Pharmacogenomics of Cisplatin-Induced Ototoxicity: Successes, Shortcomings and Future Avenues of Research

Britt I. Drögemöller[1,2], Galen E.B. Wright[2,3], Cody Lo[2,4], Tan Le[1], Beth Brooks[5], Amit P. Bhavsar[6], Shahrad R. Rassekh[2,3], Colin J.D. Ross[1,2], Bruce C. Carleton[2,3]

1. Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, British Columbia, Canada.
2. BC Children's Hospital Research Institute, Vancouver, British Columbia, Canada.
3. Division of Translational Therapeutics, Department of Pediatrics, University of British Columbia, Vancouver, British Columbia, Canada.
4. Faculty of Medicine, University of British Columbia, Vancouver, British Columbia, Canada.
5. Audiology and Speech Pathology Department, BC Children's Hospital, Vancouver, British Columbia, Canada.
6. Department of Medical Microbiology and Immunology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Alberta, Canada.

Corresponding author: Bruce C. Carleton, University of British Columbia, Vancouver, BC V6T 1Z3, Canada. Phone: 604 875 2179; E-mail: bcarleton@popi.ubc.ca

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Abstract

Cisplatin is a highly effective chemotherapeutic. Unfortunately, its use is limited by cisplatin-induced ototoxicity (CIO). Substantial research has been performed to uncover the genetic variants associated with CIO, however, there has been a lack of consistency in the results that have been reported. This article aims to provide an overview of the current state of CIO genomics research, delving into the shortcomings of past research and providing recommendations for future avenues of study.

1. Cisplatin-induced ototoxicity

Cisplatin was approved by the FDA as a chemotherapeutic agent for germ cell tumors over 40 years ago[1] and is currently used to treat a wide range of childhood and adult cancers, with survival rates as high as 80% reported for certain cancers treated with this medication[2]. The introduction of cisplatin, along with other highly effective chemotherapeutic treatments, has dramatically improved the chances of survival for cancer patients, particularly in developed countries. Unfortunately, the toxic effects that are associated with these lifesaving treatments result in severe and debilitating adverse drug reactions (ADRs). The magnitude of the burden associated with these adverse events is illustrated by the fact that at 50 years of age, the cumulative effect of chronic health conditions in survivors of childhood cancer is reported to be 99.9%, compared to only 9.2% in matched community controls[3]. Focusing specifically on cisplatin, the most frequently reported ADRs associated with this treatment are ototoxicity, nausea/emesis, neurotoxicity, myelosuppression and nephrotoxicity[4].

The occurrence of cisplatin-induced ototoxicity (CIO) is of particular concern to patients who are undergoing cisplatin treatment, as up to 95% of patients may experience this irreversible ADR[2]. Although devastating to all patients, the loss of hearing may be particularly profound in children who have yet to develop language skills. Hearing loss in children is associated with decreased educational performance

and increased social and emotional impairment[5]. The ability to predict and minimize the harm associated with the development of CIO, while maximizing anticancer effectiveness, would therefore be of great value to improve the quality of life of individuals who have already experienced the devastating consequences of cancer.

Cisplatin targets the DNA of tumor cells, which are undergoing rapid proliferation. After binding to the DNA, cisplatin induces damage, which in turn initiates DNA repair mechanisms, ultimately leading to apoptosis of the tumor cell[6]. The build-up of cisplatin and reactive oxygen species in the cochlea, as a result of these processes, has been implicated in the development of CIO[7]. Although clinical variables such as age and cumulative cisplatin dose are associated with increased risk of experiencing CIO[4], these factors do not completely account for the inter-individual variability that is observed between patients. For this reason, genetic variation is expected to play an important role in the development of CIO. Supporting this hypothesis, heritability studies have shown that 38-47% of the variability in cisplatin-induced cytotoxicity can be attributed to genetics[8] and that a large proportion (0.92 ±0.62) of cisplatin-associated ototoxicity can be attributed to common genetic variation[9].

This has led many studies investigating the genetics of CIO, with 72% of cisplatin ADR pharmacogenetics studies focusing on ototoxicity[4]. Nonetheless, there has been a lack of consistency in the results that have been published, with the majority of studies reporting conflicting results. As such, there remains a gap in knowledge regarding the contribution of robustly-associated genetic variants to the development of CIO[10].

2. The genetics of cisplatin-induced ototoxicity

2.1. Candidate gene-based approaches

As is the case with the majority of pharmacogenetics studies, investigations of the genetics of CIO began with a focus on candidate genes. The selection of candidate genes of interest was guided by what is thought to be known regarding the mechanism of action of cisplatin and was largely based on technological restrictions prior to the genomics era of research. For this reason, candidate gene studies have focused mainly on genes involved in the detoxification and transport of cisplatin, as well as DNA repair processes[6,7].

Significant associations with CIO and variants in candidate genes have been reported. However, there has been an inability to consistently replicate these candidate gene findings[7], as highlighted for six of the seven CIO candidate genes that are recorded on the curated pharmacogenomic database, PharmGKB (*GSTM1*, *GSTT1*, *LRP2*, *XPC*, *SLC22A2* and *SLC31A1*)[11]. The most promising evidence that is summarized on PharmGKB was available for rs1695 in *GSTP1*, for which significant associations are recorded from two studies[12,13]. Given the potential relevance of this gene to CIO, we further mined the literature to identify publications that may have not been captured by the PharmGKB curation process. From this search, we identified four additional publications that reported associations with CIO and rs1695[14-17], as well as two publications that did not identify significant associations[18,19]. These findings highlight that further research is needed to determine the relevance of *GSTP1* to CIO. They also serve as a reminder of the conflicting nature of results obtained from candidate gene studies and the need to perform additional manual curation when using databases such as PharmGKB. Lastly, to improve the ease of curation, pharmacogenomic publications should attempt to adhere to the standardized and structured format that has been specifically recommended for these studies[20].

2.2 Harnessing the power of unbiased genomic scans

The absence of robust findings from candidate gene-based approaches may be attributed to a lack of knowledge regarding the mechanism of action of this ADR[2], combined with the complexity of CIO[21]. These findings are in line with the level of success that has been reported for candidate gene studies that have investigated other complex traits. A paper examining the reliability of such study designs revealed that of the 166 putative candidate gene associations that were examined in more than three independent studies, only six findings were consistently replicated[22]. The inability to accurately predict which genes are involved in CIO has, to some extent, been alleviated by the advent of technologies that allow for the unbiased investigation of genetic variation across the genome. To date, four studies have applied these unbiased approaches to examine the genetics of CIO and will form the focus of this review.

2.2.1 ADME-wide association studies of cisplatin-induced ototoxicity

The first study to implement an approach that moved beyond individual candidate genes was performed in a Canadian cohort of pediatric patients diagnosed with various cancers[23]. This study examined the association between CIO and 1,949 variants located in 220 genes involved in drug absorption, distribution, metabolism and excretion (ADME). A two-stage study design was implemented, whereby variants with $P<0.01$ in both the discovery and replication cohorts were prioritized for investigation in the combined cohort (i.e. 106 cases and 56 controls). Variants were considered statistically significant if they met the Bonferroni-corrected threshold for multiple testing in this combined cohort. These analyses identified significant associations between (i) rs12201199 in *TPMT* and increased risk of CIO ($P=2.2\text{x}10^{-4}$; OR=16.89 95% CI 2.27-125.88) and (ii) rs9332377 in *COMT* and increased risk of CIO ($P=1.8\text{x}10^{-4}$; OR=5.52 95% CI 1.91-15.95) (refer to Table S1 for details).

Expanding on the ADME panel approach used by Ross *et al*.[23], a second study was performed by the researchers at the Canadian Pharmacogenomics Network for Drug Safety (CPNDS) in a cohort of adult testicular cancer patients[13]. This study examined 7,907 pharmacogenetic variants and also applied a two-stage study design, whereby variants with $P<5.0x10^{-5}$ in the discovery cohort were investigated for association with CIO in the replication cohort. Due to the reduced number of variants that were examined in the replication cohort, a less stringent significance threshold was set and variants with $P<0.05$ were carried forward for analyses in the combined cohort (37 cases and 151 controls). Variants were considered significant in the final analyses if they survived Bonferroni correction for multiple testing (i.e. $P<6.3x10^{-6}$) in the combined cohort. These analyses identified a significant association between a synonymous variant in *SLC16A5* (rs4788863, p.Leu41Leu) and decreased risk for CIO ($P=2.2x10^{-7}$; OR=0.06 95% CI 0.02-0.22) (refer to Table S1 for details). Of note, the protective effect of this variant was conferred in a dominant manner.

2.2.2 Genome-wide association studies of cisplatin-induced ototoxicity

Genome-wide association studies (GWAS) expand on the ADME approach even further, by assessing genetic variants throughout the entire genome that have been selected in an unbiased manner. The first GWAS of CIO was performed in a cohort of newly diagnosed pediatric embryonal brain tumor patients to examine the association between CIO and approximately 1.7 million genetic variants[24]. A discovery cohort (145 cases and 93 controls) was used to identify a genome-wide significant association (i.e. $P<5.0 \times 10^{-8}$) between rs1872328 in *ACYP2* and increased risk of CIO ($P=3.9x10^{-8}$; OR=4.50 95% CI 2.63-7.69). This finding was replicated in a second cohort of 68 pediatric brain tumor patients included in this study (49 cases and 19 controls; $P=6.0x10^{-3}$; OR=2.94 95% CI 1.35-6.25) (refer to Table S1 for details).

Following on from the GWAS of CIO in pediatric patients, Wheeler *et al*. performed a GWAS in 511 adult testicular cancer patients of European genetic ancestry[9]. Genotyping and subsequent imputation were used to investigate the association of approximately 5 million variants with CIO. These analyses identified a genome-wide significant association between rs62283056 in *WFS1* and increased risk of cisplatin-associated ototoxicity (P=1.4x10^{-8}; beta=-0.34+-0.06) (refer to Table S1 for details). Pseudo-replication of these results was provided by showing that decreased imputed *WFS1* gene expression in the hypothalamus was associated with increased risk of hearing loss ICD-9 codes extracted from electronic medical records in the BioVU repository. Hearing loss in this replication cohort could therefore have been caused by diverse factors such as exposure to loud noise, ear infections, injuries to the ear and genetic and congenital disorders. In addition to these analyses, Wheeler and colleagues[9] tested for an interaction between rs62283056 *WFS1* and cisplatin dose and showed that the effect of this variant on CIO was amplified at higher doses of cisplatin.

3. Validation of pharmacogenomic association results

The unbiased nature of genomic scans allows for the opportunity to uncover novel biology. However, in order to ascertain the biological significance of these findings, genetic fine-mapping and *in silico* annotation analyses are required for biological interpretation[25]. Further, to verify the findings from genomic studies, independent replication and functional validation analyses are required to ensure the validity of prioritized variants and genes. These analyses are strengthened through the use of different publicly available resources, as summarized in Figure 1. The various levels of evidence available for the genes that have been associated with CIO from genomic association analyses are detailed below and summarized in Figure 2.

3.1 Supporting evidence for the roles of *COMT* and *TPMT* in cisplatin-induced ototoxicity

To further investigate the pharmacogenomic association between *TPMT* rs12201199 and CIO, the protein coding regions of *TPMT* were sequenced[23]. These analyses revealed that rs12201199 was in linkage disequilibrium (LD) with two nonsynonymous variants that define the loss of function *TPMT*3A* allele. Similarly, sequencing of the coding regions of *COMT* revealed that rs9332377 was in LD with rs4818, a synonymous variant that has been shown to affect the activity of COMT[26]. Both *TPMT* and *COMT* are methyltransferases that are dependent on S-adenosylmethionine (SAM), a substrate that has been reported to amplify cisplatin toxicity[27]. Therefore, decreased TPMT and COMT activity may result in an increase in SAM, which in turn may increase the toxic response to cisplatin[28]. To provide functional support for the findings related to *TPMT*, Bhavsar and colleagues[28] compared the cisplatin response traits of *TPMT*3A* and functional *TPMT*1* in murine inner ear cell lines. These experiments showed that *TPMT*3A* was associated with an increase in both cisplatin biosensor response and cisplatin-induced cytotoxicity. In addition, von Stechow *et al.*[29] demonstrated that *TPMT* expression is induced by cisplatin in embryonic stem cells, providing further evidence for this drug-gene relationship. The *TPMT* and *COMT* associations have been investigated in multiple cohorts, and while some studies have replicated these associations[23,30-33], others were unable to confirm these findings[9,13,16,30,32-36] (Table 1 and Table S1). These conflicting results may in part be attributed to the heterogeneity of the cohorts under investigation, as discussed in more detail in Section 4. Of note, when excluding cohorts of patients who all received cranial irradiation[34] or which included adult patients[9,32,33,35,37], the findings from all but one study showed that *TPMT* rs12201199 was either significantly more frequent in cases when compared to controls[23,30], or trended towards significance (P=0.07)[36]. On closer examination of the small cohort of patients (n=41) that deviated from the pattern of increased risk[34], it was observed that only CIO cases carried the loss of function *TPMT*3A* variants.

3.2 Supporting evidence for the role of *SLC16A5* in cisplatin-induced ototoxicity

To investigate the *SLC16A5* region in more detail, variants with minor allele frequencies greater than 0.01 in the Exome Aggregation Consortium and 1000 Genomes Project databases were annotated with the Combined Annotation Dependent Depletion (CADD) scoring system, which is a numerical system that is used to assess the predicted deleteriousness of variants[38]. These analyses prioritized rs4788863 as the most deleterious variant (CADD=13.2) associated with CIO in the region. Interrogation of the codon usage database revealed that this synonymous variant may disrupt the translation and folding of SLC16A5[39]. In support of the protective role of the transporter, SLC16A5 has been shown to be inhibited by cimetidine, the addition of which is reported to eliminate ototoxicity caused by cisplatin in mice and rat cochlear cultures[40-42]. Functional data supporting *SLC16A5* was provided by the Shared Harvard Inner-Ear Laboratory Database (SHIELD), which showed that *Slc16a5* is expressed in murine cochlear and utricular cells, but not the surrounding cells, which is consistent with the pattern of expression of genes causing hearing loss[43,44]. Further, functional validation experiments in human cell lines showed that *SLC16A5* expression is significantly induced by cisplatin and silencing of *SLC16A5* significantly changes the viability of cells treated with cisplatin[13]. To date, only one replication study has been performed which also examined the association of *SLC16A5* with CIO using the dominant model. Although the results for rs4788863 in this study were not significant (*P*=0.14), similar to the original discovery study, carriers of the minor T-allele were more frequent in controls compared to the cases (original study: 11% in cases and 56% in controls; replication study: 37% in cases and 55% in controls)[15].

3.3 Supporting evidence for the role of *ACYP2* in cisplatin-induced ototoxicity

Similar to the analyses performed to identify causal variants in *TPMT*, the coding regions of *ACYP2* were Sanger sequenced and an additional four variants were identified as possibly contributing to CIO. Additionally, after conditioning on the lead GWAS variant (rs1872328), three variants in this region

remained significantly associated with CIO. These analyses lend support to the possibility that multiple variants in the *ACYP2* region contribute to the development of CIO. Although rs1872328 remains the most strongly associated variant, this variant was not associated with cisplatin sensitivity *in vitro* and there is currently no data demonstrating that this intronic variant has a functional impact on *ACYP2* or any of the genes within 300 kb of this variant. Nonetheless, ACYP2 has been shown to be involved in calcium homeostasis[45] and calcium signalling has been linked to hair cell damage[46]. In addition, *ACYP2* is expressed in murine ear cells[43] and decreased *ACYP2* expression is associated with increased cisplatin-induced cytotoxicity[47,48]. Further support for this variant has been provided by three independent studies that have replicated the association with *ACYP2* and CIO[36,37,49]. Two studies were, however, unable to replicate these findings[9,33] (Table S1), the results of which have not yet been recorded in the PharmGKB database.

3.4 Supporting evidence for the role of *WFS1* in cisplatin-induced ototoxicity

Annotation of *WFS1* rs62283056 revealed that this variant disrupts a transcription factor binding site[50] and is significantly associated with decreased *WFS1* expression[9]. Supporting literature for the role that *WFS1* plays in CIO was provided by the fact that coding variants, with a more deleterious impact on the WFS1 protein, cause deafness[51,52]. Thus, the less severe impact of the regulatory variant on WFS1 function may place individuals at increased risk for hearing loss when the effect of the variant is amplified in the presence of cisplatin. Functional support was provided for the gene through data which showed that *WFS1* is expressed in inner ear sensory cells[53,54] and that *WFS1* expression correlates with cisplatin-induced cytotoxicity[47,48]. Initial studies by Xu *et al*.[24] and Drögemöller *et al*.[37], which investigated the association of rs62283056 with CIO were unable to replicate these findings[24,37]. However, on closer examination, it was observed that there were substantial phenotypic differences between the patients included in the study that identified *WFS1* rs62283056 and those included in the Xu *et al*. study[24]. These include developmental (adult versus pediatric patients), disease (testicular cancer versus brain tumors) and treatment differences

(e.g. the inclusion of cranial irradiation in the pediatric cohort), which may have modified the effect of *WFS1* rs62283056 on risk of CIO. Another important aspect to consider is differences in the way in which the phenotype was measured. The importance of audiological phenotyping is demonstrated by the analyses performed by Drögemöller *et al.* in a cohort of adult testicular cancer patients. Although this study was unable to initially replicate the association between *WFS1* rs62283056 and CIO, a significant association was observed when analyses were performed using a more closely matched phenotype[37]. These results highlight the importance of phenotype matching in pharmacogenomic replication studies, which is discussed in more detail in Section 4.2.

4. Confounding factors

4.1. Cohort heterogeneity

The inconsistencies observed for pharmacogenomic associations with candidate genes and CIO, has also extended to genome-wide association analyses of CIO. This inability to consistently replicate associations between genetic variants and CIO may in part be attributed to clinical heterogeneity across cohorts. As detailed in Table S1, replication cohorts vary substantially with regards to well-known clinical variables that place individuals at increased risk for CIO (e.g. age, cumulative cisplatin dose, cranial irradiation and concomitant ototoxic medications). In many cases, studies using pediatric populations have been unable to replicate the results obtained from cohorts of adult patients, and *vice versa,* possibly due to developmental differences such as changes in gene expression levels[13].

Further accentuating the challenges associated with cohort heterogeneity, Wheeler *et al*.[9] have shown that the magnitude of risk conferred by certain genetic variants is correlated with the dose of cisplatin used. Therefore, differences in cisplatin treatments across tumor types may hamper the ability to replicate findings across cohorts examining different cancer types. Treatment protocols may also differ with regards

to the use of concomitant treatments such as the use of otoprotectants (e.g. amifostine) and ototoxic treatments (e.g. craniospinal irradiation). The development of CIO is thought to be influenced by the interaction between genetic variants and cisplatin. Therefore, it stands to reason that additional clinical variables may modify the interaction between cisplatin and risk variants. For example, the increased risk associated with the development of CIO as a result of cranial irradiation may override the effects of genetic variants that have been associated with CIO in the absence of cranial irradiation[55].

Another important aspect to consider with regards to cohort heterogeneity is the ancestry of patients. Although the allele frequencies of the variants that were identified from the genomic analyses of CIO vary widely across population groups (Figure 3), all analyses focused predominantly on European descent individuals - either through the inclusion of European only cohorts or sub-analyses performed in European only individuals, which also made up the bulk of the study cohorts. Therefore, the relevance of these variants across global populations, and the effect of population stratification on association results, remains largely unknown. The inclusion of individuals from diverse ancestral backgrounds will aid in filling these gaps in knowledge, while strengthening fine-mapping approaches. For example, in the case of the association observed between variants in the *ACYP2* region and CIO, the causal variant(s) remains to be identified. Therefore, the low linkage disequilibrium that is observed in African descent individuals may be harnessed to aid in the identification of the causal variant(s) in this region[37].

Finally, although pharmacogenomic variants have been shown to have larger effect sizes than genetic variants that influence complex diseases, the sample sizes of the studies highlighted are still relatively limited with regards to power to detect associations (largest study *n*=511, mean study *n*=292).

4.2. Inconsistencies in the assessment of cisplatin-induced ototoxicity

Genetic association studies are heavily reliant on the phenotype under investigation. Therefore, differences in the assessment of CIO can have a large impact on the results that are obtained. In this regard, several CIO designation systems have been developed (recently reviewed by Waissbluth *et al.*[56]), with each one incorporating distinct criteria (Table S2 and Figure S1). These differences are likely to affect case-control designations[57], furthering complicating attempts to replicate findings. We recently displayed the importance of carefully matching CIO phenotypes in replication analyses[37], and showed that the ability to replicate the association between *WFS1* rs62283056 and CIO was dependent on using a hearing loss related phenotyping system that matched the phenotype used in the original discovery cohort.

Of particular importance to the investigation of CIO, hearing loss may be caused by factors other than cisplatin (e.g. exposure to noise, middle ear infections, aging and inherited conditions). These confounders can in part be accounted for by obtaining detailed case histories (e.g. family history of ear disorder, noise exposure) and examining the configuration of patient audiograms. Importantly, CIO initially affects hearing in the high frequencies and is characterized by progressive, bilateral sensorineural hearing loss[21]. By harnessing this knowledge, the steeply sloping configuration of audiograms that are characteristic of CIO can be differentiated from audiograms that are typical of hearing loss caused by exposure to noise (displaying a notch effect at a single frequency) and age (displaying gradual hearing loss across frequencies) (Figure 4). In the case of middle ear infections, bone conduction tests can be performed to differentiate between inner and middle ear dysfunction. Of further note, given that CIO is predominantly bilateral in nature, in cases where the worse ear is used, hearing loss may be caused by additional factors (e.g. the presence of a tumor on the side of the worse ear). Therefore, care should be taken when determining which ear to use for case-control designation purposes.

Due to the progressive nature of CIO, the timing of the audiogram measurements that are used for case-control designation purposes is also important to consider. Specifically, individuals are more likely to be classified as cases if extensive follow-up data is available. Further, the severity of CIO may be masked by changes to treatment, which may in turn influence the results of genetic association studies. Another consideration of importance is that cisplatin initially affects hearing in the high frequency range. Therefore, designation systems which include these higher frequency measurements are more sensitive to the identification of CIO. Finally, in some cases, the details provided by certain designation systems lack clarity. For example, the Common Terminology Criteria for Adverse Events (CTCAE) v3 specifies that grading should be performed based on threshold shifts averaged at two or more contiguous frequencies. Depending on the data available, these contiguous frequencies may be interpreted as 4 and 8 kHz or as 6 and 8 kHz. Given the steeply sloping nature of CIO-related audiograms, the average across higher frequencies will result in a greater threshold shift, and consequently different case-control designations.

4.3 Differences in methods for genetic association analyses

In addition to differences in clinical factors, the manner in which genetic analyses are performed may affect the results that are obtained across studies. In line with this, each of the CIO genomic association analyses used different analyses and/or genetic models (Table 2). The differences in the analysis methods, as well as the covariates that were included in the regression analyses, may provide a partial explanation for the lack of replication that has been observed across the cohorts. This problem is further accentuated by differences in the patients that were included in association analyses. In the case of *TPMT* rs12201199 and *SLC16A5* rs4788863, initial analyses were performed excluding individuals with grade one CIO. For *TPMT* rs12201199, analyses were repeated with the inclusion of grade one CIO individuals as cases, which strengthened the association between rs12201199 and CIO. For *SLC16A5* rs4788863, ordinal logistic regression analyses were performed after the inclusion of grade one CIO individuals, which revealed that

the frequency of the minor allele inversely corresponded to the severity of CIO. These differences highlight that for all studies, analyses should be carefully documented and replication studies should match these analyses as closely as possible.

5. Future avenues of research

5.1 Standardization of phenotypes and the formation of global consortiums

As described in Section 4, there are many factors that are likely to contribute to the inconsistencies reported for CIO association analyses. These challenges may be addressed in part through the formation of global consortiums focusing on the pharmacogenomic analysis of CIO. The increased samples sizes afforded by these consortiums will result in greater power to detect associations, while also providing the opportunity to perform sub-analyses examining patients with similar treatments, tumors and ancestries. The genomic studies mentioned here have genotyped in excess of 1,100 oncology patients receiving cisplatin and represent a potential opportunity for inclusion in future cross-site studies. The availability of large cohorts of homogenous patients will also facilitate the ability to more accurately determine the effect sizes of individual variants that are associated with CIO. This increased precision will allow for the assessment of the combined effects of variants that are associated with CIO through the use of polygenic risk scores. In support of the relevance of polygenic risk scores for CIO, Wheeler *et al*. showed that a model including all investigated SNPs in their cohort explained a large proportion of the variance, lending support to the polygenic architecture of this disease[9]. Finally, the development of CIO consortiums will also facilitate the standardization of CIO designation criteria and the meta-analysis of individual grading scales. In consultation with expert audiologists, these standardization procedures may be enhanced through the incorporation of machine learning algorithms,[58] which may be used to automate the recognition of patterns of hearing loss that are consistent with ototoxicity caused by cisplatin.

As the formation of global consortiums may be challenging and time consuming, efforts to improve data sharing should be performed in parallel. To improve these harmonization initiatives, we propose that groups who are examining the genetics of CIO should perform analyses using case-control criteria and analyses that have been applied to all previous studies. The summary statistics from these diverse analyses should, upon publication, be made publicly available. As an example, the clinical and genotype data from the Wheeler *et al*. analyses have been made available through dbGaP (Accession: phs001621.v1.p1), as required by NIH-funded studies, and the related summary statistics are also present on the PGRN-RIKEN GWAS Statistics repository[59], which has greatly facilitated analyses that have been performed subsequent to the publication of these results. Through combined efforts, the gaps in our current knowledge of the genetics of CIO can begin to be filled.

5.2 The use of genomics to guide the identification of otoprotectants

Although there are currently no FDA approved agents to reduce drug-induced ototoxicity, there is substantial research investigating agents that are protective against CIO. In line with this, sodium thiosulfate has received a "fast track designation" by the FDA for reducing CIO in hepatoblastoma[4,60], highlighting the promise of this agent for certain types of cancer. Further, as reactive oxygen species have been implicated in the development of CIO, free radical scavengers were considered promising otoprotectant candidates. Unfortunately, these agents have been shown to impair antitumor activities *in vitro*[21]. To address these concerns, current research is investigating the use of local intratympanic administration of these agents to prevent systemic anticancer effects[61].

In order to uncover additional agents which may be of value to preventing ototoxicity, without compromising the antitumor activity of cisplatin, the results from pharmacogenomic studies can be used to guide the identification of otoprotectants. For example, the protective role of variants in *SLC16A5*

mirrors the effect of cimetidine, which inhibits SLC16A5[40], by preventing the occurrence of CIO in mice and rat cochlear cells[41,42]. This is of particular interest given that cimetidine does not compromise the antitumor activity of cisplatin treatment[62]. These results highlight the utility of strategies that harness the results from genomic analyses to uncover additional agents to prevent ototoxicity, the validity of which should be tested in model organisms. Already established model organisms that have been successfully used to investigate potential otoprotectants include mouse cochlear explants, as well as zebrafish and murine models[63]. Optimization of these model organisms is ongoing, and of importance to studies who aim to move towards the implementation of ototprotectants in the clinical setting, a recent publication has described the development of a CIO mouse model that is consistent with the hearing loss phenotype observed in humans experiencing CIO[64].

5.3 Expanding research beyond a single trait

A final avenue of interest would be to examine the relevance of genetic factors that are associated with CIO to other traits. Justification for these analyses is provided by the fact that genetic variants in *ACYP2* have been associated with CIO, as well as oxaliplatin-induced neuropathy[65,66], highlighting the contribution of these variants to different ADRs and platinum agents. Therefore, genetic variants that are found to be associated with CIO may be prioritized for future PheWAS analyses, to examine the relevance of these variants to other traits, particularly those related to drug outcomes. In addition, Wheeler *et al*. showed that SNPs within 50 kb of deafness genes were significantly more likely to be associated with CIO. This highlights a possible overlap between the genetics of hearing loss in the absence of cisplatin and hearing loss caused by cisplatin. Extending this further, there are more than 600 categories of drugs that are associated with the development of ototoxicity[67]. Therefore, the identification of genetic variants that are predictive of ototoxicity across drugs would increase the applicability of these findings to the prediction and prevention of these ADRs.

6. Conclusions

This review provides an in-depth summary of the findings from CIO genomic association analyses. This research has yielded novel insights into the mechanisms underlying this ADR. Although progress has been made through the application of these analyses, there remain many future avenues of research that should be considered in the next few years. Future research should focus on the harmonization of phenotypes and analyses, the investigation of the combined effects of variants and examination of gene level associations. Through combined efforts, gaps in our understanding of the genetics underlying CIO can be addressed. These results can be used to develop strategies to reduce the occurrence of this ADR, while ensuring that patients receive maximum benefit from chemotherapeutic treatments.

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Figure Legends

Figure 1: Examples of tools to investigate the functionality of identified pharmacogenomic variants and genes. A: Examples of tools that have been used to annotate variants that are associated with cisplatin-induced ototoxicity. **B**: The SHIELD database can be used to examine the pattern of gene expression in murine inner ear cells during development to determine the relevance of genes to hearing. **C:** Gene expression data from the Broad-Novartis Cancer Cell Line Encyclopedia can be combined with cisplatin cytotoxicity data from the Genomics of Drug Sensitivity in Cancer Project to infer drug-gene relationships. The depicted resources can be found at: SIFT: https://sift.bii.a-star.edu.sg/; PolyPhen-2: http://genetics.bwh.harvard.edu/pph2/; Codon Usage Database: https://www.kazusa.or.jp/codon/; GTEx: https://www.gtexportal.org/home/. Further bioinformatic resources can be found at: https://www.pgrn.org/tools.html. A: Alanine, C: Cysteine, L: Leucine, T: Threonine, Y: Tyrosine.

Figure 2: Summary of the evidence supporting the pharmacogenomic variants that have been associated with CIO in pediatric and adult populations. Red and blue boxes correspond to variants that have been associated with increased and decreased risk of CIO, respectively. CADD: Combined Annotation Dependent Depletion; CIO: Cisplatin-induced ototoxicity; eQTL: expression quantitative trait loci; SAM: S-adenosylmethionine.

Figure 3: Differences in allele frequencies of variants that have been associated with cisplatin-induced ototoxicity across global population groups. AFR: African, AMR: Admixed American, EAS: East Asian, EUR: European, SAS: South Asian.

Figure 4: Comparison of cisplatin-induced ototoxicity designation systems. Audiogram graphs representing hearing loss caused by noise (A, B and C), aging (D, E and F) and cisplatin (G, H and I), which have been graded with three schemes in pharmacogenomic studies: the Chang criteria (A, D and G), CTCAEv4.03 criteria (B, E and H) and geometric means (C, F and I). Coloured regions correspond to the following grades: green: grade 0, yellow: grade 1, orange: grade 2, red: grade 3, dark red: grade 4, grey: test frequencies not specified in grading system. In the absence of any additional data (e.g. case histories or baseline data), all three grading systems would consider these patients audiograms consistent with ≥ grade 2 CIO, with the exception of the calculation of the geometric means for noise-induced hearing loss, which would be considered grade 1 CIO. CIO: Cisplatin-induced ototoxicity.

Supplementary Information Titles

Figure S1: Comparison of case-control designation criteria.

Table S1: Summary of studies examining the association between cisplatin-induced ototoxicity and *TPMT*, *SLC16A5*, *ACYP2* and *WFS1.*

Table S2: Comparison of cisplatin-induced ototoxicity grading scales.

A Variant Annotation

Variant	Example	Annotation
Missense	*TPMT* (p.Y240C & p.A154T)	SIFT/Polyphen
Synonymous	*SLC16A5* (p.L41L)	Codon usage database
Regulatory	*WFS1* (rs62283056)	Genotype-Tissue Expression (GTEx) project

B Gene Expression in Ear Hair Cells

C Drug-Gene Relationship

Cytotoxicity ⟷ Gene Expression

Variants associated with CIO in adult populations

SLC16A5

Most likely causal variant:
rs4688873 has the highest CADD score

Supporting literature:
SLC16A5 is inhibited by cimitidine, which prevents CIO in mice and rats

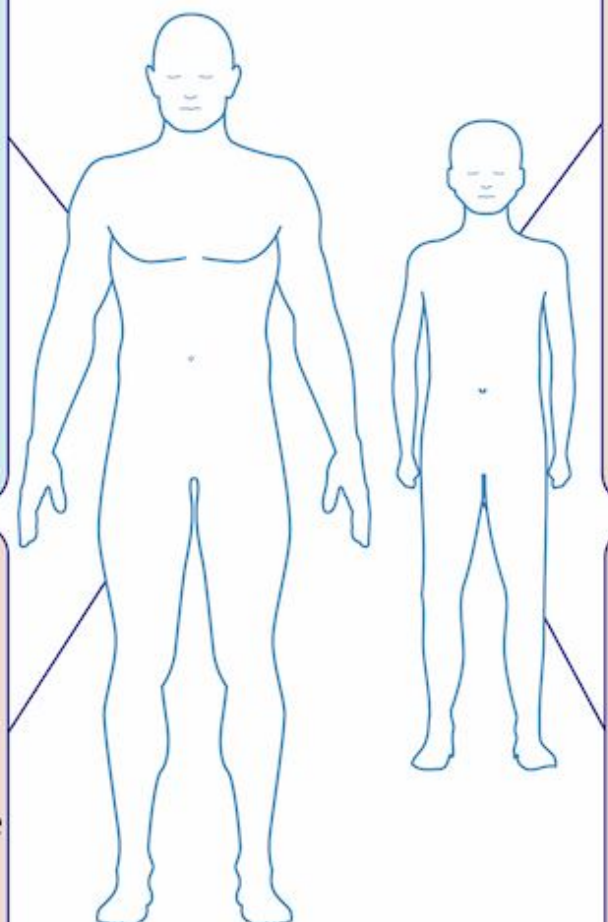
Functional validation:
SLC16A5 expression mirrors that of deafness genes & cisplatin affects the cell viability of *SLC16A5*-silenced HeLa cells

WFS1

Most likely causal variant:
rs62283056 is an eQTL of *WFS1*

Supporting literature:
Variants impacting the coding sequence of *WFS1* cause deafness

Functional validation:
WFS1 is expressed in murine inner ear cells & increased *WFS1* expression is associated with cisplatin cytotoxicity



Variants associated with CIO in pediatric populations

TPMT

Most likely causal variant:
Loss of function *TPMT*3A* allele

Hypothesized mechanism:
TPMT-mediated increase in SAM may result in an increase in toxicity

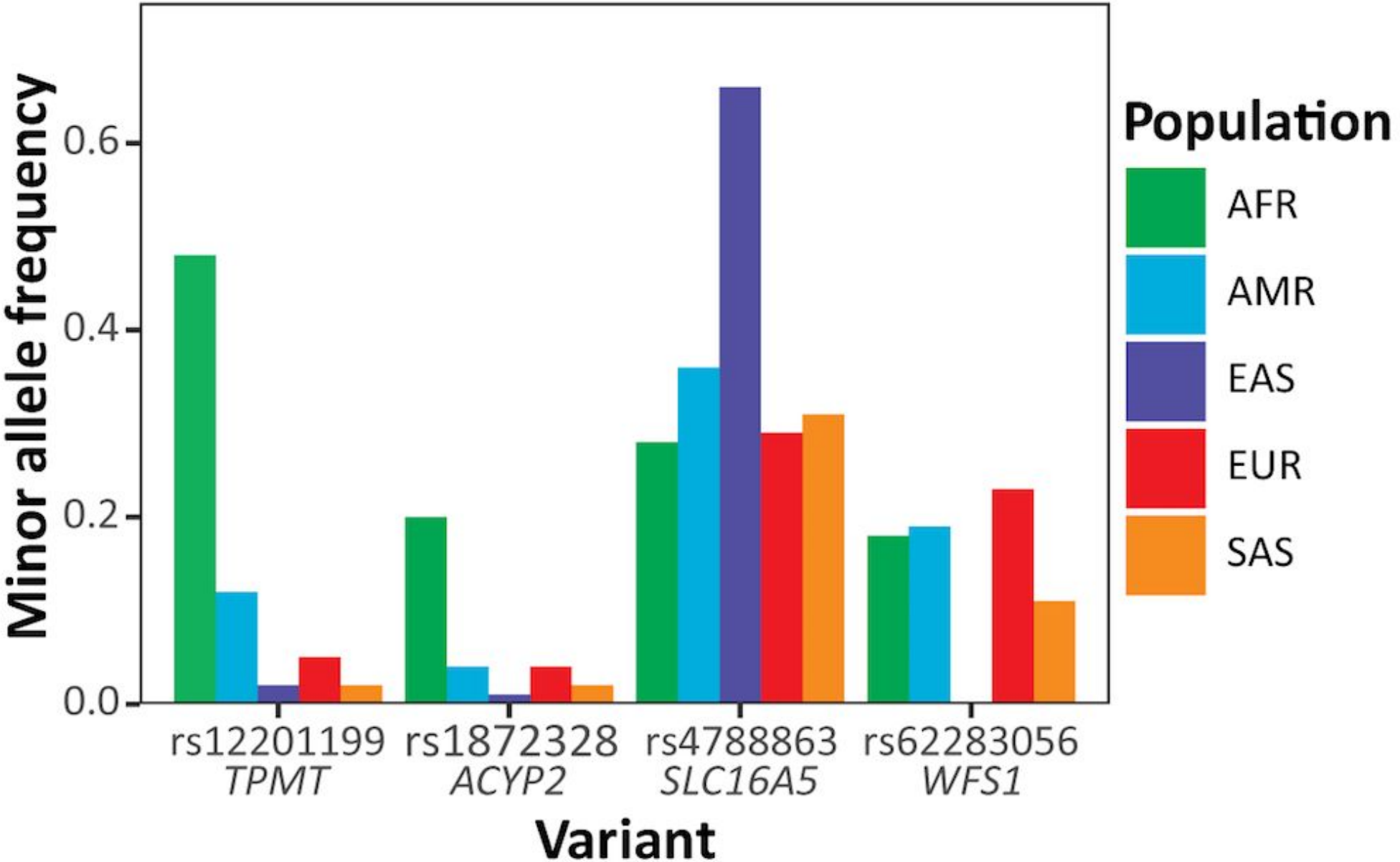
Functional validation:
*TPMT*3A* exhibits reduced cell viability & increased biosensor response after cisplatin treatment in murine inner ear cell lines

ACYP2

Most likely causal variant:
Remains to be determined

Hypothesized mechanism:
ACYP2-mediated calcium homeostasis changes may result in ear hair damage

Functional validation:
ACYP2 is expressed in murine inner ear cells & increased *ACYP2* expression is associated with cisplatin cytotoxicity


Minor allele frequency
0.0
0.2
0.4
0.6
rs12201199
TPMT
rs1872328
ACYP2
rs4788863
SLC16A5
rs62283056
WFS1
Variant
Population
AFR
AMR
EAS
EUR
SAS

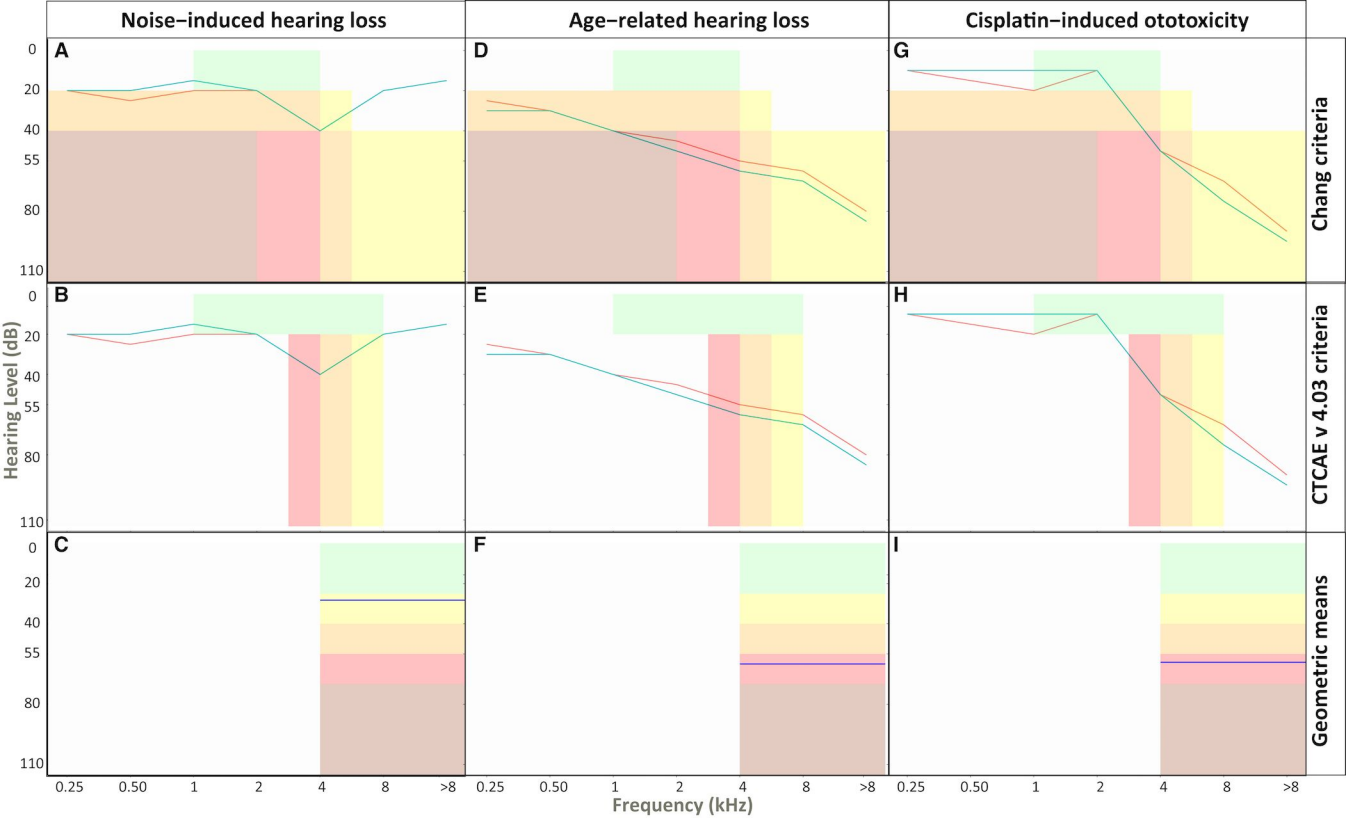

Noise–induced hearing loss
Age–related hearing loss
Cisplatin–induced ototoxicity
A
B
C
D
E
F
G
H
I
Chang criteria
CTCAE v 4.03 criteria
Geometric means
Hearing Level (dB)
0
20
40
55
80
110
0.25
0.50
1
2
4
8
>8
Frequency (kHz)

Table 1: Meta-analysis of results from studies examining variants that have been associated with cisplatin-induced ototoxicity from pharmacogenomic association analyses

SNP	Gene	Effect allele	Alternate allele	Direction*	Meta *P*	Number of samples	Cohorts
rs1872328	*ACYP2*	A	G	+ + + + - +	$6.3x10^{-8}$	1,322	Xu *et al*. 2015 (two cohorts)[24]; Vos *et al*. 2016[49]; Thiesen *et al.* 2017[36]; Wheeler *et al*. 2017[9]; Drögemöller *et al*. 2018[37]
rs62283056	*WFS1*	C	G	+ + +	$1.1x10^{-9}$	978	Xu *et al*. 2015[24]; Wheeler *et al*. 2017[9]; Drögemöller *et al*. 2018[37]
rs4788863	*SLC16A5*	T	C	- - - -	$9.6x10^{-5}$	805	Drögemöller *et al*. 2017 (two cohorts)[13]; Wheeler *et al*. 2017[9]; Lui *et al*. 2018[15]
rs9332377	*COMT*	T	C	+ + + - + - + + + +	$1.3x10^{-3}$	1,761	Ross *et al*. 2009 (two cohorts)[23]; Pussegoda *et al*. 2013[30]; Yang *et al*. 2013 (two cohorts)[34]; Hagleitner *et al*. 2014[35]; Talach *et al.* 2016[32]; Wheeler *et al.*2017[9]; Thiesen *et al.* 2017[36]; Drögemöller *et al.* 2017[13] Teft *et al.* 2019[33]
rs12201199	*TPMT*	T	A	All studies + + + - - - - + -	0.03	1,500	Ross *et al*. 2009 (two cohorts)[23]; Pussegoda *et al*. 2013[30]; Yang *et al*. 2013 (two cohorts)[34]; Hagleitner *et al*. 2014[35]; Wheeler *et al.*2017[9]; Thiesen *et al.* 2017[36]; Drögemöller *et al.* 2017[13]
				Pediatric, non-cranial irradiation studies[#] + + + - +	$1.7x10^{-6}$	478	Ross *et al*. 2009 (two cohorts)[23]; Pussegoda *et al*. 2013[30]; Yang *et al*. 2013[34]; Thiesen *et al.* 2017[36]

METAL was used to perform a fixed-effects meta-analysis of the P-values, weighted by sample size[68].
*Effect allele direction with "+" associated with increased risk of CIO "-" associated with decreased risk of CIO.
CIO: Cisplatin-induced ototoxicity
[#]Analyses were repeated excluding cohorts of patients who all received cranial irradiation[34] or which included adult patients[9,13,35].

Table 2: Statistical analysis methods applied for the identification of genetic variants that are significantly associated with cisplatin-induced ototoxicity

Gene	Variant	Statistical analyses	Model
TPMT	rs12201199	Allelic test/logistic regression	Additive
SLC16A5	rs4788863	Logistic regression	Dominant
ACYP2	rs1872328	Cox-regression	Additive
WFS1	rs62283056	Linear regression	Additive