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Variants in *WFS1* and other Mendelian deafness genes are associated with cisplatin-associated ototoxicity

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Abstract

Purpose—Cisplatin is one of the most commonly used chemotherapy drugs worldwide and one of the most ototoxic. We sought to identify genetic variants that modulate cisplatin-associated ototoxicity (CAO).

Experimental Design—We performed a genome-wide association study (GWAS) of CAO using quantitative audiometry (4–12 kHz) in 511 testicular cancer survivors of European genetic ancestry. We performed polygenic modeling and functional analyses using a variety of publicly available databases. We used an electronic health record cohort to replicate our top mechanistic finding.

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Results—One SNP, rs62283056, in the first intron of Mendelian deafness gene *WFS1* (wolframin ER transmembrane glycoprotein) and an expression quantitative trait locus (eQTL) for *WFS1* met genome-wide significance for association with CAO ($P=1.4\times10^{-8}$). A significant interaction between cumulative cisplatin dose and rs62283056 genotype was evident, indicating that higher cisplatin doses exacerbate hearing loss in patients with the minor allele (P=0.035). The association between decreased *WFS1* expression and hearing loss was replicated in an independent BioVU cohort (n=18,620 patients, Bonferroni adjusted P<0.05). Beyond this top signal, we show CAO is a polygenic trait and that SNPs in and near 84 known Mendelian deafness genes are significantly enriched for low P-values in the GWAS (P=0.048).

Conclusions—We show for the first time the role of *WFS1* in CAO and document a statistically significant interaction between increasing cumulative cisplatin dose and rs62283056 genotype. Our clinical translational results demonstrate that pre-therapy patient genotyping to minimize ototoxicity could be useful when deciding between cisplatin-based chemotherapy regimens of comparable efficacy with different cumulative doses.

Keywords

cisplatin; hearing loss; GWAS; adverse drug events; genetic architecture

Introduction

Platinum-based compounds are the most widely applied group of cytotoxic drugs worldwide. A recent multi-center investigation documented that 80% of patients experience some form of ototoxicity after cisplatin-based chemotherapy, with 18% demonstrating severe to profound hearing loss (1). For patients with advanced testicular cancer in whom cisplatin-based chemotherapy is the only curative option (2), this effect is particularly devastating, given their young median age at diagnosis (31 years) (1). While the period of testicular cancer survivorship can span upwards of 50 years, it is often accompanied by the negative impact of hearing loss on quality of life (3). Since there are currently no FDA-approved treatments for sensorineural hearing impairments, including cisplatin-associated ototoxicity (CAO), affected individuals can suffer from progressive declines in communication abilities at work and at home, often contributing to depression and an increased risk of dementia (4).

Despite over 40 years of clinical cisplatin application, the genetic underpinnings of CAO remain poorly understood (5). Previous studies of CAO have been largely conducted in small pediatric cohorts (n = 130–254) and include candidate gene investigations (6–9) with conflicting results (10–12) and some results await independent replication (7,9). In 2011, the FDA amended the cisplatin label to recommend *TPMT* (thiopurine S-methyltransferase) genotyping in children prior to cisplatin administration (10), based on the findings of Ross et al. (6). However, subsequent methodological concerns and lack of replication by independent groups (8,10) led the FDA to remove this recommendation from the drug label (12). A recent GWAS for CAO in pediatric patients identified a variant in *ACYP2* (13), which has since been replicated (14), highlighting the potential utility of genome-wide approaches. Given the critical importance of any possible inroad into the genetic underpinnings of cisplatin-associated ototoxicity, we conducted a GWAS of CAO in a

multi-center clinical cohort of testicular cancer survivors treated with homogeneous cisplatin-based chemotherapy. Extensive audiometric data as collected for this study served as the phenotype (1).

Materials and Methods

Study Design and Patients

All patients were enrolled in the Platinum Study, which includes 8 cancer centers in the U.S. and Canada (1,5). Eligibility criteria included: men with a diagnosis of histologically or serologically confirmed germ cell tumor (GCT), age <55 years at diagnosis and 18+ years at study consent, treatment with cisplatin-based chemotherapy, and no subsequent salvage chemotherapy. Study procedures were approved by the Human Subjects Review Board at each institution. All patients provided written consent for participation, including genetic analyses. For each patient, standardized research protocols and forms were used to collect demographic and clinical data, including audiometry and treatment information as described previously (1,5).

Genotyping and Imputation

DNA was extracted from the peripheral blood of 849 testicular cancer survivors. SNPs were genotyped on the Illumina HumanOmniExpressExome chip at the RIKEN Center for Integrative Medical Science (Yokohama, Japan). Samples were plated randomly with inter- and intra-plate duplicates. Standard quality control measures for GWAS genotypes were implemented using PLINK (15). Individuals with pairwise identity by descent (IBD) > 0.125 and excess heterozygosity (F inbreeding coefficient 6 standard deviations from the mean) were removed, leaving 827 individuals. Principal component analysis using HapMap populations and SMARTPCA (16) revealed 713 genetic Europeans (Supplementary Fig. S1), who underwent genotype imputation. A total of 930,450 SNPs (call rate > 0.99, in Hardy-Weinberg equilibrium ($P > 1\times10^{-6}$)) comprised the input set of SNPs for imputation, which was performed on the University of Michigan Imputation Server (17) with the following parameters: 1000G Phase 1 v3 ShapeIt2 (no singletons) reference panel, SHAPEIT phasing and the EUR (European) population. SNPs with minor allele frequency (MAF) > 0.05, in Hardy-Weinberg equilibrium ($P > 1\times10^{-6}$), imputation $R^2 > 0.8$, and INFO scores from 0.6 – 1.05 were retained for subsequent analysis. The GWAS included 5,060,354 SNPs and 511 individuals with full phenotypic data (i.e., audiometry and cumulative cisplatin dose) available. See Supplementary Fig. S2 for a flow chart of this genotype quality control process.

GWAS and Statistical Interaction Analyses

CAO was modeled as a quantitative phenotype, using the geometric mean of air conduction thresholds measured at each frequency (4, 6, 8, 10 and 12 kHz) that demonstrated a statistically significant relationship between cumulative cisplatin dose and hearing loss, after age adjustment (1). The geometric mean was rank normalized to form a normal distribution prior to association testing. Age at audiometry ($\beta = -0.06 \pm 0.004$, $P = 3.0 \times 10^{-49}$) and cumulative cisplatin dose ($\beta = -0.003 \pm 0.0006$, $P = 1.4 \times 10^{-5}$) correlated strongly with the CAO phenotype and were included as covariates in the GWAS. We have previously shown

that age at audiometry is strongly correlated with age at diagnosis (R = 0.79) and that the time between chemotherapy and audiometry did not significantly associate with CAO (P = 0.42) after adjustment for age at audiometry (1). Thus, age at diagnosis and time since chemotherapy were not included as covariates in our GWAS. SNP genotype dosages were tested for association with CAO using a linear additive model adjusted for age at audiometry, cumulative cisplatin dose, and 10 principal components of the genetic European genotype data only. Genome-wide statistical significance was assigned to SNPs with $P < 5 \times 10^{-8}$. All statistical tests were two-sided unless otherwise noted.

For the top SNP, we also tested for interaction between SNP genotype and cisplatin dose using the following model:

$$E\,(CAO) = age\,at\,audiometry + SNP * dose$$

The *dose* term is a binary variable of those with ≤300 mg/m^2 (n = 217) or >300 mg/m^2 (n = 294). Given the uniformity of treatment for testicular cancer, most patients receive a cumulative dose of either 300 or 400 mg/m^2 and this dichotomization of dose was used in prior studies of CAO (1).

The GWAS was performed using PLINK 1.90 (15) and subsequent statistical analyses were performed in R version 3.2.0 and plots were made using the R package *ggplot2* (18) and LocusZoom (19) Functional information on the top SNP was obtained from the GTEx Portal version 4 (20) and HaploReg version 4.1 (21).

Polygenic Analyses

We estimated the narrow-sense heritability for each gene using a variance-component model with a genetic relationship matrix (GRM) estimated from genotype data, as implemented using restricted maximum likelihood in GCTA (22). A Hardy-Weinberg equilibrium threshold for SNP inclusion of P > 0.05 was used and one of a pair of individuals with an estimated relatedness above 0.025 were selectively excluded to maximize sample size, leaving 4,897,434 SNPs and 464 individuals for variance component analysis. We calculated the proportion of the variance in CAO explained by all SNPs using an additive linear mixed model.

We conducted a permutation resampling analysis to test for an enrichment of the 34,095 SNPs within 50kb of 84 Mendelian nonsyndromic deafness genes (23,24) among the CAO-associated SNPs (GWAS). To this end, the patient phenotypes (CAO, cumulative dose and age vectors) were randomly shuffled while keeping the genotype data fixed to preserve linkage disequilibrium. The GWAS was then re-run for each replicate. This process was conducted 500 times to generate an expected distribution. For each of the 500 permutation replicates, we tallied the number of SNPs that had P < 0.01 in the GWAS and are in deafness genes. The distribution of the number of significant SNPs mapping to Mendelian deafness genes was compared with the observed SNP overlap to generate an empirical P value, calculated as the proportion of permutations in which the number of GWAS SNPs in deafness genes is greater than or equal to the observed number. To test the robustness of our

findings, we calculated an empirical P value across a range of inclusion thresholds for significance from $P < 0.0001$ to $P < 0.01$.

Gene Expression and Drug Toxicity Analyses in Cell Lines

WFS1 gene expression data was obtained from the Broad-Novartis Cancer Cell Line Encyclopedia (25). The dose of cisplatin, cytarabine, docetaxel and vinblastine required to inhibit 50% cell growth (IC_{50}) was obtained from the Genomics of Drug Sensitivity in Cancer Project (26). Because our GWAS results provided an expected direction of effect, Spearman correlation between *WFS1* expression and drug IC_{50} in central nervous system cancer lines (27 glioma, 3 medulloblastoma) was tested for significance using a one-sided test in GraphPad Prism Software (La Jolla, CA).

Meta-analysis with the St. Jude Cohort

We obtained summary statistics from a GWAS of cisplatin-associated hearing loss in a pediatric cohort of 238 individuals from St. Jude Children's Hospital (13). In this study, ototoxicity was modeled as a time-to-event variable, thus, we performed a fixed-effects meta-analysis of the P-values from our Platinum Study cohort and the St. Jude cohort weighted by sample size and allelic direction of effect as implemented in METAL (27). A total of 1,112,545 SNPs in 749 individuals were included in the meta-analysis.

PrediXcan in the BioVU Cohort

We estimated the genetic component of *WFS1* expression in 18,620 samples from BioVU(28), including 220 and 363 individuals with a diagnosis of "hearing loss" (PheWAS code 389) and "sensorineural hearing loss" (PheWAS code 389.1), respectively, using the PrediXcan method (29). PheWAS codes are derived from the International Classification of Disease, Ninth revision, Clinical Modification (ICD9) codes used in medical billing (28). Code 389 "hearing loss" is a "general term for the complete or partial loss of the ability to hear from one or both ears" and "causes include exposure to loud noise, ear infections, injuries to the ear, genetic, and congenital disorders" (30). ICD9 codes are hierarchical, and code 389.1 "sensorineural hearing loss" (more specific than code 389) denotes a condition that "often affects a person's ability to hear some frequencies more than others" (30). Applying genetic predictors of gene expression built via elastic net regularization (31) using transcriptome data from artery and 10 brain regions (GTEx Project) (20), we predicted the genetically regulated expression of *WFS1* in the BioVU cohort. In each tissue, to show the *WFS1* expression is amenable to genetic prediction, we estimated the proportion of *WFS1* expression that can be explained by genetic effects (heritability) using the Bayesian Sparse Linear Mixed Model (BSLMM) method (32). We tested the predicted *WFS1* expression for association with each phenotype using logistic regression.

Results

GWAS for Cisplatin-Associated Ototoxicity (CAO)

We conducted a GWAS of CAO with over 5 million common SNPs in 511 patients of European genetic ancestry (Supplementary Table S1, Supplementary Figs. S1–S2). Age at audiometry and cumulative cisplatin dose were correlated with CAO and included as

covariates in the GWAS linear regression model. One SNP, rs62283056, in the first intron of *WFS1*, which encodes wolframin ER transmembrane glycoprotein, met genome-wide significance for association with CAO ($\beta = -0.34 \pm 0.06$, $P = 1.4 \times 10^{-8}$, Fig. 1, Table 1). Mutations in *WFS1* can cause DFNA6 (deafness, autosomal dominant 6) and the recessive Wolfram syndrome, also known as DIDMOAD (Diabetes Insipidus, Diabetes Mellitus, Optic Atrophy, and Deafness) (33,34).

Polygenicity of CAO and Enrichment of Top GWAS SNPs in Mendelian Genes for Deafness

While single variant analysis revealed one genome-wide significant signal in a plausible gene, we also explored the possibility that many variants are involved in CAO by estimating the heritability explained by all common SNPs. Linear mixed modeling and variance component analysis (22) showed that all SNPs explained a large proportion of the variance (0.92 ± 0.62, $P = 0.039$).

As mutations in *WFS1* are known to cause deafness (33,34), and to test our hypothesis that related phenotypes share genetic liability with adverse drug events, we examined additional Mendelian genes that cause deafness for enrichment in the top GWAS signals. We found that SNPs within 50kb of 84 genes known to cause Mendelian nonsyndromic deafness (23,24) are significantly enriched for low P-values, as indicated by the departure from the null in the quantile-quantile plot and a permutation resampling analysis of the GWAS data ($P = 0.048$, Fig. 2). The enrichment was consistent across P-value thresholds (Supplementary Fig. S3). Of 84 autosomal deafness gene regions examined, 33 loci had at least one SNP with $P < 0.01$ (Supplementary Table S2).

Risk Allele in rs62283056 Associates with Decreased WFS1 Expression

WFS1 is expressed in a variety of tissues including inner ear sensory cells such as outer hair cells, spiral ganglion neurons, and cochlear lateral wall fibrocytes (35,36). The minor allele of rs62283056 (frequency 0.21 in the GWAS cohort) associates with both increased hearing loss (Fig. 1C) and decreased expression of *WFS1* in several human tissues (FDR < 0.05, Supplementary Fig. S4) (20). Thus, the intronic SNP is a *cis*-acting eQTL for its host gene *WFS1*. The SNP has been shown to alter a binding motif for the transcription factor groups E2F, HAND1, NANOG and POU5F1, using combined ENCODE data across cell types, indicating potential regulatory function (21).

Decreased WFS1 Expression is Correlated with Increased Sensitivity to Cisplatin

Using publicly available data from 30 central nervous system cell lines (25,26), we analyzed cisplatin IC_{50} (the concentration required for 50% cell growth inhibition) for association with *WFS1* gene expression. We found that lower levels of *WFS1* baseline gene expression correlate with greater sensitivity to cisplatin-associated cytotoxicity ($P = 0.036$), but that no relationship existed between *WFS1* levels and cytotoxicity induced by other, non-ototoxic chemotherapeutics including cytarabine, docetaxel, and vinblastine (Fig. 3). These results are in agreement with our GWAS and eQTL results, suggesting that patients with lower *WFS1* levels are more susceptible to cisplatin-induced damage, likely also resulting in greater injury to inner ear sensory cells. There are no data from testicular cancer (germ cell

tumor) cell lines in the Cancer Cell Line Encyclopedia (25), precluding assessment of any such an effect on testicular tumors.

Interaction between Cisplatin Dose and rs62283056 Genotype

Importantly, we found a significant interaction between rs62283056 genotype and cumulative cisplatin dose, indicating higher doses may further increase hearing loss in patients with the minor allele (P = 0.035, Fig. 4). For patients in the ≤ 300 mg/m^2 group, each minor allele increases hearing loss by 0.20 relative units, however in the >300 mg/m^2 category, each minor allele increases hearing loss by 0.46 relative units. Thus, each minor allele increases hearing loss 2.3 times more in patients who received >300 mg/m^2 compared to those given ≤ 300 mg/m^2 (P = 0.035). We found no significant interaction between rs62283056 genotype and age at audiometry (P = 0.36).

Meta-analysis with Pediatric Adverse Drug Event GWAS

In a meta-analysis of 1,112,545 common SNPs present in both the St. Jude Children's Hospital GWAS (13) and our GWAS, rs62283056 remained the top signal (P = 5.4×10^{-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 ($P_{St.Jude}$ = 0.18). No other SNP reached genome-wide significance (Supplementary Table S3).

Previously Reported Cisplatin-Associated Ototoxicity SNPs

Previously reported CAO SNPs in candidate gene studies of *GSTP1* (37), *TPMT* (6), *COMT* (6), *ABCC3* (9), or *SLC22A2* (7) were not replicated (Supplementary Table S4). The GWAS of cisplatin-associated hearing loss among the 238 patients treated at St. Jude Children's Hospital found that rs1872328 in *ACYP2* met genome-wide significance, a finding which was replicated in 68 children (13) and in an independent cohort of 156 pediatric and adult osteosarcoma patients (14). However, in our cohort of 511 patients, the low frequency *ACYP2* SNP (MAF = 0.02) was not significantly associated with CAO (P = 0.76).

Replication of WFS1 in BioVU via Mechanism-Based PrediXcan

We applied PrediXcan (29) to impute genetically determined *WFS1* gene expression levels in eleven relevant tissues in >18,000 genotyped individuals in the BioVU cohort (28). PrediXcan uses gene expression prediction models (31) built from genome-transcriptome datasets such as the Genotype-Tissue Expression (GTEx) Project (20). As such, this method incorporates *a priori* functional data on potential regulatory elements to provide both directionality and a mechanistic basis for association with a phenotype. The proportion of the variance in *WFS1* expression explained by SNP effects in ten brain regions and arterial tissue ranged from 0.09–0.85 (Supplementary Table S5), demonstrating there is a heritable component of *WFS1* expression amenable to genetic prediction. For validation of the *WFS1* finding, we tested the imputed genetic component of *WFS1* expression for association with two phenotypes, "hearing loss" and "sensorineural hearing loss", defined by PheWAS (ICD9-derived) codes (details in Methods) (28). Based on the number of tissues (n = 11) and the two PheWAS phenotypes tested, decreased *WFS1* expression in hypothalamus

significantly associated with common hearing loss ($P < 0.002$) after Bonferroni adjustment (Table 2).

Discussion

In the first GWAS of CAO in adults, we found that rs62283056 in the plausible gene *WFS1* was significantly associated with CAO ($P = 1.4 \times 10^{-8}$), a relationship exacerbated by increased cumulative cisplatin dose. To our knowledge, our study is the first to document an interaction ($P = 0.035$) between genotype and cisplatin dose in CAO, with important clinical implications. A major strength of our study is the homogeneity of cisplatin-based chemotherapy and collection of detailed dose information for all cytotoxic drugs. In contrast to many other cancer types, the curative cisplatin-based treatment regimens and doses for testicular cancer are standardized across the world, allowing us to assess CAO in a large number of homogeneously treated patients. Most patients with good risk advanced testicular cancer receive either 3 cycles of BEP (bleomycin, etoposide, cisplatin) or 4 cycles of EP (etoposide, cisplatin), corrresponding to 300 mg/m^2 or 400 mg/m^2 of cisplatin, respectively, with excellent equivalent survival (Table S1) (2). We previously showed that for every 100 mg/m^2 increase in cumulative cisplatin dose, a 3.2-dB decline in overall hearing threshold (4 to 12 kHz) occurred after age adjustment (1). Thus, when choosing between these therapeutically equivalent regimens, genotypes predictive of CAO could potentially be assessed pre-treatment, also taking into consideration risk factors for other adverse events such as bleomycin-associated pulmonary toxicity (2). Importantly, cisplatin is widely used in chemotherapy regimens for many other types of cancer (5), thus, robust genotypic predictors of ototoxicity would have widespread application in clinical decision making and post-treatment follow-up care.

Mutations in *WFS1* cause both autosomal dominant low-frequency sensorineural hearing loss and Wolfram syndrome, characterized by autosomal recessive hearing loss, diabetes mellitus, diabetes insipidus and optic atrophy (33,34). In addition, prior GWAS have shown *WFS1* may also play a role in type 2 diabetes susceptibility (38). Testicular cancer survivors are at increased risk of type II diabetes (3), but small numbers of diabetic patients (n = 14) in our cohort limited our ability to evaluate this association. Dominant mutations that cause low-frequency hearing loss are nearly always found in the 3′ end of *WFS1* and are usually non-inactivating (34). On the other hand, *WFS1* mutations causing the recessive Wolfram syndrome are numerous, usually loss-of-function, and distributed along the entire gene (34,39). Hearing impairment in patients with Wolfram syndrome is progressive and largely affects the high frequencies (34,40), as is also true for CAO, with hearing loss presenting in the 4–12 kHz range, which is critical for speech perception (1). The top GWAS SNP, rs62283056, is in the first intron of *WFS1* (5′ end) and the risk allele is associated with lower expression (loss-of-function) of the gene (20).

WFS1 encodes wolframin, which normally controls endoplasmic reticulum (ER) stress response through degradation of ATF6α, a key transcription factor involved in ER stress signaling (41). As shown in pancreatic β-cells from *Wfs1*$^{-/-}$ mice and human lymphocytes from Wolfram syndrome patients (36,41), dysfunction of wolframin in inner ear cells likely results in increased expression of the ER stress response genes and apoptosis. In addition to

causing DNA damage, cisplatin also induces ER stress and nucleus-independent apoptosis (42,43). Because both cisplatin and *WFS1* loss-of-function induce ER stress, increased ER stress is likely one potential mechanism for the significant interaction we found between cumulative cisplatin dose and rs62283056 genotype, with higher cisplatin dose exacerbating hearing loss in patients carrying the minor allele.

The association between decreased expression of *WFS1* and hearing loss was replicated among 18,620 patients in the BioVU cohort (28) using more general hearing loss phenotypes that encompasses multiple causes. We used two ICD9-derived PheWAS codes (28) to define the phenotypes in our replication analysis. Decreased *WFS1* expression significantly associated with case status defined using either code (Table 2). This suggests that CAO shares underlying genetic mechanisms with more general hearing loss phenotypes.

In addition to *WFS1*, we found that SNPs mapping to other genes implicated in Mendelian forms of hearing impairment (23,24) were also associated with CAO more often than expected by chance. We note that the SNPs mapping to the extended loci defined by the deafness genes are not protein-altering mutations that would cause deafness. Rather, a subset of these SNPs are likely to affect expression of the local gene, thereby leading to an increased risk of CAO and general hearing loss. Future GWAS of CAO with larger sample sizes may reveal additional variants in deafness gene loci that meet genome-wide significance. Consistent with the enrichment results in these loci, the heritability estimate for CAO (0.92 ± 0.62, $P = 0.039$) using a variance-component approach (22) provides preliminary support to the hypothesis that many common variants with small effect sizes underlie CAO; thus, it is a polygenic trait.

Lack of replication of candidate genes reported in previous smaller studies of CAO (n = 130–254 patients) also supports the hypothesis that large-effect variants for ototoxicity are unlikely. However, differences in CAO phenotypes and patient cohorts (Supplementary Table S4) could also explain inability to replicate previous findings. For example, unlike in our investigation, in the St. Jude GWAS (13), ototoxicity was prospectively modeled as a time-to-event variable in a clinical trial, which may better capture acute effects. A previous GWAS of age-related hearing impairment in a Belgian population also supported a polygenic architecture; although no individual variant attained genome-wide significance, 22% of the variance in hearing loss was explained by the collective effect of all genotyped SNPs, although the standard error was not reported (44).

Taken together, our results show for the first time a shared genetic etiology among drug-induced, Mendelian, and other types of hearing impairment. Similar examples of shared genetics have been demonstrated in GWAS of other adverse drug events. SNPs in *FGD4*, a Mendelian gene for congenital peripheral neuropathy (Charcot–Marie–Tooth disease), associated with paclitaxel-induced peripheral neuropathy (45). SNPs in *VAC14* associated with docetaxel-induced peripheral neuropathy (46), and the gene was recently shown to be mutated in pediatric-onset neurological disease (47). Beyond chemotherapy studies, genetic variants that associate with the euphoric effects of *d*-amphetamine significantly overlap with variants that decrease risk for schizophrenia and attention deficit hyperactivity disorder (48). The broad implication is that adverse drug events and related, non-drug-induced phenotypes

may have related genetic etiologies, especially in the absence of large effects from genetic variation in enzymes involved in drug absorption, metabolism, distribution or excretion.

Our approach has broad study-design implications for clinical translational research directed toward chemotherapeutic drug toxicities for which the genetic underpinnings remain unclear after standard analytic approaches and for which replication under a similar protocol in a large cohort is unlikely (49). We have shown for the first time that an adverse drug event (ototoxicity), Mendelian disorders, and multi-cause, common phenotypes share related underlying genetic etiology. Therefore, following a GWAS of an adverse drug event, a related general, multi-cause phenotype with a larger sample size, but less detailed clinical data, could be used for replication. Polygenic analyses of both the adverse drug event and the general phenotype could be performed to quantify the shared genetic architecture and to provide the basis for not only clinical risk prediction, but also preventive and interventional strategies. Thus, this type of multi-modal approach has considerable potential to identify *a priori* those patients at high risk for drug-induced toxicities, with the ultimate goal of informing clinical decision-making.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Translational Relevance

Elucidation of genetic predictors of cisplatin-associated ototoxicity (CAO, i.e., permanent, bilateral hearing loss) could inform treatment decisions to avoid unnecessary toxicity. We performed a genome-wide association study of CAO among testicular cancer survivors treated with homogenous cisplatin-based chemotherapy. One SNP in *WFS1* met genome-wide significance, with the risk allele also associated with decreased *WFS1* expression. *WFS1* mutations can cause the Mendelian disorders DFNA6 (deafness, autosomal dominant 6) and Wolfram Syndrome (with hearing loss). The significant interaction we found between cumulative cisplatin dose and the *WFS1* SNP indicates that ototoxicity in patients with the risk allele could be reduced with lower doses of cisplatin. Replication of the association between decreased *WFS1* expression and hearing loss in the BioVU cohort demonstrates that genetic findings related to an adverse drug event can be replicated among patients with a related phenotype using electronic health record data.

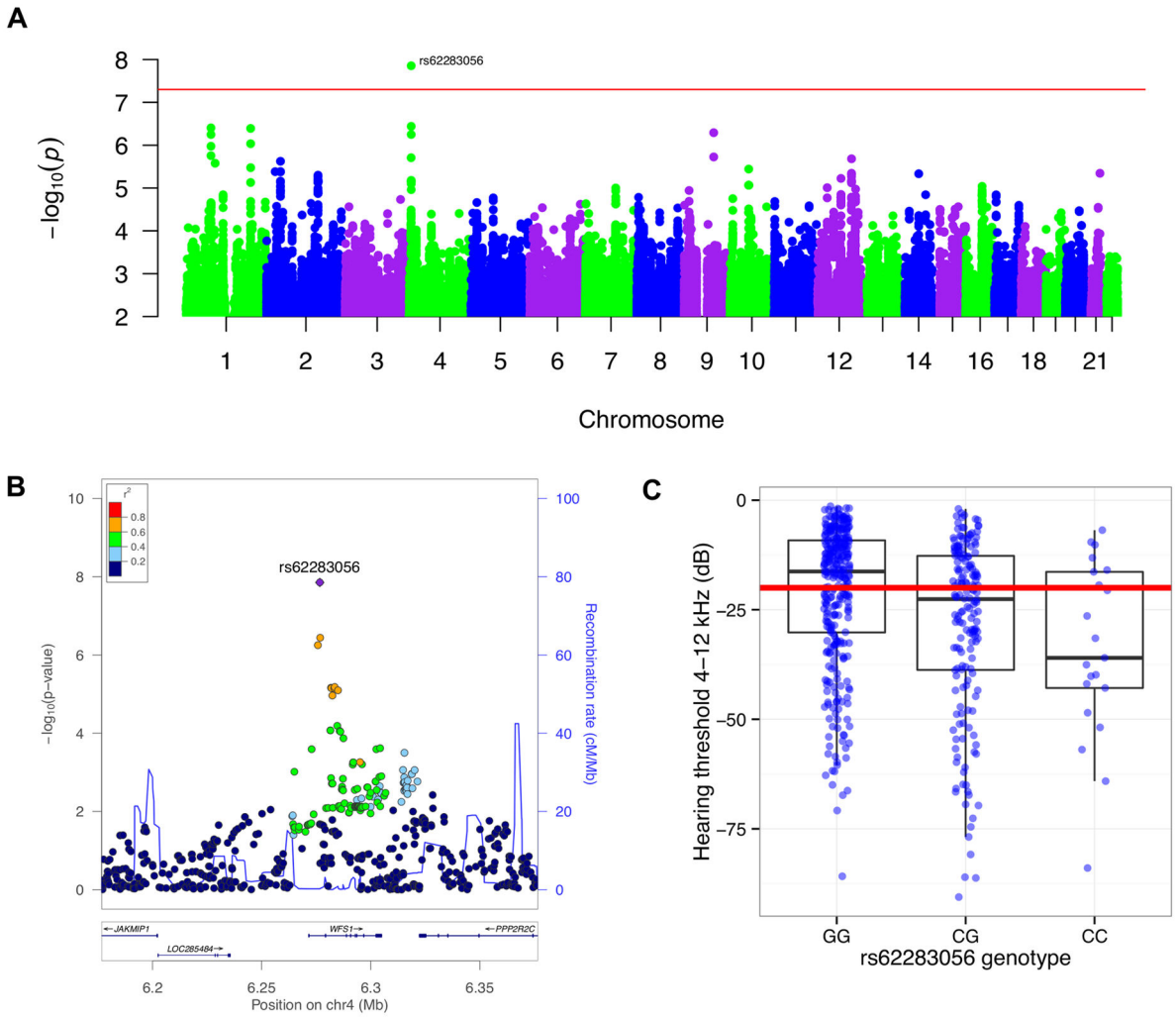

Figure 1. GWAS of cisplatin-associated ototoxicity (CAO)

(**A**) The association of SNP genotype and CAO in 511 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 the genome-wide significance threshold ($P = 5 \times 10^{-8}$). (**B**) The top GWAS signal rs62283056 is in the first intron of *WFS1* (wolframin ER transmembrane glycoprotein). The color of each dot represents the SNP's linkage disequilibrium r^2 with rs62283056 in the 1000 Genomes European populations. (**C**) For individuals with the CG and CC genotypes at rs62283056, the absolute value of median hearing threshold increases by 6 dB and 20 dB over those with the GG genotype, respectively. Hearing loss begins at thresholds below the red line (< −20 dB). Boxes define the inter-quartile range (*IQR*) and the middle horizontal line represents the median. The upper whisker extends from the third quartile to the highest value that is within 1.5 × *IQR*. The lower whisker extends from the first quartile to the lowest value within 1.5 × *IQR*.

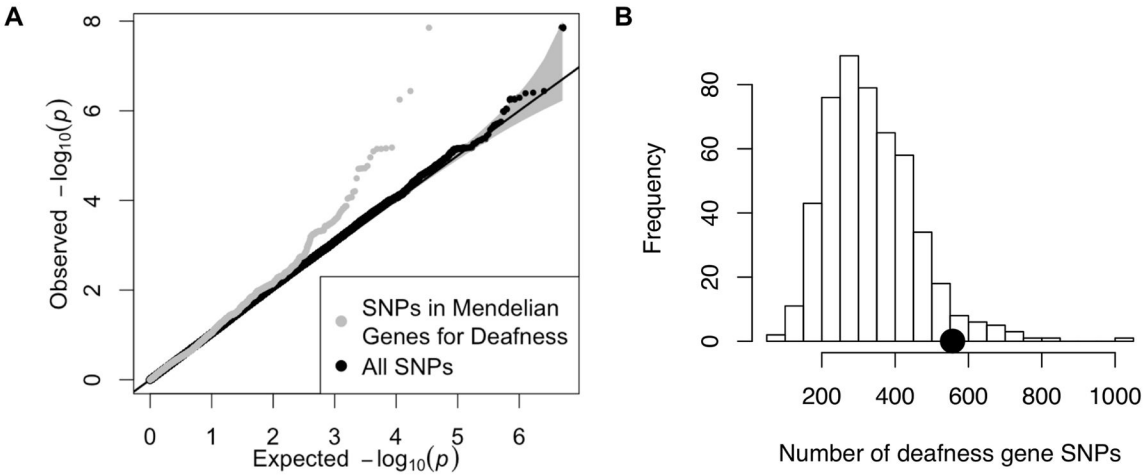

Figure 2. Enrichment of top GWAS SNPs in Mendelian deafness genes

(**A**) Quantile-quantile plot showing the distribution of P-values from all SNPs in the GWAS compared to the SNPs within 50kb of 84 Mendelian deafness genes (23,24). (**B**) Distribution of the number of top SNPs (P < 0.01) in Mendelian deafness genes based on 500 permutations of the GWAS phenotype-genotype connections. The black dot is the number of observed GWAS SNPs with P < 0.01 that are within 50kb of Mendelian deafness genes, a significant enrichment (empirical P = 0.048).

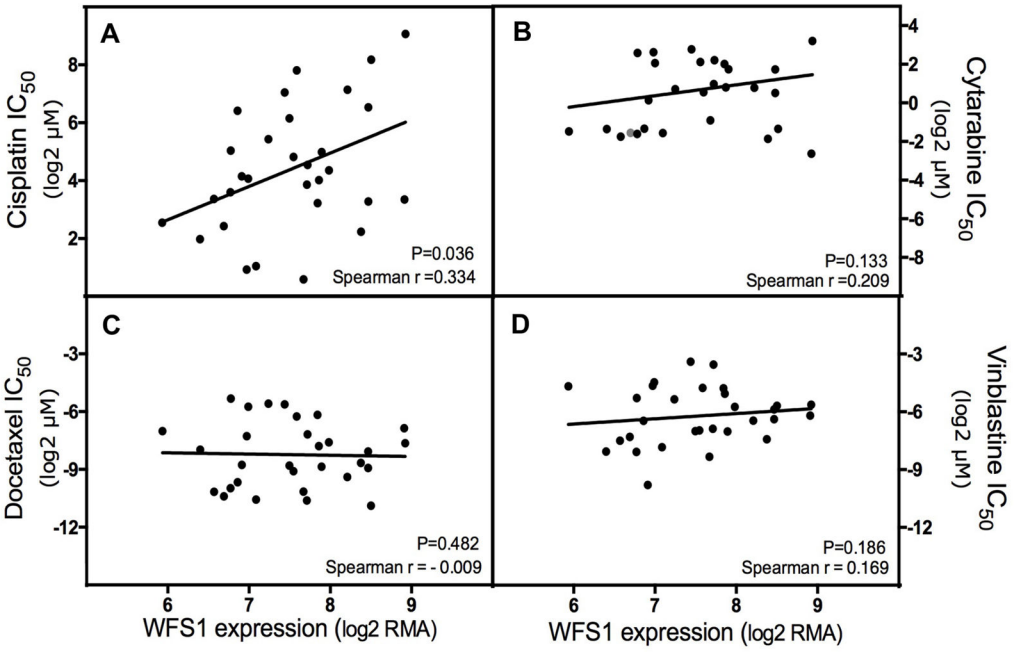

Figure 3. Lower expression of *WFS1* associates with increased cellular sensitivity to cisplatin

WFS1 gene expression data from the Cancer Cell Line Encyclopedia are plotted against cellular sensitivity ($\log_2$ IC_{50}) of CNS tumor cell lines (27 glioma and 3 medulloblastoma) to (**A**) cisplatin, (**B**) cytarabine, (**C**) docetaxel, or (**D**) vinblastine from the Genomics of Drug Sensitivity in Cancer database. Spearman's rho (r) and P-values are shown. Only cisplatin IC_{50} significantly associated with *WFS1* expression (P = 0.036).

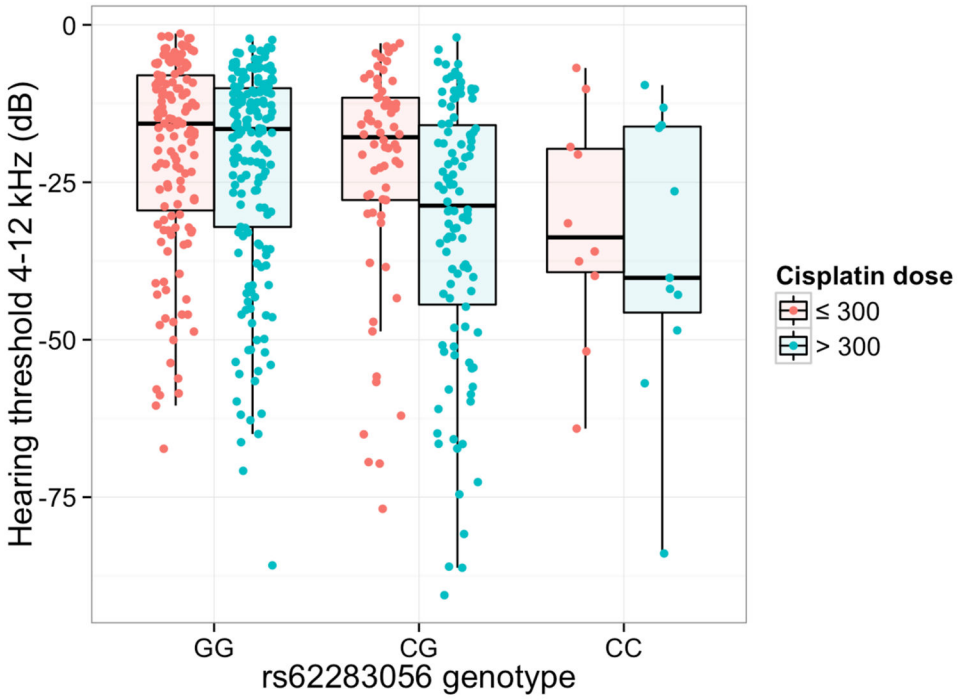

Figure 4. Interaction between rs62283056 genotype and cisplatin dose

Hearing threshold (CAO phenotype) is plotted against rs62283056 genotype dichotomized by cumulative dose group (≤ 300 mg/m^2 or > 300 mg/m^2 cisplatin). We found a significant interaction between rs62283056 genotype and cumulative cisplatin dose (P = 0.035), indicating higher doses exacerbate the hearing loss effect in patients carrying the minor (C) allele. Boxes define the inter-quartile range (*IQR*) and the middle horizontal line represents the median. The upper whisker extends from the third quartile to the highest value that is within 1.5 × *IQR*. The lower whisker extends from the first quartile to the lowest value within 1.5 × *IQR*.

Table 1

Top SNPs ($P < 1 \times 10^{-6}$) from GWAS of cisplatin-associated ototoxicity in 511 survivors of adult-onset cancer

SNP	CHR	Gene[a]	Minor (effect) allele	Other allele	MAF	Effect (SE)	P-value
rs62283056	4	*WFS1*	C	G	0.21	−0.34 (0.06)	1.4×10^{-8}
rs62283057	4	*WFS1*	T	C	0.17	−0.34 (0.07)	3.6×10^{-7}
rs1829752	1	*ST6GALNAC5*	G	A	0.45	0.26 (0.05)	3.9×10^{-7}
rs10919728	1	*LINC01221/NR5A2*	A	T	0.37	−0.26 (0.05)	4.1×10^{-7}
rs11142215	9	*SPATA31C2/SPIN1*	G	A	0.16	0.34 (0.07)	5.1×10^{-7}
rs17718958	4	*WFS1*	A	G	0.17	−0.33 (0.07)	5.6×10^{-7}
rs2143582	1	*ST6GALNAC5*	C	T	0.45	0.25 (0.05)	5.6×10^{-7}
rs10919727	1	*LINC01221/NR5A2*	G	A	0.33	−0.27 (0.06)	9.2×10^{-7}

[a]Flanking genes are listed for intergenic SNPs,

CHR=chromosome, MAF=minor allele frequency, SE=standard error, SNP=single nucleotide polymorphism

Table 2

PrediXcan replication of *WFS1* association with hearing loss in an independent cohort of 18,620 patients from the Vanderbilt University BioVU Repository

Tissue	*WFS1* PVE [95% CI][a]	PheWAS Code	Statistic[b]	P[c]	Phenotype
Brain - Hypothalamus	0.23 [0.006, 0.83]	389	−3.4	0.00066	Hearing loss
Brain - Hypothalamus	0.23 [0.006, 0.83]	389.1	−3.3	0.0011	Sensorineural hearing loss
Artery	0.09 [0.003, 0.34]	389.1	−2.1	0.036	Sensorineural hearing loss
Brain - Nucleus accumbens (basal ganglia)	0.15 [0.006, 0.66]	389	−2.0	0.044	Hearing loss

[a] PVE = proportion of variance in *WFS1* expression explained by genetic effects (SNPs within 1 Mb) using Bayesian Sparse Linear Mixed Modeling (BSLMM), CI = credible interval

[b] Negative statistic indicates decreased imputed expression is associated with increased susceptibility to phenotype

[c] P values < 0.05 are shown. The Bonferroni significance level accounting for the 22 tests performed is P < 0.002.