Published in final edited form as:
Clin Pharmacol Ther. 2013 August ; 94(2): . doi:10.1038/clpt.2013.121.

The Role of Inherited *TPMT* and *COMT* Genetic Variation in Cisplatin-induced Ototoxicity in Children with Cancer

Jun J. Yang[1], **Joshua Yew-Suang Lim**[1,2], **Jie Huang**[3], **Johnnie Bass**[4], **Jianrong Wu**[3], **Chong Wang**[3], **Jie Fang**[5], **Elizabeth Stewart**[6], **Elaine H. Harstead**[1], **E Shuyu**[1], **Giles W. Robinson**[6], **William E. Evans**[1], **Alberto Pappo**[6], **Jian Zuo**[5], **Mary V. Relling**[1], **Arzu Onar-Thomas**[3], **Amar Gajjar**[6], and **Clinton F. Stewart**[1]

[1]Department of Pharmaceutical Sciences, St. Jude Children's Research Hospital, Memphis, TN

[2]Department of Pediatrics, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

[3]Department of Biostatistics, St. Jude Children's Research Hospital, Memphis, TN

[4]Department of Pediatric Medicine, St. Jude Children's Research Hospital, Memphis, TN

[5]Department of Developmental Neurobiology, St. Jude Children's Research Hospital, Memphis, TN

[6]Deparment of Oncology, St. Jude Children's Research Hospital, Memphis, TN

Abstract

Ototoxicity is a debilitating side effect of platinating agents with substantial inter-patient variability. We sought to evaluate the association of *TPMT* and *COMT* genetic variations with cisplatin-related hearing damage in the context of frontline pediatric cancer treatment protocols. In 213 children from St. Jude Medulloblastoma-96 and -03 protocols, hearing loss was related to younger age (P=0.013) and craniospinal irradiation (P=0.001), but did not differ by *TPMT* or *COMT* variants. Results were similar in an independent cohort of 41 children from solid tumor frontline protocols. Functional hearing loss or hair cell damage was not different in *TPMT* knockout vs. wildtype mice following cisplatin treatment, and neither *TPMT* nor *COMT* variant was associated with cisplatin cytotoxicity in lymphoblastoid cell lines. In conclusion, our results indicated that *TPMT* or *COMT* genetic variation was not related to cisplatin ototoxicity in children with cancer and did not influence cisplatin-induced hearing damage in laboratory models.

Keywords

pharmacogenetics; cisplatin; ototoxicity; *TPMT*; and pediatric cancer

Corresponding author: Clinton F. Stewart, PharmD, Pharmaceutical Sciences, MS313, St. Jude Children's Research Hospital, 262 Danny Thomas Place, Memphis, TN 38105-3678, clinton.stewart@stjude.org, Phone: (901) 595-3665, FAX: (901) 525-6869.

Conflict of Interest: The authors declared no conflict of interest.

Author Contributions are: J.J.Y., A.G., C.F.S. designed the research. J.Y.S.L., E.H.H., J.B., J.F., E.S., M.V.R., G.W.R., A.P., J.Z., A.G., and C.F.S. performed the research. J.Y.S.L., J.H., J.W., C.W., J.F., and A.O.-T. analyzed the data. J.J.Y., J.Y.S.L., J.F., W.E.E., J.Z., M.V.R., A.O.-T., A.G., and C.F.S. wrote the manuscript.

INTRODUCTION

While the treatment outcomes of pediatric cancers have improved, the increase in survival has come with a price in that over 40% survivors experience long-term complications[1]. Cisplatin, a critical component of almost all contemporary pediatric brain and solid tumor treatment regimens, is associated with severe auditory side effects[2]. Bilateral and irreversible hearing loss secondary to platinating agents-containing therapy has particularly debilitating impact on developing children with possible lifelong impairment in language development and significantly diminished quality of life. More importantly, substantial variability in ototoxicity exists within individuals receiving similar cisplatin therapy, leading to the postulation that inter-individual genetic variation may contribute to the risk of cisplatin-induced ototoxicity.

Studies of cisplatin sensitivity in lymphoblastoid cells derived from family members indicate that 38–47% of variability in cisplatin cytotoxicity is mediated by inherited genetic variation[3], strongly arguing for existence of pharmacogenetic variants associated with cisplatin response in patients. However, results from candidate-gene pharmacogenetic studies have been inconsistent, e.g., *GSTM1* deletion modestly increased the risk of ototoxicity in testicular cancer survivors[4], but was not associated with cisplatin-related hearing loss in children[5]. In a more recent study of ~2,000 genetic variants in drug metabolism genes in 166 pediatric cancer patients, germline variants in the thiopurine S-methyltransferase (*TPMT*) and catechol O-methyltransferase (*COMT*) genes strongly influenced the risk of cisplatin-related hearing damage and genotype-based prediction identified 92.9% at-risk patients[5]. Subsequently, the Food and Drug Administration changed the cisplatin label to indicate the association of *TPMT* with ototoxicity.

Given the potential relevance of *TPMT* and *COMT* in cisplatin treatment individualization, it is imperative to evaluate these pharmacogenetic associations in the context of well-controlled clinical protocols of children with cancer. Thus, we sought to determine the associations of *TPMT* and *COMT* genetic variants with cisplatin ototoxicity in frontline treatment protocols of pediatric brain and solid tumors in a single-institution setting, with experimental validation using *in vitro* and *in vivo* laboratory models.

RESULTS

In 213 children from the SJMB-96 and -03 protocols, 70% of patients experienced ototoxicity (CTCAE > grade 0), the majority of which occurred between 0.5 and 6 months from the initiation of cisplatin chemotherapy (Figure 1). In univariate analyses, the risk of ototoxicity was inversely associated with age at diagnosis (P=0.0128) but did not differ by self-reported ethnicity or gender (Table 1). Ototoxicity was more common in patients receiving ≥25Gy of craniospinal irradiation than those with <25 Gy of exposure (P=0.0010), and was also more common in SJMB-96 than in the subsequent SJMB-03 protocol (P=0.0479) (Table 1). The use of amifostine was linked to reduced frequency of ototoxic events, although the differences were not statistically significant in our cohort (Table 1). Four *TPMT* variants (rs1800462, rs12201199, rs1800460 and rs1142345) and 3 *COMT* variants (rs4818, rs4646316 and rs9332377) were selected for genotyping[5]. rs1800462 and rs4818 were excluded from genotype-phenotype association analyses due to monomorphism and deviation from Hardy-Weinberg equilibrium, respectively. In the univariate analyses using an additive genetic model, none of the *TPMT* or *COMT* SNPs was significantly associated with the risk of ototoxicity at the P<0.05 level (Table 1). In fact, there was a trend for less ototoxicity in those with the variant alleles at *TPMT* SNPs rs1142345 (P=0.14, Figure 2A and Supplementary Figure S1) and rs1800460 (P=0.11, Supplementary Figure S2). Ototoxicity did not differ by genotype at rs12201199 (P=0.50, Supplementary Figure

S3). For *COMT* SNP rs4646316, the incidence of ototoxicity tended to be highest in patients with the GG genotype (73.0%), intermediate in the GA group (67.6%), and lowest in patients with the AA genotype (53.9%, Figure 2B and Supplementary Figure S4), although not statistically significant (*P*=0.15). Genotypes at *COMT* SNP rs9332377 were not associated with ototoxicity (*P*=0.78, Supplementary Figure S5). Results were similar when these analyses were stratified on self-reported ethnicity (Figure 2).

To ensure that the lack of association at *TPMT* and *COMT* SNPs was not a result of biased dichotomization of ototoxicity grades, we performed additional analyses using 5 different hearing loss classifications: CTCAE grade 0 vs. ≥2 (dichotomized); CTCAE grades 0–4 (ordinal variable); Chang grade 0 vs. >0 (dichotomized); Chang grade <2a vs. ≥2a (dichotomized); and Chang grades 0–4 (ordinal variable). Again, we did not observe any significant associations of *TPMT* or *COMT* genotypes with cisplatin-induced ototoxicity (Table 2 and Supplementary Figures S1–5). We also performed multivariate analyses including both non-genetic and genetic variables. Regardless of whether ototoxicity was defined as a dichotomous variable or as an ordinal variable, younger age at diagnosis, higher dose of craniospinal irradiation, and being enrolled on SJMB-96 protocol were independently associated with higher risk of ototoxicity (data not shown). When ototoxicity was classified as Chang <2a vs. ≥2a, male gender was linked to higher risk of ototoxicity (data not shown). However, none of the multivariate models indicated any association between *TPMT* or *COMT* SNP genotypes and ototoxicity.

Children enrolled on SJMB-96 and SJMB-03 protocols received craniospinal irradiation, a known risk factor of ototoxicity, and were treated with amifostine, an otoprotectant. To rule out confounding effects of these treatment factors, we also performed association analyses in an independent cohort of St. Jude patients with neuroblastoma and osteosarcoma who received cisplatin-containing regiments without craniospinal irradiation or amifostine (St. Jude NB-97, NB-05, and OS-08 protocols; N=41, Supplementary Table S1). Based on an ordinal regression model, ototoxicity was more severe in patients diagnosed at younger age (*P*=0.0052) and in boys (*P*=0.0265). Again, no association was observed between *TPMT* or *COMT* SNP genotypes with platinum-related hearing damage, although the statistical power was limited due to a relatively small sample size of this cohort.

Finally, we evaluated cisplatin-induced auditory damage *in vivo* using mice with different *TPMT* genotypes. After 4 consecutive daily injections of cisplatin, hearing loss was substantial as determined by the shift of auditory brainstem response (ABR) threshold at 6, 16, and 32 kHz and it was particularly profound at high frequency (Figure 3A). The loss of functional hearing was supported by frequency-specific damage of hair cells in the cochlea (Figure 3B). However, no difference in hearing damage (by either ABR or morphology) was observed between *TPMT* wildtype (N=6) and knockout mice (N=5) (Figure 3). Also, we examined the relationship between *TPMT* and *COMT* genetic variations and cisplatin cytotoxicity in lymphoblastoid cell lines (N=116, HapMap CEU and YRI cells)[6, 7]. Cisplatin IC_{50} was not associated with *TPMT* or *COMT* SNP genotypes (Supplementary Figure S6), nor with *TPMT* enzymatic activity (Supplementary Figure S7). Together, these results were consistent with our observations in patients and further argue against any direct role of *TPMT* or *COMT* in cisplatin-induced ototoxicity.

DISCUSSION

In the current study, we comprehensively examined the effects of *TPMT* and *COMT* on platinating agents-related hearing loss, in children treated on cisplatin-containing frontline protocols and in both *in vitro* and *in vivo* laboratory models. We consistently observed no association of *TPMT* or *COMT* with ototoxicity and our results argue against the hypothesis

that *TPMT* or *COMT* genetic variation is informative for the risk of clinical ototoxicity of cisplatin.

The association between *TPMT* variants and cisplatin ototoxicity described by Ross *et al.*[5] was unexpected, given that cisplatin is not a *TPMT* substrate and the fact that there are no known substrates for *TPMT* besides thiopurines[8]. *COMT* has been implicated in sensorineuronal deafness in both mouse and human[9], although the molecular mechanisms linking *COMT* to cisplatin-induced hearing damage are unclear. In fact, in children with medulloblastoma, there was a trend that G allele at *COMT* SNP rs4646316 was linked to higher incidence of ototoxicity (Figure 2B). Although this difference was not statistically significant, the directionality was consistent with the previous report[5]. Like many other drug response phenotypes, the genetic basis of cisplatin ototoxicity is likely to be complex and a number of biological pathways have been proposed based on results from animal and human studies. For example, outer hair cells and hair cells within high frequency regions in mouse cochlea are particularly susceptible to cisplatin-induced apoptosis (Figure 3), plausibly related to generation of reactive oxygen species[10] and expression of cisplatin uptake transporters (e.g., *OCT2*[11] and *CTR1*[12]). However, the exact genetic basis of cisplatin ototoxicity remains largely unclear and options for therapeutic intervention are extremely limited. The hypothesis had also been proposed[5] that TPMT/COMT deficiency (e.g., subjects with *TPMT* mutant genotype) could result in excess intracellular s-adenosylmethionine (SAM), a substrate of *TPMT* and *COMT*, and higher levels of SAM in turn potentiate the cytotoxic effects of cisplatin, explaining the link of *TPMT* and *COMT* variants with ototoxicity. However, direct measurement of SAM in red blood cells from healthy individuals showed no difference between subjects with wildtype *TPMT* (*1/*1, N=115) vs. carriers of *TPMT* loss-of-function variants (*1/*3, N=44, P=0.69)[13], challenging the notion that *TPMT* status can substantially influence SAM homeostasis. The direct relationship between COMT and SAM *in vivo* remains unclear.

Pharmacogenetic associations (relationships between genotype and drug response) are commonly confounded by non-genetic factors that can be highly variable in a clinical setting. Comparing and contrasting our current study and that by Ross *et al*[5], a number of differences in therapy are notable and could in theory contribute to the differences in the results. For example, all children in our brain tumor cohort received craniospinal irradiation prior to cisplatin therapy per SJMB protocols. Because radiotherapy is a potential risk factor for hearing loss[14, 15], questions naturally arise as to whether ototoxic events in this cohort could have been influenced by irradiation as a part of cancer treatment. To minimize the confounding effects of irradiation, we focused on early ototoxic events (i.e., within 15 months after cisplatin treatment was initiated) because hearing loss related to conformal radiotherapy has been reported to have a late onset (median time to first ototoxic event of 3.4 years post radiotherapy and minimum time to event of 18 months)[16–18]. In fact, almost all ototoxic events in the SJMB-96 and SJMB-03 cohorts occurred within a narrow window of 0.5–6 months after cisplatin treatment initiation, consistent with the temporal pattern of cisplatin ototoxicity reported previously[2]. Our additional analyses in cisplatin-treated children with solid tumors who did not receive craniospinal irradiation showed no association of *TPMT* or *COMT* with ototoxicity, further arguing against confounding effects by irradiation. Although a majority of patients enrolled on the SJMB-96 and SJMB-03 protocols were treated with amifostine as a prophylaxis for cisplatin ototoxicity, there is little biological evidence that amifostine might modify the effects of *TPMT* or *COMT* on hearing loss. Also, we did not observe any associations of ototoxicity with *TPMT* or *COMT* in children with solid tumors who did not receive amifostine, although the statistical power was limited due to the small sample size of this cohort. Nonetheless, cisplatin ototoxicity is cumulative and dose-dependent and could therefore vary substantially depending upon cisplatin regimens[19, 20].

In conclusion, our results do not support the clinical utility of *TPMT* or *COMT* genotyping to identify patient at risk of cisplatin-induced ototoxicity. Additional studies, particularly large-scale genome-wide association studies in the context of well-controlled clinical protocols, are warranted to ascertain genetic features that predispose patients to the development of drug-related hearing loss.

METHODS

Patients and treatment

This study was approved by institutional review board and informed consent was obtained from all patients, parents, or legal guardians as appropriate.

Children with brain tumors—Included in this study were 213 children with newly-diagnosed medulloblastoma enrolled on the St. Jude Medulloblastoma 96 (SJMB-96) (clincialtrials.gov: NCT00003211) or SJMB-03 (NCT00085202) protocols for whom audiology assessment was performed prior to and between 9 and 18 months from therapy initiation, and germline DNA was available. Patients with no cisplatin dose information (N=3) and/or with hearing loss before protocol enrollment (N=4) were excluded. Comparing study participants (included in the genetic analyses) with non-participants (treated on the clinical treatment protocols but not included in genetic analyses), we did not observe any significant differences in demographic or clinical features (data not shown).

SJMB-96 and SJMB-03 are two St. Jude-initiated sequential frontline treatment protocols for newly-diagnosed medulloblastoma, enrolling patients during 1996–2003 and during 2003–2012, respectively. Tumor risk classification and details of treatment plan of SJMB-96 protocol were described previously[21]. Briefly, patients with high-risk (M+ and/or non-gross totally resected) medulloblastoma underwent craniospinal radiotherapy (M0–1, 36 Gy; M2–3, 39.6 Gy) with a three-dimensional conformal boost to the tumor bed (total dose 55.8 Gy) and, where appropriate, to local sites of metastasis (total dose 50.4 Gy). Those with average-risk disease (M0 and GTR/NTR) received 23.4 Gy craniospinal radiotherapy, 36 Gy radiotherapy to the posterior fossa, and 55.8 Gy to the primary tumor bed. After a 6-week rest, all patients began 4 cycles of high-dose chemotherapy including cisplatin (75mg/m^2 per cycle). SJMB-03 protocol utilized treatment regimens nearly identical to SJMB-96 except that 1) clinical target volume margin for primary site irradiation was 1 cm for SJMB-03 and 2 cm for SJMB-96, and 2) all patients were offered amifostine as a prophylaxis for cisplatin-induced ototoxicity on SJMB-03 while patients on the SJMB-96 protocol did not have the option to receive amifostine until 2000[22].

Children with solid tumors—Forty-one children with neuroblastoma (St. Jude NB-97 [clinicaltrials.gov: NCT00186849] and NB-05 [NCT00135135] protocols) and osteosarcoma (St. Jude OS-08 protocol [NCT00667342]) were included in the genetic study based on the availability of DNA and assessment of hearing loss for at least 12 months from therapy initiation. None of children with solid tumor received any craniospinal irradiation. Similar to the brain tumor cohort, patients with pre-existing hearing damage were excluded. Treatment regimens for the solid tumor protocols have been previously published[23, 24] or described at clinicaltrials.gov.

Hearing evaluation and ototoxicity

Audiological evaluation was prospectively performed at diagnosis, immediately after radiotherapy (when applicable), after each cycle of chemotherapy, and 6 weeks, 6 months, 1 year and thereafter annually following the completion of all therapy. Age- and developmentally-appropriate audiometric testing was performed (e.g., conventional

audiometry, conditioned play, visual reinforcement audiometry, and auditory brain stem response) and thresholds were measured at 0.25, 0.5, 1, 2, 3, 4, 6, and 8 kHz. Audiograms were evaluated using two grading systems: the National Cancer Institute CTCAE version 3.0 and the Chang criteria[25]. For children on the SJMB-96 and SJMB-03 protocols, the audiology exam closest to 18 months from treatment initiation and the worse grade of two ears were used to define ototoxicity status. For children on the St. Jude NB-97, NB-05 and OS-08 protocols, we defined ototoxicity status using the worst hearing loss during the entirety of the follow-up period. Ototoxicity grade was also reviewed longitudinally to rule out temporary hearing loss (e.g., otitis).

Genotyping

TPMT and *COMT* SNP genotypes were determined in germline DNA, using previously established methods: *TPMT* rs1800462, allele-specific polymerase chain reaction[26]; *TPMT* rs12201199, sequencing; *TPMT* rs1800460 and rs1142345, PCR-restriction fragment length polymorphism[26]; *COMT* rs4818, rs4646316, and rs9332377, ABI SNaPshot Multiplex System (Applied Biosystems). SNP genotype distribution in self-reported whites was comparable to that in the HapMap CEU population and to that reported by Ross et al[5] (data not shown). The primers used are listed in Supplementary Table S2.

Cisplatin-induced hearing damage in mice

Ototoxicity was induced by consecutive cisplatin treatment and measured on the basis of auditory brainstem response (ABR), following previously established procedures with slight modifications[27–30]. Six-week-old BALB/c mice were administered 3mg/kg cisplatin by intraperitoneal injection for 4 consecutive days[27], a cumulative dose level producing plasma drug exposure in mouse comparable to that in patients receiving 100mg/m^2 cisplatin[31]. ABR at 6, 16, and 32 kHz was measured 24 hours before the first cisplatin injection and 24 hours after the final treatment of cisplatin. Functional hearing damage was defined as ABR threshold shift between post-and pre-treatment measurements[27, 28]. *TPMT* knockout mice in BALB/c background were established previously[8].

To confirm hearing loss, we also morphologically assessed hair cell damage as described previously[32]. Immediately following the post-treatment ABR, mice were euthanized and the temporal bone was dissected out, fixed, and then decalcificated for 4 days. The whole cochlear duct and corresponding medial spiral ganglion tissues were carefully divided into the basal, middle, and apical turns for whole mount staining with Alexa 546-conjugated phalloidin and Hoechst 33342. All whole-mount samples were analyzed with a Zeiss LSM 700 confocal microscope to generate the frequency map, and the high-resolution images were taken for comparison at 6, 16, and 32 kHz regions.

Cisplatin Cytotoxicity and *TPMT* activity in the HapMap Cell Lines

Cisplatin IC_{50} (concentration required to achieve 50% cell death) of 116 HapMap cell lines (57 CEU and 59 YRI parental cell lines) and *TPMT* activity of 57 HapMap CEU cell lines were previously published[6, 7] and retrieved from PharmGKB (www.pharmgkb.org PS206923 and PS206499, respectively). *TPMT* and *COMT* genotypes of HapMap samples were downloaded from the International HapMap database (release 24).

Statistical Analysis

SNP genotypes were coded according to the number of B alleles as 0, 1, and 2 for AA, AB, and BB genotype, respectively. Deviation of the genotype frequencies from those expected under Hardy-Weinberg equilibrium was assessed by a χ^2 test, or Fisher's exact test if the

expected cell count was less than 5. Ototoxicity was treated as either a dichotomized or an ordinal variable based on CTCAE or Chang grading system.

Ototoxicity as a dichotomized variable: 1) CTCAE grade 0 vs. >0; 2) CTCAE grade 0 vs. ≥2, excluding those with CTCAE grade 1[5]; 3) Chang grade 0 vs. >0; 4) Chang grade <2a vs. ≥2a. Ototoxicity as an ordinal variable: 1) CTCAE grades 0 to 4; 2) Chang grades 0 to 4.

Associations of non-genetic factors and SNP genotypes with ototoxicity were evaluated by binary (when ototoxicity was dichotomized) or ordinal (when ototoxicity was an ordinal variable) logistic regression models in both univariate and multivariate analyses. Additional analyses were performed using Fisher's exact tests and Cochran-Armitage Trend tests (when ototoxicity was dichotomized) or using Cochran-Mantel-Haenszel tests (when ototoxicity was an ordinal variable) with similar results (data not shown). The Wilcoxon paired signed-rank tests were performed to assess the differences in ABR threshold before and after cisplatin treatment in mouse at 6, 16 and 32kHz. The comparison of ABR threshold shift at each frequency between *TPMT* knockout vs. wildtype mice was evaluated by Mann–Whitney–Wilcoxon test. The associations between *TPMT* and *COMT* SNP genotype and cisplatin IC_{50} in HapMap cell lines, and between *TPMT* enzymatic activity and cisplatin IC_{50} in HapMap cell lines were evaluated by both linear regression (assuming the additive model) and by ANOVA when applicable.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Stacy Throm, PhD, for coordinating patient samples and Sarah Hughes for curating the ototoxicity data, and all patients and their parents who participated in the St. Jude protocols included in this study. This work was supported by National Institutes of Health (P30CA21765-34 and U01GM92666) and American Lebanese Syrian Associated Charities (ALSAC).

References

1. Oeffinger KC, Mertens AC, Sklar CA, et al. Chronic health conditions in adult survivors of childhood cancer. The New England journal of medicine. 2006; 355:1572–82. [PubMed: 17035650]
2. Knight KR, Kraemer DF, Neuwelt EA. Ototoxicity in children receiving platinum chemotherapy: underestimating a commonly occurring toxicity that may influence academic and social development. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2005; 23:8588–96. [PubMed: 16314621]
3. Dolan ME, Newbold KG, Nagasubramanian R, et al. Heritability and linkage analysis of sensitivity to cisplatin-induced cytotoxicity. Cancer Res. 2004; 64:4353–6. [PubMed: 15205351]
4. Oldenburg J, Kraggerud SM, Cvancarova M, et al. Cisplatin-induced long-term hearing impairment is associated with specific glutathione s-transferase genotypes in testicular cancer survivors. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2007; 25:708–14. [PubMed: 17228018]
5. Ross CJ, Katzov-Eckert H, Dube MP, et al. Genetic variants in TPMT and COMT are associated with hearing loss in children receiving cisplatin chemotherapy. Nat Genet. 2009; 41:1345–9. [PubMed: 19898482]
6. Jones TS, Yang W, Evans WE, et al. Using HapMap tools in pharmacogenomic discovery: the thiopurine methyltransferase polymorphism. Clin Pharmacol Ther. 2007; 81:729–34. [PubMed: 17329987]
7. Huang RS, Duan S, Shukla SJ, et al. Identification of genetic variants contributing to cisplatin-induced cytotoxicity by use of a genomewide approach. Am J Hum Genet. 2007; 81:427–37. [PubMed: 17701890]

8. Hartford C, Vasquez E, Schwab M, et al. Differential effects of targeted disruption of thiopurine methyltransferase on mercaptopurine and thioguanine pharmacodynamics. Cancer Res. 2007; 67:4965–72. [PubMed: 17510427]
9. Du X, Schwander M, Moresco EM, et al. A catechol-O-methyltransferase that is essential for auditory function in mice and humans. Proceedings of the National Academy of Sciences of the United States of America. 2008; 105:14609–14. [PubMed: 18794526]
10. Rybak LP. Mechanisms of cisplatin ototoxicity and progress in otoprotection. Current opinion in otolaryngology & head and neck surgery. 2007; 15:364–9. [PubMed: 17823555]
11. Ciarimboli G, Deuster D, Knief A, et al. Organic cation transporter 2 mediates cisplatin-induced oto- and nephrotoxicity and is a target for protective interventions. The American journal of pathology. 2010; 176:1169–80. [PubMed: 20110413]
12. More SS, Akil O, Ianculescu AG, et al. Role of the copper transporter, CTR1, in platinum-induced ototoxicity. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2010; 30:9500–9. [PubMed: 20631178]
13. Milek M, Smid A, Tamm R, et al. Post-translational stabilization of thiopurine S-methyltransferase by S-adenosyl-L-methionine reveals regulation of TPMT*1 and *3C allozymes. Biochemical pharmacology. 2012; 83:969–76. [PubMed: 22274639]
14. Schell MJ, McHaney VA, Green AA, et al. Hearing loss in children and young adults receiving cisplatin with or without prior cranial irradiation. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 1989; 7:754–60. [PubMed: 2715805]
15. Paulino AC, Lobo M, Teh BS, et al. Ototoxicity after intensity-modulated radiation therapy and cisplatin-based chemotherapy in children with medulloblastoma. Int J Radiat Oncol Biol Phys. 2010; 78:1445–50. [PubMed: 20231075]
16. Tuan JK, Ha TC, Ong WS, et al. Late toxicities after conventional radiation therapy alone for nasopharyngeal carcinoma. Radiother Oncol. 2012
17. Hua C, Bass JK, Khan R, et al. Hearing loss after radiotherapy for pediatric brain tumors: effect of cochlear dose. Int J Radiat Oncol Biol Phys. 2008; 72:892–9. [PubMed: 18395355]
18. Fong RS, Beste DJ, Murray KJ. Pediatric sensorineural hearing loss after temporal bone radiation. Am J Otol. 1995; 16:793–6. [PubMed: 8572144]
19. Brock PR, Knight KR, Freyer DR, et al. Platinum-induced ototoxicity in children: a consensus review on mechanisms, predisposition, and protection, including a new International Society of Pediatric Oncology Boston ototoxicity scale. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2012; 30:2408–17. [PubMed: 22547603]
20. Neuwelt EA, Brock P. Critical need for international consensus on ototoxicity assessment criteria. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2010; 28:1630–2. [PubMed: 20194840]
21. Gajjar A, Chintagumpala M, Ashley D, et al. Risk-adapted craniospinal radiotherapy followed by high-dose chemotherapy and stem-cell rescue in children with newly diagnosed medulloblastoma (St Jude Medulloblastoma-96): long-term results from a prospective, multicentre trial. Lancet Oncol. 2006; 7:813–20. [PubMed: 17012043]
22. Fouladi M, Chintagumpala M, Ashley D, et al. Amifostine protects against cisplatin-induced ototoxicity in children with average-risk medulloblastoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2008; 26:3749–55. [PubMed: 18669462]
23. Santana VM, Furman WL, Billups CA, et al. Improved response in high-risk neuroblastoma with protracted topotecan administration using a pharmacokinetically guided dosing approach. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2005; 23:4039–47. [PubMed: 15961757]
24. Furman WL, McGregor LM, McCarville MB, et al. A single-arm pilot phase II study of gefitinib and irinotecan in children with newly diagnosed high-risk neuroblastoma. Invest New Drugs. 2011
25. Chang KW, Chinosornvatana N. Practical grading system for evaluating cisplatin ototoxicity in children. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2010; 28:1788–95. [PubMed: 20194861]

26. Yates CR, Krynetski EY, Loennechen T, et al. Molecular diagnosis of thiopurine S-methyltransferase deficiency: genetic basis for azathioprine and mercaptopurine intolerance. Ann Intern Med. 1997; 126:608–14. [PubMed: 9103127]
27. Kim HJ, Oh GS, Lee JH, et al. Cisplatin ototoxicity involves cytokines and STAT6 signaling network. Cell Res. 2011; 21:944–56. [PubMed: 21321603]
28. Kermany MH, Parker LL, Guo YK, et al. Identification of 17 hearing impaired mouse strains in the TMGC ENU-mutagenesis screen. Hear Res. 2006; 220:76–86. [PubMed: 16949226]
29. Wu X, Gao J, Guo Y, et al. Hearing threshold elevation precedes hair-cell loss in prestin knockout mice. Brain Res Mol Brain Res. 2004; 126:30–7. [PubMed: 15207913]
30. Schutz M, Scimemi P, Majumder P, et al. The human deafness-associated connexin 30 T5M mutation causes mild hearing loss and reduces biochemical coupling among cochlear non-sensory cells in knock-in mice. Hum Mol Genet. 2010; 19:4759–73. [PubMed: 20858605]
31. Filipski KK, Mathijssen RH, Mikkelsen TS, et al. Contribution of organic cation transporter 2 (OCT2) to cisplatin-induced nephrotoxicity. Clin Pharmacol Ther. 2009; 86:396–402. [PubMed: 19625999]
32. Liu Z, Owen T, Zhang L, et al. Dynamic expression pattern of Sonic hedgehog in developing cochlear spiral ganglion neurons. Dev Dyn. 2010; 239:1674–83. [PubMed: 20503364]

STUDY HIGHLIGHTS

What is the current knowledge on the topic?

Ototoxicity is a dose-limiting adverse effect of cisplatin with particularly debilitating impact on children with cancer, and inherited genetic variation in *TPMT* and *COMT* was recently linked to inter-patient variability in cisplatin-related hearing loss.

What question this study addressed?

What this study adds to our knowledge?

In the current study, we evaluated the effects of *TPMT*and *COMT* variants in 254 children enrolled on frontline pediatric cancer treatment protocols with cisplatin-containing therapy, and observed no association between these genetic variants and ototoxicity. Consistently, further functional experiments in laboratory models did not show any effects of *TPMT* or *COMT* on cisplatin-induced hearing damage *in vitro* or *in vivo*.

How this might change clinical pharmacology and therapeutics?

Our findings indicated that *TPMT* or *COMT* genetic variants were not associated with cisplatin ototoxicity and challenged the clinical utility of these genetic markers in individualizing cisplatin treatment.

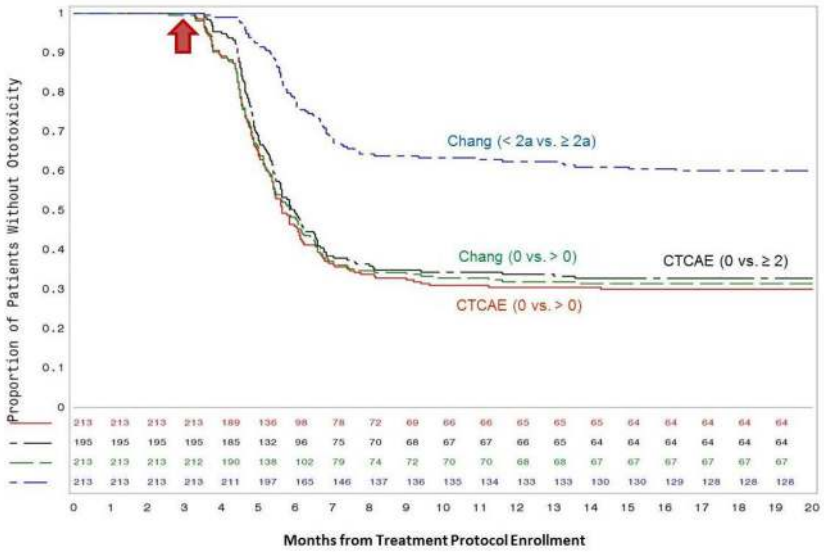

Figure 1. The Kaplan-Meier plot of cisplatin-induced ototoxicity in SJMB-96 and SJMB-03 cohorts

The majority of the ototoxicity events occurred between 3.5 and 9 months from treatment protocol enrollment (i.e., between 0.5 and 6 months from when cisplatin chemotherapy was started [indicated by red arrow]). Each curve represents different ototoxicity definition: CTCAE > 0 (red), CTCAE ≥2 (black), Chang > 0 (green), and Chang ≥2a (blue).

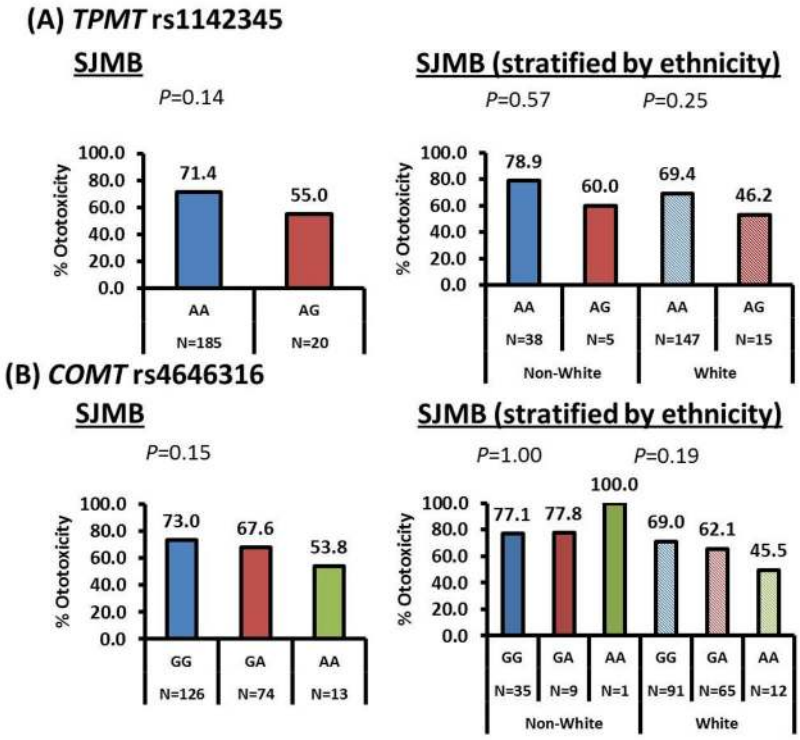

Figure 2. The distribution of cisplatin-induced ototoxicity by *TPMT* and *COMT* SNP genotype

The incidence of ototoxicity is plotted for each genotype at *TPMT* (rs1142345, A) and *COMT* (rs4646316, B) SNPs. For each SNP, results from 3 analyses are depicted: the Ross *et al.* study, the SJMB-96 and SJMB-03 cohort, the SJMB cohort stratified by ethnicity. *P*-values were determined by Fisher's exact allelic test in the Ross *et al.* cohort, by univariate logistic regression models in the SJMB cohort, and by Cochran Armitage trend test in the SJMB cohort stratified by ethnicity, respectively.

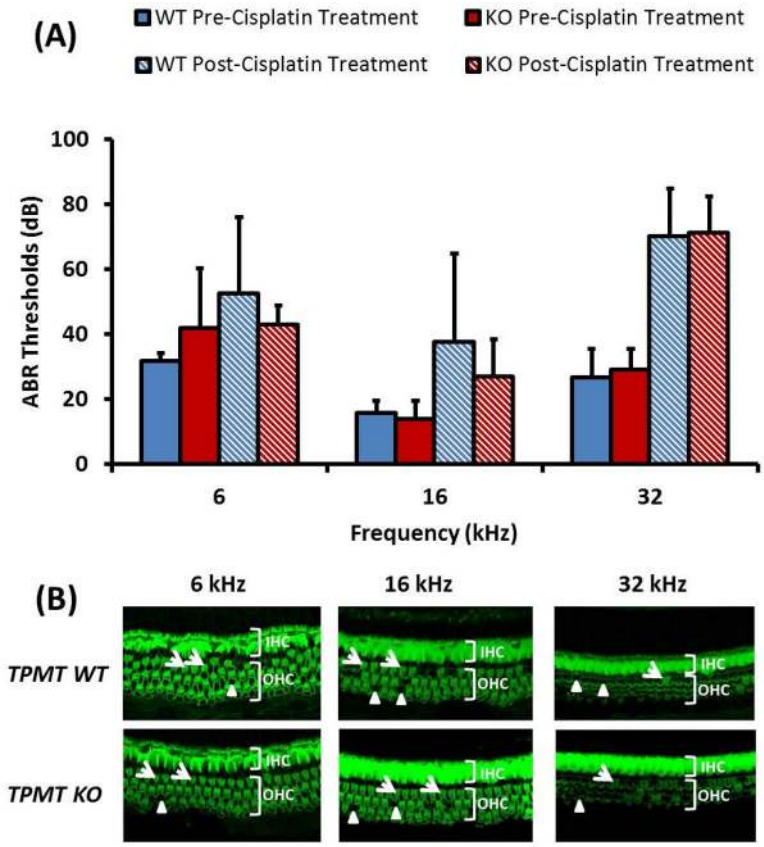

Figure 3. Cisplatin-induced hearing loss in *TPMT* knockout and wildtype mice

Panel A: The auditory brainstem response (ABR) thresholds before (solid bars) and after (striped bars) cisplatin treatment (daily injection of 3 mg/kg for 4 consecutive days) in *TPMT* wildtype (WT, blue) and knockout (KO, red) mice at 6, 16 and 32 kHz. Differences in ABR before and after cisplatin treatment were statistically significant ($P<0.05$, Wilcoxon paired signed-rank test) but not between *TPMT* knockout vs. wildtype at 6, 16 and 32kHz. Panel B: representative images of hair cell loss (inner hair cells [IHC] and outer hair cells [OHC]) in each frequency region of the cochlea (6, 16, and 32 kHz) in *TPMT* WT and KO mice following cisplatin treatment (see Methods). The whole mount staining of the organ of Corti was performed using Alexa 546-conjugated phalloidin. The presence and absence of green fluorescence indicate intact (arrows) and apoptotic (arrow-heads) hair cells, respectively. Note that phalloidin also labels remaining supporting cell processes surrounding the lost hair cells.

Table 1

Patient Characteristics of the SJMB-96 and SJMB-03 Cohort[a]

	No Ototoxicity CTCAE = Grade 0 (n = 64)	Ototoxicity CTCAE > Grade 0 (n = 149)	*P*-value
Age at diagnosis (years) [median (min, max)]	10.16 (3.29, 19.75)	7.58 (3.11, 21.56)	0.0128*
Self-reported ethnicity			
White	54 (32.1%)	114 (67.9%)	0.20
Non-White	10 (22.2%)	35 (77.8%)	
Gender			
Male	44 (31.2%)	97 (68.8%)	0.61
Female	20 (27.8%)	52 (72.2%)	
Cumulative cisplatin dose (mg/m^2) [median (min, max)]	299.59 (79.01, 311.97)	300.06 (76.50, 312.64)	0.59
Study protocol			
SJMB-96	12 (20.0%)	48 (80.0%)	0.0479*
SJMB-03	52 (34.0%)	101 (66.0%)	
Craniospinal irradiation			
< 25 Gy	54 (37.5%)	90 (62.5%)	0.0010*
≥25 Gy	10 (14.5%)	59 (85.5%)	
Amifostine			
Yes	61 (31.6%)	132 (68.4%)	0.14
No	3 (15.0%)	17 (85.0%)	
TPMT			
rs12201199			
TT	46 (28.6%)	115 (71.4%)	0.50
TA	14 (34.1%)	27 (65.9%)	
AA	1 (33.3%)	2 (66.7%)	
rs1800460			
GG	53 (28.2%)	135 (71.8%)	0.11
GA	8 (47.1%)	9 (52.9%)	
AA	0 (0.0%)	0 (0.0%)	
rs1142345			
AA	53 (28.6%)	132 (71.4%)	0.14
AG	9 (45.0%)	11 (55.0%)	
GG	0 (0.0%)	0 (0.0%)	
COMT			
rs4646316			
GG	34 (27.0%)	92 (73.0%)	0.15
GA	24 (32.4%)	50 (67.6%)	
AA	6 (46.1%)	7 (53.9%)	
rs9332377			
GG	45 (30.4%)	103 (69.6%)	0.78
GA	17 (30.9%)	38 (69.1%)	

	No Ototoxicity CTCAE = Grade 0 (n = 64)	Ototoxicity CTCAE > Grade 0 (n = 149)	*P*-value
AA	2 (22.2%)	7 (77.8%)	

Abbreviation: SJMB – St Jude Medulloblastoma

[a] Data are presented as number (%) of patients unless otherwise indicated.

* indicates that the variable is statistically significant at $P<0.05$.

P-values were determined by the univariate logistic regression model and an additive model was assumed for genetic variables. Similar results were obtained when a dominant or recessive model was considered.

Table 2

Summary of Statistical Analyses Using Different Ototoxicity Criteria in the SJMB-96 and SJMB-03 Cohort

Variables (Reference)	Ototoxicity Criteria									
	CTCAE				Chang					
	Dichotomized Scale Grade = 0 vs. ≥2		Ordinal Scale Grade 0–4		Dichotomized Scale Grade = 0 vs. > 0		Dichotomized Scale Grade < 2a vs. ≥2a		Ordinal Scale Grade 0–4	
	Estimate	*P*-value	Estimate	*P*-value	Estimate	*P*-value	Estimate	*P*-value	Estimate	*P*-value
Age at diagnosis	−0.12	0.0028*	−0.11	0.0008*	−0.10	0.0076*	−0.10	0.0061*	−0.10	0.0012*
Self-reported ethnicity (Non-White)	−0.28	0.16	−0.30	0.32	−0.44	0.26	−0.12	0.72	−0.25	0.42
Gender (Female)	−0.09	0.59	0.30	0.25	−0.26	0.41	0.61	0.0478*	0.30	0.26
Cumulative cisplatin dose	0.00	0.64	0.00	0.58	0.00	0.51	0.00	0.41	0.00	0.44
Study protocol (SJMB-96)	−0.38	0.0428*	−0.87	0.0022*	−0.94	0.0114*	−0.86	0.0054*	−1.00	0.0004*
Craniospinal irradiation (< 25 Gy)	0.65	0.0008*	1.22	<0.0001*	0.95	0.0071*	0.93	0.0020*	1.08	<0.0001*
Amifostine (No)	−0.52	0.11	−1.12	0.0131*	−1.03	0.11	−1.14	0.0207*	−1.18	0.0082*
TPMT										
rs12201199 (TT)	−0.16	0.64	0.19	0.50	−0.25	0.44	0.31	0.32	0.18	0.51
rs1800460 (GG)	−0.81	0.12	−0.39	0.40	−0.77	0.13	0.05	0.92	−0.28	0.54
rs1142345 (AA)	−0.78	0.12	−0.47	0.27	−0.86	0.0703	−0.19	0.70	−0.50	0.24
COMT										
rs4646316 (GG)	−0.39	0.11	−0.38	0.0621	−0.15	0.54	−0.26	0.26	−0.26	0.21
rs9332377 (GG)	0.13	0.64	0.11	0.61	0.00	0.99	−0.02	0.95	0.02	0.94

Abbreviation: SJMB – St Jude Medulloblastoma

* indicates that the variable is statistically significant at $P<0.05$.

Estimates and *P*-values were determined on the univariate logistic regression models. Binary logistic regression models were used for dichotomized ototoxicity outcomes, and ordinal logistic regression models were used for ordinal ototoxicity outcomes. For genetic variables, SNP genotypes were treated as continuous variables under the assumption of an additive model. Similar results were obtained when a dominant or recessive model was considered. Reference variables are indicated in parentheses.

Clin Pharmacol Ther. Author manuscript; available in PMC 2014 August 01.