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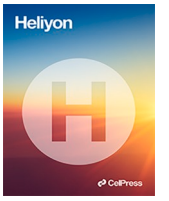

The rs9402373 polymorphism of CTGF gene may not be related to inflammatory bowel disease susceptibility in Chinese population based on ARMS-PCR genotyping

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ABSTRACT

Background: It has been confirmed that the connective tissue growth factor (CTGF) gene rs9402373 polymorphism is associated with fibrotic and inflammatory diseases. However, studies on the relationship between polymorphisms in CTGF rs9402373 and inflammatory bowel disease (IBD) remain rare. Therefore, the aim of this study was to assess the association between the CTGF rs9402373 polymorphism and IBD susceptibility in a Chinese population.

Materials and methods: To establish an amplification refractory mutation system (ARMS) PCR technology for genotyping CTGF gene rs9402373 polymorphism, we designed two specific forward primers for the wild and mutant types by placing the allele-specific nucleotide at the penultimate position of the '3′ end of the primer. Then, 10 samples were randomly selected and rechecked by DNA sequencing to verify the accuracy of this method. We further used the established method to detect specimens collected from 191 patients with inflammatory bowel disease, including 120 Crohn's disease (CD) and 71 ulcerative colitis (UC), and 110 healthy Han Chinese individuals.

Results: We successfully established the ARMS-PCR method for genotyping, and the results of 10 randomly selected samples were completely consistent with DNA sequencing. The rs9402373 G allele frequencies in UC and CD cases were 38.03% and 43.75%, respectively, and in controls, they were 41.82%. No significant difference was found in minor allele frequencies between the UC or CD and control groups ($P = 0.473$, $P = 0.676$). Genotype analysis demonstrated that there was no relationship between CTGF rs9402373 polymorphism and the risk of IBD regardless of the inheritance mode ($P > 0.05$).

Conclusions: In this preliminary study, we successfully developed a simple, efficient and cost-effective method for genotyping CTGF rs9402373 polymorphism. The polymorphism may not be related to IBD susceptibility in the Chinese Han population.

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1. Introduction

Inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), is a chronic nonspecific intestinal inflammatory disease and usually causes persistent inflammation of the mucosa. It usually has a long disease course accompanied by the characteristics of alternating clinical relapse and remission, requiring long-term medication, which greatly affects the quality of life of patients. Recent epidemiological data show that with the development of industrialization, the incidence of IBD has been increasing year by year in the Asia-Pacific region, which was considered a low-incidence area in the past [1]. Although the morbidity and prevalence rates in China are still lower than those in most Western countries, the overall trend is on the rise [2–4]. However, the exact pathogenesis is not yet fully clear; currently, most scholars believe that the interaction between genetic susceptibility, environmental factors and immune factors leads to innate and adaptive immune response disorders [5]. With the rapid development of genetic technology, IBD genetic research has been developed extensively and deeply. To date, more than 200 susceptibility loci have been identified through candidate genes, linkage, genome-wide association and targeted association mapping studies, and some important disease-related pathways have been clarified, such as innate and acquired immune system pathways and autonomic pathways [6–8]. Multiple susceptible loci have been verified by experiments and clinical practice.

The connective tissue growth factor (CTGF) gene is located on human chromosome 6q23.2 and consists of 5 exons, which encode a protein with 349 amino acids. CTGF is a crucial signal–regulating molecule that plays an important role in the process of cell adhesion, migration, proliferation, angiogenesis and tissue repair. Previous studies have indicated that it is related to the occurrence and development of a number of diseases, including cancer, fibrotic diseases and inflammatory diseases [9]. Studies have confirmed that single nucleotide polymorphisms (SNPs) in the CTGF gene are associated with susceptibility to diseases such as systemic sclerosis, type 1 diabetic nephropathy, and cardiovascular disease [10–12]. The base mutation of the CTGF gene at the rs9402373 locus may influence nuclear factor binding and alter its transcriptional activity or transcript stability , thereby affecting the expression of CTGF protein. Several studies [13–16] have shown that both protein and mRNA levels of CTGF are overexpressed in the intestinal tissues of IBD patients. Dessen et al. [17] reported that CTGF rs9402373 was associated with hepatic fibrosis. Similar to liver fibrosis, a feature of IBD is intestinal fibrosis, which damages the intestinal epithelium and connective tissue due to the abnormal proliferation of intestinal fibroblasts. Therefore, we hypothesize that the polymorphisms of CTGF rs9402373 may be associated with susceptibility to IBD. A study on the European population demonstrated that the rs9402373CC genotype is associated with a nonstricturing, nonpenetrating disease phenotype in CD patients [18]. However, to the best of our knowledge, the rs9402373 SNP of the CTGF gene in the Chinese population with IBD has not been reported until now.

Taking into account the abovementioned facts, the aim of our study was to investigate whether the rs9402373 SNP of the CTGF gene is associated with IBD in the Chinese population by amplification refractory mutation system (ARMS) PCR technology for genotyping CTGF gene polymorphisms.

2. Materials and methods

2.1. Subjects

A total of 191 consecutive patients with IBD (120 with CD and 71 with UC) and 110 healthy controls of the Chinese population were recruited from the Second Affiliated Hospital of Zhejiang University School of Medicine between March 2021 and September 2021. All patients were diagnosed by senior physicians on the basis of the Chinese consensus on the diagnosis and treatment of inflammatory bowel disease (2018, Beijing). Controls were randomly selected from healthy subjects who had undergone routine health examination in our hospital during the same period. The demographic data and clinical characteristics of the patients were collected from hospital electronic medical records. The principle of double blind was implemented. The person who did not participate in the experiment was responsible for numbering the samples and the subjects were anonymous to the experimenters.

This study was conducted in conformity with the guidelines of the Declaration of Helsinki and approved by the Institutional Review Committee of the Second Affiliated Hospital of Zhejiang University School of Medicine. All participants signed informed consent forms before blood sampling.

2.2. Sample collection and DNA extraction

Venous blood (2 ml) was collected from all subjects by vacuum blood collection with EDTA anticoagulation and then immediately transferred into an Eppendorf tube. The samples were stored in a −80 °C refrigerator until use. The genomic DNA of the sample was extracted according to the manufacturer's instructions of the commercial kit (Cwbio, BloodGen Mini Kit, China). All extracted DNA samples were used to determine the concentration and purity with a microultraviolet spectrophotometer, and the 260/280 ratio was maintained between 1.6 and 2.0.

2.3. Primer design

All primers were designed employing the genomic sequences in GenBank (http://www.ncbi.nlm.nih.gov) and synthesized by Sangon Biotech, Inc. (Shanghai, China). We designed two forward primers with nucleotide variation at the penultimate '3' position, each of which was specific for the wild-type or mutant type, and one reverse primer (Fig. 1). In addition, a pair of primers was designed on the conservative sequence of the gene as an internal control. There were at least 4 primers in the same PCR reaction at the same

time. The Primer-BLAST was used to verify the specificity of the primers we designed and the OligoAnalyzer Tool (https://sg.idtdna.com/calc/analyzer) was used to check the formation of dimers between primers. The primer sequences are listed in Table 1.

2.4. ARMS PCR assay

Each sample was assessed by ARMS PCR using a thermal cycler (LifeTouch, Bioer, China) in two tubes, one for wild-type and the other for mutant. Each PCR amplification reaction system consisted of a 25 μL of reaction mixture containing 12.5 μL of PCR Master Mix (Takara, Dalian, China), 320 nM wild-type forward primer (or mutant forward primer), 320 nM reverse primer, 320 nM internal control forward primer, 320 nM internal control reverse primer and 2 μL template DNA. The PCR procedure was as follows: after an initial denaturation step at 95 °C for 5 min, 10 cycles of 98 °C for 5 s, 63 °C for 30 s, and 72 °C for 30 s were performed, followed by amplification with 30 cycles of 98 °C for 5 s, 58.7 °C for 30 s, and 72 °C for 30 s. After amplification, the products were analyzed for the presence or absence of PCR specific to the particular alleles by 2% agarose gel electrophoresis followed by Gelred staining. In order to reduce the sample bias, we tested the sample which has been tested in the last batch of the experiment again including the whole process of extraction, amplification, electrophoresis in the next batch of experiments. To determine the accuracy of ARMS-PCR, 10 samples were randomly selected for sequencing verification.

2.5. Statistical analysis

The Hardy-Weinberg equilibrium was performed to examine the distributions of mutation genotype frequency, and the SPSS 22.0 (SPSS, Chicago, IL, USA) software package was used for statistical analysis. Independent *t*-test and χ2 test were used to compare the difference between average age and sex. The differences in genotype frequency and allele frequency distribution between groups were compared by the χ^2 test. The relationship between CTGF gene polymorphisms and the clinicopathological characteristics of IBD patients was also analyzed by the χ^2 test or Fisher's exact test. Odds ratios (Ors) and 95% confidence intervals (Cis) were calculated to evaluate the relative risk. Values of $P < 0.05$ were considered statistically significant. The G*power was used to calculate the power analysis.

3. Results

3.1. Amplification bands of the different genotypes and DNA sequencing

In this study, to avoid false negative results caused by amplification failure and improve the reliability of the experiment, we added a pair of primers to each amplification tube as an internal control to monitor the PCR. If the internal control is not amplified, the experiment fails, and the sample needs to be retested. Gratifyingly, using the allele-specific primers we designed, the mutant and wild-type amplification products of rs9402373 containing the target region were successfully amplified, and the internal control of each sample was also amplified. It can be clearly observed from the electropherogram that an amplified fragment of 254 bp was the target region product, and a fragment of 362 bp was the internal control product (Fig. 2). In addition, the results of 10 randomly selected

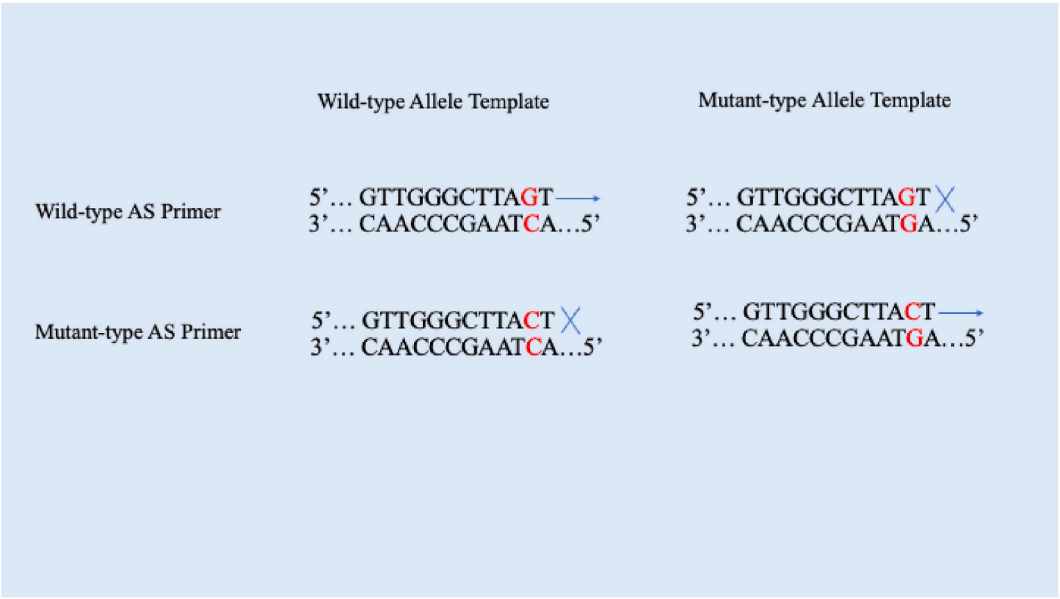


Fig. 1. Schematic diagram of primers synthesis principle. " " means inhibition of amplification.

Table 1
Sequences of primers.

SNP	Primer	Sequence	Amplificon length
rs9402373	Wildtype forward prime	5′-CAATCTGAGTTGGGCTTAGT-3′	254
	Mutant forward primer	5′-CAATCTGAGTTGGGCTTACT-3′	254
	Common reverse primer	5′-AATACCATTAGGTGGAGCCT-3′	
	Internal control forward primer	5′-CAAAGACCCTAGTGAAGCTG-3′	362
	Internal control reverse primer	5′-TCCTCAAGATGCCTACCTGT-3′	

samples in which genotypes were confirmed by ARMS-PCR were completely consistent with the sequencing results (Table 2).

3.2. Demographic data of subjects

A total of 191 IBD patients (71 with UC and 120 with CD) and 110 healthy controls were enrolled in the present research. The demographic data of these subjects are illustrated in Table 3. No significant differences were found in age between the UC group or the CD group and the healthy group ($P = 0.543$, $P = 0.153$). There was no significant difference between UC patients and healthy controls in terms of sex ($P = 0.126$), but there was a statistically significant difference between CD patients and healthy controls ($P = 0.015$, $\chi 2$ test). The minor allele frequency G in UC and CD patients was 38.03% and 43.75%, respectively, when compared with the healthy group, and neither UC nor CD had a significant difference ($P = 0.473$, $P = 0.676$).

3.3. Distribution of the CTGF rs9402373 allele and genotype frequencies in IBD patients and controls

In this study, the distributions of CTGF rs9402373 genotypes in the control group and IBD group were both in accordance with the Hardy-Weinberg equilibrium test results ($P > 0.05$). The distributions of allele and genotype frequencies in IBD patients and controls are shown in Table 4. Sixty (31.41%) IBD patients and 38 (34.55%) healthy controls carried the CC genotype (wild type), while these values were 103 (53.93%) and 52 (47.275%) for the CG heterozygous genotype and 28 (14.66%) and 20 (18.18%) for the GG mutant homozygous genotype in the IBD and control groups, respectively. We found no statistically significant association in any case of the model. When we divided IBD into the UC group and CD group, 24 (33.80%) UC and 36 (30.0%) CD patients carried the CC genotype,

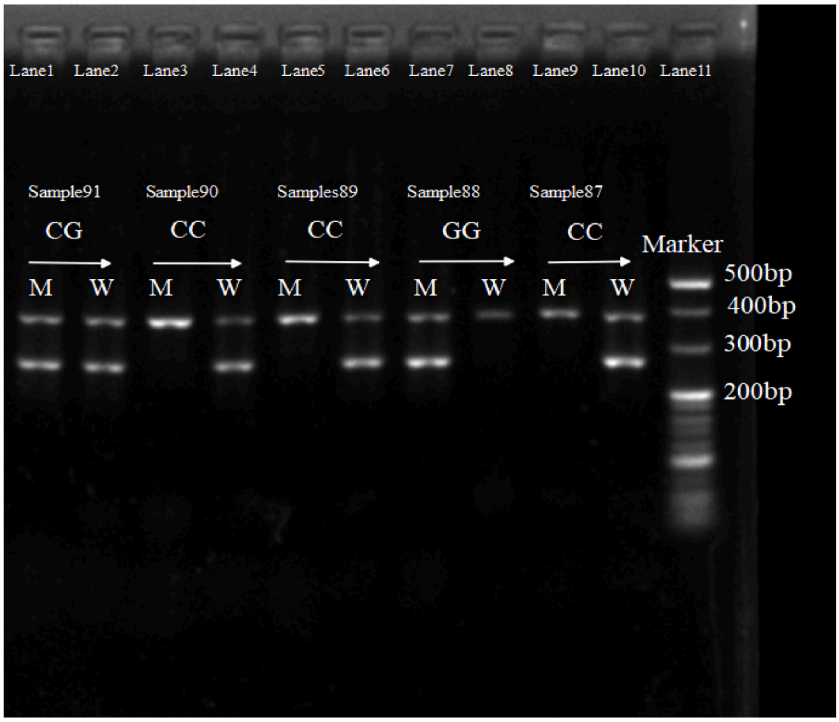
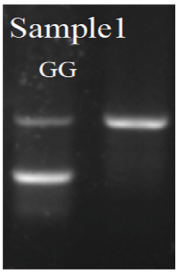
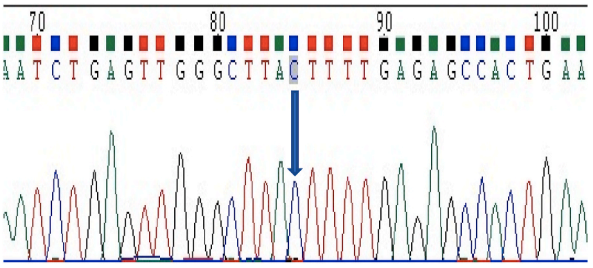
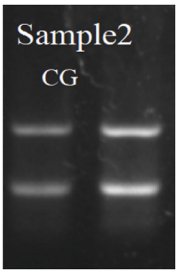
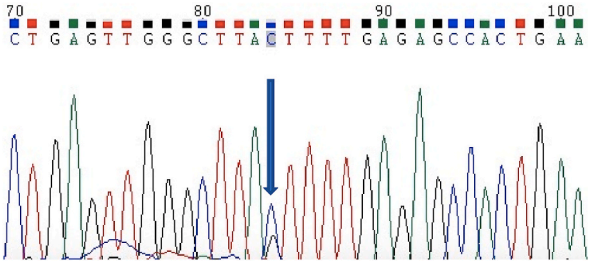
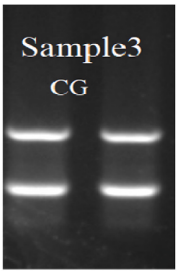
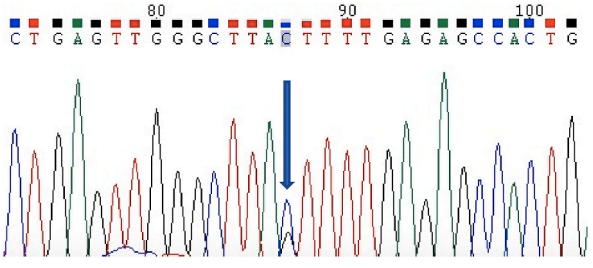
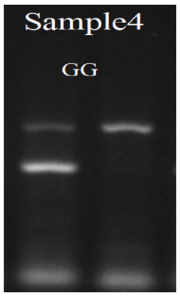
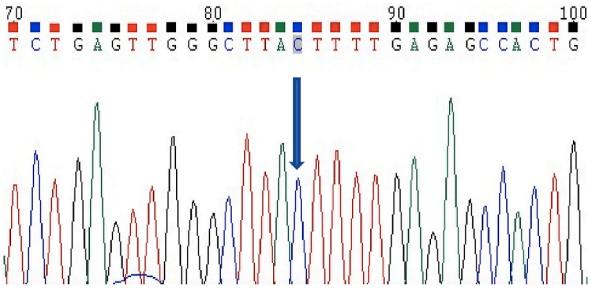
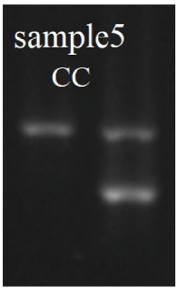
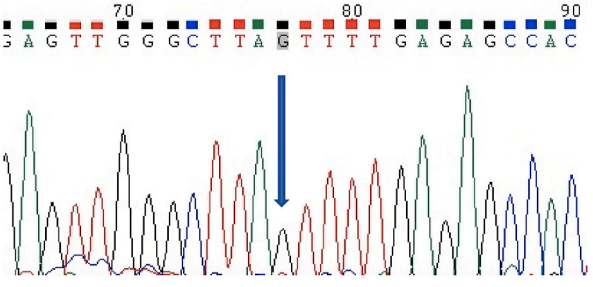


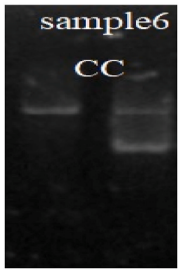
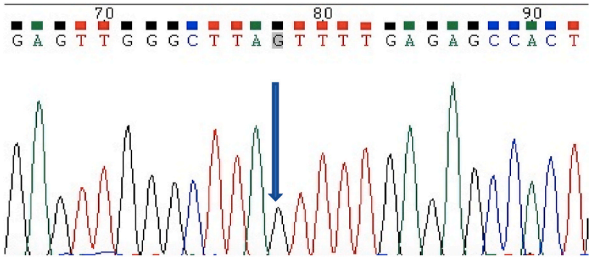
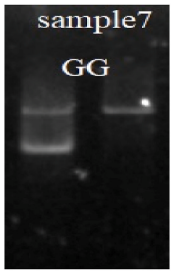
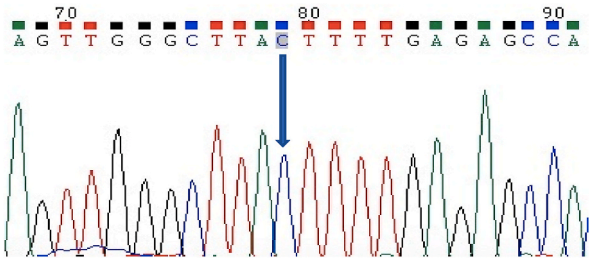
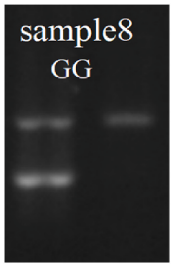
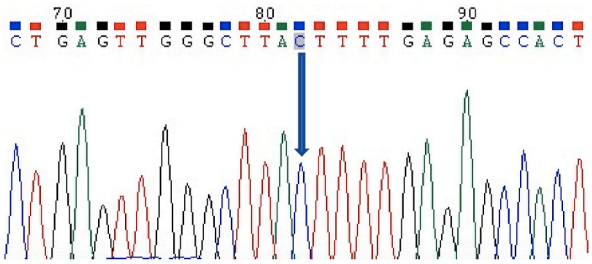
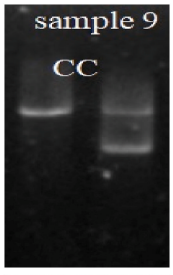
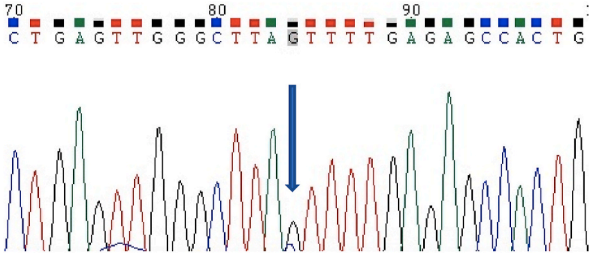
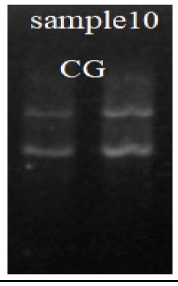
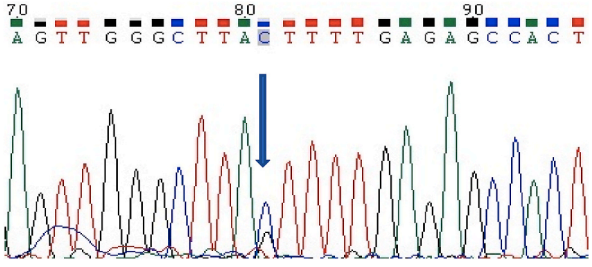
Fig. 2. Amplification bands of the different genotypes of SNP rs9402373.
Note: Every two lanes represent one sample. M represents tube of mutant primers; W represents tube of wildtype primers. Internal control amplificon length is 362bp; target gene amplificon length is 254bp. Lane 1–2 indicate CG genotype: Both the tube of mutant and wildtype primers have internal control and target amplification bands; Lane 3–4, 5–6 and 9–10 indicates CC genotype: Only the tube of wildtype primers has target amplification band; Lane 7–8 indicate GG genotype: Only the tube of mutant primers has target amplification band; Lane 11 indicates DNA marker.

Table 2
The Gel electrophoretogram and Sequencing map of 10 randomly selected samples.

Sample NO.	Genotype	Amplification bands	Sequencing map
Sample 1	GG		
Sample 2	CG		
Sample 3	CG		
Sample 4	GG		
Sample 5	CC		

(*continued on next page*)

Table 2 (*continued*)

Sample NO.	Genotype	Amplification bands	Sequencing map
Sample 6	CC		
Sample 7	GG		
Sample 8	GG		
Sample 9	CC		
Sample 10	CG		

Notes: The sequenced DNA strand is the complementary strand of the template DNA strand. The C base on the sequence map corresponds to the GG genotype in the electrophoresis map, and the G base on the sequence map corresponds to the CC genotype in the electrophoresis map.

while these values were 40 (56.34%) and 63 (52.50%) for the CG heterozygous genotype and 7 (9.86%) and 21 (17.5%) for the GG mutant homozygous genotype in the UC and CD groups, respectively. There were also no significant associations in any case of the model. We then performed a stratified analysis of genotype frequencies in CD patients and healthy controls according to sex because there was a statistically significant difference between CD patients and healthy controls in terms of sex. We still found no significant differences in any case of the model (data not shown). The power calculation analysis was performed. Effect Size $w = 0.3$, $\alpha = 0.05$, $Df = 1$, Total sample size $= 301$, Power $= 0.999$.

Table 3
Demographic data of IBD patients and controls.

	UC (n = 71)	CD (n = 120)	Controls (n = 110)	P1	P2
Gender (male/female)	45/26	81/39	57/53	0.126	**0.015***
Age (years)	43.13 ± 14.37	33.82 ± 12.74	45.02 ± 13.93	0.543	0.153
G allele frequency	38.03%	43.75%	41.82%	0.473	0.676

P1: compared with UC; P2: compared with CD. Bold font means P value less than 0.05. *: χ^2 test was used for the statistical analyses.

Table 4
Distribution of allele and genotype frequencies in IBD patients and controls.

Patients	Model	Allele/Genotype	Cases (n, %)	Controls (n, %)	OR (95%CI)	P
IBD (n = 191)		CC	60 (31.41)	38 (34.55)	1	
	Additive model 1 (CG vs. CC)	CG	103 (53.93)	52 (47.27)	1.254 (0.742–2.122)	0.398
	Additive model 2 (GG vs. CC)	GG	28 (14.66)	20 (18.18)	0.887 (0.439–1.791)	0.737
	Dominant model (CG + GG vs. CC)	CC	60 (31.41)	38 (34.55)	1	
		CG + GG	131 (68.59)	72 (65.45)	1.152 (0.700–1.896)	0.577
	Recessive model (GG vs. CC + CG)	CC + CG	163 (85.34)	90 (81.82)	1	
		GG	28 (14.66)	20 (18.18)	0.773 (0.412–1.450)	0.422
	Allele	C	223 (58.38)	128 (58.18)	1	
		G	159 (41.62)	92 (41.82)	0.992 (0.709–1.389)	0.963
UC(n = 71)		CC	24 (33.80)	38 (34.55)	1	
	Additive model 1 (CG vs. CC)	CG	40 (56.34)	52 (47.27)	1.218 (0.632–2.348)	0.566
	Additive model 2 (GG vs. CC)	GG	7 (9.86)	20 (18.18)	0.554 (0.204–1.508)	0.245
	Dominant model (CG + GG vs. CC)	CC	24 (33.80)	38 (34.55)	1	
		CG + GG	47 (66.20)	72 (65.45)	1.034 (0.551–1.940)	0.918
	Recessive model (GG vs. CC + CG)	CC + CG	64 (90.14)	90 (81.82)	1	
		GG	7 (9.86)	20 (18.18)	0.492 (0.196–1.233)	0.125
	Allele	C	88 (61.97)	128 (58.18)	1	
		G	54 (38.03)	92 (41.82)	0.854 (0.554–1.315)	0.473
CD (n = 120)		CC	36 (30.00)	38 (34.55)	1	
	Additive model 1 (CG vs. CC)	CG	63 (52.50)	52 (47.27)	1.279 (0.712–2.296)	0.410
	Additive model 2 (GG vs. CC)	GG	21 (17.50)	20 (18.18)	1.108 (0.517–2.378)	0.792
	Dominant model (CG + GG vs. CC)	CC	36 (30.00)	38 (34.55)	1	
		CG + GG	84 (70.00)	72 (65.45)	1.231 (0.708–2.143)	0.461
	Recessive model (GG vs. CC + CG)	CC + CG	99 (82.50)	90 (81.82)	1	
		GG	21 (17.50)	20 (18.18)	0.955 (0.486–1.876)	0.893
	Allele	C	135 (56.25)	128 (58.18)	1	
		G	105 (43.75)	92 (41.82)	1.082 (0.748–1.566)	0.676

3.4. The genotype of rs9402373 in CTGF and clinical features of IBD

The correlations of the rs9402373 polymorphism with the clinical features of UC and CD are presented in Table 5 and Table 6. We observed that the rs9402373 polymorphism was not associated with the age of onset in UC patients ($P > 0.05$) and was also not associated with the age of onset, location of the disease or disease behavior in CD patients ($P > 0.05$). However, in UC patients, six of the seven patients with UC who carried the GG genotype had extensive colitis (OR = 0.083, 95% CI = 0.009–0.815, P = 0.028, Fisher's exact test), which suggested that the GG genotype may be correlated with a more extensive disease pattern of UC in Chinese patients.

4. Discussion

In this paper, we obtained three main findings. First, we successfully established the ARMS-PCR method for genotyping, and the

Table 5
The relationship between genotype and the clinical characteristics of UC.

	Genotype			
Group	CC	CG	GG	GG + CG
Age of onset				
≤40/>40	12/12	24/16	4/3	28/19
OR (95%CI); P	1	0.667 (0.240–1.849); 0.435	0.750 (0.137–4.095); 1.000*	1.474 (0.548–3.965); 0.442
Disease extension				
E3/E1+E2	8/16	19/21	6/1	25/22
OR (95%CI); P	1	0.533 (0.193–1.581); 0.267	**0.083(0.009–0.815);0.028***	0.440 (0.158–1.225); 0.113

E1: Proctitis; E2: Left-side colitis; E3: Extensive colitis; Bold font means P value less than 0.05. *: Fisher's exact test was used for the statistical analyses.

Table 6
The relationship between genotype and the clinical characteristics of CD.

	Genotype			
Group	CC	CG	GG	GG + CG
Age of onset				
A3/A1+A2	4/32	13/50	9/48	22/98
OR (95%CI); P	1	2.080 (0.623–6.943); 0.227	2.500 (0.589–10.607); 0.205	2.182 (0.682–6.979); 0.181
Location of the disease				
L1/L2+L3	9/27	21/42	6/15	27/57
OR (95%CI); P	1	1.500 (0.599–3.758); 0.385	1.200 (0.358–4.026); 0.768	1.421 (0.588–3.435); 0.434
Disease behavior				
B1/B2+B3	18/18	29/34	12/9	41/43
OR (95%CI); P	1	0.853 (0.376–1.936); 0.704	1.333 (0.451–3.940); 0.602	0.953 (0.437–2.082); 0.905

A1: less than 16 years; A2:17–40 years; A3: more than 40 years.
L1 indicates terminal ileum; L2, colon, L3, ileocolon; L4, upper gastrointestinal tract.
B1 indicates nonstricturing and nonpenetrating; B2, stricture formation; B3, internally penetrating.

results were completely consistent with DNA sequencing. Second, the distributions of CTGF rs9402373 genotypes in the control group and the UC and CD groups was in accordance with the Hardy-Weinberg equilibrium test results. The minor allele frequencies (MAFs) in the Chinese population with UC and CD were 38.03% and 43.75%, respectively, and in controls, the MAF was 41.82%. Finally, no significant difference was found in minor allele frequency between the UC or CD and control groups. Genotype analysis demonstrated that there was no relationship between the CTGF rs9402373 polymorphism and the risk of IBD regardless of the inheritance mode.

Due to the characteristics of simple operation, time savings, high sensitivity and low cost, ARMS-PCR technology has been widely used to detect single base mutations [19–21]. In previous reports, the allele-specific nucleotide was usually designed at the 3′ end of the primer, and an intentionally mismatched base was introduced at the 3′ penultimate position of the primer. However, unfortunately, when we designed two specific forward primers for the wild and mutant types in this method, we found that the target gene was not successfully amplified. Placing the allele-specific nucleotide in the 3′-end of the primer resulted in many non-specific amplicons in the case of mutant-type allele template. However, when the allele-specific nucleotide was placed at the penultimate position of the 3′ end of the primer, the non-specific amplifications were reduced. It was theoretically practicable to place the allele-specific nucleotide at the penultimate position of the 3′-end of the primer or at the 3′-end of the primer. From the thermodynamic point of view, one mismatched base will affect the stability of a total of three bases on its left and right side. We speculated that in our case the sequence of the three base pairs at the end of the primer was TGA/AGT or TGA/ACT, and the value of standard free energy change ($\triangle G^{\circ}_{37}$) was greater than zero ($\triangle G^{\circ}_{37}>0$) which decreases non-specific amplification [22]. This result provides new insights into ARMS-PCR primer design. In addition, we performed preamplification with 10 cycles at an annealing temperature of 63 °C and then amplification with 30 cycles at an annealing temperature of 58.7 °C. Through touchdown PCR, the amplification efficiency and specificity were improved [23]. In addition, in our study, a pair of primers was designed on the conservative sequence of the gene as an internal control, and the reliability of the method was greatly ameliorated.

To the best of our knowledge, this is the first study to report the CTGF rs9402373 polymorphism in Chinese patients with UC and CD. In this study, we observed that the distributions of CTGF rs9402373 genotypes in the control and IBD groups were in accordance with the Hardy-Weinberg equilibrium test results. The MAFs in the UC group, CD group and control group (0.3803, 0.4375 and 0.4182, respectively) were in line with the corresponding SNP MAF (0.4504) of the population in East Asia in the 1000 Genomes Project database, but they were inconsistent with the MAF (0.2147) of the population in Europe (https://www.ncbi.nlm.nih.gov/snp/rs9402373#frequency_tab). These data illustrated that there were differences in the genetic background of IBD between Eastern and Western populations.

IBD is a chronic relapse and migration disease characterized by intestinal stenosis, intestinal shortening and intestinal fibrosis caused by the abnormal proliferation of intestinal fibroblasts. Previous studies found that as a downstream mediator of transforming growth factor (TGF)-β1, CTGF exerted its profibrotic effect through the SMAD pathway to cause the remodeling and destruction of intestinal tissues, and CTGF was overexpressed at both the protein and mRNA levels in the intestinal tissues of patients with CD [15, 16]. Another study showed that the expression of CTGF in the intestinal mucosa of UC patients was also increased, and it was found that the inflammatory response and intestinal flora were improved and that DSS-induced UC was partially reversed by inhibiting CTGF [24]. However, only CTGF gene expression and protein levels have been analyzed in most previous studies, and little is known about the role of CTGF polymorphisms in inflammatory bowel disease.

In our current study, we examined the rs9402373 SNP of the CTGF gene in 191 IBD patients (71 patients with UC and 120 patients with CD) and 110 healthy controls in a Chinese population. The interesting results indicated that the CTGF rs9402373 polymorphism was not correlated with the IBD group or with either the UC subgroup or CD subgroup. Since there was a significant difference in sex between the selected cases in the CD group and the healthy control group, we performed a stratified analysis according to sex and found that the CTGF rs9402373 polymorphism was also not correlated with the UC group regardless of the male subgroup or the female subgroup. In addition, our research demonstrated that there was also no relationship between the CTGF genotype and the clinical characteristics of CD. Contrary to our results, Burke JP et al. [18] reported that the CTGF rs9402373CC genotype was positively related to B1 (nonstricturing and nonpenetrating) disease and negatively related to B2 (stricturing) disease in Irishman CD patients. This result revealed that the genetic background in the Chinese population with IBD may be different from that in European

populations since they have different epidemiologies, incidences and phenotypes. It is a common phenomenon that the genetic susceptibility of one gene with a specific disease in one population usually cannot be reproduced in another due to racial heterogeneity. It is intriguing that the CTGF rs9402373 GG genotype may be correlated with a more extensive disease pattern of UC in Chinese patients. Based on the published literature [17,24] the expression level of CTGF in patients with GG genotype was lower and it was related to the severity of UC disease, which was not consistent with our results. In our study, only one case in a group of more extensive disease pattern carried the GG genotype, which may make it prone to bias in data analysis. In addition, the power calculation analysis illustrates that the possibility of false negative results is very small and our results are credible. Although rs9402373 was not a risk gene in the Chinese IBD population, we investigated the prevalence of the gene, which could provide a strong reference for CTGF gene studies in other populations or in other diseases.

There are several limitations in our study. First, in our study, the sample size was relatively small, which makes it prone to bias in cohort selection and data analysis. Second, the study population was limited to Zhejiang Province, China, resulting in a slightly narrow research area. Third, this study was not designed to carry out experiments corresponding to gene expression and function. Therefore, further prospective studies with larger cohort sizes or from different populations are needed to provide more valuable information for in-depth interpretation of the genetic immunological pathogenesis of IBD.

5. Conclusion

In summary, we successfully developed a simple, efficient and cost-effective method for genotyping the CTGF rs9402373 polymorphism. Moreover, to the best of our knowledge, this was the first study to report the CTGF rs9402373 polymorphism in Chinese patients with UC and CD. Although the polymorphism may not be related to IBD susceptibility in the Chinese population, we investigated the prevalence of the gene, which could provide a strong reference for CTGF gene studies in other populations or in other diseases.

Author contribution statement

Yiyi Xie: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.
Ying ping; Pan Yu: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.
Weiwei Liu; Qi Wang: Contributed reagents, materials, analysis tools or data.
Xingan Chen: Performed the experiments.
Yuhua Chen: Analyzed and interpreted the data.
Xiuzhi Duan: Conceived and designed the experiments.
Xuchu Wang: Conceived and designed the experiments; Wrote the paper.

Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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