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Association between gallstone diseases and six gene SNPs linked to lipid traits in the Chinese Population

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Abstract

Background: The association between six gene SNPs, namely PCSK9 p. Arg93Cys (rs151193009), LDLR p. Arg257Trp (rs200990725), CETP p. Asp459Gly (rs2303790), PKD1L3 p. Thr429Ser (rs7185272), PNPLA3 p. Ile148Met (rs738409), and TEAD2 p. Asp12Asn (rs142665148), and lipid metabolism has been reported in Journal of Nature Communications. Abnormal lipid metabolism is an important risk factor for gallstone disease. Gallstones are categorized into three sub-types: cholesterol, pigment, and mixed gallstones, based on composition. In this study, the association between these six gene SNPs and gallstone disease was investigated, and differences in the degree of mutations among the three subtypes of gallstone were determined.

Method: Venous blood (5 ml) and gallstones were collected from 100 patients who were treated as gallstone group. In addition, 5 ml of samples of venous blood were taken from 30 healthy volunteers who served as control group. Fourier transform infrared spectroscopy was used to determine gallstone components. There were two gallstone groups: cholesterol-type and non-cholesterol-type gallstone groups. Then, Mass ARRAY mass spectrometer was used to identify the mutants of the loci in the blood samples, and also for analysis of differences between the groups.

Results: 1. Significant difference was found between the gallstone and control groups in the degree of mutation in TEAD2 (rs142665148; $p=0.039<0.05$). The degree of mutation in TEAD2 (rs142665148) in the gallstone group was significantly higher than in the control group. No significant differences were found in the degree of mutations in PKD1L3 (rs7185272), PNPLA3 (rs738409), PCSK9 (rs151193009), LDLR (rs200990725), and CETP (rs2303790) between the gallstone and control groups ($p>0.05$). 2. Significant differences were found in the degrees of mutations in PKD1L3 (rs7185272) and PNPLA3 (rs738409) between the cholesterol-type and non-cholesterol-type gallstone groups ($p <0.05$). The degrees of mutation in PKD1L3 (rs7185272) and PNPLA3 (rs738409) in the cholesterol-type gallstone group were significantly higher than those in the non-cholesterol-type gallstone group. 3. No significant differences were found in degree of mutations in PCSK9 (rs151193009), LDLR (rs200990725), CETP (rs2303790), and TEAD2 (rs142665148) between the cholesterol-type and non-cholesterol-type gallstone groups ($p >0.05$).

Conclusion: 1. SNPs in TEAD2 (rs142665148), PKD1L3 (rs7185272), and PNPLA3 (rs738409) are associated with gallstone diseases. 2. Associations of SNPs in PKD1L3 (rs7185272) and PNPLA3 (rs738409) are more significant in the cholesterol-type gallstones than in the non-cholesterol-type type. There are significant differences in association of SNPs in TEAD2 (rs142665148) between the cholesterol-type and non-cholesterol-type gallstones.

Keywords: Lipid trait, gallstone disease, Fourier transform infrared spectrum, MassARRAY mass spectrometer, SNP.

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1. Introduction

Gallstone disease is a disease with high incidence of 10–15% in developed countries (1). In China, the incidence of gallstone disease is approximately 10% (2). Treatments for gallstone diseases are expensive. Studies have shown that in 2004, the conservative cost of treatment for gallstone disease in the US was approximately 4 billion USD, while the cost of surgical treatment reached more than 6.5 billion USD (3-4). Therefore, studies on gallstone diseases are very important. Gallstone consists mostly of cholesterol, bile pigment, phospholipids, proteins, bacteria, metal ions, and other components (5). However, cholesterol and bile pigments account for the largest proportions. Gallstone has been classified conventionally into three types: cholesterol gallstone, pigment gallstone, and mixed gallstone. The classification criteria are based on cholesterol content. The cholesterol and pigment types have cholesterol content of more than 70 and less than 30%, respectively. Other components constitute mixed type gallstones. Pigment and mixed gallstones are also called non-cholesterol gallstones. Several hypotheses have suggested the possible causes of gallstones. Evidence from studies have suggested that abnormal lipid metabolism may be responsible for gallstone formation. Abnormal lipid metabolism induces lipid metabolism disorders, excessive cholesterol secretion and supersaturation, cholesterol crystallization, mucus overproduction, and imbalance between nucleating and anti-nucleation factors. These phenomena lead to gallstone formation (6).

Abnormal lipid metabolism is related to several factors such as age, sex, and eating habits, as well as polymorphism in lipid metabolism loci. Thus, studies on polymorphism of lipid metabolism loci could enhance understanding of the pathogenesis of gallstone disease. In 2013, a meta-analysis identified 157 loci associated with blood lipid levels in European ancestry, of which 62 loci were not previously associated with blood lipids (7). In 2015, the University of Hong Kong conducted a large-scale screening of lipid metabolism gene loci for Asians (7).

Six loci: *PKD1L3* (rs7185272), *PNPLA3* (rs738409), *PCSK9* (rs151193009), *LDLR* (rs200990725), *CETP* (rs2303790), and *TEAD2* (rs142665148), were selected, out of which *CETP* (rs2303790), *LDLR* (rs200990725), and *PCSK9* (rs151193009) were Asian-specific (8).

The *PNPLA3* locus affects regulation of lipid metabolism, and it is strongly correlated with non-alcoholic fatty liver disease (9). A previous study showed that *PNPLA3* affects the secretion of very low-density lipoprotein (VLDL) and lipid metabolism in hepatocytes (10). Moreover, PCSK modulates *LDLR* which plays an important role in lipid metabolism, while *CETP* promotes exchange between cholesterol and triglycerides. The *TEAD2* locus affects the synthesis of phospholipids, which are critical components needed in synthesis of HDL. These genes are closely related to lipid metabolism (11). Although most studies have suggested that *PKD1L3* is associated with acid taste receptors, the results from a study by the University of Hong Kong showed that *PKD1L3* is closely related to lipid metabolism. As important pathogenic factors in gallstone diseases, it can be speculated that these six genes may affect gallstone formation.

Cholesterol is involved a vast majority of cholesterol gallstones. Although cholesterol is also their main component, non-cholesterol gallstone also contains bile pigment. There may be differences among the aforementioned six genetic polymorphisms affecting lipid metabolism. It has been reported that the six gene loci are related to lipid metabolism in Chinese population. Indeed, *LDLR* has been correlated with gallstone disease (12), but the relationship between gallstone disease and *PKD1L3* (rs7185272), *TEAD2* (rs142665148), and *PNPLA3* (rs738409) has not been reported. To study the relationship between these genetic loci and gallstone disease, a new screening index should be used for screening Chinese populations for groups susceptible to gallstone disease. This would lead to elucidation of the mechanism of cholesterol gallstone disease, leading to establishment of a new perspective and target for treating the disease.

Many methods such as electrophoresis, enzyme digestion, direct sequencing, bioinformatics, and DNA chip, are used to detect single nucleotide polymorphism (SNP) (13). Given the large sample size, the experiment needed a detection method that can simultaneously detect multiple samples and multiple indices. Thus, MassArray mass spectrometry was chosen. It yields accurate typing

results, has flexible experimental design, and is an ideal platform for parallel detection of polygenes. We collaborated with Huada Gene Company for analysis of polymorphism of the gene loci using mass spectrometry.

In this study, Fourier transform infrared spectroscopy (FTIR) was used to classify the gallstones. It has a unique advantage in stone analysis, because a small number of samples can be used in this process to determine the components of stones and the content and distribution of different components in the stone (14). It has been reported that no differences were found between the qualitative analysis of gallstone classification using FTIR, and quantitative analysis with UV spectrophotometer. Several studies outside China have reported good accuracy of FTIR in stone analysis (15). Therefore, FTIR was adopted in this study. Cholesterol, bile pigment, and other components have characteristic peaks which can be considered if the measured peaks basically coincide with these characteristic peaks.

In this study, gallstones were classified using FTIR, and the SNPs in the venous blood of patients and healthy volunteers were analyzed using MassArray mass spectrometry. Then, a statistical method was used to analyze the possible associations between the six gene loci polymorphisms and gallstone disease. The possibility of differences in the mutations in the various types of gallstone was also determined.

2. Materials and methods

The experimental flow chart is shown in figure 1.

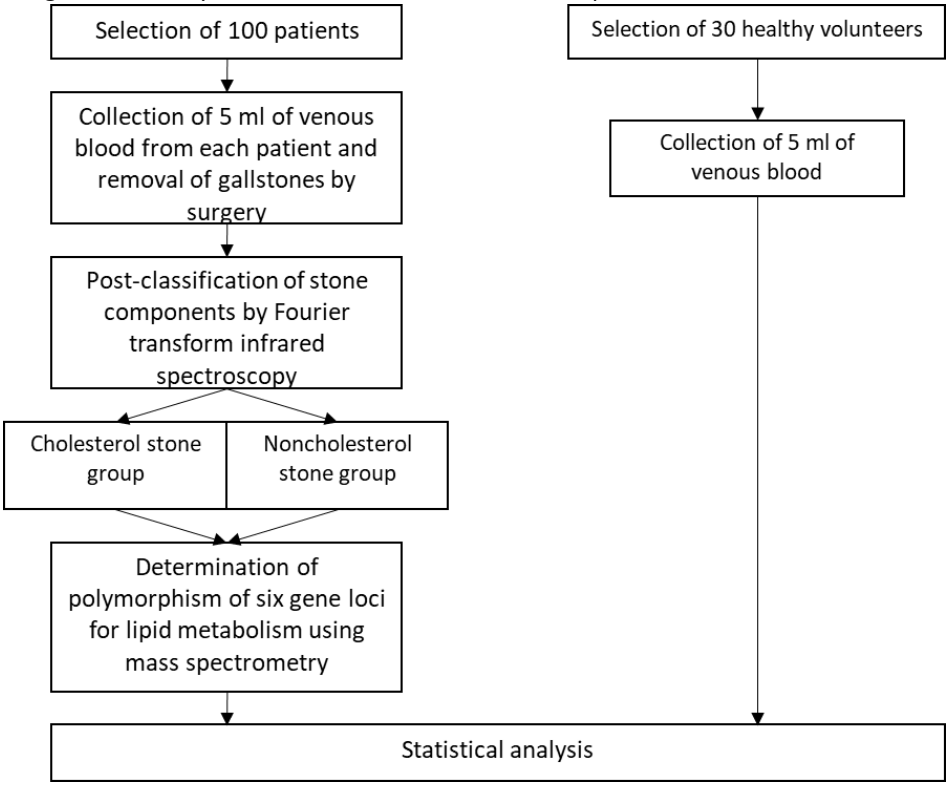

Figure 1: *Flow chart depicting the set-up of the study*

Study population

A total of 100 patients who were scheduled for laparoscopic cholecystectomy under general anesthesia were recruited from October 2015 to June 2016. A total of 100 gallstones were collected from the patients, and 5 ml of venous blood was obtained from each participant. In addition, 5 ml of venous blood was collected from 30 healthy volunteers. Prior to the study, written informed consent was obtained from all participants.

Participants were included or rejected from the study based on established inclusion and exclusion criteria. The inclusion criterion was Han Chinese ethnicity. Pregnant women and individuals with blood diseases, replacement of heart valve, long-term use of oral contraceptives, and individuals who were on long-term estrogen replacement therapy were excluded from the study.

Experimental procedures

The venous blood samples (5-ml) were put into test tubes containing citric acid antacid. Gallstones were collected from patients undergoing cholecystectomy. Samples were also collected from 30 healthy volunteers.

Detection of gallstone by FTIR

The gallstones were ground into powder, and 1–2 mg of the gallstone powder was finely mixed with 200 mg of pure KBr. The mixture was placed in an infrared pressing die and pressed. The resulting mixture was then pressed into a transparent sheet at a pressure of $5–10\times10^7$ Pa. The KBr and gallstone powder were dried and ground to less than 2 μm. Pure KBr was used to produce a blank piece of KBr, using the above method. The pressed KBr blank was put on the sample frame in the sample bin of the FTIR spectrometer, and the lid was closed. After recording the reference background, the gallstone specimens were analyzed one by one according to the requirements, and the spectra were recorded.

Determination of DNA in blood samples

DNA extraction

Whole blood tablet was taken from a 96-well plate with BSD punching instrument. Then, 80 μl of 5% Chelex-100 solution was added. The resulting solution was heated to 100 °C for 10 min, cooled, and then centrifuged at 4000 rpm for 10 min. Then, 30 μl of the resultant supernatant was used for analysis.

Multiple PCR detection by mass spectrometry

The ADS2.0 software (Agena Bioscience Company) was used to design the primers, and the sequences are listed in Table 1. The primers were synthesized by Liuhe Huada Gene Technology Co., Ltd., Beijing. In this process, DNA (1 μl) was obtained and mixed with 0.95 μl of water, 0.625 μl of PCR buffer (containing 15 mM $MgCl_2$, 0.325 μl of 25 mM $MgCl_2$), 1 μl of 2.5 mM dNTP, 1 μl of PCR primers, and 0.1 μl of HotStar Taq enzyme. Then, the PCR reaction was performed under the following conditions: 45 cycles of 94 °C for 15 min, 94 °C for 20 sec, 56 °C for 30 sec, and 72 °C for 1 min, and finally at 72 °C for 3 min. The remaining dNTP was dephosphorylated. The reaction system consisted of 1.53 μl of water, 0.3 ul alkaline phosphatase, and 0.17 μl of SAP buffer. The reaction was performed at 37 °C for 40 min, and then at 85 °C for 5 min to inactivate alkaline phosphatase. The SNP single nucleotide extension primers were reacted with the following solution: 0.75 μl of water, 0.2 μl of 10x iPLEX buffer, 0.2 μl of iPLEX enzyme termination mixture, and 0.804 μl of 10 μM of primer extension. The single-base extension reaction condition was as follows: 94 °C for 30 sec; 94 °C for 5 sec; 52 °C for 5 sec; 80 °C for 5 sec, with 5 cycles (a total of 40 cycles). The reaction was terminated after 72 °C for 3 min.

Mass spectrometry detection

The PCR product board was centrifuged for 5 min at 4000 rpm. Ultrapure water (19 μl) was added to each reaction well, and the solution was centrifuged for 1 min. Then, the resin was coated and allowed to stand at room temperature for 15–30 min. The resin was added to the product board, and the film was sealed and placed on a quiet mixer, followed by mixing for approximately 40 to 60 min. The samples were loaded onto the SpectroCHIP using MassARRAY Nanodispenser. A co-crystallized film of chip matrix and sample was prepared and analyzed with a mass spectrometer. Data were read using MassARRAY RT software system, while the genotypes were analyzed using MassARRAY Typer software.

Statistical analysis

The data were sorted out with SPSS19.0, and four-grid χ^2 test was used for statistical analysis. In addition, $\alpha=0.05$ was the test standard, and the 2-tailed probability value of $p< 0.05$ was used as the criterion to judge statistical significance.

The blood of the 100th patient was contaminated. Thus, this sample was not tested using MassArray mass spectrometer. No result was obtained for this patient.

3. Results

TEAD2 (rs142665148)

The four-grid table χ^2-test (Fisher's exact test) was used to determine statistical significant difference in degree of mutation between the gallstone and control groups. The degree of mutation in the gallstone group was significantly higher than that in the control group ($p<0.05$). However, no statistical difference was found in degree of mutation between the cholesterol and non-cholesterol gallstone groups (p =0.212>0.05). **Table 1.**

PCSK9 (rs151193009)

The four-grid table χ^2-test (Fisher's Exact Test) was used to determine differences in degree of mutation between the gallstone and control groups, and the results showed no statistically significant difference (p =0.412>0.05). The same text also revealed no statistically significant differences in degree of mutation between the cholesterol and

non-cholesterol stone groups (*P* =0.788>0.05). Table 1.

Table 1: *TEAD2* and *PCSK9* expression

Influence of shikonin on incidence and severity of arthritis in CIA mice

Group	*TEAD2*				*PCSK9*			
	Positive	Negative	χ^2	P	Positive	Negative	χ^2	P
Gallstone	58	42	13.33	0.003	47	53	0.46	0.50
Control	6	24			12	18		
Cholesterol gallstone	45	26	0.08	0.78	37	42	0	0.95
Non-choloesterol gallstone	13	8			10	11		

PKD1L3 (rs7185272)

The four-grid table χ^2- test (Continuity Correction) was used to compare mutation rates between the gallstone and control groups. The results showed no statistically significant difference (χ^2=0.84, *p*=0.36>0.05). The four-grid table χ^2-test (Fisher's Exact Test) was used to test the degree of mutation in the cholesterol and non-cholesterol stone groups. The degree of mutation in the non-cholesterol stone group was significantly higher than that in the cholesterol stone group (*p*=0.04<0.05). Thus, further analyses and comparison of difference between the non-cholesterol gallstone and control groups were carried out. The degree of mutation in the non-cholesterol stone group was significantly higher than that in the control group (*p* <0.05). **Table 2.**

PNPLA3 (rs738409)

The four-grid table χ^2 test (Continuity Correction) was used to analyze differences in the degrees of mutation between the gallstone and control groups. The results showed no statistically significant difference between the two groups (χ^2=0.111, *p* =0.739>0.05). The four-grid table χ^2-test (Continuity Correction) was used analyze the degrees of mutation between the cholesterol and non-cholesterol gallstone groups. Mutation in the non-cholesterol stone group was significantly higher than that in the cholesterol stone group (χ^2=0.855, *p* =0.015<0.05). Further analysis and comparison were carried out between the non-cholesterol gallstone and control groups. The results revealed that the degree of mutation in the non-cholesterol stone group was significantly higher than that in the control group (*P* <0.05). Table 2.

Table 2: *PKD1L3* and *PNPLA3* expression

Group	*PNPLA3*				*PKD1L3*			
	Positive	Negative	χ^2	P	Positive	Negative	χ^2	P
Gallstone	55	45	2.08	0.15	57	43	1.73	0.19
Control	12	18			13	17		
Cholesterol gallstone	48	31			44	21		
Non-choloesterol gallstone	7	14	5.04	0.02	8	13	5.82	0.02

LDLR (rs200990725)

All mass spectrometer tests were positive when the gallstone and control groups, as well as the cholesterol and non-cholesterol stone groups, were compared. No statistically significant differences were found in both comparisons (*p* >0.05). Table 3.

CETP (rs2303790)

The four-grid table χ^2-test (Fisher's Exact Test) showed no statistically significant difference in the degree of mutation between the gallstone and control groups (*p* =0.767>0.05). Moreover, using the same test, there were no statistically significant differences in the degree of mutation between the

cholesterol and non-cholesterol gallstone groups (p =0.788>0.05). Table 3.

Table 3: *LDLR* and *CETP* expression

Group	*LDLR*				*CETP*			
	Positive	Negative	χ^2	P	Positive	Negative	χ^2	P
Gallstone	52	48	0.69	0.41	46	54	2.77	0.10
Control	13	17			19	11		
Cholesterol gallstone	39	40			35	44		
Non-choloesterol gallstone	13	7	1.56	0.21	11	10	0.44	0.51

4. Discussion

Gallstone disease has high incidence, and it involves high cost during hospitalization. The etiology of gallstones still needs to be further elucidated. Gallstone formation involves many risk factors such as obesity, dyslipidemia, diabetes hyperinsulinemia, hypertension, estrogen, family history, and hematologic disease (16). Dyslipidemia, abnormal blood glucose, hyperinsulinemia, hypertension, and obesity are correlated to each other, and they lead to metabolic syndrome (17-19). Approximately 25% of the world's population have metabolic syndrome (20). Abnormal lipid metabolism is an important risk factor for metabolic syndrome. When lipid metabolism disorder occurs, blood lipid levels rise, and hepatic hypersecretion of cholesterol causes supersaturation and crystallization of cholesterol. These processes eventually lead to gallstone formation. Moreover, lipid metabolism disorder is often closely correlated with genes related to lipid metabolism. Only three of the six loci in the current study were related to gallstone disease. However, no statistically significant differences were found in *PCSK9* (rs151193009), *LDLR* (rs200990725), and *CETP* (rs2303790) between the gallstone and control groups. In contrast, a study from the University of Hong Kong showed that these loci are related to lipid metabolism abnormalities in Chinese population. Moreover, the sample size in the control group was small. In addition, these three loci were both highly expressed in the experimental and control groups. Thus, it could not be established whether the three loci were related to gallstone disease or not. In future studies, an attempt will be made to increase the sample size and further explore the relationship between these six loci and gallstone disease.

Association between TEAD2 and gallstone disease

TEAD2 is generally considered conversion enhancer 4. The gene load test showed that this gene is an HDL-related gene which may affect the peroxisome proliferator activated receptor. This receptor is important for fatty acid oxidation. It also affects the metabolism of phospholipids which are substrates in the synthesis of HDL-C (21). The results showed that *TEAD2* (rs142665148) was associated with gallstone disease, but no statistically significant difference was found between the cholesterol and non-cholesterol gallstone groups. Therefore, this gene locus is involved in the pathogenesis of two kinds of gallstone disease at the same time. The influence of *TEAD2* on HDL synthesis and lipid metabolism abnormality, and its participation in the pathogenesis of gallstone may give an insight into the mechanism involved in its effect. This mechanism may be a common mechanism in the formation of the two kinds of gallstones.

Association between PNPLA3 and PKD1L3 and non-cholesterol gallstone disease

The protein encoded by *PNPLA3* is expressed in the liver, and it is involved in lipid metabolism. This protein is considered an important factor in fat accumulation in the liver (22). The protein encoded by *PNPLA3* has been hypothesized as a hepatocyte membrane protein of approximately 53 kDa (23, 24). The protein encoded by the wild-type has also been shown to exhibit glycerol esterase and acylglyceryl transferase activities. In addition, the mutant type has been demonstrated to have reduced glycerol esterase activity, which leads to the accumulation of glycerol, lipid metabolism disorder, and ultimately fatty liver. Moreover, the mutant type affects lipid metabolism and ability to synthesize phospholipids (25). The protein encoded by the wild-type may play a stronger role in lipid metabolism in the liver, while

the mutant-type may have lost this function, leading to lipid accumulation. These hypotheses suggest that the mutant type can cause lipid disorders. The results show that this locus was significantly higher in patients with non-cholesterol gallstone. The degree of mutation in the non-cholesterol gallstone group was higher than that in the control group. Thus, *PNPLA3* can be reasonably assumed to be involved in abnormal lipid regulation, which leads to non-cholesterol gallstone disease.

The *PKD1L3* is often associated with acid taste receptor which is expressed in the liver (26). The mechanism underlying the participation of PDK1L3 in the regulation of lipid metabolism remains unclear. However, mutation in *PKD1L3* occurs in a particular region with the transcription factor binding signals of TCF7L2 and FOXA2 (27). Studies have revealed that TCF7L2 is a type 2 diabetes gene that directly disrupts blood sugar and insulin homeostasis (28). This process is often a part of metabolic syndrome which affects lipid metabolism. Indeed, FOXA2 influences the function of islet β-cells and directly affects homeostasis of liver blood glucose and lipid metabolism in the short term. Moreover, FOXA2 exerts some influence on the differentiation of islet β-cells, as well as insulin secretion. *PKD1L3* may be directly involved in lipid metabolism in the liver (29), while the mutant type disrupts TCF7L2 and FOXA2, thereby disrupting homeostasis of lipid metabolism. These processes eventually lead to gallstone formation.

No statistically significant difference was found in the expression of *PKD1L3* (rs7185272) and *PNPLA3* (rs738409) between the gallstone and control groups. However, the non-cholesterol gallstone group had significantly higher expression of these genes than the cholesterol gallstone group. In addition, the degree of mutation was higher in the non-cholesterol gallstone group than in the control group. Thus, these two loci are involved in formation of non-cholesterol gallstone. Moreover, although cholesterol is the main component of these two gallstones, the gene sites involved in their roles in the pathogenesis of gallstones have not yet been identified. It is known that *PNPLA3* exhibits triacylglycerol lipase activity, and also affects the level of blood glucose. The gallstones may also be related to blood glucose and triglyceride levels. Hypercholesterolemia, hypertriglyceridemia, and hyperglycemia are associated with metabolic syndrome. This syndrome includes various metabolic abnormalities, such as hypertension. hypertriglyceridemia, hypercholesterolemia, hyperinsulinemia, and hyperglycemia. These abnormalities are interrelated. Thus, triglycerides and blood glucose may also be associated with gallstone disease.

Association among LDLR, CETP, PCSK9, and non-cholesterol gallstone disease

The *LDLR* which is expressed in the hepatocytes is a housekeeping gene which plays an important role in lipid metabolism. Through receptor-mediated endocytosis, cholesterol-rich LDL is transferred into hepatocytes. Mutations in *LDLR* often compromise cholesterol metabolism, which leads to hypercholesterolemia. Hypercholesterolemia is a high risk factor for gallstone disease (30). The *LDLR* is a candidate gene for gallstone disease, which is an intergenic disease with multiple genes. Mutation in *LDLR* is highly expressed in the Chinese population, and may not play a major role in the two gallstone diseases. However, this gene may necessarily cooperate with other genes to participate in gallstone disease.

The *CETP* encodes cholesteryl ester transfer protein involved in the triglyceride and cholesterol ester pathways (31), It mediates the exchange of triglyceride and cholesterol ester by HDL or LDL (32). The relationship between *CETP* and hypercholesterolemia is relatively clear, and cholesterol ester transfer protein blockers have been developed (33).

Studies have shown that *PCSK9* is an important protein in the pathway of LDL cholesterol metabolism. It degrades *LDLR*, inhibits intracellular circulation, and reduces the production of *LDLR* (34). Thus, it generally reduces the amount of *LDLR* and seriously weakens its role in lipid metabolism (35). These effects are enhanced by mutation in PCSKk9 gene, leading to hypercholesterolemia and other dyslipidemia.

The three loci of *LDLR* (rs200990725), *CETP* (rs2303790), and *PCSK9* (rs151193009) are all specific to the Chinese population. The results showed that 128 out of 129 samples were AA homozygotes, while only one was AG heterozygote. Moreover, *CETP* (rs2303790) was highly expressed in the Chinese population. The other two loci were similar to *CETP* (rs2303790), and such high expression will, to some extent mask the mechanism involved. Given the small number of experimental cases, it was not possible to determine whether or not these three loci are involved in the formation of gallstone. Thus, studies should be carried out to investigate whether a Chinese population with low expression exists, and whether this population has a

low incidence of gallstone disease. However, the high expression in all samples also shows the importance of these three lipid metabolic gene loci in Chinese population.

All loci were found to regulate *LDLR.* Given that *LDLR* is a candidate gene for gallstone disease and is related to gallstone formation, *CETP*, *PCSK9*, and *LDLR* can be reasonably speculated to have a common mechanism. This mechanism influences the metabolism of cholesterol by affecting the number or structure of LDL receptors. These three gene mutation loci are unique to the Chinese population. This population has three unique loci that affect *LDLR* and thus increase cholesterol secretion in the liver cells. These results may facilitate the development of targeted drugs for these three loci for treating hypercholesterolemia. A new choice for the prevention and treatment of gallstone in Chinese population may be provided.

Summary

Gallstone disease is a complex disease involving many genes. The main components of gallstone are cholesterol, gallbladder pigment, protein, and hydroxyapatite (15). Although cholesterol remains the main component of gallstones, genetic differences between cholesterol and non-cholesterol gallstone groups are also significant. The formation of cholesterol gallstone is different from that of the non-cholesterol gallstone. Many high-risk factors are involved in gallstone disease, among which abnormal lipid metabolism is prominent. The polymorphism in the six gene loci reported by the University of Hong Kong reflects the lipid characteristics of the Chinese population. Three of these six gene loci are specific to Asia, which is of great significance for studies of lipid metabolism in the Chinese population. The relationship between these three gene loci and gallstone disease had not been investigated in China and abroad. The present study revealed the relationship between gallstone and the 6 gene loci viz: *PCSK9* p.arg93cys (rs151193009), *LDLR* p.arg257trp (rs200990725), *CETP*p.asp459gly (rs2303790), *PKD1L3*p.thr429ser (rs7185272), *PNPLA3*p.ile148met (rs738409), and *TEAD2* p.asp12asn (rs142665148). The relationship among the different types of gallstone reveals the mechanism of gallstone formation, and reflects the differences and relationship between the formation of two kinds of gallstone. Moreover, the results provide a new therapeutic target and angle for gallstone disease, especially in Chinese population, and also provides a new index for screening susceptible populations.

5. Conclusion

SNPs in *TEAD2* (rs142665148), *PKD1L3* (rs7185272), and *PNPLA3* (rs738409) were associated with gallstone diseases.

The association of SNPs in *PKD1L3* (rs7185272) and *PNPLA3* (rs738409) were more significant in cholesterol-type gallstones than in non-cholesterol-type gallstones. There were significant differences between cholesterol-type and non-cholesterol-type gallstones, with respect to association of SNPs in *TEAD2* (rs142665148).

Acknowledgements

None.

Conflict of Interest

There are no conflicts of interest in this study.

Author's contribution

All work was done by the author named in this article and the authors accept all liability resulting from claims which relate to this article and its contents. The study was conceived and designed by Yue Zhang; Gang Zou, Xiaojun Huang, Yuehua Guo, Liping Liu, Yue Zhang collected and analysed the data; Gang Zou and Xiaojun Huang wrote the text and all authors have read and approved the text prior to publication.

Gang Zou and Xiaojun Huang contributed equally to this work and should be considered as co-first authors.

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