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 x 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.
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.

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 R3 (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 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 × 10−5) and replication (OR, 0.02; 95% CI, 0.00-0.38; P = 7.10 × 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 × 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 × 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 GSTPI, 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,
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 non-targeting 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, and mutations in genes uniquely expressed in ear hair cells are likely to cause deafness. Second, previous research has shown that genetic variants in other SLC genes exert protection from CIO in adult patients. Importantly, SLC16A5 is inhibited by...
cimetidine, and the addition of cimetidine to cisplatin treatments prevented the occurrence of CIO in rat cochlear cultures and mice without compromising the antitumor activity of cisplatin treatment.

The association of rs4788863 with CIO in 2 independent cohorts was corroborated 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. 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 rs1695, in GSTPI (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 priori patients at higher genomic risk for CIO and guide important research into intervention strategies to mitigate hearing loss from cisplatin treatment.

Conflict of Interest Disclosures: Dr Monzon has participated in speakers’ bureau and consulting and/or advisory roles for Bristol-Myers Squibb, Celegene, Merck, Novartis, and Roche. Ms Brooks has received research funds from Oticon. Dr Liu has participated in consulting and/or advisory roles and received honoraria from Pfizer, AstraZeneca, Millennium Pharmaceuticals Inc, the Takeda Oncology Company, Roche, and Novartis. Dr Renouf has participated in consulting and/or advisory roles and received honoraria from Baxalta and Celgene. Dr Hayden has been employed, been compensated for leadership roles, and has ownership interest in Teva Pharmaceuticals. Dr Ross has received research funds from Teva Pharmaceuticals. Dr Carleton has received research funds from Pfizer. No other conflicts are reported.

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REFERENCES