

Replication of *TPMT* and *ABCC3* Genetic Variants Highly Associated With Cisplatin-Induced Hearing Loss in Children

K Pussegoda^{1,2}, CJ Ross^{1,2,3}, H Visscher^{1,2}, M Yazdanpanah^{1,2,4}, B Brooks⁵, SR Rassekh^{2,6}, YF Zada⁷, M-P Dubé⁷, BC Carleton^{2,3,8}, MR Hayden^{1,2}, and the CPNDS Consortium

¹Department of Medical Genetics, Centre for Molecular Medicine and Therapeutics, University of British Columbia, Vancouver, British Columbia, Canada

²Child and Family Research Institute, University of British Columbia, Vancouver, British Columbia, Canada

³Division of Translational Therapeutics, Department of Pediatrics, University of British Columbia, Vancouver, British Columbia, Canada

⁴British Columbia Clinical Genomics Network, University of British Columbia, Vancouver, British Columbia, Canada

⁵Department of Audiology and Speech Pathology, British Columbia Children's Hospital, Vancouver, British Columbia, Canada

⁶Division of Pediatric Hematology/Oncology/Bone Marrow Transplant, Department of Pediatrics, British Columbia Children's Hospital, Vancouver, British Columbia, Canada

⁷Montreal Heart Institute Research Centre and Université de Montreal, Montreal, Quebec, Canada

⁸Pharmaceutical Outcomes Programme, British Columbia Children's Hospital, Vancouver, British Columbia, Canada

Abstract

Cisplatin is a widely used chemotherapeutic agent for the treatment of solid tumors. A serious complication of cisplatin treatment is permanent hearing loss. The aim of this study was to replicate previous genetic findings in an independent cohort of 155 pediatric patients. Associations were replicated for genetic variants in *TPMT* (rs12201199, $P = 0.0013$, odds ratio (OR) 6.1) and

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Correspondence: MR Hayden (mrh@cmm.ubc.ca).
C.J.R. and H.V. contributed equally to this work.

AUTHOR CONTRIBUTIONS

M.R.H., K.P., C.J.R., H.V., and B.C.C. wrote the manuscript. M.R.H., C.J.R., and B.C.C. designed the research. K.P., H.V., B.B., and S.R.R. performed the research. K.P., M.Y., Y.F.Z., and M.-P.D. analyzed data. M.R.H., C.J.R., and B.C.C. contributed new reagents/analytical tools.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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ABCC3 (rs1051640, $P=0.036$, OR 1.8). A predictive model combining variants in *TPMT*, *ABCC3*, and *COMT* with clinical variables (patient age, vincristine treatment, germ-cell tumor, and cranial irradiation) significantly improved the prediction of hearing-loss development as compared with using clinical risk factors alone (area under the curve (AUC) 0.786 vs. 0.708, $P=0.00048$). The novel combination of genetic and clinical factors predicted the risk of hearing loss with a sensitivity of 50.3% and a specificity of 92.7%. These findings provide evidence to support the importance of *TPMT*, *COMT*, and *ABCC3* in the prediction of cisplatin-induced hearing loss in children.

Cisplatin is one of the most effective chemotherapeutic agents for children with solid tumors, including hepatoblastoma, brain tumors, and germ-cell tumors, and has contributed to a dramatic increase in the survival rate. Cisplatin has shown efficacy in standard-risk hepatoblastoma and can be used as monotherapy with a >80% 3-year event-free survival.¹ A major complication that limits the use of cisplatin is the risk of drug-induced ototoxicity,² which manifests as permanent, bilateral hearing loss in about 10–25% of adults and 26–90% of children depending on dose and treatment regimen.^{3–8} In children, even mild losses in hearing can significantly influence speech and language development and increase the risk of learning difficulties.^{9,10} In adults, the rate of hearing loss may be higher than reported due to a lack of baseline and follow-up audiometry in cisplatin protocols.

Interindividual variability of cisplatin-induced effects on hearing in patients receiving the same dose of cisplatin is considerable, from no hearing loss to high-frequency hearing loss often progressing to severe hearing impairment in the speech frequencies.^{10–12} Furthermore, patients show no improvement in hearing and often the progression of hearing loss continues long after the end of therapy.¹³ Higher cumulative cisplatin dose,^{3,14,15} younger age,^{3,14,16} cranial irradiation,^{15,17} and concomitant use of aminoglycosides and vincristine^{14,18,19} are known to influence cisplatin-induced ototoxicity.¹¹ However, the debate over ototoxicity of vincristine continues; case reports suggest that vincristine may be ototoxic^{20,21} or transiently ototoxic at high doses¹⁴ whereas larger systematic clinical trials have reported that vincristine, alone, is not ototoxic.^{22,23} Because of the limited number of large studies, there is insufficient evidence to either support or refute the hypothesis that vincristine is an ototoxic agent. The interindividual variability in hearing loss suggests that clinical factors alone are insufficient predictors of safety.

At present, no standard methods exist to identify individuals who are at increased risk of developing hearing loss. Genetic factors involved in drug biotransformation, transport, and receptors have been recognized to influence patient drug response and susceptibility to adverse drug events, including ototoxicity.^{24–26} The identification of genetic markers that increase susceptibility to cisplatin-induced ototoxicity has important implications for improving patient care during cisplatin treatment.

Recently, a candidate gene study in children receiving cisplatin identified genetic variants in thiopurine *S*-methyltransferase (*TPMT*) (rs12201199, rs1800460, rs1142345), catechol *O*-methyltransferase (*COMT*) (rs9332377, rs4646316), and several other variants, including the ATP-binding cassette transporter C3 (*ABCC3*) (rs1051640) as conferring increased risk of developing cisplatin-induced hearing loss.⁹

For clinical application, it is essential to replicate these genetic findings in independent cohorts of patients so as to reduce the number of false-positive results and to ensure that the genetic risk prediction is consistent.²⁷ It is now well recognized that replication of genetic associations is required before any causal inferences can be drawn.²⁷ However, several studies have reported that consistent replication is challenging and difficult to achieve. The aim of this study was to investigate replication of genetic risk factors for cisplatin-induced hearing loss in an independent cohort of patients recruited from across Canada, as well as to evaluate a predictive multi-SNP (single-nucleotide polymorphism) model.

RESULTS

Patient characteristics

Patient characteristics of the initial cohort,⁹ current replication cohort, and the combined cohort are listed in Table 1. Adverse drug reaction surveillance clinicians routinely update information in the Canadian Pharmacogenomics Network for Drug Safety (CPNDS) patient database as new, clinically relevant information (i.e., relapse and concomitant medication use) becomes available. For the initial cohort, we included additional demographic data, including relevant information on the clinical course of these patients in the time since the study was first reported;⁹ we have also included additional concomitant medication details.²⁸ In the current replication cohort, 87 (56%) of the 155 pediatric oncology patients developed hearing loss. Concomitant vincristine treatment was significantly higher in cases than in controls in the replication cohort (66.7% vs. 27.9%, $P = 2.1 \times 10^{-6}$), as well as in the initial cohort (50.9% vs. 17.9%, $P = 4.1 \times 10^{-5}$; combined cohort $P = 1.1 \times 10^{-9}$). Fewer patients with osteosarcoma in the replication cohort developed hearing loss (11.5% in cases vs. 29.4% in controls, $P = 0.0073$), but the difference was not significant in the initial cohort (22.6% in cases vs. 28.6% in controls, $P = 0.45$). Furthermore, follow-up after therapy was longer in cases than in controls in the replication cohort (5.0 years vs. 2.0 years, $P = 2.1 \times 10^{-4}$), but the difference was not significant in the initial cohort (3.0 years vs. 2.0 years, $P = 0.10$).

Genetic results

In the replication cohort, all genetic variants assessed in *TPMT* (rs12201199, rs1142345, and rs1800460) showed a significant association with cisplatin-induced hearing loss (Table 2), rs12201199 being the most strongly associated variant ($P = 0.0013$, odds ratio (OR) 6.1). The risk allele (A) was observed in 21 (12%) cases and 3 (2%) controls. The analysis of the combined cohort of all 317 patients showed a stronger association with rs12201199 ($P = 8.7 \times 10^{-7}$, OR 8.9) than in the initial cohort alone.⁹ The other *TPMT* variants, rs1142345 and rs1800460, also showed a stronger association with cisplatin-induced hearing loss in the combined cohort than in the initial cohort (Table 2). Moreover, in the combined cohort, all three *TPMT* variants remained significantly associated with cisplatin-induced hearing loss, after correcting for clinical factors including patient age, vincristine treatment, germ-cell tumor, and cranial irradiation.

The protective “A” allele of *COMT*rs4646316 was observed in 33 (19%) cases and 32 (24%) controls in the replication cohort. However, the effect of this variant in the replication

cohort, although in the same direction as that of the initial cohort, was smaller (OR 1.3) and not significantly associated with hearing loss ($P = 0.33$). A similar effect was seen for *COMT*rs9332377 ($P = 0.28$, OR 1.4).

Next, we assessed whether other genetic variants could be replicated, thereby improving the model in predicting the risk of cisplatin-induced hearing loss. Three of the six variants showed an effect in the same direction as the initial cohort (Table 2, Supplementary Table S1 online). One synonymous variant in *ABCC3* (rs1051640) (E1503E) was significantly associated with cisplatin-induced ototoxicity in the current replication cohort ($P = 0.036$, OR 1.8), and showed a stronger association in the combined cohort ($P = 7.8 \times 10^{-4}$, OR 2.0; Table 2). The protective “A” allele of *ABCC3* rs1051640 was observed in 28 (16%) cases and 35 (26%) controls in the replication cohort. The association of *ABCC3* rs1051640 with cisplatin-induced hearing loss remained significant in the combined cohort after adjusting for clinical factors ($P = 0.0033$).

By principal component analysis, a majority (80%) of patients were found to have European genetic ancestry²⁹ (Supplementary Figure S1 online). With the aim to reduce potential bias caused by population stratification, we assessed whether associations in the replication cohort remained significant in a more homogeneous subset of patients of only European ancestry ($n = 124$). For this subset, the findings were similar (Supplementary Table S2 online).

We generated a model that combines the effects of associated risk genotypes and clinical risk factors, and receiver operating characteristic analyses were performed. Three independent SNPs (*TPMT*rs12201199, *ABCC3* rs1051640, and *COMT*rs4646316) were selected to construct a genetic-only model based on forward logistic regression. Although no significant association with variants in *COMT* in the current replication cohort was found, the combined cohort logistic regression retained *COMT*rs4646316 because it added significantly to the prediction model. The clinical-only model used the dichotomized variables age (median patient age cutoff: <8 years or >8 years), vincristine treatment, germ-cell tumor, and cranial irradiation. The combination of clinical and genetic variables significantly improved the prediction of cisplatin ototoxicity (Figure 1), yielding a higher area under the curve (AUC 0.786) than that of the clinical-only model (AUC 0.708, $P = 0.00048$).

A predictive multimarker model based on these genetic variants could stratify patients by risk of ototoxicity. Using this model, we defined risk groups according to predictive values (PVs) as at lower (PV < 0.4), intermediate (0.4–0.8), and high (>0.8) risk. Accordingly, 30 (9.5%) individuals were classified as at lower risk, 245 (77.3%) at intermediate risk, and 42 (13.2%) at high risk (Table 3). In the high-risk group, 39 (92.9%) individuals developed ototoxicity (positive predictive value (PPV) 92.9%) as compared with 3 (7.1%) controls, conferring a specificity of 97.6% (Table 4). In the lower-risk group, 8 (26.7%) individuals developed ototoxicity (PPV 73.3%) as compared with 22 (73.3%) controls, conferring a sensitivity of 95.9% (Table 4).

Individuals in the high-risk group also had a significantly higher risk of cisplatin-induced ototoxicity as compared with the lower- to intermediate-risk group ($P = 1.3 \times 10^{-4}$, OR 11.0; Table 4). Severity of hearing loss also increased with increasing risk-group status (Table 3). A Kaplan–Meier plot (Figure 2) shows that the intermediate- and high-risk groups have a significantly increased risk of hearing loss over time ($P_{\text{trend}} = 5.3 \times 10^{-9}$).

The model combining the effect of *TPMT*, *ABCC3*, and *COMT* was, however, able to distinguish patients as at lower or high risk better than the model based on *TPMT* alone (Table 4). Patients at lower risk could therefore be identified with more certainty if *ABCC3* and *COMT* are included in the model. However, the ability to identify patients at high risk was similar between the *TPMT*-only model and the combined model, which includes *TPMT*, *ABCC3*, and *COMT* (PPV 91.5 vs. 92.9%) (Table 4). As illustrated by a Kaplan–Meier plot, we were able to identify 97 (50.3%) patients at high risk using a model combining both clinical and genetic variables, as compared with only 39 (20.2%) patients using a model based on genetics alone (Figure 3, Table 4). In the high-risk group, 97 (91.5%) individuals developed hearing loss (PPV 91.5%) as compared with 9 (8.5%) controls, conferring a specificity of 92.7% and a sensitivity of 50.3% (Table 4).

DISCUSSION

The development of hearing loss after treatment with cisplatin leads to serious lifelong disability, particularly in children.² A method to identify patients at high risk of developing serious cisplatin-induced hearing loss would significantly improve the safety of pediatric cancer therapy.

Recently, we identified genetic variants associated with cisplatin-induced hearing loss in children after cancer therapy.⁹ The replication of genetic associations for validation in independent populations is critical before development and clinical implementation of guidelines. In this replication study, we recruited a large independent cohort of pediatric patients from across Canada who received cisplatin therapy. We confirmed the associations of genetic variants in *TPMT* and *ABCC3* with moderate to severe cisplatin-induced hearing loss. Genetic variants in *COMT* exhibited smaller effect sizes in the replication cohort (OR 2.5 vs. 1.3 and 5.5 vs. 1.4; previous vs. new cohorts). We generated a novel predictive model combining *TPMT*, *ABCC3*, and *COMT* genetic risk factors with clinical risk factors. The combined model was more predictive than a model based on clinical risk factors alone (AUC 0.786 vs. 0.708; $P = 0.00048$). The most highly associated *COMT* variant was included because it statistically significantly added to the predictive model with *TPMT* and *ABCC3*. The *COMT* and *ABCC3* variants do not contribute to the prediction of patients at high risk of cisplatin-induced ototoxicity significantly; instead they stratify patients between intermediate and lower risk. The model was based on results from the combined cohort; further studies in children are needed to validate the model and assess its clinical utility.

Our study is the first to replicate the reported association between *TPMT* (rs12201199, rs1142345, and rs1800460) and cisplatin-induced hearing loss in a large independent cohort of pediatric patients treated for a variety of malignancies. There is evidence that patients who are heterozygous or homozygous for these *TPMT* variants have an increased risk of

cisplatin-induced hearing loss, probably through reduced activity of the gene.⁹ The loss of function of *TPMT* is likely to increase the risk of cisplatin toxicity by inactivating the binding of the compound to purines in DNA, thereby regulating cisplatin cross-linking. Another potential mechanism of cisplatin toxicity is the accumulation of *S*-adenosylmethionine due to reduced activity of *TPMT*.^{9,30–32}

The association between the synonymous variant in *ABCC3* rs1051640 (E1503E) and cisplatin-induced hearing loss was also significantly replicated. Patients who carried the G allele of rs1051640 were at increased risk of developing hearing loss after cisplatin treatment. This is the first study to describe the role of genetic variation in *ABCC3* in the context of cisplatin ototoxicity. *ABCC3* is a transporter that mediates the efflux of organic anions, xenobiotics, and glutathione *S*-conjugates.^{33,34} One of the mechanisms by which cancer chemotherapies, including platinum drugs, are detoxified is through conjugation of the active metabolite to glutathione, making the compound more anionic,^{35,36} thus enabling the compounds to be more readily exported from cells through an ATP-dependent pump. Studies in rat hepatocyte cell lines have shown that both *ABCC2* and *ABCC3* protein levels and mRNA expression increased after treatment with cisplatin.^{37,38} Studies in lung cancer cells lines have also shown that *ABCC3* mRNA expression levels are significantly correlated with resistance to cisplatin and other platinum drugs.^{39,40} Reduction in *ABCC3* activity can affect the detoxification pathway, resulting in ineffective transport of toxic compounds out of the cell, which leads to toxicity. In turn, polymorphisms may regulate *ABCC3* levels or affect transporter function. Further studies are required to assess the exact mechanisms by which variants in *ABCC3* are associated with cisplatin-induced hearing loss.

The allele frequencies observed in our cohort were consistent with reported population frequencies of mainly European and Asian descent. For example, the minor allele frequency of *TPMT**3C ranges from 0.7 to 2.7% in Europeans and Asians, in line with our findings of 8.9% in cases and 1.6% in controls (HapMap). Furthermore, the minor allele frequency of rs1051640 ranges from 19% in the European population to 3.6% in Asian populations, in line with the observed 15% in cases and 26% in controls (HapMap).

Vincristine is a chemotherapy drug that is sometimes administered to patients receiving cisplatin. Whether vincristine itself is ototoxic is currently unclear. Case reports suggest that vincristine may be ototoxic^{20,21} or transiently ototoxic at high doses,¹⁴ whereas systematic clinical trials have reported that vincristine alone is not ototoxic.^{22,23} Bokemeyer *et al.* (1998) reported an increase in the prevalence of ototoxic symptoms in patients receiving vincristine together with cisplatin.¹⁴ To examine the effect of vincristine on the genetic associations with cisplatin-induced ototoxicity, binary logistic regression analyses were conducted with and without vincristine as a covariate. The regression analyses revealed that concomitant vincristine did not affect the associations of *TPMT*, *COMT*, and *ABCC3* variants with cisplatin-induced ototoxicity (Supplementary Table S3 online).

Based on our *a priori* power calculations considering the effect sizes and genotype frequencies of the original studies, we had sufficient power for all polymorphisms to find similar effect sizes in the replication cohort. However, several SNPs previously reported to have an association could not be replicated, limiting the use of these variants in the risk

prediction model. This is not surprising; many original studies have reported stronger genetic associations with larger effect sizes than in follow-up studies.^{41,42} There are several other potential reasons for nonreplication of initial findings. Significant interstudy heterogeneity can arise due to differences in the cohorts such as those related to population diversity, gender, and treatment protocols.^{41,42} This could lead to differences in the effects of variants in specific subpopulations or masking of the effects by factors that have not been sufficiently controlled for. We aimed to control for these factors by first evaluating power to ensure that the sample size is large enough to detect an association, then adjusting for clinical variables in the cohort, and finally carrying out a subgroup analysis in patients of only European ancestry. Nevertheless, a combination of these factors may explain why we could not significantly replicate the association between *COMT*, as well as other polymorphisms, and cisplatin-induced hearing loss. Another limitation of this study is the longer follow-up of cases than controls in the current replication cohort as compared with the initial cohort. This could introduce a potential source of bias but is likely to underestimate the positive results seen because rarely do controls have late adverse events of cisplatin therapy that were not uncovered during treatment or the 2-year period following treatment.

Combining the effect of *TPMT*, *ABCC3*, and *COMT* with clinical factors significantly improved the ability of the model to predict risk as compared with the model using clinical factors alone (AUC 0.786 vs. 0.708, $P = 0.00048$). By combining clinical risk factors and genetic markers, we could identify more patients at high risk as compared to using genetic factors alone (50.3% for combined vs. 20.2% for genetic alone). This is of particular importance in children, for whom even mild losses in hearing can cause difficulties in school performance.^{43,44} Several strategies have been proposed to prevent hearing loss in individuals at high risk of adverse effects from cisplatin. Patients might be placed on alternative medications that are less ototoxic (e.g., carboplatin, which is less ototoxic in older patients),^{16,45} receive increased monitoring for hearing loss, or be given otoprotective agents. However, the possibility of compromise of antitumor activity has raised concern.^{46,47} Hearing aids or cochlear implantation are also options used to manage cisplatin-induced hearing loss.

In conclusion, this study confirms previous findings and provides further evidence in an independent patient cohort for the associations of *TPMT* and *ABCC3* with cisplatin-induced hearing loss and for the association of *COMT* with cisplatin-induced hearing loss in the combined cohort. The combination of *TPMT*, *ABCC3*, and *COMT* with clinical variables provides a novel method that promises to improve the risk prediction of hearing loss from cisplatin therapy. With the discovery of additional variants through genome-wide association studies, the current predictive model may be improved and refined further. The combination of clinical and genetic risk factors can potentially improve risk classifications for hearing loss and may allow for individualized treatment.

METHODS

Patients

Study participants were recruited through the CPNDS, a national multicenter surveillance consortium for studying adverse drug reactions.⁴⁸ Between June 2008 and March 2011, a new independent replication cohort of 155 pediatric patients with cisplatin-induced hearing loss and drug-matched control patients were recruited. The previous cohort (discovery and replication) included in the combined analyses has been described previously.⁹

Cisplatin-induced hearing loss was diagnosed on the basis of audiometric findings using criteria described by the CTCAE v3 (Common Terminology Criteria for Adverse Events).⁴⁹ To better differentiate between cases and controls, subjects with serious cisplatin-induced hearing loss were defined as with grade 2 or higher hearing impairment. Control patients were defined as those with normal audiometric data after cisplatin therapy (grade 0). Patients with grade 1 ($n = 8$) hearing loss were excluded from the analysis. Serious hearing impairment (grades 2–4 hearing loss) was defined as the point at which chemotherapy protocols recommend reducing or terminating cisplatin treatment. Only the most recent audiological assessment was used. Informed written consent or assent was obtained from each subject or his or her parents/legal guardians. The study was approved by the University of British Columbia/Children & Women's Health Centre of British Columbia Research Ethics Board (H04-70358).

Genotyping

DNA of patients was extracted from blood, saliva, or buccal swabs using the QIAmp DNA purification system (Qiagen, Toronto, ON, Canada) according to the manufacturer's protocol. We selected 11 SNPs to assess whether the prediction of risk of hearing loss could be improved. We selected genetic variants for genotyping on the basis of evidence of a significant association ($P < 0.01$) in the initial combined cohort⁹ as well as in either the initial discovery or replication cohorts.⁹ We first assessed whether associations with variants in *TPMT* (rs12201199, rs1800460, and rs1142345) and *COMT* (rs9332377 and rs4646316) could be replicated in the current replication cohort. We then assessed whether the associations with other variants in *ABCC3* (rs1051640), *MTHFR* (rs3737964), *VKORC1* (rs17884333 and rs8050894), and *SLCO1A2* (rs4115170 and rs2306231) could be replicated. Patient DNA was genotyped using a custom Illumina GoldenGate SNP genotyping assay (Illumina, San Diego, CA). This assay included additional nonstudy SNPs for principal component analysis for the determination of patient ancestry and for quality-control purposes. All SNP genotype data were clustered manually using GenomeStudio software (Illumina, San Diego, CA). Samples with a call rate below 95% were excluded. The average genotyping call rate for included samples was 98.3%. The overall genotype call rate of the 11 study SNPs was 99.9% and all SNPs were in Hardy–Weinberg equilibrium ($P > 0.05$).

Statistical analysis

Clinical characteristics of patients with and without cisplatin-induced hearing loss were compared using the Wilcoxon–Mann–Whitney U -test for continuous variables and Fisher's

exact test for categorical variables. Hardy–Weinberg equilibrium was confirmed in cases and controls using the permutation version of the exact test of Hardy–Weinberg of Guo and Thompson.⁵⁰

The study had a statistical power between 65 and 99% (mean 86%) based on the previous effect sizes and genotype frequencies, in order to replicate previously associated polymorphisms with cisplatin-induced hearing loss at $P=0.05$. Two genetic variants in *SLCO1A2* (rs11045913 and rs11045912) were excluded prior to genotyping due to inadequate power (<10%) in detecting an association. Homozygous and heterozygous odds ratios (ORs) were calculated using the homozygous genotype of the protective allele as identified in the initial cohort as a reference.⁹ ORs in the case of empty cells was calculated by adding 0.5 to the empty cells. The association between genetic polymorphisms and cisplatin-induced hearing loss was assessed by computing Fisher's exact test for unadjusted (nonregression) P -values and adjusted P -values determined by logistic regression. Independent clinical factors for the prediction of cisplatin-induced hearing loss in the combined cohort were identified by forward logistic regression. The following factors were included: the first principal component, patient age, cisplatin dose, treatment duration, vincristine treatment, and germ-cell tumor. None of the variables was retained in the combined cohort at a threshold of 0.10 except concomitant vincristine treatment ($P=1.4 \times 10^{-9}$). However, we also included variables that are known to increase risk of hearing loss and that were significantly different ($P=0.05$) between cases and controls. Therefore, patient age, concomitant vincristine treatment, germ-cell tumor, and cranial irradiation were included as covariates. The treatment of all non-CNS germ-cell tumors is unique because these patients are treated at low individual doses of 20 mg/m² over 5 days and are known to be at less risk of hearing loss.⁵¹ In an additional analysis, we combined the initial cohort⁹ and the current cisplatin replication cohort of patients to determine the overall significance of associations. Associations with variants were considered to be statistically significant if they had $P < 0.05$ in the new replication cohort.

Population stratification in the data set was assessed by principal component analysis using SVS/HelixTree software.^{29,52} Patients of African ancestry ($n=8$) were excluded from the analysis to match the ancestry of the previous cohort.^{9,52} A graphical display of principal components was constructed using GraphPad Prism 5 software (GraphPad Software, San Diego, CA). A secondary stratified analysis of individuals of only European ancestry was based on the first two principal components.

Genetic variants were further evaluated in a multivariate logistic regression model including clinical variables using an additive model. A risk score was calculated by multiplying each variable with the estimated β (log odds ratio) from the current combined cohort. The clinical-only model included dichotomized variables. Therefore, age (median patient age: <8 years or >8 years), vincristine treatment, germ-cell tumor, and cranial irradiation were included in the model. The genetic-only model included variants (*TPMT* rs12201199, *ABCC3* rs1051640, and *COMT* rs4646316) that were selected based on statistical evidence from a forward logistic regression model in the combined cohort.

The association between genetic risk score and cisplatin-induced hearing loss was assessed using logistic regression. Three prediction models were investigated: (i) clinical variables only, (ii) genetic variables only, and (iii) clinical and genetic variables. The contribution of genetic and/or clinical risk scores to the prediction of cisplatin-induced hearing loss was investigated by comparing the area under the receiver operating characteristic curves of the prediction models. AUC estimates were obtained using the receiver operating characteristic plot function on the basis of the linear predictors obtained from the logistic regression model for clinical factors and genotypic scores. The statistical difference between the curves was calculated using DeLong's method⁵² implemented in the *R* package pROC.⁵³ Two-sided *P*-values < 0.05 were considered statistically significant.

Risk groups were defined on the basis of the PVs for each patient sample from the multi-SNP logistic regression model that included three SNPs (*TPMT*rs12201199, *ABCC3*rs1051640, and *COMT*rs4646316). The threshold for genetic variables used to determine lower risk was the median PV of controls minus one standard deviation (PV < 0.4); high risk was defined as the median PV of cases plus one standard deviation (>0.8). Intermediate risk was defined as a PV between 0.4 and 0.8. The threshold for the combination of clinical and genetic variables for lower risk was defined as the median PV of controls (PV < 0.45), and high risk was defined as the median PV of cases (>0.8). Intermediate risk was defined as a PV between 0.45 and 0.8. We calculated sensitivity, specificity, PPV, and negative predictive value for each defined threshold. Kaplan–Meier curves were generated for each risk group using time from start of treatment to the time of first evidence of toxicity or last audiogram. Log-rank test was used to compare the trends of survival curves. Hazard ratios for each curve were calculated using the Cox regression model considering the lower risk group as the reference.

Statistical genetic analyses were performed using SNP and Variation Suite 7.4.5 (Golden Helix, Bozeman, MT), SPSS Statistics 19 (SPSS, Chicago, IL), and R 2.13.0 (R Development Core Team).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

- Cisplatin is a widely used chemotherapeutic agent in the standard treatment of a variety of solid tumors. A serious complication of cisplatin treatment is permanent hearing loss. A previous study identified genetic variants in *TPMT* and *COMT* that were highly associated with cisplatin-induced hearing loss in children.

WHAT QUESTION DID THIS STUDY ADDRESS?

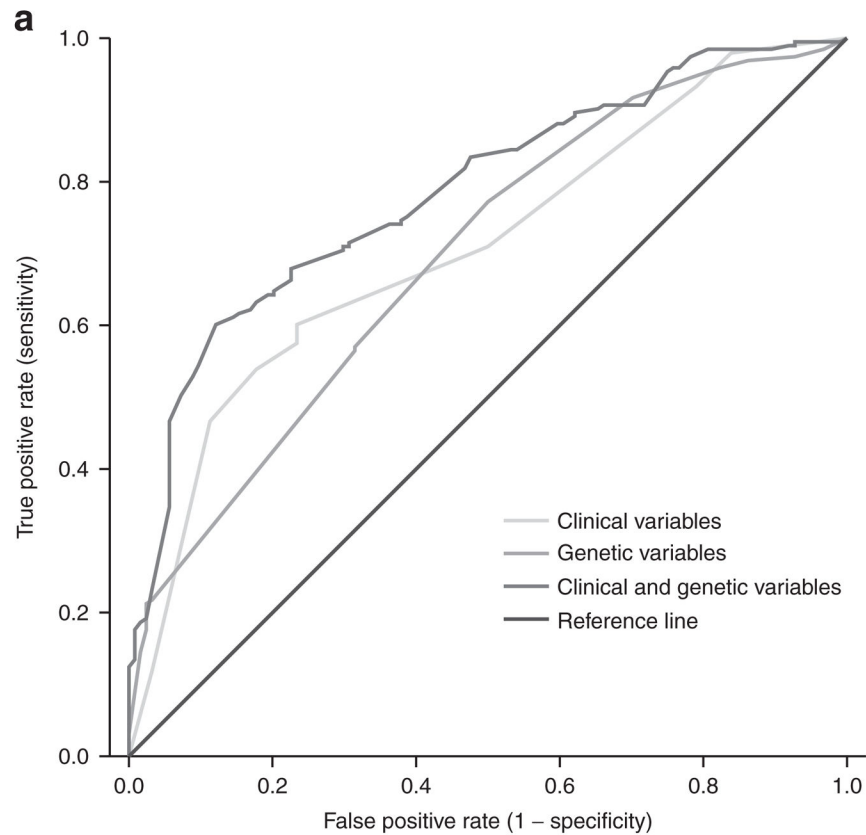
- The aim of this study was to replicate previously described genetic factors and identify others that increase susceptibility to cisplatin-induced hearing loss, as well as to develop predictive models for the identification of patients at risk of toxicity.

WHAT THIS STUDY ADDS TO OUR KNOWLEDGE

- This study strengthens the evidence of previous findings and provides evidence for novel genetic variants in the prediction of cisplatin-induced hearing loss in children. Furthermore, the study demonstrates that predictive models combining clinical and genetic factors can classify patients based on predicted risk of cisplatin-induced hearing loss.

HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY AND THERAPEUTICS

- The findings have the potential to influence treatment decisions toward improving safety and efficacy of cisplatin use in children.



b

Model	AUC (95% CI)	<i>P</i> -value
Clinical variables	0.708 (0.651–0.765)	–
Genetic variables (three SNPs)	0.696 (0.638–0.754)	0.77
Clinical and genetic variables	0.786 (0.736–0.836)	0.00048

Figure 1. Receiver operating characteristic curves of clinical and genetic variables for the prediction of cisplatin-induced hearing loss in the combined cohort ($n = 317$). **(a)** Clinical variables are age, vincristine treatment, germ-cell tumor, and cranial irradiation, whereas genetic variables combine the effect of *TPMT*rs12201199, *COMT*rs4646316, and *ABCC3*rs1051640. **(b)** The area under the curve for the combined cohort for each model. The *P*-values indicate the statistical significance between the curves for the combination of genetic and clinical variables as compared with clinical variables alone. CI, confidence interval; SNP, single-nucleotide polymorphism.

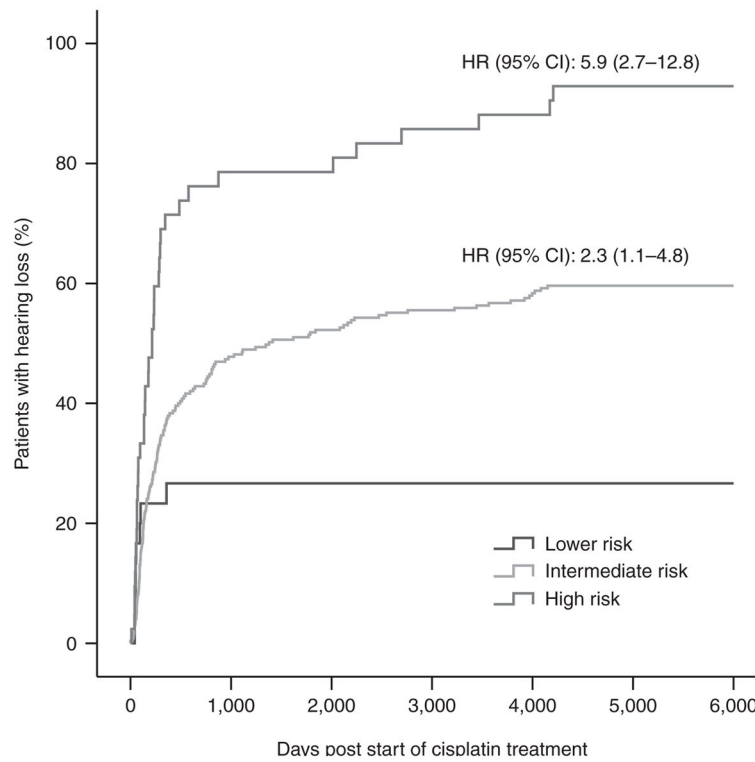


Figure 2. Kaplan–Meier curve of cisplatin-induced hearing loss in three different risk groups (Table 3) combining genetic factors (*TPMT*rs12201199, *ABCC3*rs1051640, and *COMT*rs4646316). The curves show that the incidence of hearing loss increases with increasing risk group status. Hazard ratios (HRs) were used to compare curves with that of the lower risk group and were adjusted for clinical variables ($P_{\text{trend}} = 5.3 \times 10^{-9}$). CI, confidence interval.

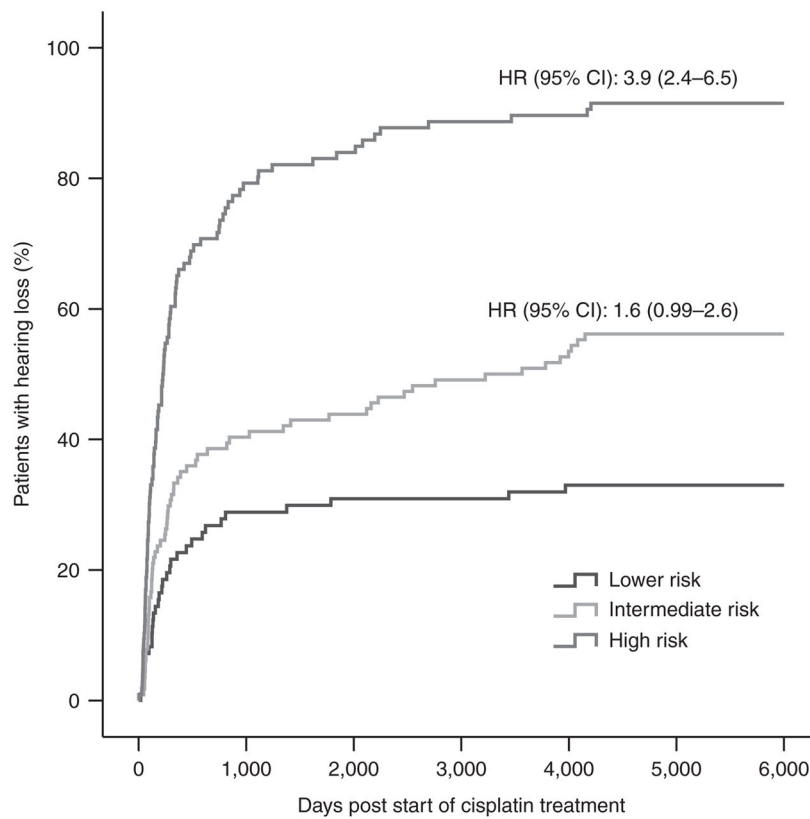


Figure 3.

Kaplan–Meier curve of cisplatin-induced hearing loss in three different risk groups (Table 3) combining clinical and genetic information. Clinical variables are age, vincristine treatment, germ-cell tumor, and cranial irradiation, whereas genetic variables combine the effect of *TPMT* rs12201199, *COMT* rs4646316, and *ABCC3* rs1051640. The curves show that the incidence of hearing loss increases with increasing risk group status. Hazard ratios (HRs) were used to compare curves with that of the lower risk group and were adjusted for clinical variables ($P_{\text{trend}} = 3.4 \times 10^{-19}$). CI, confidence interval.

Table 1

Patient demographics

	Initial cohort ^a (n = 162)		Replication cohort (n = 155)		Combined cohort (n = 317)		P-value
	Cases (n = 106)	Controls (n = 56)	Cases (n = 87)	Controls (n = 68)	Cases (n = 193)	Controls (n = 124)	
Age, years (median (min, max))	6.0 (0, 16)	9.0 (0, 19)	6.0 (1, 25)	11.0 (0, 18)	6.0 (0, 25)	10.0 (0, 19)	0.012
Dose, cumulative mg/m ² (median (min, max))	400 (120, 720)	400 (100, 720)	400 (92, 800)	400 (20, 768)	400 (92, 800)	400 (20, 768)	0.71
Treatment duration, months (median (min, max))	5.0 (1, 12)	4.0 (1, 11)	4.0 (0, 14)	4.0 (0, 15)	4.0 (0, 14)	4.0 (0, 15)	0.85
Gender (male, n (%))	71 (67.0%)	28 (50.0%)	43 (49.4%)	34 (50.0%)	114 (59.1%)	62 (50.0%)	0.13
Caucasian ethnicity ^b (n (%))	80 (75.5%)	48 (85.7%)	70 (80.5%)	54 (79.4%)	150 (77.7%)	102 (82.3%)	0.39
Concomitant medication (n (%))							
Tobramycin	32 (30.2%)	15 (26.8%)	23 (26.4%)	15 (22.1%)	55 (28.5%)	30 (24.2%)	0.44
Vancomycin	25 (23.6%)	11 (19.6%)	26 (29.9%)	14 (20.6%)	51 (26.4%)	25 (20.2%)	0.23
Vincristine	54 (50.9%)	10 (17.9%)	4.1 × 10 ⁻⁵ 58 (66.7%)	19 (27.9%)	2.1 × 10 ⁻⁶ 112 (58.0%)	29 (23.4%)	1.1 × 10⁻⁹
Gentamicin	21 (19.8%)	7 (12.5%)	21 (24.1%)	19 (27.9%)	42 (21.8%)	26 (21.0%)	0.89
Tumor type (n (%))							
Brain tumor	25 (23.6%)	8 (14.3%)	26 (29.9%)	11 (16.2%)	51 (26.4%)	19 (15.3%)	0.026
Endodermal sinus tumor of thymus	0	1 (1.8%)	0	1 (1.5%)	0	2 (1.6%)	0.15
Germ-cell tumor	7 (6.6%)	15 (26.8%)	7 (8.0%)	11 (16.2%)	14 (7.3%)	26 (21.0%)	0.00046
Hepatoblastoma	22 (20.8%)	5 (8.9%)	17 (19.5%)	7 (10.3%)	39 (20.2%)	12 (9.7%)	0.013
Lymphoma	0	1 (1.8%)	1 (1.1%)	2 (2.9%)	1 (0.5%)	3 (2.4%)	0.30
Nasopharyngeal carcinoma	1 (0.9%)	0	0	2 (2.9%)	1 (0.5%)	2 (1.6%)	0.56
Neuroblastoma	26 (24.5%)	9 (16.1%)	24 (27.6%)	12 (17.6%)	50 (25.9%)	21 (16.9%)	0.073
Osteosarcoma	24 (22.6%)	16 (28.6%)	10 (11.5%)	20 (29.4%)	34 (17.6%)	36 (29.0%)	0.019
Other sarcoma	1 (0.9%)	1 (1.8%)	0	1 (1.5%)	1 (0.5%)	2 (1.6%)	0.56
Other carcinoma	0	0	0	1 (1.5%)	0	1 (0.8%)	0.39
Retinoblastoma	0	0	1 (1.1%)	0	1 (0.5%)	0	1.00
Mesenchymal tumor of the liver	0	0	1 (1.1%)	0	1 (0.5%)	0	1.00

	Initial cohort ^d (n = 162)		Replication cohort (n = 155)		Combined cohort (n = 317)	
	Cases (n = 106)	Controls (n = 56)	Cases (n = 87)	Controls (n = 68)	Cases (n = 193)	Controls (n = 124)
Follow-up, years (median (min, max))	3 (0, 18)	2 (0, 15)	5 (0, 25)	2 (0, 16)	4 (0, 25)	2 (0, 16)
Cranial irradiation (n (%))	23 (21.7%)	7 (12.5%)	20 (23.0%)	8 (11.8%)	43 (22.3%)	15 (12.1%)
					0.00021	0.025
					0.093	

For age, dose, treatment duration, and follow-up, the Wilcoxon–Mann–Whitney test with normal approximation was used. For gender, ethnicity, concomitant medication, tumor type, and cranial irradiation, the Fisher's exact test was used. Boldface numbers are statistically significant values at $P < 0.05$ type I error rate.

Max, maximum; min, minimum.

^aResults from the initial combined cohort⁹ compared with the current replication cohort as well as all cohorts combined.

^bCaucasian ethnicity assessed by principal component analysis.

Table 2

Genetic variants associated with cisplatin-induced hearing loss

SNP	Allele	Initial cohort ^a (n = 162)			Replication cohort (n = 155)			Combined cohort (n = 317)						
		Case	Ctrl	OR (95% CI)	P-value	Case	Ctrl	OR (95% CI)	P-value	Case	Ctrl	OR (95% CI)	P-value	P-value ^b
<i>TPMT</i>														
rs12201199	A	28	1	16.9 (2.3–125.9)	0.00022	21	3	6.1 (1.8–20.9)	0.0013	49	4	8.9 (3.2–24.9)	8.7 × 10⁻⁷	4.0 × 10⁻⁵
	T	184	111			153	133			337	244			
rs1142345	G	19	1	10.9 (1.4–82.7)	0.0017	16	3	4.5 (1.3–15.7)	0.011	35	4	6.1 (2.1–17.3)	0.00014	0.00039
	A	193	111			158	133			351	244			
rs1800460	A	16	0	18.0 (1.1–302.7)	0.0031	13	3	3.6 (1.0–12.8)	0.038	29	3	6.6 (2.0–21.8)	0.00043	0.00073
	G	196	110			161	133			357	243			
<i>COMT</i>														
rs4646316	G	176	74	2.5 (1.5–4.3)	0.00055	141	104	1.3 (0.8–2.3)	0.33	317	178	1.8 (1.2–2.6)	0.0021	0.0068
	A	36	38			33	32			69	70			
rs9332377	A	36	4	5.5 (1.9–16.0)	0.00018	38	23	1.4 (0.8–2.4)	0.28	74	27	1.9 (1.2–3.1)	0.0054	0.043
	G	176	108			136	113			312	221			
<i>ABCC3</i>														
rs1051640	G	182	83	2.1 (1.2–3.8)	0.0092	146	101	1.8 (1.0–3.3)	0.036	328	184	2.0 (1.3–2.9)	0.00078	0.0033
	A	30	29			28	35			58	64			

Boldface numbers are results more significant in current combined cohort than in previous combined cohort (ref. 9).

CI, confidence interval; Ctrl, control; OR, odds ratio; SNP, single-nucleotide polymorphism.

^aResults from the initial combined cohort (ref. 9) compared with the current replication cohort as well as all cohorts combined.

^b Adjusted for age, vincristine treatment, germ-cell tumor, and cranial irradiation.

Table 3

Risk group comparisons and grade of hearing loss

	Patients with hearing loss (number (%^a))	Normal-hearing controls (number (%^a))	Total (number (%^b))	Grade of hearing loss (mean \pm SEM)
Lower risk (<0.4)	8 (26.7%)	22 (73.3%)	30 (9.5%)	0.77 \pm 0.24
Intermediate risk (0.4–0.8)	146 (59.6%)	99 (40.4%)	245 (77.3%)	1.69 \pm 0.092
High risk (>0.8)	39 (92.7%)	3 (7.1%)	42 (13.2%)	2.71 \pm 0.13

^aPercentage in risk group.^bPercentage of total.

Table 4

Comparison of risk groups

Model	Risk group	Ototox. (number, %)	Controls (number, %)	OR (95% CI) ^d	P-value ^d	Sens	Spec	PPV	NPV
<i>TPMT</i> only	Risk haplotype carriers ^b	43 (91.5%)	4 (8.5%)	9.3 (3.1, 27.4)	5.5×10^{-5}	22.3%	96.8%	91.5%	44.4%
	Non-carriers	150 (55.6%)	120 (44.4%)						
<i>TPMT, ABCC3, COMT</i>	High (>0.8)	39 (92.9%)	3 (7.1%)	11.0 (3.2, 37.6)	1.3×10^{-4}	20.2%	97.6%	92.9%	44.0%
	Low plus intermediate (<0.8)	154 (56.0%)	121 (44.0%)						
<i>TPMT, ABCC3, COMT</i>	High plus intermediate(>0.4)	185 (64.5%)	102 (35.5%)	4.8 (1.9, 11.9)	7.1×10^{-4}	95.9%	17.7%	64.5%	73.3%
	Low (<0.4)	8 (26.7%)	22 (73.3%)						
Clinical + <i>TPMT, ABCC3, COMT</i>	High (>0.80)	97 (91.5%)	9 (8.5%)	8.8 (4.0, 19.2)	5.5×10^{-8}	50.3%	92.7%	91.5%	54.5%
	Low plus intermediate (<0.8)	96 (45.5%)	115 (54.5%)						
Clinical + <i>TPMT, ABCC3, COMT</i>	High plus intermediate(>0.45)	161 (73.2%)	59 (26.8%)	3.1 (1.6, 5.9)	4.3×10^{-4}	83.4%	52.4%	73.2%	67.0%
	Low (<0.45)	32 (33.0%)	65 (67.0%)						

CI, confidence interval; NPV, negative predictive value; OR, odds ratio; Ototox, ototoxicity; PPV, positive predictive value; Sens, sensitivity; Spec, specificity.

^a Adjusted for age, vincristine treatment, germ-cell tumor, and cranial irradiation.

^b Risk haplotype carriers are individuals that are heterozygous or homozygous for *TPMT* rs12201199 risk variant.