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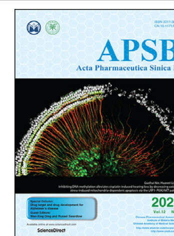

SHORT COMMUNICATION

Genome-wide analysis identify novel germline genetic variations in *ADCY1* influencing platinum-based chemotherapy response in non-small cell lung cancer

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KEY WORDS

Pharmacogenomics;
NSCLC;
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ADCY1;
Precious medicine;
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Abstract To explore the pharmacogenomic markers that affect the platinum-based chemotherapy response in non-small-cell lung carcinoma (NSCLC), we performed a two-cohort of genome-wide association studies (GWAS), including 34 for WES-based and 433 for microarray-based analyses, as well as two independent validation cohorts. After integrating the results of two studies, the genetic variations related to the platinum-based chemotherapy response were further determined by fine-mapping in 838 samples, and their potential functional impact were investigated by eQTL analysis and *in vitro* cell experiments. We found that a total of 68 variations were significant at $P < 1 \times 10^{-3}$ in cohort 1 discovery stage, of which 3 SNPs were verified in 262 independent samples. A total of 541 SNPs were significant at $P < 1 \times 10^{-4}$ in cohort 2 discovery stage, of which 8 SNPs were verified in 347 independent samples. Comparing the validated SNPs in two GWAS, *ADCY1* gene was verified in both independent studies. The results of fine-mapping showed that the G allele carriers of *ADCY1* rs2280496 and C allele carriers of rs189178649 were more likely to be resistant to platinum-based chemotherapy. In conclusion, our study found that rs2280496 and rs189178649 in *ADCY1* gene were associated the sensitivity of platinum-based chemotherapy in NSCLC patients.

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1. Introduction

Platinum drugs are one of the most widely used anti-cancer drugs worldwide[1]. Currently, platinum-based chemotherapy is the first-line drug regimen for various tumors such as lung, ovarian and testicular cancer[2]. However, resistance and adverse drug reactions are two major obstacles in successful platinum-based therapy. Pharmacogenomics (PGx) studies have shown that gene variations are one of the most critical factors affecting both drug response and adverse drug reactions[3]. Several genetic variants have been showed to be associated with cisplatin-induced ototoxicity. For example, TPMT was annotated in the clinical guideline of cisplatin prescription in pediatric cancer patients by the Canadian Pharmacogenomics Network for Drug Safety (CPNDS)[4−6].

However, the genetic underpinnings of platinum sensitivity remain poorly understood[7]. Previously, a large number of candidate gene association studies were used to screen for single-nucleotide polymorphisms (SNPs) related to platinum-based chemotherapy response[8,9]. However, there has been a lack of consistency in these results. Our meta-analysis found that these SNPs contributed little to sensitivity of platinum-based chemotherapy in non-small-cell lung carcinoma (NSCLC)[10]. Currently, several studies have employed the genome-wide association studies (GWAS) to screen genetic variations affecting the efficacy of platinum-based chemotherapy, but most of them took overall survival as the primary endpoint and no consistent results have been obtained[11−17].

Lung cancer is the type of cancer with the highest incidence rate worldwide, and NSCLC accounted for more than 80% of lung cancer cases. Unfortunately, most patients are diagnosed at an advanced stage due to the absence of symptoms in the early stage. Platinum-based chemotherapy is one of the important first-line chemotherapy regimens for these patients with improving overall survival and life quality[18]. The responses of patients, with similar clinical characteristics, to platinum-based drugs vary enormously[19]. Therefore, we conduct a GWAS on the sensitivity of platinum-based chemotherapy in NSCLC patients and aim to discover potential PGx biomarkers.

2. Methods

2.1. Study population and data collection

All patients in this study were recruited from Xiangya Hospital of Central South University and Hunan Cancer Hospital (Changsha, China), from 2012 to 2019. All patients were diagnosed with NSCLC by histopathological examination and confirmed the absence of driver genetic alterations that could be targeted. All patients received first-line platinum-based chemotherapy regimens for 2−6 cycles: cisplatin (75 mg/m^2) or carboplatin (AUC 5), which were both administered on Day 1 every 3 weeks, in combination with pemetrexed (500 mg/m^2) on Day 1 every 3 weeks, gemcitabine (1250 mg/m^2) on Days 1 and 8 every 3 weeks, paclitaxel (175 mg/m^2) on Day 1 every 3 weeks, docetaxel (75 mg/m^2) on Day 1 every 3 weeks, or navelbine (25 mg/m^2) on Days 1 and 8 every 3 weeks. Patients did not undergo surgery, targeted therapy, radiotherapy, or other anti-tumor therapy before chemotherapy. A physical examination as well as a detailed inquiry into each patient's medical history was carried out. Patients with serious concomitant diseases that might greatly affect their physical condition were excluded. The study protocol was approved by the Ethics Committee of Institute of Clinical Pharmacology, Central South University (Changsha, China), and all subjects were provided with written informed consents. We applied this study for clinical admission in the Chinese Clinical Trial Register (registration number: ChiCTR-RO-12002873, registered 21 December 2012, http://www.chictr.org.cn/showprojen.aspx?proj=6681).

Chemotherapy response of patients was assessed according to response evaluation criteria in solid tumors (RECIST) 1.1 version. Patients with a complete response (CR) or a partial response (PR) were defined as platinum responders or platinum sensitivity, and patients having stable disease (SD) or progressive disease (PD) were defined as platinum non-responders or platinum resistance. However, more stringent enrollment criteria were used for the discovery stage of cohort 1. For the platinum sensitivity group, the sum of maximum diameters of the target lesion needed to be reduced by ≥50% after platinum-based chemotherapy compared with that before chemotherapy, and no new lesions should be

found. For the platinum resistance group, the sum of maximum diameters of the target lesion did not decrease after platinum-based chemotherapy or was increased compared with that before chemotherapy, or new lesions were found.

As indicated in Supporting Information Fig. S1, this was a two-cohort, three-stage study. A total of 1230 patients were enrolled, and excluded patients without efficacy assessment ($n = 140$), failed DNA quality control ($n = 107$), failed library construction ($n = 23$) and failed genotyping quality control ($n = 21$).

2.2. Whole exome sequencing (WES) and quality control

Whole blood was collected from patients at the time of enrollment, and genomic DNA was extracted from peripheral blood by standard procedures using Wizard® Genomic DNA Purification Kit (Promega). Peripheral blood DNA samples were quality checked and quantified by Qubit (Invitrogen), and then paired-end sequenced (PE101) by HiSeq2000 (Illumina). Supporting Information Table S1 shows the quality measure of WES. Variants were included in further analysis only if they fulfilled all the following criteria: (1) variants were mapped on autosomal chromosomes; (2) variants were covered by at least 8 reads; (3) variants were covered by at least 4 variant-supporting reads and without strand bias; (4) the percentage of reads supporting the variant was at least 20%. A heterozygous variant was defined when the percentage of reads supporting the variant was 20%−80%; a homozygous variant was defined when the percentage of reads supporting the variant was at least 80%. A total of 155,829 variants passed the initial quality control. Then, variants were excluded if call rate <90%, or Minor Allele Frequency (MAF) <0.01, or Hardy Weinberg Equilibrium (HWE) test $P < 1 \times 10^{-5}$. Finally, 142,805 variants were included in association analysis.

2.3. Genotyping, quality control and whole genome imputation

For GWAS, genotyping was performed using the Illumina BeadChip Array Global Screening Array-24+ v1.0 (Illumina) for 688,783 SNPs. Genotype calls were determined using GenomeStudio software from Illumina. The systematic quality control was performed on the raw genotyping data to filter both unqualified samples and SNPs using PLINK (1.9 version). Samples were excluded if they failed genotyping in more than 10% of variants. SNPs were excluded if: (1) SNPs were not mapped on autosomal chromosomes; (2) SNPs had a call rate <90%; (3) SNPs had MAF<0.01; or (4) SNPs deviated significantly from HWE ($P < 1 \times 10^{-5}$). IMPUTE2 was used to perform phasing and imputation on the filtered unphased genotypes to impute untyped SNPs based on 1000 Genomes Phase 3 v4 reference[20]. The reference panel contains 84.4 million variants of 2504 individuals from 26 populations. Imputed genotypes with IMPUTE2 info lower than 0.4 were discarded for association analysis. Finally, 6,411,261 unique SNPs were included in association analysis for microarray genotyping.

In validation and fine-mapping stage, SNP genotyping was performed using the Sequenom MassARRAY system (Sequenom) according to the manufacturers' instructions. The exclusion criteria included: (1) samples that failed genotyping in more than 10% of variants; (2) SNPs had a call rate <90%.

2.4. Genome-wide association analysis

All patients from the discovery stage were used to perform the principal component analysis (PCA). The population substructure was determined by EIGENSTRAT (6.1.4 version)[21]. The first five principal components (PC) were incorporated in the association analysis as covariates. The genomic inflation factor of population stratification λ was calculated by PLINK (1.9 version)[22]. GWAS was conducted under the additive model by multivariate logistic regression analysis, adjusted by age, sex, smoking status, histology, stage, chemotherapeutic regimen and the first five principal components (PC1−PC5, Supporting Information Fig. S2) as covariates.

2.5. The linkage disequilibrium (LD) and haplotype block estimations

The linkage disequilibrium and haplotype block estimations analyses were performed using PLINK (1.9 version) and Haploview (4.2 version)[23], according to algorithm proposed by Gabriel et al.[24]. Regional plots were generated using the online tool LocusZoom (http://locuszoom.sph.umich.edu//)[25].

2.6. In vitro cell experiments

2.6.1. Materials

ADCY1 and β-actin antibodies were purchased from Sigma−Aldrich. Cisplatin was obtained from Meilunbio. Cell culture media and reagents were from Corning or Invitrogen. Scramble siRNA and *ADCY1* siRNA were purchased from Santa Cruz, and *SEPT7PP2* siRNA was from Bioegene.

2.6.2. Cell culture and siRNA/plasmid transfection

H1299, A549, A549/DDP, SK-MES1 and HBE were cultured in RPMI-1640 medium supplemented with 10% FBS. MRC-5 was cultured in MEM medium supplemented with 10% FBS. The cells were maintained at 37 °C in humidified 5% CO_2 atmosphere. For siRNA transfection, H1299 and A549 cells transfected with 80 nmol/L *ADCY1* or 100 nmol/L *SEPT7P2* or scrambled control siRNA using Lipofectamine RNAiMAX Invitrogen according to the instructions. For plasmid transfection, A549/DDP cells transfected with 1 μg of *ADCY1* overexpression pReceiver-M98 plasmid or vector control plasmid in 6-well plate by Lipofectamine LTX Invitrogen. The cells were then incubated for 24 h for survival or 48 h for gene expression detection.

2.6.3. Real-time PCR and Western blot

The total RNA was extracted by using Trizol (Invitrogen). 1 μg of total RNAs were reverse transcribed by PrimeScript Reverse Transcribe reagent kit with gDNA Eraser (Takara). Quantitative real-time PCR was carried out on the LightCyder 480II system (Roche Diagnostics) using SYBR Green PCR Master Mix (Takara). Relative mRNA expression level of *ADCY1* and *SEPT7P2* were determined by using Ct value and β-actin was used as internal control.

For Western blot, protein was extracted by RIPA buffer and concentration was measured by BCA protein assay kit (Beyotime). Samples were separated by 10% SDS-PAGE and transferred to a PVDF membrane (Millipore). The membranes were incubated with primary antibodies at 4 °C for overnight after blocking, and then incubated with secondary antibodies for 1 h at room temperature (RT). The bands were detected by ChemiDoc imaging system (Bio-Rad).

2.6.4. Survival assay

H1299 and A549 cells were transfected with *ADCY1* or *SEPT7P2* or Scramble siRNA in 6-well plates. A549/DDP was transfected

with *ADCY1* overexpression or vector control plasmid. Then, the cells were planted into 96-well plates (3000 cells/well) after cultured for 24 h. The cells were following treated with different concentration of cisplatin for 48 h. Culture medium was removed and CCK8 (Bimake) was added to each well, and incubated for 1−4 h at 37 °C. Absorbance was measured at 450 nm by using a microplate reader (Bio-Rad). GraphPad software was used to form the dose-response curves and calculate IC_{50} values.

2.7. Statistical analysis

Pearson's chi-square test or Fisher's exact test was used to assess the relationship between clinical characteristics and response of platinum-based chemotherapy. The influences of genetic variants on the platinum sensitivity were examined using multivariable logistic regression by calculating odds ratios (ORs) and their 95% confidence intervals (CIs). The P values were two-sided, and $P < 0.05$ was considered statistically significant. All these analyses were performed by PLINK (1.09 version), SPSS (20.0 version), and R.

3. Results and discussion

3.1. Association analysis of cohort 1

Thirty-four patients with obvious phenotypes from Xiangya Hospital of Central South University were recruited into the discovery stage of cohort 1, including 17 patients with significant sensitivity and 17 patients with significant resistance. The validation stage of cohort 1 included 262 patients recruited from Xiangya Hospital of Central South University and Hunan Cancer Hospital. Of these, 82 (31.30%) were sensitive to platinum-based chemotherapy, and 180 (68.70%) were resistant to platinum-based chemotherapy. Supporting Information Table S2 summarizes the demographic characteristics of cohort 1 discovery and validation stages.

Peripheral blood DNA samples from 34 patients in the discovery stage of cohort 1 were used for WES. After quality control, 142,805 variations were included for association analysis. The regional plots (Fig. 1A) and Manhattan plot (Supporting Information Fig. S3A) show the results. A total of 68 variations were significant at $P < 0.001$. After pruning SNPs for strong LD ($r^2 > 0.8$), 45 variations were selected for MassArray genotyping in the 262 validation stage samples. Association analysis was conducted under the additive model by multivariate logistic regression analysis, adjusted by histology as a covariate. Three SNPs with $P < 0.05$ were identified (Supporting Information Table S3): rs9937572 in *CES5A* gene ($P = 0.030$), rs3792594 in *PDCD6IP* gene ($P = 0.031$) and rs2293106 in *ADCY1* (adenylate cyclase 1) gene ($P = 0.046$). When combined data from the discovery and validation stages, statistically significant differences still existed for these three SNPs. Supporting Information Table S4 shows the results of the stratified analysis by histological type.

3.2. Association analysis of cohort 2

The discovery stage of cohort 2 included 433 patients recruited from Xiangya Hospital of Central South University, of which 206 (47.58%) were sensitive and 227 (52.42%) were resistant to platinum-based chemotherapy. The validation stage of cohort 2 included 347 patients recruited from Xiangya Hospital, of which 145 were sensitive and 202 were resistant to platinum-based chemotherapy. Supporting Information Table S5 summarizes the clinical characteristics of patients in cohort 2 discovery and validation stages.

The whole-genome microarray analysis was performed on peripheral blood DNA samples from 433 patients in the discovery stage of cohort 2. After quality control and genotype imputation, a total of 6,411,261 SNPs passed quality screening and were included in the association analysis. A quantile-quantile plot (Supporting Information Fig. S4) was created to evaluate the data quality. Deviation from the expected P-value distribution was evident only in the tail region. For the results of the association analysis, the genomic inflation factor λ was 1.021. It shows that the patient population was ethnically homogeneous, and the observed associations were not driven by potential population substructure. The regional plots (Fig. 1B) and Manhattan plot (Fig. S3B) shows the genome-wide association analysis results. A total of 4960 SNPs were significant with $P < 1 \times 10^{-3}$ and 541 SNPs were significant with $P < 1 \times 10^{-4}$, of which, 31 SNPs were significant with $P < 1 \times 10^{-5}$.

After LD pruning among the SNPs with $P < 1 \times 10^{-4}$ at the discovery stage, 88 SNPs were selected for MassArray genotyping in 347 validation stage samples. Association analysis was conducted under the additive model by multivariate logistic regression analysis, adjusted by histology as a covariate. 8 SNPs were found with $P < 0.05$ (Supporting Information Table S6): rs11157691 ($P = 0.003$), rs189178649 ($P = 0.006$), rs7142582 ($P = 0.015$), rs16922098 ($P = 0.017$), rs1470518 ($P = 0.023$), rs7820895 ($P = 0.024$), rs6069858 ($P = 0.039$) and rs2740168 ($P = 0.043$). After combination of the discovery and validation stages, 5 of the SNPs still showed statistically significant differences. The results of stratified analysis by histological types are listed in Supporting Information Table S7. It is interesting to note that EPHX1 polymorphisms were reported to be associated with cisplatin induced nephrotoxicity in ovarian cancer[26]. Moreover, cisplatin treatment would increase mRNA level of *EPHX1* for 15-fold[27].

3.3. Fine-mapping

Above results show that only *ADCY1* gene variations are significantly correlated with platinum-based chemotherapy efficacy in both cohorts (Table 1). Therefore, we conducted LD and haplotype block estimation for the genomic regions of *ADCY1* rs2293106 and rs189178649. Data from the cohort 2 discovery stage was used to calculate the haplotype blocks of the two target SNPs (Fig. 2). The haplotype block containing rs189178649 has a range of 36.4 kb, which is located in the *ADCY1* gene. The haplotype block containing rs2293106 has a range of 134.6 kb, which is located across the *ADCY1* and *SEPT7P2* (septin 7 pseudogene 2) genes.

To further confirm the functional variations in these two blocks, which might be associated with platinum sensitivity, we conducted a fine-mapping study in a larger cohort. First, we examined whether there were any missense variations in strong LD with the target SNPs in 2 Mb regions before and after the two blocks (chr7 43.5 Mb−48.0 Mb). No SNPs were selected. Following that, we screened potential functional variations in these two blocks. There were two criteria: variations needed to be located in the coding region, 3′UTR or a non-coding transcript, and the MAF≥0.01 in East Asian

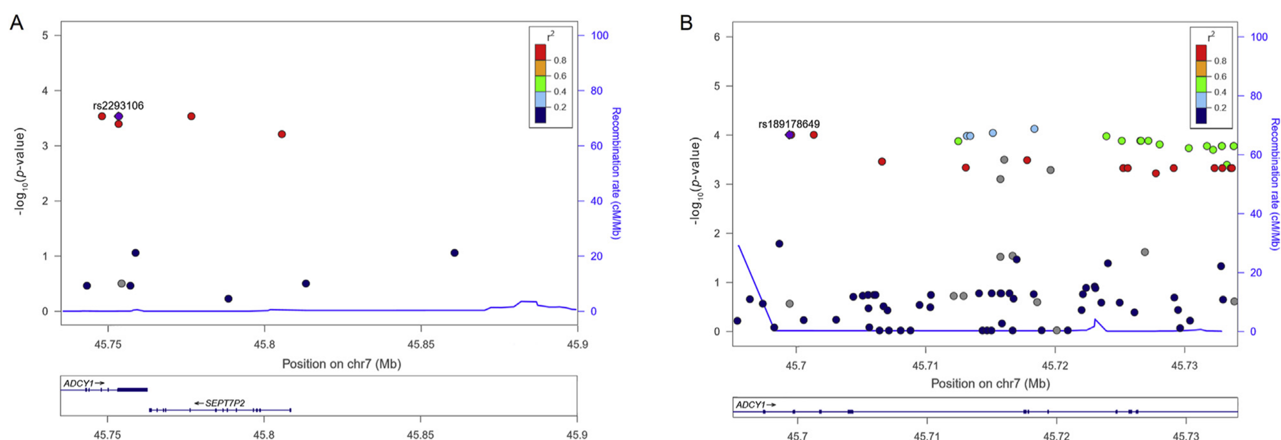

Figure 1 Association results of *ADCY1* gene in the discovery stage. (A) and (B) The regional plot for rs2293106 and rs189178649. The regional plots were constructed using LocusZoom (http://locuszoom.sph.umich.edu//). P values [$-\log_{10}$(P values); y axis] in cohort 1 (A) and cohort 2 (B) discovery stage were plotted against the respective chromosomal position of each SNP (x axis). Colors indicate LD (r^2) with rs2293106 and rs189178649 in 1000 Genomes Project East Asian populations.

Table 1 The SNPs associated with platinum-based chemotherapy response in ADCY1 gene.

Cohort	SNP	Gene	Allele	MAF	Frequency	Discovery stage		Validation stage		Combine stage	
						P value	OR (95%CI)	P value	OR (95%CI)	P value	OR (95%CI)
Cohort 1	rs2293106	ADCY1	G/A	0.121	0.128	2.92E-04	NA[a]	0.046	2.11 (1.01−4.40)	1.91E-03	3.08 (1.51−6.26)
Cohort 2	rs189178649	ADCY1	T/C	0.121	0.129	9.87E-05	2.70 (1.63−4.46)	0.006	1.91 (1.20−3.03)	1.30E-06	2.29 (1.64−3.20)

[a]All mutant allele carriers of rs2293106 in ADCY1 were non-responders, the specific OR value could not be calculated.

population. Thus, 36 variations were enrolled, which were combined with the target SNP rs189178649 and rs2293106 as 38 variations. They were used as candidate variations for fine-mapping. After LD pruning, 16 SNPs were finally used for MassArray genotyping.

The cohort sample used for fine-mapping included 838 patients from Xiangya Hospital of Central South University and Hunan Cancer Hospital; of these, 380 (45.35%) patients were sensitive, and 458 (54.65%) patients were resistant to platinum-based chemotherapy. Supporting Information Table S8 shows the

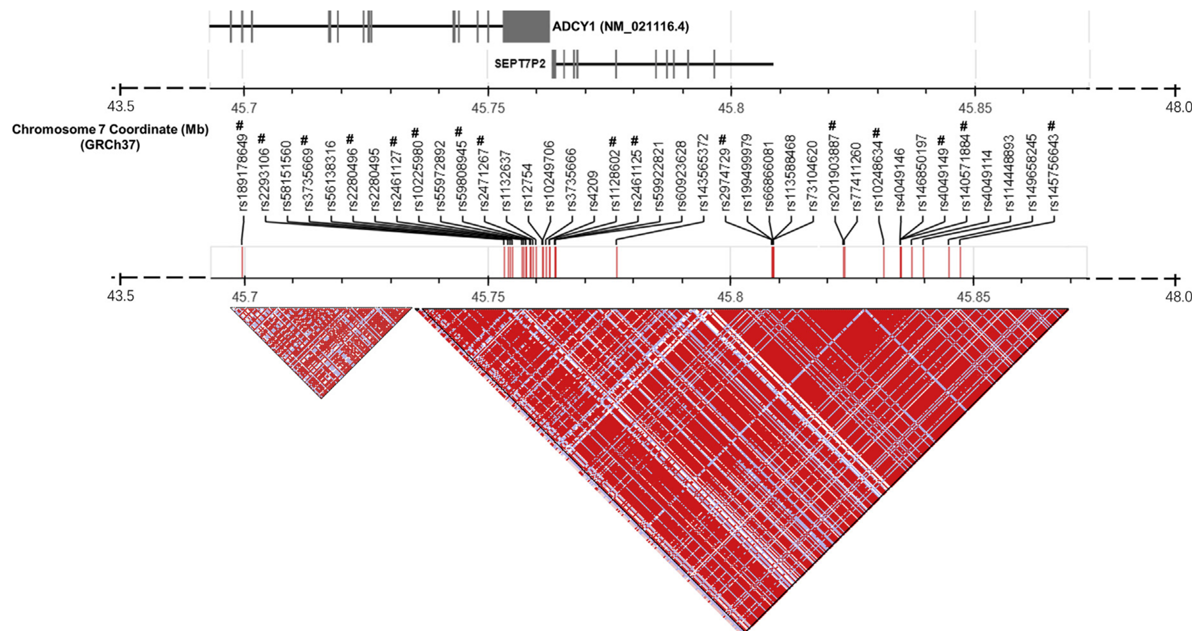

Figure 2 The haplotype block and research area of fine-mapping. The genomic positions of all candidate variations are shown, and the SNPs genotyped by MassArray are labeled with #.

clinical and pathological characteristics of this cohort. Genotyping of the 16 candidate SNPs was conducted in this cohort, and association analysis was conducted under the additive model by multivariate logistic regression analysis, adjusted by age, sex, smoking status, histology, stage, chemotherapeutic regiment as covariates. Table 2 shows the results: rs189178649 and its strongly linked rs2280496 finally show significant differences between the responder and non-responder groups.

3.4. *ADCY1 variants associated with platinum-based chemotherapy sensitivity*

Next, we conducted a further stratified analysis of the histology types for rs189178649 and rs2280496. The patient distribution of rs2280496 and rs189178649 genotypes is displayed in Fig. 3. The mutant allele carrier had a higher proportion of non-responders compared with wild-type carriers in both adenocarcinoma and squamous cell carcinoma. In addition, we employed the dominant and recessive models for association analysis (Table 2). In the dominant and additive model, rs189178649 and rs2280496 showed a significant correlation with the sensitivity to platinum-based chemotherapy in the total samples and the subgroups of adenocarcinoma and squamous cell carcinoma. In the recessive models, rs189178649 and rs2280496 only showed a significant correlation with the sensitivity to platinum-based chemotherapy in the total samples. In addition, they showed the same trends in the adenocarcinoma and squamous cell carcinoma subgroups that did not reach statistical significance (Fig. 3). Thus, *ADCY1* rs189178649 and rs2280496 were associated with platinum-based chemotherapy sensitivity.

Currently, no effective predictive biomarkers have been found for platinum response. Some genetic variants were widely studied in candidate gene association investigations, including *ERCC1* rs11615, *XPD* rs1799793, rs13181, *GSTP1* rs1695, etc. However, their results were inconsistent[10]. These variants also didn't reach statistically significant in our current study. These results together suggest that there were other PGx biomarkers remained to be discovered. Previously, most studies investigated the relationship between genetic variations and overall survival at genome-wide scale[12,14,17]. Response is also a very important endpoint for platinum drugs, however, it was less investigated. As overall survival is confounded by other factors, such as other underlying diseases, tumor heterogeneity, and other treatment modalities, the RECIST response evaluation method employed in this study could standardize drug response evaluation and provide more sensitive result phenotypes. Two investigations conducted GWAS for platinum drug response in small cell lung cancer and cervical cancer respectively, but the sample size was relatively small[11,16]. In this study, we largely expanded the sample size in NSCLC patients and discovered that genetic variations in *ADCY1* influence platinum-based chemotherapy response, which is unknown previously.

It should be noteworthy that the MAF of rs189178649 showed obvious differences in allele frequencies among different ethnic groups, it was a common mutation only in East Asian population (Fig. 4). In addition, rs189178649 and rs2280496 were in strong LD only in East Asian population. We thus concluded that G allele carriers of rs2280496 and C allele carriers of rs189178649 tended to develop resistance to platinum-based chemotherapy. A lot of previous studies indicated that there were ethnic differences for PGx biomarkers in many drugs[10,28,29]. Our results provide new evidence for ethnic differences in platinum drugs. rs189178649 was a common mutation only in East Asian population. In addition, rs189178649 and rs2280496 were in strong LD only in East Asian population. As this study was carried out in the Chinese patients, it is rational to discover the potential PGx biomarker only existing in East Asian populations. Thus, SNPs identified in this study might only play a role in patients with similar genetic background to Chinese, such as East Asian populations. The subsequent validation should also be carried out in these populations. In addition, it was possible that there were other biomarkers remained to be discovered in other populations with different races and ethnicities.

3.5. *Possible mechanisms of ADCY1 variants in platinum response*

As indicated in Fig. 2, both rs2280496 and rs189178649 were located in *ADCY1* and *SEPT7P2* gene region. We proposed that they might affect ADCY1 and SEPT7P2 expression. eQTL analysis was conducted in Genotype-Tissue Expression (GTEx) database. rs189178649, rs2280496 and other 56 variations in strong LD with them were analyzed in 47 tissue types (Supporting Information Fig. S5). As indicated, these variations were significantly correlated with the expression of SEPT7P2 and ADCY1 in some tissues. Therefore, we speculated that these SNPs might exert their effects by changing the expression levels of ADCY1 and SEPT7P2. We then predicted miRNA binding sites in genomic region of rs2280496 and its strongly linked SNPs by

Table 2 The SNPs associated with platinum-based chemotherapy response in the fine-mapping cohort.

CHR	SNP	Gene	Allele	MAF	Frequency	Pathology	Additive		Dominant		Recessive	
							P value	OR (95%CI)	*P* value	OR (95%CI)	*P* value	OR (95%CI)
7	rs2280496	ADCY1	C/G	0.116	0.134	All	0.013	2.22 (1.18−4.17)	9.22E-05	1.94 (1.39−2.71)	0.023	4.32 (1.23−15.20)
						Adenocarcinoma	0.013	1.74 (1.12−2.71)	0.023	1.75 (1.08−2.82)	0.130	5.01 (0.62−40.46)
						Squamous	0.004	1.88 (1.22−2.91)	0.005	1.98 (1.23−3.19)	0.202	2.93 (0.56−15.31)
7	rs189178649	ADCY1	T/C	0.121	0.144	All	0.012	2.04 (1.17−3.55)	8.53E-05	1.92 (1.39−2.66)	0.022	3.64 (1.20−11.01)
						Adenocarcinoma	0.015	1.68 (1.10−2.56)	0.018	1.75 (1.10−2.79)	0.255	2.48 (0.52−11.83)
						Squamous	0.005	1.83 (1.20−2.78)	0.010	1.86 (1.16−2.98)	0.077	4.18 (0.86−20.38)

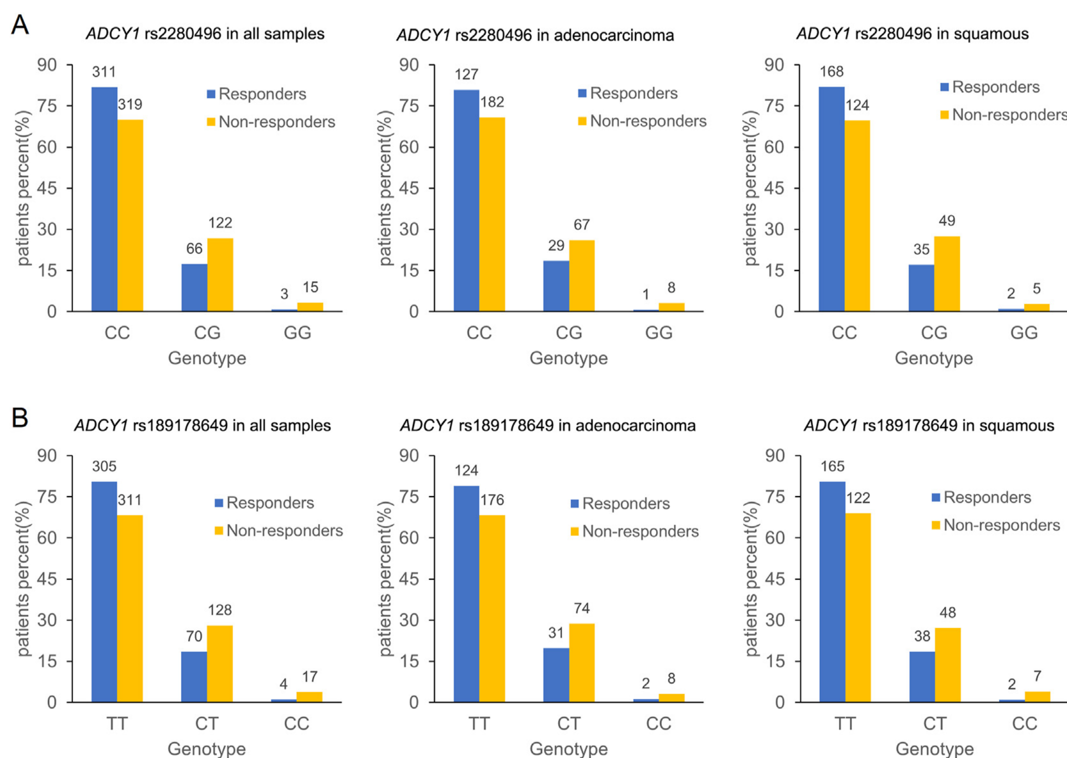

Figure 3 Patients distribution of rs2280496 (A) and rs189178649 (B) genotypes according to the sensitivity of platinum-based chemotherapy.

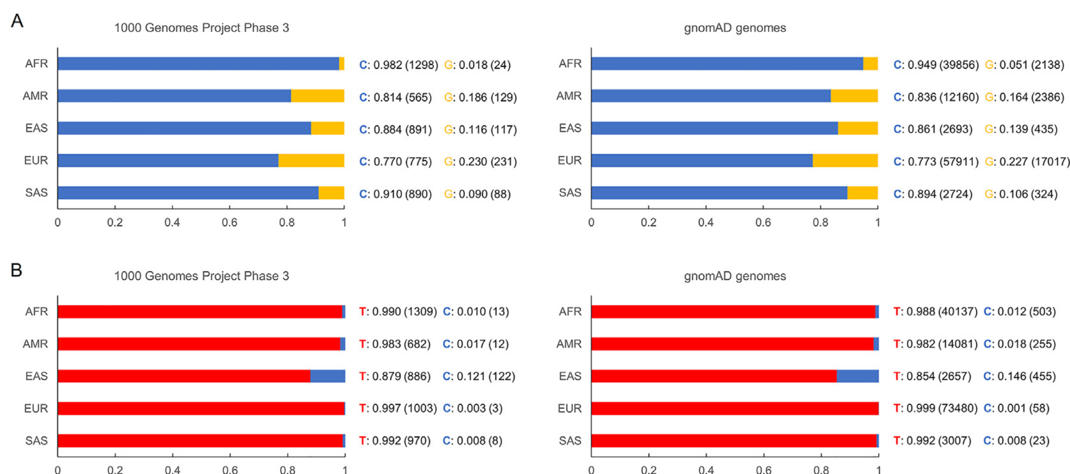

Figure 4 The MAF of rs2280496 (A) and rs189178649 (B) in different ethnic populations.

miRWalk, TargetScan and miRDB. The results show that 2 of these variations (rs2280495 and rs1132637) were predicted to be miRNA binding site SNPs, and 21 miRNAs could target regions of them (Supporting Information Table S9). It suggested that rs2280495 and rs1132637 might influence ADCY1 expression by affecting the miRNA binding activity.

Next, we explored the role of ADCY1 and SEPT7P2 expression in regulating cell response to cisplatin. First, ADCY1 expression in several NSCLC and HBE cells lines were detected. As indicated in Supporting Information Fig. S6A, ADCY1 was overexpressed in lung cancer cell lines, and its expression level was higher in H1299 and A549 lung adenocarcinoma cell lines than in SK-MES1 lung squamous cell carcinoma. Moreover, low expression of ADCY1 in cisplatin resistance A549 (A549/DDP) cell line when compared with A549 cell lines (Fig. S6B). To examine the effects of ADCY1 on cisplatin sensitivity in NSCLC, H1299 and A549 cells were transfected with *ADCY1* siRNA. ADCY1 were significantly downregulated, and sensitivity to cisplatin was decreased in ADCY1 knockdown cells (Fig. S6C and S6D). After the transfection of *ADCY1* overexpression plasmid in A549/DDP cells, ADCY1 was overexpressed, and cisplatin sensitivity was increased by 1.31-fold change compared to cells transfected with vector control plasmid ($P = 0.020$) (Fig. S6E). We also examined SEPT7P2 expression in several

NSCLC cell lines and MRC-5 cells. SEPT7P2 was overexpressed in lung cancer cell lines, and the expression level was higher in H1299 and A549 lung adenocarcinoma cell lines than in SK-MES1 lung squamous cell carcinoma (Fig. S6F). Moreover, low expression of SEPT7P2 in cisplatin resistance A549 (A549/DDP) cell line when compared with A549 cell line (Fig. S6G). In order to investigate the effects of SEPT7P2 on cisplatin sensitivity, SEPT7P2 was significantly downregulated by the transfection of *SEPT7P2* siRNA in H1299 and A549 cell lines. However, there was no significant change observed on sensitivity to cisplatin in the cell lines (Fig. S6H and S6I). Our results show that SEPT7P2 had no effect on cisplatin response.

Based on our previous summarization, ADCY1 is a main regulator of cAMP signaling pathway by catalyzing ATP to cAMP. As a second messenger, cAMP participates in many important cellular activities, including DNA damage, proliferation, and apoptosis, which were contributed to drug resistance[30]. It should be noteworthy that although ADCY1 expression level reduction was associated with increased cisplatin resistance, its role was minimal. Therefore, the specific molecular mechanism of rs189178649 and rs2280496 affecting platinum sensitivity has not been fully elucidated. It is possible that ADCY1 is unlikely the main cause, but other variations in the same haplotype are mainly responsible for platinum response. It was also not clear to what extent ADCY1 impacted platinum sensitivity. More *in vitro* and *in vivo* studies are needed to be conducted in further investigations.

Taken these results together, we thought this study had several limitations. Firstly, more solid invalidations of *in vitro* studies were needed to investigate the effects of polymorphisms in ADCY1 expression and platinum sensitivity, including knock-in the two SNPs in H1299 and A549 cell lines to provide more direct evidence of these two SNPs in mediating the sensitivity of platinum-based treatment. Secondly, *in vivo* animal models are needed to demonstrate the role of the SNPs in NSCLC.

4. Conclusions

To the best of our knowledge, this is the largest sample size GWAS for platinum drug response. We discovered novel germline genetic variations in ADCY1 that affect the sensitivity of platinum-based chemotherapy on NSCLC patients. G allele carriers of rs2280496 and C allele carriers of rs189178649 were more resistant to platinum-based chemotherapy.

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Author contributions

Chenxue Mao, Juan Chen, Jiye Yin and Zhaoqian Liu designed and guided the experiments. Ting Zou, Yuankang Zhou· Junyan Liu, Xiangping Li, Min Li, Pinhua Pan, Wei Zhuo, Yang Gao, Shuo Hu, Desheng Xiao, Lin Wu and Zhan Wang collected the samples, Chenxue Mao, Xi Li, Jiye Yin, Juan Chen performed the GWAS and WES data analyses, Juan Chen and Ting Zou conducted the *in vitro* experiments. The manuscript was written by Chenxue Mao and Juan Chen, and revised by Heng Xu, Wen Yang, Yingjie Xu, Haihua Xiao, Kazuhiko Hanada, Wei Zhang, Honghao Zhou, Jiye Yin and Zhaoqian Liu.

Conflicts of interest

The authors declare no conflicts of interest.

Appendix A. Supporting information

Supporting data to this article can be found online at https://doi.org/10.1016/j.apsb.2021.10.007.

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