Research Article

The intragenic epistatic association of ADD3 with biliary atresia in Southern Han Chinese population

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Biliary atresia (BA) is a multifactorial pathogenic disease with possible genetic components. As a member of membrane skeletal proteins in the liver and bile ducts, a haplotype composed by five single nucleotide polymorphisms (SNPs) on adducin 3 (ADD3) has been identified as associated with BA. However, limited study was designed to further elaborate the mutual relationship amongst those replicated SNPs to disease. We selected three susceptibility SNPs in ADD3 and conducted a replication study using 510 BA cases and 1473 controls to evaluate the individual function of the SNPs and further stratified the potential roles with disease and its subclinical features. Two SNPs in ADD3 were replicated as associated with BA (1.60E-04 ≤ P ≤ 1.70E-04, 1.33 ≤ odds ratio (OR) ≤ 1.58 for rs17095355, 2.10E-04 ≤ P ≤ 5.30E-04, 1.26 ≤ OR ≤ 1.57 for rs2501577). Though we failed to replicate the individual association of rs10509906 to disease, the intragenic epistatic effect between rs10509906 and rs2501577 was suggested as exhibiting susceptibility to BA, further cross-validated by multifactor dimensionality reduction (MDR) (P = 0.068, OR = 1.37), which may explain extra hidden heritability of ADD3 to BA. Furthermore, through subclinical stratification, we also observed the association of risk to disease mainly came from the female patients.

Introduction

Biliary atresia (BA) is a multifactorial pathogenic disease exclusively seen in infants. It is characterized by exacerbating jaundice and acholic stools caused by progressive intra- and extraportal bile duct fibrosis. The incidence of the disease varies amongst populations, ranges from 1 in 5000 in Asians to 1 in 18000 in Caucasians [1-3]. The etiology of BA is still unclear, several hypotheses have been raised, including perinatal virus infection, environmental toxins, and genetic defects [4,5].

Growing evidence indicates that BA is caused by genetic pre-exposures and later an environmental secondary strike [6]. Previous genome-wide association studies (GWASs) had identified common genetic variant (rs17095355) in 10q24.2 associated with BA [7]. Subsequently, Cheng et al. [8] revealed five single nucleotide polymorphisms (SNPs) in the intergenic region between X-prolyl aminopeptidase P (XPNPEP1) and adducin 3 (ADD3) as associated with BA, including rs17095355, rs10509906, rs2501577, rs6584970, and rs708605. Further, they found the risk haplotype may modulate the ADD3 expression level which varies in different status of the disease. However, whether the haplotype association acquired according to the linkage equilibrium or potential intragenic effect was not clear.

In the present study, we conducted a replication study of three SNPs in ADD3 using 510 BA cases and 1473 controls. The association of two SNPs in ADD3 was further replicated including rs17095355 and rs2501577. However, we failed to replicate the individual effect of SNP rs10509906 to disease. Haplotype analysis amongst the three included SNPs presented stronger association with disease than each individual SNP alone. We further identified intragenic epistasis between rs10509906 and rs2501577 which may elevate the risk of disease.
Materials and methods

Study subjects
Tissue samples were taken during surgical procedures from 510 sporadic BA patients recruited from 2000 to 2015. They were all claimed as unrelated Southern Han Chinese. The blood samples of 1473 geographically and ethnically matched controls were collected with no history of BA and related hepatobiliary disorders. The samples included in the current study were collected from Guangzhou Women and Children’s Medical Center. The study was approved by the institutional review board of the hospital. Written informed consents were provided by guardians of all patient subjects. All cases have undergone ultrasonography evaluation before surgical procedures. Diagnosis was made by intraoperation cholangiography and confirmed by pathology after surgery.

SNP genotyping and quality control
According to the previous study by Cheng et al. [8], five SNPs in ADD3 were reported as associated with BA, SNPs were annotated and scored according to RegulomDB (Supplementary Table S1). The detailed clinical information adopted for further analysis to the selected SNPs was summarized in Supplementary Table S2. Three SNPs (rs17095355, rs10509906, and rs2501577) in ADD3 were included according to the relative higher annotation score and lower linkage disequilibrium (LD, \( r^2 < 0.8 \)) in between as shown in Supplementary Figure S1. The samples were genotyped by MassARRAY iPLEX Gold system (Sequenom) based on the manufacturer’s instructions. The quality control steps were carried out as follows: all three SNPs passed the quality control with less than 10% missing data. SNPs were removed if Hardy–Weinberg equilibrium (HWE) \( P<0.05 \) calculated by control subjects.

Association analysis
SNP association were analyzed in a logistic model to detect the association between all SNPs and BA risk by comparing the allele frequency in patients and controls (basic allelic test) using PLINK1.9. Odds ratios (ORs) and corresponding 95% confidence intervals (CIs) were calculated from the logistic regression model. Genotype test of \( 3 \times 2 \) contingency tables, Cochran–Armitage trend test, test of the additive model, dominant and recessive model tests were also performed by PLINK1.9. [9].

Independence testing
HaploView was utilized to generate LD patterns and values in our study. Independent contribution tests for SNPs in ADD3 toward disease associations were done using logistic regression adjusting for the effect of a specific SNP in the same locus (SNPTEST v2.5b). [10].

Haplotype association analysis
First, all founders were phased using the E-M algorithm implanted in PLINK, which will generate haplotype-specific tests (1 df) for both disease and quantitative traits; an omnibus association statistic was also computed \( (P{\text{-}}\text{omnibus}) \). In all the cases, the tests were based on the expected number of haplotypes each individual had. Then the association was performed on all the most likely haplotype assignments as SNPs and use all the standard analytic options \( (P) \). The case/control omnibus test is a \( H-1 \) degree of freedom test, if there are \( H \) haplotypes.

Genetic epistasis effect analysis
Epistasis test (case–control analysis) was performed using parametric logistic regression (PLINK1.9) [11]. To further clarify the epistasis effect, we also used multifactor dimensionality reduction (MDR) analysis [12] to perform the pairwise non-parametric epistasis test. This method includes a cross-validation (CV) procedure as well as a permutation test which minimizes false positive results by multiple examinations of the data. By comparing the average prediction error from the observed data with the distribution of average prediction errors under the null hypothesis, the statistical significance was determined. The MDR analysis was carried out using version 2.0 of the open-source MDR software package (http://www.epistasis.org).

Results

Association of ADD3 SNPs with BA
Three previously identified SNPs in ADD3 were selected for replication using 510 patients and 1473 controls from South Chinese population (see selection details in ‘Materials and methods’ section). All the SNPs did not violate the HWE in the control subjects, further association analysis was performed as shown in Table 1. The association
Table 1 Replication results on three SNPs in ADD3 in Southern Han Chinese population using 510 cases and 1473 controls

<table>
<thead>
<tr>
<th>SNP</th>
<th>CHR Gene</th>
<th>Left_gene</th>
<th>Right_gene</th>
<th>Cheng et al. (Hepatology, 2013) [8]</th>
<th>BP</th>
<th>A1/A2</th>
<th>Cases</th>
<th>Controls</th>
<th>OR (CI 0.95)</th>
<th>P</th>
<th>P_adj</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs17095355</td>
<td>10 NA XPNPEP1</td>
<td>ADD3</td>
<td></td>
<td>MAF (case/control): 0.54/0.39</td>
<td>T/C</td>
<td>461/483</td>
<td>1212/1680</td>
<td>1.33 (1.15–1.54)</td>
<td>1.60E-04</td>
<td>1.20E-04</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>OR: 1.83 (1.45–2.31)</td>
<td>ADD</td>
<td>-</td>
<td>-</td>
<td>1.34 (1.15–1.56)</td>
<td>1.50E-04</td>
<td>2.58E-04</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>RISK allele: T</td>
<td>DOM</td>
<td>358/114</td>
<td>964/482</td>
<td>1.58 (1.24–2.00)</td>
<td>1.70E-04</td>
<td>2.27E-04</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P-value: 5.32E-06</td>
<td>REC</td>
<td>103/369</td>
<td>248/1198</td>
<td>1.35 (1.04–1.75)</td>
<td>0.02</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>rs10509906</td>
<td>10 NA XPNPEP1</td>
<td>ADD3</td>
<td></td>
<td>MAF (case/control): 0.52/0.31</td>
<td>G/A</td>
<td>461/479</td>
<td>1237/1671</td>
<td>1.31 (1.13–1.51)</td>
<td>3.60E-04</td>
<td>4.03E-04</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>OR: 1.61 (1.28–2.03)</td>
<td>ADD</td>
<td>-</td>
<td>-</td>
<td>1.30 (1.12–1.51)</td>
<td>5.30E-04</td>
<td>4.29E-04</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>RISK allele: G</td>
<td>DOM</td>
<td>357/113</td>
<td>972/482</td>
<td>1.57 (1.24–2.00)</td>
<td>2.10E-04</td>
<td>2.17E-04</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P-value: 8.28E-05</td>
<td>REC</td>
<td>104/366</td>
<td>265/1189</td>
<td>1.26 (0.98–1.63)</td>
<td>0.07</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>rs2501577</td>
<td>10 ADD3 XPNPEP1</td>
<td>MX1</td>
<td></td>
<td>*MAF (case/control): 0.54/0.42</td>
<td>G/A</td>
<td>461/479</td>
<td>1237/1671</td>
<td>1.31 (1.13–1.51)</td>
<td>3.60E-04</td>
<td>4.03E-04</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>OR: 1.61 (1.28–2.03)</td>
<td>ADD</td>
<td>-</td>
<td>-</td>
<td>1.30 (1.12–1.51)</td>
<td>5.30E-04</td>
<td>4.29E-04</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>RISK allele: G</td>
<td>DOM</td>
<td>357/113</td>
<td>972/482</td>
<td>1.57 (1.24–2.00)</td>
<td>2.10E-04</td>
<td>2.17E-04</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P-value: 8.28E-05</td>
<td>REC</td>
<td>104/366</td>
<td>265/1189</td>
<td>1.26 (0.98–1.63)</td>
<td>0.07</td>
<td>0.06</td>
<td></td>
</tr>
</tbody>
</table>

A1/A2 indicate the risk allele and protective allele to disease. F_AF_L indicates risk allele frequency of the SNP in cases or controls. The P-value indicate the significance based on allelic association tests. The calculation of OR is also based on the risk allele of each SNP. Abbreviations: BP, base pair where the SNP is located; CHR, chromosome; Gene.refgene, the gene where the SNP is located at; OR, odds ratio.

Table 2 Independence test by adjusting for the effects of other SNPs in the ADD3 region

<table>
<thead>
<tr>
<th>SNP</th>
<th>SNPs whose effects were adjusted*</th>
<th>SNPs whose effects were adjusted*</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs17095355</td>
<td>rs10509906</td>
<td>rs2501577</td>
</tr>
<tr>
<td>rs17095355</td>
<td>NA</td>
<td>P=5.3E-05</td>
</tr>
<tr>
<td></td>
<td>1.42 (1.20–1.68)</td>
<td>1.38 (1.03–1.86)</td>
</tr>
<tr>
<td>rs10509906</td>
<td>P=0.13</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>1.17 (0.95–1.44)</td>
<td>1.18 (0.95–1.46)</td>
</tr>
<tr>
<td>rs2501577</td>
<td>P=0.86</td>
<td>P=9.5E-05</td>
</tr>
<tr>
<td></td>
<td>0.97 (0.73–1.30)</td>
<td>1.41 (1.19–1.68)</td>
</tr>
</tbody>
</table>

*The data in each column represent the remaining effect of association (P-values) after adjusting for the effect of SNP(s) on the top row of each column.

of SNP rs17095355 was successfully replicated (OR = 1.33, P=1.6E-04), consistent with the previous GWAS [7,8]. We also replicated the association of SNP rs2501577, showing a comparable significance with SNP rs17095355 (OR = 1.31, P=3.6E-04); but failed to replicate the association of SNP rs10509906 (P=0.58). To better understand any potential genetic inherited pattern, we specified the association following additive, dominant, and recessive models. For two replicated SNPs in ADD3, larger effect was observed in both SNPs under the dominant models (OR = 1.58 for rs17095655 and OR = 1.57 for rs2501577, respectively).

The LD amongst the three SNPs based on Guangzhou samples were examined. The LD pattern was consistent with the 1000G data, showing limited LD of SNP rs10509906 with other two replicated SNPs (r² < 0.2). SNP rs17095355 and rs2501577 showed moderate LD with each other (r² = 0.72). According to the pairwise independence test (Table 2), we observed rs17095355 remains significance after adjusting for the effect of SNP rs2501577 (P=0.032); however, SNP rs2501577 was not significant conditioning on the effect of SNP rs17095355 (P=0.86), which may reflect the association mainly derived from SNP rs17095355.

Haplotype analysis

Haplotype was estimated using the standard E-M algorithm and Chi-square test was performed. We tested the association of different combinations on the three SNPs including rs17095355, rs10509906, and rs2501577 to BA (Table 3). More than one haplotype were observed as risk combinations associated with BA, the omnibus association was...
Table 3 The association of haplotypes derived from three SNPs on ADD3

<table>
<thead>
<tr>
<th>SNP combination</th>
<th>Haplotype</th>
<th>F_A</th>
<th>F_U</th>
<th>DF</th>
<th>P</th>
<th>OR (CI 0.95)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs17095355—rs10509906—rs2501577</td>
<td>OMNIBUS</td>
<td>NA</td>
<td>NA</td>
<td>2</td>
<td>4.23E-04</td>
<td>-</td>
</tr>
<tr>
<td>Risk</td>
<td>TG</td>
<td>0.48</td>
<td>0.41</td>
<td>1</td>
<td>2.23E-04</td>
<td>1.32 (1.15–1.53)</td>
</tr>
<tr>
<td>Protective</td>
<td>CG</td>
<td>0.32</td>
<td>0.38</td>
<td>1</td>
<td>5.07E-04</td>
<td>0.76 (0.65–0.88)</td>
</tr>
<tr>
<td>rs10509906—rs2501577</td>
<td>OMNIBUS</td>
<td>NA</td>
<td>NA</td>
<td>2</td>
<td>3.97E-04</td>
<td>-</td>
</tr>
<tr>
<td>Risk</td>
<td>CG</td>
<td>0.49</td>
<td>0.43</td>
<td>1</td>
<td>2.25E-04</td>
<td>1.32 (1.15–1.53)</td>
</tr>
<tr>
<td>Protective</td>
<td>GA</td>
<td>0.30</td>
<td>0.36</td>
<td>1</td>
<td>4.80E-04</td>
<td>0.75 (0.65–0.88)</td>
</tr>
<tr>
<td>rs17095355—rs2501577</td>
<td>OMNIBUS</td>
<td>NA</td>
<td>NA</td>
<td>3</td>
<td>2.67E-04</td>
<td>-</td>
</tr>
<tr>
<td>Risk</td>
<td>TG</td>
<td>0.46</td>
<td>0.39</td>
<td>1</td>
<td>4.82E-05</td>
<td>1.37 (1.18–1.58)</td>
</tr>
<tr>
<td>Protective</td>
<td>CG</td>
<td>0.02</td>
<td>0.04</td>
<td>1</td>
<td>0.028</td>
<td>0.59 (0.38–0.93)</td>
</tr>
<tr>
<td>Protective</td>
<td>CA</td>
<td>0.49</td>
<td>0.54</td>
<td>1</td>
<td>3.25E-03</td>
<td>0.80 (0.69–0.92)</td>
</tr>
<tr>
<td>rs17095355—rs10509906—rs2501577</td>
<td>OMNIBUS</td>
<td>NA</td>
<td>NA</td>
<td>4</td>
<td>2.19E-04</td>
<td>-</td>
</tr>
<tr>
<td>Risk</td>
<td>TG</td>
<td>0.47</td>
<td>0.39</td>
<td>1</td>
<td>1.88E-05</td>
<td>1.38 (1.19–1.59)</td>
</tr>
<tr>
<td>Protective</td>
<td>CG</td>
<td>0.02</td>
<td>0.04</td>
<td>1</td>
<td>0.047</td>
<td>0.64 (0.41–0.99)</td>
</tr>
<tr>
<td>Protective</td>
<td>CGA</td>
<td>0.29</td>
<td>0.34</td>
<td>1</td>
<td>1.79E-03</td>
<td>0.78 (0.66–0.91)</td>
</tr>
</tbody>
</table>

F_A indicates risk allele frequency of the SNP in each subclinical group. F_U indicates protective allele frequency of the SNP in each subclinical group. Abbreviation: DF, degree of freedom.

Table 4 Pairwise epistatic interacting results amongst three independent variants in ADD3 done by logistic regression and MDR

<table>
<thead>
<tr>
<th>SNP</th>
<th>ADD3 rs17095355</th>
<th>ADD3 rs10509906</th>
<th>ADD3 rs2501577</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs17095355</td>
<td>MDR</td>
<td>Logistic regression</td>
<td>P_{jnt}=0.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P_{jnt}=0.54</td>
</tr>
<tr>
<td>rs10509906</td>
<td>NA</td>
<td>Logistic regression</td>
<td>P_{jnt}=0.068</td>
</tr>
<tr>
<td>rs2501577</td>
<td></td>
<td></td>
<td>P=0.0001</td>
</tr>
</tbody>
</table>

Cross-validation consistency (CVC) reflects the number of times MDR analysis identified the same model as the data were divided into different segments.

adopted to calculate the overall association of haplotypes to disease. For the replicated two SNPs (rs17095355 and rs2501577), the risk allele combination T-G showed more significant association with disease than each individual SNPs alone (OR equal to 1.37 and P equal to 4.82E-05 for the haplotype compared with OR equal to 1.33 and P equal to 1.6E-04 for the most associated SNP rs17095355). Interestingly, we also observed SNP rs10509906(G) may play potential role with SLE. We observed T-G-G formed by the risk allele of the three SNPs was the strongest risk haplotype with a combined OR of 1.38 and P-value equal to 1.88E-05. Thus, SNP rs10509906 may play unknown role, such as intragenic epistasis, which may elevate the risk to disease.

Intragenic epistasis in ADD3 to BA

Based on the haplotype association results, we speculated potential genetic epistasis amongst the three SNPs in ADD3 involved in the present study may affect the risk to BA. Pairwise epistasis test was performed using logistic regression implemented in PLINK. We observed marginal epistatic association for rs10509906 and rs2501577 with BA as shown in Table 4 (P=0.068, OR = 1.37). This piece of data was consistent with the findings in haplotype association that risk haplotype combination slightly improved the association with disease. However, due to sample size limitation and larger sample size requirement for epistasis validation, the results failed to reach the statistical significance (P < 0.05). To conquer this shortcoming, we adopted the classic non-linear regression analysis method, MDR, to cross-validate the findings through logistic regression. Significant pairwise interaction between rs10509906 and rs2501577 was observed as shown in Table 4 (left bottom panel).
Clinical stratification of risk haplotype and replicated SNPs in ADD3 with BA

The presence of cystic structure at the hilus was usually considered as cystic BA (CBA), which involved improved bile drainage and better native liver survival rate after surgery [13,14]. The existence of the subclinical symptoms might have underlying genetic background. Consistent with the previous epidemiological finding reported the presence of CBA in 5–10% of the patients [13,15], in our study there are 8.7% (44 out of 506 patients) patients diagnosed as CBA [14]. There was also a report showing slightly higher female predominance in BA with an incidence ratio equal to 1.4 compared with 1 [16,17]. We examined our BA samples, the female to male ratio was opposite to the previous study equal to 1 compared with 1.4. We further examined whether there exist unrevealed associations between the specific subclinical presentation and the major risk haplotypes. We examined the replicated SNPs in ADD3 through subphenotype-control analysis including CBA compared with controls, non-CBA compared with controls and patients with different genders. As shown in Table 5, we failed to observe any tremendous differences between classified subclinical types. Interestingly, we found the risk of T-G-G to disease mainly came from the female patients ($P=9.10E-04$) other than male patients ($P=0.59$). Consistently, the association of individual replicated SNP to disease in female patients was more significant compared with males. This result may partially give us some clue on the mild difference genders to BA, of course, further functional validation was required for clearly understanding the mechanism of disease.

### Discussion

BA is known as a multifactorial pathogenic disease with genetic components. The development of GWAS offers a different way of insight into the etiology of the disease. Garcia-Barcelo et al. [7] found several SNPs including rs17095355 on chromosome 10q24.2 strongly associated with BA. This SNP is in the intergenic area and flanked by the XPNPEP1 and ADD3 genes. ADD3 was found widely expressed in the liver and biliary epithelial cells and played roles in remodeling of membrane cytoskeleton at points of cell–cell contact. XPNPEP1 is also found in liver epithelial cells which is important in bile acid excretion. Cheng et al. [8] from the same team fine-mapped the region in a larger case–control group and found the SNP rs17095355 achieved significance with a $P$-value of $10^{-10}$. A risk haplotype comprising five SNPs (rs17095355, rs10509906, rs2501577, rs6584970, and rs7086057) was identified strongly correlating with BA. Furthermore, the risk haplotype tagged by 17095355 was proved to be a cis-regulator of ADD3, presented the haplotype significantly down-regulated ADD3 gene expression in BA liver. We further examined the functional role of three included SNPs according to the public available Genotype Tissue Expression (GTEx) project as genetic consequences of molecular characterization. As shown in the Supplementary Figures S2 and S4, we observed the two replicated SNPs serve as liver-specific expression quantitative trait loci (eQTL) which exhibited the risk alleles were associated with the abnormal expression of ADD3 ($P=2.8E-03$ for rs17095355, $P=3.4E-05$ for rs2501577). SNP rs10509906 showed no evidence of correlation with Add3 expression in liver, which was consistent with the lack of evidence of individual association for the SNP to disease in current study (Supplementary Figure S3).

So far, the association studies of ADD3 SNPs were investigated in the context of LD, the independent effects and intragenetic epistasis of the SNPs has not been insighted before. We replicated the result of haplotypes of ADD3 with the risk of BA in Southern Han Chinese population. Consistent with previous studies, the association of rs17095355 and rs2501577 were successfully replicated, however, the association of 10509906 was not significant in our cohort.
On the other hand, by comparing the haplotypes with single individual SNP, we found that the risk allele rs17095355(T) and rs2501577(G) combination showed more significant association with disease than each SNP alone. Interestingly, the combination of SNP rs10509906(G) and rs2501577(G) exhibited a stronger effect size to disease by comparing with SNP rs2501577 alone. These results indicate that epistasis effects amongst SNPs may play an important role in the onset of BA. Intra-genetic epistasis was observed between rs10509906 and rs2501577. More and more evidence supporting the theory of interlocus interaction strongly influencing the risk of complex trait [18]. These findings may indicate the existence of synergy effect amongst rs10509906 and its nearby SNPs.

CBA is a special subclinical type of BA. It was usually diagnosed prenatally. Besides the characteristic cystic lesion at the hilus, the patient with CBA has better jaundice clearance rate and longer native liver survival time. Whether these distinctions are caused by a genetic variation or environmental interactions remain largely unknown. In the present study, we replicated SNPs in ADD3 through CBA-control analysis, but we failed to observe any tremendous difference between subclinical types. Further efforts should be taken in finding variants associated with CBA in a study with larger sample size before any conclusion could be drawn.

Conclusion

Although the mechanism of SNPs associated with BA risk still remains largely undetermined, we successfully replicated two common variants as associated with BA, our study proposed a possibility that epistasis effect may play roles in BA pathogenesis. Further investigation could focus on verification of such effect on transcription and gene expression level.

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Competing interests

The authors declare that there are no competing interests associated with the manuscript.

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Author contribution

All the individuals involved in the present study gave informed consents for research publication. The study was approved by the Institutional Review Board. All the data involved in the study can be supplied upon request. H.X., Y.Z., R.-Z.Z., and J.Z. designed the study and revised the manuscript. Z.W., Y.Z., and J.Z. analyzed, interpreted the data, and drafted the manuscript. Z.W., X.X., Y.L., and W.Z. performed the surgical operations and collected clinical samples. Z.W., X.X., M.F., and W.Z. collected the clinical information and took charge of the clinical sample arrangement.

Abbreviations

ADD3, adducin 3; BA, biliary atresia; CBA, cystic BA; GWAS, genome-wide association study; HWE, Hardy–Weinberg equilibrium; LD, linkage disequilibrium; MDR, multifactor dimensionality reduction; OR, odds ratio; SNP, single nucleotide polymorphism; XPNPEP1, X-prolyl aminopeptidase P.

References


