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ARTICLE

Genetic variation of cisplatin-induced ototoxicity in non-cranial-irradiated pediatric patients using a candidate gene approach: The International PanCareLIFE Study

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Abstract

Ototoxicity is a common side effect of platinum treatment and manifests as irreversible, high-frequency sensorineural hearing loss. Genetic association studies have suggested a role for SNPs in genes related to the disposition of cisplatin or deafness. In this study, 429 pediatric patients that were treated with cisplatin were genotyped for 10 candidate SNPs. Logistic regression analyses revealed that younger age at treatment (≤5 years vs >15 years: OR: 9.1; 95% CI: 3.8–21.5; $P = 5.6 \times 10^{-7}$) and higher cumulative dose of cisplatin (>450 vs ≤300 mg/m^2: OR: 2.4; 95% CI: 1.3–4.6; $P = 0.007$) confer a significant risk of ototoxicity. Of the SNPs investigated, none of them were significantly associated with an increase of ototoxicity. In the meta-analysis, *ACYP2* rs1872328 (OR: 3.94; 95% CI: 1.04–14.03; $P = 0.04$) and *SLC22A2* rs316019 (OR: 1.46; 95% CI: 1.07–2.00; $P = 0.02$) were associated with ototoxicity. In order to increase the understanding of the association between SNPs and ototoxicity, we propose a polygenic model, which takes into account multiple interacting genes of the cisplatin pathway that together confer an increased risk of ototoxicity.

Introduction

Ototoxicity is a well-known side effect of platinum treatment in both adult and pediatric cancer patients [1–3]. Ototoxicity significantly impacts development due to its effects on speech and language development and potential learning difficulties [4–7]. The degree of cisplatin-induced ototoxicity is variable. Previous epidemiological studies have identified several environmental and clinical risk factors that can increase the risk of platinum-induced ototoxicity, including the type of platinum agent and drug-dosing conditions, such as single and cumulative cisplatin dose [8–10]. Cisplatin-induced hearing loss is particularly serious in pediatric and elderly patients [8, 9, 11]. In addition, concomitant cranial radiation may aggravate cisplatin-induced ototoxicity [9, 12, 13].

Since the same cisplatin dose may be ototoxic for one person but not for another of the same age, genetic factors may underlie susceptibility to cisplatin-related ototoxicity. Accordingly, research has been conducted to identify evidence for a genetic predisposition, focusing on gene variants producing alterations in cisplatin pharmacokinetics and pharmacodynamics. To date, several candidate

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gene studies and two genome-wide association studies (GWAS) have investigated the role of genetics in platinum-induced ototoxicity in pediatric and adult cancer patients [14]. These studies found several genetic variants in genes related to deafness (otospiralin, *OTOS*) or those that encode for drug-metabolizing enzymes (thiopurine S-methyltransferase, *TPMT*), transporters (organic cation transporter 2, *SLC22A2*; monocarboxylic acid transporter, *SLC16A5*; megalin, *LRP2*; ATP binding cassette transporter subfamily C member 3, *ABCC3*), detoxifying and metabolizing enzymes (superoxide dismutase 2, *SOD2*; glutathione S-transferase, *GSTP1*; acylphosphatase 2, *ACYP2*), or transcription factors (nuclear factor erythroid 2-related factor 2, *NFE2L2*).

The majority of genetic association studies were performed with <50 case-patients (i.e., patients with hearing loss after cisplatin treatment) in their respective discovery cohorts. Results from initial studies correlated only modestly with subsequent research on the same association for several genetic markers of cisplatin-associated ototoxicity [15–30]. In some studies that reported an association with a specific SNP, independent confirmatory studies are lacking; therefore, the strength of this evidence is unclear [28, 29]. Moreover, functional studies to understand the biological plausibility underlying the association are also lacking for many candidate genes and polymorphisms. Due to limited statistical power and missing replication studies, substantiated associations between candidate genetic markers and cisplatin-induced ototoxicity remain unknown.

The aim of the current study was to replicate previously identified genetic associations for cisplatin-induced ototoxicity in an independent cohort of non-cranially irradiated primarily cisplatin-treated pediatric cancer survivors recruited from across Europe within the EU-funded PanCareLIFE project. In addition, we performed a meta-analysis of the current study together with previously published studies to evaluate cumulative evidence for the presence or absence of the association of these SNPs with cisplatin-induced ototoxicity.

Patients and methods

Patients

This multinational retrospective study within the PanCareLIFE consortium was performed in a cohort of cisplatin-treated non-cranially irradiated pediatric cancer patients treated between April 1980 and April 2017. This study was approved by the local medical ethical committees of Switzerland (Bern, KEK-BE 362/2016), Italy (Genova, 507REG2014), Czech Republic (Brno and Prague, 18-36-54-60), Denmark (Copenhagen, H-1-2014-125), Germany (Münster and Lübeck, D-48147, 15-172, Austria (Graz, 27-015 ex 14/15), and The Netherlands (Amsterdam, Rotterdam, Utrecht and Groningen (2015_202#A201581, MEC-2014-633, 2015/JD/0019).

Participants were 19 years or younger at the time of diagnosis. Eligible patients met the following inclusion criteria: (1) started treatment with cisplatin (either solely cisplatin throughout the entire course of treatment or changed from cisplatin to carboplatin, but did not receive carboplatin initially), (2) normal hearing before start of cisplatin therapy, (3) no radiotherapy administered to the brain or inner ear, (4) at least one pure-tone audiometry had been performed within 5 years following the end of chemotherapy, and (5) saliva or blood available for DNA extraction, and genotyping passed quality control. Patients were enrolled after approval was obtained from local review boards, and written informed consent was obtained from patients, parents, or legal guardians. Further description of the PanCareLIFE cohort recruitment was described previously [31].

Audiological classification and phenotyping

All audiograms were classified by two raters according to the Münster classification system (Table 1), with this categorization functioning as an assessment of the severity of hearing loss [32]. To be classified, each audiogram must be legible, use standard or clearly-documented symbols, show unaided threshold measurements, be correctly masked where necessary, be at least within the frequencies 2 or 3, 4

Table 1 Münster classification of platinum-induced ototoxicity used in the PanCareLIFE study

Grade	Parameters	Functional relevance
0	≤10 dB HL at all frequencies	No considerable damage
1	>10 and ≤20 dB HL at one or more frequencies, or tinnitus	Questionable, commencing damage
2	>20 dB HL at 4 kHz and above	Moderate damage
2a	>20–≤40 dB	
2b	>40–≤60 dB	
2c	>60 dB	
3	>20 dB HL at <4 kHz	Impairment compensable with hearing aid
3a	>20–≤40 dB	
3b	>40–≤60 dB	
3c	>60 dB	
4	≥80 dB HL at <4 kHz	Loss of function, compensable by cochlear implantation

Scale is based on sensorineural hearing thresholds in dB hearing level

HL hearing loss

& 6 or 8 kHz (air-conduction), demonstrate no conductive hearing loss, and have no suggestion of significant test artifact (such as atypical air-bone configuration).

After the audiograms were classified, two pediatric audiologists from the audiological reference center assessed data from patients for whom audiogram data and platinum cycle treatment data were available. This assessment defined the kinetic course of hearing loss for each patient. The minimum data requirement for phenotype assessment included availability of a normal pre-treatment audiogram or a normal audiogram before the third platinum cycle and at least one post-treatment audiogram within 15 months after the last chemotherapy cycle. Sound field audiometry was also accepted if ear-specific pure-tone audiometry was subsequently performed.

In the current study, three phenotype groups were defined as follows: no hearing loss, minor hearing loss, and clinically-relevant hearing loss at end of treatment. Patients were assigned to the no hearing loss group if post-treatment audiograms were exclusively or mostly Münster class 0 and no audiogram was classified as Münster >1. Patients were assigned to the clinically-relevant hearing loss group if follow-up audiograms indicated hearing loss of at least Münster class 2b. All other patients were classified as part of the minor hearing loss group. All patients were phenotyped separately by two pediatric audiologists. After completion, all cases that had been phenotyped differently by the two pediatric audiologists were discussed between them and an agreement was made.

Genotyping and imputation

Genomic DNA was extracted from peripheral blood or saliva and was genotyped for 686,085 SNPs using the Illumina Infinium© Global Screening Array (Illumina, San Diego, CA). Standard quality control procedures were performed followed by imputations to the Haplotype Reference Consortium (HRC r1.1) reference panel using the Michigan Imputation Server, which increased the number of SNPs available for analysis to ~40 million [33, 34]. Imputation quality for the included SNPs was high ($r^2 > 0.98$), indicating a high certainty of imputed genotypes.

In this study, SNPs previously identified to be associated with platinum-induced ototoxicity were examined. Candidate SNPs were selected based on a literature search for genetic association studies on cisplatin-induced ototoxicity. To control for alpha inflation with minimal loss in statistical power, the number of candidate genes was limited to ten with one SNP genotyped per gene. Candidate genes/SNPs were selected based on expected allele frequencies, previously reported odds ratios, and quality criteria, such as the sample size of the previous studies or homogeneity of previous replication results. The SNPs were selected as follows: *ACYP* (rs1872328), *LRP2* (rs2075252), *NFE2L2* (rs6721961), *OTOS* (rs2291767), *TPMT* (rs12201199), *SOD2* (rs4880), *SLC22A2* (rs316019), *GSTP1* (rs1695), *ABCC3* (rs1051640), and *SLC16A5* (rs4788863) (Table 2).

Study selection for meta-analysis

A MEDLINE search was conducted (last update December 2018) to identify publications on genetic susceptibility factors associated with ototoxicity in pediatric and adult cancer patients. The following search terms were used: (“cisplatin”[MeSH Terms] OR “cisplatin”[All Fields]) AND (ototoxicity[All Fields] OR “hearing loss”[MeSH Terms] OR “hearing loss”[All Fields]) AND ((“polymorphism, genetic”[MeSH Terms]) OR ACYP2[All

Table 2 Candidate SNPs evaluated

Gene	Chromosome	Position GRCh37	rs #	Reference allele	Variant allele	Variant allele frequency (1000 genomes)	Consequence
ACYP2	2	54395259	rs1872328	G	A	0.07	Intron variant
LRP2	2	170010985	rs2075252	T	C	0.78	Missense variant (p.Lys4094Glu)
NFE2L2	2	178130037	rs6721961	T	G	0.85	2KB upstream variant
OTOS	2	241080132	rs2291767	T	C	0.07	5′ UTR variant
TPMT	6	18139802	rs12201199	A	T	0.16	Intron variant
SOD2	6	160113872	rs4880	A	G	0.41	Missense variant (p.Val16Ala)
SLC22A2	6	160670282	rs316019	A	C	0.86	Missense variant (p.Ser270Ala)
GSTP1	11	67352689	rs1695	A	G	0.35	Missense variant (p.Ile105Val)
ABCC3	17	48768486	rs1051640	A	G	0.10	Synonymous variant
SLC16A5	17	73089852	rs4788863	T	C	0.63	Synonymous variant

Fields] OR LRP2[All Fields] OR NFE2L2[All Fields] OR (OTOS[All Fields] OR "OTOS protein, human"[Supplementary Concept]) OR TPMT[All Fields] OR SOD2 [All Fields] OR (SLC22A2[All Fields] OR "Organic Cation Transporter 2"[Mesh]) OR ("glutathione s-transferase pi"[MeSH Terms] OR ("glutathione"[All Fields] AND "s-transferase"[All Fields] AND "pi"[All Fields]) OR "glutathione s-transferase pi"[All Fields] OR "gstp1"[All Fields]) OR ABCC3[All Fields] OR SLC16A5[All Fields]).

Studies were screened for inclusion in the meta-analysis. We included studies if (1) pediatric and/or adult cancer patients were treated with cisplatin-based chemotherapy (with or without cranial radiation), (2) the data were original (independence among studies), and (3) genotype or allele frequencies were provided in case and control patients.

Statistical analysis

Our primary aim was to replicate previously reported associations of SNPs with platinum-induced hearing loss. When planning the study, we calculated the sample size as follows: assuming a frequency of the risk allele in the European population of 5–10% and control to case ratio of 1, 151–272 case-patients are needed to detect an odds ratio of ≥2.5 with power = 80% and a type I error alpha = 5%.

Statistical analyses were performed using IBM SPSS (SPSS 24.0, Chicago, IL, USA). The three phenotype groups, patients without hearing loss, patients with minor hearing loss (Münster class 1, 2a) and patients with clinically-relevant hearing loss (Münster class ≥ 2b), were compared using descriptive statistics. A Chi-square test was used to determine whether there was a significant difference between the expected and observed frequencies between patient groups. The Kruskal–Wallis test was used to analyze the differences between patient groups for continuous variables. Cohen's kappa coefficient was used to measure inter-rater agreement for qualitative (categorical) items. Test-retest reliability was assessed using Kendall's Tau-b coefficients.

Association analysis of the 10 candidate SNPs with platinum-induced ototoxicity was performed using unadjusted and adjusted logistic regression with ototoxicity (Münster class ≥ 2b) vs no ototoxicity (Münster class 1, 2a) as outcome measure. For these analyses, adjustments were made for age at diagnosis (with four predefined age categories: ≤5 years, >5 and ≤10 years, >10 and ≤15 years, and >15 years), total cumulative dose of cisplatin (with three predefined dose levels: ≤300 mg/m^2, >300 mg/m^2 and ≤450 mg/m^2, and >450 mg/m^2), and the first four genetic principal components. We defined a family-wise alpha level of 0.05. To account for multiple testing, the Bonferroni correction was applied, resulting in a per comparison alpha level of 0.005.

Meta-analysis of previously published study results combined with our current results was performed using Review Manager 5.3. Heterogeneity between studies was assessed using the I^2 statistic and the Chi-squared statistic [35, 36]. If significant heterogeneity was identified ($I^2 >$ 50% and P value from the chi-squared test < 0.05), a random effects meta-analysis model was performed to adjust accordingly.

Results

Patient characteristics

In the PanCareLIFE study, 1,347 pediatric cancer survivors, from whom SNP array analysis was performed and genotype data had passed quality control, were screened for eligibility. Five hundred participants qualified for inclusion and 428 patients were included in the final analyses; 72 patients were excluded from the final analysis because their cumulative platinum dose was unknown or because the quality of audiometry was not sufficient (see Consort flow diagram; Fig. 1).

The median age at diagnosis was 10.7 years (range: 0–19.0 years), and the median follow-up time between the end of treatment and audiometric testing was 3 months (0–4.9 years) (Table 3). The most frequent diagnoses were osteosarcoma and (soft-tissue) sarcoma (50.5%), germ cell tumors (14.3%), and neuroblastoma (15.2%). Patients received a median cumulative cisplatin dose of 480 mg/m^2 (range: 64–950 mg/m^2) at a median duration of 4.5 months (minimum: 2 days; maximum: 3 years). Concomitant carboplatin chemotherapy was given to 96 patients (22.4%), either according to the protocol or due to cisplatin-induced adverse drug reactions, such as allergic reactions, renal dysfunction, or hearing loss.

Quality of audiogram classification and audiological phenotyping

One hundred and twelve randomly chosen audiograms (110 ears and two free-field audiograms) from 57 patients were classified independently by both raters to measure the degree of agreement in the classification of audiological data. We found a Cohen's Kappa coefficient of 1.00, which means that the results of the rating were unaffected by the raters. The test-retest reliability was examined by comparing the classifications described above with those given to the same 112 audiograms within the previous few months. With only one single difference between the first and the second classification, we found a nearly perfect correlation with Kendall's Tau-b coefficient of 0.993, indicating high test-retest reliability. Two raters independently classified

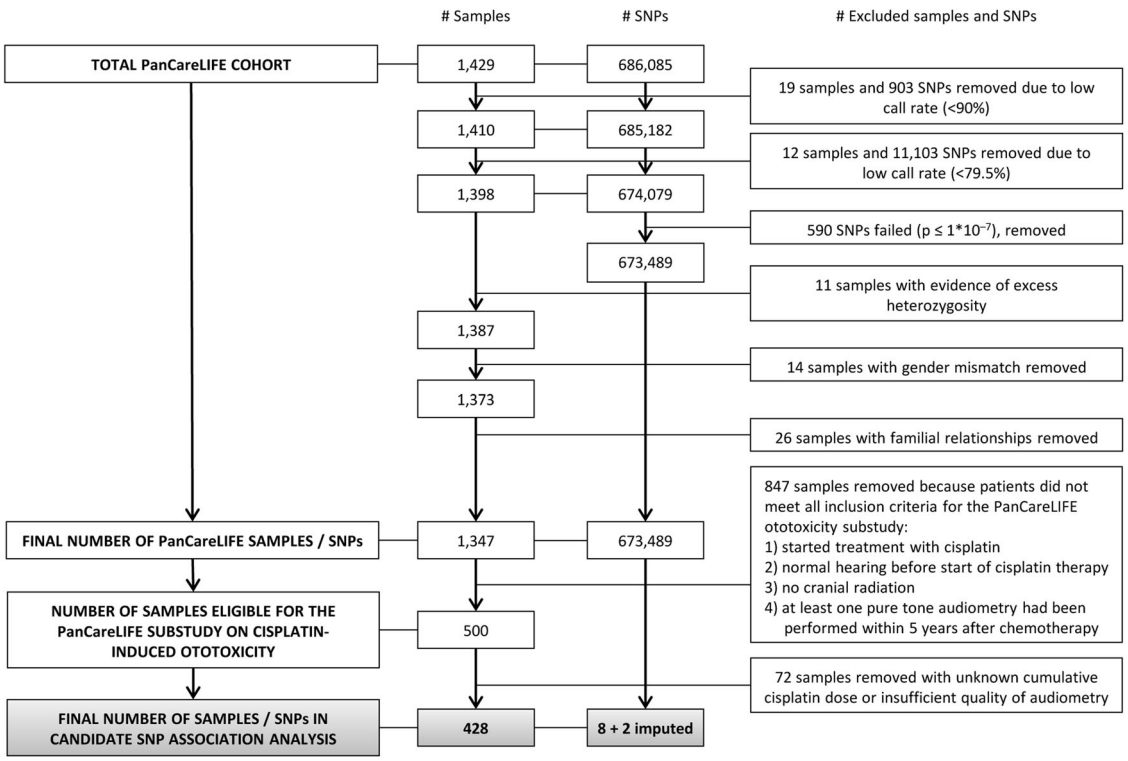

Fig. 1 Consort trial participant flow diagram. SNP, single nucleotide polymorphism

100 patients and inter-rater reliability was calculated to measure the degree of agreement in the phenotyping of audiological data. The Cohen's Kappa coefficients were 0.95 and 1.00 for the phenotypes of no hearing loss and clinically-relevant hearing loss, respectively.

Non-genetic risk factors of ototoxicity

In our cohort ($n = 428$), 229 (54%) patients developed minor hearing loss and 95 (22%) patients developed clinically-relevant hearing loss after cisplatin treatment. Logistic regression analyses were performed to ascertain the effects of age, gender, and cumulative cisplatin dose on the likelihood that participants developed any hearing loss or clinically-relevant hearing loss (Fig. 2a, b). Both logistic regression models were statistically significant ($P = 3.7 \times 10^{-8}$ and $P = 4.8 \times 10^{-4}$, respectively), and explained 15% (Nagelkerke R^2) of the variance in hearing loss, correctly classifying 76% and 64% of cases, respectively.

Compared with children >15 years of age, children under 5 years were more likely to develop platinum-associated hearing loss (Münster class ≥1: odds ratio, OR: 9.1; 95% confidence interval, CI: 3.8–21.5; $P = 5.6 \times 10^{-7}$; Münster class ≥ 2b: OR: 3.9; 95% CI: 1.4–11.2; $P = 0.012$). Patients who received a cumulative cisplatin dose of >450 mg/m^2 had a 2.4 higher odds of developing platinum-associated hearing loss (any hearing loss: OR: 2.4; 95% CI: 1.3–4.6; $P = 0.007$; clinically-relevant hearing loss: OR: 5.0; 95% CI: 1.8–14.5; $P = 0.003$) than patients treated with low cumulative cisplatin doses (≤300 mg/m^2). Gender was not associated as a risk factor.

SNP associations

None of the ten candidate SNPs from ten different genes was significantly associated with platinum-induced ototoxicity. Table 4 shows the calculated ORs and *P*-values of the univariable and multivariable association analyses by applying an additive effects model of variant allele dosage. In the multivariable model, adjustments were made for age at diagnosis, total cumulative cisplatin dose, and the four genetic principal components (to account for genetic ancestry differences). The observed ORs ranged from 0.43 to 1.74.

Meta-analyses

Meta-analyses were carried out for each SNP investigated in our study. The literature search retrieved 51 references. After screening titles and abstracts, 21 references remained for further evaluation and data extraction. Two studies were excluded because allele or genotype frequencies were not fully reported and authors did not respond to our request for additional information on these data [37, 38]. Pussegoda

Table 3 Demographic and clinical characteristics of the study cohort across phenotypes

Characteristics	No hearing loss ($n = 104$)	Minor hearing loss ($n = 229$)	Clinically-relevant hearing loss ($n = 95$)	*P* value
Age at diagnosis (years), median (min, max)	14.0 (0.9–18.3)	8.4 (0.0–19.0)	10.4 (0.8–17.4)	<0.001
Age at start of platinum treatment (years), median (min, max)	14.0 (0.9–18.3)	8.4 (0.2–19.3)	10.4 (0.8–17.6)	<0.001
Cisplatin cumulative dose (mg/m^2), median (min, max)	480 (64–770)	480 (100–950)	480 (83–800)	0.042
Concomitant carboplatin chemotherapy—*n* (%)	8 (8%)	50 (22%)	38 (40%)	<0.001
Carboplatin dose, cumulative (mg/m^2), median (min, max)	2,525 (1,500–8,250)	1,500 (253–6,178)	1,500 (120–6,545)	0.023
Male sex—*n* (%)	60 (58%)	121 (53%)	51 (54%)	0.707
Tumor type—*n* (%)				
CNS tumor	2 (1.9%)	5 (2.2%)	1 (1.1%)	0.794
Germ cell tumor	25 (24)	33 (14.4%)	3 (3.2%)	<0.001
Hepatoblastoma	3 (2.9%)	31 (13.5%)	5 (5.3%)	0.003
Hodgkin lymphoma	0	3 (1.3%)	0 (0%)	0.273
Neuroblastoma	4 (3.8%)	38 (16.6%)	23 (24.2%)	<0.001
Osteosarcoma and (soft-tissue) sarcoma	57 (45.8%)	102 (44.5%)	57 (60%)	0.018
Other	5 (4.8%)	10 (4.4%)	5 (5.3%)	0.872
Missing	8 (7.7%)	7 (3.1%)	1 (1.1%)	0.032
Country—*n* (%)				
Austria	2 (1.9)	4 (1.7)	1 (1.1)	0.873
Switzerland	19 (18.3)	39 (17.0)	13 (13.7)	0.662
Czech Republic	30 (28.8)	40 (17.5)	16 (16.8)	0.037
Germany	33 (31.7)	73 (31.9)	36 (37.9)	0.542
Denmark	6 (5.8)	18 (7.9)	7 (7.4)	0.791
Italy	1 (1.0)	6 (2.6)	0 (0.0)	0.197
The Netherlands	13 (12.5)	49 (21.4)	22 (23.2)	0.103

HL hearing loss

et al. reported implausible allele frequencies of rs1051640 which were clarified with the authors [17]. The corrected data were then included in our meta-analysis.

Supplementary Table 1 summarizes the study characteristics and reported allele frequencies. Forest plots and statistical results are shown in Fig. 3, Supplementary Figures 1 and 2. The studies are heterogeneous with respect to participant age, cranial radiotherapy, patient diagnosis, and the use of other platinum compounds in addition to cisplatin such as carboplatin. These differences are reflected by the I^2 statistic with $r\ I^2 > 50\%$ for the meta-analyses of seven SNPs; therefore, the random effects approach was applied.

Four independent studies investigated the association between the rs1872328 SNP in *ACYP2* and ototoxicity [22, 23, 27, 30]. A meta-analysis of the present study together with the four previous studies, comprising a total of 709 case and 476 control patients, indicated a nominally significant association (test for overall effect: $P = 0.04$) between the *ACYP2* rs1872328 variant and cisplatin ototoxicity, with an OR of 3.94 (95% CI: 1.04–14.93; random effects model).

In the meta-analysis of the effect of *SLC22A2* rs316019, four studies including the current were combined, comprising a total of 644 ototoxicity cases and 309 controls. The overall association was significant ($P = 0.02$), with an OR of 1.46 (95% CI: 1.07–2.00; fixed effects model).

Discussion

The prevalence and severity of cisplatin-induced ototoxicity are highly variable across patients. Extensive research has been conducted to identify potential risk factors that may

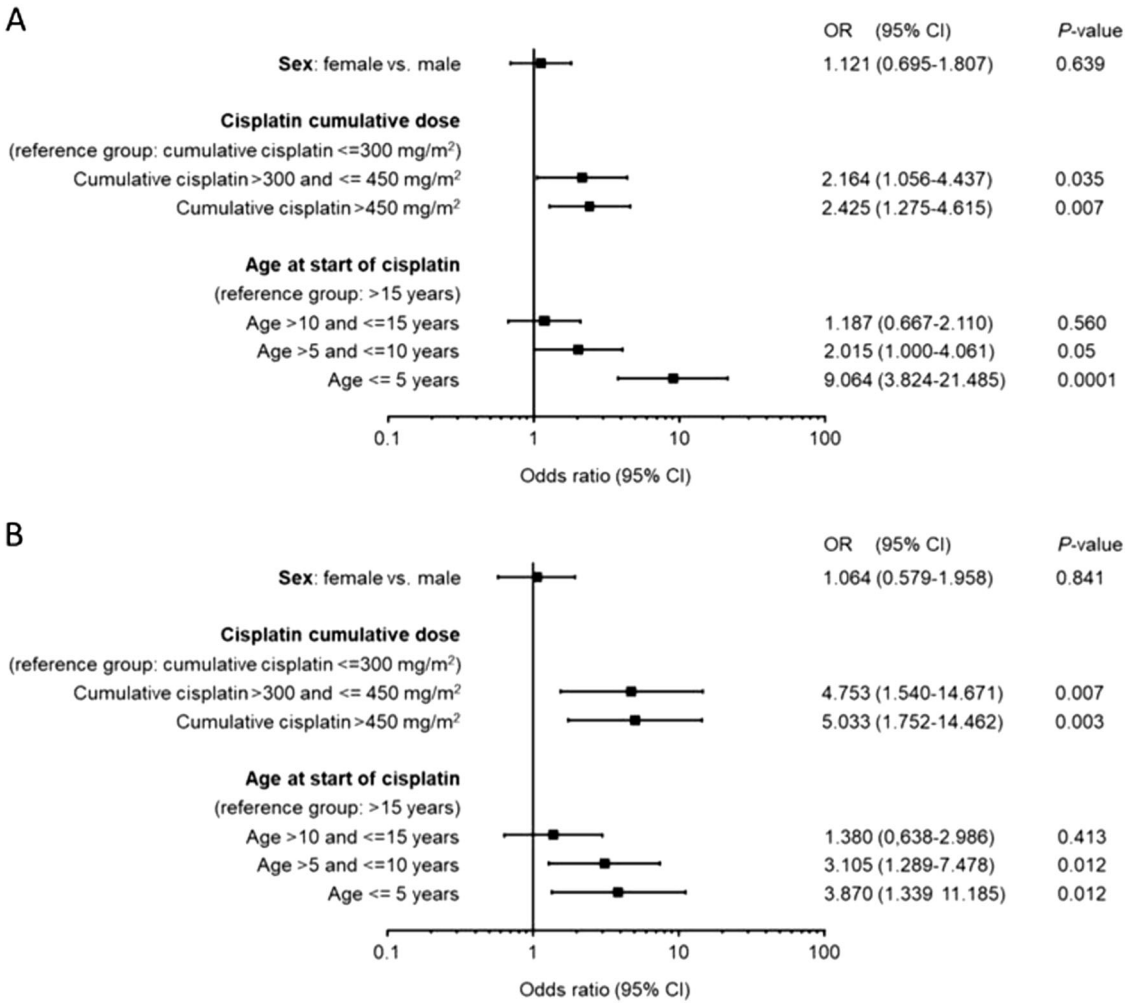

Fig. 2 Non-genetic risk factors. **a** Odds ratios of developing hearing loss according to gender, age at diagnosis, and cumulative cisplatin dose; **b** odds ratios of developing clinically-relevant hearing loss (grade Münster ≥ 2b) according to gender, age at diagnosis, and cumulative cisplatin dose

explain this variability and could be used as predictive markers of platinum-induced hearing loss. Several non-genetic risk factors, such as cisplatin dose, age, concomitant use of other ototoxic drugs, and cranial irradiation, have been identified.

Our study confirmed the effect of age and cumulative cisplatin dose on the risk of ototoxicity, as the OR per 5-year increase in age was ~0.5 ($P < 0.001$) and patients who received a cumulative cisplatin dose of >450 mg/m^2 had a 2.4 higher odds ($P < 0.01$) of developing platinum-associated hearing loss than patients treated with low cumulative cisplatin doses (≤300 mg/m^2). These factors, however, only partially explain the inter-individual variability in ototoxic responses to platinum. In our study, age and cumulative cisplatin dose together explained no more than 15% of the variance in hearing loss.

The considerable inter-individual variability in the prevalence and severity of ototoxicity among patients receiving similar treatment indicate that genetic factors may render certain individuals more susceptible to these effects. To date, several studies investigated associations between genetic variations within pre-specified genes of the platinum pathway with ototoxicity. In many of these initial discovery studies, a very strong association between the respective risk allele and cisplatin-induced ototoxicity was reported, for example, for *TPMT* rs12201199 (OR: 17.0), *ACYP2* rs1872328 (OR: 4.5), and *SLC16A5* rs4788863 (OR: 16.7) [16, 23, 26]. These promising results suggested that certain markers could help to identify patients at risk. However, prior to widespread clinical application, multiple clinical studies must be conducted to confirm how well the respective genetic variant is associated with the risk of ototoxicity (i.e., clinical validity) and the usefulness of genetic testing for the marker in patients treated with cisplatin (i.e., clinical utility).

To this end, we re-evaluated the strength of associations between previously identified genetic markers with cisplatin-induced ototoxicity. Of note, for some of the

Table 4 Association between candidate SNPs and cisplatin-induced ototoxicity

Gene, rs#, REF | VAR allele	Genotypes	Phenotype groups			Univariable logistic regression		Multivariable logistic regression[a]	
		No hearing loss	Minor hearing loss	Clinically-relevant hearing loss	Hearing loss of any degree vs. no hearing loss	Clinically-relevant hearing loss vs. no hearing loss	Hearing loss of any degree vs. no hearing loss	Clinically-relevant hearing loss vs. no hearing loss
		($n = 104$)	($n = 229$)	($n = 95$)	(OR; 95% CI; *P* value)	(OR; 95% CI; *P* value)	(OR; 95% CI; *P* value)	(OR; 95% CI; *P* value)
ACYP2	G/G	98	218	88	0.96	1.30	0.82	1.63
rs1872328	G/A	6	11	7	(0.37–2.49)	(0.42–4.01)	(0.29–2.34)	(0.47–5.67)
G | A	A/A	0	0	0	0.93	0.65	0.71	0.44
LRP2	T/T	7	21	7	0.95	0.94	0.96	0.96
rs2075252	T/C	37	75	35	(0.67–1.34)	(0.6–1.46)	(0.66–1.39)	(0.59–1.57)
T | C	C/C	60	133	53	0.76	0.77	0.83	0.88
NFE2L2	T/T	0	2	2	1.08	0.74	0.98	0.71
rs6721961	T/G	24	38	24	(0.65–1.78)	(0.4–1.36)	(0.58–1.67)	(0.36–1.39)
T | G	G/G	80	189	69	0.76	0.33	0.95	0.31
OTOS	T/T	99	218	93	0.83	0.43	1.04	0.32
rs2291767	T/C	5	11	2	(0.29–2.38)	(0.08–2.25)	(0.32–3.34)	(0.04–2.29)
T | C	C/C	0	0	0	0.73	0.31	0.95	0.26
TPMT	A/A	96	202	83	1.66	1.74	1.36	1.61
rs12201199	A/T	8	26	12	(0.76–3.62)	(0.68–4.45)	(0.58–3.2)	(0.56–4.62)
A | T	T/T	0	1	0	0.21	0.25	0.49	0.38
SOD2	A/A	25	58	17	0.96	1.10	0.94	1.12
rs4880	A/G	51	117	54	(0.70–1.32)	(0.73–1.65)	(0.66–1.34)	(0.71–1.77)
A | G	G/G	28	54	24	0.8	0.65	0.72	0.63
SLC22A2	A/A	3	1	2	1.36	1.11	1.61	1.2
rs316019	A/C	23	43	20	(0.85–2.16)	(0.63–1.96)	(0.97–2.68)	(0.64–2.26)
A | C	C/C	78	185	73	0.2	0.71	0.07	0.57
GSTP1	A/A	51	94	43	1.14	1.17	1.04	1.15
rs1695	A/G	40	114	36	(0.81–1.58)	(0.79–1.73)	(0.74–1.48)	(0.75–1.77)
A | G	G/G	13	21	16	0.45	0.42	0.81	0.52
ABCC3	A/A	72	161	64	1.02	1.04	1.08	1.13
rs1051640	A/G	29	58	29	(0.68–1.54)	(0.61–1.77)	(0.69–1.68)	(0.63–2.04)
A | G	G/G	3	10	2	0.92	0.88	0.74	0.69
SLC16A5	T/T	8	19	5	0.96	1.12	0.95	1.13
rs4788863	T/C	41	97	38	(0.67–1.36)	(0.71–1.76)	(0.64–1.41)	(0.68–1.88)
T | C	C/C	55	113	52	0.81	0.62	0.82	0.63

Shown are genotype frequencies and univariable and multivariable binary logistic regression analysis of genotype–phenotype associations

CI confidence interval, *OR* odds ratio, *REF* reference allele, *VAR* variant allele

[a]Adjusted for age at diagnosis, total cumulative cisplatin dose, and four genetic principal components

previously described genetic markers, such as *NFE2L2* rs6721961 and *OTOS* rs229767, our study is the first to independently replicate these findings. To control for radiation-related toxicity, which could bias genetic association analysis, we excluded patients who also received cranial radiotherapy. To detect an effect as small as an OR

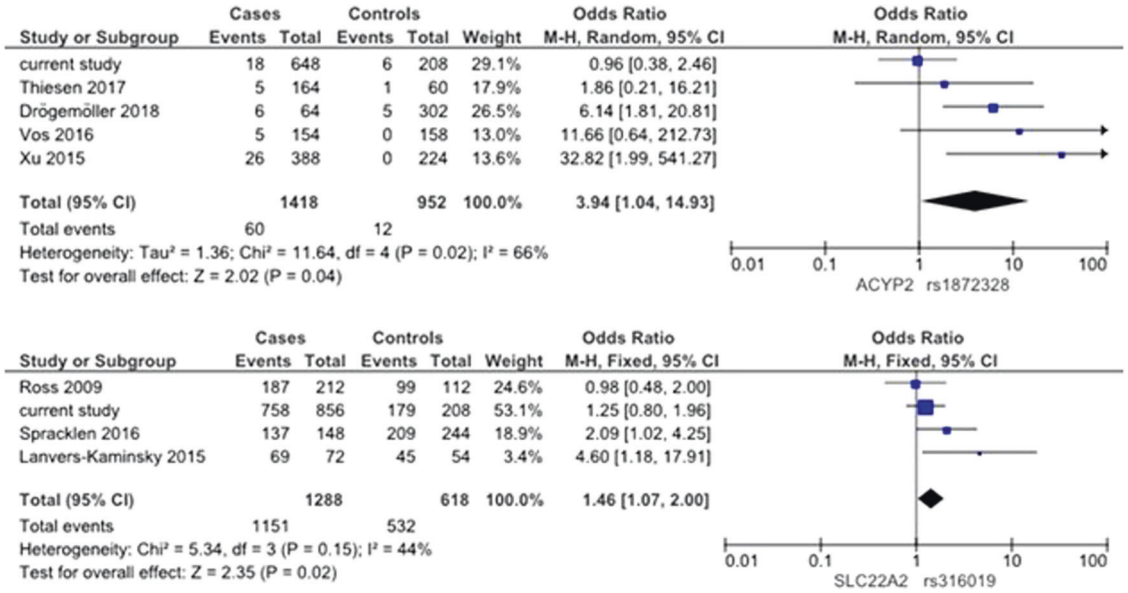

Fig. 3 Random effects model meta-analyses of the associations between *ACYP2* rs1872328 and cisplatin-induced hearing loss

of 2.5, we evaluated a large cohort of patients in a pan-European multi-center collaboration.

For all SNPs investigated, we found the observed ORs of cisplatin-induced ototoxicity to be considerably lower than in the initial discovery studies. The ORs, as a measure of the strength of the association between the respective genetic marker and ototoxicity, did not exceed a value of 2.3. Given that our study was designed to detect an OR > 2.5 with sufficient statistical power, the non-significance of the results was not surprising. The type of comparison (patients with any degree of hearing loss compared with controls, or patients with clinically significant hearing loss compared with controls) or method of analysis (unadjusted or adjusted for age, cumulative dose, and ancestry) essentially did not affect the results.

Failure to replicate the results of the discovery cohorts might be explained by the so-called "winner's curse", which in genetic association studies appear as upward bias in the estimated effect size of a newly identified risk allele. The winner's curse manifests mostly in (genome-wide) association studies in which several thousand single-nucleotide polymorphisms are tested and when the design of the discovery study lacks sufficient statistical power [39].

The results from our study do not provide evidence that any of the evaluated risk alleles imposes a more than 2.5-fold odds of developing ototoxicity in patients treated with cisplatin. However, larger prospective studies with increased statistical power may indeed detect small associations of these SNPs with the development of ototoxicity. As the association between single genetic markers and ototoxicity is weak, we propose a polygenic model, which takes into account multiple interacting genes of the cisplatin pathway that together confer an increased risk of ototoxicity. The proof of this concept, however, requires larger cohort studies.

To derive a pooled estimate of the effects of the SNPs investigated in our study, we performed meta-analyses by combining previous genetic association studies with our study results. It is important to note that there was vast between-study heterogeneity for most SNPs in our meta-analysis. This heterogeneity may be due to many factors, such as differences in the ototoxicity grading systems, age, and ancestry of study participants, whether patients had cranial radiation, cisplatin dose levels, or the use of oto-protectants. In the meta-analysis, only *ACYP2* rs1872328 (OR: 3.94; 95% CI: 1.04–14.93; $P = 0.04$; random effects) and *SLC22A2* rs316019 (OR: 0.69; 95% CI: 0.50–0.94; $P = 0.02$; fixed effects) were significantly associated with cisplatin-induced ototoxicity.

The intron variant rs1872328 in *ACYP2* has previously been described as a risk marker for ototoxicity in a GWAS, including 238 cisplatin-treated and cranial-irradiated brain tumor patients [23]. *ACYP2* encodes an acylphosphatase, which is expressed in the brain and cochlea and may induce hair cell damage via dysregulated calcium homeostasis [40]. The initially observed association between rs1872328 and ototoxicity has been replicated in an independent cohort of brain tumor patients and in three subsequent studies, one study in pediatric osteosarcoma patients, one study in pediatric patients with different cancer types, and one study with adult testicular cancer survivors [22, 27, 30].

The *SLC22A2* gene encodes a polyspecific organic cation transporter (OCT2). rs316019 in *SLC22A2* (c.808T>G, p.Ser270Ala) is the most common coding variant of *SLC22A2* with a frequency of the minor T allele of ~15% in many

populations; it has also been reported to affect OCT2 activity. Compared with 270Ala, 270Ser of OCT2 is associated with altered V_{max} (maximal uptake rate) and K_m (the concentration at which half the maximal uptake occurs) for OCT2 substrates, such as 1-methyl-4-phenylpyridinium, dopamine, norepinephrine, and propranolol [41]. A study showed that comedication with the OCT2 inhibitor cimetidine or knockout of Oct1/2 protected mice from cisplatin-induced ototoxicity [42]. Given that in humans, as in other species, OCT2 is expressed in the cochlea and the 270Ser variant of OCT2 also affects cisplatin uptake, it is possible that in rs316019 T carriers accumulation of cisplatin in the inner ear is reduced and, thus, cisplatin-induced ototoxicity is prevented.

In conclusion, there is a need to identify genetic biomarkers for individualized patient management of cisplatin treatment. Information on the association between *TPMT* polymorphisms with cisplatin-induced hearing loss is included in the FDA drug label. However, researchers have debated the association. Our study confirmed two potential genetic markers, rs1872328 in *ACYP2* and rs316019 in *SLC22A2*. Due to the heterogeneity of results from genetic association studies performed so far, the evidence seems not yet sufficient to recommend screening for specific markers. Advances in the understanding of the pathophysiologic mechanisms of cisplatin-induced ototoxicity, as well as future genome-wide association studies, may help identify suitable genetic markers.

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