

JAG1 Variants Confer Genetic Susceptibility to Thyroid Dysgenesis and Thyroid Dyshormonogenesis in 813 Congenital Hypothyroidism in China

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Background and Objective: Congenital hypothyroidism (CH) is indeed a prevalent neonatal endocrine disorder, affecting approximately 1 in 2000–3000 newborns worldwide, and 1 in 2400 newborns in China. Despite its high incidence, the genetic causes of CH, particularly those related to thyroid dysgenesis (TD), are still not well understood. However, previous studies have suggested that JAG1 may be a potential susceptibility gene for congenital thyroid defects. To explore the association between JAG1 and CH, we screened JAG1 variants in a large cohort of 813 CH patients.

Methods: We performed genetic analysis of JAG1 using next-generation sequencing in 813 CH cases. The pathogenicity of the variants was assessed by bioinformatics softwares, protein sequence conservation analysis, and hydrophobic analysis. Further genetic analysis was conducted targeting 20 CH-related genes in these 25 JAG1 variant carriers.

Results: We identified 10 pathogenic missense mutations (p.V45L, p.V272I, p.P552L, p.G610E, p.G852D, p.A891T, p.E1030K, p.R1060W, p.A1131T, p.P1174L) carried by 25 patients, the mutation rate of JAG1 in CH was 3.08%. Among these 25 patients, 16 with 1 variant, 6 with 2 variants, and the other 3 with 3 variants. Our findings indicated that JAG1 variants confer genetic susceptibility to both TD and DH, but with different inheritance models. JAG1 variants lead to TD mainly through monogenic model, while for DH cases, both monogenic mechanisms and oligogenic mechanisms play a pivotal role. Oligogenicity may contribute to the disease severity of DH.

Conclusion: JAG1 is a shared genetic factor in TD and DH, with a detection rate of 3.08% in Chinese individuals with CH. A comparison between the oligogenic and monogenic groups suggests a gene dosage effect in CH. Patients with the same JAG1 mutation exhibit diverse clinical phenotypes, indicating complex mechanisms underlying phenotypic heterogeneity.

Keywords: congenital hypothyroidism, JAG1, pathogenic variant, inheritance model, phenotypic heterogeneity

Introduction

Congenital hypothyroidism (CH) characterized by primary thyroid hormone deficiency, is the most common and preventable neonatal endocrine disorder, with an incidence of 1 in 2000–3000 newborns worldwide, and 1 in 2400 in China.^{1,2} Extensive research in human and experimental models has confirmed the genetic origin of CH.³ CH can be classified into two groups based on the pathogenesis: dyshormonogenesis (DH) and thyroid dysgenesis (TD). DH is associated with genes such as *DUOX2*, *DUOXA2*, *DUOX1*, *IYD*, *SLC5A5*, *TG*, *TPO*, *SLC26A4*, and *SLC26A7*, which are inherited in an autosomal-recessive manner.⁴ In contrast, the pathogenesis of TD is more complex, with well-established pathogenic genes including *NKX2.1*, *PAX8*, *TSHR*, and *FOXE1*. Next-generation sequencing has led to the identification of additional candidate genes for TD, such as *NKX2.5*, *GLIS3*, *CDCA8*, *NTN1*, *TUBB1*, *THR3*, *NNT*, *DUOX2*, and *JAG1*.^{5,6} TD caused by these genetic defects can occur in isolation or in conjunction with extrathyroidal abnormalities,

but the risk is significantly higher in the context of syndromes. Therefore, exploring TD pathogenesis from the perspective of syndromic TD pathogenic genes may be a more efficient strategy.

JAG1 (NM_000214.3) is located on human chromosome 20p12.2 and encodes a ligand of the Notch signaling pathway. It is expressed on the cell surface and activates Notch signaling by interacting with receptors on adjacent cells. Defects in *JAG1* have been identified as the main cause of Alagille syndrome (ALGS; OMIM 118450), a multisystemic disorder that affects the liver, heart, skeleton, eyes, and facial features.⁷ *JAG1* is known to play a role in the development of various organ systems, including the thyroid. Luca Persani et al confirmed the importance of the jagged1-Notch signal in thyroid development and function using zebrafish models. They found that loss of *jag1* function resulted in thyroid hypoplasia.⁸ Subsequently, they screened 100 cases of CH and identified four carriers of *JAG1* variants, three of which had TD, suggesting that *JAG1* is a novel susceptibility gene for congenital thyroid defects. Subsequently, they screened 100 cases of CH and identified four carriers of *JAG1* variants, three of which had TD, suggesting that *JAG1* is a novel susceptibility gene for congenital thyroid defects.⁹ However, further clinical and experimental data are needed to explore the association between *JAG1* and CH.

In this study, we performed *JAG1* analysis in a larger cohort of 813 CH patients using next-generation sequencing, and identified 10 likely pathogenic mutations carried by 25 patients. We further analyzed 20 CH-related genes in these 25 carriers, assessed the pathogenicity of variants and the association between genotype and phenotype of *JAG1* variants. Our findings revealed that the *JAG1* variants may confer genetic susceptibility to both TD and DH, but with different inheritance model.

Method

Patients

Eight hundred and thirteen CH patients in Henan Province and Shandong Province from January 2012 to Mar 2019 were included in the study. Newborns with TSH levels of >10 μ IU/mL were recalled for the re-examination of serum TSH and FT4 levels by electrochemiluminescence assay. A diagnosis of CH was made on the basis of elevated TSH (TSH ≥ 10 mIU/l) level and decreased free T4 (fT4 < 12 pmol/l) level. To ensure the accuracy of the study, patients with other congenital malformations were excluded from the analysis. Additionally, all patients underwent thyroid ultrasonography and technetium thyroid scans for classification purposes. Generally, after 2–3 years of L-T4 replacement therapy, patients were assessed as permanent congenital hypothyroidism (PCH) or transient congenital hypothyroidism (TCH) by retesting TSH and FT4 one month after drug withdrawal. This study was approved by the Ethics Committee of the Affiliated Hospital of Qingdao University (QDFY WZLL 28515) and the Third Affiliated Hospital of Zhengzhou University, and complied with the Declaration of Helsinki. Written informed consent had been obtained from the parents of all participants.

Next-Generation Sequencing and Data Analysis

We extracted the genomic DNA from peripheral blood leukocytes using the Tiangen DNA extraction kit (TIANGEN, Beijing, China). A total of 10 ng qualified DNA per sample was randomly fragmented into 180 ~ 250 bp by Sonication (Covaris S2, Covaris, USA) for the DNA libraries preparation. The exons and 10 bp exon–intron boundaries of *JAG1* were selected by a gene capture strategy, using the GenCap custom enrichment kit (MyGenostics, Beijing, China) for mutational screening. Sequencing of amplicon libraries was carried out using the MiSeq system (Illumina, CA, USA). Surecall software (V3.0.1.4, Agilent) was used for data analysis and variant annotation.

We detected 111 variants in *JAG1* in 813 CH patients. Variants located in introns or synonymous variants were excluded. Forty-one variants with MAF < 0.01 in all database (1000 Genome, ExAC, esp6500siv2, PopFreqMax) and located in exons and splicing site were retained. And then the functional impact of variants were tested by 4 online programs (SIFT, Polyphen2_HDIV, MutationTaster), CADD (threshold ≥ 20), we regard the variants predicted to be possibly damaging by ≥ 2 programs as pathogenic variants. In the end, a total of 12 pathogenic variants were identified through screening, and out of those, 10 pathogenic variants were confirmed through Sanger sequencing in 25 patients.

For these 25 *JAG1* variant carriers, we performed further genetic analysis targeting 20 CH-related genes (*TG*, *TPO*, *DUOX1*, *DUOXA1*, *DUOX2*, *DUOXA2*, *IYD*, *SLC26A4*, *SLC26A5*, *TSHR*, *NKX2.1*, *FOXE1*, *PAX8*, *HHEX*, *THRB*, *GLIS3*,

CDCA8, NKX2.5, NTN1, TUBB1). With the same method of variant detection and filtering, we finally validated 7 pathogenic variants in *DUOX1*, 1 in *DUOX2* in 9 patients.

Sanger Sequencing

2 × Rapid Taq Master Mix (Vazyme, Nanjing, China) was used for PCR amplification. BigDye[®] Terminator Cycle Sequencing Kit and automated sequencer ABI 3730XL were used for sequencing reaction of the PCR products. The sequencing results were interpreted using Chromas software V.2.5.

Statistical Analysis

The statistical significance was assessed using the Mann–Whitney *U*-test and chi-square test. Differences were considered significant at $P < 0.05$. All analyses were performed using SPSS.

Results

Clinical Characteristics of Patients

The present study enrolled a total of 813 CH patients consisting of 224 TD and 589 DH, the ratio of male to female was 1:1.07. Except for 306 patients without classification, the other 296 patients were diagnosed as PCH and 211 were TCH. We summarized the clinical characteristics of all subjects and made a comparison between all subjects and *JAG1* variant carriers, indicating that the carriers had lower levels of T3, T4, fT3 and fT4 in diagnostic serum, but only the fT4 level was

Table 1 The Clinical Characteristics of All CH Subjects and *JAG1* Variant Carriers

		All Subjects	<i>JAG1</i> Variant Carriers	p value
Gender (males/females)(n)		393/420	12/13	0.973
Birth weight, median (IQR) (kg)		3.4(3.1–3.7)	3.5(3.1–3.6)	0.787
Diagnostic serum	TSH level, median (IQR) (mIU/L)	>75(44.3–75)	>75(47.5–79)	0.558
	T3 level, median (IQR) (nmol/L)	1.7(1.0–2.3)	1.6(1.0–2.2)	0.560
	T4 level, median (IQR) (nmol/L)	34.4(12.9–66.3)	26.5(14.45–59.7)	0.653
	fT3 level, median (IQR) (pmol/L)	5.0(3.2–6.0)	4.2(3.56–5.7)	0.616
	fT4 level, median (IQR) (pmol/L)	6.2(3.9–9.6)	4.7(3.0–7.1)	0.043
CH severity (N, percentage)	severe (<5 pmol/L)	333, 41.0%	14, 56%	0.306
	moderate (5 to 10 pmol/L)	268, 33.0%	8, 32.0%	
	mild (>10 pmol/L)	181, 22.3%	3, 12.0%	
Pathogenesis (N, percentage)	thyroid dysgenesis	224, 27.6%	8, 32.0%	0.287
	thyroid dysmorphogenesis	589, 72.4%	17, 68.0%	
CH phenotype (N, percentage)	PCH	296,	12,	0.183
	TCH	211,	4,	
	unclassified	306	9	
Other clinical features (N)	Prolonged jaundice	149	3	
	Abdominal distension	81	5	
	Umbilical hernia	40	2	
	Low heart sounds	32	2	
	Hoarse cry	21	1	
	Drowsiness	9	0	
	Cardiac murmurs	4	0	
	Protruding tongue	1	0	
	High muscular tension in both lower limbs	1	0	

Notes: Mann Whitney *U*-test and chi-square test were used for statistical analysis.

Abbreviations: IQR, interquartile range; PCH, permanent congenital hypothyroidism; TCH, transient congenital hypothyroidism; NBS, newborn screening; L-T4, L-thyroxine.

statistically different ($p < 0.05$). Based on European Society for Paediatric Endocrinology criteria, serum fT4 concentration correlates with CH severity, which can be classified into mild (>10 pmol/L), moderate (5 to < 10 pmol/L), and severe (<5 pmol/L). The *JAG1* variant carriers appear to have a higher distribution in severe phenotypes (56% vs 41%). [Table 1](#) records additional symptoms associated with CH, including prolonged jaundice, abdominal distension, umbilical hernia, low heart sounds, hoarse cry, and drowsiness, among others. Among the patients, 12 had a family history of CH, while the remaining cases were sporadic.

Variant Detection and Mutation Spectrum

After data analysis and verification by Sanger sequencing, we identified 10 *JAG1* pathogenic variants in 25 patients, the mutation rate is 3.08%. The mutant p.V45L was the most common variant, accounting for 8 cases, followed by mutant p.P552L (5 cases), p.A1131T (3 cases), p.G610E (2 cases), p.P1174L (2 cases) and the others (p.V272I, p.G852D, p.A891T, p.E1030K, p.R1060W). All of the identified variants were missense mutations. Among them, the p.G852D and p.E1030K variants were first reported by your research team, while the remaining variants were potential disease-causing mutations previously identified in patients with ALGS or pituitary stalk interruption syndrome. Currently, only the mutant p.P1174L was described in hypothyroidism. In addition, we screened 20 CH associated genes (*TG*, *TPO*, *DUOX1*, *DUOX1A1*, *DUOX2*, *DUOX2A2*, *IYD*, *SLC26A4*, *SLC26A5*, *TSHR*, *NKX2.1*, *FOXE1*, *PAX8*, *HHEX*, *THRB*, *GLIS3*, *CDC48*, *NKX2.5*, *NTN1*, *TUBB1*) in these 25 patients in the same way, and found that 1 with *DUOX2* monoallelic variant, 5 with *DUOX2* monoallelic variant, and 3 with *DUOX2* biallelic variants ([Table 2](#)). Specifically, we screened out 7 pathogenic variants in *DUOX2* and 1 in *DUOX1A2*, all these variants had been reported in previous study of CH ([Supplementary Figure 1](#), [Supplementary Table 1](#)). It is worth noting that all the pathogenic variants identified in this study were found to be heterozygous. Detailed information regarding these variants, as well as relevant data, can be found in [Supplementary Table 1](#).

Pathogenicity Assessment

We assessed all variants pathogenicity using SIFT, PolyPhen2, Mutation Taster and CADD (threshold ≥ 20), the pathogenic variants were predicted to be deleterious by at least 2 bioinformatics software. To assess the conservation of the amino acid site where the missense mutation is located, a multiple sequence alignment was performed using ClustalW2 across eight different species (*Homo sapiens*, *Mus musculus*, *Rattus norvegicus*, *Sus scrofa*, *Pan troglodytes*, *Bos Taurus*, *Macaca mulatta* and *Felis catus*). The results revealed that all the amino acid sites were highly conserved except for the 1174 site of *JAG1*, 407 and 967 site of *DUOX2* ([Figure 1](#)). Considering the effect of amino acid hydrophobicity on protein structure and stability, we analyzed the changes of hydrophobic parameters caused by amino acid substitution, most of these substitution lead to significant increase (R>C, P>L, R>G, R>W) or decrease (I>T, G>D, G>E, I>M, V>F) of hydrophobic parameters, a few with minor changes (R>Q, V>I, V>L, E>K) ([Figure 2](#)). According to American College of Medical Genetics and Genomics (ACMG) guidelines, 1 variants were classified as pathogenic, 6 were likely pathogenic, and the other 11 were variants of uncertain significant (VUS) ([Supplementary Table 1](#)).

Genotype and Phenotype Relationship

Among these 25 patients, 16 carried with 1 variant, 6 with 2 variants, and the other 3 with 3 variants ([Table 2](#)). The distribution of the number of variants carried by each patients in TD and DH groups showed that TD is mainly caused by monogenic mutation (7/8), while for DH, monogenic mutation (9/17) and multiple mutations (8/17) account for about half of the cases respectively ([Figure 3A](#)). In this study, the patients were divided into two groups based on the number of variants they carried: the monogenic group and the oligogenic group. The concentration of fT4 was then compared between these two groups to assess any differences. The results showed that the fT4 concentration in oligogenic group was lower than that in monogenic group, though it was not statistically significant ($P=0.192$) ([Figure 3B](#)). Further analysis was conducted on the basis of CH classification. For TD patients, only one case in oligogenic group, which cannot be used for statistical analysis. For DH patients, the concentration of fT4 in oligogenic group was significantly lower than that in monogenic group ($P=0.043$) ([Figure 3C](#)). Compared with DH cases, TD cases showed a more severe phenotype. In monogenic group, the level of fT4 in TD was lower ($P=0.185$) ([Figure 3D](#)), and in subjects with classification, all TD patients were diagnosed as PCH ([Table 2](#)). We conducted the burden testing of the *JAG1* variants we identified using

Table 2 Clinical Characteristics of Patients with Potential Pathological Variants

Patient	Sex	Diagnostic Evaluation					CH Severity	CH Phenotype	Thyroid Morphology	Other Clinical Features	Variant 1 in <i>JAG1</i>	Variant 2	Variant 3
		FT3	FT4	T3	T4	TSH							
		pmol/L		nmol/L		mIU/L							
1	M	9.17	6.5	2.65	37.7	74.5	moderate	PCH	Agenesis				
2	M	3.65	12	1.8	110	37.7	mild	NA	Normal				
3	F	5.4	4.39	1.4	20.8	>75	severe	NA	Normal	Prolonged jaundice Abdominal distension			
4	F	1.9	<3.86	<0.62	<12.9	>75	severe	PCH	Hypoplasia	Prolonged jaundice Abdominal distension Umbilical hernia Low heart sounds Hoarse cry			
5	M	6	5.1	1.8	22.3	50.3	moderate	TCH	Goiter				
6	F	3.9	0.42	2.21	2.43	83	severe	PCH	Normal		DUOX2 p.R1485G		
7	F	2.5	10.3	0.6	36	50.3	mild	NA	Goiter		DUOX2 p.R1110Q		
8	M	4.6	4.71	1.6	17.2	>75	severe	NA	Goiter	Abdominal distension	DUOX2 p.R1110Q	DUOX2 p.V407F	
9	M	5.5	6.34	1.7	32.6	>75	moderate	NA	Normal		p.V272I		
10	M	5	6.24	2.17	27.2	100	moderate	PCH	Normal		p.P552L		
11	F	1.4	1.61	0.5	20	100	severe	PCH	Agenesis				
12	M	3.47	3.86	1.04	14.5	100	severe	PCH	Hypoplasia				
13	M	7.3	7.26	4.3	59.3	>75	moderate	NA	Goiter				
14	M	<1	<3	<0.4	<10	>75	severe	TCH	Normal	Prolonged jaundice		DUOXA2 p.I26M	
15	M	3.76	1.27	1.43	89.4	12.75	severe	NA	Normal		p.G610E		
16	F	8.7	0.05	2.2	180	21.8	severe	PCH	Goiter			DUOX2 p.R1110Q	
17	F	3.8	4.7	1.2	20.8	>75	severe	PCH	Agenesis		p.G852D		
18	M	4.1	9.2	1.1	61.8	44.6	moderate	PCH	Hypoplasia		p.A891T		
19	F	5.4	7.16	2.5	52.5	39.7	moderate	TCH	Normal	Abdominal distension Umbilical hernia	p.E1030K		
20	M	6.99	5	2	26.5	100	severe	NA	Normal		p.R1060W	DUOX2 p.P76L	DUOX2 p.R625X
21	F	1.8	3	<0.4	<10	>75	severe	PCH	Agenesis	Abdominal distension Low heart sounds	p.A1131T		
22	F	5.9	7.6	2.4	60.1	42.7	moderate	TCH	Normal				
23	F	4.7	<7	2.23	66.7	100	moderate	PCH	Agenesis			DUOX2 p.I967T	
24	F	3.8	<3.86	1	<12.9	>75	severe	NA	Goiter		p.P1174L	DUOX2 p.R1110Q	
25	F	4.2	<3	1.3	14.4	>75	severe	PCH	Goiter			DUOX2 p.R82C	

Abbreviations: PCH, permanent congenital hypothyroidism; TCH, transient congenital hypothyroidism.

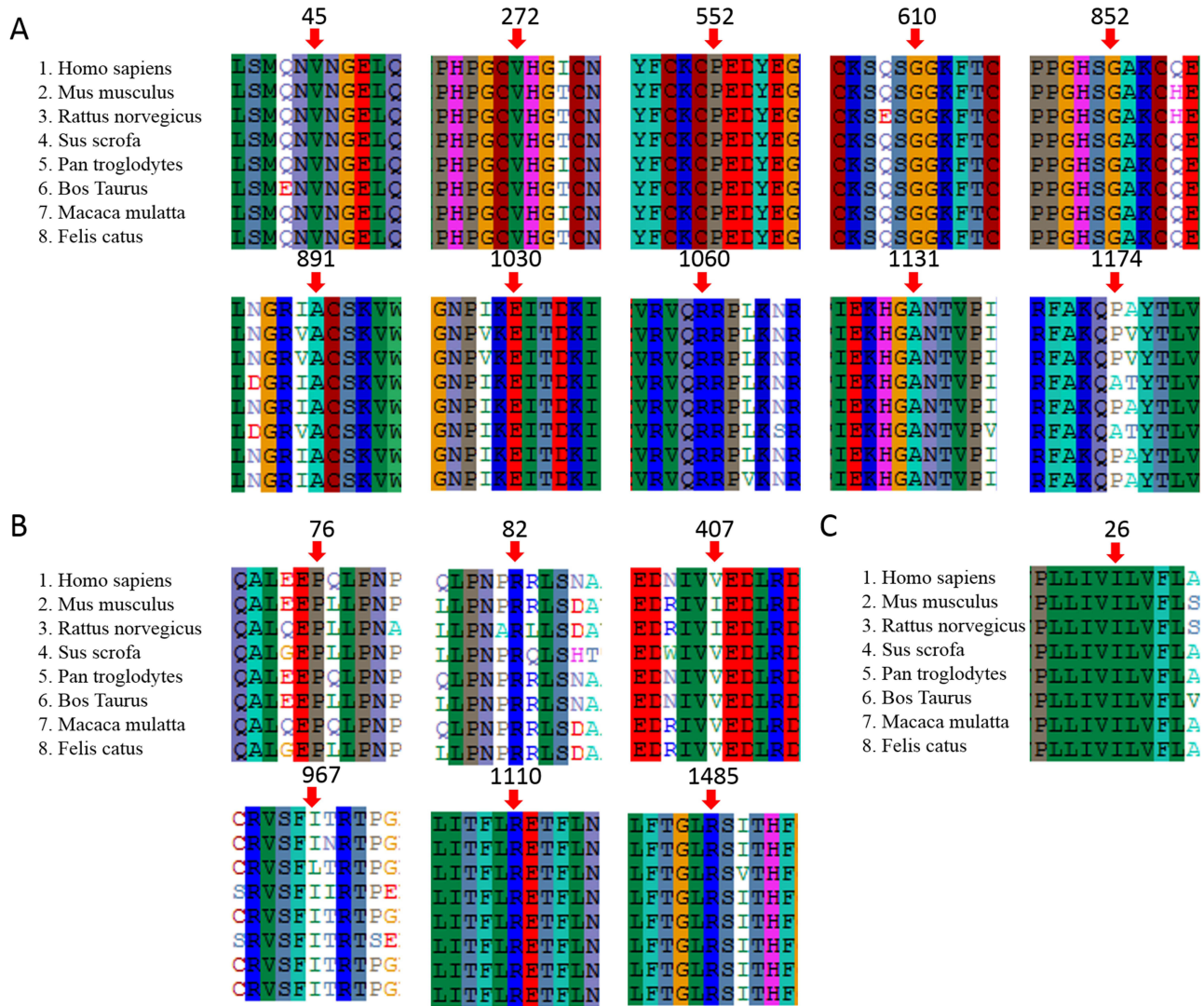


Figure 1 Multiple sequences alignment. Multiple sequences alignment were conducted using ClustalW2 among Homo sapiens, Mus musculus, Rattus norvegicus, Sus scrofa, Pan troglodytes, Bos Taurus, Macaca mulatta and Felis catus. (A) Multiple sequences alignment of JAG1; (B) Multiple sequences alignment of DUOX2; (C) Multiple sequences alignment of DUOX2A2. The mutation sites were marked by red arrows.

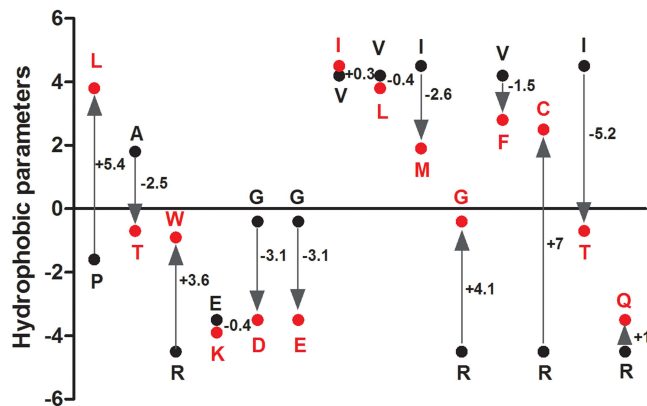


Figure 2 The changes of hydrophobic parameters caused by amino acid substitution. Wild-type amino acids were marked with black, substituted amino acids were marked with red; "+" means increased hydrophobicity, "-" means decreased hydrophobicity.

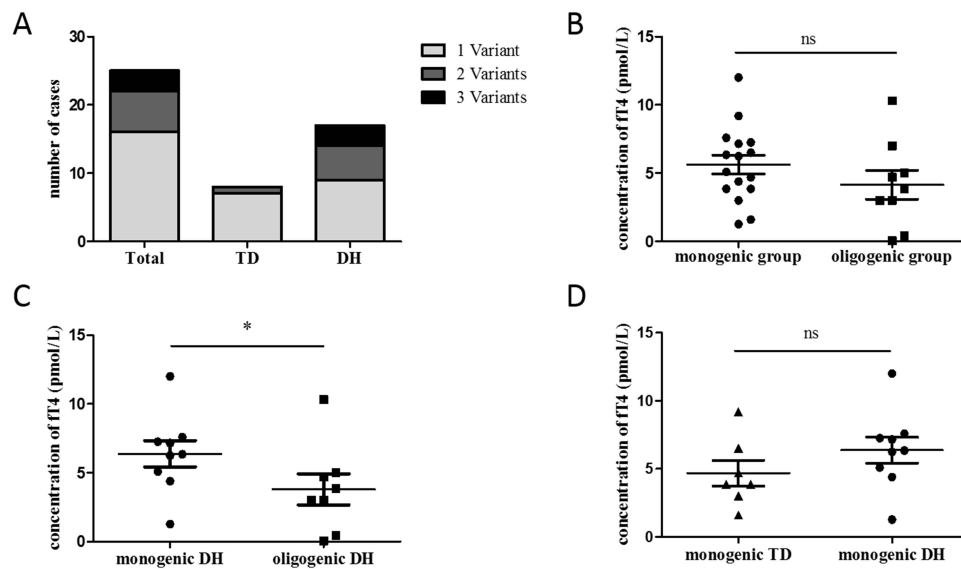


Figure 3 Analysis of genotype and phenotype relationship (A) The distribution of the number of variants carried by each patients; (B) The comparison of fT4 concentration between monogenic group and oligogenic group; (C) The comparison of fT4 concentration between monogenic DH and oligogenic DH; (D) The comparison of fT4 concentration between monogenic TD and monogenic DH. "ns" means $P > 0.05$, "*" means $P < 0.05$.

publicly available data in GnomAD ($n=60,146$) as control, the results indicated the association between *JAG1* variants and CH ($OR = 4.003$, $p = 0.000$). Although the pathogenic mechanisms and clinical manifestations of *JAG1* variant in TD and DH are different, there is no difference in its distribution in TD and DH patients ($P=0.287$) (Table 1).

Discussion

In this study, we performed NGS targeting *JAG1* in a large cohort of 813 subjects with CH, and then identified 10 pathogenic missense mutations (p.V45L, p.V272I, p.P552L, p.G610E, p.G852D, p.A891T, p.E1030K, p.R1060W, p.A1131T, p.P1174L) carried by 25 patients, the mutation rate of *JAG1* in CH was 3.08%.

JAG1 is expressed widely during mammalian development and plays a crucial role in the development of organ systems associated with Alagille syndrome (ALGS) and the thyroid.^{7,10} In the context of ALGS, *JAG1* mutations have been characterized, with frameshift mutations being the most common (37%), followed by nonsense mutations (22%), large gene deletions (13%), missense mutations (13%), splice site mutations (12%), and other types (<3%).¹¹ Missense variants in *JAG1* tend to cluster within the first 6 exons of the gene, and approximately 25% of these variants result in the gain or loss of

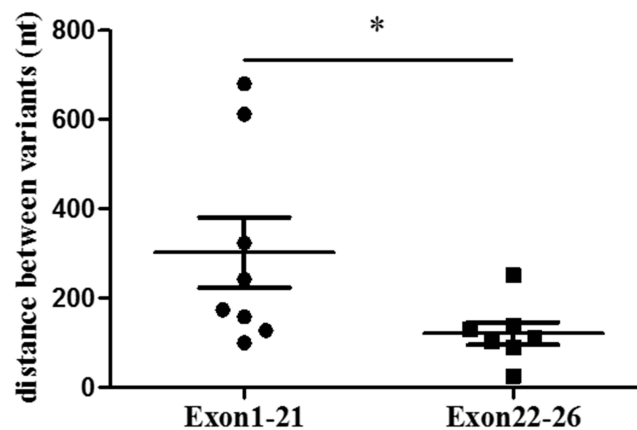


Figure 4 Clustering analysis of *JAG1* variants in CH. Distance in nucleotides between missense mutations within exons 1–21 and exons 22–26. Statistical significance was calculated using an unpaired, two-tailed *t* test, "*" means $P < 0.05$.

a cysteine within the EGF-like domain, which is statistically significant.¹¹ In the genetic screening of *JAG1* variants in cases of CH, Filippis et al identified seven missense variants (p.P476T, p.F509L, p.Y663C, p.R744Q, p.R937Q, p.I1021T, p.R1094W).^{9,12} In your study, 10 missense variants were found in CH patients. Notably, all the *JAG1* variants detected in CH were missense mutations, and they were significantly concentrated in exons 22–26 ($p=0.049$) (Figure 4), which differs from the pattern observed in ALGS.¹¹ Phenotypic effects of *JAG1* mutations are highly penetrant, but there is considerable variability in expressivity, which is consistent with the findings of your research. Patients harboring the same pathogenic variant can exhibit a high degree of variable expressivity. This observation led to the hypothesis that a second gene could act as a modifier of the phenotype. Candidate genetic modifiers, such as glycosyltransferases (Lunatic Fringe, Radical Fringe, Manic Fringe, and POGLUT1) and THBS2, have been reported.^{13–15} However, whether the hypothesis is applicable for the pathogenic mechanism of *JAG1* in CH still needs further verification.

Among these 813 CH patients we enrolled, 224 were classified as TD and the other 589 were DH. Our data showed once again that DH is a more common cause of CH than TD in China. Combined with the genetic screening of 20 genes associated with CH in these 25 *JAG1* variants carriers, we further analyzed the correlation between genotype and phenotype, the results revealed that the *JAG1* variants confer genetic susceptibility to both TD and DH, but with different inheritance model.

In TD cases, 7 of 8 were monogenic *JAG1* variant carriers, the other 1 with heterozygous variant in *JAG1* and *DUOX2*, indicating that *JAG1* variants lead to TD mainly through monogenic model. Currently, most knowledge on TD genetics has been accumulated on monogenic forms of TD, which concern mutations in genes *TSHR*, *NKX2-1*, *PAX8*, *FOXE1* and *NKX2-5*. However, as causative mutations have been identified in less than 5% of cases, and the genetics of TD do not follow simple Mendelian patterns, the transmission mode of TD remains a topic of debate. Polygenic and epigenetic mechanisms have been proposed alongside the monogenic model.^{16,17} The polygenic origin of TD was initially supported by mouse models, as *Nkx2-1*^{+/-} and *Pax8*^{+/-} mice displayed normal thyroid gland, while [*Nkx2-1*, *Pax8*]^{+/-} mice showed hypoplasia or hemiagenesis.¹⁸ Polygenic variants were also identified in very few TD patients, the combination of variants in genes reported in previous studies were *TSHR* and *TPO/DUOX2/GANS/PAX8/JAG1*, *PAX8* and *NKX2-5*, *TG* and *PAX8/TPO/JAG1*. Notably, one patient with variants in both *JAG1* (p.R937Q) and *TSHR* (p.S526G) exhibited thyroid hypoplasia, and another patient carried *JAG1* (p.R744Q) and biallelic *TSHR* variants (p.R1250L, p.R1136Q) and was diagnosed with thyroid ectopy.^{12,17,19} Additionally, a *JAG1* variant in combination with a *DUOX2* variant was found in a CH patient without further classification.²⁰ In this study, only 1 patient with agenesis was detected with digenic variants in *JAG1* (p.A1131T) and *DUOX2* (p.I967T), the low incidence of polygenic mutations indicating it possess a marginal role in TD.

In DH cases, both monogenic mechanisms (9/17, 52.9%) and oligogenic mechanisms (8/17, 47.1%) play a pivotal role. It is worth noting that DH caused by monogenic variants of *JAG1* is not inherited in the classic autosomal dominant manner, as all parents of affected individuals were euthyroid. Although biallelic and monogenic mutations are considered the most common causes of DH, oligogenicity has been found in 20–43.5% of patients with CH, particularly in association with *DUOX2* and *DUOX2A2*.^{20,21} *DUOX2* is the primary gene responsible for DH, and the detection rate of *DUOX2* mutations in China is as high as 28%–44%,²² which is consistent with the detection rate obtained in this study (44%). In our patients with DH, 2 out of 9 cases in monogenic group were severe CH, while for oligogenic group, 7 out of 8 cases were severe CH. Pearson chi-square analysis showed that oligogenic inheritance was significantly associated with severe CH ($P=0.007$), and the finding is supported by previous study.²³ To examine the correlation between variant dosage and severity, the study compared the free thyroxine (fT4) levels in the monogenic and oligogenic groups. The results indicated that oligogenicity may contribute to the disease severity of DH, which is consistent with the findings of a study by Takeshi et al.²⁰ Therefore, the oligogenic model may partly account for the differences in the penetrance and expressivity of CH.

The role of *JAG1* in thyroid development has been investigated in zebrafish, where it was found to affect the proliferation and survival of thyroid follicular cells, possibly through a permissive effect on the TSH signal. In the biosynthesis of thyroid hormone, *JAG1* may play a role by regulating *SLC5A5* through the Notch effector *HES1*.^{8,10} Interestingly, there is an overlap of genetic causes between TD and thyroid DH, as identified in many studies. Patients with the same mutation can exhibit a wide range of clinical phenotypes.^{12,20,24} This suggests that the genetics of CH are complex, and the mechanisms involved in its phenotypic heterogeneity remain unclear.

In conclusion, we screened out 25 *JAG1* variant carriers in 813 CH patients, the mutation rate is 3.08%. Further analysis revealed that the *JAG1* variants confer genetic susceptibility to both TD and DH, but with different inheritance model. The comparison between oligogenic group and monogenic group indicated that CH may exhibit a gene dosage effect. There are two limitations in the present study should be noted. First, patients with other congenital malformations were excluded when collecting samples, as a result, we could not evaluate the effects of these *JAG1* variants on other organ systems. Second, we did not carry out functional studies of these *JAG1* variants.

Data Sharing Statement

The data used to support the findings of this study are available from the corresponding author upon request.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no potential conflicts of interest in this work.

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