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Original Research

Usefulness of current candidate genetic markers to identify childhood cancer patients at risk for platinum-induced ototoxicity: Results of the European PanCareLIFE cohort study

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Abstract ***Background:*** Irreversible sensorineural hearing loss is a common side effect of platinum treatment with the potential to significantly impair the neurocognitive, social and educational development of childhood cancer survivors. Genetic association studies suggest a genetic predisposition for cisplatin-induced ototoxicity. Among other candidate genes, thiopurine methyltransferase (*TPMT*) is considered a critical gene for susceptibility to cisplatin-induced hearing loss in a pharmacogenetic guideline. The aim of this cross-sectional cohort study was to confirm the genetic associations in a large pan-European population and to evaluate the diagnostic accuracy of the genetic markers.

Methods: Eligibility criteria required patients to be aged less than 19 years at the start of chemotherapy, which had to include cisplatin and/or carboplatin. Patients were assigned to three phenotype categories: no, minor and clinically relevant hearing loss. Fourteen variants in eleven candidate genes (*ABCC3, OTOS, TPMT, SLC22A2, NFE2L2, SLC16A5, LRP2, GSTP1, SOD2, WFS1* and *ACYP2*) were investigated. Multinomial logistic regression was performed to model the relationship between genetic predictors and platinum ototoxicity, adjusting for clinical risk factors. Additionally, measures of the diagnostic accuracy of the genetic markers were determined.

Results: 900 patients were included in this study. In the multinomial logistic regression, significant unique contributions were found from *SLC22A2* rs316019, the age at the start of platinum treatment, cranial radiation and the interaction term [platinum compound]* [cumulative dose of cisplatin]. The predictive performance of the genetic markers was poor compared with the clinical risk factors.

Conclusions: PanCareLIFE is the largest study of cisplatin-induced ototoxicity to date and confirmed a role for the polyspecific organic cation transporter *SLC22A2*. However, the predictive value of the current genetic candidate markers for clinical use is negligible, which puts the value of clinical factors for risk assessment of cisplatin-induced ototoxicity back into the foreground.

1. Introduction

The anti-neoplastic drugs cisplatin and carboplatin are effective treatments for a wide variety of malignancies. However, their anti-tumour efficacy comes at the price of ototoxicity, which can include permanent hearing loss and tinnitus that is secondary to sensorineural degradation [1]. The incidence of ototoxicity is higher in children and more prevalent after cisplatin compared to carboplatin treatment. With rising survival rates of childhood cancers, the impact on survivorship quality of life by affecting neurocognitive, social and educational development of childhood cancer survivors has become a major concern [1,2].

The occurrence of cisplatin-induced ototoxicity seems unpredictable, even among patients receiving similar treatment regimens. Although several non-genetic risk factors have been identified, including age, cumulative dose and head (cochlear) radiation therapy, these factors only partially explain the inter-individual variability in ototoxic responses to platinum, suggesting that genetic variation may also contribute [1]. Accordingly, in recent years, there have been several efforts to identify genetic factors that predispose individuals to platinum ototoxicity. Specifically, genetic variants in metabolic enzymes (thiopurine methyltransferase [*TPMT*], glutathione-S-transferase pi [*GSTP1*]), transporters (solute carrier family 22 member 2 [*SLC22A2*], monocarboxylate transporter 6 [*SLC16A5*], ATP-binding cassette subfamily C member 3 [*ABCC3*], megalin [*LRP2*]), constituents in apoptotic signaling and oxidative stress (sodium dismutase [*SOD2*], NF-E2-related factor 2 [*NFE2L2*]) and deafness-associated genes (otospiralin [*OTOS*]) have been linked to cisplatin-induced ototoxicity in candidate gene association studies [3–10].

Genome-wide association studies (GWAS) also identified markers in the acylphosphatase 2 gene (*ACYP2*) or the wolframin gene (*WFS1*) [11,12]. Validation of key genetic determinants, however, is hampered by the lack of replication and the conflicting study results for many of the genetic markers [13]. An important limitation of most previous association studies is their low statistical power, with fewer than 100 patient cases in their (discovery) cohorts [3–10].

One marker gene that has been the focus of some association studies is *TPMT*. Based on available evidence, information on the association of *TPMT* polymorphisms with cisplatin-induced hearing loss was included in the FDA-approved drug label for cisplatin, stating that 'genetic factors such as variants in *TPMT* may contribute to the cisplatin-induced ototoxicity' [14]. A guideline was developed by the Canadian Pharmacogenomics Network for Drug Safety (CPNDS), which recommends pharmacogenetic testing for the *TPMT* *2, *3A, *3B and *3C defective alleles when prescribing cisplatin to paediatric cancer patients [15].

When evaluating the appropriate use of new pharmacogenetic tests, clinicians and health care policymakers must consider the accuracy with which a test for marker SNPs identifies the patient's ototoxicity risk. However, the clinical validity of the candidate genetic markers for cisplatin-induced ototoxicity in a real, unselected population of cancer survivors has not been well established. The primary aim of our study was to investigate the predictive value of candidate genetic markers in a large unselected paediatric population of cancer patients across Europe that were treated with cisplatin and/or carboplatin. To this end, we investigated the replication of 14 SNPs in 11 candidate genes for platinum-induced hearing loss in this largest cohort for this type of investigation. As a secondary objective, we determined the diagnostic accuracy of the genetic markers with a particular focus on *TPMT* markers that are currently recommended in a pharmacogenetic guideline [15]. This study was carried out as part of the PanCareLIFE European project [16–19].

2. Methods

2.1. Study design and participants

Background and methods of the European multicenter PanCareLIFE study have been described elsewhere [16,18,19]. Patients were enrolled after approval was obtained from local review boards (Supplementary Methods), and written informed consent was obtained from patients, parents or legal guardians. Participants were enrolled both retrospectively and prospectively (i.e. chemotherapy was started and finished during the 5-year term of PanCareLIFE). Eligibility criteria were: 1) age at diagnosis <19 years, 2) treatment with cisplatin, carboplatin or both, 3) at least one pure tone audiometry within 5 years after the end of chemotherapy. Exclusion criteria were: 1) non-consent and 2) hearing loss before the start of platinum treatment. Patients of this larger ototoxicity cohort participated in the pharmacogenetic study if there was additional consent for the genetic analyses and biomaterial was provided.

2.2. Genotyping

Biosamples were sent to the PanCareLIFE genotyping center. Genomic DNA (gDNA) was isolated from EDTA blood samples with a QIAamp DNA Blood Kit (Qiagen, Hilden, Germany) or from saliva samples (Oragene DNA collection kit, DNA Genotec, Ottawa, ON, Canada) using the prepIT L2P reagent (DNA Genotec, Ottawa, ON, Canada). All gDNA samples isolated were tested for quality (A260/A280 ratio of >1.9 and agarose gel electrophoresis) before any further work on DNA analysis. Samples were genotyped for 14 SNPs (Supplementary Table S2) by TaqMan SNP

genotyping using predesigned primers and probes (Applied Biosystems, Foster City, CA, USA). In order to not lose too much statistical power, the number of candidate genes was limited to 11 with one SNP each except for TPMT, for which 4 SNPs were examined. The candidate SNPs were selected on the basis of the available evidence of association, taking into account the sample size of the discovery cohort and the effect size.

Laboratory assistants were blinded to the audiological phenotype of the patients. Multiple positive and negative controls and replicate samples were included in the genotyping assays and plates. No genotype discordance of replicate samples was observed. Ten samples were finally excluded due to the genotype call rate per sample <100%.

2.3. Audiological classification and phenotyping

Patients were assigned to one of three phenotypes based on their audiograms, hereinafter referred to as no hearing loss, minor hearing loss (post-treatment audiograms indicated hearing loss of Münster class 1 or 2a), and clinically relevant hearing loss (post-treatment audiograms indicated a hearing loss of at least Münster class 2b). A detailed description of the phenotyping is provided in the Supplementary Methods.

2.4. Statistical analysis

Departure from Hardy–Weinberg equilibrium (HWE) was defined as p-HWE<0.0045 (after Bonferroni correction of the nominal p-value set at 0.05) and tested by χ^2 test of goodness of fit between the observed and expected genotypes.

Power for a logistic regression model, assuming a true additive genetic effect, was calculated using the genpwr R package. By assuming a case rate of 50%, an additive genetic effect, and a total cohort of 900 patients, our study would be able to detect an odds ratio of between 1.4 and 2.3, depending on the frequency of the minor allele, with power = 80% and a type I error alpha equal to 0.45% (after Bonferroni correction of the nominal p-value set at 0.05) (Supplementary Fig. S1).

Consistent with previous reports, in this study, an additive mode of inheritance was assumed with SNPs coded 0, 1, or 2 to represent wild-type homozygotes, heterozygotes and mutant-homozygotes, respectively. The outcome measure was ototoxicity with three categories – no, minor and clinically relevant hearing loss – and the explanatory variables included genotypes of the 14 candidate SNPs, cranial radiation, sex, age at the start of treatment (groups at 5-year intervals), cumulative dose of cisplatin ($\leq$300 mg/m^2, >300 mg/m^2 and $\leq$450 mg/m^2, and >450 mg/m^2), platinum compound (the use of carboplatin, cisplatin, or both), and concomitant ototoxic medication with aminoglycoside antibiotics or vinca alkaloids (none, a drug from one of the two groups, and drugs from each of the two groups). Since carboplatin is recommended as a second-line treatment in several protocols when patients do not tolerate cisplatin due to side effects such as ototoxicity or when the cumulative cisplatin dose has already exceeded a certain threshold, we assumed a potential interaction between the variables 'cumulative dose of cisplatin' and 'platinum compound', and therefore, constructed the interaction term from both variables. We planned to analyze the total cohort of patients treated with cisplatin and/or carboplatin and the subcohort of patients treated with cisplatin (with or without carboplatin). We planned to perform an ordered logistic regression if the proportional odds assumption was not violated. Because we observed that ordered logit coefficients were not equal across the levels of the outcome, we fitted a multinomial logit model. In a first step, the predictors' unique contributions to the multinomial logistic regression were tested. We then excluded all predictors that did not have significant unique effects at the traditional 0.05 criterion of statistical significance, and parameter estimates for the reduced multinomial logit model were calculated. A family-wise alpha level of 0.05 was defined. For the accounting of multiple testing, the Benjamini-Hochberg False Discovery Rate (FDR) was used.

3. Results

3.1. Patient characteristics

In PanCareLIFE, 2696 paediatric cancer survivors were enrolled in the larger ototoxicity study. Of these, 1112 patients provided biomaterial for genotyping, and therefore were screened for participation in the pharmacogenetic part of the study, and 900 participants qualified for inclusion (Consort flow diagram, Fig. 1). The patient characteristics, according to the degree of hearing loss, are listed in Table 1.

3.2. Genotypes

Genotype frequencies for the candidate SNPs are summarised in Supplementary Table S3. All SNPs passed the HW test at $p > 0.05$.

3.3. Regression analysis

Multinomial logistic regression was performed in the total cohort (treatment with carboplatin and/or cisplatin; N = 900) and in the subcohort of cisplatin-treated patients (with or without carboplatin; N = 741) to model the relationship between the predictors and membership in the three phenotype groups (no hearing loss, minor hearing loss and clinically relevant hearing loss).

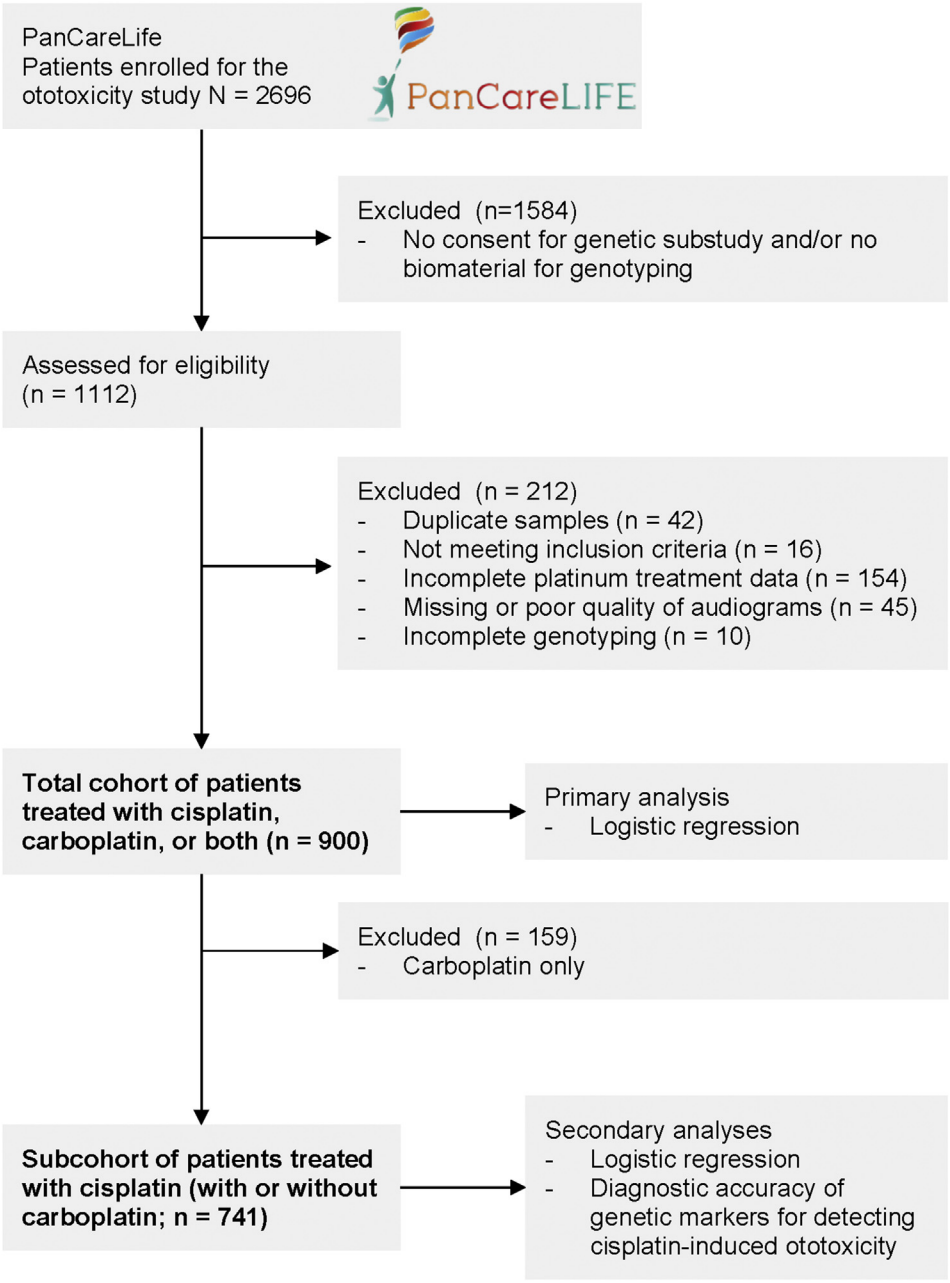

Fig. 1. Flowchart of patients' inclusion/exclusion.

The modeling results, using a simultaneous entry method, are summarised in Table 2 and Supplementary Table S4. Of the genetic markers, only rs316019 in *SLC22A2* significantly contributed to the multinomial logit model. Furthermore, the clinical variables age, cranial irradiation and the interaction term [platinum compound]*[cumulative cisplatin dose] contributed significantly. Sensitivity analysis by the exclusion of all patients with cranial irradiation confirmed a contribution of rs316019 in *SLC22A2* to the multinomial logit model (likelihood ratio test $p = 0.020$, after the correction for multiple testing p(FDR) = 0.033).

A reduced multinomial logit model was calculated after exclusion of all predictors that did not have significant unique effects (Fig. 2 and Supplementary Table S5). The Nagelkerke-R square indicated that 25% and 21% of the total variations in platinum-induced ototoxicity in the total cohort and the cisplatin-treated subcohort, respectively, were due to variations in the predictor variables. *SLC22A2* rs316019 was a weak independent predictor of minor hearing loss (total cohort: OR 1.51, 95% CI 1.02–2.25, $p = 0.042$, after the correction for multiple testing p(FDR) = 0.051; cisplatin-treated subcohort: OR 1.79, 95% CI 1.15–2.77, $p = 0.009$, p(FDR) = 0.026).

Table 1
Patient characteristics.

Characteristics	No hearing loss (n = 222)	Hearing loss (n = 678)	Minor hearing loss (n = 481)	Clinically- relevant hearing loss (n = 197)
Male sex – n (%)	131 (59.0%)	391 (57.7%)	270 (56.1%)	121 (61.4%)
Age at diagnosis – months, median (min, max)	138.0 (0–220)	93.0 (0–225)	80.5 (0–225)	110.0 (4–220)
Age at start of platinum treatment – months, median (min, max)	138.0 (1–220)	94.5 (0–224)	78.0 (0–224)	113.0 (7–220)
Age group of the beginning of platinum treatment – n (%)				
≤ 5 years	40 (18%)	262 (39%)	217 (45%)	45 (23%)
[5 years; 10 years]	54 (24%)	145 (21%)	82 (17%)	63 (32%)
[10 years; 15 years]	78 (35%)	180 (27%)	119 (25%)	61 (31%)
>15 years	50 (23%)	91 (13%)	63 (13%)	28 (14%)
Platinum compound – n (%)				
Cisplatin	128 (57.7%)	376 (55.5%)	279 (58.0%)	97 (49.2%)
Carboplatin	66 (29.7%)	93 (13.7%)	82 (17.0%)	11 (5.6%)
Cisplatin + carboplatin	28 (12.6%)	209 (30.8%)	120 (24.9%)	89 (45.2%)
Cisplatin cumulative dose – mg/m^2, median (min, max)	421.0 (30–1100)	408.0 (60–1650)	400.0 (60–1650)	443.5 (83–800)
Cisplatin dose group – n (%)				
>450 mg/m^2	73 (33%)	271 (40%)	181 (38%)	90 (46%)
[300 mg/m^2; 450 mg/m^2]	41 (18%)	160 (24%)	110 (23%)	50 (25%)
[0 mg/m^2; 300 mg/m^2]	42 (19%)	154 (23%)	108 (22%)	46 (23%)
0 mg/m^2	66 (30%)	93 (14%)	82 (17%)	11 (6%)
Carboplatin cumulative dose – mg/m^2, median (min, max)	3000 (800–12,600)	1700 (120 –13,750)	1800 (253–13,750)	1560 (120–13,000)
Cranial radiation – n (%)	43 (19.4%)	175 (25.8%)	104 (21.6%)	71 (36.0%)
Tumour type (ICD-10) – n (%)				
Osteosarcoma (C40, C41)	70 (31.5%)	201 (29.6%)	135 (28.1%)	66 (33.5)
Malignant neoplasm of brain (C71)	38 (17.1%)	176 (26.0%)	110 (22.9%)	66 (33.5%)
Malignant germ cell tumours (C52, C53, C56, C57, C62)	34 (15.3%)	32 (4.7%)	26 (5.4%)	6 (3.0%)
Neuroblastoma (C74)	4 (1.8%)	51 (7.5%)	36 (7.5%)	15 (7.6%)
Hepatoblastoma (C22)	5 (2.3%)	43 (6.3%)	36 (7.5%)	7 (3.6%)
Ganglioneuroma (C47)	1 (0.5%)	26 (3.8%)	19 (4.0%)	7 (3.6%)
Retinoblastoma (C69)	5 (2.3%)	15 (2.2%)	13 (2.7%)	2 (1.0%)
Other	48 (21.6%)	95 (14.0%)	80 (16.6%)	15 (7.6%)
Missing	17 (7.7%)	39 (5.8%)	26 (5.4%)	13 (6.6%)
Concomitant ototoxic medication (ATC) – n, (%)				
Aminoglycoside antibacterials (J01G)	45 (20.3%)	158 (23.3%)	114 (23.7%)	44 (22.3%)
Vinca alkaloids (L01CA)	102 (45.9%)	290 (42.8%)	197 (41.0%)	93 (47.2%)
Country – n (%)				
Austria	8 (3.6%)	19 (2.8%)	16 (3.3%)	3 (1.5%)
Czech Republic	57 (25.7%)	158 (23.3%)	113 (23.5%)	45 (22.8%)
Denmark	15 (6.8%)	74 (10.9%)	57 (11.9%)	17 (8.6%)
Germany	87 (39.2%)	243 (35.8%)	160 (33.3%)	83 (42.1%)
Italy	1 (0.5%)	6 (0.9%)	6 (1.2%)	0 (0.0%)
The Netherlands	13 (5.9%)	71 (10.5%)	49 (10.2%)	22 (11.2%)
Switzerland	41 (18.5%)	107 (15.8%)	80 (16.6%)	27 (13.7%)

The Münster classification was used to define the three phenotype groups: no hearing loss (Münster grade 0), mild hearing loss (Münster grade 1 or 2a) and clinically relevant hearing loss (Münster grade ≥2b) [18,41].

3.4. Diagnostic accuracy of the genetic markers for detecting cisplatin-induced ototoxicity

The discriminative potential of genetic markers for detecting cisplatin-induced ototoxicity can be quantified by the measures of diagnostic accuracy. The negative likelihood ratio for the presence of the variant *SLC22A2* rs316019 C allele for detecting cisplatin-induced ototoxicity was 0.44. Thus, a negative test result (i.e. absence of the rs316019 C allele) decreases the probability of cisplatin-induced ototoxicity about 17% (pretest probability 79% versus posttest probability 62%). Overall, the test performance was poor due to the low specificity (0.9%, 95% CI 0.3%–2.0%). The receiver operating characteristic (ROC) curve analysis of the clinical variables (i.e. age at the start of platinum treatment, cranial radiation and [platinum compound]* [cumulative dose of cisplatin]) for prediction of hearing loss of any degree is shown in Fig. 3. Inclusion of the genetic marker *SLC22A2* rs316019 in the ROC analysis did not significantly increase the area under the curve (0.730 versus 0.733).

Table 2
Predictors' Unique Contributions in the Multinomial Logistic Regression (Analysis of the Total Study Cohort, n = 900).

Effect	Chi-square	df	p(unc.)	FDR
ABCC3 rs1051640	0.01	2	0.994	0.994
ACYP2 rs1872328	0.97	2	0.615	0.835
GSTP1 rs1695	3.42	2	0.181	0.491
LRP2 rs2075252	0.67	2	0.716	0.872
NFE2L2 rs6721961	1.72	2	0.422	0.819
OTOS rs2291767	7.12	2	0.028	0.106
SLC16A5 rs4788863	0.39	2	0.824	0.921
SLC22A2 rs316019	10.92	2	4.26×10^{-3}	0.020
SOD2 rs4880	0.62	2	0.734	0.872
TPMT rs1142345	1.62	2	0.445	0.819
TPMT rs12201199	1.73	2	0.421	0.819
TPMT rs1800460	1.49	2	0.474	0.819
TPMT rs1800462	5.38	2	0.068	0.215
WFS1 rs62283056	0.05	2	0.978	0.994
Sex	1.25	2	0.536	0.835
Age at start of platinum	82.84	6	9.23×10^{-16}	8.77×10^{-15}
Cranial radiation	29.36	2	4.22×10^{-7}	2.67×10^{-6}
[Cisplatin cumulative dose] * [Carboplatin use]	109.26	12	8.40×10^{-18}	1.60×10^{-16}
Concomitant aminoglycosides or vinca alkaloids	1.03	2	0.598	0.835

p(unc.), uncorrected p-value; FDR, p corrected for multiple testing by a Benjamini-Hochberg false discovery rate methodology.

We also investigated the diagnostic performance of the combined testing for functional *TPMT* *2, *3a, *3B and *3C alleles, as recommended by the CPNDS guideline. Diagnostic performance indicators of the pharmacogenetic test for predicting a hearing loss of any degree or clinically relevant hearing loss are summarised in Supplementary Tables S6 and S7, respectively. The likelihood ratios were close to 1, indicating a lack of diagnostic value.

4. Discussion

In PanCareLIFE, 75% of children treated with cisplatin and/or carboplatin suffered audiometrically detectable hearing loss, and 22% had clinically relevant ototoxicity (Münster grade ≥2b). Ototoxicity has a detrimental impact on survivorship quality of life by affecting social and occupational function and is expected to cost society approximately $300,000 (index year 2000) over the lifetime of each individual [16,20]. Thus, early identification and/or aggressive medical intervention should have a substantial benefit.

Efforts have been made to identify genetic markers for preemptive testing to identify patients at risk for platinum-induced ototoxicity [21]. Our study confirms the association observed in some previous studies (Table 3) between rs316019, a non-synonymous SNP in *SLC22A2*, and platinum-induced ototoxicity. Several lines of evidence support the biological plausibility of the association. *SLC22A2* encodes the polyspecific organic cation transporter 2 (OCT2), which is primarily expressed in renal proximal tubule cells but is also found in other tissues such as the cochlea [22]. OCT2 translocates a variety of cationic compounds, including cisplatin, across the plasma membrane and maytherefore, be critical for the influx of cisplatin into the cell as the first step in its toxicity [23]. As confirmation, it was observed that co-medication with the OCT2 inhibitor cimetidine or knockout of the mouse homolog of human *SLC22A2* protected mice from cisplatin-induced ototoxicity [24]. Moreover, the missense SNP rs316019 (p.Ser270Ala) has been shown to affect the transport function of human OCT2 [25].

It is important to note that rs316019 was associated only with mild ototoxicity, suggesting that this coding SNP in *SLC22A2* is a hypomorphic or low-penetrance variant. In the clinically relevant hearing loss, the contribution of rs316019 is dominated by the much higher risk associated with factors such as cranial radiation or high cumulative dose of cisplatin. However, with an OR of 1.51, the variant rs316019 had only a small effect on the ototoxicity risk, and the test specificity was too low to support the use of rs316019 as a diagnostic marker in the clinic. Although having no clinical importance, the moderate evidence of an association of *SLC22A2* may be useful for understanding the biology and etiology of platinum-induced ototoxicity.

The CPNDS guideline recommends pharmacogenetic testing for the defective *TPMT* alleles *2, *3A, *3B and *3C in paediatric patients expected to receive cisplatin [15]. For carriers of at least one defective allele, the guideline authors encourage the use of otoprotectants or alternative treatments to cisplatin [15]. The recommendation does not contain any restrictions regarding tumour diagnosis, cranial radiation, ethnicity or the cisplatin treatment regimen of the patient. Our study is the first to evaluate the ability of combined testing for the *TPMT* alleles *2, *3A, *3B and *3C to correctly classify patients as having cisplatin-induced hearing loss in a large unselected cohort, and we found that these

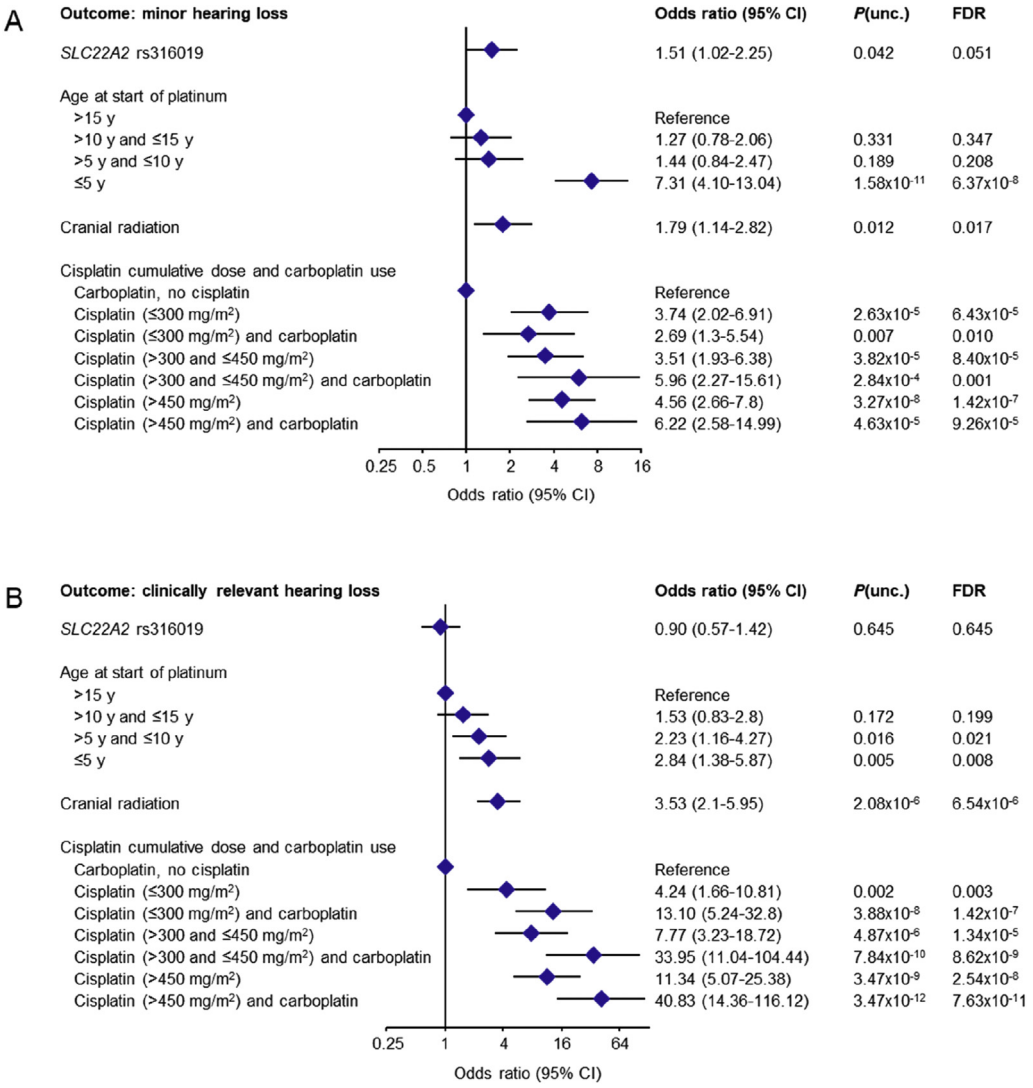

Fig. 2. Multinomial logistic regression model: adjusted odds ratios (OR) and 95% confidence intervals (CI) for platinum-induced minor hearing loss (A) and platinum-induced clinically relevant hearing loss (B). The total study cohort (cisplatin and/or carboplatin treatment; n = 900) was analyzed. p(unc.), uncorrected p-value; FDR, p corrected for multiple testing by a Benjamini-Hochberg false discovery rate methodology.

alleles have no diagnostic value. The functional role of *TPMT* in cisplatin-induced hearing loss is controversial. Overexpression of the dysfunctional *TPMT*∗3A haplotype in murine inner ear cell lines was found to be associated with an increase in cisplatin-induced cytotoxicity compared to cell lines that overexpress wild-type TPMT, suggesting a *TPMT*-cisplatin relationship [26]. However, no difference in cisplatin-induced hearing damage was identified in *Tpmt* wild-type and knockout mice, suggesting that no TPMT-cisplatin relationship exists [27].

Association studies of *TPMT* yielded inconsistent results (Table 3), and it had been speculated whether genetic associations are specific to certain treatment protocols [3,27–35]. Comparing the protocols of the discovery study of Ross *et al.* and the study of Yang *et al.*, in which replication failed, the use of cranial irradiation was a striking difference in both study protocols [3,33]. Because radiotherapy is a well-known independent risk factor for hearing loss, the question naturally arises as to whether ototoxic events may have been influenced by radiation as part of the treatment protocol [33]. We observed no significant association of the *TPMT* tag SNP rs12201199 with cisplatin-induced ototoxicity in patients without radiotherapy [17], which is in line with findings of Yang *et al.* in a small cohort of children with solid tumours who did not receive cranial irradiation [33]. Overall, there is no convincing evidence to suggest that the genetic association of *TPMT* with cisplatin-induced ototoxicity is specific only to patients who have not been irradiated cranially. Given the still unexplained heterogeneity

Table 3

Study	Population, age group	Ototoxicity phenotype definition	Sample size, number of cases/controls in the analysis	Cumulative dose of cisplatin (mg/m^2) in cases/controls	Patients with cranial radiation (%)	Diagnosis	Effect size [OR (95% CI)]				
							TPMT rs12201199	TPMT rs1142345	TPMT rs1800460	TPMT rs1800462	SLC22A2 rs316019
Ross *et al.* (2009) [3]	Canadian, paediatric	CTCAE (v3.0) ≥2	33/20 (discovery cohort); 73/36 (replication cohort)	360/360 (median, discovery cohort) 400/410 (median, replication cohort)	17 (discovery) 19 (replication)	Different cancer types	14.29 (0.81, 251.74) (discovery); 9.98 (1.31, 76.36) (replication)	11.03 (0.61, 197.64) (discovery); 5.79 (0.73, 45.72) (replication)	11.03 (0.61, 197.64) (discovery); 8.12 (0.46, 143.37) (replication)	–/–	Not reported
Pussegoda *et al.* (2013) [43]	Canadian, paediatric	CTCAE (v3.0) ≥2	87/68	400/400 (median)	18	Different cancer types	8.9 (3.2–24.9)	6.1 (2.1–17.3)	6.6 (2.0–21.8)	–/–	–/–
Yang *et al.* (2013) [33]	USA, paediatric	CTCAE (v3.0) ≥1; Chang ≥22a	144/61	300/300 (median)	100	Brain tumour	0.80 (0.42 –1.52)	0.50 (0.20 –1.24)	0.46 (0.17 –1.22)	Excluded from analysis	–/–
Lanvers-Kaminsky *et al.* (2014) [44]; Lanvers-Kaminsky *et al.* (2015) [5]	German, paediatric	Münster ≥1	36/27	412/418 (mean)	Not reported	Different cancer types	1.56 (0.45 –5.49)	–/–	–/–	–/–	4.60 (1.18 –17.91)
Hagleitner *et al.* (2014) [32]	Dutch, Spanish, adult and paediatric	CTCAE (v3.0) ≥2; SIOP Boston ≥2	19/53 (Dutch cohort), 18/16 (Spanish cohort)	500/480 (median, Dutch cohort), 504/515 (median, Spanish cohort)	0	Osteo-sarcoma	0.74 (0.19 –2.81) (Dutch) 6.79 (0.34 –136.71) (Spanish)	0.68 (0.14 –3.45) (Dutch) 4.69 (0.22 –101.72) (Spanish)	0.29 (0.04 –2.44) (Dutch) 2.58 (0.10 –65.61) (Spanish)	–/–	–/–
Olgun *et al.* (2016) [34]	Turkish, paediatric	Münster ≥2; Brock ≥2	30/42	Not reported; 19% of patients with additional carboplatin	21	Different cancer types	OR not reported (association was not significant)	–/–	–/–	–/–	–/–
Spracklen *et al.* (2016) [9]	South-African, paediatric and adult	ASHA; Chang ≥1; CTCAE (v4.0) ≥1	74/122	300/238 (median)	0	Different cancer types	–/–	–/–	–/–	–/–	2.09 (1.02 –4.25)
Thiesen *et al.* (2017) [35]	UK, paediatric	CTCAE (v4.0) ≥1	90/26	360–480/344–350 (range); 17% of patients with additional carboplatin	35	Different cancer types	0.24 (0.13 –0.44)	0.48 (0.26 –0.90)	0.76 (0.38 –1.51)	–/–	–/–
Mironowich *et al.* (2018) [28]	Russian, paediatric	Audiometry	16/34	428/396 mg/kg (mean)	14	Different cancer types	2.20 (0.30 –16.37)	–/–	–/–	–/–	–/–

Teft *et al.* (2019) [27]	Canadian, adult	CTCAE (v4.0) ≥grade 2 change from baseline	130/76	274/273 (median); switch to carboplatin allowed	100	Head and neck squamous cell carcinoma	OR not reported (association was not significant)	OR not reported (association was not significant)	OR not reported (association was not significant)	OR not reported (association was not significant)	OR not reported (association was not significant)
Driessen *et al.* (2019) [31]	Dutch, adult	CTCAE (v4.0) ≥2	7/85	240 (median, total cohort)	100	locally advanced head and neck cancer	OR not reported (association was not significant)	OR not reported (association was not significant)	OR not reported (association was not significant)	–/–	OR not reported (association not investigated)

OR, odds ratio; 96% CI, 95% confidence interval; –/–, not genotyped; ASHA, American Speech-Language-Hearing Association; CTCAE, Common Terminology Criteria for Adverse Events.

Fig. 3. Receiver operating characteristic (ROC) curve analysis of the clinical variables (age at the start of platinum treatment, cranial radiation and the interaction term [platinum compound]* [cumulative dose of cisplatin]), either alone or in combination with *SLC22A2* rs316019 genotypes for the prediction of minor hearing loss or clinically relevant hearing loss. AUC, the area under the curve.

across study results, the poor diagnostic performance of the *TPMT* markers, and the weak biological evidence linking *TPMT* with cisplatin-induced ototoxicity [26,27], we cannot recommend *TPMT* genetic testing for the management and prevention of cisplatin-induced hearing loss.

Although our study was well powered to detect an odds ratio of only 1.4 to 2.3, depending on the frequency of the minor allele, we could not replicate an association with cisplatin-induced ototoxicity for any candidate SNP except *SLC22A2* rs316019. Replication failure might be explained (in part) by the so-called 'winner's curse', which in GWAS appears as an upward bias in the estimated effect size of a newly identified risk allele [36]. Another factor that complicates replication may be heterogeneity across study populations in terms of the treatment protocol (including concomitant ototoxic medication or cranial radiation), ethnicity, and age. The methodological limitations of the association studies contributing to the lack of replication or between-study heterogeneity have been the subject of scientific debate [29,37]. Insufficient adjustment for non-genetic risk factors, which explain an important part of the ototoxicity risk, as shown in our study, may have inflated the significance of associations [37]. Access to study data at the patient level for a patient-level meta-analysis could help to reconcile the conflicting results of these studies.

There is currently no standardised grading scale of platinum-induced hearing loss, so the inconsistent use of scales for phenotyping and inconsistent threshold definitions for dichotomizing the ototoxicity phenotype (i.e. the definition of normal hearing versus hearing loss) may also lead to discrepancies in research results. The PanCareLIFE consortium used the Münster Classification for several reasons. This scale was developed to specifically classify platinum induced ototoxicity in children [38]. In contrast to all other scales, it also takes tinnitus into account. The Münster Classification was not inferior to other grading scales in comparative studies and appears to be more sensitive than other scales in the early detection of hearing loss [39–41].

Advances in the understanding of the pathophysiological mechanisms underlying cisplatin-induced ototoxicity, as well as future large-scale GWAS, may help to identify suitable genetic markers that can be used either alone or in combination to identify patients at risk for this permanent, treatment-related side effect. Overall, the genetic association studies on the ototoxicity of cisplatin provide preliminary support for the hypothesis that many common variants (with small effect sizes) underlie cisplatin-induced ototoxicity, i.e. that it is a polygenic trait [12,42]. This assumption is consistent with the experimental results of Dolan *et al.* who investigated the genetic determinants that explain the variation in cytotoxicity of cisplatin in lymphoblastoid cell lines [42]. Sensitivity to the cytotoxic effects of cisplatin was under the marked genetic influence (heritability was about 0.47), with several loci with low locus-specific heritability contributing to the trait [42].

As long as no suitable genetic markers are available for clinical use, patient-related and treatment-related risk factors continue to be of particular importance. Assessment of the individuals' age during treatment, the cumulative cisplatin dose and cranial/ear radiation provided fair diagnostic performance (area under the ROC curve: 0.73) for the prediction of platinum-induced ototoxicity. The results of the European PanCareLIFE study form the basis for the development or update of guidelines and will facilitate future monitoring and counselling with regards to platinum-related long-term effects [16].

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Appendix A. Supplementary data

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