HHS Public Access

Author manuscript

Pharmacogenomics J. Author manuscript; available in PMC 2013 August 01.

Published in final edited form as:

Pharmacogenomics J. 2013 February ; 13(1): 35–43. doi:10.1038/tpj.2011.38.

Genome-wide Meta-analysis Identifies Variants Associated with Platinating Agent Susceptibility Across Populations

Heather E. Wheeler[1], **Eric R. Gamazon**[2], **Amy L. Stark**[3], **Peter H. O'Donnell**[1], **Lidija K. Gorsic**[1], **R. Stephanie Huang**[1], **Nancy J. Cox**[2], and **M. Eileen Dolan**[1,*]

[1]Section of Hematology/Oncology, Department of Medicine, University of Chicago, Chicago, IL 60637, USA

[2]Section of Genetic Medicine, Department of Medicine, University of Chicago, Chicago, IL 60637, USA

[3]Department of Human Genetics, University of Chicago, Chicago, IL 60637, USA

Abstract

Platinating agents are used in the treatment of many cancers, yet they can induce toxicities and resistance that limit their utility. Using previously published and additional world population panels of diverse ancestry totaling 608 lymphoblastoid cell lines (LCLs), we performed meta-analyses of over 3 million SNPs for both carboplatin- and cisplatin-induced cytotoxicity. The most significant SNP in the carboplatin meta-analysis is located in an intron of *NBAS* ($p = 5.1 \times 10^{-7}$). The most significant SNP in the cisplatin meta-analysis is upstream of *KRT16P2* ($p = 5.8 \times 10^{-7}$). We also show that cisplatin-susceptibility SNPs are enriched for carboplatin-susceptibility SNPs. Most of the variants that associate with platinum-induced cytotoxicity are polymorphic across multiple world populations; therefore, they could be tested in follow-up studies in diverse clinical populations. Seven genes previously implicated in platinating agent response, including *BCL2*, *GSTM1*, *GSTT1*, *ERCC2*, and *ERCC6* were also implicated in our meta-analyses.

Keywords

meta-analysis; pharmacogenomics; cisplatin; carboplatin; cross-population

Introduction

Platinum compounds comprise a class of chemotherapeutic agents that are used worldwide as essential components of many anticancer treatment regimens. In particular, carboplatin and cisplatin are commonly used to treat cancers such as lung, head and neck, colorectal, testicular, ovarian, cervical, and relapsed lymphoma[1-6]. Intrastrand and interstrand cross-

*Corresponding Author: 900 East 57th St, University of Chicago, Chicago, IL 60637. Phone: (773) 702-4441; Fax: (773) 702-9268; edolan@medicine.bsd.uchicago.edu.

Conflict of interest The authors declare no conflict of interest.

Supplementary information is available at *The Pharmacogenomics Journal* website.

links are thought to be the covalent cytotoxic lesions introduced onto DNA by platinating agents[4]. Both agents are associated with particular toxicities, predominantly myelosuppression for carboplatin and nephrotoxicity and ototoxicity for cisplatin[4,7,8]. However, there are currently no reliable means to identify patients at high risk for developing significant platinum-related toxicities, nor means to predict antitumor response. Heterogeneity in sensitivity is consistent with a role for genetic variation in explaining differences in response and toxicity to platinating agents. The similar mechanisms of action of carboplatin and cisplatin could suggest that the same genetic variants might contribute to platinum response. However, different predominant toxicities could mean the two drugs also have independent variants involved in susceptibility to each drug.

Candidate gene investigations of cisplatin-related ototoxicity, focusing on the role of genetic variants in *GSTM3*[9], *GSTP1*[10], mitochondrial DNA[11], or megalin[12] were inconclusive, possibly due to the use of single gene approaches and small patient cohorts. Another analysis focused on pathways associated with reactive oxygen species induction, were restricted to glutathione *S*-transferase (*GST*) genotypes, and identified variants in *GSTP1* and *GSTM1* as risk factors for ototoxicity in cisplatin-treated testicular cancer survivors[13]. Ross *et al.* tested variants in 220 drug-metabolism genes in children for association with cisplatin-induced hearing loss and found variants in methyltransferase genes (*COMT* and *TPMT*) with large 8- to 16-fold risks[14]. A recent genome-wide association study in non-small cell lung cancer patients receiving platinum treatment identified a SNP in *CMKLR1* associated with overall survival, but it was not significant after multiple-testing correction[15].

Patient populations of adequate size treated with the same chemotherapeutic dosage regimen are rare, making genome-wide association (GWA) studies of chemotherapeutic response in clinical settings challenging. To avoid confounders such as comorbidities, concomitant medications and diet, LCL models have been developed as useful discovery tools in germline genetic studies of chemotherapeutic susceptibility[16-20]. Recently, some SNPs associated with chemotherapeutic susceptibility in LCL studies have been replicated in patient populations by associating with phenotypes like tumor response and overall survival, demonstrating the potential utility of this model[21,22].

Several GWA studies using LCLs from different population panels of the International HapMap Project[23,24] have been performed to find variants and genes associated with platinum cytotoxicity. Previous studies identified variants associated with carboplatin[17] and cisplatin[18] cytotoxicity that also associated with gene expression in the initial (phase I/II) YRI (Yoruba from Ibadan, Nigeria) and CEU (Northern and Western European ancestry from Utah) HapMap panels. Taking an innovative approach that considered cytotoxicity-associated SNPs in cell lines derived from the population most sensitive to platinating agents (ASN, Japanese from Tokyo and Chinese from Beijing), O'Donnell *et al.* then identified those that replicated in a combined YRI and CEU population[25]. Although each of these studies found suggestive variants associated with platinating agent response, the top findings did not always replicate when examined in additional populations.

In this study, our goal was to identify variants that associate with platinating agent-induced cytotoxicity across populations. We believe that, once validated, such cross-population

variants could be used to identify individuals who are likely to be sensitive or resistant to carboplatin and/or cisplatin regardless of genetic ancestry. In addition to the population panels mentioned in the studies above, we collected platinating agent cytotoxicity data from the HapMap phase III YRI, CEU, ASW (African ancestry from the Southwestern United States) and CHD (Chinese ancestry from Denver) panels[26]. Using a meta-analysis approach[27,28], we combined the results of GWA studies for carboplatin- or cisplatin-induced cytotoxicity in each of 7 population panels. We identified SNPs associated with each of the two drug phenotypes and an enrichment of carboplatin-associated SNPs in the top cisplatin-associated SNPs. Most of the identified SNPs were common in all 7 panels, but several were specific to a population class. Seven genes previously implicated in platinating response through candidate studies were also implicated in our meta-analyses.

Materials and Methods

Lymphoblastoid Cell Lines

International HapMap Project LCLs from 7 panels were purchased from the Coriell Institute for Medical Research. The panels included 176 genotyped individuals from the Yoruba in Ibadan, Nigeria (YRI1/2 [HAPMAPPT03] and YRI3 [HAPMAPPT04], 83 individuals of African ancestry from the Southwestern United States (ASW [HAPMAPPT07]), 85 individuals of Han Chinese ancestry from Denver, Colorado (CHD [HAPMAPV11]), 90 Japanese from Tokyo and Han Chinese from Beijing (ASN [HAPMAPPT02]), and 174 Utah residents with Northern and Western European ancestry (CEU1/2 [HAPMAPPT01] and CEU3 [HAPMAPPT06]) for which genotype data is available (HapMap r27). Family structure of the panels is indicated in Table 1. Cell lines were maintained in RPMI 1640 (Mediatech, Herndon, VA, USA) supplemented with 15% fetal bovine serum (HyClone Laboratories, Logan, UT, USA) and 1% L-glutamine (Invitrogen, Carlsbad, CA, USA). Cell lines were diluted 3 times per week at a concentration of 3.5×10^5 cells/mL and incubated at 37°C with 5% CO_2 and 95% humidity.

Cytotoxicity Assays

Cells were treated with carboplatin[17] and cisplatin[18] as previously described. The concentration required to inhibit 50% of cell growth (IC_{50}) was calculated for each carboplatin- and cisplatin-treated cell line. All IC_{50} values were either $\log_2$- or rank-transformed to normality (*rntransform* function in the *GenABEL* R library) before statistical modeling. If the $\log_2$-transformed data was not consistent with normality (Shapiro-Wilk test $p < 0.05$), the phenotype was rank-transformed to normality. The ASW phenotypes were rank-transformed; the phenotypes from the other panels were $\log_2$-transformed.

Genome-wide Association Analyses of Individual Panels

GWA studies of carboplatin- and cisplatin-induced cytotoxicity were performed on each of the seven panels individually. Studies of carboplatin- and cisplatin-susceptibility in the YRI1/2[17,18], CEU1/2[18], and ASN[25] panels have been previously published. To increase genome coverage of YRI3 and CEU3 (HapMap r27), ungenotyped makers were imputed using the BEAGLE software using YRI1/2 and CEU1/2 (HapMap r22) as reference, respectively[29]. BEAGLE imputes ungenotyped markers for parent-offspring trios by

modeling the family structure in the analysis. To measure the accuracy of the imputation at each SNP locus, R^2 was calculated as described following 100 imputations of the data[29]. Imputed SNP genotypes with $R^2 > 0.80$ were carried through the rest of the analysis. For YRI1/2, YRI3, CEU1/2, and CEU3, greater than 2 million SNPs (minor allele frequency (MAF) > 0.05 within the panel, no Mendelian errors and in Hardy-Weinberg equilibrium ($p > 0.001$)) were tested for association with carboplatin $\log_2(IC_{50})$ and cisplatin $\log_2(IC_{50})$ using the quantitative trait disequilibrium test (QTDT) total association model[30].

To control for population structure in the admixed ASW population (HapMap r27), local ancestry at each genotyped SNP locus was estimated using the HAPMIX software[31]. Phased genotypes from the YRI1/2 and CEU1/2 populations were used as the two parental populations to estimate the ancestry of the ASW population. For each individual, the algorithm estimated the number of CEU chromosomes (0-2) at each SNP locus. To increase genome coverage of the ASW, ungenotyped makers were imputed using the BEAGLE software[29], using both YRI1/2 and CEU1/2 as reference populations. Local ancestry for each imputed SNP was inferred by using the predicted number of CEU chromosomes from nearest genotyped SNP. GWA studies in the ASW population were performed between carboplatin and cisplatin IC_{50} phenotypes rank-transformed to normality and greater than 2 million SNPs using the quantitative trait disequilibrium test (QTDT) total association model[30]. Local ancestry (continuous predicted number of CEU chromosomes at each locus) was included as a covariate in the model for each drug.

To increase genome coverage of the CHD (HapMap r27), ungenotyped makers were imputed using the MaCH software with the ASN population (HapMap r27) as reference[32]. Imputed SNP genotypes with $R^2 > 0.80$ were carried through the rest of the analysis. For CHD and ASN, PLINK software was used to test greater than 2 million SNPs (MAF > 0.05 and in Hardy-Weinberg equilibrium ($p > 0.001$)) for linear association with carboplatin $\log_2(IC_{50})$ and cisplatin $\log_2(IC_{50})$[33].

Genomic control lambda (λ_{GC}) values[34] were calculated for the GWA study of each population panel-drug phenotype combination. Studies with λ_{GC} values greater than 1.00 were corrected for residual inflation of the test statistic by dividing the observed test statistic at each SNP by the λGC[34] and then the corresponding p-values were carried through the meta-analyses.

Meta-analysis of Seven GWA Studies

To determine which SNPs associate with carboplatin- and cisplatin-induced cytotoxicity across populations, we performed a meta-analysis to combine the results of the individual GWA studies from the 7 panels. We used the software METAL, which combines SNP p-values across studies taking into account a study specific weight (sample size) and direction of effect (positive or negative beta)[28]. This approach converted the direction of effect and p-value observed in each study into a signed Z-score such that very negative Z-scores indicate a small p-value and an allele associated with lower IC_{50}, whereas large positive Z-scores indicate a small p-value and an allele associated with higher IC_{50}. Z-scores for each SNP were combined across studies in a weighted sum, with weights proportional to the square-

root of the sample size for each study[28]. Q-Q plots of the corresponding p-values are shown in Supplementary Figure S1. Gene region plots of top SNPs were made with LocusZoom[35].

Results

Population Panel Characteristics

Carboplatin- and cisplatin-induced cytotoxicity was measured in 608 LCLs from seven HapMap panels. The panels included YRI1/2[17,18], YRI3, ASW, CHD, ASN[25], CEU1/2[18,36], and CEU3. Percent survival data at several concentrations (0-80 μM carboplatin or 0-20 μM cisplatin) were used to calculate the IC_{50} for each cell line and drug. Table 1 displays the means and standard deviations of carboplatin and cisplatin IC_{50} for each panel. For both drugs, the IC_{50} values are in the range of the plasma platinum concentrations observed in patients after treatment. The mean IC_{50} values ranged from 19.7-36.8 μM (7.3-13.7 μg/ml) for carboplatin and 4.5-10.2 μM (1.4-3.1 μg/ml) for cisplatin in the LCL panels. In previous pharmacokinetic studies, plasma concentrations of platinum a few hours after administration ranged from 5-20 μg/ml in patients treated with carboplatin[37-39] and from 1-10 μg/ml in patients treated with cisplatin[38,40].

Meta-analysis reveals SNPs associated with platinating agent cytotoxicity

GWA studies of carboplatin- and cisplatin-induced cytotoxicity were performed in each of the seven panels individually. Each study tested 2-2.5 million genotyped or imputed SNPs for association with carboplatin IC_{50} or cisplatin IC_{50}. To determine which SNPs associate with carboplatin IC_{50} or cisplatin IC_{50} across populations, we performed a meta-analysis to combine the results of the individual GWA studies from the 7 panels. Some SNPs are unique to a particular panel or subset of panels and therefore ~3 million SNPs were tested in the meta-analysis. At the suggestive threshold of $p < 10^{-4}$, 322 SNPs associated with carboplatin IC_{50} and 334 SNPs with cisplatin IC_{50} (Figure 1, Supplementary Table S1). Most of the SNPs at this threshold were common (MAF > 0.05) in all 7 HapMap panels (Figure 2).

The most significant SNP in the carboplatin IC_{50} meta-analysis was rs7572081, which is located in an intron of *NBAS* (Table S1, $p = 5.1 \times 10^{-7}$). The most significant SNP in the cisplatin IC_{50} meta-analysis was rs7210837 (Table S1, $p = 5.8 \times 10^{-7}$). The most significant missense SNP in the meta-analyses was rs244903, which associated with carboplatin IC_{50} and is located in the first exon of *RARS* ($p = 5.4 \times 10^{-6}$, Figure 3).

Cisplatin-associated SNPs are enriched for carboplatin-associated SNPs

To compare the top SNP associations for the two drug phenotypes, we examined the Z-score distributions of the top cisplatin IC_{50} SNPs at various thresholds in the carboplatin IC_{50} meta-analysis results and vice versa (Figure 4). We verified that the directions of effect of the top SNPs are the same between the two drugs. That is, when the minor allele of a particular SNP is associated with increased resistance to carboplatin, the minor allele is also associated with increased resistance to cisplatin. Therefore, cisplatin-associated SNPs are enriched for carboplatin-associated SNPs and common genetically mediated mechanisms may influence the effect of both chemically related drugs. The Pearson correlation between

carboplatin IC_{50} and cisplatin IC_{50} varied across HapMap panels (YRI1/2 = 0.84, YRI3 = 0.75, ASW = 0.67, CHD = 0.82, ASN = 0.76, CEU1/2 = 0.58, CEU3 = 0.60).

Genes previously implicated in platinating agent cytotoxicity replicated by meta-analysis

We compiled a list of 41 genes previously implicated in platinating agent response (Supplementary Table S2). This list contained the genes that make up the PharmGKB platinum pathway[41], the two methyltransferases found associated with cisplatin-induced hearing loss[14], and several genes from a review of platinating agents[4]. None of the top SNPs ($p < 10^{-4}$) from both the carboplatin and cisplatin meta-analyses were located within these 41 genes. However, 28 of the 41 genes are expressed in LCLs[42] and 7 of these 28 genes were expression targets of top meta-analysis SNP eQTLs[43] (Supplementary Table S2).

Included in these 7 genes are *BCL2* and *GSTM1*, which are both targeted by YRI1/2 eQTLs from the same region of chromosome 14 that also associate with both carboplatin and cisplatin IC_{50} (Figure 5). Also included in the previously implicated genes are *GSTT1*, *ERCC6*, and *ERCC2*. rs2191934 is associated with cisplatin IC_{50} (meta-$p = 8.3 \times 10^{-5}$) and the expression of *GSTT1* and *ERCC6* (Supplementary Figure S2). rs9527419 is associated with cisplatin IC_{50} (meta-$p = 5.8 \times 10^{-6}$) and the expression of *ERCC2* (Supplementary Figure S3).

Discussion

We performed a meta-analysis of the results of GWA studies for carboplatin- and cisplatin-induced cytotoxicity in 7 HapMap panels that included a total of 608 LCLs. We identified 322 SNPs that associate with carboplatin IC_{50} and 334 SNPs that associate with cisplatin IC_{50} at the suggestive threshold of $p < 10^{-4}$. About half of the identified SNPs were common in all 7 panels. By the nature of the meta-analysis, this indicates that the allelic relationship with IC_{50} was the same in most if not all of the populations. Therefore, if these variants are confirmed in clinical cohorts, they could be used to predict chemotherapeutic response in individuals from most world populations. However, several SNPs were specific to a population class and therefore were not interrogated in as many individuals. For these population-specific SNPs, additional cell lines from the appropriate population are needed to confirm these findings.

We identified an enrichment of carboplatin-associated SNPs in the top cisplatin-associated SNPs. This is somewhat expected given that the phenotypes are correlated. The correlation between carboplatin and cisplatin IC_{50} within a HapMap panel ranges from 0.58 in CEU1/2 to 0.84 in YRI1/2. Both carboplatin and cisplatin are platinating agents that act through the formation of intrastrand and interstrand DNA cross-links, which result in DNA strand breaks leading to cell death[4]. Our results support that common genetic mechanisms may influence the effects of both drugs. However, there are also likely unique genetic mechanisms contributing to the different clinical toxicities observed between the two drugs. Although findings in LCL studies have been replicated by associating with patient phenotypes like tumor response and overall survival[21,22], studies testing top LCL findings for association with patient toxicity phenotypes are also needed to fully understand the utility of the LCL model.

Several genes connected to top SNPs associated with platinum susceptibility have been implicated in tumorigenesis. For instance, the most significant SNP in the carboplatin IC_{50} meta-analysis, rs7572081, is located in an intron of *NBAS* (neuroblastoma amplified sequence). Carboplatin is often used in neuroblastoma treatment regimens[44,45] and increased *NBAS* expression has been associated with poorer outcome in patients greater than 18 months old[46]. The most significant missense SNP in the meta-analyses was rs244903, which associated with carboplatin IC_{50} and is located in the first exon of *RARS* (arginyl-tRNA synthetase). Since RARS is necessary for protein synthesis, it has been recognized as a potential chemotherapeutic drug target[47]. The *RARS* SNP is also an eQTL associated with the expression of five genes in YRI1/2, including *NOL1* and *CCNG2*[43]. Reduced expression of *NOL1*, also known as p120, reduced cell growth in the human breast cancer line MCF-7[48]. *CCNG2* is a negative regulator of cell cycle progression and decreased expression of the gene has been observed in oral cancers[49].

Seven genes previously implicated in platinating response out of 28 tested were also implicated in our meta-analyses. These genes included *BCL2* and *GSTM1*, which are both targeted by eQTLs located in the same region of chromosome 14 that also associate with both carboplatin and cisplatin IC_{50}. Inhibition of apoptosis by increased *BCL2* expression has been shown to lead to cisplatin resistance[50]. Here, the opposite effect was observed: the eQTL minor alleles associated with decreased *BCL2* expression[42] and increased IC_{50}. Further studies are necessary to elucidate how *BCL2* may be functioning in LCLs in response to platinating agents. *GSTM1* is an enzyme that contributes to the detoxification of platinating agents[41]. Here, alleles associated with increased *GSTM1* expression[42] were also associated with increased IC_{50}. Correlations have been observed between high levels of the related protein GSTP1 and cisplatin resistance in colon, lung adenocarcinoma, and glioblastoma tumor cell lines; however, results in other studies are inconsistent[4].

GSTT1, another glutathione S-transferase, was also implicated in our meta-analysis. rs2191934 associated with cisplatin IC_{50} and the expression of *GSTT1* and *ERCC6*. rs9527419 associated with cisplatin IC_{50} and the expression of *ERCC2*. *ERCC2* and *ERCC6* are components of the platinum pathway involved in nucleotide excision repair[41]. Platinating agent DNA adduct repair occurs primarily through nucleotide excision repair[4]. Here alleles associated with increased expression of *ERCC2* and *ERCC6* were also associated with cisplatin resistance (increased IC_{50}). Cisplatin resistance is correlated with the increased expression of several nucleotide excision repair genes; in ovarian cancer, *XPA* and *ERCC1* were shown to have increased expression in tumors of patients resistant to platinum treatment[51,52]. Similarly, a study of gastric cancer patients showed a correlation between cisplatin resistance and *ERCC1* levels[53]. The observation that numerous candidate genes are not replicated here is not necessarily surprising since our unbiased GWA approach might not be expected to identify prior candidate genes, since it makes no assumption that these are the most important. In addition, some of the SNPs within candidate genes have a MAF <0.05 and therefore would not be tested in this model.

One previous study from our laboratory identified six SNPs that contribute to cisplatin-induced cytotoxicity through their effects on the expression of 8 genes in the combined CEU1/2 and YRI1/2 population[18]. Although not one of our top findings, one of these six

SNPs remained associated with cisplatin IC_{50} when the additional panels were added in the current study (rs2136241, meta-p = 6.9×10^{-4}). This SNP was present and the direction of effect was the same in all seven HapMap panels. The association signal for the other five SNPs was lost, due to discordant directions of effect when the additional panels were added. The previous study examined 176 individuals, so power to detect associations was limited. Another study from our group identified cytotoxicity-associated SNPs in cell lines derived from the population most sensitive to platinating agents (ASN, n=90) and showed that 13 of the SNPs also associated with either carboplatin or cisplatin IC_{50} in a combined CEU1/2 and YRI1/2 population (n=106)[25]. One of these SNPs, rs6691275, remained associated with carboplatin IC_{50} in the current study (meta-p = 3.1×10^{-4}) and the direction of effect was the same in six of the seven tested HapMap panels. The association signal for the other 12 SNPs was lost, due to discordant directions of effect when the additional panels were added, again likely due to limited power in the initial study. However, the direction of effect was the same in the two panels of Asian ancestry (ASN, CHD) for 10 of the 13 SNPs, indicating the SNPs could associate with cytotoxicity in a population specific manner.

Our results show that many genes and variants may be involved in cellular response to platinating agents. Since most of the variants that associate with platinum-induced cytotoxicity are polymorphic across multiple world populations (African, Asian, European), they can be tested in follow-up studies in both LCL and tumor cell line panels from multiple populations, or in diverse clinical populations. Our cell line models allow us to select the most promising SNPs for testing in clinical studies. We plan to clinically validate cytotoxicity-associated variants in a cohort of patients treated with carboplatin or cisplatin to determine their roles in patient response and toxicity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study is supported by NIH/NIGMS Pharmacogenomics of Anticancer Agents grant U01GM61393 (NJC, MED), the University of Chicago Breast Cancer SPORE P50 CA125183 (RSH, NJC, MED), NIH/NIGMS grant K08GM089941 (RSH) and University of Chicago Cancer Center Support Grant P30 CA14599 (RSH, MED). The authors would like to thank the Pharmacogenomics of Anticancer Agents Cell Line Core for assistance in providing and maintaining these cell lines. In addition, the authors thank Marleen Welsh for phenotyping some of the ASW and CEU3 cell lines and Wei Zhang for performing initial imputation analyses in the CHD.

References

1. Borghaei H, Langer CJ, Millenson M, Ruth KJ, Litwin S, Tuttle H, et al. Phase II study of paclitaxel, carboplatin, and cetuximab as first line treatment, for patients with advanced non-small cell lung cancer (NSCLC): results of OPN-017. J Thorac Oncol. 2008; 3:1286–1292. [PubMed: 18978564]
2. McWhinney SR, Goldberg RM, McLeod HL. Platinum neurotoxicity pharmacogenetics. Mol Cancer Ther. 2009; 8:10–16. [PubMed: 19139108]
3. Micheletti E, Face B La, Bianchi E, Cagna E, Apostoli P, Ruggeri G, et al. Continuous infusion of carboplatin during conventional radiotherapy treatment in advanced squamous carcinoma of the cervix uteri IIB-IIIB (UICC). A phase I/II and pharmacokinetic study. Am J Clin Oncol. 1997; 20:613–620. [PubMed: 9391552]

4. Rabik CA, Dolan ME. Molecular mechanisms of resistance and toxicity associated with platinating agents. Cancer Treat Rev. 2007; 33:9–23. [PubMed: 17084534]
5. Siddik ZH. Cisplatin: mode of cytotoxic action and molecular basis of resistance. Oncogene. 2003; 22:7265–7279. [PubMed: 14576837]
6. Wang D, Lippard SJ. Cellular processing of platinum anticancer drugs. Nat Rev Drug Discov. 2005; 4:307–320. [PubMed: 15789122]
7. Celik I, Kars A, Ozyar E, Tekuzman G, Atahan L, Firat D. Major toxicity of cisplatin, fluorouracil, and leucovorin following chemoradiotherapy in patients with nasopharyngeal carcinoma. J Clin Oncol. 1996; 14:1043–1044. [PubMed: 8622011]
8. van Glabbeke M, Renard J, Pinedo HM, Cavalli F, Vermorken J, Sessa C, et al. Iproplatin and carboplatin induced toxicities: overview of phase II clinical trial conducted by the EORTC Early Clinical Trials Cooperative Group (ECTG). Eur J Cancer Clin Oncol. 1988; 24:255–262. [PubMed: 3281842]
9. Peters U, Preisler-Adams S, Hebeisen A, Hahn M, Seifert E, Lanvers C, et al. Glutathione S-transferase genetic polymorphisms and individual sensitivity to the ototoxic effect of cisplatin. Anticancer Drugs. 2000; 11:639–643. [PubMed: 11081456]
10. Oldenburg J, Kraggerud SM, Brydoy M, Cvancarova M, Lothe RA, Fossa SD. Association between long-term neuro-toxicities in testicular cancer survivors and polymorphisms in glutathione-s-transferase-P1 and -M1, a retrospective cross sectional study. J Transl Med. 2007; 5:70. [PubMed: 18162130]
11. Peters U, Preisler-Adams S, Lanvers-Kaminsky C, Jurgens H, Lamprecht-Dinnesen A. Sequence variations of mitochondrial DNA and individual sensitivity to the ototoxic effect of cisplatin. Anticancer Res. 2003; 23:1249–1255. [PubMed: 12820379]
12. Riedemann L, Lanvers C, Deuster D, Peters U, Boos J, Jurgens H, et al. Megalin genetic polymorphisms and individual sensitivity to the ototoxic effect of cisplatin. Pharmacogenomics J. 2008; 8:23–28. [PubMed: 17457342]
13. Oldenburg J, Kraggerud SM, Cvancarova M, Lothe RA, Fossa SD. Cisplatin-induced long-term hearing impairment is associated with specific glutathione s-transferase genotypes in testicular cancer survivors. J Clin Oncol. 2007; 25:708–714. [PubMed: 17228018]
14. Ross CJ, Katzov-Eckert H, Dube MP, Brooks B, Rassekh SR, Barhdadi A, et al. Genetic variants in TPMT and COMT are associated with hearing loss in children receiving cisplatin chemotherapy. Nat Genet. 2009; 41:1345–1349. [PubMed: 19898482]
15. Wu X, Ye Y, Rosell R, Amos CI, Stewart DJ, Hildebrandt MA, et al. Genome-Wide Association Study of Survival in Non-Small Cell Lung Cancer Patients Receiving Platinum-Based Chemotherapy. J Natl Cancer Inst. 2011
16. Hartford CM, Duan S, Delaney SM, Mi S, Kistner EO, Lamba JK, et al. Population-specific genetic variants important in susceptibility to cytarabine arabinoside cytotoxicity. Blood. 2009; 113:2145–2153. [PubMed: 19109566]
17. Huang RS, Duan S, Kistner EO, Hartford CM, Dolan ME. Genetic variants associated with carboplatin-induced cytotoxicity in cell lines derived from Africans. Mol Cancer Ther. 2008; 7:3038–3046. [PubMed: 18765826]
18. Huang RS, Duan S, Shukla SJ, Kistner EO, Clark TA, Chen TX, et al. Identification of genetic variants contributing to cisplatin-induced cytotoxicity by use of a genomewide approach. Am J Hum Genet. 2007; 81:427–437. [PubMed: 17701890]
19. Li L, Fridley BL, Kalari K, Jenkins G, Batzler A, Weinshilboum RM, et al. Gemcitabine and arabinosylcytosin pharmacogenomics: genome-wide association and drug response biomarkers. PLoS One. 2009; 4:e7765. [PubMed: 19898621]
20. Watters JW, Kraja A, Meucci MA, Province MA, McLeod HL. Genome-wide discovery of loci influencing chemotherapy cytotoxicity. Proc Natl Acad Sci U S A. 2004; 101:11809–11814. [PubMed: 15282376]
21. Niu N, Schaid DJ, Fridley BL, Kalari K, Li L, Jenkins G, et al. Oral Session III-B (OIII-B) Molecular Targets and Genetics in Oncology 9:30 am - 10:30 am. Clin Pharmacol Ther. 2011; 89:S66–S68.

22. Ziliak D, O'Donnell PH, Im HK, Gamazon ER, Chen P, Delaney S, et al. Germline polymorphisms discovered via a cell-based, genome-wide approach predict platinum response in head and neck cancers. Transl Res. 2011; 157:265–272. [PubMed: 21497773]
23. Altshuler D, Brooks LD, Chakravarti A, Collins FS, Daly MJ, Donnelly P. A haplotype map of the human genome. Nature. 2005; 437:1299–1320. [PubMed: 16255080]
24. Frazer KA, Ballinger DG, Cox DR, Hinds DA, Stuve LL, Gibbs RA, et al. A second generation human haplotype map of over 3.1 million SNPs. Nature. 2007; 449:851–861. [PubMed: 17943122]
25. O'Donnell PH, Gamazon E, Zhang W, Stark AL, Kistner-Griffin EO, Huang R Stephanie, et al. Population differences in platinum toxicity as a means to identify novel genetic susceptibility variants. Pharmacogenet Genomics. 2010; 20:327–337. [PubMed: 20393316]
26. Altshuler DM, Gibbs RA, Peltonen L, Dermitzakis E, Schaffner SF, Yu F, et al. Integrating common and rare genetic variation in diverse human populations. Nature. 2010; 467:52–58. [PubMed: 20811451]
27. de Bakker PI, Ferreira MA, Jia X, Neale BM, Raychaudhuri S, Voight BF. Practical aspects of imputation-driven meta-analysis of genome-wide association studies. Hum Mol Genet. 2008; 17:R122–128. [PubMed: 18852200]
28. Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. Bioinformatics. 2010; 26:2190–2191. [PubMed: 20616382]
29. Browning BL, Browning SR. A unified approach to genotype imputation and haplotype-phase inference for large data sets of trios and unrelated individuals. Am J Hum Genet. 2009; 84:210–223. [PubMed: 19200528]
30. Abecasis GR, Cookson WO, Cardon LR. Pedigree tests of transmission disequilibrium. Eur J Hum Genet. 2000; 8:545–551. [PubMed: 10909856]
31. Price AL, Tandon A, Patterson N, Barnes KC, Rafaels N, Ruczinski I, et al. Sensitive detection of chromosomal segments of distinct ancestry in admixed populations. PLoS Genet. 2009; 5:e1000519. [PubMed: 19543370]
32. Li Y, Willer CJ, Ding J, Scheet P, Abecasis GR. MaCH: using sequence and genotype data to estimate haplotypes and unobserved genotypes. Genet Epidemiol. 2010; 34:816–834. [PubMed: 21058334]
33. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007; 81:559–575. [PubMed: 17701901]
34. Devlin B, Roeder K. Genomic control for association studies. Biometrics. 1999; 55:997–1004. [PubMed: 11315092]
35. Pruim RJ, Welch RP, Sanna S, Teslovich TM, Chines PS, Gliedt TP, et al. LocusZoom: regional visualization of genome-wide association scan results. Bioinformatics. 2010; 26:2336–2337. [PubMed: 20634204]
36. Huang RS, Kistner EO, Bleibel WK, Shukla SJ, Dolan ME. Effect of population and gender on chemotherapeutic agent-induced cytotoxicity. Mol Cancer Ther. 2007; 6:31–36. [PubMed: 17237264]
37. Harland SJ, Newell DR, Siddik ZH, Chadwick R, Calvert AH, Harrap KR. Pharmacokinetics of cis-diammine-1,1-cyclobutane dicarboxylate platinum(II) in patients with normal and impaired renal function. Cancer Res. 1984; 44:1693–1697. [PubMed: 6367971]
38. Lerza R, Vannozzi MO, Tolino G, Viale M, Bottino GB, Bogliolo G, et al. Carboplatin and cisplatin pharmacokinetics after intrapleural combination treatment in patients with malignant pleural effusion. Ann Oncol. 1997; 8:385–391. [PubMed: 9209670]
39. Oguri S, Sakakibara T, Mase H, Shimizu T, Ishikawa K, Kimura K, et al. Clinical pharmacokinetics of carboplatin. J Clin Pharmacol. 1988; 28:208–215. [PubMed: 3283185]
40. Peng B, English MW, Boddy AV, Price L, Wyllie R, Pearson AD, et al. Cisplatin pharmacokinetics in children with cancer. Eur J Cancer. 1997; 33:1823–1828. [PubMed: 9470840]
41. Marsh S, McLeod H, Dolan E, Shukla SJ, Rabik CA, Gong L, et al. Platinum pathway. Pharmacogenet Genomics. 2009; 19:563–564. [PubMed: 19525887]

42. Duan S, Huang RS, Zhang W, Bleibel WK, Roe CA, Clark TA, et al. Genetic architecture of transcript-level variation in humans. Am J Hum Genet. 2008; 82:1101–1113. [PubMed: 18439551]
43. Gamazon ER, Zhang W, Konkashbaev A, Duan S, Kistner EO, Nicolae DL, et al. SCAN: SNP and copy number annotation. Bioinformatics. 2010; 26:259–262. [PubMed: 19933162]
44. Kushner BH, Kramer K, Modak S, Cheung NK. High-dose carboplatin-irinotecan-temozolomide: treatment option for neuroblastoma resistant to topotecan. Pediatr Blood Cancer. 2011; 56:403–408. [PubMed: 21049517]
45. Veal GJ, Cole M, Errington J, Pearson AD, Gerrard M, Whyman G, et al. Pharmacokinetics of carboplatin and etoposide in infant neuroblastoma patients. Cancer Chemother Pharmacol. 2010; 65:1057–1066. [PubMed: 19701749]
46. Kaneko S, Ohira M, Nakamura Y, Isogai E, Nakagawara A, Kaneko M. Relationship of DDX1 and NAG gene amplification/overexpression to the prognosis of patients with MYCN-amplified neuroblastoma. J Cancer Res Clin Oncol. 2007; 133:185–192. [PubMed: 17028906]
47. Bence AK, Crooks PA. The mechanism of L-canavanine cytotoxicity: arginyl tRNA synthetase as a novel target for anticancer drug discovery. J Enzyme Inhib Med Chem. 2003; 18:383–394. [PubMed: 14692504]
48. Saijo Y, Perlaky L, Valdez BC, Busch RK, Henning D, Zhang WW, et al. The effect of antisense p120 construct on p120 expression and cell proliferation in human breast cancer MCF-7 cells. Cancer Lett. 1993; 68:95–104. [PubMed: 8443798]
49. Kim Y, Shintani S, Kohno Y, Zhang R, Wong DT. Cyclin G2 dysregulation in human oral cancer. Cancer Res. 2004; 64:8980–8986. [PubMed: 15604262]
50. Eliopoulos AG, Kerr DJ, Herod J, Hodgkins L, Krajewski S, Reed JC, et al. The control of apoptosis and drug resistance in ovarian cancer: influence of p53 and Bcl-2. Oncogene. 1995; 11:1217–1228. [PubMed: 7478541]
51. Dabholkar M, Vionnet J, Bostick-Bruton F, Yu JJ, Reed E. Messenger RNA levels of XPAC and ERCC1 in ovarian cancer tissue correlate with response to platinum-based chemotherapy. J Clin Invest. 1994; 94:703–708. [PubMed: 8040325]
52. Li Q, Yu JJ, Mu C, Yunmbam MK, Slavsky D, Cross CL, et al. Association between the level of ERCC-1 expression and the repair of cisplatin-induced DNA damage in human ovarian cancer cells. Anticancer Res. 2000; 20:645–652. [PubMed: 10810335]
53. Metzger R, Leichman CG, Danenberg KD, Danenberg PV, Lenz HJ, Hayashi K, et al. ERCC1 mRNA levels complement thymidylate synthase mRNA levels in predicting response and survival for gastric cancer patients receiving combination cisplatin and fluorouracil chemotherapy. J Clin Oncol. 1998; 16:309–316. [PubMed: 9440758]

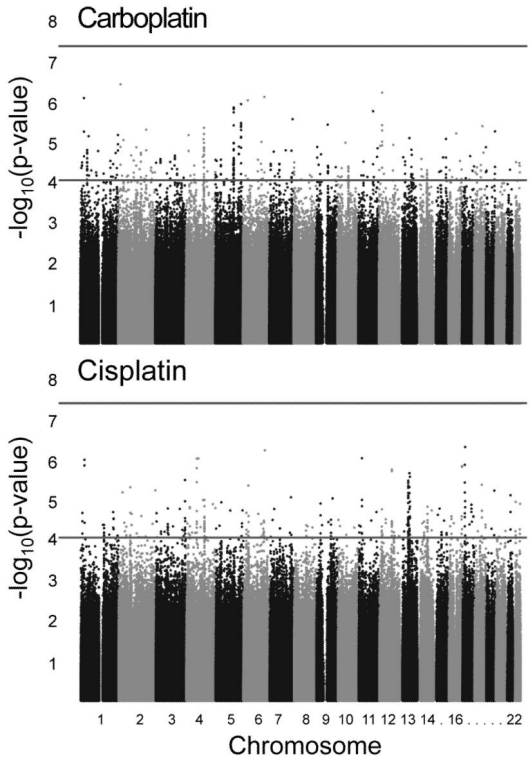

Figure 1. Meta-analysis results of 7 genome-wide association studies of platinum-induced cytotoxicity

Each point represents a SNP. Horizontal lines are at the suggestive significance threshold of $p = 10^{-4}$ and the genome-wide significance threshold of $p = 5 \times 10^{-8}$.

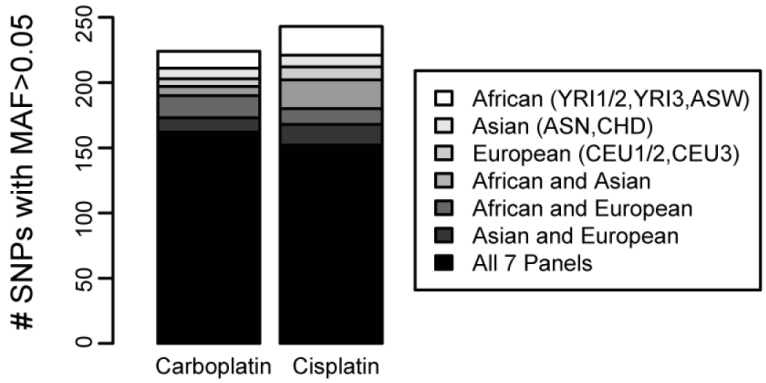

Figure 2. Population class distribution of the top meta-analysis hits

The number of SNPs with a meta p-value < 10^{-4} that have a MAF > 0.05 in each population panel included in the listed population classes. Most (50.3% for carboplatin and 45.5% for cisplatin) of the top SNPs ($p < 10^{-4}$) were present in all 7 panels. For the vast majority of these SNPs present in all 7 panels, the direction of effect was the same for either 7/7 or 6/7 panels (98.1% for carboplatin, 96.7% for cisplatin). SNPs not included in this bar chart were present in a subset of panels not included as one of the listed population classes.

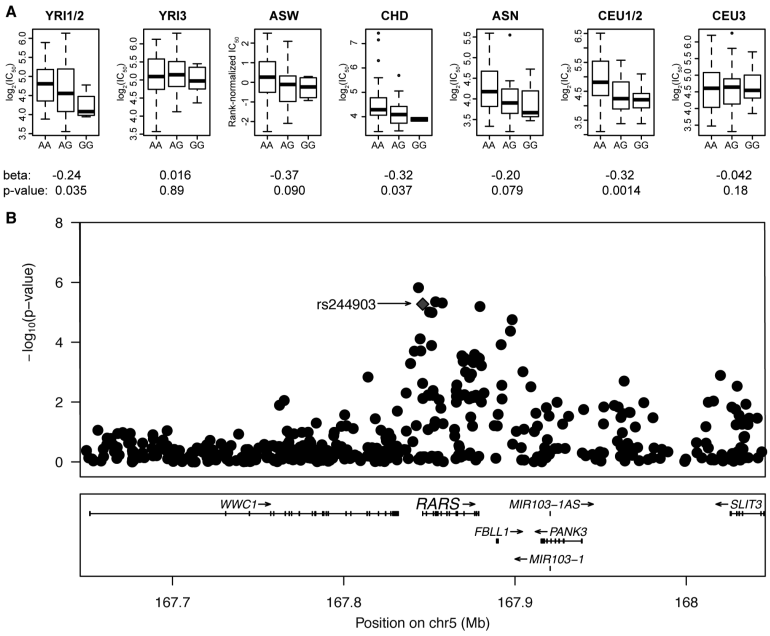

Figure 3. rs244903 is the most significant missense SNP ($p = 5.4 \times 10^{-6}$) in the metaanalysis

(A) Boxplots of carboplatin IC_{50} versus rs244903 genotype, beta terms, and p-values are shown for each population panel. Notice six of seven betas are in the same direction. (B) rs244903 (diamond) is located in the first exon of *RARS* (arginyl-tRNA synthetase). The DNA change A to G corresponds to the amino acid change isoleucine to valine. The SNP is an eQTL associated with the expression of five genes in YRI[43] ($p < 10^{-4}$). P-values are from the carboplatin IC_{50} meta-analysis.

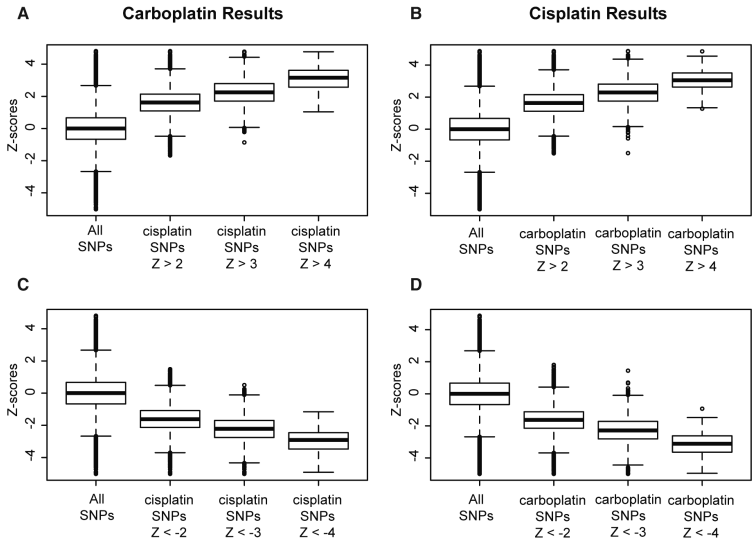

Figure 4. Meta-analysis Z-score distribution comparison between the two platinating-agents

The Z-scores for the top cisplatin IC_{50}-associated SNPs at various positive (A) and negative (C) Z-score thresholds were pulled from the overall list of ~3 million SNPs tested for association with carboplatin IC_{50}. The Z-scores for the top carboplatin IC_{50}-associated SNPs at various positive (B) and negative (D) Z-score thresholds were pulled from the overall list of ~3 million SNPs tested for association with cisplatin IC_{50}. The means of the "all SNPs" classes differed from the means of the other classes for both carboplatin and cisplatin ($p < 10^{-16}$, Student's t-test).

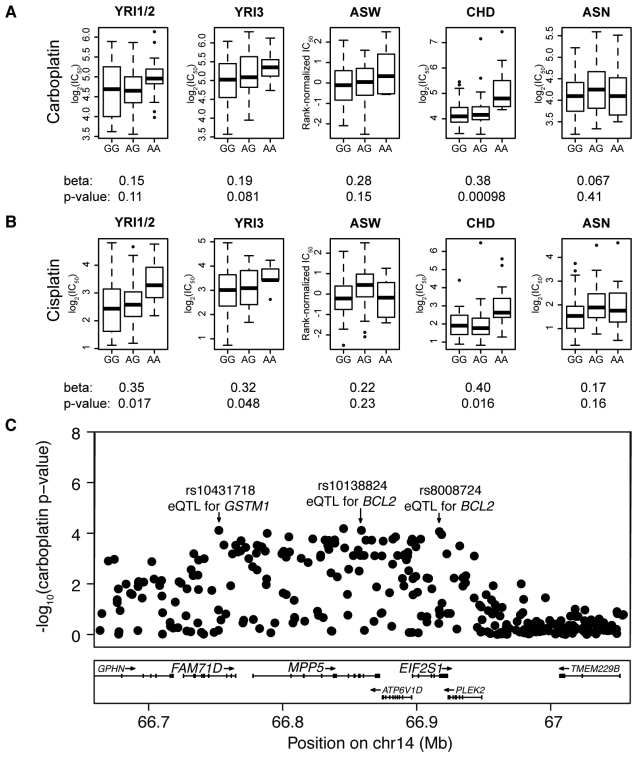

Figure 5. 200kb region on chr14 is associated with carboplatin IC_{50}, cisplatin IC_{50}, and the expression of two genes previously implicated in platinum response

Boxplots of (A) carboplatin (meta-p = 8.2×10^{-5}) or (B) cisplatin IC_{50} (meta-p = 3.6×10^{-5}) versus rs10138824 genotype, beta terms, and p-values are shown for each population panel. Only the five panels with MAF > 0.05 were included in the meta-analysis. Notice all betas are in the same direction. (C) rs10138824 is located in an intron of *MPP5* (membrane protein, palmitoylated 5) and is associated with the expression of *BCL2* (B-cell CLL/lymphoma 2) in YRI[43] ($p = 10^{-4}$). The two other indicated SNPs in the region are also associated with both carboplatin and cisplatin IC_{50} ($p < 10^{-4}$). rs10431718 is located in an intron of *FAM71D* (family with sequence similarity 71, member D) and is associated with the expression of *GSTM1* (glutathione S-transferase mu 1) in YRI[43] ($p = 10^{-4}$). rs8008724 is located in an intron of *EIF2S1* (eukaryotic translation initiation factor 2, subunit 1 alpha, 35kDa) and is associated with the expression of *BCL2* in YRI[43] ($p = 10^{-4}$). P-values are from the carboplatin IC_{50} meta-analysis.

Table 1

Characteristics and mean responses to carboplatin and cisplatin of the HapMap panels included in the meta-analyses.

	YRI1/2	YRI3	ASW	CHD	ASN	CEU1/2	CEU3
N	**90**	**86**	**83**	**85**	**90**	**90**	**84**
family structure	30 trios	27 trios, 1 duo, 3 singletons	10 trios, 20 duos, 13 singletons	unrelated	unrelated	30 trios	22 trios, 5 duos, 8 singletons
carboplatin IC_{50}[1] (μM)	30.0 (17.4)	36.8 (14.1)	20.1 (7.1)	25.1 (23.7)	19.7 (8.2)	24.5 (13.9)	28.2 (14.0)
cisplatin IC_{50}[1] (μM)	8.3 (6.5)	10.2 (6.1)	4.6 (4.1)	6.7 (11.5)	4.5 (3.7)	7.8 (8.5)	8.1 (5.6)

[1] mean (SD)