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# Association Between *SLC16A5* Genetic Variation and Cisplatin-Induced Ototoxic Effects in Adult Patients With Testicular Cancer

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**IMPORTANCE** Cisplatin-induced ototoxic effects are an important complication that affects testicular cancer survivors as a consequence of treatment. The identification of genetic variants associated with this adverse drug reaction will further our mechanistic understanding of its development and potentially lead to strategies to prevent ototoxic effects.

**OBJECTIVE** To identify the genetic variants associated with cisplatin-induced ototoxic effects in adult testicular cancer patients.

**DESIGN, SETTING, AND PARTICIPANTS** This retrospective study was performed by the Canadian Pharmacogenomics Network for Drug Safety using patients recruited from 5 adult oncology treatment centers across Canada. Male patients who were 17 years or older, diagnosed with germ cell testicular cancer, and previously treated with cisplatin-based chemotherapy were recruited from July 2009 to April 2013 using active surveillance methodology. Cisplatin-induced ototoxic effects were independently diagnosed by 2 audiologists. Patients were genotyped for 7907 variants using a custom pharmacogenomic array. Logistic regression was used to identify genetic variants that were significantly associated with ototoxic effects. The validity of these findings was confirmed through independent replication and cell-based functional assays.

**EXPOSURES** Cisplatin-based chemotherapy.

**MAIN OUTCOMES AND MEASURES** Cisplatin-induced ototoxic effects.

**RESULTS** After exclusions, 188 patients (median [interquartile range] age, 31 [24-39] years) were enrolled in this study to form the discovery and replication cohorts. Association and fine-mapping analyses identified a protein-coding variant, rs4788863 in *SLC16A5*, that was associated with protection against cisplatin-induced ototoxic effects in 2 independent cohorts (combined cohort: odds ratio, 0.06; 95% CI, 0.02-0.22; $P = 2.17 \times 10^{-7}$). Functional validation of this transporter gene revealed that in vitro *SLC16A5*–silencing altered cellular responses to cisplatin treatment, supporting a role for *SLC16A5* in the development of cisplatin-induced ototoxic effects. These results were further supported by the literature, which provided confirmatory evidence for the role that *SLC16A5* plays in hearing.

**CONCLUSIONS AND RELEVANCE** This study has identified a novel association between protein-coding variation in *SLC16A5* and cisplatin-induced ototoxic effects. These findings have provided insight into the molecular mechanisms of this adverse drug reaction in adult patients with germ cell testicular cancer. Given that previous studies have shown that cimetidine, an SLC16A5-inhibitor, prevents murine cisplatin-induced ototoxic effects, the findings from this study have important implications for otoprotectant strategies in humans.

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Supplemental content

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Cisplatin, a chemotherapeutic agent used in the management of several cancers, is a key component in the treatment of testicular cancer—the most common malignancy among young men. Unfortunately, the use of this drug is complicated by the development of high-frequency hearing loss, which occurs in 20% to 40% of patients with testicular cancer treated with cisplatin.[1]

Studies performed in pediatric populations have enhanced our understanding of the pharmacogenetic variants involved in cisplatin-induced ototoxic effects (CIO).[2] However, whether these same genetic variants influence CIO in adult populations is unknown. The aim of this study was therefore to perform a comprehensive examination of the effects of variation in drug absorption, distribution, metabolism, and excretion (ADME) genes on the development of CIO in adult patients with testicular cancer treated with cisplatin.

### Key Points

**Question** Do genetic polymorphisms contribute to the development of cisplatin-induced ototoxic effects?

**Findings** In this pharmacogenomic case-control association study of adult patients with testicular cancer treated with cisplatin, patients carrying a genetic variant in *SLC16A5* were significantly less likely to experience ototoxic effects. These findings were validated through independent replication, 2 functional assays, and literature reporting that cimetidine, an SLC16A5-inhibitor, prevents cisplatin-induced ototoxic effects in mice.

**Meaning** To our knowledge, this is the first report of an association between a genetic variant in *SLC16A5* and cisplatin-induced ototoxic effects; this variant can be used to aid in predicting risk of ototoxic effects.

## Methods

### Patient Cohorts and Audiological Assessments

A total of 260 patients were recruited to take part in this study. After exclusion of patients according to specified criteria (eFigure 1.1 in the Supplement), 188 patients were included in the discovery cohort from Ontario (n = 96; 23 cases and 73 controls) and replication cohort from British Columbia (n = 92; 14 cases and 78 controls). All patients were men 17 years or older who were diagnosed with germ cell testicular cancer and previously treated with cisplatin-based chemotherapy. Cisplatin-induced ototoxic effects were independently diagnosed by 2 audiologists (Section 2 in the Supplement). Written informed consent was obtained from each patient and the study was approved by the ethics committee of each participating center.

### Genotyping and Statistical Analyses

Samples were genotyped for 7907 variants located within ADME gene regions using a custom Illumina Infinium Panel (Illumina). Using these data, genetically determined ancestry was calculated (Section 3 in the Supplement) for inclusion in the logistic regression model, along with clinical variables that were significantly associated with CIO (**Table**). All 3 models of inheritance were investigated to identify genetic variants that were significantly associated with CIO in the discovery and replication cohorts (Section 4.1 in the Supplement). These variants were prioritized for fine-mapping analyses (Section 5.1 in the Supplement) and subsequent genotyping using TaqMan Genotyping Assays (ThermoFisher Scientific). Variants associated with CIO in previous studies were extracted from PharmGKB, and association analyses were performed in the combined cohort. Statistical analyses were performed using R[3] (R Foundation) and SVS (Golden Helix Inc).

### Cell Viability and Relative Gene Expression Assays

For cell viability assays, *SLC16A5* gene silencing was performed in HeLa cells (Section 6.2 in the Supplement), after which cells were treated with cisplatin (316 nM-316 μM) and dissolved in phosphate buffered saline for 48 hours. Cell viability was assayed using an MTT assay (Sigma-Aldrich) and

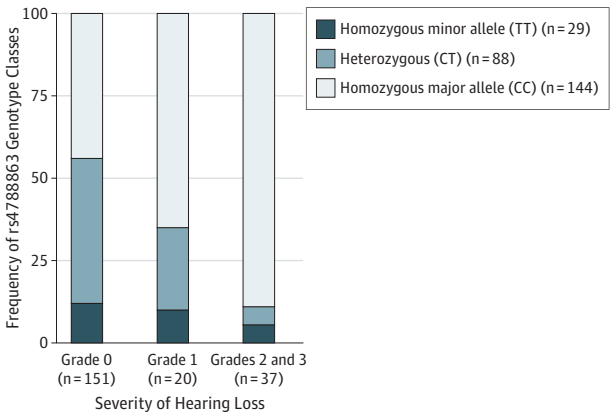
Table. Summary of Patient Characteristics

| | Discovery (n = 96) | | | Replication (n = 92) | | |
|---|---|---|---|---|---|---|
| Characteristic | Cases (n = 23) | Controls (n = 73) | *P* Value | Cases (n = 14) | Controls (n = 78) | *P* Value |
| Age at time of treatment, median (IQR), y | 39 (32-47) | 29 (23-34) | $3.82 \times 10^{-4}$ | 47 (39-52) | 30 (24-35) | $1.59 \times 10^{-4}$ |
| Cumulative cisplatin dose, median (range), mg/m$^2$ | 400 (300-800) | 300 (200-800) | .04 | 400 (300-920) | 400 (200-700) | .06 |
| Cycles of cisplatin treatment, No., median (range) | 4 (3-8) | 3 (2-8) | .03 | 4 (3-10) | 4 (2-7) | .05 |
| Cancer type, seminoma, No. (%) | 4 (17.4) | 5 (6.8) | .21 | 8 (57.1) | 22 (28.2) | .06 |
| Concomitant ototoxic medicationa, No. (%)[a] | 0 | 2 (2.7) | >.99 | 1 (7.1) | 2 (2.6) | .39 |
| Cranial irradiation, No. (%) | 1 (4.3) | 0 | .24 | 0 | 2 (2.6) | >.99 |
| Cancer treatment protocol, No. (%) | | | .06 | | | .07 |
| BEP | 14 (60.9) | 59 (80.8) | .09 | 3 (21.4) | 41 (52.6) | .04 |
| EP | 4 (17.4) | 8 (11.0) | .47 | 9 (64.3) | 24 (30.8) | .03 |
| VIP2 | 0 | 2 (2.7) | >.99 | 1 (7.1) | 4 (5.1) | .57 |
| Combination | 5 (21.7) | 4 (5.5) | .03 | 1 (7.1) | 9 (11.5) | >.99 |

Abbreviations: BEP, 20 mg/m$^2$ cisplatin, 100 mg/m$^2$ etoposide, 30 units bleomycin for 5 days per cycle; EP, 20 mg/m$^2$ cisplatin, 100 mg/m$^2$ etoposide for 5 days per cycle; IQR, interquartile range; max, maximum; min, minimum; VIP2, 20 mg/m$^2$ cisplatin, 75 mg/m$^2$ etoposide, 1500 mg/m$^2$ ifosfamide, 300 mg/m$^2$ mesna for 5 days per cycle.

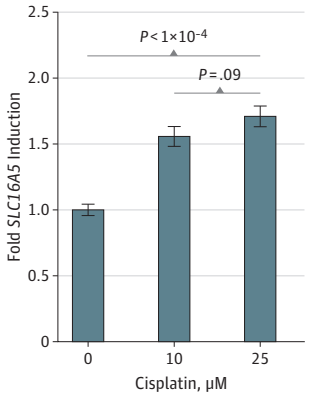
[a] Tobramycin, vancomycin, vincristine, furosemide. Significance was set at $P < .05$. Pearson correlation tests revealed that cumulative cisplatin dose was significantly correlated with the number of cycles of cisplatin treatment ($r = 0.94$ and $r = 0.95$ in discovery and replication cohorts, respectively; $P < 2.2 \times 10^{-16}$).

Figure 1. Correlation of *SLC16A5* rs4788863 Genotype Distribution and Severity of Hearing Loss




The minor allele (T) of rs4788863 exerted a protective effect against cisplatin-induced ototoxic effects and was enriched in controls, depleted in cases experiencing moderate-to-severe cisplatin-induced ototoxic effects, and occurred at an intermediate frequency in patients experiencing mild cisplatin-induced ototoxic effects. Due to the small number of individuals with grade 2 hearing loss (n = 9), these patients were grouped with patients experiencing grade 3 hearing loss.

Figure 2. Cisplatin Treatment and Increase in *SLC16A5* Expression




*SLC16A5* expression was measured in HeLa cells after treatment with the indicated concentrations of cisplatin. Shown are aggregate data (n = 18) from 2 independent experiments. Comparisons to untreated cells were analyzed by 1-way analysis of variance, while comparison between the treated cells were analyzed by unpaired *t* test.

absorbance was read on a POLARstar Omega plate reader (BMG Labtech). For *SLC16A5* expression experiments, HeLa cells were treated with cisplatin (0, 10, and 25 μM) for 24 hours, after which total RNA was purified for complementary DNA synthesis and subsequent quantitative polymerase chain reaction reactions (Section 6.3 in the Supplement).

## Results

### Genetic Association and Annotation Analyses

Association analyses identified a synonymous variant in *SLC16A5*, rs4788863 (p.Leu41Leu), that exerted a dominant protective effect on the development of CIO in both the discovery (odds ratio [OR], 0.05; 95% CI, 0.01-0.28; $P = 2.03 \times 10^{-5}$) and replication (OR, 0.02; 95% CI, 0.00-0.38; $P = 7.10 \times 10^{-4}$) cohorts (eTable 4.1 in the Supplement). This association remained significant after Bonferroni correction in the combined cohort (OR, 0.06; 95% CI, 0.02-0.22; $P = 2.17 \times 10^{-7}$). These results were further substantiated through the inclusion of individuals with grade 1 CIO (n = 20), demonstrating that the frequency distribution of rs4788863 was correlated with the severity of CIO ($P = 8.35 \times 10^{-6}$) (**Figure 1**; eTable 4.3 in the Supplement). Partitioning of the cohort according to patient ancestry and clinical characteristics revealed that rs4788863 was protective against CIO in all subanalyses (eTable 4.2 in the Supplement). Lastly, of the variants extracted from PharmGKB, only rs1695, in *GSTP1,* was significantly associated with CIO in the combined cohort (OR, 2.97; 95% CI, 1.02-8.66; $P = .049$) (eTable 4.4 in the Supplement).

Annotation of variants with minor allele frequency (MAF) greater than 0.01 within the *SLC16A5* gene region revealed that rs4788863 (p.Leu41Leu) was assigned the highest Combined Annotation Dependent Depletion score (13.2) indicating that this variant is predicted to be the most deleterious common variant in *SLC16A5*. In addition, rs4788863 was predicted to alter the rate of codon usage at this position (frequency per thousand: 39.6 for CUG vs 12.9 for UUG). One additional variant in the *SLC16A5* region was prioritized for further investigation—missense variant, rs4789134 (p.Arg32Lys). However, subsequent annotation and association analyses did not support the role of this variant in the development of CIO (Section 5.2 in the Supplement).

### Biological Interaction of SLC16A5 and Cisplatin In Vitro

Statistically significant ($P < 1.0 \times 10^{-4}$) differences in cell viability were observed between *SLC16A5*-silenced cells and nontargeting siRNA-treated cells, which was attributable to a larger magnitude Hill slope for *SLC16A5*-silenced cells (eTable 6.1 in the Supplement). In addition, expression analyses revealed that *SLC16A5* was significantly induced by cisplatin in a dose-dependent manner ($P < 1.0 \times 10^{-4}$) (**Figure 2**).

## Discussion

This study identified an association between a synonymous variant (rs4788863, p.Leu41Leu) in *SLC16A5* and CIO (OR, 0.06; 95% CI, 0.02-0.22; $P = 2.17 \times 10^{-7}$). To our knowledge, this is the first study to identify a relationship between *SLC16A5* and CIO, providing important insight into the biological mechanisms underlying this adverse drug reaction. There are several lines of evidence supporting the role of *SLC16A5* in CIO. First, murine *Slc16a5* is uniquely expressed in the cochlear and utricle hair cells, but not the surrounding cells,[3] and mutations in genes uniquely expressed in ear hair cells are likely to cause deafness.[4] Second, previous research has shown that genetic variants in other *SLC* genes exert protection from CIO

in adult patients.[5,6] Importantly, SLC16A5 is inhibited by cimetidine,[7] and the addition of cimetidine to cisplatin treatments prevented the occurrence of CIO in rat cochlear cultures and mice[8,9] without compromising the antitumor activity of cisplatin treatment.[10]

The association of rs4788863 with CIO in 2 independent cohorts was corroborated by in silico analyses, which revealed that rs4788863 is predicted to be the most deleterious common variant (MAF>0.01) in the *SLC16A5* region (Combined Annotation Dependent Depletion score, 13.2), with additional annotation analyses suggesting that this variant may disrupt accurate protein translation.[11-14] The evidence for a drug-gene interaction between cisplatin and *SLC16A5* is strengthened by in vitro data, which demonstrate that *SLC16A5* was significantly induced by cisplatin and that *SLC16A5* exerts a significant impact on cisplatin-induced cell death.

In addition to the novel association of *SLC16A5* with CIO, we corroborated the association of a previously reported ADME variant[15] (rs1695, in *GSTP1* [OR, 2.97; $P$ = .049]). Interestingly, this is the only study listed on the curated pharmacogenomics database, PharmGKB, that matched our cohort in terms of age, sex, and cancer type (eTable 4.4 in the Supplement). These results highlight the importance of considering clinical and demographic differences in patient cohorts and highlight the need for future studies to examine the relevance of rs4788863 in other tumor types treated with cisplatin to determine whether these results extend to additional clinical scenarios.

### Limitations

Although this study has played an important role in uncovering genetic risk factors for CIO in patients with testicular cancer, limitations to this study should be acknowledged. These include the retrospective case-control design, limited number of baseline audiograms and a relatively small sample size. The findings reported in this study would be strengthened by replication studies in large prospective cohorts of adult patients with testicular cancer, the use of which would also facilitate the discovery of additional genetic variants with smaller effect sizes.

## Conclusions

This study identified a variant in *SLC16A5* as a novel genetic risk factor for CIO in patients with testicular cancer, the validity of which was substantiated by replication in an independent cohort, supporting literature, and functional validation. The identification of this variant will inform the development of pharmacogenomic tests to predict a priori patients at higher genomic risk for CIO and guide important research into intervention strategies to mitigate hearing loss from cisplatin treatment.

ARTICLE INFORMATION

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**Author Contributions:** Dr Carleton had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analyses. Drs Drögemöller and Monzon contributed equally to this work. Drs Ross, Gelmon, and Carleton contributed equally to the supervision of the work.
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