

Further Investigation of the Role of *ACYP2* and *WFS1* Pharmacogenomic Variants in the Development of Cisplatin-Induced Ototoxicity in Testicular Cancer Patients

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Abstract

Purpose: Adverse drug reactions such as ototoxicity, which occurs in approximately one-fifth of adult patients who receive cisplatin treatment, can incur large socioeconomic burdens on patients with testicular cancer who develop this cancer during early adulthood. Recent genome-wide association studies have identified genetic variants in *ACYP2* and *WFS1* that are associated with cisplatin-induced ototoxicity. We sought to explore the role of these genetic susceptibility factors to cisplatin-induced ototoxicity in patients with testicular cancer.

Experimental Design: Extensive clinical and demographic data were collected for 229 patients with testicular cancer treated with cisplatin. Patients were genotyped for two variants, *ACYP2* rs1872328 and *WFS1* rs62283056, that have previously been associated with hearing loss in cisplatin-treated patients. Analyses were performed to investigate the association of these variants with ototoxicity in this cohort of adult patients with testicular cancer.

Results: Pharmacogenomic analyses revealed that *ACYP2* rs1872328 was significantly associated with cisplatin-induced ototoxicity [$P = 2.83 \times 10^{-3}$, OR (95% CI):14.7 (2.6–84.2)]. *WFS1* rs62283056 was not significantly associated with ototoxicity caused by cisplatin ($P = 0.39$); however, this variant was associated with hearing loss attributable to any cause [$P = 5.67 \times 10^{-3}$, OR (95% CI): 3.2 (1.4–7.7)].

Conclusions: This study has provided the first evidence for the role of *ACYP2* rs1872328 in cisplatin-induced ototoxicity in patients with testicular cancer. These results support the use of this information to guide the development of strategies to prevent cisplatin-induced ototoxicity across cancers. Further, this study has highlighted the importance of phenotypic differences in replication studies and has provided further evidence for the role of *WFS1* rs62283056 in susceptibility to hearing loss, which may be worsened by cisplatin treatment. *Clin Cancer Res*; 24(8); 1866–71. ©2018 AACR.

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Introduction

Cisplatin is a chemotherapeutic agent that is integral to the treatment of many cancers (1); however, the development of ototoxicity is a significant limitation of this therapy (2). It is estimated that the lifetime costs per patient associated with this adverse drug reaction (ADR) range from \$300,000 in adults to over \$1,000,000 in children (3). Although clinical variables such as age and dose of cisplatin have been shown to contribute to the development of cisplatin-induced ototoxicity (CIO), there is a large body of evidence showing that genetic variation plays an important role in the development of this ADR (4, 5). Identifying genetic variants that increase the risk of experiencing this ADR, as well as confirming their importance in independent replication cohorts, will provide valuable information to guide the development of strategies to prevent the occurrence of CIO.

Genome-wide association studies (GWAS) offer the opportunity for the large-scale investigation of the role that common genetic variation plays in the development of CIO. To date, two GWAS have been published examining the genetics of CIO (6, 7). The first GWAS identified a significant association with a genetic variant (rs1872328) in *ACYP2* and CIO in pediatric embryonal

Translational Relevance

Testicular cancer is the most common malignancy in young men, and the majority of patients are cured with cisplatin-based treatment. Unfortunately, approximately one-fifth of these patients develop ototoxicity as a result of this treatment, placing large socioeconomic burdens on these young adults. This study has provided the first evidence for the contribution of a genetic variant in *ACYP2* to the development of cisplatin-induced ototoxicity in patients with testicular cancer. Furthermore, this study has shown that individuals carrying a variant in *WFS1* are more likely to experience hearing loss, which is likely to be worsened by cisplatin treatment. These results provide evidence that inclusion of these pharmacogenomic variants in predictive genotyping tests may play an important role in the prevention of cisplatin-induced ototoxicity.

patients with brain tumors, the results of which were replicated in a second cohort of pediatric patients with brain tumors (6). Subsequent to this publication, two studies have replicated the association of *ACYP2* rs1872328 with CIO in patients with osteosarcoma (8) and pediatric patients with cancer (9), while a second GWAS of CIO, which was performed in a cohort of patients with testicular cancer, was unable to replicate this association. This GWAS did, however, identify another variant (rs62283056) in *WFS1* that was associated with hearing loss in patients treated with cisplatin. Although the association with this variant and CIO has not been replicated to date, the authors did report a significant association with predicted *WFS1* expression and hearing loss (7).

Testicular cancer is the most common malignancy in young men, and the majority of patients are cured with cisplatin-based treatment (10). Therefore, understanding the genetic variants contributing to the development of CIO in survivors of testicular cancer will be of great value to improving the quality of life of these individuals. We have previously examined the contribution of genetic variants in drug absorption, distribution, metabolism, and excretion (ADME) genes to CIO in patients with testicular

cancer. These analyses identified an association with a genetic variant (rs4788863) in *SLC16A5* and CIO, the results of which were validated by replication, functional assays, and supporting literature (11). Because *ACYP2* and *WFS1* are not traditional ADME genes, they were not assessed in our previous study. The current study therefore aimed to investigate the role that genetic variants in *WFS1* and *ACYP2* play in the development of CIO in adult patients with testicular cancer.

Materials and Methods

Patient cohorts

A total of 229 ≥ 17 -year-old male patients with germ cell testicular cancer who were previously treated with cisplatin-based chemotherapy, were available for inclusion in this study. Written informed consent was obtained from each patient, and the ethics committee of each participating center approved the study, in accordance with the Helsinki Declaration as revised in 2008.

For case-control assignment, we utilized two different approaches (Table 1). In the first approach, which we term *clinically determined CIO*, two audiologists independently reviewed the patient audiograms (0.25–8 kHz) and relevant clinical data (noise exposures, age, concomitant ototoxic medications, and timing of cisplatin treatment) to define CIO as previously described (11). Discrepant cases were discussed and resolved through discussion between the two audiologists in conjunction with a clinical pharmacologist.

In the second approach, the geometric mean of the hearing thresholds at 4, 6, and 8 kHz was calculated for each individual as previously described (7, 12). This second set of hearing loss definitions was used to match the hearing loss phenotype that identified the association with *WFS1* rs62283056 as closely as possible. The calculated geometric mean values were subsequently used for case-control assignment, which was based on American Speech-Language-Hearing Association Degree of Hearing Loss criteria (ref. 13; Table 1).

Genotyping

Genomic DNA samples for all patients were genotyped for rs62283056 in *WFS1* (Assay ID: C_88555782_10) and

Table 1. Case-control assignment for cohort using two different criteria

	Clinically determined CIO (N = 229)	Geometric mean of hearing thresholds (N = 229)
	<i>Moderate-severe CIO (N = 37)^a</i>	<i>Moderate-profound hearing loss (N = 33)^a</i>
Cases	Audiogram configuration consistent with CIO; hearing threshold ≥ 25 dB at frequencies < 8 kHz	Geometric mean hearing thresholds > 40 dB
Controls	<i>No CIO (N = 153)^{b, c}</i> Audiograms show no evidence of ototoxicity or audiogram configurations show that hearing loss is clearly ascribable to a cause other than CIO (e.g., flat or upsloping sensorineural hearing loss)	<i>No hearing loss (N = 167)^{b, c}</i> Geometric mean hearing thresholds ≤ 25 dB
Exclusions	<i>Mild CIO (N = 20)</i> Audiogram configuration consistent with CIO; hearing threshold ≥ 25 dB at frequencies ≥ 8 kHz <i>Ambiguous (N = 19)</i> Audiogram configuration does not provide sufficient information to allow for classification as cases or controls (e.g., hearing loss possibly due to aging)	<i>Mild hearing loss (N = 19)</i> Geometric mean hearing threshold 26–40 dB <i>Asymmetrical hearing loss^d (N = 10)</i> Audiograms show ear asymmetry (geometric mean difference > 20 dB between the two ears)

Abbreviations: CIO: cisplatin-induced ototoxicity, dB: decibels, kHz: kilohertz.

^aTwenty-six individuals were assigned case status by both assignment systems.

^bOne hundred forty-four individuals were assigned control status by both assignment systems.

^cGenotyping failed in two individuals.

^dTo ensure consistency between the current cohort and the cohort utilized by Wheeler et al. (7), which reported only two patients with ear asymmetry, we excluded patients with ear asymmetry.

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rs1872328 in *ACYP2* (Assay ID: C_11643398_10) using TaqMan Genotyping Assays (ThermoFisher Scientific), according to the manufacturer's instructions.

Statistical analyses

The association of clinical variables and genetically determined ancestry, as ascertained by ADMIXTURE (14), with CIO case-control status for both designation systems (Table 1) was assessed as previously described (11). Clinical variables that were significantly associated with CIO ($P < 0.05$) were included as covariates in the subsequent genetic association analyses. In addition, principal component analyses of the genetic data were performed using EIGENSOFT v5.0 (15), including the 1000 Genomes Project phase III samples as a reference. In addition to the clinical covariates, principal components 1 to 4 were included as covariates in the genetic association analyses. To investigate the degree of population differentiation for the two variants under investigation, fixation index (F_{ST}) statistics were calculated for the 1000 Genomes Project populations, as previously described (16). Further to this, European individuals were identified through visual inspection of the first two principal components and were included in European only subset analyses.

Minor allele frequencies (MAFs) and deviations from Hardy-Weinberg equilibrium (HWE) were determined for the genotyped SNPs. Annotation of variants was performed using the Combined Annotation Dependent Depletion (CADD) scoring system, which ranks the predicted deleteriousness of variants using a Phred-like scale from 1 to 99, with higher scores corresponding to more deleterious variants (17). To match the analyses that identified the association with *WFS1* rs62283056 as closely as possible, the rank-normalized geometric means of hearing thresholds from 4 to 8 kHz were included in linear regression analyses, and the interaction between SNP genotype and cisplatin dose was tested as previously described (7). Normality of the data was tested with the Shapiro-Wilk test. In addition, logistic regression was used to investigate the association of the genetic variants with case-control status as described in Table 1. Consistent with the previous reports (6, 7), the additive genetic model was used in all genetic association analyses. Statistical analyses were performed using either R (18) or the SNP and Variation Suite (SVS) v8.3 (Golden Helix, Inc.). $P < 0.05$ was considered statistically significant.

Meta-analysis of *ACYP2* rs1872328

To identify studies previously investigating the relationship between *ACYP2* or *WFS1* and CIO, a systematic literature search was conducted on November 1, 2017, using Embase (studies between 1980 and 2017 October 31) and Ovid Medline (1946 to present with daily update) on all published, peer-reviewed English-language articles. All articles containing the search terms "ACYP2" and "cisplatin" or "WFS1" and "cisplatin" were reviewed for inclusion in a meta-analysis and the number of cases and controls for each genotype group were extracted from the studies. A meta-analysis of these data was performed using the software package Review Manager 5.3 (Cochrane Collaboration, 2014). The pooled allelic odds ratio (OR) for the dichotomous trait (ototoxicity, as defined by each of the studies) across studies was estimated using the Mantel-Haenszel random-effects method, with studies weighted according to the reciprocal of their variance. Heterogeneity across studies was assessed using the I^2 statistic.

Results

Genotyping of *ACYP2* rs1872328 and *WFS1* rs62283056 was successful for 99% of the samples, with each SNP failing in two samples designated as controls both for clinically determined CIO and for hearing loss as ascertained from geometric mean hearing thresholds. Each polymorphism was in HWE ($P = 0.68$ and $P = 0.73$, respectively).

Investigation of the clinical variables revealed that age at cisplatin treatment initiation and cumulative cisplatin dose were associated with CIO case-control status for both designation systems (Table 2). In addition, cancer treatment protocol was significantly different between cases and controls in the geometric mean of hearing threshold cohort and trended toward significance in the clinically determined CIO cohort. Therefore, age at cisplatin treatment initiation, cumulative cisplatin dose, and cancer treatment protocol were included as covariates in all subsequent genetic association tests. Upon examination of ancestry, it was observed that proportion European genetic ancestry, as calculated by ADMIXTURE, was significantly different between cases and controls (Table 2). While proportion of East Asian genetic ancestry was not significantly different, inspection of the second principal component revealed that East Asian genetic ancestry was unequally distributed between cases and controls (Supplementary Fig. S1). Although F_{ST} values greater than 0.1 were observed for both variants in the 1000 Genomes Project superpopulations, all F_{ST} values were less than 0.05 in the European subpopulations (ranging from 0.00 to 0.03 for *ACYP2* rs1872328 and 0.00 to 0.02 for *WFS1* rs62283056). Therefore, in addition to including principal components 1 to 4 in the genetic association analyses, analyses were repeated including only European individuals (Supplementary Fig. S1) to account for the effects of population stratification.

On examination of the genetic association results, it was observed that *ACYP2* rs1872328 was significantly associated with clinically determined CIO ($P = 2.83 \times 10^{-3}$, OR (95% CI) = 14.7 (2.6–84.2); Table 3). This association remained significant when analyses were repeated in the European only cohort [$P = 1.04 \times 10^{-3}$, OR (95% CI) = 29.2 (3.8–221.9); Supplementary Table S1]. To further investigate the association of *ACYP2* rs1872328 with CIO, systematic review of the literature identified 6 reports including *ACYP2* and cisplatin—two conference abstracts, one erratum, and three original research articles. The three original research articles were included along with the current study in a meta-analysis, which showed that the pooled OR for *ACYP2* rs1872328 was statistically significant [$P = 0.0002$; OR (95% CI) = 5.50 (2.25–13.46); Fig. 1].

Although the association of *WFS1* rs62283056 with clinically determined CIO was not statistically significant ($P = 0.39$), the frequency of this variant was higher in cases compared with controls (Table 3). Therefore, in line with the study performed by Wheeler and colleagues (7), analyses were repeated to investigate the association of the genetic variants with the geometric means of the hearing thresholds. A Shapiro-Wilk test confirmed that the rank-normalized geometric means of hearing thresholds were normally distributed ($P = 1.0$). Although linear regression analyses of these data did not identify a significant association with *WFS1* rs62283056 or *ACYP2* rs1872328 and hearing loss ($P = 0.10$ and $P = 0.17$, respectively), individuals homozygous for *WFS1* rs62283056 or heterozygous for *ACYP2* rs1872328 exhibited worse geometric mean hearing thresholds (Supplementary

Table 2. Summary of patient characteristics

	Clinically determined CIO (N = 190)			Geometric mean of hearing thresholds (N = 200)		
	Cases (N = 37)	Controls (N = 153)	P value	Cases (N = 33)	Controls (N = 167)	P value
Age at time of treatment (years), median (IQR)	40 (32-49)	29 (23-35)	3.00 × 10⁻⁷	40 (31-51)	30 (24-35)	3.11 × 10⁻⁷
Cumulative cisplatin dose (mg/m ²), median (min, max)	400 (300-920)	400 (300-800)	0.005	400 (200-920)	400 (200-900)	0.012
Concomitant ototoxic medication, ^a n (%)	1 (2.7)	4 (2.6)	1.000	1 (3.0)	6 (3.6)	1.000
Cranial irradiation, n (%)	1 (2.7)	2 (1.3)	0.480	1 (3.0)	1 (0.6)	0.304
Cancer treatment protocol			0.078			0.012
BEP, n (%)	17 (45.9)	102 (66.7)	0.024	14 (42.4)	114 (68.3)	0.009
EP, n (%)	13 (35.1)	32 (20.9)	0.085	11 (33.3)	32 (19.2)	0.038
VIP2, n (%)	1 (2.7)	6 (3.9)	1.000	1 (3.0)	8 (4.8)	0.357
Combination, n (%)	6 (16.2)	13 (8.5)	0.217	7 (21.2)	13 (7.8)	0.028
Proportion ancestry	0.85 (0.81-0.90)	0.81 (0.70-0.87)	0.018	0.85 (0.81-0.91)	0.81 (0.70-0.87)	0.036
European, median (IQR)						
East Asian, median (IQR)	0.02 (0.00-0.04)	0.01 (0.00-0.05)	0.636	0.02 (0.00-0.04)	0.01 (0.00-0.05)	0.190
South Asian, median (IQR)	0.08 (0.03-0.14)	0.07 (0.02-0.12)	0.564	0.08 (0.03-0.14)	0.07 (0.02-0.12)	0.253
American, median (IQR)	0.01 (0.00-0.05)	0.02 (0.00-0.05)	0.167	0.01 (0.00-0.05)	0.02 (0.00-0.05)	0.102
African, median (IQR)	0.02 (0.00-0.03)	0.03 (0.02-0.05)	0.075	0.02 (0.00-0.03)	0.03 (0.01-0.05)	0.077

BEP: 20 mg/m² cisplatin, 100 mg/m² etoposide, 30 units bleomycin for 5 days per cycle; EP: 20 mg/m² cisplatin, 100 mg/m² etoposide for 5 days per cycle, IQR: interquartile range; max: maximum; min: minimum; VIP2: 20 mg/m² cisplatin, 75 mg/m² etoposide, 1500 mg/m² ifos, 300 mg/m² mesna for 5 days per cycle.

^aTobramycin, vancomycin, vincristine, furosemide. Significant P values (P < 0.05) are bolded. Proportion ancestry was calculated using ADMIXTURE, including five ancestral components.

Table 3. Association results for ACYP2 rs1872328 and WFS1 rs62283056

Variant	Gene	MAF (cases)	MAF (controls)	P value ^a	Adjusted OR (95% CI) ^a
Association with clinically determined CIO					
rs1872328	ACYP2	0.08	0.02	2.83 × 10 ⁻³	14.73 (2.58-84.24)
rs62283056	WFS1	0.26	0.17	0.39	1.36 (0.67-2.77)
Association with geometric mean of hearing thresholds					
rs1872328	ACYP2	0.03	0.02	0.97	1.05 (0.10-11.42)
rs62283056	WFS1	0.33	0.16	5.67 × 10 ⁻³	3.22 (1.35-7.67)

Abbreviations: CI: confidence interval; CIO: cisplatin-induced ototoxicity; MAF: minor allele frequency, OR: odds ratio.

^aP values and ORs are based on association results that have been corrected for the following covariates: age at cisplatin treatment initiation, cumulative cisplatin dose, treatment type, and principal components 1-4.

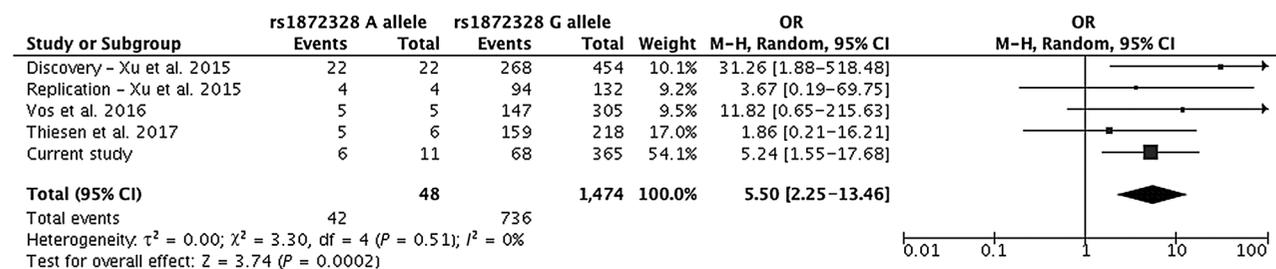
Fig. S2). Interestingly, this observed association was heightened in individuals receiving >300 mg/m² doses of cisplatin (Supplementary Fig. S3).

To further explore this association, individuals were assigned case-control status based on Table 1 definitions, and logistic regression was performed. These analyses identified a significant association with WFS1 rs62283056 and hearing loss [P = 5.67 × 10⁻³, OR (95% CI) = 3.2 (1.4-7.7)], which remained significant upon exclusion of non-European individuals [P = 7.11 × 10⁻³, OR (95% CI) = 3.4 (1.3-9.0)]. This association was not observed

for ACYP2 rs1872328 (P = 0.97; Table 3 and Supplementary Table S1). A systematic review of the literature for WFS1 and CIO identified only one article (7); therefore, a meta-analysis of these data was not performed.

Discussion

This study has provided the first evidence for the role of ACYP2 rs1872328 in the development of CIO in patients with testicular cancer. This is an important finding because platinum-based

**Figure 1.**

Meta-analysis of ACYP2 rs1872328 showing the pooled odds ratio for developing ototoxicity for the A allele (variant) vs. the G allele (wild-type). CI, confidence interval, M-H: Mantel-Haenszel. *ORs are based on association results that are not corrected for clinical or demographic covariates.

drugs are among the most frequently used chemotherapeutic (1), and 20% of patients with testicular cancer experience moderate to severe CIO (11). Therefore, understanding the genetic predictors of this ADR will have important socioeconomic implications for patients undergoing cancer treatments.

These data have added to the growing body of evidence for the role that *ACYP2* rs1872328 plays in the development of CIO across cancers (6, 8, 9). Further, another variant (rs843748) located within the *ACYP2* region has been associated with oxaliplatin neuropathy (19, 20), possibly implicating this region in additional chemotherapy-related ADRs. Supporting the involvement of *ACYP2* in these ADRs, this gene encodes an acylphosphatase, which has been postulated to effect calcium homeostasis (21), the dysregulation of which has been implicated in both hair cell damage (22) and oxaliplatin-induced central neuropathy (23). In addition, *ACYP2* is expressed in the cochlear (24) and brain regions (25). This points toward a mechanistic link between *ACYP2* and chemotherapy-induced ototoxicity and neuropathy. Nonetheless, neither rs1872328 nor rs843748 is predicted to be deleterious (CADD scores <5) and both variants are located within intronic regions of *ACYP2* with no known functional significance (6, 19). Therefore, it is possible that both of these variants are proxy markers for other causal variants and future studies are needed to provide further insight into the mechanistic link between variation in the *ACYP2* region and CIO.

A key strength of our study relates to the incorporation of the expertise of two independent audiologists and the fact that our case-control designation procedure accounts for external influences on hearing (e.g., aging and noise exposure), which is particularly important in the investigation of CIO in adults. Using these criteria, we were unable to identify a significant association with clinically determined CIO and *WFS1* rs62283056. However, additional analyses using the geometric means of hearing thresholds for case-control designation identified a significant association ($P = 5.67 \times 10^{-3}$, OR = 3.2) with rs62283056 and hearing loss. This aligns with the fact that mutations in *WFS1* are known to cause deafness (26, 27) and rs62283056 is associated with a decreased expression of *WFS1* (25). The association of *WFS1* and hearing loss also mirrors the analyses that were described in the original paper (7), which replicated the association of *WFS1* with hearing loss, but were unable to replicate this finding in an independent cohort examining CIO. These findings highlight that rs62283056 may play an important role in hearing loss, the effect of which may be amplified in the presence of cisplatin (Supplementary Figs. S2 and S3).

The inability to replicate the association of rs62283056 *WFS1* with clinically determined CIO in our cohort may be attributed to a relatively small sample size. Although the collection of large cohorts of patients that are independently reviewed by two audiologists may not be feasible in all settings, in cases where there are multiple etiologies of hearing loss, consideration of audiogram configuration (i.e., the shape of the audiogram; Supplementary Fig. S4) and independent review by two audiologists is invaluable for accurate phenotyping. Furthermore, although 12-kHz readings were not included in the current study, given the sloping nature of high-frequency hearing loss, future studies investigating CIO may benefit from the inclusion of these data.

As has been brought to light previously (28), the findings presented here illustrate that it is essential to carefully match CIO phenotypes to ensure that appropriate replication studies are performed in pharmacogenomics research. There is large

variability among studies investigating the genetics of CIO with respect to phenotype definitions, treatment protocols, and patient demographics (29). Furthermore, examination of clinically relevant pharmacogenetic variants and drug-gene interaction networks has reported that cisplatin traits are affected by multiple genetic variations (30), providing further evidence for the polygenic nature of CIO (7). If CIO pharmacogenomic variants are to impact the lives of patients, future research will need to focus on uncovering the factors that contribute to differences and similarities in genetic susceptibility across cohorts of patients. The quantification of the individual effects of genetic variants in different patient cohorts will facilitate the development of strategies to prevent the occurrence of this ADR across cohorts of patients treated with cisplatin.

Of specific relevance to the current study, both variants investigated are rare in East Asian populations (MAF of *ACYP2* rs1872328: 0.011; MAF of *WFS1* rs62283056: 0.003). Although there were only 10 East Asian individuals in the current cohort, none of these patients experienced CIO. To confirm that the observed association between the variants and CIO was not driven by population stratification, analyses were repeated excluding all non-European individuals. These analyses confirmed that the variants remained significantly associated with CIO in the European cohort. Of further interest, *ACYP2* rs1872328 is common in African descent individuals (MAF African: 0.199; MAF European: 0.039), and while no patients of African ancestry were included in this cohort, future research should investigate the contribution of *ACYP2* rs1872328 and *WFS1* rs62283056 to CIO in cohorts of different ancestries. Particularly, given the low linkage disequilibrium present in African populations, the inclusion of African populations in CIO association studies may aid in fine-mapping strategies to identify causal variants linked to *ACYP2* rs1872328. Further, the inclusion of diverse cohorts in future pharmacogenomic studies is especially important to ensure that the benefits of genomic medicine are realized across the globe (16, 31).

This study has added to the growing body of evidence for the role that *ACYP2* rs1872328 plays in CIO across different cancers. These results support the utility of this genetic variant in predicting CIO in both adults and children. This information will ultimately provide clinicians with predictive information to aid in the quantification of patients' risk of experiencing this severe adverse reaction. In addition, although *WFS1* rs62283056 was not directly implicated in CIO, this study has provided further evidence for the role that this variant plays in susceptibility to hearing loss, which may be worsened by cisplatin treatment. In conclusion, identification of patients carrying risk variants for CIO and hearing loss will allow for strategies to reduce hearing loss in these patients, including increased monitoring of hearing and the consideration of otoprotectant strategies.

Disclosure of Potential Conflicts of Interest

B. Brooks is a co-investigator for a grant received from the Ida Institute in Denmark for a psychosocial study of cisplatin-related hearing loss in children. No potential conflicts of interest were disclosed by the other authors.

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