Whole-exome sequencing identifies the novel mutations in the ABC transporters' genes are associated with intrahepatic cholestasis of pregnancy disease: a case-control study

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Abstract

Objectives To identify the novel pathogenic genetic variants associated with intrahepatic cholestasis of pregnancy (ICP) disease by whole-exome sequencing (WES) approach. Design WES the DNA, and conduct association between genetic variants and total bile acids. Setting Jiangxi. Samples 151 ICP patients. Methods DNA samples from 151 ICP patients were subjected to WES. Rare novel exonic variants (minor allele frequencies: MAF $< 1\%$) were performed for subsequent analysis. Main outcome measures Association of genetic variants with ICP and other clinical disorders. Results We detected 42 were novel. We classified these loci as four panels according to the prediction results, of which, 7 novel possible pathogenic mutations were identified which located in the known functional genes including ABCB4, ABCB11 and ABCC2 for first reported in damaging group. Besides, compare to reference, ABCC2 Ser1342Tyr modified protein structure showed a slight change in the chemical bond lengths of ATP-ligand binding amino acid side chains. And in placental tissue, the expression level of ABCC2 gene in ICP patients was significantly higher than healthy pregnant women. Moreover, the patients with two mutations in ABC family genes have higher average value of TBA, AST, DBIL, CHOL, TG and HDL compared to the patients which have one mutation, no mutation in ICP and local controls. Conclusion Our results provide new insights into the genetic architecture of ICP disease. They may contribute to genetic diagnose of ICP disease, and provide new treatment for ICP patients. Keywords WES, TBA, ABC transporters' genes, novel variants, ICP, ABCC2 gene, Ser1342Tyr mutation

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Running title: ABC genes novel mutations in 151 ICP patients

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Conclusion Our results provide new insights into the genetic architecture of ICP disease. They may contribute to genetic diagnose of ICP disease, and provide new treatment for ICP patients.

KeywordsWES, TBA, ABC transporters' genes, novel variants, ICP, *ABCC2* gene, Ser1342Tyr mutation; gene expression

Tweetable abstract Novel mutations in the ABC transporters' genes are associated with intrahepatic cholestasis of pregnancy disease.

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Introduction

Intrahepatic cholestasis of pregnancy (ICP) is a reversible pregnancy-specific liver disease, which characterized by pruritus and abnormal liver function, such as elevated liver enzymes and increased the serum total

bile acids (TBA) ([?] 10 μmol/L), appeared in the second and third trimester of the pregnancy and resolved completely after delivery in the early postpartum period.[1] The incidence of ICP disease reported between 0.1% and 15.6% depending on geographical differences.[1, 2] ICP has associated with the adverse fetal outcomes, including spontaneous preterm birth, respiratory distress, low Apgar scores, fetal distress and fetal death.[3-7] It is noted that approximately 2%-4% of ICP pregnancies are affected by fetal mortality.[8, 9] And the level of TBA increased the risk of fetal morbidity and stillbirth.[10-12]Therefore, untangling the genetic mechanisms of ICP and is associated with the fetal complications is very important.

The abnormal synthesis, metabolism transport, secretion and excretion of bile acids may lead to ICP disease.[13] Therefore, the etiology contributing to the development of ICP disease is complex that depend on multiple factors, including hormonal, genetic and environmental backgrounds.[14]Familial clustering analysis in pedigree studies, showed a high incidence in mothers and sisters of patients with ICP, implicating that a genetic predisposition for the condition.[15-18] Among which, the gene mutations in the hepatocellular transporters of bile salts playing a pivotal role in the pathogenesis of ICP.[19]

Bile salts transport is the key physiological function of ATP-binding cassette (ABC) membrane proteins covered seven distinct members: ABCA, ABCB, ABCC, ABCD, ABCE, ABCF and ABCG. Of these genes, *ABCB4* ,*ABCB11* and *ABCC2* are functional known genes having an effect on the development of ICP. Except for *ABCB4* , *ABCB11*and *ABCC2* genes, the role of other ABC transporters' genes seems to less study. By taking advantage of the high-throughput genotyping technologies in a larger scale population, WES approach that combined genotype data for all patients has proved to be far more efficient to anchor all mutations for the target gene at once. In particular, the method can greatly accelerate to screen for new potential pathogenicity sites of all the mutations. Therefore, examining exonic variants across ICP disease groups likely augments the excavation of novel loci. However, until now, to our best knowledge, there have no reports of use of WES to identify the genetic variants in the ABC series genes of bile acids transporter for ICP disease.

Given this background, we hypothesized that the genetic variation in ABC transporters confers susceptibility to ICP. Therefore, we performed WES to investigate extensively the presence of mutations, especially for the discovery of new functional variants, of ABC series genes involved in bile acids transport in 151 patients with ICP disease and related them to the clinical data and pregnancy outcomes.

Methods

Patients and clinical data

151 pregnancy women were excluded other liver disease, diagnosed as ICP on the basis of skin pruritus in combination with abnormal liver biochemistry indexes, e.g. TBA, ALT and AST, etc. In addition, 26 clinical features covering six main indicators of maternal and neonatal data, including patient basic features (age, gestational age and body mass index: BMI), ion concentration (K, Na, Cl, Ca, Mg, P), routine blood test (the counts of white blood cell: WBC, red blood cell: RBC, platelet: PLT, red blood cell distribution width.SD: RDW.SD), liver function index (TBA, ALT, AST, total bilirubin: TBIL, direct bilirubin: DBIL, indirect bilirubin: IDBIL), lipid index (CHOL, TG, high-density lipoprotein: HDL, low-density lipoprotein: LDL, uric acid) and outcomes of pregnant women and newborns (birth weight and bleeding amount), were recorded. Besides, we also recruited 1029 pregnancy women without ICP as negative controls.

Whole exome sequencing

151 genomic DNA was exacted from peripheral blood by Axy Prep Blood Genomic DNA Mini prep Kit (Item No. 05119KC3). To fulfill the requirements for genotyping, the concentration and integrality of the all the exacted DNA samples were determined by Nanodrop-1000 spectrophotometer (Thermo Fisher, USA) and gel electrophoresis, respectively. A total of 151 ICP patients samples were subjected to carry out exome capture with BGI Exon Kit according to manufacturer's protocols. DNA library were constructed using combinatorial Probe Anchor Ligation (cPAL) method. Each resulting qualified captured library was then loaded on BGISEQ-500 sequencing platforms.

Variant annotations, filtering and prioritizing

The bioinformatics analysis began with the sequencing data. First, joint contamination and low quality variants (read depth < 15 and genotype quality score < 20) were removed. The reads were mapped to the reference human genome (UCSC GRCh37/hg19) using BWA (Burrows-Wheeler Aligner) software. After that, variants calls were conducted by the GATK (v3.7). Finally, ANNOVAR tool was applied to perform a series of annotations, such as the frequency in the databases, conservative prediction and pathogenicity prediction with available website tools for variants.

The work flow of quality control were carried out as shown in Figure 1. First, we remove variants with a MAF more than 0.01 in the 1000 Genomes Project (http://www.internationalgenome.org/), Exome Aggregation Consortium (ExAC) (http://exac.broadinstitute.org/), dbSNP (https://www.ncbi.nlm.nih.gov/snp/) in databases. Second, missense, damaging or loss of function variants of ABC genes were embraced for the subsequent analyses. Third, we concentrated on novel variants which were filtered by NCBI and Ensembl. In addition, we prioritize to pay attention to the novel variants that would likely have functional effect. For instance, a variant will be highlighted when it predicted to be simultaneously deleterious by SIFT, Mutation Taster and FATHMM software.

Statistical analysis

We applied to *sapply* function to make descriptive statistics for clinical data. The Pearson correlations coefficients estimation between clinical data were evaluated by using *cor* function. Using*cor.test* function examined the significance of correlation coefficients. *TukeyHSD* function was conducted to analyze the differences in average value of clinical data among groups.*Fisher* test is used to test the significance of frequency. Moreover, *pie* function was used to draw the percentage of all ABC mutations. All the analysis were preformed using R software. In addition, we used three web-available tools SIFT, Mutation Taster and FATHMM to predict protein damage. Predictions were defined as damaging, probable damaging, possible damaging, neutral when three in all prediction tools, two out of three, one out of three and none reached damaging, respectively.

Verification of novel candidate sites by Sanger sequencing

Totally thirteen pairs of primers were designed for random sequencing of some sites in four groups. The details of PCR primers and its optimum annealing temperatures were showed in Table S1.

Evolutionary conservation analysis

The evolutionary conservation analysis of the amino acids encoded by the new pathogenicity sites in ABC genes were performed among vertebrates, including Gibbon, Macaque, Olive baboon, Gelada, Marmoset, Mouse, Rat, Cow, Sheep, Pig, Chicken, Zebra finch, Zebrafish, etc, using the genomic alignments of the Ensembl Genome Browser.

Protein structure modeling

Protein structure modelling involves in two steps. First, the reference and the modified (*ABCC2* Ser1342Tyr) protein structure were submitted to SWISS-MODEL (http://swissmodel.expasy.org/) software for structure modeling. Then, these two protein models were compared simultaneously using Chimera 1.14rc package.

Results

Whole-exome sequencing result of the variants of ABC transporters' genes in 151 ICP samples

Totally ABC transporters covering 44 genes went through targeted WES to identify variants in a cohort of 151 patients with ICP disease. Overall, we identified 2953 genetic variants, including 1254 mutations in 12 ABCA genes which consisted of *ABCA1 -ABCA10* ,*ABCA12 -ABCA13* , 479 variants in *ABCB1* ,*ABCB4 -ABCB11* , 812 genetic variants in eleven genes covering *ABCC1 -ABCC6* , *ABCC8 -ABCC12* , 408 variants in ABCD-ABCG series genes. These types of variants covered 2057 intron, 297 synonymous, 76 splice, 13 stop gained /start lost, 36 3' primer UTR, 21 5' primer UTR, 10 downstream gene, 32 upstream gene, 3

structural interaction and 408 missense. The percentage of these types of variants is shown in Figure S1A (See Supplementary material). After quality control (MAF < 0.01 in all databases), 42 out of 320 variants were detected which was first reported, including 17 out of 146 variants in ABCA genes, 10 out of 59 in ABCB, 9 out of 71 in ABCC and 6 out of 44 in ABCD-ABCG genes (See Supplementary material, Figure S1B).

For these 42 novel mutations, we classified these mutations as four echelons (damaging, probable damaging, possible damaging and neutral) according to prediction results (Table 1). Damaging group have fifteen mutation, which contained 7 mutations in three known functional genes associated with ICP disease, such as *ABCB4* Trp708Ter, Gly527Glu and Lys386Glu, *ABCB11* Gln1194Ter, Gln605Pro and Leu589Met, and*ABCC2* Ser1342Tyr (Data has shown in our previous). Besides, 8 functional mutations in other ABC genes, i.e. *ABCA4* Phe754Ser,*ABCA12* Cys2440Tyr, *ABCA13* Ser3286Ter, *ABCB1*Pro693Ser, *ABCB5* Ser925Ile, *ABCC3* Ile1147Thr, *ABCC9*Ala456Thr and *ABCG2* Leu646Met. The frequency of these mutations in 151 ICP samples reached 10.60% (16/151). Moreover, they are absent in 1029 local healthy women. Therefore, significantly difference in frequency between cases and control groups (P = 1.10E-14).

Twenty-one mutations were assigned to the second tier (probable damaging), including 9 in ABCA genes (*ABCA2* Asp1108Glu and Ala583Val, *ABCA5* Val997Ala, *ABCA7* Pro449His, *ABCA8*Val8Ala, *ABCA10* Leu260Ser, *ABCA12* Ile1022Thr,*ABCA13* Leu4624Val and Thr4912Ala), Glu386Lys and Ile339Val in all in ABCB9 gene, 6 in ABCC series genes (*ABCC1* Ser497Gly,*ABCC3* Leu137Phe, *ABCC5* Gln851Pro, *ABCC6* His1043Gln,*ABCC9* Glu1034Val and *ABCC12* Pro37Ser), *ABCD4*Ser395Phe and Thr385Ala, *ABCF2* Gly47Ser and *ABCG1*Ile273Val in ABCD-ABCG series genes. These mutations were also absent in controls. The MAF in these mutations between cases and controls showed a significant difference (P = 2.20E-16).

We identified five possible potential pathogenic mutations associated with ICP disease, including *ABCA3* Ser593Gly, *ABCA10*Leu823Val, *ABCA12* Asn2492Ser, *ABCA13* Ser3111Asn and*ABCG1* Thr378Ile. Only one mutation was divided into neutral group. 42 mutations in all were absent in the databases, including 1000 Genome Project, ExAC, dbSNP, ChinaMAP and 1029 local controls.

Confirmation of the novel variants by Sanger sequencing

We used Sanger sequencing to confirm 13 possible candidate novel pathogenicity loci in the ABC family genes form four lines. The result (See Supplementary material, Figure S2) were all consistent with WES.

Evolutionary conservation analysis

We performed evolutionary conservative analysis of twelve loci, which were also sequenced by Sanger. The result suggested these mutations were highly conserved among vertebrate species, including rat, sheep, cow, pig, dog, horse, chicken and so on (See Supplementary material, Figure S3).

Comparison of protein structural model of *ABCC2*Ser1342Tyr mutation

ABCC2 have consisted of 32 exons involved in bile formation which mediates hepatobiliary excretion of numerous organic anions and conjugated organic anions such as methotrexate and also transports sulfated bile salt such as taurolithocholate sulfate.[20, 21] This gene has two main nucleotide binding domains which located at position 671-678 and 1334-1341 (Figure 2A), separately. This location of this variant (Ser1342Tyr) in this study is closely to the ATP binding functional domain (1334-1341).

To further investigate the possible effects of the missense variant on protein structure, the reference and the modified protein structure of*ABCC2* gene were compared simultaneously using UCSF Chimera 1.14rc. The result showed that, compared reference molecular structure, the 3D model of mutation have a slight change in the chemical bond lengths of ATP-ligand binding amino acid side chains at positions Ser1342, Ser678 and Gln706 (Figure 2B). The change in the amino side chains could affect the binding efficiency of the ATP molecular.

To further analysis the genetic basis of *ABCC2* , we analyzed the mRNA expression level of *ABCC2* gene in placental tissue between 2 healthy and four ICP patients using the GEO datasets derived from NCBI (GEO accession: GSE46157), when having no liver targeted tissue. The significant ($P < 0.05$) difference in the gene expression was observed between the two groups (Figure 2C). And the expression of *ABCC2* upregulated in ICP group. Besides, we also detected that the expression of other three genes, such as *ABCC6* ,*ABCE1* and *ABCG5* , have changed in placental tissue (See Supplementary material, Figure S4).

Correlations among clinical data

We examined the correlation coefficients (See Supplementary material, Figure S5) among these 26 clinical data and found that TBA was significant negative correlations with gestation days (r = -0.34), birth weight (r = -0.30), RDW.SD (r = -0.16), and significant positive correlations with TBIL (r = 0.52), DBIL (r = 0.56), IDBIL (r = 0.18), ALT (r = 0.17), AST (r = 0.18). And there was also a close relationship among liver function indexes. Such as, ALT highly positively correlated with AST (r = 0.93). ALT, AST positively correlated with TBIL, DBIL. TBIL positively correlated with DBIL (r = 0.89). Besides, CHOL, TG, LDL and uric acid also positively with ALT and AST. In addition, the concentration of Ca, Mg ion positively correlated with AST. The correlations between the above mentioned clinical data were significant ($P < 0.05$).

Biochemical and clinical features of ICP cases with variants

Descriptive statistics of 26 clinical data for ICP patients with 42 new mutations are shown in Table S2. For all the clinical data, the level of ALT, AST, TBA, DBIL, CHOL and TG of 151 patients with ICP disease were higher than reference level. Notably, the average value of ABC novel mutations individuals had a fourfold higher TBA and a twofold higher ALT, AST, TG than the reference value, which confirms that the ICP disease presents with abnormal liver functions and elevated bile acids associated with the abnormal lipid metabolism.

Additionally, we found that six ICP patients contained both two mutations exhibited higher TBA, AST, DBIL, CHOL, TG and HDL than 31 patients with one mutation, 113 no mutation in ICP samples and local controls in 414 healthy population (Figure 3). In particular, TBA, as a clinical characteristic of ICP, the trend of the average value of which measured in ICP with mutations of ABC transporters' genes and healthy local controls were ranked: ICP with two mutations > ICP with one mutation > ICP with no mutation > healthy controls.

Discussion

Main findings

This study is the first to use whole-exome sequencing technology to uncover potential novel pathogenic mutations in ABC family genes involved in bile acid transport. In all, we identified 42 new loci covering 44 ABC genes in 151 patients which were diagnosed with ICP disease.

Strengths andlimitations

The present study has 4 major strengths: first, following the advent of WES technology, which has proved to be efficient to unearth sequence variation across the MAF spectrum in obstetric and gynecological diseases.[22, 23] Using this method, we successfully identified novel candidate pathogenicity loci with known functional genes *ABCB4* , *ABCB11* and *ABCC2* . In addition to these three genes, we also dig some novel variations in other ABC series genes. Our results investigated the genetic mutations of the first WES-identified ABC family genes associated with ICP disease. Second, to date, no studies have unraveled the genetic mutations in ABC genes of hepatic disease among pregnancy women from a relatively large scale of nationally representative sample (n=151) in China. Moreover, the clinical data of these patients are relatively complete, providing data support for association analysis between mutations and clinical data and subsequent functional verification. Finally, combine WES and clinical data, it is in favor of deciphering of molecular mechanism of ICP disease.

Certainly, our study also have limitations. First, WES approach needs to more sample size to target the low-frequency and rare variants. Otherwise, it may lead to miss some valuable variants. Moreover, it also bring out inaccuracy MAF of rare variants. However, the strict exclusion conditions guaranteed the selection of the defined cohort, such as employing the MAF in the databases, 151 ICP cases and 1029 controls derived from locals and combined with predicted tools. Second, the samples in this study were all derived from Jiangxi Province, which lack of geographic diversity, may limit the applicability of the generality of these results. However, this study is still valuable since the high incidence (˜ 1%˜3%) of ICP in Jiangxi. Finally, the causality between these potential pathogenic candidate loci and ICP disease needs to be verified by validation functional experiments.

Interpretation

Previous studies have detected genetic mutations to ICP primarily by Sanger sequencing or offspring studies based on a limited number of individuals. The rare (MAF < 0.01) variations that affect ICP have been more challenging to access. Fortunately, WES emerged as an efficient approach to dig for all the mutations of targeted genes. Exonic variants, particularly missense, nonsense, loss-of-function variants, incline to show the most dramatic effect sizes, possessing the greater power for detection. In recent years, in obstetrics and gynecology, there are a number of examples of using WES method to search for the key candidate genes and causative variants. For instance, Huusko et al employed WES revealing *HSPA1L* as a genetic risk factor for spontaneous preterm birth.[22] To our knowledge, this study is the largest analysis to date of the role of mutations of ABC series genes in 151 ICP susceptibility.

Intriguingly, seven novel pathogenic loci in *ABCB4* , *ABCB11*and *ABCC2* gene was simultaneously assigned to damaging group, suggesting that the accuracy of our results. Several studies have shown that the heterozygous missense mutations in *ABCB4* , especially low-frequency and rare variations, is common responsible for the occurrence of ICP disease, which is consistent with our result.[24-26] After filtering the frequency of the data, we identified a total of eight mutations having a MAF < 0.01, seven of which were heterozygous missense and one was nonsense in *ABCB4* . The role of*ABCB11* in ICP has also been clearly identified, although its contribution seems less than *ABCB4* . A comprehensive analysis of multiple previous studies have concluded that up to 5% of ICP cases harbor a monoallelic mutations in *ABCB11* .[27] Our study confirmed the role of *ABCB11* and further expanded the role of ABCB11 gene. Previous studies have shown that the presence of Arg696Trp mutation in ICP samples, which was confirmed in our samples.

The attention and genetic contribution of *ABCC2* to ICP was less than that of *ABCB4* and *ABCB11* . We didn't detected any mutations which were previously reported in *ABCC2* gene associated ICP in this cohort. The reasonable explanation of this discrepancy may be due to distinct genetic background and genetic heterogeneity of populations. Our results demonstrated a small number of variations, especially that one novel pathogenic heterozygous (*ABCC2*Ser1342Tyr) variant was detected. The function of *ABCC2* involved in bile formation. In placental tissue, the expression of *ABCC2*upregulated, indicating that *ABCC2* is also likely overexpressed in the liver tissue of ICP patients, resulting in increased bile formation, thus, implying that Ser1342Tyr most likely belonged to function-gained mutation. More et al also found that the expression of*ABCC2* was more closely related in the livers from alcohol cirrhosis cohort, indicating that *ABCC2* expression changed in liver related disease.[28], which is consistent with the fact that ICP is a liver disease. As for other three genes which expressed differently, we found *ABCC6* having a novel mutation His1043Gln which was located in probable damaging group. Therefore, the gene *ABCC6* and the loci His1043Gln was of in interest in the development of ICP disease. In our study, we failed to detect the novel mutations *ABCE1* and *ABCG5* genes, therefore, we conjectured it is probably due to the fact that the known mutations in these two genes associated with ICP.

Because there have been relatively few studies on the genetic basis of ICP diseases, which leaded to many functional genes have not been mined. However, based on previous studies, several researchers found some evidence that the genes may predispose to liver or ICP disease, such as*ABCB9* associated with Hepatocellular Carcinoma and*ABCG2*associated with ICP.[29, 30] Therefore, we hypothesized that the genetic mutations in these unknown function genes excluding*ABCB4* , *ABCB11* and *ABCC2* confers susceptibility to ICP,

especially damaging group. These findings extended the role of mutations and strengthens the understanding of genetic basis of ICP disease, along with many other ABC family genes.

Bacq et al suggested that the elevations in serum TBA, ALT and AST activity were identified among ICP patients who have mutations in ABC transporter genes.[31] In agreement with that, our results confirmed that the mutation group containing 42 novel variants was associated with higher TBA, AST, DBIL, CHOL, TG and HDL (Table S2; and Figure 3). Furthermore, Piatek K et al suggested that the analysis of genotypes' co-existence pointed to the possibility of the mutated variants of polymorphism of genes having a summation effect on the development of ICP disease.[32] Consistent with this result, in our study, we have six ICP patients contain two mutations at once. Notably, ICP harbored both two mutations increased the average TBA level by 17.33, 20.22 and 50.32 μmol/L, respectively, based on the difference in average TBA levels in ICP with two mutations, one mutation, no mutation in ABC transporter genes and no mutation in healthy controls. As expected, so did the AST, DBIL, CHOL, TG and HDL. The reasonable explanation of this situation is that double mutation has additive effect on TBA level, and this accumulation of TBA level results in ICP exacerbation.

Moreover, the significant difference between wild type and mutant type was biochemical index, which suggested that the accumulation of TBA could lead to lipid abnormality. Consistent with this result, researchers found that, bile acids (BA), which well known for its amphipathic nature, is essential to lipid absorption and energy balance in human.[33-35] Thus, taken together, our study provides additional support for effect of mutations in the ABC family genes on ICP disease and lipid metabolism.

Not surprisingly, to date, there are now only a handful of reports genetic analysis studies for ICP disease. The present findings not only enrich the molecular basis of the known functional genes (such as*ABCB4* , *ABCB11* and *ABCC2*) but also expand the new candidate genes (ABCA, ABCB, ABCC, ABCD-ABCG) associated with ICP. Our results support the facts the mutations in ABC transporters' genes lead to ICP disease. Therefore, further work on the genetic mutations involved in ICP pathogenesis has potential to inspire novel therapies for ICP patients.

Recently, there is currently much interest in whether variation in bile salt concentrations and mutations statue of *ABCB4* /*ABCB11* /*ABCC2* /other new candidate genes could be a biomarker for various forms of drug-induced liver injury.[36] Furthermore, considering the negative effect of the ABC mutations on mother and fetal outcomes, it is important to genotyped these mutations to timely genetic diagnosed, which, in order to get immediate attention and treatment for ICP susceptible person. Our study provides the evidence that these mutations have potential contribution to the ICP disease.

Conclusion

We detected 42 novel potential pathogenic mutations in 44 altogether ABC family genes. Among them, seven loci were identified in *ABCB4* ,*ABCB11* and *ABCC2* , and the remaining 34 loci were in other genes. We performed validation and bioinformatics analysis on some of these novel pathogenic sites. The results showed that most loci were conservative. Moreover, we found the detection of genetic variants that are significantly associated with six biochemical index, including TBA, ALT, AST, DBIL, CHOL and TG ($P < 0.05$). Nevertheless, their functional validation needs to be further investigated. Our findings provide new valuable insights into the genetic basis of ICP disease and suggest potential candidate variant for clinical diagnose.

Disclosure of interests

The authors have declared that no potential conflicts of interest exist.

Contribution to authorship

XL and HL performed the experiments, analyzed the data, prepared the figures and drafted the manuscript. SX, XZ, LN, ZL and MW collected samples. LZM, JZ and YZ contributed to the concept and design of the

study. JZ and YZ performed the experiments, analyzed the data and revised the manuscript. All authors read and approval the final manuscript.

Details of ethical approval

The present study followed the tenets of the Helsinki Declaration and the ethics approval was approved by the Institutional Review Board of Jiangxi Provincial Maternal and Child Health Hospital in China, and each participated woman gave informed consent.

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Table 1. Forty-two potential pathogenic ABC novel mutations were identified in 151 Han Chinese with ICP disease.

ID	Gene	Patients	Chr	Position	Alleles	Protein change	SIFT[d]	Mutation Taster[e]	FATHMM
1	*ABCA4*	ICP76	chr1	94522278	A/G	Phe754Ser	0.001 (D)	0.999 (D)	-4.24 (D)
2	*ABCA12*[a,b]	ICP75	chr2	215809749	C/T	Cys2440Tyr	0.041 (D)	1 (D)	-3.67 (D)
3	*ABCA13*[a]	ICP112	chr7	48354004	C/G	Ser3286Ter	-	1 (A)	-
4	*ABCB1*	ICP121	chr7	87173579	G/A	Pro693Ser	0.031 (D)	1 (D)	-2.21 (D)
5	*ABCB4*	ICP21	chr7	87053310	C/T	Trp708Ter	-	1 (A)	-
6	*ABCB4*[a,b]	ICP154	chr7	87069134	C/T	Gly527Glu	0.0 (D)	1 (D)	-2.35 (D)
7	*ABCB4*[a,b]	ICP133,135	chr7	87073053	T/C	Lys386Glu	0.002 (D)	1 (D)	-2.65 (D)
8	*ABCB5*[a,b]	ICP47	chr7	20767985	G/T	Ser925Ile	0.002 (D)	0.786 (D)	-2.5 (D)
9	*ABCB11*	ICP115	chr2	169783704	G/A	Gln1194Ter	-	1 (A)	-
10	*ABCB11*	ICP118	chr2	169826057	T/G	Gln605Pro	0.006 (D)	0.972 (D)	-1.99 (D)
11	*ABCB11*[a,b]	ICP2	chr2	169826599	G/T	Leu589Met	0.0 (D)	0.994 (D)	-2.82 (D)
12	*ABCC2*[a,b,c]	ICP79	chr10	101605418	C/A	Ser1342Tyr	0.0 (D)	0.999 (D)	-2.95 (D)
13	*ABCC3*[a,b]	ICP93	chr17	48755166	T/C	Ile1147Thr	0.0 (D)	1 (D)	-2.57 (D)
14	*ABCC9*[a,b]	ICP124	chr12	22061100	C/T	Ala456Thr	0.001 (D)	1 (D)	-2.58 (D)
15	*ABCG2*[a,b]	ICP6	chr4	89013418	G/T	Leu646Met	0.01 (D)	0.998 (D)	-2.33 (D)
16	*ABCA2*[a,b]	ICP64	chr9	139910497	G/C	Asp1108Glu	0.043 (D)	0.999 (N)	-3.27 (D)
17	*ABCA2*	ICP19	chr9	139913419	G/A	Ala583Val	0.351 (T)	0.999 (D)	-2.17 (D)
18	*ABCA5*	ICP102	chr17	67266794	A/G	Val997Ala	0.527 (T)	0.961 (D)	-1.77 (D)
19	*ABCA7*	ICP108	chr19	1045131	C/A	Pro449His	0.015 (D)	1 (N)	-4.44 (D)
20	*ABCA8*	ICP47	chr17	66938153	A/G	Val8Ala	0.009 (D)	0.984 (N)	-2.41 (D)
21	*ABCA10*[a,b]	ICP35	chr17	67212035	A/G	Leu260Ser	0.038 (D)	1 (N)	-2.28 (D)
22	*ABCA12*	ICP95	chr2	215865543	A/G	Ile1022Thr	0.093 (T)	0.994 (D)	-3.77 (D)
23	*ABCA13*	ICP18	chr7	48559709	C/G	Leu4624Val	0.017 (D)	0.954 (N)	-2.477 (D
24	*ABCA13*	ICP107	chr7	48634399	A/G	Thr4912Ala	0.001 (D)	0.958 (N)	-5.88 (D)
25	*ABCB9*	ICP112	chr12	123430667	C/T	Glu386Lys	0.891 (T)	0.682 (D)	-2.4 (D)
26	*ABCB9*	ICP140	chr12	123433209	T/C	Ile339Val	0.176 (T)	0.999 (D)	-2.6 (D)
27	*ABCC1*	ICP98	chr16	16149964	A/G	Ser497Gly	0.268 (T)	0.972 (D)	-2.71 (D)
28	*ABCC3*	ICP57	chr17	48734467	C/T	Leu137Phe	0.011 (D)	1 (D)	0.85 (T)
29	*ABCC5*	ICP49	chr3	183670989	T/G	Gln851Pro	0.026 (D)	0.999 (D)	0.95 (T)
30	*ABCC6*	ICP57	chr16	16259657	G/T	His1043Gln	0.029 (D)	0.999 (N)	-3.35 (D)
31	*ABCC9*	ICP36	chr12	21968809	T/A	Glu1304Val	0.111 (T)	0.999 (D)	-2.61 (D)
32	*ABCC12*	ICP155	chr16	48180227	G/A	Pro37Ser	0.217 (T)	0.812 (D)	-2.76 (D)
33	*ABCD4*	ICP95	chr14	74757137	G/A	Ser395Phe	0.033 (D)	0.983 (N)	-2.77 (D)
34	*ABCD4*	ICP142	chr14	74757168	T/C	Thr385Ala	0.254 (T)	0.990 (D)	-2.63 (D)
35	*ABCF2*	ICP84	chr7	150923406	C/T	Gly47Ser	0.156 (T)	0.999 (D)	-2.77 (D)
36	*ABCG1*[a,b]	ICP113	chr21	43704752	A/G	Ile273Val	0.002 (D)	1 (D)	0.52 (T)
37	*ABCA3*	ICP22	chr16	2348506	T/C	Ser593Gly	0.126 (T)	1 (N)	-3.3 (D)
38	*ABCA10*	ICP64	chr17	67181648	G/C	Leu823Val	0.09 (T)	1 (N)	-2.19 (D)
39	*ABCA12*	ICP71	chr2	215802301	T/C	Asn2492Ser	0.403 (T)	0.958 (N)	-2.28 (D)
40	*ABCA13*	ICP111	chr7	48349554	G/A	Ser3111Asn	0.071 (T)	0.999 (N)	-2.11 (D)
41	*ABCG1*	ICP22	chr21	43708158	C/T	Thr378Ile	0.21 (T)	1 (N)	-1.98 (D)
42	*ABCA13*	ICP52	chr7	48318169	A/G	Ile2460Val	0.916 (T)	1 (N)	0.66 (T)

[a] This loci was validated by Sanger sequencing.

[b] This loci was performed by evolutionary conservative analysis.

[c] The effect of the change of this mutation on the encoded protein structure.

[d] D: disease-causing, T: tolerated.

[e] N: polymorphism, A: disease causing automatic.

[f] Pro damaging: probable damaging; Pos damaging: Possible damaging.

[g] GERP++NR: GREP++ conservation score. The higher the value, the more conservative the loci.

[h] PhastCons100way_ver: conservative prediction in vertebrates in 100 vertebrates. The scores of PhastCons score ranged from 0 to 1, and the higher the values, the more conservative the loci.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Primes used for confirmed the variants in ABC transporters' genes.

Table S2. Descriptive statistics of 26 clinical data of ICP individuals associated with these mutations.

Figure S1. The distribution and numbers of genetic variants from WES data for ICP. (A) The percentage of the types of genetic variants in ABC series genes. (B) The total number of genetic variants before and after quality control, and the novel variants. TSNPs: Total SNPs before quality control; QCSNPs: Total SNPs after quality control; NSNPs: Novel SNPs.

Figure S2. Sanger sequencing to validate the novel variants in ABC series genes. (A) ABCA genes; (B) ABCB genes; (C) ABCC genes; (D) ABCD-ABCG genes.

Figure S3. The evolutionary conservative analysis of ABC novel representative mutations. There mutations were highly conserved in vertebrate species.

Figure S4. The comparison of the expression level of*ABCC6* , *ABCE1* and *ABCG5* genes between two healthy and 4 ICP patients in placental tissue.

Figure S5. Correlation coefficients among twenty-six clinical features. The dots indicate the significant ($P < 0.05$) correlation coefficients between each pair of features. Its size and colors separately represent the degree and direction of correlation coefficients.