). Testicular cancer survivors with tinnitus had significantly worse hearing at every frequency (0.25 kHz-12 kHz, P<0.0001, Figure 2A). Tinnitus was also associated with self-reported hearing loss (OR=6.38[4.9-8.5], P<0.0001, Figure 2B), problems hearing in a crowd (OR=8.28[5.5-12.5], P<0.0001, Figure 2C), and persistent dizziness or vertigo (OR=6.40[3.2-12.9], P<0.0001, Figure 2D), as well as symptoms of sensory neuropathy (OR range 1.66-2.72, P-values<0.0001, Figure 2E). In multivariate analyses, tinnitus was significantly associated with hypertension (OR=1.79, P=0.03), noise exposure either at work (OR=1.96, P=0.0004) or outside of work (OR=2.31, P<0.0001), and long-term tobacco use (OR=2.09, P=0.008). We found that additive SNP effects explained a large fraction of CisIT variance (h2=0.81±0.42, P=0.006). In GWAS, no SNP met genome-wide significance. The first independent signal identified (rs7532231, OR = 0.45, P = 1.1x10-6) is 116 kilobases upstream of the lncRNA *RP11-364B6.1*. The second (rs6671895, OR = 4.32, P = 1.2x10-6) is intronic to the lncRNA *RP5-884C9.2*. The third (rs7606353, P=1.9x10-6, Figure 3A) is a SNP 14 kilobases downstream of *OTOS* (otospiralin). In GTEx, *OTOS* was expressed in pituitary and thyroid tissues. We found that *OTOS* eQTLs were significantly enriched in GWAS independently from rs7606353 (P=0.018). One haplotype block containing six thyroid/pituitary eQTLs was driving this enrichment. The minor allele (A) of the most significant variant within this block (rs10190781, 1.5% frequency) was associated with higher cisplatin induced tinnitus risk (OR=3.42[1.5-9.5], P=0.007, Figure 3B) and lower *OTOS* expression (Figure 3C). Tinnitus risk increased with the number of risk alleles in either locus (OR=3.77[2.3-6.2], P=9.5 x 10-8, Figure 3D). We tested the association between *OTOS* expression and cisplatin resistance in central nervous system tumor lines and found a significant positive correlation (Spearman Rho=0.46, P=0.03, Figure 3E), further supporting a protective *OTOS* function against cisplatin-induced damage. Interestingly, *OTOS* has been previously implicated in CAO, as a previous study investigated the association of exonic variants in the gene with a baseline vs. follow-up audiometric phenotype, and identified a significant additive protective effect of two variants (19). Another study used adenoviral transfection to up-regulate *OTOS* in spiral ligament fibrocytes (a cell type in the cochlea known to normally express the gene) which markedly decreased apoptosis following cisplatin treatment when compared to non-transfected cells, thereby indicating *OTOS* may have a protective role in the cochlea (20).  |
| **Outline of Project** | We have identified some shared genetic underpinnings for congenital, general, and cisplatin-induced hearing loss (6). **We hypothesize that cisplatin-induced tinnitus and tinnitus in the general population may similarly share genetic etiologies.** The Electronic Medical Records and Genomics (eMERGE) Network would enable us to test this hypothesis and elucidate the genetic underpinnings and mechanisms of tinnitus. Specifically, we will utilize ICD-9-CM diagnosis codes for tinnitus (unspecified, subjective; Table 1, created 10/2/17) to define our case-control phenotype (n­cases = 4,882, ncontrols > 80,000). We will exclude cancer patients that potentially have a prior history of cisplatin exposure (individuals identified by ICD-9-CM Diagnosis Codes of cancers known to be treated with cisplatin, n = 690; Table 2, created 10/2/17) because the proposed GWAS is intended to compare SNPs/genes that are highly associated with general tinnitus to genetic variants we previously characterized in our GWAS of cisplatin-induced tinnitus. We will exclude those ICD-9 codes that denote diseases with diseases and disorders that may cause tinnitus (Table 3, created 10/2/17). We will investigate associations with demographic characteristics (age, sex, self-reported race) and potentially related traits such as hearing loss, vertigo, and dizziness. We will perform a GWAS with the appropriate covariates. We will investigate the extent to which GWAS findings of cisplatin-induced and general tinnitus are shared. We will compare effect sizes of top variants in the two GWAS, perform permutation resampling-based enrichment analyses, and train predictive models in one set to be tested in the other. PrediXcan is a gene-based method that uses reference transcriptome (genotype-gene expression) data to generate models used to ‘impute’ gene expression data from genotype data and associate the predicted gene expression with phenotypes of interest (21). 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. We will then incorporate PrediXcan analyses to evaluate overlap of genes associated with tinnitus with those we have identified for cisplatin induced tinnitus (*OTOS*) and the gene associated with cisplatin-associated hearing loss (*WFS1*). Finally, to identify potential molecular mechanisms of validated genes that either prevent or potentiate cisplatin associated ototoxicity, we will alter the expression levels of the gene through knockdown (siRNA), knockout (CRISPR-Cas9) and overexpression (transfection) in normal cell model systems of ototoxicity (HEI-OC1; House Ear Institute-Organ of Corti 1) mouse cells, human induced pluripotent derived neurons, as well as several relevant neoplastic cell lines to evaluate whether the manipulation of gene expression can mitigate toxicity to normal cells without inhibiting antineoplastic activity.  |
| **Desired Variables** *(essential for analysis**indicated by* ***\*****)* | * Patient demographics
	+ Year of birth
	+ Sex
	+ Race
	+ Ethnicity
* Phenotypes (Table 1)
	+ Tinnitus code status, age at diagnosis
	+ Hearing loss (either requiring or not requiring hearing aids) status, age at diagnosis
* Surrogate for Cisplatin Administration:
	+ ICD-9-CM code status for malignancies likely treated with cisplatin (Table 2)
	+ age at diagnosis
* Exclusion Criteria (ICD9 code status for the following disorders; Table 3):
	+ Ménière's disease, age at diagnosis
	+ Head or neck injuries age at diagnosis
	+ Temporomandibular joint dysfunction, age at diagnosis
	+ Vestibular Schwannoma, age at diagnosis
* Genotypes from eMERGE-3 imputed data
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| **Desired Data** | EMERGE 3 (merged I-III GWAS dataset) |
| **Planned Statistical Analyses** | All GWAS will be performed in PLINK (22) using a logistic regression model. model and assuming linear additive effects. The genome-wide significance threshold will be set at α = 5 x 10-8. Multiple hypothesis testing in other contexts will use Bonferroni correction to define significance thresholds. Appropriate covariates will be incorporated into the regression model, including non-heritable traits that are correlated with the phenotype such as age, and ancestry principal components. We will use genotype data to impute gene expression levels in individuals using PrediXcan (21). All z-statistic p-values less than a Bonferroni-corrected threshold based on number of genes evaluated and number of tissues investigated will be considered statistically significant. The burden of multiple testing is decreased due to fewer total tests of association in this gene-based analysis compared to variant-based GWAS. Genes with statistically significant predicted tissue-specific dysregulation can be evaluated in downstream functional analyses.Permutation resampling-based enrichment analyses will be performed by creating an empirical null distribution of SNP overlaps between the two GWAS. Individual phenotypes and covariates will be randomly shuffled to genotypes and a null GWAS will be performed. This will be repeated for 1,000-10,000 random permutations to generate an expected distribution of SNP overlaps in the absence of genetic associations. A frequency distribution will be used to create a null distribution and the observed overlap will be compared to it to generate an empirical p-value of enrichment as described in our previous study exploring the enrichment between a cisplatin associated hearing loss GWAS and Mendelian deafness genes (6). All functional studies will be performed with appropriate technical and biological replicates, and corresponding applicable statistical analyses. Statistical significance will be considered at α = 0.05 unless a more conservative approach is required.  |
| **Ethical Considerations** | All data will be de-identified, therefore no ethical concerns. |
| **Available Funding or Resources** | All dry-lab analyses relevant to this request will be funded by R01 (CA157823, PI: Travis). |
| **Milestones\*\*** | **June 2018** **Receipt of eMERGE data****June 2018 – September 2018:** * Perform a GWAS of patients diagnosed with unspecified, subjective or objective tinnitus in eMERGE.
* Assess whether there are novel associations between cisplatin-induced tinnitus in the Testicular Cancer Survivor cohort (“The Platinum Study”) and general tinnitus in eMERGE.
* Perform a genome-wide meta-analysis of cisplatin-induced tinnitus in the Testicular Cancer Survivor cohort and general tinnitus patients in eMERGE.
* Evaluate associations between genetically determined gene expression and tinnitus using PrediXcan to determine the directionality of effect and potential molecular mechanisms.

**October 2018 – December 2018:**  **Publication of joint manuscript with eMERGE collaborators describing the above findings.****Potential future experiments based on results.** * Investigate potential molecular mechanisms of the genes of interest that either protect or potentiate cisplatin-associated cytotoxicity by altering the expression levels of the gene through knockdown, knockout, and overexpression in normal cell model systems of ototoxicity (HEI-OC1; House Ear Institute-Organ of Corti 1) mouse cells, human induced pluripotent derived neurons, as well as several relevant neoplastic cell lines to evaluate whether the manipulation of gene expression can mitigate cisplatin-induced ototoxicity without inhibiting antineoplastic activity.
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***\*\**** *This section should include the timeline for completion of project, including: approval, project duration, first and second draft of the paper and submission.*

**Figure 1. Self-reported Health and Use of Psychotropic Medications by Tinnitus Status.** **A.** Bar plot of the distribution of self-reported health according to tinnitus status. Patients were asked: “How would you rate your overall health?” and responded on a poor-excellent ordinal scale, which was converted to a numeric scale (0 = poor/fair, 1 = good, 2 = very good, 3 = excellent). Logistic regression revealed significant negative correlation (OR=0.54[0.4-0.7], P<0.0001). **B.** Bar plot of psychotropic medication use by tinnitus status shows significantly higher prevalence of psychotropic medication use in tinnitus cases (OR= 2.4[1.3-4.4], P=0.003). Patients were dichotomized to “Yes” and “No” psychotropic medication use based on receiving medications from a list of frequently prescribed antidepressants, anxiolytics, and antipsychotics (Supplemental Methods, [51]). The number of subjects per category is presented on the x-axis. Error bars represent the binomial 95% CIs for each subgroup.

****Figure 2. Associations of Tinnitus with Subjective and Objective Hearing Loss, Problems Hearing, Vertigo, and Neurological Symptoms. A.** Box plot of audiometric hearing thresholds (y-axis, dB) across all tested audiometric frequencies (x-axis, kHz) showing significantly worse hearing in tinnitus cases at each frequency (P < 0.0001 at each frequency). Box centers indicate medians, hinges indicate interquartile regions (IQRs), and whiskers indicate 1.5x IQRs. Points outside the range of 1.5x IQR are shown**.** Bar plots of self-reported **B.** difficulty hearing (OR = 6.36[4.8-8.5], P < 0.0001), **C.** problems hearing in crowd (OR = 8.2[5.5-12.5[, P<0.0001), and **D.** persistent dizziness or vertigo (OR = 6.40[3.2-12.9], P<0.0001). Error bars represent the binomial 95%CIs for subgroup. **E.** Forest plot showing the odds ratio (OR, center points) and 95% confidence intervals (error bars) of the association between tinnitus and neurotoxic symptoms from the EORTC-CIPN20. “H” denotes hands/fingers. “F” denotes feet/toes. Responses to EORTC-CIPN20 items were converted from a none-very much Likert scale to a numerical ordinal scale (0-3). Associations in B-E were evaluated using logistic regression adjusted for age at diagnosis.

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**Figure 3) GWAS of Cisplatin-Induced Tinnitus Reveals Genetic Loci Near *OTOS* gene.** **A.** LocusZoom plot of the third most significant GWAS signal near two adjacent genes: *OTOS* and *MYEOV2*. Each point represents a SNP. The x-axis indicates chromosomal position. The left y-axis shows –log10(p-value) of association with CisIT and the right y-axis and blue line indicate the recombination rate in centimorgans/megabase (cM/MB). The color of each variant indicates the linkage disequilibrium R2 with the top signal in the region, rs7606353 (purple). **B.** Bar plot of CisIT frequency by genotype of *OTOS* eQTL, rs10190781. The minor allele (G) increases CisIT risk (OR = 3.42, P = 0.007). Error bars represent the binomial 95% CIs. **C.** Boxplot of *OTOS* expression in thyroid by rs10190781 genotype indicates that the minor allele is associated with lower expression of *OTOS*. Data were obtained from the GTEx Portal on 04/28/18 **D.** Bar plot of number of risk alleles for both rs7606353 and rs10190781 shows additive allele effects (OR = 3.77[2.3-6.2], P=9.5 x 10-8). **E.** Scatter plot of cisplatin resistance as a function of normalized *OTOS* expression. Cisplatin resistance, measured as the area under the cisplatin dose-response curve, for all central nervous system tumor lines (19 glioma and 4 neuroblastoma lines) was extracted from CancerRX and normalized *OTOS* expressions were downloaded from the Cancer Cell Line Encyclopedia. Correlation was assessed non-parametrically using Spearman’s Rank method (Rho = 0.46, P = 0.03).

**Table 1: ICD-9-CM diagnosis codes for phenotypes of interest.**

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| **Diagnosis** | **ICD-9-CM Code** |
| **Tinnitus** | **388.3** |
| Unspecified tinnitus | 388.30 |
| Subjective tinnitus | 388.31 |
| **Hearing loss** | **389** |
| **Conductive hearing loss** | **389.0** |
| Conductive hearing loss, unspecified Non-specific | 389.00 |
| Conductive hearing loss, external ear | 389.01 |
| Conductive hearing loss, tympanic membrane | 389.02 |
| Conductive hearing loss, middle ear | 389.03 |
| Conductive hearing loss, inner ear | 389.04 |
| Conductive hearing loss, unilateral | 389.05 |
| Conductive hearing loss, bilateral | 389.06 |
| Conductive hearing loss of combined types | 389.08 |
| **Sensorineural hearing loss** | **389.1** |
| Sensorineural hearing loss, unspecified Non-specific | 389.10 |
| Sensory hearing loss, bilateral | 389.11 |
| Neural hearing loss, bilateral | 389.12 |
| Neural hearing loss, unilateral | 389.13 |
| Central hearing loss | 389.14 |
| Sensorineural hearing loss, unilateral | 389.15 |
| Sensorineural hearing loss, asymmetrical | 389.16 |
| Sensory hearing loss, unilateral | 389.17 |
| Sensorineural hearing loss, bilateral | 389.18 |
| **Mixed conductive and sensorineural hearing loss** | **389.2** |
| Mixed hearing loss, unspecified Non-specific | 389.20 |
| Mixed hearing loss, unilateral | 389.21 |
| Mixed hearing loss, bilateral | 389.22 |
| **Deaf, nonspeaking, not elsewhere classifiable** | **389.7** |
| **Other specified forms of hearing loss** | **389.8** |
| **Unspecified hearing loss** | **389.9** |
| **Dizziness and giddiness** | **780.4** |
| **Vertigo** | **438.85** |
| Epidemic vertigo | 078.81 |
| Peripheral vertigo NOS | 386.10 |
| Peripheral vertigo NEC | 386.19 |
| Benign parxysmal vertigo | 386.11 |
| Central origin vertigo | 386.2 |
| Other and unspecified peripheral vertigo | 386.1 |

**Table 2: ICD-9-CM diagnosis codes for cancers that are treated with cisplatin.**

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| **Primary Diagnosis** | **ICD-9-CM Code** |
| Cervical Cancer | 180 |
| Endometrial Cancer | 179, 182 |
| Bladder Cancer | 188 |
| Stomach Cancer | 151 |
| Head and Neck Cancer | 195.0 |
| Lung Cancer | 162 |
| Esophageal Cancer | 150 |
| Pancreatic Cancer | 157 |
| Osteosarcoma | 170 |
| Ovarian Cancer | 183 |
| Testicular Cancer | 186 |
| Breast Cancer | 174 |
| Hodgkins Lymphoma | 201 |
| **Pediatric Malignancies** |  |
| Germ Cell Tumors | 183, 186 |
| Hepatoblastoma | 155 |
| Medulloblastoma | 191.7 |
| Neuroblastoma | 194 |
| Osteosarcoma | 170 |

**Table 3: ICD-9-CM diagnosis codes for exclusion criteria.**

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| **Diagnosis** | **ICD-9-CM Code** |
| **Meniere's disease** | **386.0** |
| Ménière's disease NOS | 386.00 |
| Actv Ménière, cochlvestib | 386.01  |
| Active Ménière, cochlear | 386.02  |
| Actv Ménière, vestibular | 386.03  |
| Inactive Ménière's dis  | 386.04 |
| **Head injury, unspecified** | 959.01 |
| **Injury of face and neck** | 959.09 |
| **Temporomandibular joint disorders** | 524.6  |
| Temporomandibular joint disorders, unspecified | 524.60  |
| Temporomandibular joint disorders, adhesions and ankylosis (bony or fibrous) | 524.61  |
| Temporomandibular joint disorders, arthralgia oftemporomandibular joint | 524.62  |
| Temporomandibular joint disorders, articular disc disorder (reducing or non-reducing) | 524.63  |
| Temporomandibular joint sounds on opening and/or closing the jaw | 524.64  |
| Other specified temporomandibular joint disorders | 524.69  |
| **Benign neo cranial nerve** | **225.1** |
| **Malignant neoplasm of cranial nerves** | **192.0** |

Table 4. Patients in eMERGE with Tinnitus



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