The genetic vulnerability to cisplatin ototoxicity: a systematic review

Evangelia Tserga1, Tara Nandwani2, Niklas K. Edvall1, Jan Bulla3,4, Poulam Patel5, Barbara Canlon1, Christopher R. Cederroth1 & David M. Baguley6,7

Ototoxicity is one of the major side-effects of platinum-based chemotherapy, in particular cisplatin (cis-diammine dichloroplatinum II). To our knowledge, no systematic review has previously provided a quantitative summary estimate of the impact of genetics upon the risk of developing hearing loss. We searched Embase, Medline, ASSIA, Pubmed, Scopus, and Web of Science, for studies documenting the genetic risk of ototoxicity in patients with cancer treated with cisplatin. Titles/abstracts and full texts were reviewed for inclusion. Meta-analytic estimates of risk (Odds Ratio) from the pooled data were calculated for studies that have been repeated twice or more. The search identified 3891 papers, of which 30 were included. The majority were retrospective (44%), ranging from n = 39 to n = 317, some including only patients younger than 25 years of age (33%), and some on both genders (80%). The most common cancers involved were osteosarcoma (53%), neuroblastoma (37%), prostate (17%) and reproductive (10%). Most studies performed genotyping, though only 5 studies performed genome-wide association studies. Nineteen single-nucleotide polymorphisms (SNPs) from 15 genes were repeated more than twice. Meta-analysis of group data indicated that rs1872328 on ACYP2, which plays a role in calcium homeostasis, increases the risk of ototoxicity by 4.61 (95% CI: 3.04–7.02; N = 696, p < 0.0001) as well as LRP2 rs4668123 shows a cumulated Odds Ratio of 3.53 (95% CI: 1.48–8.45; N = 118, p = 0.0059), which could not be evidenced in individual studies. Despite the evidence of heterogeneity across studies, these meta-analytic results from 30 studies are consistent with a view of a genetic predisposition to platinum-based chemotherapy mediated ototoxicity. These new findings are informative and encourage the genetic screening of cancer patients in order to identify patients with greater vulnerability of developing hearing loss, a condition having a potentially large impact on quality of life. More studies are needed, with larger sample size, in order to identify additional markers of ototoxic risk associated with platinum-based chemotherapy and investigate polygenic risks, where multiple markers may exacerbate the side-effects.

Early detection and modern treatments for cancer have contributed to improved survival rates for many types and sites of disease, such that there are presently 14.5 million cancer survivors in the United States alone1. As the number of cancer survivors increases, so does the need to understand and moderate the factors that may adversely impact quality of life in survivorhood. One such factor is that of hearing loss, which has been shown in general populations to have adverse consequences for cognition2, and mental health3, if untreated. Specifically, hearing loss is a significant risk factor for dementia4. Given the vulnerability of many cancer survivors, the understanding of ototoxicity arising from cancer therapies is of high importance.

Treatment with cisplatin (cis-diaminedichloroplatinum II or CDDP) chemotherapy continues to be a mainstay of curative therapy for many common cancers including breast, testis, and ovarian cancer in adults, and...
paediatric neuroblastoma. The propensity for cisplatin to instigate cochlear dysfunction, and hence deficits in auditory sensitivity and discrimination abilities has long been known, although this is still not clearly communicated to the cancer patient in the current medical practice. The prevalence of hearing loss following cisplatin treatment is dependent upon cumulative dose, and has been reported as being up to 90%. Given the recent classification of hearing problems as the 4th leading cause in years lived with disability by the WHO, interventions that cause hearing problems as a side-effect can have significant adverse consequences for quality of life. Whilst cisplatin administration leads to changes in auditory function that are detectable during and immediately after treatment, specifically bilateral progressive and irreversible high frequency hearing loss, the long-term persistent presence of the platinum compounds in the cochlea can increase the vulnerability to subsequent insults (age related metabolic change, noise, or viral, for example) and cumulate towards greater social communication impediments and burden.

The anti-cancer actions of cisplatin are mainly due to its interference with tumour cell proliferation. Through its binding to nuclear DNA, cisplatin blocks transcription and causes double-strand breaks leading to cell cycle arrest. However, since cells from the cochlea do not proliferate, it is thought that platination of mitochondrial DNA is a more likely cause of hearing loss than nuclear DNA damage. It is generally known that cisplatin ototoxicity has 3 major targets, hair cells, spiral ganglion neurons and the stria vascularis (the metabolic hub of the cochlea). Several molecular mechanisms have been described as mediators of cisplatin-induced ototoxicity. Cisplatin has been shown to target the NOX3 anti-oxidant system by causing the formation of reactive oxidative species (ROS), which in turn triggers inflammatory pathways in the cochlea and promotes apoptotic and necrotic cell death. Downstream of ROS generation is the JNK pathway, which activates STAT-1 mediated inflammatory pathways, leading to the induction of apoptotic cascades involving caspase 3 and 9. Elegant in vitro and in vivo animal experiments have recently evidenced the involvement of the ATM-Chk2-p53 signaling pathway in cisplatin-mediated hair cell damage. Interestingly, the stria vascularis retains platinum-based compounds for a long period of time, leading to subsequent alterations in potassium homeostasis and in the generation of the endocochlear potential, both being essential for normal hearing function. A genetic predisposition has also been proposed based upon observations of substantial inter-individual variability in the prevalence and severity of ototoxicity. Whilst there are multiple potential pathways for ototoxic hearing loss associated with cisplatin, the possibility of genetic susceptibility to ototoxic side effects is of interest from a number of perspectives. The identification of polymorphisms that render individuals vulnerable to chemotherapy induced hearing loss is an important precursor step to precision individualized medicine approaches that might titrate a chemotherapy regimen such that hearing loss was less likely or severe. Further, such knowledge would support translational genomic approaches in this area. Additionally, it has been suggested that ototoxicity may act as a valid surrogate marker for other, less well defined health tissue damage associated with platinum-based chemotherapy.

The aim of the present study was to perform a systematic review and meta-analysis of the literature pertaining to potential genetic predisposition to ototoxicity associated with cisplatin chemotherapy in humans.

Methods

Search strategy. A systematic search of the literature was conducted by two of the authors (E. T. & T. N.) from 6 different databases: Embase, Medline, ASSIA, Pubmed, Scopus and Web of Science. For each database, the search was performed using the key terms: (Gene OR genotype OR genetic) AND (tinnitus OR ototoxic OR hearing loss OR hearing impairment OR hearing disorder OR cochleotoxicity OR deafness) AND (Cisplatin OR cisplatinum OR platamin OR neoplatin OR cismaplatin OR cis-diamminedichloroplatinum OR carboplatin OR paraplatin OR oxaliplatin OR platinum AND chemotherapy). Literature searches were conducted in October 2017 and updated in September 2018 (Fig. 1).

Criteria for considering studies for this review. All studies written in English were considered eligible for this review. There was no restriction on participant age since studies with both children and adults were included. All different study designs were taken into account. Studies that were not available in English were excluded as we did not have the resources to translate them. Both adults and children were included in the review as many of the studies have been in children and since it is known that cisplatin causes more severe ototoxicity in children than in elders. In vitro and in vivo studies were excluded because cell lines and animals are not fully representative of the ototoxic effects of platinum-based chemotherapy that could have on humans.

Data Extraction and Management. Data extracted included study design, demographic characteristics, intervention and genetic association. Data extraction tables were developed and piloted for this purpose. Where data were missing or unclearly reported, an attempt was made to contact the relevant corresponding author of the study. Three articles were excluded after reading the full text. One paper was excluded because platinum-based chemotherapy was only studied by in vitro methods. Another was excluded because there was no association between cisplatin ototoxicity and the mitochondrial mutations, which they analysed and there is no report of ototoxicity grade. A third paper was excluded because the statistical results are based on comparison with craniospinal radiation. A study by Upadhya et al. reported that 31.4% of patients had sensorineural hearing loss 6 months after radiation of the ear. Radiation causes ototoxic effects, therefore is a confounding factor when investigating the ototoxic effect of chemotherapy. Studies by Brown et al., Drögemöller et al., Olgun et al. and Wheeler et al. are included in the socio-demographic and the cisplatin intervention tables but not in the forest.
plots since not all values about patients with or without ototoxicity in relationship with the genetic profile were available.

The data from each article was extracted and summarized in an extraction form (Table 1 & Sup. Table S1). The extraction form includes socio-demographic data of the study participants, details of the treatment intervention and audiological assessment and the results of the statistical analysis of the genes examined. The Critical Appraisal Skills Program checklist was used to assess the validity and results of each article included in the systematic review (CASP Critical Appraisal Skills Program Oxford UK, 2017).

Risk of bias (Quality assessment). Risk of bias assessment was conducted by four authors (E.T., T.N., N.E. & C. R. C) on those study records included in the meta-analysis. Risk of bias criteria that were taken into consideration in this review were the study population (age, gender, ethnicity), type of cancer (any type of malignancies), type of intervention (other ototoxic drugs, irradiation, prior hearing loss) and measurement of hearing outcome. All these criteria were taken into account in the interpretation of the results.

Meta-analysis. Forest plots were created using the Forest Plot add-in (version 8.0) for JMP 13.2.1 data analysis software to visually summarize the results from each study included in this review. The forest plots display the odds ratios (OR) and 95% confidence intervals that demonstrate the association between ototoxicity and the various genes and single-nucleotide polymorphisms (SNPs) reported in the literature. One forest plot was created to demonstrate the results for the genes tested in a single study. A second forest plot was created to compare the findings of different studies examining the same genes and SNPs and provide an estimate of the combined result of these studies. This is a meta-analysis of the data. The combined odds ratios and 95% confidence intervals were calculated using the values for the number of variant SNPs and controls in the patient groups with normal hearing and with ototoxicity after chemotherapy.

Statistical analysis. The number of cases from the included publications were extracted to four groups: Ototoxicity with SNP variant (OtSNP), Ototoxicity no SNP variant (Ot), Normal hearing with SNP variant (NhSNP), Normal hearing no SNP variant (Nh), and arranged the groups in a contingency table. Since the number of observations in all contingency tables considered is not too large, Fisher’s exact test serves for hypothesis
testing. Although Fisher’s exact test is preferable whenever the computational power allows to carry it out, we also report results from the commonly used chi-squared ($\chi^2$) test to ensure comparability with the literature. Furthermore, we report odds ratios (OR) for quantifying the risk of ototoxicity. The OR for being affected by ototoxicity if also having the SNP variant was then calculated as:

$$OR = \frac{OtSNP/NhSNP}{Ot/Nh}$$

Employing the Woolf method$^{34,35}$, the 95% confidence interval (CI) of the odds ratio is given by:

$$95\% CI = \exp(\text{ln}(OR) \pm 1.96 \times SE)$$

where $SE = \sqrt{\frac{1}{N_{\text{SNP}}} + \frac{1}{N_{\text{hSNP}}} + \frac{1}{N_{\text{Ot}}} + \frac{1}{N_{\text{Nh}}}}$.

For tables containing empty cells (i.e. if no research subjects populated a group), we applied the Haldane-Anscombe correction$^{36,37}$, that is we added 0.5 to all cells in the table for that publication. The statistical procedures were carried out using JMP 13.2.1. Values of $p < 0.05$ were considered significant.

### Results

From the 30 included papers, 44% were retrospective with a sample size ranging from 39 to 317 (Table 1). Some of them (33%) were performed on patients younger than 25 years of age$^{30,37-47}$ and 80% on both genders. The ethnicity was rather broad with Northern America and Norwegians representing together the majority of the articles (14%)$^{31,41,48-50}$. However, 12 of the papers did not specify the ethnicity of the patients$^{32,33,37-40,47,51-55}$ and 4 papers did not include age$^{32,36,54,56}$, which is a known risk factor for ototoxicity$^{22}$.

Supplementary Table S1 presents medical aspects reported in the studies. Fifty-three percent of the studies were performed on osteosarcoma$^{32,37-40,42-44,46,47,51,56-60}$, medulloblastoma in 33%$^{30,32,38,41,44-46,56}$, while testicular cancer was studied in 17% of them$^{31,33,48-50,53}$. How the dose was reported varied between the studies with 17

| Record                             | Study design   | Sample size | Ethnicity                                                                 | Median age (min, max) | Gender ratio (m/f) |
|-----------------------------------|----------------|-------------|---------------------------------------------------------------------------|-----------------------|-------------------|
| Peters, 2000$^{38}$               | N/S            | 39          | N/S                                                                       | (3–22)               | 22/17             |
| Peters, 2003$^{39}$               | N/S            | 39          | N/S                                                                       | (3–22)               | 23/16             |
| Oldenburg, 2007$^{38}$            | retrospective  | 173         | Norwegian                                                                 | 42 (24–73)           | 173/0             |
| Oldenburg, 2007$^{39}$            | retrospective  | 238         | Norwegian                                                                 | 29.3 (14.6–63.6)     | 238/0             |
| Riedemann, 2008$^{40}$            | N/S            | 50          | N/S                                                                       | (5–22)               | 27/23             |
| Barahmami, 2009$^{41}$            | N/S            | 42          | Hispanic, Non-Hispanic white, African American, Other                      | 6.8 (1.6–18)         | 34/8              |
| Caronia, 2009$^{39}$              | retrospective  | 91          | N/S                                                                       | 14.9 (3.7–34)        | 51/40             |
| Ross, 2009$^{42}$                 | case-control   | 162         | Caucasian                                                                 | 7.5 (0–19)           | 99/83             |
| Xu, 2012$^{43}$                   | prospective    | 204         | N/S                                                                       | 55 (33–77)           | 143/61            |
| Choeyprasert, 2013$^{44}$         | case-control   | 68          | Thai                                                                      | N/S                   | 40/28             |
| Khokhrin, 2013$^{35}$             | N/S            | 87          | Yak Russian                                                               | N/S                   | 0/87              |
| Pussegoda, 2013$^{44}$            | case-control   | 317         | Caucasian                                                                 | 8.5 (0–25)           | 77/78             |
| Yang, 2013$^{45}$                 | retrospective  | 213         | White, Non White                                                          | (3.11–21.56)         | 141/72            |
| Xu, 2013$^{46}$                   | retrospective  | 282         | Han Chinese                                                               | 56 (34–76)           | 192/90            |
| Haglittner, 2014$^{47}$           | retrospective  | 148         | Dutch, Spanish                                                            | (4–40)               | 76/72             |
| Spracklen, 2014$^{48}$            | prospective    | 100         | Caucasian, Cape mixed, Black African, Indian, Unknown                      | 46.5 (14–75)         | 73/27             |
| Brown, 2015$^{49}$                | N/S            | 71          | Non-Hispanic white, Hispanic, Other                                       | (0.7–18)             | 52/19             |
| Lanvers-Kaminsky, 2015$^{50}$     | retrospective  | 64          | pediatric & 66 adults                                                     | N/S                   | pediatric 38/26 adult 32/34 |
| Xu, 2015$^{51}$                   | retrospective  | 306         | N/S                                                                       | N/S                   | 148/90            |
| Olgun, 2016$^{52}$                | prospective    | 72          | N/S                                                                       | N/S                   | 40/32             |
| Talach, 2016$^{53}$               | prospective    | 55          | N/S                                                                       | 35                    | 52/3              |
| Vos, 2016$^{54}$                  | retrospective  | 156         | Dutch                                                                    | (3.4–43.9)           | 84/72             |
| Brown, 2017$^{55}$                | retrospective  | 80          | White, Hispanic, Other                                                    | (3.7–18.2)           | 57/23             |
| Drogemöller, 2017$^{56}$          | retrospective  | 188         | North American                                                            | 31 (24–39)           | 188/0             |
| Lopes-Aguar, 2017$^{57}$          | prospective    | 90          | Caucasian, Indigenous N/S                                                 | 56 (27–74)           | 83/7              |
| Spracklen, 2017$^{58}$            | N/S            | 222         | African, Indian, mixed ancestry                                          | 48 (14–75)           | 158/64            |
| Thiesen, 2017$^{59}$              | retrospective  | 116         | White, Asian, African                                                    | (0–19)               | 74/42             |
| Wheeler, 2017$^{60}$              | N/S            | 511         | N/S                                                                       | (18–55)              | 51/10             |
| Drogemöller, 2018$^{61}$          | N/S            | 229         | European, East Asian, South Asian, American, African                     | (23–49)              | N/S               |
| Lui, 2018$^{62}$                  | retrospective  | 106         | N/S                                                                       | 2.5 (0.2–16.9)       | 49/57             |

Table 1. Description of the socio-demographic data from the collected literature. *N/S: Not specified.
studies reporting median values of cisplatin administration (from 100 to 525.5 mg/m²)\textsuperscript{30,31,36,42–44,47–50,52,54–59}, and reporting mean values (from 328.2 to 425.5 mg/m²)\textsuperscript{32,38,39,45}. Seven studies only reported the range\textsuperscript{33,37,40,46,51,53,60}, and the dose per cycle\textsuperscript{41,60,61}. Only 9 studies reported the number of cycles/courses\textsuperscript{31,41,44,48,51,52,54,59,61}. Regarding auditory measures, there was also a large heterogeneity. Information on the tests and metrics used was missing in 9 studies\textsuperscript{36,41–43,51,52,55,58,61}. Sixty-three percent of the studies used pure-tone audiometry (PTA), of which 5 included auditory brainstem responses (ABR)\textsuperscript{32,37,39,44,54} and 4 included distortion products of otoacoustic emissions (DPOAE)\textsuperscript{32,37,39,40}. However, 33\% did not measure hearing at baseline, which makes the changes in hearing difficult to assess\textsuperscript{30,33,42,43,48,49,51,55,56,58}. The average percent of patients with ototoxicity (>grade 2) was 41.8\%, ranging from 8 to 75\%. There were 6 different grading systems of ototoxicity used across the literature: Brock classification, CTCAE, Boston classification, Chang classification, ASHA, Muenster classification and the Standard National Cancer Institute classification. Nevertheless, in 10 papers there was no clarification of the ototoxicity grading system used\textsuperscript{30,31,36,38,41,45,48–51,55}.

Radiotherapy or other ototoxic drugs (such as aminoglycosides and vincristine) were used as part of the treatment of patients in 24 of the included studies. Six papers did not specify whether other ototoxic drugs or radiotherapy were used\textsuperscript{33,37–40,52}. Radiation to the head and neck causes ototoxic effects and is a confounding factor when investigating the ototoxic effect of chemotherapy\textsuperscript{25}. Only 8 studies adjusted the statistical analysis to relevant clinical variables, such as age at diagnosis, gender, ethnic group, cumulative cisplatin dose, vincristine treatment and craniospinal irradiation doses\textsuperscript{43,47–50,55,59,60}.

Twenty SNPs of 9 genes were investigated once without having been repeated (Fig. 2). Three of the SNPs were shown to be otoprotective\textsuperscript{36,38,60}. Epoxides are among the many targets of GSTs. Converging this pathway, the Epoxide Hydrolase 1 (EPXH1) rs2234922 was related to otoprotection (OR: 0.05; 95\% CI: 0.00–0.94; n = 84; p = 0.004)\textsuperscript{36}. Spracklen et al. identified two SNPs predictors of cisplatin otoprotection\textsuperscript{40}; rs6721961 of NFE2L2 gene, involved in the protection of cells against oxidative stress (OR: 0.34; 95\% CI: 0.15–0.81; n = 222; p = 0.019) and rs10950831 of ABCB5 gene, which contributes on the cellular efflux of cisplatin (OR: 0.30; 95\% CI: 0.12–0.73; n = 222; p = 0.008).

Nineteen SNPs of 15 genes were investigated at least twice and the meta-analysis is shown in Figs 3 and 4. Seven of these SNPs showed no overall effect, namely the copper transport protein 1 (CTR1) rs10981694, GSTM1 and T1 deletions, GSTP1 rs1695, SLC16A5 rs4788863, XPC rs2228001 [a component of nucleotide excision repair (NER)] and XPD rs1799793. Albeit, XPD rs1799793 did not present an overall effect, was significantly ototoxic in one study (OR: 2.621; 95\% CI: 1.13–6.10; n = 106; p = 0.034)\textsuperscript{47}. The low-density lipoprotein-related protein 2 (LRP2) encoding the protein megalin was shown positively associated with ototoxicity on 2 SNPs (rs2075252 and rs4668123, Fig. 4). Interestingly, while the latter did not appear significant in hypothesis testing in 2 studies\textsuperscript{40,46,47}, the accumulated data supports a positive association (OR: 3.532; 95\% CI: 1.48–8.45; n = 118; p = 0.0059), likely due to an increase in the statistical power. Three SNPs (rs12201199, rs1142345, rs1800460) on the thiopurine S-methyltransferase gene (TPMT) were found significant in 2 studies\textsuperscript{43} and not in others\textsuperscript{44,46,57}. However, the overall pattern of the 5 studies merged together displayed significant associations with increased ototoxic risk from OR 2.47 to 2.82, with a total sample size of 786 (p < 0.0001). Another variant in COMT rs9332377

Figure 2. Forest plot describing the genes/SNPs tested in one study in alphabetic order. Black indicates a non-significant association with ototoxicity, red indicates a significant association with otoprotection, and blue indicates a significant association with ototoxicity. The square is centred on the odds ratio and the horizontal line represents the 95\% confidence interval. n = sample size. The asterisk (*) identifies studies in which the p value to reach significance differed between Fishers and \chi^2 tests.
showed mixed results with 2 studies showing positive associations\(^42,53\), and 4 others not\(^43,44,46,57\), while Hagleitner \(\text{et al.}\) presented an otoprotective effect of this SNP (OR: 0.395; 95% CI: 0.19–0.83; \(n = 148; p = 0.014\))\(^57\). The meta-analysis showed an overall positive association but with the smallest risk of all genes (OR: 1.55; 95% CI:

---

**Figure 3.** Forest plot describing \(\text{ABCC3 rs1051640, ACYP2 rs1872328, COMT rs4646316, COMT rs9332377, CTR1 rs10981694, GSTM1 del, GSTM3 rs1799735, GSTP1 rs1695, GSTT1 del tested in multiple studies. Black indicates a non-significant association with ototoxicity, blue indicates a significant association with ototoxicity and red a significant association with otoprotection. The square is centred on the odds ratio and the horizontal line represents the 95% confidence interval of each study. The diamond summarises each SNP average OR and the horizontal shows the 95% confidence interval. \(n = \) overall sample size. The asterisk (*) identifies studies in which the \(p\) value to reach significance differed between Fishers and \(\chi^2\) tests.**
Figure 4. Forest plot describing LRP2 rs2075252, LRP2 rs4668123, SLC16A5 rs4788863, SLC22A2 rs316019, SOD2 rs4880, TPMT rs1142345, TPMT rs12201199, TPMT rs1800460, XPC rs2228001, XPD/ERCC2 rs1799793 tested in multiple studies. Black indicates a non-significant association with ototoxicity, blue indicates a significant association with ototoxicity and red a significant association with otoprotection. The square is centred on the odds ratio and the horizontal line represents the 95% confidence interval of each study. The diamond summarises each SNP average OR and the horizontal shows the 95% confidence interval. n = overall sample size. The asterisk (*) identifies studies in which the p value to reach significance differed between Fishers and χ² tests.
The acylphosphatase-2 ACYP2 variant rs1872328, showed in Vos et al.\(^{58}\) and in Xu et al.\(^{54}\) reports a significant association (\(p = 0.0274\) and \(p < 0.0001\), respectively, by Fisher's test), in spite of an extremely large confidence interval (Fig. 3). High OR of this SNP was also presented in another study showing the strong relation with cisplatin ototoxicity.\(^{59}\) The combined data reveals a strong positive association with an overall risk of 4.618 (95% CI: 3.04–7.02; \(n = 696\); \(p < 0.0001\)). Another gene playing an important role in oxidative stress, superoxide dismutase 2, mitochondrial (SOD2), with rs4880 showing a positive association with cisplatin ototoxicity in one of the two studies,\(^{45}\) but also in the overall meta-analysis with an OR of 1.917 (significant with \(\chi^2\), but not with Fisher's test, Fig. 4).

In contrast, some genes presented overall otoprotective associations in this meta-analysis such as the antioxidant polymorphism GSTM3*B (rs1799735) was associated with increased otoprotection (OR: 0.275; 95% CI: 0.13–0.59; \(n = 145\); \(p = 0.001\)), a drug clearing transporter, namely the solute carrier SLC22A2 rs316019 (OR: 0.485; 95% CI: 0.27–0.86; \(n = 286\); \(p = 0.017\) and rs1051640 of ABCG3 gene (OR: 0.557; 95% CI: 0.39–0.798; \(n = 539\); \(p = 0.0017\)). Although the COMT rs4646316 variant appeared ototoxic in one study,\(^{42}\) no significant associations were found in the other studies,\(^{43,44,46,57}\) and the overall meta-analysis instead presented otoprotective associations with an OR of 0.620 (\(p = 0.0008\)).

| Gene     | SNP            | OR   | CI Low | CI High | Sample size | \(p (\chi^2)\) | \(p\) of Fisher |
|----------|----------------|------|--------|---------|-------------|----------------|----------------|
| TPMT     | rs12201199     | 2.822| 2.06   | 3.86    | 786         | <0.0001        | <0.0001        |
| LRP2     | rs2075252      | 2.80 | 1.25   | 6.28    | 118         | 0.010          | 0.013          |
| TPMT     | rs1142345      | 2.618| 1.93   | 3.56    | 786         | <0.0001        | <0.0001        |
| LRP2     | rs1800460      | 2.472| 1.82   | 3.35    | 786         | <0.0001        | <0.0001        |
| SOD2     | rs4880         | 1.917| 1.01   | 3.61    | 177         | 0.04           | 0.05           |
| COMT     | rs9332377      | 1.553| 1.18   | 2.05    | 847         | <0.0001        | <0.0001        |

**Table 2.** Summary of all ototoxic associations from repeated studies, listed from the greatest OR to the smallest.

A striking finding from this systematic review is that studies with non-significant findings in isolation reached sufficient power when combined to show increased risk of developing cisplatin-induced ototoxicity. This is the case for LRP2 rs4668123, which emphasizes the need of considering larger sample sizes when performing such studies in order to provide more statistically solid evidence. Although our meta-analysis did not use individual data nor included adjustments (for instance for age, sex, the ethnic group, and the cumulative cisplatin dose), the summarized analysis emphasizes the need of large sample sizes to reveal biologically relevant associations that would otherwise been underestimated or missed.

We identified 8 different SNPs from 5 different genes (including rs4668123 from LRP2) from repeated studies showing significant associations with cisplatin ototoxicity (Table 2). These genes are mainly related to anti-oxidant regulation, neurotransmission or to auditory function. ACYP2 encodes the acylphosphatase-2 expressed in the cochlea that hydrolyses phosphoenzyme intermediates of membrane pumps that affect Ca\(^{2+}\) ion homeostasis.\(^{64}\) While ATP-dependent Ca\(^{2+}\) signalling has been shown to be involved in hair cell development, the exact role of ACYP2 on the cochlea remains unknown.\(^{65}\) Interestingly this ACYP2 polymorphism showed the highest average risk (OR: 4.618), which suggests its major involvement in cisplatin ototoxicity and opens the possibility for more investigations addressing the contribution of this polymorphism in cisplatin-mediated ototoxicity.

With an OR ranging from 2.8 to 3.53, the LRP2 rs2075252 and rs4668123 polymorphisms also appear as important risk factors for developing cisplatin-mediated ototoxicity. LRP2 or megalin is currently the only gene important risk factors for developing cisplatin-mediated ototoxicity.\(^{65}\) It was recently demonstrated that HEI-OC1 and UB/OC-1 cells derived from the cochlea are more sensitive to cisplatin when expressing the TPMT*A variant instead of the wild-type TPMT.*\(^{65}\) In contrast, Tpmpt knock-out mice do not display an increased sensitivity to...
cisplatin when administered at comparable levels as found in humans, however this result might not be surprising given the known resistance of mice to cisplatin ototoxicity when compared to rats or guinea pigs.

Of the two polymorphisms tested for COMT, only rs9332377 appeared as an important risk factor, although displaying the smallest OR of all validated studies (OR: 1.553). Mutations in COMT genes are implicated in sensorineural deafness. Hearing loss is less severe in subjects with COMT Met allele, possible due to the protective effect of dopamine on the hearing system. While COMT has not been described in the cochlea, a homolog sharing 30% sequence identity, Comt2 was found expressed in hair cells and mice homozygous for a missense mutation in Comt2 showed sensorineural deafness due to degeneration of hair cells. Overall, there are strong indications that catecholamines play a potential role in the auditory function. Thus, a greater vulnerability to cisplatin ototoxicity may arise when the function of the auditory system is already weakened.

Another important polymorphism related with increased risk of cisplatin ototoxicity is SOD2 rs4880 presented an overall OR of 1.917. SOD2 catalyses the metabolism of the highly toxic superoxide anion to less but still toxic hydrogen peroxide. The SNP rs4880, which results in an exchange of valine against alanine, increases the catalytic activity of SOD2, leading to the accumulation of hydrogen peroxide and secondary ROS generation. It is thus possible that altered mitochondrial function in the cochlea may increase the vulnerability to cisplatin ototoxicity. Notably, SOD2 polymorphisms (IVS3-23T/G; IVS3-60T/G; and V16A) have also been implicated in noise induced hearing loss (NIHL).

Three polymorphisms, which have been evaluated twice, were found with a significant oto-protective effect, namely ABCC3 rs1051640, GSTM3 rs1799735 and SLC22A2 rs316019. ABCC3 is an ATP-binding cassette member of the MRP subfamily which is involved in multi-drug resistance. This transporter regulates the efflux of organic anions, glutathione S-conjugates and xenobiotics. MRP expression in cancer cells correlates with resistance to cisplatin. The mechanisms by which ABCC3 regulates cisplatin-induced hearing loss are unclear, but some studies suggest it may act upstream of GST. Indeed, consistent with the otoprotective effects of the ABCC3 variant, GSTM3 rs1799735 shown to be otoprotective by Peters et al. but also in the overall analysis. GSTM3 variants are indeed thought to alter the susceptibility to potential carcinogens and toxins. SLC22A2 is a solute carrier that encodes CTR1, which is a plasma-membrane transport-protein that has an essential role in cisplatin uptake into cochlea hair cells. Since cisplatin accumulates in the stria vascularis from the cochlea, polymorphism that positively affects monocarboxylate transporter function may improve the strial function affected by cisplatin.

Only two studies have evaluated polygenic effects on the vulnerability to cisplatin ototoxicity. Oldenburg et al. evaluated the cumulated risks of combinations in variants of GSTT1, GSTM1 and GSTP1. Such an approach makes sense when considering that the overall results for GSTM1 and T1 appeared inconclusive (Fig. 3). However, the combination of GSTM1 null, T1 null and P1 Ile105/Ile105 alleles had a major impact on the risk for severe hearing impairment. These findings are consistent with the known association of GSTM1 null, T1 null and P1 Ile105/Ile105 genotypes with greater vulnerability of developing NIHL and an 8.88-fold increase in the risk of developing presbyacusis (sensorineural hearing loss caused by natural ageing). The incapacity of these individuals to conjugate certain metabolites may ultimately cause oxidative stress and damage to the cochlea, which would be exacerbated in presence of cisplatin.

There are a number of limitations to be noted in the present study. First, our meta-analysis was performed using group data and not individual data, which pre-empted the possibility of adjusting for e.g. age at diagnosis, gender, ethnic group, cumulative cisplatin dose. Second, in all studies reviewed, hearing loss associated with cisplatin chemotherapy was assessed immediately or soon after treatment. Given that cisplatin persists indefinitely in the human cochlea after such treatment, the possibility of longer-term cochlear vulnerability (and hence progressive hearing loss) cannot be discounted. Third, ototoxic effects do not only lead to hearing loss, but also tinnitus, and vestibular toxicity, which were not assessed in the present review, may help determining additional impacts on the auditory system that cannot be revealed with traditional audiometry. There are numerous ototoxicity grading scales used across the different studies. In the clinical trial setting, standardization is vital and the variability between different studies makes analysis more challenging. Currently, there are 2 main categories of ototoxicity assessment criteria: (1) those measuring a change of hearing from baseline, such as the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), and (2) those measuring absolute hearing levels, such as the Brock or Chang classifications. Interestingly, Spracklen et al. used the CTCAE and Chang grading scales, but also the American Speech-Language-Hearing Association criteria (ASHA), and the resulting associations differed depending on which scale is used. As a matter of fact NFE2L2 polymorphisms presented as significantly ototoxic in ASHA and CTCAE scales (the latter of which was included in Fig. 2) but not when Chang scale is used. As a consequence, the selection of the grading scale can have a dramatic impact on the outcome of the study. These findings highlight the needs of determining the most sensitive measures in order to standardise the methodologies into the context of genetic testing in ototoxic vulnerability.

Finally, whilst a genetic predisposition to cisplatin mediated sensorineural hearing loss has been identified and may help identifying cancer patients with greater ototoxic risk, the specific mechanisms remain elusive. This would be an essential precurious step to the development of oto-protective therapy together with cisplatin interventions. It has recently been demonstrated that specifically targeting the p53 pathway protects from cisplatin ototoxicity while still maintaining cancer treatment efficacy. Knowing the genetic predisposition to cisplatin is an important advancement for improving clinical treatment but new new therapies that target specific pathways are being developed to protect against cisplatin-induced ototoxicity.
References
1. Burstein, H. J. et al. Clinical Cancer Advances 2017: Annual Report on Progress Against Cancer From the American Society of Clinical Oncology. J Clin Oncol 35, 1341–1367, https://doi.org/10.1200/JCO.2016.71.5292 (2017).
2. Thomson, R. S., Auduong, P., Miller, A. T. & Gurgel, R. K. Hearing loss as a risk factor for dementia: A systematic review. Laryngoscope Investig Otolaryngol 2, 69–79, https://doi.org/10.1002/lio2.65 (2017).
3. Greenzang, K. A. Hearing Loss. J Clin Oncol 36, 94–95, https://doi.org/10.1200/JCO.2017.75.2212 (2018).
4. Frisina, R. D. et al. Comprehensive Audiometric Analysis of Cisplatin-Based Chemotherapy in Survivors of Adult-Onset Cancer. J Clin Oncol 34, 2712–2720, https://doi.org/10.1200/JCO.2016.66.8822 (2016).
5. Lippman, A. J., Helson, C., Henson, L. & Krakoff, I. H. Clinical trials of cis-diaminedichloroplatinum (NSC-119875). Cancer Chemother Rep 57, 191–200 (1973).
6. Landier, W. Ototoxicity and cancer therapy. Cancer 122, 1647–1658, https://doi.org/10.1002/cncr.29779 (2016).
7. The L. Hearing loss: time for sound action. Lancet 390, 2414, https://doi.org/10.1016/S0140-6736(17)33097-0 (2017).
8. Wilson, B. S., Tucci, D. L., Merson, M. H. & D’Onofrio, G. M. Global hearing health care: new findings and perspectives. Lancet 390, 2503–2515, https://doi.org/10.1016/S0140-6736(17)31073-5 (2017).
9. Breglio, A. M. et al. Cisplatin is retained in the cochlea indefinitely following chemotherapy. Nat Commun 8, 1654, https://doi.org/10.1038/s41467-017-01853-1 (2017).
10. Groisman, S., Mukherjea, D., Rybak, I. P. & Ramkumar, V. Mechanisms of Cisplatin-Induced Ototoxicity and Otoprotection. Front Cell Neurosci 11, 338, https://doi.org/10.3389/fncel.2017.00338 (2017).
11. Wang, D. & Lippard, S. J. Cellular processing of platinum anticancer drugs. Nat Rev Drug Discov 4, 307–320, https://doi.org/10.1038/nrd1691 (2005).
12. Siddik, Z. H. Cisplatin: mode of cytotoxic action and molecular basis of resistance. Oncogene 22, 7265–7277, https://doi.org/10.1038/sj.onc.1206933 (2003).
13. Groisman, S., Mukherjea, D., Jajoo, S. & Ramkumar, V. Cisplatin ototoxicity and protection: clinical and experimental studies. Tohoku J Exp Med 219, 177–186 (2009).
14. Wang, J. et al. Caspase inhibitors, but not c-Jun NH2-terminal kinase inhibitor treatment, prevent cisplatin-induced hearing loss. Cancer Res 64, 9217–9224, https://doi.org/10.1158/0008-5472.CAN-04-1581 (2004).
15. Benkafadar, N. et al. Reversible p53 inhibition prevents cisplatin ototoxicity without blocking chemotherapy efficacy. EMBO Mol Med 9, 7–26, https://doi.org/10.15252/emmm.201606230 (2017).
16. Gao, J. P., Lauterjung, J., Liebert, R., Seiler, F. & Thomale, J. High accumulation of platinum-DNA adducts in spiral marginal cells of the cochlea is an early event in cisplatin but not carboplatin ototoxicity. Mol Pharmacol 70, 23–29, https://doi.org/10.1124/mol.106.022244 (2006).
17. Yancey, A. et al. Risk factors for cisplatin-associated ototoxicity in pediatric oncology patients. Pediatr Blood Cancer 59, 144–148, https://doi.org/10.1002/pbc.24138 (2012).
18. Grodin, Y. et al. Genetic Polymorphisms Associated with Hearing Threshold Shift in Subjects during First Encounter with Occupational Impulse Noise. PLoS One 10, e0130827, https://doi.org/10.1371/journal.pone.0130827 (2015).
19. Gauvin, D. V., Yoder, J., Zimmermann, Z. J. & Tapp, R. Ototoxicity: The Radical Drum Beat and Rhythm of Cochlear Hair Cell Life and Death. Int J Toxicol 37, 195–206, https://doi.org/10.1080/10548528.2018.1471128 (2018).
20. Travis, L. B. et al. Chemotherapy-induced peripheral neurotoxicity and ototoxicity: new paradigms for translational genomics. J Natl Cancer Inst 106, https://doi.org/10.1093/jnci/dju014 (2014).
21. Oldenburg, J. & Gietema, J. A. The Sound of Silence: A Proxy for Platinum Toxicity. J Clin Oncol 34, 2687–2689, https://doi.org/10.1200/JCO.2016.68.2476 (2016).
22. Knoll, C., Smith, R. J., Shores, C. & Blatt, J. Hearing genes and cisplatin deafness: a pilot study. Laryngoscope 116, 72–74, https://doi.org/10.1097/01.jlou.0000172262.02070.d2 (2006).
23. Redman, S., Scheurer, M. E., Adesina, A., Lau, C. C. & Okcu, M. F. Glutathione S-transferase P1 single nucleotide polymorphism predicts permanent ototoxicity in children with medulloblastoma. Pediatr Blood Cancer 60, 593–598, https://doi.org/10.1002/pbc.24366 (2013).
24. Ueda, Y., Jariwala, N. & Datar, J. Ootoxic effects of irradiation. Indian J Otolaryngol Head Neck Surg 63, 151–154, https://doi.org/10.1007/s12070-011-0422-9 (2011).
25. Grodin, Y. et al. DNA methylation of a novel PAK4 locus influences ototoxicity susceptibility following cisplatin and radiation therapy for pediatric embryonal tumors. Neuro Oncol 19, 1372–1379, https://doi.org/10.1093/neuonc/nox076 (2017).
26. Drogemoller, B. I. et al. Association Between SLC16A5 Genetic Variation and Cisplatin-Induced Otoxic Effects in Adult Patients With Testicular Cancer. JAMA Oncol 3, 1558–1562, https://doi.org/10.1001/jamaoncol.2017.0502 (2017).
27. Olum, Y. et al. Analysis of genetic and non genetic risk factors for cisplatin ototoxicity in pediatric patients. Int J Pediatr Otolaryngology90, 64–69, https://doi.org/10.1016/j.ijpedit.2016.09.001 (2016).
28. Wheeler, H. E. et al. Variants in WFS1 and Other Mendelian Deafness Genes Are Associated with Cisplatin-Associated Ototoxicity. Clin Cancer Res 23, 3325–3333, https://doi.org/10.1158/1078-0433.CCR-16-2809 (2017).
29. Robertson, J. M. & Neuhäuser, M. Review of alternative approaches to calculation of the odds ratio for a 2 × 2 contingency table. Methods in Ecology and Evolution, 9–13 (2013).
30. Lawrenz, R. Small sample confidence intervals for the Odds Ratio. Communications in Statistics - Simulation and Computation 33, 1095–1113 (2004).
31. Kaplan, K. et al. Glutathione S-transferase genetic polymorphisms and individual sensitivity to the ototoxic effect of cisplatin. Pharmacogenomics J 8, 23–28, https://doi.org/10.1038/sj.tpj.6500455 (2008).
32. Barahmani, N. et al. Glutathione S-transferase M1 and T1 polymorphisms may predict adverse effects after therapy in children with medulloblastoma. Neuro Oncol 11, 292–300, https://doi.org/10.1215/15228517-2008-089 (2009).
77. Lin, C. Y.
71. Fortunato, G.
76. Checa-Rojas, A.
74. Young, L. C.
66. Liu, C.
67. Poirrier, A. L.
64. Asadov, C., Aliyeva, G. & Mustafayeva, K. Thiopurine S-Methyltransferase as a Pharmacogenetic Biomarker: Significance of Testing
62. Li, Q.
60. Spracklen, T. F., Vorster, A. A., Ramma, L., Dalvie, S. & Ramesar, R. S. Promoter region variation in NFE2L2 influences susceptibility
61. Xu, X.
59. Spracklen, T. F.
57. Hagleitner, M. M.
55. Lopes-Aguiar, L.
53. Talach, T.
51. Caronia, D.
49. Oldenburg, J.
48. Oldenburg, J., Kraggerud, S. M., Cvancarova, M., Lothe, R. A. & Fossa, S. D. Cisplatin-induced long-term hearing impairment is
46. Thiesen, S.
transport: role of the MRP/CFTR/ABCC and OATP/SLC21A families of membrane proteins.
Hear Res
threshold shift.
et al.
https://doi.org/10.18632/oncotarget.24796 (2018).
14609–14614, https://doi.org/10.1073/pnas.0807219105 (2008).
workers.
et al.
2012–2018, https://doi.org/10.1373/clinchem.2004.037788 (2004).
platinum drugs in lung cancer.
Int J Cancer
89–105, https://doi.org/10.4067/S0716-97602011000100012 (2011).
Cancer Res
platinum-induced ototoxicity in children with cancer.
Clin Pharmacol Ther
238–255, https://doi.org/10.1016/j.heares.2009.07.008 (2009).
Shen, H. et al. Genetic variation in GSTM1 is associated with susceptibility to noise-induced hearing loss in a Chinese population.
J Occup Environ Med
1157–1162, https://doi.org/10.1097/JOM.0b013e31825902ce (2012).

79. Manche, S. K., Jangala, M., Putta, P., Koralla, R. M. & Akka, J. Association of oxidative stress gene polymorphisms with presbycusis. *Gene* **593**, 277–283, https://doi.org/10.1016/j.gene.2016.08.029 (2016).
80. Callejo, A. et al. Dose-dependent cochlear and vestibular toxicity of trans-tympanic cisplatin in the rat. *Neurotoxicology* **60**, 1–9, https://doi.org/10.1016/j.neuro.2017.02.007 (2017).
81. King, K. A. & Brewer, C. C. Clinical trials, ototoxicity grading scales and the audiologist's role in therapeutic decision making. *Int J Audiol*, 1–10, https://doi.org/10.1080/14992027.2017.1417644 (2017).
82. Schlee, W. et al. Innovations in Doctoral Training and Research on Tinnitus: The European School on Interdisciplinary Tinnitus Research (ESIT) Perspective. *Front Aging Neurosci* **9**, 447, https://doi.org/10.3389/fnagi.2017.00447 (2017).

**Acknowledgements**
The work was supported by an independent research program funded under the Biomedicine and Molecular Biosciences European Cooperation in Science and Technology (COST) Action framework (TINNET BM1306). DB is funded through the NIHR Biomedical Research Centre program, however the views expressed are those of the authors and not of the NIHR nor the UK Department of Health. BC has received funding from the Swedish Medical Research Council K2014-99X-22478-01-3, Karolinska Institutet and Tysta Skolan. CRC has received funding from Hörselforskningsfonden, Tysta Skolan, Karolinska Institutet as well as support from the European Union’s Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No. 722046.

**Author Contributions**
D.B. designed the study; E.T. and T.N. collected data; E.T., T.N., N.E. and C.R.C., analysed data; E.T., T.N., N.E. and C.R.C., generated figures; J.B., P.P., D.B., C.R.C. and B.C. helped to develop the scientific arguments and contributed to data interpretation. All authors played a role in writing the manuscript and approved the final version.

**Additional Information**
Supplementary information accompanies this paper at https://doi.org/10.1038/s41598-019-40138-z.

**Competing Interests:** The authors declare no competing interests.

**Publisher's note:** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© The Author(s) 2019