ORIGINAL PAPER / OBSTETRICS

Associations between *ALDOB* polymorphisms and intrahepatic cholestasis of pregnancy susceptibility in the Chinese Han population

Xianxian Liu, Siming Xin, Jiusheng Zheng, Huai Liu, Yang Zeng, Xiaoying Wu, Feng Zhang, Xiaoming Zeng, Yang Zou, Hua Lai

Jiangxi Maternal and Child Health Hospital, Jiangxi p*rovincial Maternal and Child Health Hospital, Nanchang, China*

Corresponding author:
Xianxian Liu
Jiangxi Maternal and Child Health Hospital, Jiangxi Provincial Maternal and Child Health Hospital, 330006 Nanchang, China
e-mail: 510718301@qq.com

ABSTRACT

Objectives: To research the associations between fructose-bisphosphate aldolase B (*ALDOB*) gene polymorphisms and intrahepatic cholestasis of pregnancy (ICP) risk.
Material and methods: Whole-genome sequencing (WGS) was performed to detect *ALDOB* polymorphisms. Five web-available tools were employed to predict the effect of the site variant on the protein. Protein structure comparisons between the reference and *ALDOB*-modified samples were performed by SWISS-MODEL and Chimera 1.14rc, respectively.
Results: We identified 28 genetic variants in the *ALDOB* gene. When the cut-off value of minor allele frequency (MAF) of loci was 0.001 in four databases, five missense variants, including rs747604233, rs759204107, rs758242037, rs371526091

and rs77718928, were reserved for subsequent analysis. These variants were absent from the 1029 control individuals. The influence of all five variants on protein function was predicted to be damaging by the abovementioned five prediction software programs. Bioinformatics analysis demonstrated that these five missense variants were highly conserved among vertebrates. Compared to the wild-type protein structure, all five mutated protein structures showed a slight change in the chemical bond lengths of the enzyme activity domains. The combined clinical data indicate that the variant group had a significantly older age ($p = 0.038$), a higher level of indirect bilirubin (IDBIL, $p = 0.033$), and lower counts of white blood cells (WBCs, $p = 7.38E-05$) and platelets (PLTs, $p = 0.018$) than the wild-type group.

Conclusions: This is the first study to examine the associations between *ALDOB* polymorphisms and ICP disease in 249 Chinese patients with ICP. Our present study expands the understanding of the pathogenesis of ICP.

Keywords: intrahepatic cholestasis of pregnancy; whole-genome sequencing; fructose-Bisphosphate Aldolase B; polymorphisms; risk

INTRODUCTION

Intrahepatic cholestasis of pregnancy (ICP) is one of the most common hepatic diseases that appears in the second and early third trimesters of pregnancy. This disease is characterized by skin pruritus, abnormal liver functions and elevated serum total bile acid (TBA, ≥ 10 μmol/L) levels. All the symptoms and biochemical abnormalities usually dissolve spontaneously within 48 hours after delivery. The incidence of ICP ranges from 0.1% to 25%, with obvious geographical distributions and ethnic differences [1]. The recurrence rate of ICP in subsequent pregnancies has been reported to be between 40% and 60%. ICP is associated with an increased risk of preeclampsia, diabetes, hepatobiliary disease, and hypothyroidism in pregnant women [2, 3]. In addition, ICP has been associated with adverse obstetrical outcomes, including amniotic fluid faecal infection, foetal distress, preterm birth and death [4, 5]. The foetal complications in ICP are believed to be high levels of bile acid in the foetal serum [6, 7]. Therefore, untangling the pathogenesis of ICP and its association

with foetal complications is very important.

Intrahepatic cholestasis of pregnancy is a complicated obstetrical disease whose mechanisms remain poorly understood. Until now, it has been generally accepted that ICP is influenced by a combination of genetic, endocrine hormones, microorganisms, ages, seasons, nutrition and environmental factors. Recently, accumulated evidence has shown that ICP is associated with an abnormal metabolic profile, including glucose intolerance and dyslipidaemia. Maternal cholestasis during pregnancy programs metabolic disease in offspring [8]. Interestingly, it is worth noting that the *ALDOB* gene is a key enzyme that catalyses the cleavage of fructose-1,6-bisphosphate to glyceraldehyde-3-phosphate and dihydroxyacetone phosphate in glycolysis [9]. It is abundantly expressed in the liver and kidneys and plays a crucial role in glycolysis, fructolysis, the synthesis of glyceraldehyde and ATP, and the maintenance of lipid metabolism [10]. Therefore, because of the crucial role of this molecule in glycogen and lipid metabolism, certain genomic alteration events and the expression levels of *ALDOB* might cause metabolic disorders and further lead to human disease. Indeed, multiple previous studies have reported that *ALDOB* gene variants or its aberrant expression are observed in several human diseases and cancer types, including hereditary fructose intolerance (HFI) [11], colorectal adenocarcinoma [12], hepatocellular carcinoma (HCC) [13], and non-alcoholic fatty liver disease (NAFLD) [14]. Furthermore, prior studies revealed that patients with NAFLD had significantly higher levels of circulating or toxic TBA in the liver than healthy pregnant women [15]. Bile acids have similarly been studied as potential therapeutic targets in NAFLD [14]. Coincidently, ICP is a severe liver disease with the unique biochemical characteristic of an elevated level of TBA. Thus, we speculated that the abnormalities of *ALDOB* might be associated with TBA levels in ICP disease.

Considering that women with ICP exhibit elevated serum bile acid levels and that *ALDOB* abnormalities are associated with fluctuations in TBA levels, we speculated that variants in *ALDOB* might be present in women with ICP. Therefore, we performed targeted gene sequencing of the *ALDOB* gene in a total of 249 Han Chinese women with ICP and related them to clinical data and pregnancy outcomes in

current study.

MATERIAL AND METHODS

Samples and characteristics

A total of 249 pregnant women who did not have other liver diseases and were diagnosed with ICP disease based on skin pruritus in combination with abnormal liver laboratory investigations, *e.g.*, TBA, alanine transaminase (ALT) and aspartate transaminase (AST) levels, were recruited. The cut-off value of TBA was 10 μmol/L. All peripheral blood samples were collected from the Department of Obstetrics at Jiangxi Provincial Maternal and Child Health Hospital (Nanchang, Jiangxi, China) between 2018 and 2022. Moreover, we recorded 32 available clinical and laboratory characteristic data. These data included the age at diagnosis, gestational age, body mass index (BMI), gravidity, parity, the levels of ion concentrations for K, Na, Cl, Ca, Mg and P, the WBC, red blood cell (RBC), and PLT counts, red blood cell distribution width SD (RDW-SD), the levels of serum biochemical indices, including TBA, cholyglycine (CG), ALT, AST, γ-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), total bilirubin (TBIL), direct bilirubin (DBIL), IDBIL, total cholesterol (CHOL), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and uric acid (UA), and the outcomes of pregnant women and newborns, including birth weight, Apgar score and bleeding amount. All clinical parameters were measured as previously described [16–18]. Briefly, the ion concentrations and serum biochemical indices were determined by an AU5800 automatic biochemical analyser (Beckman Coulter, Inc., USA). Routine blood tests were performed using a Sysmex-xn-2000 automatic blood cell analyser (Sysmex Corporation, Japan). The birth weight, gestational age, and Apgar score were recorded by two experienced neonatologists. Additionally, 1029 local individuals without ICP disease were also recruited in the same period. This study was carried out in accordance with the Declaration of Helsinki and was approved by the institutional review board of Jiangxi Provincial Maternal and Child Health Hospital. Each participating woman gave informed consent.

Variant analysis

Two hundred forty-nine genomic DNA samples were isolated from the peripheral blood with an Axy Prep Blood Genomic DNA Mini Prep Kit (Item No. 05119KC3, Axygen Scientific, Inc., Union City, CA, USA) according to the manufacturer's instructions. The concentration and integrity of the DNA samples were examined by a Nanodrop-1000 spectrophotometer (Thermo Fisher, USA) and gel electrophoresis, respectively. After quality control, 249 ICP samples were subjected to targeted exome sequencing for the *ALDOB* gene. Briefly, a series of oligonucleotide probes were designed to enrich the genomic regions, including nine exons and the intron–exon boundaries of the *ALDOB* gene, by hybridizing the probes with whole genomic DNA fragments. Then, the constructed DNA library was sequenced on BGISEQ-500 platforms. All steps were performed following the provider's guidelines. The clean reads were aligned to the human reference genome (UCSC Genome Browser GRCh37/hg19) using Burrows–Wheeler Aligner (BWA) software [19]. Then, variant calls and annotations were conducted by the genome Analysis Toolkit (GATK) and ANNOVAR tools [20, 21]. All identified variants were filtered against public databases, including the 1000 Genomes Project (1000G_ALL, http://www.internationalgenome.org/), the Exome Aggregation Consortium (ExAC, http://exac.broadinstitute.org/), Trans-Omics for Precision Medicine (TOPMed, https://www.nhlbi.nih.gov/science/precision-medicine-activities), the Genome Aggregation Database (gnomAD_exome, http://gnomad-sg.org/) and the 1029 local individuals. The in silico prediction tools SIFT, PolyPhen2, Mutation Taster, FATHMM and CADD were used to predict the effect of the protein change.

Sanger analysis

After filtering, five possibly pathogenic variants in the *ALDOB* gene were validated by Sanger sequencing with an ABI 3730XL DNA sequencer (Applied Biosystems, Foster City, CA, USA). Primers were designed by Primer Premier 5 software. The details of the PCR primers are shown in Table 1.

Evolutionary conservative analysis

Evolutionary conservation analyses among multiple species, including chimpanzee, gibbon, macaque, olive baboon, gelada, marmoset, prairie vole, mouse, rat, rabbit, domestic yak, cow, goat, sheep, sperm whale, Arabian camel, dog, dingo, cat, leopard, horse, elephant, zebrafish and zebra finch, were performed by the genomic alignments of the Ensembl Genome Browser.

Protein structural modelling

In this study, we compared the protein structures of the wild-type and 5 *ALDOB* gene mutants. Briefly, the reference and modified (*ALDOB* p.G75R, p.G76S, p.V105G, p.R331L and p.A338V) protein sequences were submitted to SWISS-MODEL (https://swissmodel.expasy.org/) software for structure modelling. Then, two protein structure models were compared simultaneously using the Chimera 1.14rc package.

Statistical analysis

The *t.test* function was performed to analyse the potential associations of 32 clinical points between 249 samples with or without the five possible pathogenic variants of *ALDOB*. All the p *value*s were two-tailed, and p values < 0.05 were considered significant. We estimated the effect of the *ALDOB* polymorphisms on the ICP using logistic regression analysis, and this was reported as the odds ratio (OR) and 95% confidence interval (CI). Furthermore, we performed an receiver operating characteristic (ROC) analysis to assess the predictive value, sensitivity and specificity levels of TBA, ALT and AST for premature birth. All the above-mentioned analyses were carried out with R software.

RESULTS

***ALDOB* variants**

In this study, we performed WGS to identify *ALDOB* variants in a cohort of 249

patients with ICP disease. We identified a total of 28 genetic variants, including 4 3_prime_UTR, 18 intron and 6 missense variants, in the *ALDOB* gene (Supplementary material — Tab. S1). Quality control procedures were carried out to remove variants with an MAF more than 0.001 in the 1000G_ALL, ExAC, TOPMed and gnomAD_exome databases. The variants, which were predicted to be harmful to the protein according to the prediction results of five website available tools, were conserved for further analysis. After quality control, five missense variants, including rs747604233, rs759204107 and rs758242037 in exon 3 and rs371526091 in exon 8 and rs77718928 in exon 9, were highlighted (Tab. 2). We used Sanger sequencing to confirm five candidate possibly pathogenic variants in the *ALDOB* gene. The results (Fig. 1) were all consistent with WGS.

The frequency of these variants in 249 patients with ICP disease reached 3.2% (8/249). All five of these missense variants were absent from the 1029 control individuals. Furthermore, four out of five variants were absent from the 1000G_ALL database and had a lower frequency in the ExAC database. These frequencies ranged from 1.65E-05 to 1.15E-04. ORs and 95% CIs were used to assess the association between *ALDOB* polymorphisms and ICP disease risk. The results showed that rs747604233, rs759204107 and rs758242037 were significantly associated with susceptibility to ICP ($p < 0.012$). Additionally, the OR values of the 249 samples in three databases (the ExAC, TOPMed and gnomAD_exome databases) were all higher, ranging from 33.92 to 369.76 (Table 3). In addition, rs371526091 (OR: 3.36, 95% CI: 0.064–42.03, $p = 0.32$) and rs77718928 (OR: 15.69, 95% CI: 0.38–94.44, $p = 0.064$) also demonstrated an association with an increased risk of ICP.

Clinical characteristics of ICP patients with *ALDOB* variants

The clinical and biochemical data of the eight patients with the five *ALDOB* gene variants are shown in Table 4. Of the eight babies of the ICP patients, three (ICP114, 203, 21) were born prematurely (gestational weeks < 37), and the other three were born only a few days after 37 weeks. Of the eight patients, six gave birth to their babies by caesarean section. Serum bile acid and TG levels were elevated in all eight

patients. The serum bile acid and TG levels of the patients with the five missense variants ranged from 16.3 μmol/L to 75.4 μmol/L (reference value: 0–10 μmol/L) and from 2.55 mmol/L to 5.94 mmol/L (reference value: 0.34–1.69 mmol/L), respectively. Six out of eight patients had higher levels of ALT and AST than the reference value. Notably, the patient (ICP21) harbouring the rs77718928 (p.A338V) variant had the highest bile acid (75.4 μmol/L) and ALT (447 U/L) levels and gave birth to a 2.25 kg baby at 34 + 2 weeks of gestation. Furthermore, the concentrations of CHOL for the seven patients with *ALDOB* variants were higher (5.88–9.6 mmol/L) than the reference value (0–5.2 mmol/L).

Evolutionary conservative analysis

Evolutionary conservation analysis showed that these five variants were highly conserved among 25 vertebrate species, e.g., rats, cows, sheep, dogs and horses (Fig. 2).

Comparison of the protein structural model of the five *ALDOB* variants

The human *ALDOB* gene is located on chromosome 9q22.3 and consists of nine exons. The first exon is untranslated and represents the promoter region. Exons 2–9 encode a 364-amino acid polypeptide (type B monomer) (Fig. 3A).

To further investigate the possible effects of these five missense variants on protein structure, the reference and modified protein structures of the *ALDOB* gene were compared using UCSF Chimera 1.14rc software. Taking the p.G75R variant as an example, compared with the reference molecular structure, the 3D model of the variant had a slight change in the chemical bond lengths of amino acid side chains at positions ARG43, LYS108, ARG149, GLU188, GLU190, LYS230, SER272 and ARG304 (Fig. 3B). In addition, the other four variants also caused changes (Fig. 3C–F). The changes may cause considerable structural perturbations and further have a remarkable impact on its protein–protein interactions, substrate binding or enzyme activity.

Correlations between variants and clinical data

Descriptive statistics of 32 clinical characteristics for patients with ICP with the five missense variants are presented in Table 5. In the 249 patient samples, regardless of whether the difference was significant, the variant group tended to be associated with higher age, BMI, gravidity, parity, bleeding count and levels of ALT, AST, TBIL, IDBIL, and TG and lower levels of Ca+, WBCs, and PLTs. Remarkably, the variant group had a significantly higher age ($p = 0.038$), higher level of IDBIL ($p = 0.033$), and lower level of WBCs ($p = 7.38E-05$) and PLTs ($p = 0.018$) than the wild-type group.

Additionally, we carried out ROC curve analysis and used the area under the curve (AUC) for the quantitative evaluation of TBA, ALT and AST levels for predicting premature birth (Fig. 4A). Previously, we calculated the predictive value of TBA for preterm birth in 151 ICP samples. The cut-off value of preterm delivery was 46.05 μmol/L [16]. When the number increased to 318 individuals, TBA, ALT and AST levels had a higher predictive value for the incidence of preterm birth, with AUCs of 0.649 (95% CI: 0.462–0.833), 0.642 (95% CI: 0.590–0.670) and 0.644 (95% CI: 0.599–0.659), respectively (Fig. 4B–D). The preterm delivery need increased at a TBA cut-off value of 47.35 μmol/L (Fig. 4B).

DISCUSSION

ALDOB belongs to the aldolase family, which plays various roles in human disease and cancers [22]. In recent years, a growing body of evidence has suggested that aldolase is an independent clinical prognostic marker in human cancers [23]. Most previous studies have reported that *ALDOB* dysfunction or deficiency can lead to HFI [24]. Recently, using high-throughput screening and omics database integration, it has also been suggested that *ALDOB* is associated with the hazard ratios of liver diseases, such as NAFLD and HCC. For example, Niu et al. [14] revealed six proteins demonstrating statistically significant changes, including ALDOB, APOM, LGALS3BP, PIGR, VTN, and AFM, when performing plasma proteome profiling of 48 patients with and without cirrhosis or NAFLD. He et al. [13] reported that the loss of ALDOB activates Akt and promotes hepatocellular carcinogenesis by destabilizing

the Aldob/Akt/PP2A protein complex. Interestingly, Nimer et al. [15] suggested that plasma levels of bile acids are elevated among subjects with NAFLD compared to control individuals. Based on the abovementioned studies, we conclude that the abnormalities of ALDOB are related to liver diseases, which is consistent with the result that ICP is a liver disease.

Combined with clinical data analyses, our present results found that the age in the variant group was significantly higher than that in the wild group. This implies that age was associated with an increased risk of developing ICP, which is consistent with the results of Heinonen et al. [25]. In addition, several studies have confirmed that ICP is characterized by glucose intolerance and dyslipidaemia [26, 27]. For instance, Jin et al. [28] suggested that high maternal TG concentrations during late pregnancy were independently and significantly associated with a greater risk of ICP. Consistent with this research, our present study found that, compared to the wild group, the average levels of CHOL and TG were higher in the variant group. This finding implies that ALDOB dysfunction is associated with lipid abnormalities.

To date, most previous studies have shown that variants in the *ALDOB* gene lead to HFI, which is a rare disease and an inborn error carbohydrate metabolism in humans [29]. This disease often occurs in newborns and is associated with vomiting and difficulty feeding [30]. The estimated incidence of the disease is 1 in 20000, and the number of cases is increasing [24, 31]. To date, multiple variants, including R46W, I74T, C135R, A150P, A175D, C178R, P185R, V222F, L229P, L257P, L284P, R304Q, R304W, N335K, A338V and Y343H, have been reported in the *ALDOB* gene [9, 11, 32, 33]. In this study, these eight patients did not present with HFI symptoms. Interestingly, we also found a 338V variant, which has previously been found in HFI patients, in ICP21. The eight patients with this variant had the highest levels of TBA, which were more than 7-fold higher than the upper limit of normal pregnancy. In addition, the ALT and AST activities of ICP21 patients exceeded 10-fold and 6-fold, respectively. A reasonable explanation of this situation may be that the pathogenic mechanism of this locus is different in different populations or pleiotropy of gene loci. To the best of our knowledge, four other missense variants, G75R, G76S, V105G and

R331L, of the *ALDOB* gene that were identified in 249 ICP cases have not been reported in HFI patients. Therefore, combining the significant differences in frequency between different groups, the prediction results of variant-induced protein damage with correlations between variants and clinical data indicated that five *ALDOB* variants might be associated with the risk of ICP.

In addition, all these variants cause structural perturbations in the protein. For example, the p.G75R variant affects the chemical bond lengths of amino acid side chains at positions ARG43, LYS108, ARG149, LYS230, and ARG304 with the active site pocket of ALDOB and plays an important role in substrate binding and catalysis [34, 35]. However, it is not clear whether these variants contribute to ICP disease by influencing ALDOB enzyme activity or by nonenzymatic actions. Therefore, the causality between these five potential pathogenic candidate loci and ICP disease needs to be verified by validation functional experiments.

Our present study has several strengths. First, the greatest strength of this study is the large size of the cohort. We probably collected the highest number of ICP patients ever analysed genetically (249). Next, the focus of the analysis was extended beyond the metabolic ICP-related gene *ALDOB*, thus making it possible to find a potential pathogenic locus. This is the first study to explore the associations between metabolism-related gene polymorphisms of *ALDOB* and ICP risk in a Chinese population. Previously, most researchers made more efforts to identify the genetic variants of well-known ICP-related transporter genes, such as *ABCB4*, *ABCB11*, and *ABCC2*, and the nuclear receptor gene *NR1H4*, which contribute to the development of ICP. We also reported the identification of the *ANO8* gene as a genetic risk factor for ICP [16]. More importantly, from a clinical perspective, patients with the five candidate damaging variants demonstrated significantly higher levels of IDBIL and other biochemical markers. Therefore, it is important to genotype these five variants to genetically diagnose them and provide immediate treatment for ICP-susceptible individuals. Certainly, the ALDOB-based signalling pathways and networks that affect biochemical indices and contribute to ICP still need more exploration.

CONCLUSIONS

In summary, we reported five potential damaging variants (p.G75R, p.G76S, p.V105G, p.R331L and p.A338V) in the *ALDOB* gene in eight out of 249 Chinese patients with ICP for the first time. Our findings provide valuable insights into the genetic architecture of ICP disease and suggest potential candidate pathogenic variant targets for ICP clinical treatment.

Article information and declarations

Ethics statement

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

Acknowledgments

The authors would like to thank the patients who participated in this study.

Conflict of interests

The authors declare that they have no competing interests.

Supplementary material

Table S1. https://journals.viamedica.pl/ginekologia_polska/article/view/92931

REFERENCES

1. Geenes V, Williamson C. Intrahepatic cholestasis of pregnancy. World J Gastroenterol. 2009; 15(17): 2049–2066, doi: 10.3748/wjg.15.2049, indexed in Pubmed: 19418576.
2. Rezai S, Lora I, Henderson CE. Severe intrahepatic cholestasis of pregnancy is a risk factor for preeclampsia in singleton and twin pregnancies. Am J Obstet Gynecol. 2015; 213(6): 877, doi: 10.1016/j.ajog.2015.08.007, indexed in Pubmed: 26264824.
3. Liu C, Gao J, Liu J, et al. Intrahepatic cholestasis of pregnancy is associated with an increased risk of gestational diabetes and preeclampsia. Ann Transl Med. 2020; 8(23): 1574, doi: 10.21037/atm-20-4879, indexed in Pubmed: 33437773.

4. Yayi Hu, Danqing W, Shuyun L, et al. Immunologic abnormality of intrahepatic cholestasis of pregnancy. Am J Reprod Immunol. 2010; 63(4): 267–273, doi: 10.1111/j.1600-0897.2009.00798.x, indexed in Pubmed: 20085564.
5. Lee RH, Kwok KM, Ingles S, et al. Pregnancy outcomes during an era of aggressive management for intrahepatic cholestasis of pregnancy. Am J Perinatol. 2008; 25(6): 341–345, doi: 10.1055/s-2008-1078756, indexed in Pubmed: 18509787.
6. Brouwers L, Koster MPH, Page-Christiaens GC, et al. Intrahepatic cholestasis of pregnancy: maternal and fetal outcomes associated with elevated bile acid levels. Am J Obstet Gynecol. 2015; 212(1): 100.e1–100.e7, doi: 10.1016/j.ajog.2014.07.026, indexed in Pubmed: 25046809.
7. Glantz A, Marschall HU, Mattsson LA. Intrahepatic cholestasis of pregnancy: Relationships between bile acid levels and fetal complication rates. Hepatology. 2004; 40(2): 467–474, doi: 10.1002/hep.20336, indexed in Pubmed: 15368452.
8. Papacleovoulou G, Abu-Hayyeh S, Nikolopoulou E, et al. Maternal cholestasis during pregnancy programs metabolic disease in offspring. J Clin Invest. 2013; 123(7): 3172–3181, doi: 10.1172/JCI68927, indexed in Pubmed: 23934127.
9. Esposito G, Imperato MR, Ieno L, et al. Hereditary fructose intolerance: functional study of two novel ALDOB natural variants and characterization of a partial gene deletion. Hum Mutat. 2010; 31(12): 1294–1303, doi: 10.1002/humu.21359, indexed in Pubmed: 20848650.
10. Salvatore F, Izzo P, Paolella G. Aldolase gene and protein families: structure, expression and pathophysiology. Horiz Biochem Biophys. 1986; 8: 611–665, indexed in Pubmed: 3530956.
11. Esposito G, Santamaria R, Vitagliano L, et al. Six novel alleles identified in Italian hereditary fructose intolerance patients enlarge the mutation spectrum of the aldolase B gene. Hum Mutat. 2004; 24(6): 534, doi: 10.1002/humu.9290, indexed in Pubmed: 15532022.
12. Li Q, Li Y, Xu J, et al. Aldolase B Overexpression is Associated with Poor Prognosis and Promotes Tumor Progression by Epithelial-Mesenchymal Transition in Colorectal Adenocarcinoma. Cell Physiol Biochem. 2017; 42(1): 397–406, doi: 10.1159/000477484, indexed in Pubmed: 28558381.
13. He X, Li M, Yu H, et al. Loss of hepatic aldolase B activates Akt and promotes hepatocellular carcinogenesis by destabilizing the Aldob/Akt/PP2A protein complex. PLoS Biol. 2020; 18(12): e3000803, doi: 10.1371/journal.pbio.3000803, indexed in Pubmed: 33275593.

14. Niu L, Geyer PE, Wewer Albrechtsen NJ, et al. Plasma proteome profiling discovers novel proteins associated with non-alcoholic fatty liver disease. Mol Syst Biol. 2019; 15(3): e8793, doi: 10.15252/msb.20188793, indexed in Pubmed: 30824564.

15. Nimer N, Choucair I, Wang Z, et al. Bile acids profile, histopathological indices and genetic variants for non-alcoholic fatty liver disease progression. Metabolism. 2021; 116: 154457, doi: 10.1016/j.metabol.2020.154457, indexed in Pubmed: 33275980.

16. Liu X, Lai H, Zeng X, et al. Whole-exome sequencing reveals ANO8 as a genetic risk factor for intrahepatic cholestasis of pregnancy. BMC Pregnancy Childbirth. 2020; 20(1): 544, doi: 10.1186/s12884-020-03240-z, indexed in Pubmed: 32942997.

17. Liu X, Lai H, Xin S, et al. Whole-exome sequencing identifies novel mutations in ABC transporter genes associated with intrahepatic cholestasis of pregnancy disease: a case-control study. BMC Pregnancy Childbirth. 2021; 21(1): 110, doi: 10.1186/s12884-021-03595-x, indexed in Pubmed: 33546617.

18. Liu X, Zheng J, Xin S, et al. Whole-exome sequencing expands the roles of novel mutations of organic anion transporting polypeptide, ATP-binding cassette transporter, and receptor genes in intrahepatic cholestasis of pregnancy. Front Genet. 2022; 13: 941027, doi: 10.3389/fgene.2022.941027, indexed in Pubmed: 36046230.

19. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics. 2009; 25(14): 1754–1760, doi: 10.1093/bioinformatics/btp324, indexed in Pubmed: 19451168.

20. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res. 2010; 38(16): e164, doi: 10.1093/nar/gkq603, indexed in Pubmed: 20601685.

21. McKenna A, Hanna M, Banks E, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 2010; 20(9): 1297–1303, doi: 10.1101/gr.107524.110, indexed in Pubmed: 20644199.

22. Chang YC, Yang YC, Tien CP, et al. Roles of Aldolase Family Genes in Human Cancers and Diseases. Trends Endocrinol Metab. 2018; 29(8): 549–559, doi: 10.1016/j.tem.2018.05.003, indexed in Pubmed: 29907340.

23. Yamamoto T, Kudo M, Peng WX, et al. Identification of aldolase A as a potential diagnostic biomarker for colorectal cancer based on proteomic analysis using formalin-fixed paraffin-embedded tissue. Tumour Biol. 2016; 37(10): 13595–13606, doi: 10.1007/s13277-016-5275-8, indexed in Pubmed: 27468721.

24. Santer R, Rischewski J, von Weihe M, et al. The spectrum of aldolase B (ALDOB) mutations and the prevalence of hereditary fructose intolerance in Central Europe. Hum Mutat. 2005; 25(6): 594, doi: 10.1002/humu.9343, indexed in Pubmed: 15880727.

25. Heinonen S, Kirkinen P. Pregnancy outcome with intrahepatic cholestasis. Obstet Gynecol. 1999; 94(2): 189–193, doi: 10.1016/s0029-7844(99)00254-9, indexed in Pubmed: 10432125.

26. Ahmad TR, Haeusler RA. Bile acids in glucose metabolism and insulin signalling - mechanisms and research needs. Nat Rev Endocrinol. 2019; 15(12): 701–712, doi: 10.1038/s41574-019-0266-7, indexed in Pubmed: 31616073.

27. Menżyk T, Bator M, Derra A, et al. The role of metabolic disorders in the pathogenesis of intrahepatic cholestasis of pregnancy. Clin Exp Hepatol. 2018; 4(4): 217–223, doi: 10.5114/ceh.2018.80122, indexed in Pubmed: 30603668.

28. Jin WY, Lin SL, Hou RL, et al. Associations between maternal lipid profile and pregnancy complications and perinatal outcomes: a population-based study from China. BMC Pregnancy Childbirth. 2016; 16: 60, doi: 10.1186/s12884-016-0852-9, indexed in Pubmed: 27000102.

29. Oppelt SA, Sennott EM, Tolan DR. Aldolase-B knockout in mice phenocopies hereditary fructose intolerance in humans. Mol Genet Metab. 2015; 114(3): 445–450, doi: 10.1016/j.ymgme.2015.01.001, indexed in Pubmed: 25637246.

30. Tran C. Inborn Errors of Fructose Metabolism. What Can We Learn from Them? Nutrients. 2017; 9(4), doi: 10.3390/nu9040356, indexed in Pubmed: 28368361.

31. James CL, Rellos P, Ali M, et al. Neonatal screening for hereditary fructose intolerance: frequency of the most common mutant aldolase B allele (A149P) in the British population. J Med Genet. 1996; 33(10): 837–841, doi: 10.1136/jmg.33.10.837, indexed in Pubmed: 8933337.

32. Brooks CC, Tolan DR. A partially active mutant aldolase B from a patient with hereditary fructose intolerance. FASEB J. 1994; 8(1): 107–113, doi: 10.1096/fasebj.8.1.8299883, indexed in Pubmed: 8299883.

33. Lau J, Tolan DR. Screening for hereditary fructose intolerance mutations by reverse dot-blot. Mol Cell Probes. 1999; 13(1): 35–40, doi: 10.1006/mcpr.1998.0208, indexed in Pubmed: 10024431.

34. Dalby AR, Tolan DR, Littlechild JA. The structure of human liver fructose-1,6-bisphosphate aldolase. Acta Crystallogr D Biol Crystallogr. 2001; 57(Pt 11): 1526–1533, doi: 10.1107/s0907444901012719, indexed in Pubmed: 11679716.

35. Choi KH, Shi J, Hopkins CE, et al. Snapshots of catalysis: the structure of fructose-1,6-(bis)phosphate aldolase covalently bound to the substrate dihydroxyacetone phosphate. Biochemistry. 2001; 40(46): 13868–13875, doi: 10.1021/bi0114877, indexed in Pubmed: 11705376.

Table 1. Five pairs of primers were used to sequence the 5 missense variants of the human *ALDOB* gene

Rs#	Patient	PCR Products [bp]	Forward primer [5'–3']	Reverse primer [5'–3']
rs747604233	ICP114	101	GCAGTTCCGAGAAATCCTCTT	CCTGGCTGTCCTTCTGGTAG
rs759204107	ICP58, ICP95	597	TGGCTTGCTCCTTATGCT	CTGCCTTCCAAAGTGCTG
rs758242037	ICP63, ICP79, ICP203	597	TGGCTTGCTCCTTATGCT	CTGCCTTCCAAAGTGCTG
rs371526091	ICP8	182	CTCTACCAAAGCCCTGGAAA	GTCAAGCCCCAAATGTGAAC
rs776189280	ICP21	461	CAGACAGGGTCAAGGTGG	TTGGATGAGGAGCCGATA

ICP — intrahepatic cholestasis of pregnancy

Table 2. Pathogenic prediction for 5 variants of the *ALDOB* gene in 249 Han Chinese people with intrahepatic cholestasis of pregnancy (ICP) disease

Rs#	Chr	Position	Alleles	Protein change	SIFT	PolyPhen2	Mutation taster	FATHMM	CADD
rs747604233	9	104192138	C/T	Gly75Arg	0.001 (D)	0.974 (D)	1 (D)	–2.07(D)	24.9
rs759204107	9	104192135	C/T	Gly76Ser	0.001 (D)	0.997 (D)	1 (D)	–3.9 (D)	24.7
rs758242037	9	104192047	A/C	Val105Gly	0.002 (D)	0.982 (D)	1 (D)	–2.07(D)	29.0
rs371526091	9	104187132	C/A	Arg331Leu	0.035 (D)	0.88 (P)	1 (D)	–2.2(D)	25.7

rs77718928	9	104184173	G/A	Ala338Val	0.0 (D)	0.994 (D)	1 (D)	–2.67(D)	29.9

Table 3. The frequencies of five possibly pathogenic variants in four public databases

Rs#	**MAF in samples**	**MAF in 1000G_ALL**	**OR (95% CI, p value)**[1]	**MAF in ExAC**	**OR (95% CI, p value)**[2]	**MAF in TOPMed**	**OR (95% CI, p value)**[3]	**MAF in gnomAD_exome**	**OR (95% CI, p value)**[4]
rs7476042233	2.008E-03	–	–	1.65E-05	122.58 (2.07–2385.85, 0.012)	1.89E-05	106.58 (2.25–977.17, 0.011)	1.19E–05	167.71 (3.21–2231.84,7.88E-03)
rs7592041074	4.016E-03	–	–	1.15E-04	35.05 (3.85–154.25, 1.92E-03)	4.16E-05	97.29 (10.43–454.09, 2.70E-04)	1.19E–04	33.92 (3.91–134.97, 1.86E-03)
rs7582420376	6.024E-03	–	–	5.77E-05	105.53 (17.53–470.70, 7.91E-06)	2.27E-05	369.76 (43.23–1240.12, 5.45E-07)	5.60E–05	108.76 (19.93–394.34, 5.17E-06)
rs371526091	2.008E-03	5.99E-04	3.36 (0.064–42.03, 0.32)	–	–	–	–	–	–
rs77718928	2.008E-03	–	–	–	–	1.28E-04	15.69 (0.38–94.44, 0.064)	–	–

MAF — minor allele frequency; OR— odds ratio; CI — confidence interval; [1–4] the OR and 95% CI were used to evaluate the correlation between *ALDOB* polymorphisms and ICP risk in different groups, including 249 samples and 1000G_ALL database, 249 samples and ExAC database, 249 samples and TOPMed database, 249 samples and gnomAD_exome database

Table 4. Clinical and biochemical data of the 8 patients with the five variants in the *ALDOB* gene

	rs7476042 33	**rs759204107**	**rs758242037**	**rs3715260 91**	**rs7771892 8**

Characteristics[1]	ICP114	ICP58	ICP95	ICP63	ICP79	ICP203	ICP8	ICP21
Basic information								
Age [years]	35	34	30	38	32	32	30	32
Gestational age [days]	245	272	260	262	277	233	262	240
BMI [kg/m^2]	27.3	30.3	28	27.9	22.2	22.5	38.5	22.7
Gravidity [times]	5	2	1	5	1	3	8	2
Parity [times]	2	1	0	1	0	1	3	1
Serum biochemical index								
K [3.5–5.1, mmol/L]	4.2	3.9	4.6	4	4.2	4	4	4
Na [135–145, mmol/L]	137	143	134	137	139	135	137	142
Cl [96–108, mmol/L]	103	108	104	105	103	104	102	107
Ca [2.1–2.9, mmol/L]	2.4	2.31	2.3	2.15	2.41	2.6	2.27	2.15
Mg [0.6–1.1, mmol/L]	0.7	0.82	0.8	0.89	0.91	0.7	0.77	0.83
P [0.85–1.51, mmol/L]	1.1	1.31	1.2	1.04	0.72	1.6	1.44	0.96
WBC [3.69–9.16, ×10^9/L]	6.83	7.58	7.06	7.04	7.92	7.73	8.16	6.74
RBC [3.68–5.13, ×10^{12}/L]	4.81	3.87	3.74	3.27	4.54	3.47	3.32	3.8
PLT [101–320, ×10^9//L]	118	93	196	107	168	269	75	152
RDW–SD [37–54, fL]	41.1	49.2	41.5	49.2	42.2	45.2	62.4	43.2
TBA [0–10, μmol/L]	67.3	16.3	21.5	33.3	34.9	29.9	26	75.4
CG [0–2.7, mg/L]	8.9	1.9	2.6	4.5	10.3	23.8	5.7	8.9
ALT [0–35, U/L]	104	7	45	94	12	142	301	447
AST [0–35, U/L]	92	13	46	63	22	104	244	221
GGT [9–64, U/L]	45	7	20	13	9	13	24	47
ALP [45–125, U/L]	279	110	115	138	109	174	209	187
TBIL [3.4–20.5, μmol/L]	16	11.2	9.8	11	13.3	32.1	22	16.4

DBIL [0–5, μmol/L]	6.1	2	4.3	2.8	3.1	15.8	8.7	6.9
IDBIL [0–14, μmol/L]	9.9	9.2	5.5	8.2	10.2	16.3	13.3	9.5
CHOL [0–5.2, mmol/L]	9.6	3.75	5.88	6.12	6.18	6.4	6.01	7.55
TG [0.34–1.69, mmol/L]	3.75	5.94	4.07	3.28	3.74	3.61	2.55	2.71
HDL [0.9–2, mmol/L]	1.93	0.92	1.61	1.82	2.16	1.95	1.85	1.47
LDL [0–3.74, mmol/L]	5.97	0.13	2.42	2.81	2.32	2.81	3	4.85
UA [155–357, μmol/L]	249	300	269	489	266	232	350	314
Outcomes of pregnancy women and newborn baby								
Birth weight [kg]	2.7	3.75	3.2	3.75	2.95	–	3.54	2.25
Apgar score [1–10]	9	9	9	10	10	–	10	9
Bleeding count [mL]	300	300	400	300	150	–	260	200

ALP — alkaline phosphatase; ALT — alanine transaminase; AST — aspartate transaminase; BMI — body mass index; CG — cholyglycine; CHOL — total cholesterol; DBIL — direct bilirubin; GGT — γ-glutamyl transpeptidase; HDL — high-density lipoprotein; ICP — intrahepatic cholestasis of pregnancy; IDBIL — indirect bilirubin; LDL — low-density lipoprotein; PLT — platelet; RBC — red blood cell; RDW-SD — red blood cell distribution width SD; TBA — total bile acid; TBIL — total bilirubin; TG — triglyceride; UA — uric acid; WBC — white blood cell

Table 5. The potential correlations between *ALDOB* variants and clinical and laboratory data in 249 Han Chinese patients with intrahepatic cholestasis of pregnancy (ICP) disease

Characteristics	**ICP without *ALDOB* variants**	**ICP with *ALDOB* variants**	**p value[3]**
Basic information			
Age [years]	29.09 ± 5.90 (n = 237)[1]	32.88 ± 2.52 (n = 8)	0.038
Gestational age [days]	263.68 ± 15.25 (n = 220)	256.37 ± 14.53 (n = 8)	0.19

BMI [kg/m^2]	25.74 ± 3.28 (n = 231)	27.43 ± 5.07 (n = 8)	0.41
Gravidity [times]	2.36 ± 1.45 (n = 236)	3.38 ± 2.29 (n = 8)	0.28
Parity [times]	0.64 ± 0.77 (n = 236)	1.13 ± 0.93 (n = 8)	0.08
Serum biochemical index			
K [mmol/L]	4 (3.8–4.2, 241)[2]	4 (4–4.2, 8)	0.34
Na [mmol/L]	137 (136–139, 241)	137 (136.50–139.75, 8)	0.35
Cl [mmol/L]	104 (102–105, 241)	104 (103–105.5, 8)	0.54
Ca [mmol/L]	2.38 (2.23–2.5, 241)	2.31 (2.24–2.40, 8)	0.41
Mg [mmol/L]	0.8 (0.7–0.83, 241)	0.81 (0.75–0.85, 8)	0.99
P [mmol/L]	1.18 (1–1.3, 241)	1.15 (1.02–1.34, 8)	0.92
WBC [×10^9]	8.07 (6.85–9.75, 241)	7.32 (6.99–7.78, 8)	7.38E-05
RBC [×10^{12}]	3.79 (3.52–4.1, 241)	3.77 (3.43–4.04, 8)	0.76
PLT [×10^9]	193 (156–236, 241)	135 (103.5–175, 8)	0.018
RDW-SD [fL]	45.4 (42.9–48.8)	44.20 (42.03–49.20)	0.67
TBA [μmol/L]	29.2 (19.2-52.35, 240)	31.60 (24.88–43.00, 8)	0.76
CG [mg/L]	7.6 (3.73–12, 190)	7.3 (4.03–9.25, 8)	0.58
ALT [U/L]	33 (10–175, 241)	99 (36.75–181.75, 8)	0.32
AST [U/L]	33 (18–129, 241)	77.50 (40–133.25, 8)	0.64
GGT [U/L]	18 (10–36, 236)	16.50 (12–29.25, 8)	0.18
ALP [U/L]	151 (115–219.75, 236)	156 (113.75–192.50, 8)	0.75
TBIL [μmol/L]	12.3 (9.9–16.6, 237)	14.65 (11.15–17.80, 8)	0.48
DBIL [μmol/L]	4.7 (3.5–7.5, 237)	5.20 (3.03–7.35, 8)	0.79
IDBIL [μmol/L]	7.2 (5.5–9, 237)	9.70 (8.95–10.98, 8)	0.033
CHOL [mmol/L]	6.17 (5.41–7.12, 233)	6.15 (5.98–6.69, 8)	0.86
TG [mmol/L]	3.18 (2.47–4.05, 233)	3.68 (3.14–3.83, 8)	0.71
HDL [mmol/L]	1.89 (1.64–2.25, 233)	1.84 (1.58–1.94, 8)	0.17
LDL [mmol/L]	2.82 (2,08–3.82, 233)	2.81 (2.40–3.46, 8)	0.87
UA [μmol/L]	315.5 (268.25–400, 238)	284.50 (261.75–323, 8)	0.45
Birth weight [kg]	3.1 (2.7-3.40, 218)	3.2 (2.83–3.65, 7)	0.54

Apgar score [1–10]	9 (9–10, 212)	9 (9–10, 7)	0.79
Bleeding count [mL]	240 (200–300, 214)	300 (230–300, 7)	0.78

ALP — alkaline phosphatase; ALT — alanine transaminase; AST — aspartate transaminase; BMI — body mass index; CG — cholyglycine; CHOL — total cholesterol; DBIL — direct bilirubin; GGT — γ-glutamyl transpeptidase; HDL — high-density lipoprotein; IDBIL — indirect bilirubin; LDL — low-density lipoprotein; PLT — platelet; RBC — red blood cell; RDW-SD — red blood cell distribution width SD; TBA — total bile acid; TBIL — total bilirubin; TG — triglyceride; UA — uric acid; WBC — white blood cell; [1]Mean ± standard deviation (n = number of samples); [2]Median (25^{th} percentile–75^{th} percentile, number of samples); [3]Significant differences are underlined

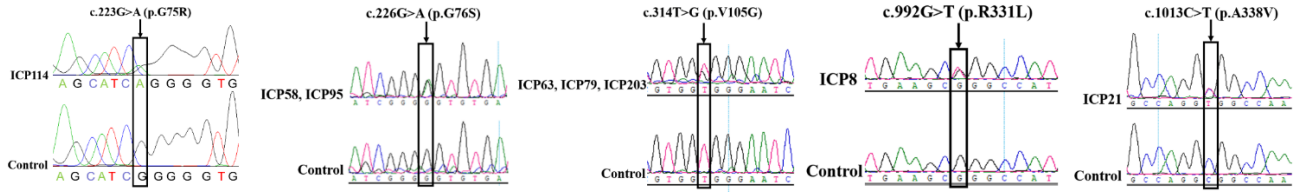

Figure 1. Sanger sequencing to validate the five missense variants, p.G75R, p.G76S, p.V105G, p.R331L and p.A338V, in the *ALDOB* gene. The variant location is marked with an arrow

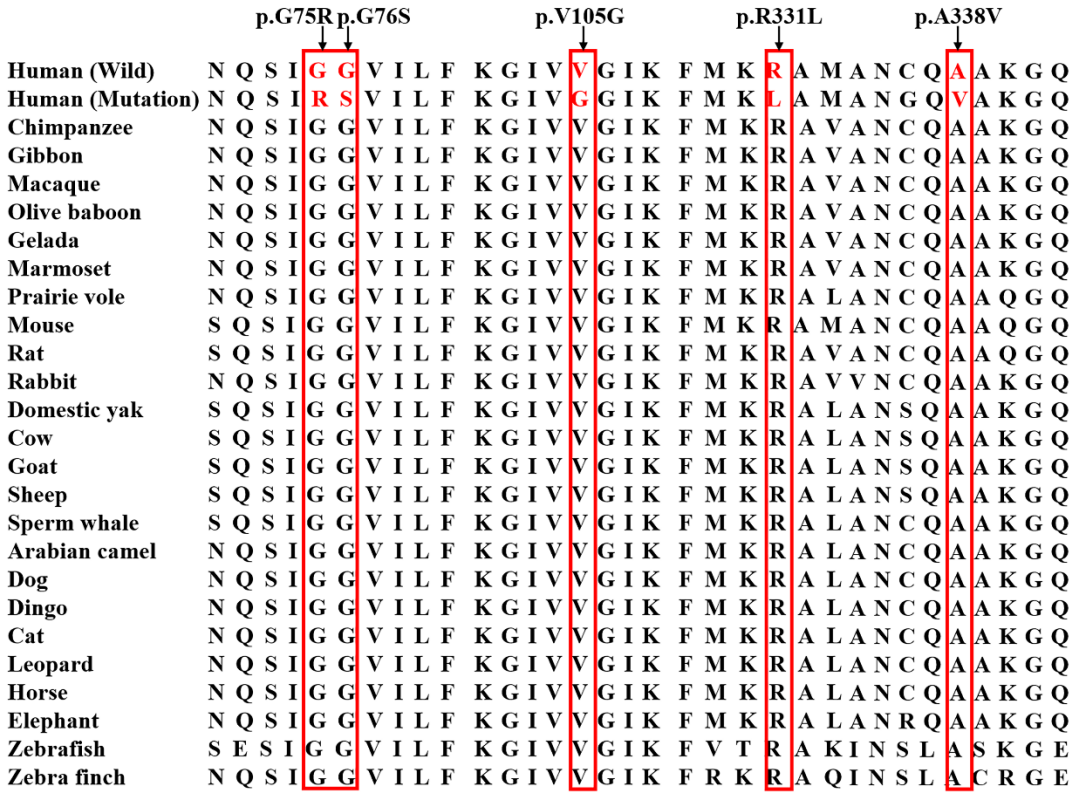

Figure 2. Evolutionary conservation analysis of the *ALDOB* p.G75R, p.G76S, p.V105G, p.R331L and p.A338V variants among 25 vertebrates, ranging from human to zebra finch. These five amino acids in the red horizontal line were highly conserved

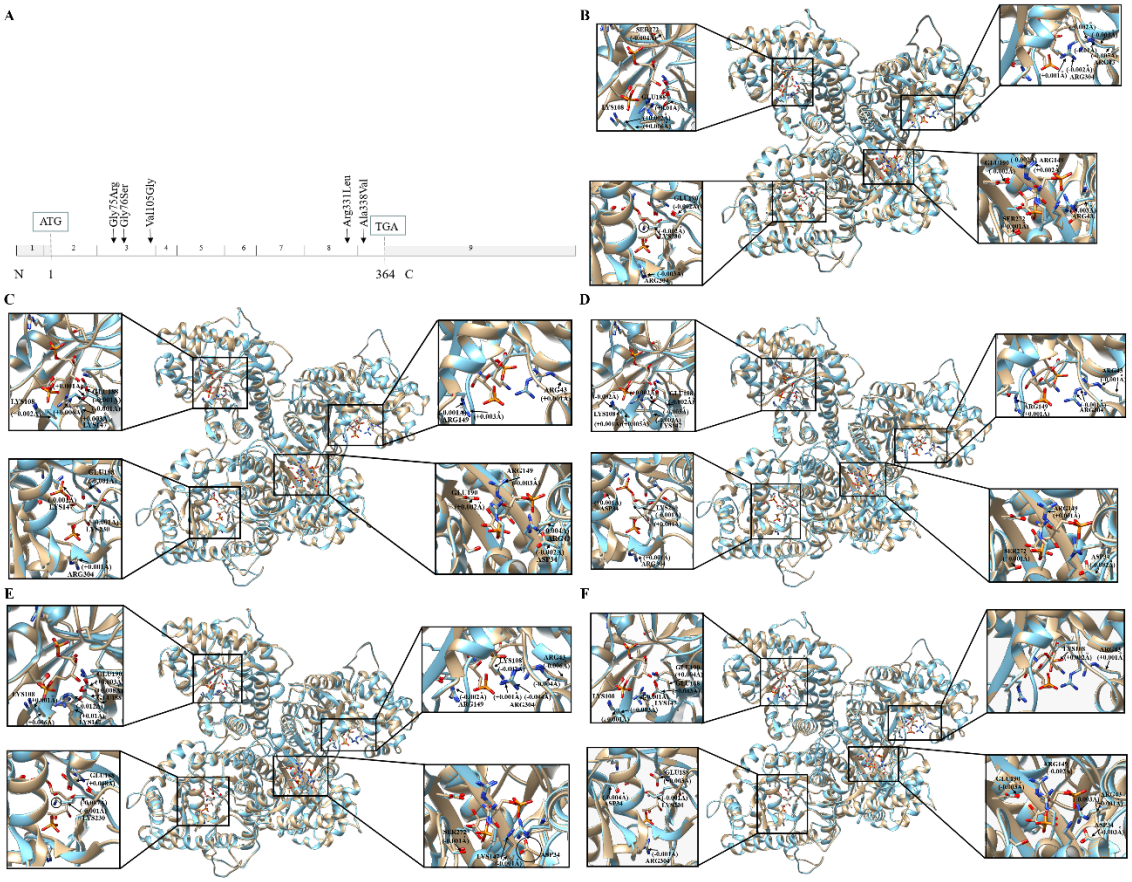

Figure 3. Effects of the five *ALDOB* variants on the protein sequence and structure; **A.** The distribution of five *ALDOB* variants. *ALDOB* is a 364-amino acid protein containing four functional domains that play important roles in substrate binding or catalysis. The locations of five *ALDOB* variants from WGS are shown in the protein sequence; **B.** Effects of the *ALDOB* p.G75R; **C.** p.G76S; **D.** p.V105G; **E.** p.R331L; **F.** p.A338V variants on the protein structure. The reference and modified molecules are presented as gold and blue rounded structures, respectively. The enlarged image shows that the four functional domains have small changes in the chemical bond lengths

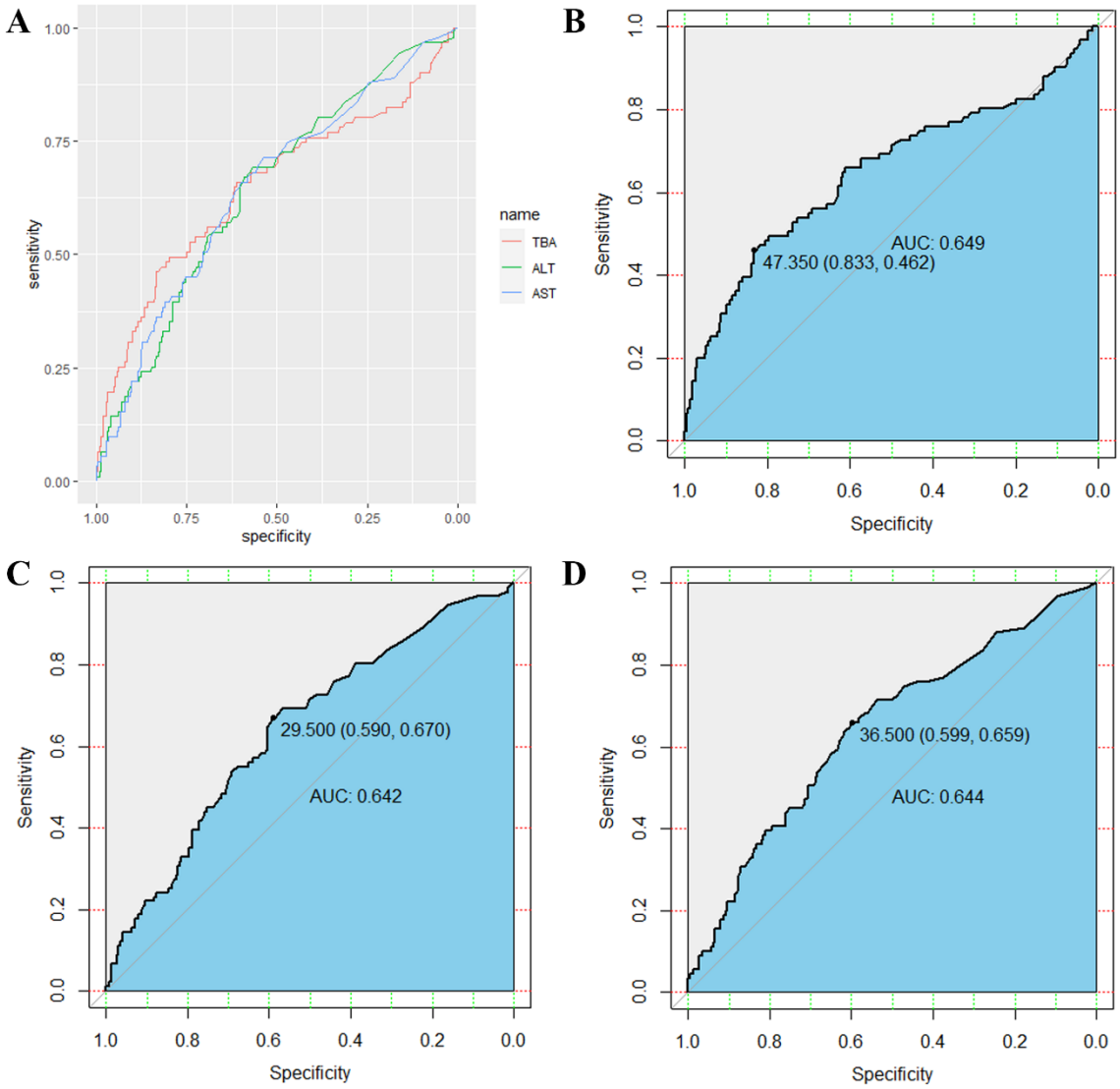

Figure 4. Receiver operating characteristic (ROC) curves for the associations between premature birth and three serum biochemical markers; **A.** Associations between premature birth and total bile acid (TBA), alanine transaminase (ALT), and aspartate transaminase (AST) levels; **B.** Association between premature birth and the TBA level; **C.** ALT level; **D** AST level; AUC — area under curve