Published in final edited form as:
Pharmacogenomics. 2011 July ; 12(7): 1039–1050. doi:10.2217/pgs.11.48.

Pharmacogenomics of cisplatin-induced ototoxicity

Debashree Mukherjea[†,1] and **Leonard P Rybak**[1]

[1]Department of Surgery, Division of Otolaryngology, Southern Illinois University, School of Medicine, Springfield, IL, USA

Abstract

Cisplatin ototoxicity affects different individuals in a widely variable manner. These variations are likely to be explained by genetic differences among those affected. It would be highly advantageous to identify genetic variants that predispose to cisplatin ototoxicity in order to minimize the risk to susceptible subgroups. Although this area of research is very important, only a few studies have rigorously examined the genetic basis for cisplatin-induced susceptibility to hearing loss. This article addresses recent progress in clarifying the incidence of cisplatin ototoxicity and the risk factors and controversies regarding the identifcation of genetic variants associated with cisplatin-induced hearing loss.

Keywords

audiometry; cisplatin; COMT; genome-wide screening; glutathione; GST; GSTM1; GSTP1; megalin; ototoxicity; pharmacogenomics; SNP; TMPT; XPC

Cisplatin is an effective and widely used chemotherapeutic agent for the treatment of solid tumors including ovarian, testicular, cervical, lung, head and neck and bladder cancers in adult patients. It is used as a standard therapy for many types of cancer in children, including neuroblastoma, osteosarcoma and hepatoblastoma. Cisplatin is one of the most effective chemotherapeutic agents for children with a cure rate of 85% [1]. Dose-limiting side effects of cisplatin include nephrotoxicity, neurotoxicity and ototoxicity. Although nephrotoxicity may be prevented by saline hydration as well as mannitol diuresis, there are no known cures or preventative treatments available for ototoxicity and neurotoxicity. Reports demonstrate some degree of hearing loss in 75–100% of cisplatin-treated patients [1]. Cisplatin-induced hearing loss is usually bilateral and irreversible, and is particularly serious in the pediatric population (age 6 months and onwards) with cancers requiring irradiation of the base of the skull or brain [2]. A total of 41–61% of children treated with cisplatin may experience hearing loss [3,4]. Kushner *et al.* [5] divided pediatric patients with neuroblastoma into three groups: group 1 had hearing tested after induction, which included two cycles of high-dose platinum with etoposide (cumulative cisplatin dose of 400 mg/m^2); group 2 had hearing tested after induction, which included three cycles of high-dose platinum with etoposide (cumulative cisplatin dose of 600 mg/m^2); group 3 had hearing tested following carboplatin-containing myeloablative therapy administered after induction, which included two cycles of high-dose platinum with etoposide include cumulative dose. Hearing was tested after

[†]Author for correspondence: Tel.: +1 217 545 2214, Fax: +1 217 545 0145, dmukherjea@siumed.edu.

Financial & competing interests disclosure

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

clinical recovery from induction and/or myeloablative therapy using air and bone conduction testing (at octave intervals 250–8000 Hz and 250–4000 Hz, respectively), speech visual reinforcement, play audiometry and distortion product otoacoustic emissions. The testing varied with age and degree of cooperation. Ototoxicity was scored according to the method of Brock *et al.* [6], with a threshold level of 40 dB at targeted frequencies of 1000, 2000, 4000 and 8000 Hz. Grades 3 and 4 correlate with hearing loss in the speech frequency range. All three groups had similar clinical characteristics, including median age at diagnosis of approximately 3 years. Severe (grade 3/4) deficits affected 25% of group 1, 54% of group 2 and 50% of group 3 patients. Patients younger than 5 years of age experienced greater ototoxicity than older patients [6]. Loss of hearing at an early developmental stage hampers speech, cognitive and social development of the child. Although ototoxicity caused by cisplatin may occur within 72 h to days after drug administration [7], delayed ototoxicity from cisplatin may occur in children. Pediatric patients treated with cisplatin at cumulative doses approaching 400 mg/m^2 demonstrated worsening of their hearing long after treatment. Audiograms demonstrated hearing loss in 5% of patients before the end of therapy. After more than 2 years of follow-up, 44% had significant hearing loss [8]. The median time for detection of the first significant decrease in hearing was 135 days in children. Additional follow-up for 6–44 months demonstrated further progression of hearing loss of 10–15 dB after completion of therapy [9].

High-frequency audiometric thresholds are often affected first. However, hearing impairment may progress to involve the middle frequencies when doses in excess of 100 mg/m^2 are used. If ultra-high frequency audiometric testing is used, up to 100% of patients receiving high-dose cisplatin (150–225 mg/m^2) may demonstrate some degree of hearing loss [10].

Symptoms of ototoxicity include subjective hearing loss, ear pain and tinnitus [11]. Hearing loss following cisplatin chemotherapy appears to be quite variable and it may be related to dose. Ototoxicity has been demonstrated to be more severe in patients receiving higher cumulative and individual doses, and was most noticeable in patients receiving bolus injections of cisplatin [11]. The age of the patient (young children, appear to be more susceptible than adults [3]), and other factors, such as noise exposure [12], exposure to other ototoxic drugs, depleted nutritional state including low serum albumin and anemia [10], and cranial irradiation [13], all appear to play a role. Ross *et al.* [14] hypothesized that because there is significant interindividual variability in hearing loss in patients receiving similar doses of cisplatin, genetic factors may be responsible for toxicity.

Mechanisms of cisplatin ototoxicity

A simplistic overview of the mechanism for cisplatin ototoxicity is the acute and chronic generation of reactive oxygen species (ROS), in addition to DNA damage. Increased ROS generation has been demonstrated in all three subregions of the cochlea: organ of Corti, lateral wall (stria vascularis, spiral ligament) and spiral ganglionic cells. This ROS overload leads to the depletion of the cochlear antioxidant enzyme system (e.g., glutathione-*S*-transferase [GST], glutathione peroxidase, glutathione reductase, superoxide dismutase, catalase, amongst others), that scavenge and neutralize the superoxides generated [15]. Therefore, polymorphisms in any of the antioxidant enzymes or scavengers for ROS leads to cisplatin sensitivity and ototoxicity. Cisplatin-induced chronic increase in ROS generation causes increased proinflammatory cytokine formation and superoxide generation in the cochleae. This uncontrolled increase in ROS generation leads to the activation of proapoptotic pathways (both caspase dependent and independent). A review of drugs in clinical trials for the amelioration of cisplatin ototoxicity reveals that anti-inflammatory antioxidants and ROS scavengers are in the pipeline [16].

Megalin

Megalin (low-density lipoprotein-related protein 2), a multifunctional receptor, is the largest of the seven members of the low-density lipoprotein family with a size of approximately 600 kDa. It was initially described as a scavenger receptor because it can bind multiple ligands. It was discovered to be the antigenic determinant for Heymann nephritis in rats [17]. It was subsequently demonstrated to be critical for the reabsorption of various molecules in the proximal renal tubule. However, it was also demonstrated that megalin has additional roles in cell signaling [17]. Megalin is classified as an endocytic receptor that binds and internalizes a number of ligands including: proteins that carry vitamins and hormones; proteases and proteaseinhibitor complexes; and lipoproteins [18]. Thus, megalin acts as a cargo transporter for lipophilic vitamins and steroid hormones bound to carrier proteins. Megalin is localized to the top of epithelial cells that border lumina and can also be present within endosomes of embryonic cells and in adult gastrointestinal cells [17].

Megalin & hearing

Megalin appears to play an important role in hearing. In the cochlea, megalin is strongly expressed within the marginal cells of the stria vascularis. Megalin was localized using a post-embedding immunogold method in the rat cochlear duct. Marginal cells of the stria vascularis expressed megalin on the apical, but not on the basolateral surface. This pattern resembled that observed in kidney proximal tubule cells. Immunoreactivity was also detected on epithelial cells of the spiral prominence and Reissner's membrane. Intermediate and basal cells of the stria vascularis, mesothelial cells of Reissner's membrane or fibrocytes in the lateral wall did not demonstrate megalin immunoreactivity. It is interesting to note that sensory cells and supporting cells of the organ of Corti were also not labeled [19]. Megalin expression was also demonstrated in the neonatal rat inner ear by immunohistochemistry and immunofluorescence. In the cochlea, the pattern of labeling was similar to that noted above. In addition, intense labeling was detected in the vestibular dark cells flanking the crista ampullaris and those in the utricle. These findings suggest that megalin may play a crucial role in the development of the inner ear. Receptor–ligand interaction analysis using surface plasmon resonance revealed that a series of six ototoxic aminoglycoside antibiotics bind to megalin with dissociation constants in the range of 1–3 mM [18].

Homozygous megalin mutant mice exhibit profound hearing loss at 3 months of age associated with features of presbycusis, enrichment of lipofuscin granules and a reduced number of microvilli in marginal cells of the stria vascularis. Megalin-deficient mice were found to have a reduced uptake of fluorescein isothiocyanate-labeled estrogen into marginal cells. Previous studies had demonstrated that estrogen receptors are expressed in marginal cells that express megalin. This suggests that estrogen may be a ligand for megalin and may function through megalin in the inner ear. Megalin acts as a cargo transporter for steroid hormones bound to carrier proteins. A crucial role of megalin in hearing should be entertained and the megalin/estrogen interaction needs to be considered as a player in early presbycusis in estrogen-deficient humans and mice [20].

Megalin polymorphisms & ototoxicity

Megalin has been demonstrated to bind aminoglycoside antibiotics [18]; it is also possible that megalin binds cisplatin. A study of 74 pediatric cancer patients was performed to ascertain whether megalin SNPs were associated with risk of hearing loss from cisplatin. A total of 24 patients were excluded from analysis owing to the presence of other risk factors for hearing loss or because the dose of cisplatin was deemed to be insufficient to cause hearing impairment. Of the remaining 50 subjects, half of these developed hearing loss after

cisplatin. The other 25 patients did not suffer hearing loss after receiving cisplatin. The mean age of both groups was similar (13.6 and 12.6 years of age), respectively and the mean cumulative dose of cisplatin at the end of therapy was equivalent in patients who did or did not develop hearing loss after cisplatin. The authors compared the distribution of megalin SNPs between the two groups. The A allele SNP rs2075252 occurred more frequently in the group susceptible to hearing loss compared with the group without hearing loss after cisplatin ($p < 0.016$). On the other hand, the allele distribution of SNP rs4668123 of megalin was no different in patients who developed hearing loss after cisplatin therapy compared with those who maintained their hearing [21]. Audiologic assessments were performed prior to therapy and after each course of cisplatin treatment and again at the follow-up. These assessments consisted of pure-tone audiometry with air and bone conduction, tympanometry and transient click-evoked and distortion product otoacoustic emissions (TEOAE and DPOAE). In some cases, brain stem audiometry was performed. The extent of hearing impairment was classified according to the Muenster classification [21]. A pure-tone audiogram grade of 0 was required for entry into the study. There were 50 patients eligible for analysis in this study. They were classified into two groups. Group 0 consisted of 25 patients with no significant hearing impairment after therapy (grade ≤1); group S comprised 25 patients (grade ≥2) following cisplatin treatment. Genotyping was carried out with peripheral blood mononuclear cells using the validated genotyping assays for two SNPs, rs2075252 and rs4668123), out of 757 SNPs reported for the megalin gene since they were nonsynonymous. The rs2075252 SNP has a G to A exchange in position 12,384 of megalin mRNA, resulting in an amino acid exchange of glutamic acid for lysine. The rs4668123 SNP has a nucleotide transition of A to G in position 8718 of megalin mRNA, yielding an amino acid exchange of threonine for alanine at position 2872 of the megalin protein. The allele distributions of the rs2075252 SNP between the two groups of patients with or without hearing loss following cisplatin were significantly different. The frequency of occurrence of the A allele in group S was higher than in group 0, in the whole group of patients and in a reference group. The difference between the two groups of patients was statistically significant. The allele distributions of the rs4668123 SNP were not significantly different between the two groups. The frequency of the T allele was not significantly different between groups. However, the T/T genotype of the rs4668123 SNP distribution was significantly higher in group S compared with group 0. The distribution of C/C and C/T + T/T genotypes was not significantly different between the two groups. It is interesting to note that there were neither T/T homozygotes of rs4668123 nor A/A homozygotes of rs2075252 in group 0 as opposed to group S, which included five T/T homozygotes and three A/A homozygotes. Furthermore, all three A/A homozygotes of rs2075252 were also homozygous for T/T in rs4668123.

Although the A allele of the rs2075252 SNP was found with a significantly greater frequency in patients with cisplatin ototoxicity than in those who did not experience hearing loss, it was not found in every patient suffering cisplatin-induced hearing loss. The data from this study suggests that megalin may be involved in the transport of cisplatin or its adducts and that polymorphisms of the megalin gene could be associated with susceptibility to cisplatin-induced ototoxicity. On the other hand, since megalin has only been localized to the marginal cells of the stria vascularis, Reissner's membrane, endolymphatic sac and dark cells of the vestibular apparatus [18,19] as well as the kidney, but not in the sensory cells of the organ of Corti or the spiral ganglion cells, it is difficult to explain why these latter two groups of key target cells for cisplatin ototoxicity could be directly affected by megalin polymorphisms [21]. Perhaps the ototoxicity occurs via an indirect mechanism through the cells of the stria vascularis.

By contrast, a more recent study of a large group of Canadian children failed to identify significant associations with cisplatin ototoxicity and the megalin rs2075252 A allele [14].

The contrast between the findings of this latter study and the report by Riedemann *et al.* [21] could be owing to ethnic differences. The latter study was carried out in 74 German pediatric patients, and the data obtained could be related to ethnic differences in genetic background. Additional studies of larger samples of patients experiencing hearing loss from cisplatin are needed to confirm or refute these findings.

Glutathione-*S*-transferases

Glutathione is a water soluble antioxidant tripeptide compound, consisting of glycine, glutamic acid and cysteine molecules, synthesized *de novo* in mammalian cells. Glutathione conjugation is considered to be an innate protective mechanism, developed to protect the body from potentially damaging electrophilic compounds. GSTs are a complex group of isoenzymes which catalyze the conjugation of potentially damaging electrophiles with glutathione. Compounds metabolized by GSTs include environmental pollutants, pesticides, carcinogens, drugs, drug metabolites and byproducts of oxidative stress – all of which represent electrophilic threats to the body [22,23]. GSTs exist in the soluble cytosolic form as well as in membrane-bound forms. Membrane-bound microsomal isoforms differ from cytosolic GSTs structurally and play key roles in the endogenous metabolism of leukotrienes and prostaglandins. Soluble mammalian GSTs exist as dimers and based on their amino acid similarities they have been classified into eight known classes: κ, μ, Ω, π, σ, θ and ζ [24–30,101]. GSTs are expressed in virtually every tissue of the body including the brain [31].

Genetic polymorphisms in GSTs in the population have been demonstrated to render individuals with certain genotypes more susceptible to cancers and to side effects from chemotherapeutic drugs like cisplatin [25,32–34]. Individuals with inherited homozygous deletions or null variants of the *GSTT1* or *GSTM1* gene demonstrate no enzymatic activity [35,36]. However, *GSTP1* polymorphism consists of a single nucleotide substitution (A→G) at position 313 resulting in the replacement of isoleucine with valine at codon 105, which in turn substantially decreases the catalytic activity of the *GSTP1* enzyme [29,36,37].

Glutathione-S-transferase subclasses M1, T1 and P1 have been demonstrated to influence the outcome of chemotherapy with platinum compounds and are directly involved in their detoxification [37–41]. This can result in the development of resistance by cancer cells or may lead to enhanced therapeutic response to platinum drug chemotherapy.

GSTM1 is a member of the GSTμ class of isoenzymes, is polymorphic and has three alleles *GSTM1*A*, *GSTM1*B* and *GSTM1*0* in humans [42–44]. Amino acid exchange from lysine to asparagine at the 172 position constitutes the difference in the **A* and **B* alleles [45], while the deletion of the entire gene is designated as *GSTM1*O* [46]. Approximately 42–60% of the Caucasian population and 42–54% of the Asian population is deficient for the *GSTM1* gene (*GSTM1*0*: null genotype), and do not express the GSTM1 protein [47–51].

GSTT1 is a member of the GSTθ class of GSTs and is polymorphic. Between 13–16% of the Caucasian population and 35–52% of the Asian population [26,49] has been reported to be deficient or lacking function of this enzyme owing to homozygous deletions. Interestingly, a recent report demonstrated that the deletion of 37 kb in the *GSTT2B* gene created a potentially null allele for *GSTT2* as seen in all three HapMap populations tested [52]. Furthermore the researchers discovered that there was nonrandom association of *GSTT2B/GSTT1* deletions in humans while chimpanzees retained both the *GSTT1* and *GSTT2B* genes.

GSTP1 is a paralog of the GSTπ class, and is a SNP as discussed above. Thus a SNP resulting in an A to G transition at position +313 (amino acid 105) and a C to T transition at position +341 (amino acid 114), leads to *GSTP1*A* (105Ile/114Ala), *GSTP1*B* (105Val/114Ala) and *GSTP1*C* (105Val/114Val) genotypes [29,53,102,103]. Another class,

*GSTP1*D* (105Ile/114Val), has also been identified, but no clinical significance has been reported [37,104].

Glutathione-S-transferase polymorphisms and their effect on platinum drug therapy have been studied for more than two decades in animal and human cell lines, animal models and in humans [42,54–56]. However, very few reports have studied GST polymorphisms in cisplatin-induced ototoxicity.

GST polymorphisms & cisplatin ototoxicity

The GSTM and GSTP proteins are expressed in the inner ear [57–59], and along with GSTT1 have been implicated in the development of cisplatin ototoxicity. The most frequent polymorphisms occurring in the GST isoforms related to platinum drug ototoxicity are controlled by the *GSTP1, GSTT1* and *GSTM1* genes which either decrease or completely abolish enzymatic activity of GST.

Peters *et al.* [60] were the first group to publish on GST polymorphisms that were associated with cisplatin ototoxicity. They reviewed hearing assessments (which included pure-tone audiometry from both ears, tympanometry, measurements of transient evoked and distortion product emissions) from 71 young adults (3–22 years of age) diagnosed with sarcoma, germ cell tumor, neuroblastoma and brain tumor who received cisplatin chemotherapy. Patients were grouped as group N (n = 19) with intact hearing and group H (n = 20) who were those patients that suffered early hearing loss >20 dB at 4 kHz. GST polymorphisms assessed were the *GSTM (GSTM1*A, GSTM1*B, GSTM3*A, GSTM3*B* and *GSTM1*0* homozygotes), *GSTT (GSTT1* and *GSTT1*0* homozygotes), GSTP *(GSTP1*A, GSTP1*B* and *GSTP1*C*) and *GSTZ* alleles (*GSTZ1*A, GSTZ1*B* and *GSTZ1*C*). Frequency of occurrence of GST- M1, T1, P1 and Z1 mutations between the two groups failed to demonstrate any associations of statistical significance. However, the *GSTM3*B* allele was present in group N (frequency: 0.18) with normal hearing, at higher frequency than in the group H (frequency: 0.025) – the group with significant hearing loss – and therefore was reported as otoprotective. This was the first evidence of link between GST polymorphism in cisplatin induced hearing loss in the pediatric population, albeit in a small sampling of patients. In 2007, Oldenburg *et al.* published a larger study wherein 173 testicular cancer survivors (TCS) treated with cisplatin revealed a clear association between protection from cisplatin-induced ototoxicity and the expression of certain *GSTP1* and *GSTM1* alleles [53]. Audiometry was performed by measuring air and bone conduction thresholds at nine frequencies ranging from 250 to 8000 Hz. These audiometric threshold results were categorized at a 4 kHz frequency into six categories according to decibel thresholds for five percentiles for decadal age groups: 10th, 25th, 50th, 75th and 90th percentiles at 4 kHz as was depicted by Engdahl *et al.* in 23,446 males [61]. The three GST polymorphisms tested were *GSTT1, GSTM1* and *GSTP1* (homozygous deletions in *GSTT1* and *GSTM1* constitute nonfunctional enzymes, while the A315G SNP in *GSTP1* leads to 105Ile or 105Val). Association between these three GST polymorphisms and the six categories linked protection from cisplatin-induced hearing loss to homozygous *GSTP1* GG, as opposed to *GSTP1* AA or AG. Notably, out of 28 TCS with the protective *GSTP1* GG, only two patients (7%) reported severe hearing loss. *GSTP1* AA or AG were reported in 145 TCS of which 34 (23.4%) reported severe hearing loss. Conversely, excellent hearing was reported in 32% of TCS with *GSTP1 GG* and in only 5.5% of TCS with *GSTP1 AA* or *AG*. Interestingly, the presence of *GSTM1* was found to be detrimental for cisplatin ototoxicity. Combination genotypes of pattern 1 (*GSTT1+, GSTM1+* and 105Ile/105Ile-*GSTP1*) were found to be associated with impaired hearing ability (17 out of 30 TCS had severe hearing loss), while pattern 2 (*GSTT1+, GSTM1+* and 105Val/105Val-*GSTP1*) had better hearing ability (three out of 20 TCS had severe hearing loss) than TCS without these patterns.

Contradictory findings of *GSTM1* being detrimental for hearing ability after cisplatin treatment was explained by the authors as *GSTM1* and *GSTP1* competing for glutathione and therefore the deletion of *GSTM1* leads to increased availability of glutathione for *GSTP1*; and *GSTM1* binds and inactivates apotosis signal-regulating kinase [62]. Thus in the absence of *GSTM1*, activation of apotosis signal-regulating kinase may lead to increased activity of the JNK/p38 pathway thereby increasing the susceptibility to cisplatin-induced apoptosis in the organ of Corti. The protective effect of *GSTP1 GG* was explained as 105Val-*GSTP1* when expressed in *Escherichia coli* not only conferring the ability to tolerate higher doses of cisplatin compared with 105Ile-*GSTP1* but also having a higher substrate specificity for cisplatin [63]. The Oldenburg *et al.* study [53] has been corroborated by the 238 TCS who self-reported tinnitus and hearing impairment [64].

In their study on medulloblastoma and GST polymorphisms Barahmani *et al.* [51] did not find any relationship between development or time-to-development of ≥grade 3 ototoxicity. Serial hearing evaluations for 34 patients were reviewed: 19 developed ≥grade 3 ototoxicity. All patients were treated with craniospinal radiation followed by chemotherapy including cisplatin. However it was reported that the *GSTM1T1* polymorphism was associated with several adverse effects including ≥grade 3 toxicities owing to treatment [51]. This was a small group study with 34 patients and only ≥grade 3 toxicities were reported; 15 patients out of 34 did not demonstrate grade 3 ototoxicity. The method of analyses was different and more attention was given to the development of grade 3 toxicities such as myelosuppression, nephrotoxicity, neurotoxicity, ototoxicity and intellectual impairment. This study was not geared towards determining cisplatin-induced hearing loss; several different treatment regimens were followed although all seemed to include cisplatin. Cumulative dosage for cisplatin was not reported, nor were toxicities grade 3. In addition, neither hearing loss gradation nor adjustments for decadal age groups were performed.

On the other hand, Ross *et al*. genotyped 1949 SNPs using the Illumina Goldengate assay to determine genetic variation in 220 key genes involved in absorption, distribution, metabolism and elimination, but did not find any significant association of *GSTP1* or *GSTM1* with cisplatin ototoxicity [14]. The study consisted of 54 children in the initial cohort with 33 children exhibiting cisplatin-induced hearing loss and 22 children on cisplatin treatment with normal hearing. The second independent replicate cohort of 112 children treated with cisplatin was recruited (73 children suffered from moderate–severe ototoxicity). A total of 192 children with hearing loss unrelated to cisplatin were recruited to determine the frequency of genetic variants contributing to cisplatin ototoxicity.

TMPT & catechol-*O*-methyl transferase

DNA samples were obtained from patients in Canada who were treated with cisplatin and were genotyped for approximately 2000 SNPs in order to evaluate the genetic variation in 220 genes involved in drug disposition. Severity of hearing loss was classified based on the Cancer Therapy Evaluation Program Common Terminology Criteria for Adverse Events Criteria. A multistage approach was employed to increase the power to detect clinically relevant genetic variants. An initial discovery cohort of 54 children treated with cisplatin among which 33 (61%) suffered serious ototoxicity from a single hospital were genotyped. This was followed by a second cohort of 112 children of whom 73 (66%) had experienced serious ototoxicity, recruited through the National Surveillance for Adverse Drug Reactions in Canada. Male gender was moderately associated with ototoxicity (67 vs 50% in females) [14,65].

Two genetic variants in thiopurine *S*-methyltransferase (TPMT; rs12201199) and catechol-*O*-methyl transferase (*COMT*; rs9332377) were found to be highly associated with cisplatin-

induced hearing loss in both groups [14]. The data from the discovery cohort was combined with that of the replication group. The risk allele was not present in controls who did not experience ototoxicity in the discovery group and was only found in one control patient who did not experience ototoxicity in the replication cohort, whereas it was present in nine of the discovery cohort and in 16 of the replication group of patients. This resulted in an odds ratio of 15.9 in the discovery cohort and 9.82 in the replication group. The Bonferroni corrected p-value was 0032 [14]. The *COMT* risk allele, rs9332377, was found in 14 out of 16 discovery patients with ototoxicity and 22 out of 24 patients in the replication cohort group, and a total of 36 out of 40 patients in the combined group. The Bonferroni corrected p-value was 0.026. In a Kaplan–Meier plot, the combined effect of an increasing number of *TMPT* rs1220199 and *COMT* rs9332377 risk alleles was associated with earlier onset and increased severity of cisplatin-induced hearing loss [14]. A combination of risk genotypes accounted for 48% of children who experienced ototoxicity [14].

How do the genetic variants in these enzymes relate to hearing loss caused by cisplatin? The endogenous substrate of TPMT is unknown; this enzyme inactivates exogenous purine compounds such as the metabolites of azathioprine [14]. Cisplatin binds to purines and forms intra- and inter-strand crosslinks with DNA leading to cell death. It is possible that decreased TPMT enzyme activity may reduce the inactivation of cisplatin-bound purines, resulting in increased cisplatin crosslinking of DNA, thus increasing cisplatin toxicity [14]. Ototoxicity may also result from increased *S*-adenosylmethionine (SAM) levels owing to reduced TPMT and COMT enzyme activity. TPMT and COMT are methyltransferases that require SAM, a methyl donor substrate in the methionine pathway. A study conducted in mice administered SAM did not demonstrate SAM toxicity. The administration of cisplatin resulted in moderate nephrotoxicity. However, the combination of SAM and cisplatin increased nephrotoxicity significantly [66]. These findings suggest that the ototoxicity associated with cisplatin may be related to an accumulation of SAM substrate, resulting from a reduction in the activity of TMPT or COMT in the presence of cisplatin [14]. Recent studies of LRTOMT2, an enzyme that has 60% similarity with COMT, including the substrate-binding region, demonstrate that it functions as a COMT and is essential for proper auditory function in both mice and humans [14,67–69]. A novel gene, *COMT2*, was recently reported which produces a unique isoform of COMT–COMT2 which is expressed in the inner and outer hair cells of the cochlea [68]. These findings suggest that a loss of COMT may result in hearing loss when patients are treated with cisplatin [65].

Other candidate genes

Peters *et al.* [70] were able to characterize clustering of mitochondrial DNA mutations for haplotype by restriction analysis. They reported that out of 39 patients on cisplatin treatment, 20 patients had hearing impairment and 19 patients did not have hearing loss. Patients in the rare European haplogroup J clustered more frequently (five out of 20 in the hearing loss group) compared with those with normal hearing (one out of 19 in the nonhearing loss group). The European mitochondrial haplotype J is also associated with Leber's Hereditary Optic Neuropathy and may form the basis for genetically predisposing this group of individuals to cisplatin-induced hearing loss. Neither the mitochondrial mutation A7445G, nor the 7472insC insertion or the A1555G mutation were identified in any of the patients [70].

Knoll *et al.* [71] screened for the *GJB2* (codes for connexin) and *SLC26A4* (codes for pendrin, an anion transporter) genes as well as three mitochondrial mutations in buccal washes of 11 patients diagnosed with osteosarcoma, soft tissue sarcoma and CNS tumors who developed severe hearing loss and received a cumulative dose of 400 mg/m^2 cisplatin chemotherapy. The three mitochondrial mutations tested were A1555G (progressive

nonsyndromic high-frequency sensorineural hearing loss), A3243G and A7445G (abnormal glutathione transport or oxidative stress), and were found to be associated with drug-mediated ototoxicity (aminoglycoside). No mutant alleles for any of the five genes tested were found [71]. The group size for this study was very small (n = 11), and larger studies are required to rule out the involvement of polymorphisms of these genes.

Caronia *et al.* [72] analyzed eight SNPs in the *ERCC2*, *XPC*, *XPA*, *ERCC1*, *ERCC4* and *ERCC5* genes in 91 patients with osteosarcoma who had been treated with cisplatin. Audiometric data was available for 32 patients (15 with ototoxicity and 17 with normal hearing). Association with cisplatin ototoxicity was detected with the rs2228001 SNP (*XPC* gene). Genotype *XPC* CC predisposed 80% of the patients to ototoxicity (four out of five), *XPC AA* rendered 50% of the patients (eight out of 16) carrying this mutation with hearing loss while the *XPC AA* allele was observed in 27% (three out of 11 patients). Furthermore AA, AC and CC genotype frequencies in this SNP were 20, 53 and 27% respectively in the 15% of patients with hearing loss and 47, 47 and 6% respectively in 17 patients with normal hearing [72]. The results of various pharmacogenomic studies of cisplatin ototoxicity are listed in Table 1.

An organic cation transporter has been implicated in the cellular uptake of cisplatin by the kidney. In mice, deletion of Oct1 and Oct2 resulted in significantly impaired urinary excretion of cisplatin without an apparent influence on plasma levels. Furthermore, the Oct1/Oct2-deficient mice were protected from severe cisplatin-induced renal tubular damage. Subsequently, it was found that a nonsynonymous SNP in the *OCT2* gene *SLC22A2* (rs316019) was associated with reduced cisplatin-induced nephrotoxicity in patients. Collectively, these results indicate the critical importance of OCT2 in the renal handling and subsequent renal toxicity of cisplatin [73]. OCT2 is expressed in hair cells of the cochlea. OCT1/2 double-knockout (KO) mice demonstrated no sign of ototoxicity and only mild nephrotoxicity after cisplatin treatment of KO mice compared with wild-type mice [74]. To date, no studies of the effects of mutations in the *OCT2* gene on cisplatin ototoxicity in humans have been reported. In the future, it would be extremely interesting to study patients with the nonsynonymous SNP in the *OCT2* gene *SLC22A2* (rs316019) to determine whether they are protected from cisplatin ototoxicity.

Latest trends in the identification of polymorphisms: the genome-wide approach

Chemotherapeutic cytotoxicity as well as efficacy have been attributed to genetic factors and identification of the genetic variants that confer susceptibility to the cytotoxic effects can help tailor drug therapy to decrease adverse events. Sequencing of the human genome and the international haplotype map (HapMap) project provide valuable resources for the expansion of pharmacogenetic studies and for genome-wide studies. The International HapMap project was initiated in 2002 to help characterize common genetic variations in DNA sequence among four different populations and to construct haplotype maps. Cell lines derived from individuals from four different populations were extensively genotyped [105]:

- A total of 30 trios (mother, father and child), that is, 90 Utah (USA) residents with ancestry from northern and western Europe (CEU);
- A total of 30 trios (mother, father and child), that is, 90 Yoruba in Ibadan, Nigeria (YRI);
- A total of 15 trios, that is, 45 Japanese in Tokyo, Japan (JPT);
- A total of 15 trios, that is, 45 Han Chinese in Beijing, China (CHB).

In this genome-wide approach the entire genome is analyzed without bias towards a particular gene or pathway [75]. Dolan *et al.* published a study on Epstein–Barr virus-transformed B-lymphoblastoid cell lines from ten Caucasian Utah Centre d'Etude du Polymorphisme Humain (CEPH) families (1331, 1332, 1333, 1346, 1347, 1362, 1408, 1413, 1416 and 1423) and reported that genetic components mediate 38–47% of variation in cisplatin-induced cytotoxicity in humans [76]. In an attempt to further identify the genetic variants in cisplatin cytotoxicity, the same group used cell lines derived from 30 trios of the European descent (CEU) and 30 trios of Yoruba African descent (YRI). Cytotoxicity and gene expression was performed using cell growth inhibition and the Affymetrix Genechip Human exon 1.0 ST array. These researchers identified several SNPs in the populations tested that were deemed important in 'cisplatin cytotoxicity'. In the combined population six SNPs exerted their effects on eight genes; in the CEPH population two SNPs regulated two genes; and in the Yoruban (YRI) populations nine SNPs regulate 16 gene expressions. These genetic variants thus explain the 27, 29 and 45% variation in cisplatin sensitivity in the above mentioned populations, respectively [77]. The same group then studied linkage-directed association analysis of 86 CEPH HapMap samples to narrow down the linkage regions contributing to cisplatin-induced cytotoxicity. A total of 20 SNPs were found to contribute to cisplatin cytotoxicity, ten of the these SNPs were located in five genes, and four of these 20 SNPs contributed to over 10% of the variation observed in cisplatin-induced apoptosis [78].

Conclusion

Genetic polymorphisms and their effect on cisplatin ototoxicity have been reported; however, several controversies exist as to the importance of the different genes and their variants on cisplatin ototoxicity. Phase II metabolic enzymes such as GST are documented as being important contributors to cisplatin ototoxicity as they are not only involved in xenobiotic detoxification but are also expressed in the organ of Corti. Several studies have reported different genetic variants of GST contributing to cisplatin ototoxicity; however, the protective effect of *GSTP1* and *GSTM1* on cisplatin ototoxicity is not well established and reports from different groups are contradictory. Different reports have used variable criteria, methods of analyses, statistics and interindividual variation and there is always the inherent variability in chemotherapy regimens, cumulative dosage of cisplatin and different tumor types. This trend seems to hold with most of the other polymorphisms in genes linked to cisplatin ototoxicity such as megalin, *TPMT*, *COMT*, *XPC* amongst others. The genome-wide approach is a novel method for analyzing and determining a large number of SNPs and multiple genes and thus pathways associated with cisplatin ototoxicity, and seems to be the way forward. However, it has its own drawbacks; high false discovery rate and the probability of overlooking rare genetic variants. Additional pharmacogenomic research with larger and well-defined cohorts are urgently needed to provide robust risk assessments for cisplatin ototoxicity in combination with the functional validation of genes. One may postulate a monogenetic cause for cisplatin induced ototoxicity; however, owing to the complexity of cisplatin toxicology for the auditory system, several genes may be responsible for the susceptibility to hearing loss. This is implied by the studies of Oldenburg *et al.* [53,64] and Ross *et al.* [14]. No single polymorphism was found in all affected individuals.

Future perspective

Sequencing of the human genome and the international HapMap project has provided a wealth of genotypic information based on more than 6 million SNPs for each cell line derived from the four major haplotype groups. This has revolutionized the field of pharmacogenetics. Several groups are racing to perform genome-wide search for genetic variants of genes responsible for drug sensitivities for various diseases. This budding field is

still in its infancy and researchers are developing techniques for analysis of whole genome data like the gene sequence enrichment analysis [79] and methods for validation of the genes identified by these studies. Thus it is reasonable to foresee that in the next few years' genotyping people may be carried out in order to customize chemotherapy and to minimize adverse drug effects and optimize therapeutic benefit.

Executive summary

Incidence

- A wide variability of hearing loss resulting from cisplatin exists ranging from 10 to 100%, depending on dose and risk factors.
- Young children are highly susceptible and can suffer effects that have severe consequences for future functioning.

Phenotyping

- Symptoms of cisplatin ototoxicity include otalgia, hearing loss and tinnitus.
- Documentation of hearing loss should be made by performing auditory testing before, during and following the completion of cisplatin treatment.
- Delayed onset of ototoxicity may occur.
- High-frequency audiometry and the measurement of otoacoustic emissions appear to offer earlier indications of cisplatin-induced hearing loss than do routine audiometric tests.

Genome-wide approach

- The entire genome has been analyzed without any bias towards a particular gene to determine gene variants and SNPs related to cisplatin ototoxicity.
- Several genetic variants revealed were able to explain interindividual differences in cisplatin ototoxicities.
- In the future, it will be important to genotype patients to provide tailored chemotherapy to maximize drug efficacy with minimal side effects.
- With large amounts of data being generated, bioinformatic programs are still being developed to analyze the results.
- The downsides include: high false discovery rate, contradictory findings in the initial studies reported and targets, which still have to be validated in study groups.

Acknowledgments

The authors were supported by the National Institutes of Health (NIH), the National Institute for Deafness and other Communicative Disorders (NIDCD) grants to Debashree Mukherjea (F32-DC 009950) and Leonard P Rybak (R01 DC 02396) during the preparation of this manuscript.

Bibliography

Papers of special note have been highlighted as:

▪▪ of considerable interest

1. McKeage MJ. Comparative adverse effect profiles of platinum drugs. Drug Saf. 1995; 13:228–244. [PubMed: 8573296]
2. Chen WC, Jackson A, Budnick AS, et al. Sensorineural hearing loss in combined modality treatment of nasopharyngeal carcinoma. Cancer. 2006; 106:820–829. [PubMed: 16421885]
3. Li Y, Womer RB, Silber JH. Predicting cisplatin ototoxicity in children: the influence of age and the cumulative dose. Eur. J. Cancer. 2004; 40:2445–2451. [PubMed: 15519518]
4. Coradini PP, Cigana L, Selistre SG, Rosito LS, Brunetto AL. Ototoxicity from cisplatin therapy in childhood cancer. J. Pediatr. Hematol. Oncol. 2007; 29:355–360. [PubMed: 17551394]
5. Kushner BH, Budnick A, Kramer K, Modak S, Cheung NK. Ototoxicity from high-dose use of platinum compounds in patients with neuroblastoma. Cancer. 2006; 107:417–422. [PubMed: 16779793]
6. Brock PR, Bellman SC, Yeomans EC, Pinkerton CR, Pritchard J. Cisplatin ototoxicity in children: a practical scoring system. Med. Pediatr. Oncol. 1991; 19:295–300. [PubMed: 2056973]
7. Bührer C, Weinel P, Sauter S, Reiter A, Riehm H, Laszig R. Acute onset deafness in a 4-year-old girl after a single infusion of cis-platinum. Pediatr. Hematol. Oncol. 1990; 7(2):145–148. [PubMed: 2206855]
8. Bertolini P, Lassalle M, Mercier G, et al. Platinum compound-related ototoxicity in children: long-term follow-up reveals continuous worsening of hearing loss. J. Pediatr. Hematol. Oncol. 2004; 26:649–655. [PubMed: 15454836]
9. Knight KR, Kraemer DF, Neuwelt EA. Ototoxicity in children receiving platinum chemotherapy: underestimating a commonly occurring toxicity that may influence academic and social development. J. Clin. Oncol. 2005; 23:8588–8596. [PubMed: 16314621]
10. Kopelman J, Budnick AS, Sessions RB, Kramer MB, Wong GY. Ototoxicity of high-dose cisplatin by bolus administration in patients with advanced cancer and normal hearing. Laryngoscope. 1988; 98:858–864. [PubMed: 3398663]
11. Reddel RR, Kefford RF, Grant JM, Coates AS, Fox RM. Ototoxicity in patients receiving cisplatin: importance of dose and method of drug administration. Cancer Treat. Rep. 1982; 66:19–23. [PubMed: 7198012]
12. Bokemeyer C, Berger CC, Hartmann JT, et al. Analysis of risk factors for cisplatin-induced ototoxicity in patients with testicular cancer. Br. J. Cancer. 1998; 77:1355–1362. [PubMed: 9579846]
13. Huang E, Teh BS, Strother DR, et al. Intensity-modulated radiation therapy for pediatric medulloblastoma: early report on the reduction of ototoxicity. Int. J. Radiat. Oncol. Biol. Phys. 2002; 52:599–605. [PubMed: 11849779]
14. Ross CJD, Katzov-Eckert H, Dube MP, et al. Genetic variants in *TPMT* and *COMT* are associated with hearing loss in children receiving cisplatin therapy. Nat. Genet. 2009; 41:1345–1349. [PubMed: 19898482] ▪▪ This study reports that two genetic variants in thiopurine-*S*-methyl-transferase (*TMPT*) and catechol-*O*-methyl-trsansferase were found to be highly associated with cisplatin-induced hearing loss in case-control cohorts of pediatric oncology patients.
15. Rybak LP, Mukherjea D, Jajoo S, Ramkumar V. Cisplatin ototoxicity and protection: clinical and experimental studies. Tohoku J. Exp. Med. 2009; 219(3):177–186. [PubMed: 19851045]
16. Mukherjea D, Rybak LP, Sheehan K, et al. The design and screening of drugs to prevent acquired sensorineural hearing loss. Expert Opin. Drug Discov. 2011; 6(5):1–15.
17. May P, Woldt E, Matz RL, Boucher P. The LDL receptor related protein (LRP) family: an old family of proteins with new physiological functions. Ann. Med. 2007; 39:219–228. [PubMed: 17457719]
18. Tauris J, Christensen EI, Nykjaer A, Jacobsen C, Petersen CM, Ovesen T. Cubilin and megalin co-localize in the neonatal inner ear. Audiol. Neurootol. 2009; 14(4):267–278. [PubMed: 19202329]
19. Mizuta K, Saito A, Watanabe T, et al. Ultrastructural localization of megalin in the rat cochlear duct. Hear. Res. 1999; 129:83–91. [PubMed: 10190754]
20. Koenig O, Ruettiger L, Mueller M, et al. Estrogen and the inner ear: megalin knockout mice suffer progressive hearing loss. FASEB J. 2008; 22:410–417. [PubMed: 17846082]

21. Riedemann L, Lanvers C, Deuster D, et al. Megalin genetic polymorphisms and individual sensitivity to the ototoxic effect of cisplatin. Pharmacogenomics J. 2008; 8(1):23–28. [PubMed: 17457342]
22. Hoban PR, Robson CN, Davies SM, Hall AG, Cattan RA, Hickson ID. Reduced topoisomerase II and elevated a class glutathione *S*-transferase expression in a multidrug resistant CHO cell line highly cross-resistant to mitomycin C. Biochem. Pharmacol. 1992; 43:685–693. [PubMed: 1311588]
23. Hayes JD, Pulford DJ. The glutathione *S*-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. Crit. Rev. Biochem. Mol. Biol. 1995; 30(6):445–600. [PubMed: 8770536]
24. Kasahara M, Matsumura E, Webb G, Board PG, Figueroa F, Klein J. Mapping of class a glutathione *S*-transferase 2 *(GST-2)* genes to the vicinity of the d locus on mouse chromosome 9. Genomics. 1990; 8(1):90–96. [PubMed: 1981995]
25. Meyer DJ, Coles B, Pemble SE, Gilmore KS, Fraser GM, Ketterer B. Theta: a new class of glutathione transferases purified from rat and man. Biochem. J. 1991; 274:409–414. [PubMed: 1848757]
26. Pemble S, Schroeder KR, Spencer SR. Human glutathione *S*-transferase Theta (*GSTT1*): cDNA cloning and characterization of a genetic polymorphism. Biochem J. 1994; 300:271–276. [PubMed: 8198545]
27. Ross VL, Board PG. Molecular cloning and heterologous expression of an alternatively spliced human Mu class glutathione *S*-transferase transcript. Biochem J. 1993; 294(Pt 2):373–380. [PubMed: 8373352]
28. Board PG, Baker RT, Chelvanayagam G, Jermiin LS. Zeta, a novel class of glutathione transferases in a range of species from plants to humans. Biochem. J. 1997; 328:929–935. [PubMed: 9396740]
29. Ali-Osman F, Akande O, Antoun G, Mao JX, Buolamwini J. Molecular cloning, Characterization, and expression in Escherichia coli of full-length cDNAs of three human glutathione *S*-transferase Pi gene variants. Evidence for differential catalytic activity of the encoded proteins. J. Biol. Chem. 1997; 272(15):10004–10012. [PubMed: 9092542]
30. Board PG, Coggan M, Chelvanayagam G, et al. Identification, characterization, and crystal structure of the Omega class glutathione transferases. J. Biol. Chem. 2000; 275(32):24798–24806. [PubMed: 10783391]
31. Terrier P, Townsend AJ, Coindre JM, Triche TJ, Cowan KH. An immunohistochemical study of pi class glutathione *S*-transferase expression in normal human tissue. Am. J. Pathol. 1990; 137(4): 845–853. [PubMed: 1977319]
32. Chao CC, Huang YT, Ma CM, Chou WY, Lin-Chao S. Overexpression of glutathione *S*-transferase and elevation of thiol pools in multidrug-resistant human colon cancer cell line. Mol. Pharmacol. 1992; 41:69–75. [PubMed: 1346333]
33. Moscow JA, Fairchild CR, Madden MJ, et al. Expression of anionic glutathione *S*-transferase and P-glycoprotein genes in human tissues and tumors. Cancer Res. 1989; 49:1422–1428. [PubMed: 2466554]
34. Tsuchida S, Sato K. Glutathione transferases and cancer. Crit. Rev. Biochem. Mol. Biol. 1992; 27:337–384. [PubMed: 1521461]
35. London SJ, Yuan JM, Chung FL, Gao YT, Coetzee GA, Ross RK. Isothiocyanates, glutathione *S*-transferase M1 and T1 polymorphisms, and lung-cancer risk: a prospective study of men in Shanghai, China. Lancet. 2000; 356:724–729. [PubMed: 11085692]
36. Stoehlmacher J, Park D, Zhang W, et al. Association between glutathione *S*-transferase P1, T1, and M1 genetic polymorphism and survival of patients with metastatic colorectal cancer. J. Natl Cancer Inst. 2002; 94(12):936–942. [PubMed: 12072547]
37. Watson MA, Stewart RK, Smith GB, Massey TE, Bell DA. Human glutathione *S*-transferase P1 polymorphisms: relationship to lung tissue enzyme activity and population frequency distribution. Carcinogenesis. 1998; 19:275–280. [PubMed: 9498276]

38. Hengstler JG, Arand M, Herrero ME, Oesch F. Polymorphisms in *N*-acetyltransferases, glutathione *S*-transferases, microsomal epoxide hydrolase and sulfotransferases: influence on cancer susceptibility. Recent Results Cancer Res. 1998; 154:47–85. [PubMed: 10026993]
39. Ban N, Takahashi Y, Takayama T, et al. Transfection of glutathione *S*-transferase (GST)-pi antisense complementary DNA increases the sensitivity of a colon cancer cell line to adriamycin, cisplatin, melphalan, and etoposide. Cancer Res. 1996; 56:3577–3582. [PubMed: 8758929]
40. Goto S, Iida T, Cho S, Oka M, Kohno S, Kondo T. Overexpression of glutathione *S*-transferase pi enhances the adduct formation of cisplatin with glutathione in human cancer cells. Free Radic. Res. 1999; 31:549–558. [PubMed: 10630679]
41. Sadzuka Y, Shimizu Y, Takino Y. Role of glutathione *S*-transferase isoenzymes in cisplatin-induced nephrotoxicity in the rat. Toxicol. Lett. 1994; 70:211–222. [PubMed: 8296325]
42. Board PG. Gene deletion and partial deficiency of the glutathione *S*-transferase (ligandin) system in man. FEBS Lett. 1981; 135(1):12–14. [PubMed: 7319029]
43. DeJong JL, Chang CM, Whang-Peng J, Knutsen T, Tu CP. The human liver glutathione *S*-transferase gene superfamily: expression and chromosome mapping of an Hb subunit cDNA. Nucleic Acids Res. 1988; 16:8541–8554. [PubMed: 3419925]
44. Seidegård J, Pero RW. The hereditary transmission of high glutathione transferase activity towards *trans*-stilbene oxide in human mononuclear leukocytes. Hum. Genet. 1985; 69:66–68. [PubMed: 3967891]
45. Widersten M, Pearson WR, Engström A, Mannervik B. Heterologous expression of the allelic variant μ-class glutathione transferases μ and ψ. Biochem. J. 1991; 276:519–524. [PubMed: 2049077]
46. Seidegård J, Vorachek WR, Pero RW, Pearson WR. Hereditary differences in the xpression of the human glutathione transferase active on *trans*-stilbene oxide are due to a gene deletion. Proc. Natl Acad. Sci. USA. 1988; 85:7293–7297. [PubMed: 3174634]
47. McLellan RA, Oscarson M, Alexandrie AK, et al. Characterization of a human glutathione *S*-transferase mu cluster containing a duplicated *GSTM1* gene that causes ultrarapid enzyme activity. Mol. Pharmacol. 1997; 52(6):958–965. [PubMed: 9415705]
48. Board P, Coggan M, Johnston P, Ross V, Suzuki T, Webb G. Genetic heterogeneity of the human glutathione transferases: a complex of gene families. Pharmacol. Ther. 1990; 48:357–369. [PubMed: 2084706]
49. Garte S, Gaspari L, Alexandrie AK, et al. Metabolic gene polymorphism frequencies in control populations. Cancer Epidemiol. Biomarkers Prev. 2001; 10(12):1239–1248. [PubMed: 11751440]
50. Arand M, Muhlbauer R, Hengstler J, et al. A multiplex polymerase chain reaction protocol for the simultaneous analysis of the glutathione *S*-transferase *GSTM1* and *GSTT1* polymorphisms. Anal. Biochem. 1996; 236(1):184–186. [PubMed: 8619490]
51. Barahmani N, Carpentieri S, Li XN, et al. Glutathione *S*-transferase M1 and T1 polymorphisms may predict adverse effects after therapy in children with medulloblastoma. Neuro. Oncol. 2009; 11(3):292–300. [PubMed: 18952980]
52. Zhao Y, Marotta M, Eichler EE, Eng C, Tanaka H. Linkage disequilibrium between two high-frequency deletion polymorphisms: implications for association studies involving the glutathione *S*-transferase *(GST)* genes. PLoS Genet. 2009; 5(5):e1000472. [PubMed: 19424424]
53. Oldenburg J, Kraggerud SM, Cvancarova M, Lothe RA, Fossa SD. Cisplatin-induced long-term hearing impairment is associated with specific glutathione *S*-transferase genotypes in testicular cancer survivors. J. Clin. Oncol. 2007; 25(6):708–714. [PubMed: 17228018] ▪▪ Largest association study in testicular cancer survivors on the expression of *GSTP1* and *GSTM1* in protection from cisplatin-ototoxicity.
54. Nakagawa K, Yokota J, Wada M, et al. Levels of glutathione *S*-transferase pi mRNA in human lung cancer cell lines correlate with the resistance to cisplatin and carboplatin. Jpn J. Cancer Res. 1988; 79(3):301–304. [PubMed: 2836347]
55. Puchalski RB, Fahl WE. Expression of recombinant glutathione *S*-transferase pi, Ya, or Yb1 confers resistance to alkylating agents. Proc. Natl Acad. Sci. USA. 1990; 87(7):2443–2447. [PubMed: 2320566]

56. Green JA, Robertson LJ, Clark AH. Glutathione *S*-transferase expression in benign and malignant ovarian tumours. Br. J. Cancer. 1993; 68(2):235–239. [PubMed: 8347477]
57. el Barbary A, Altschuler RA, Schacht J. Glutathione *S*-transferases in the organ of corti of the rat: enzymatic activity, subunit composition and immunohistochemical localization. Hear Res. 1993; 71:80–90. [PubMed: 8113147]
58. Touliatos JS, Neitzel L, Whitworth C, Rybak LP, Malafa M. Effect of cisplatin on the expression of glutathione-*S*-transferase in the cochlea of the rat. Eur. Arch. Otorhinolaryngol. 2000; 257(1):6–9. [PubMed: 10664037]
59. Fujimura T, Suzuki H, Udaka T, et al. Immunoreactivities for glutathione *S*-transferases and glutathione peroxidase in the lateral wall of pigmented and albino guinea pig cochlea. Med. Mol. Morphol. 2008; 41(3):139–144. [PubMed: 18807139]
60. Peters U, Preisler-Adams S, Hebeisen A, et al. Glutathione *S*-transferase genetic polymorphisms and individual sensitivity to the ototoxic effect of cisplatin. Anticancer Drugs. 2000; 11(8):639–643. [PubMed: 11081456] ▪▪ First study to determine association of genetic polymorphisms and cisplatin ototoxicity.
61. Engdahl B, Tambs K, Borchgrevink HM, Hoffman HJ. Screened and unscreened hearing threshold levels for the adult population: results from the Nord-Trøndelag hearing loss study. Int. J. Audiol. 2005; 44(4):213–230. [PubMed: 16011050] ▪▪ Calculated percentiles of the decibel thresholds for each decadal age group for eight different frequencies.
62. Adler V, Yin ZM, Fuchs SY, et al. Regulation of JNK signaling by GSTp. EMBO J. 1999; 18:1321–1334. [PubMed: 10064598]
63. Oldenburg J, Fosså SD, Ikdahl T. Genetic variants associated with cisplatin-induced ototoxicity. Pharmacogenomics. 2008; 9(10):1521–1530. [PubMed: 18855538]
64. Oldenburg J, Kraggerud SM, Brydøy M, Cvancarova M, Lothe RA, Fossa SD. Association between long-term neuro-toxicities in testicular cancer survivors and polymorphisms in glutathione-*S*-transferase-P1 and -M1, a retrospective cross sectional study. J. Transl. Med. 2007; 5:70. [PubMed: 18162130]
65. Pussegoda K. Genetic variants associated with cisplatin-induced hearing loss. Clin. Genet. 2010; 78:33–35. [PubMed: 20597921]
66. Ochoa B, Bobadilla N, Arrelin G, et al. *S*-adenosyl-*L*-mehtionine increases serum BUN and creatinine in cisplatin-treated mice. Arch. Med. Res. 2009; 40:54–58. [PubMed: 19064128]
67. Ahmed ZM, Masmoudi S, Kalay E, et al. Mutations of *LRTOMT*, a fusion gene with alternative reading frames, causes nonsyndromic deafness in humans. Nat. Genet. 2008; 40:1335–1340. [PubMed: 18953341]
68. Du X, Schwander M, Moresco EM, et al. A catechol-*0*-methyltransferase that is essential for auditory function in mice and humans. Proc. Natl Acad. Sci. USA. 2008; 105:14609–14614. [PubMed: 18794526]
69. Duman D, Sirmaci A, Cengiz FB, Ozdag H, Tekin M. Screening of 38 genes identifies mutations in 62% of families with nonsyndromic deafness in Turkey. Genet. Test Mol. Biomarkers. 2011; 15(1–2):29–33. [PubMed: 21117948]
70. Peters U, Preisler-Adams S, Lanvers-Kaminsky C, Jürgens H, Lamprecht-Dinnesen A. Sequence variations of mitochondrial DNA and individual sensitivity to the ototoxic effect of cisplatin. Anticancer Res. 2003; 23(2B):1249–1255. [PubMed: 12820379]
71. Knoll C, Smith RJ, Shores C, Blatt J. Hearing genes and cisplatin deafness: a pilot study. Laryngoscope. 2006; 116(1):72–74. [PubMed: 16481813]
72. Caronia D, Patiño-García A, Milne RL, et al. Common variations in *ERCC2* are associated with response to cisplatin chemotherapy and clinical outcome in osteosarcoma patients. Pharmacogenomics J. 2009; 9(5):347–353. [PubMed: 19434073] ▪▪ This is the first pharmacogenomic investigation of osteosarcoma patients demonstrating that ototoxicity was associated with the CC genotype of *XPC*.
73. Filipski KK, Mathijssen RH, Mikkelsen TS, Schinkel AH, Sparreboom A. Contribution of organic cation transporter 2 (OCT2) to cisplatin-induced nephrotoxicity. Clin. Pharmacol. Ther. 2009; 86:396–402. [PubMed: 19625999]

74. 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. Am. J. Pathol. 2010; 176(3): 1169–1180. [PubMed: 20110413]

75. Hartford CM, Dolan ME. Identifying genetic variants that contribute to chemotherapy-induced cytotoxicity. Pharmacogenomics. 2007; 8(9):1159–1168. [PubMed: 17924831] ▪▪ Provides an overview of methods for identification of genetic variants in chemotherapy-induced cytotoxicity.

76. Dolan ME, Newbold KG, Nagasubramanian R, et al. Heritability and linkage analysis of sensitivity to cisplatin-induced cytotoxicity. Cancer Res. 2004; 64(12):353–4356.

77. 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(3):427–437. [PubMed: 17701890]

78. Shukla SJ, Duan S, Badner JA, Wu X, Dolan ME. Susceptibility loci involved in cisplatin-induced cytotoxicity and apoptosis. Pharmacogenet. Genomics. 2008; 18(3):253–262. [PubMed: 18300947] ▪▪ Used genome-wide approach to identify SNPs and important genetic variants in cisplatin cytotoxicity.

79. Subramanian A, Kuehn H, Gould J, Tamayo P, Mesirov JP. GSEA-P: a desktop application for gene set enrichment analysis. Bioinformatics. 2007; 23(23):3251–3253. [PubMed: 17644558]

Websites

101. Drug metabolism. www.drugmetabolism.co.uk/gst.aspx

102. The Pharmacogenomics Knowledge Base. www.pharmgkb.org

103. Protein knowledgebase. www.uniprot.org

104. The Signaling Gateway. www.signaling-gateway.org

105. International HapMap Consortium. www.ncbi.nlm.nih.gov/pubmed/16255080

Table 1

Brief overview of cisplatin-induced ototoxicity and genetic polymorphisms.

Author (year)	Genetic polymorphisms tested	Patients (n)	Observation	Hearing testing	Ref.
Peters *et al.* (2000)	*GSTM1*, *GSTM3*, *GSTT1*, *GSTP1*, *GSTZ1*	39	Protective effects of *GSTM3*B* ($p = 0.023$)	Audiometry	[60]
Oldenburg *et al.* (2007)	*GSTT1*, *GSTM1*, *GSTP1*	173	Protective effects of ^{105}Val-*GSTP1* ($p < 0.001$) Detrimental effects of *GSTM1* positivity ($p < 0.022$)	Audiometry	[53]
Oldenburg *et al.* (2007)	*GSTT1*, *GSTM1*, *GSTP1*	238	Protective effects of *GSTP1G* ($p = 0.008$) Detrimental effects of *GSTM1* ($p = 0.025$)	Self-reported tinnitus	[64]
Barahmani *et al.* (2009)	*GSTM1*, *GSTT1*, *GSTM1T1*	34	No associations found	Audiometry	[51]
Riedemann *et al.* (2008)	Megalin, SNPs rs2075252, rs4668123	50	Detrimental effects of A allele of rs2075252 ($p < 0.02$) No association with SNP rs4668123	Audiometry	[21]
Ross *et al.* (2009)	Megalin, *TPMT* (rs1220119), *COMT* (rs9332377), *GSTP1* (rs1695G–G), *GSTM1*	166	Detrimental effects of *TPMT* (rs1220119); ($p = 0.00018$) and *COMT* (rs9332377); ($p = 0.00109$) No association with megalin, *GSTP1* ($p = 0.61$) or *GSTM1* ($p = 0.51$)	Audiometry	[14]
Peters *et al.* (2003)	Mitochondrial DNA mutations: A7445G mut, 7472insC, A1555G mut, nucleotide variations in D-loop region, European haplogroup J	39	Hearing loss more frequent in European haplogroup J	Audiometry	[70]
Knoll *et al.* (2006)	*GJB2*, *SLC26A4*, mitochondrial DNA mutations: A1555G, A3243G, A7445G	11	No associations found	Self-reported	[71]
Caronia *et al.* (2009)	*ERCC2*, *XPC*, *XPA* *ERCC1*, *ERCC4*, *ERCC5*	32	Detrimental effects of XPC	Audiometry	[72]

ins: Insertion; mut: Mutation; XPC: Xeroderma pigmentosum type C.