INTRODUCTION

As an inflammatory bowel disease (IBD), Crohn's disease (CD) is known for its complex etiology and serious family life impairment, including psychological health. It was once recognized as no known cure for inflammation that may involve any part of the gastrointestinal tract. CD was reported with a high incidence in western countries. In recent years, the incidence of CD has been gradually increasing in China. Despite the low incidence of CD in China, the rising momentum of this complex disease in children still raises public concerns in the country. Because young CD patients, especially pediatric patients, are at higher risk of obstinacy or recurrence.

Till now, the exact genetic mechanism of CD remains unclear. Given many clues in hand, a deep insight into high-risk genes is urgently needed. The disease is featured with familial aggregation, which means the disease appears more frequently than expected in specific families. According to European and American literature statistics, the first-blood relatives of patients with CD or ulcerative
coliitis are at 10 times higher risk of developing the same disease.9 Although the pathogenesis of CD is not well understood, it is well accepted that CD is highly related to genetic predisposition, environmental triggers, and the interaction between infection and the immunity system.

Thanks to large-scale international cooperation, more than 200 genes were found as high-risk genes for CD (or IBD) by using the technology of genome-wide association analysis.10,11 The IL-10−/− mice were more susceptible to CD.12–14 Kucharzik et al.15 revealed the relationship between the IL-10 level and the activity of CD and ulcerative colitis through gene studies, and Wu et al.16 also confirmed the association between IL-10 polymorphism and IBD. Schmit et al.17 found that recombinant human IL-10 could downregulate the secretion of TNFα by lamina propria monocytes in patients with CD. The evidence suggests that the expression regulation of IL-10 strongly correlates with the incidence of CD. Here, we explored the association of 3 SNPs located in the promoter region of IL-10 with pediatric CD in China.

2 MATERIALS AND METHODS

2.1 Subjects

A total of 86 CD patients and 142 healthy controls were enrolled in this study from February 2019 to February 2021. All 86 cases from the Children’s Hospital of Zhejiang University School of Medicine were diagnosed with CD using standard criteria18 and their disease site and behavior classification were determined according to Montreal19 and Pairs criteria.20 Among the 86 CD patients with a mean age of 10.2 ± 3.6 years. There was an obvious male preponderance in the patients (68.6%). Most children had ileocolonic disease (L3 ± L4, 61.6%) and inflammatory disease (B1, 94.2%) at diagnosis. The detailed clinical characteristics of patients and controls together are provided in Table 1.

2.2 Genotyping

DNA isolation, SNP selection, and SNP genotyping were performed according to the instruction as described by Xu et al.21 Briefly, DNA was extracted from 200 μl blood in EDTA vials following the DNA extraction kit instruction (Tissuebank Biotechnology co., LTD). SNP genotyping was finished on the sequence Mass ARRAY platform (sequence). PCR primers sequences for IL-10 rs1800872 were 5’-GTGGGCTAAATATCCTCAAAGTTC-3’ (sense) and 5’-AGCATATAAGAAGCTTTCAGCAAG-3’ (antisense), The primers for IL-10 rs3790622 were 5’-ACGTTGGATGGCACTCTACATGGAGGAAAC-3’ (sense) and 5’-ACGTTGGATGTTCTCCCCACTGTAGACATC-3’ (antisense), and the primers for IL-10 rs1800896 were 5’-AAAGTTTAAAAATGGGTTGGAAG-3’ (sense) and 5’-CTTACCTTCTACACACACACAC-3’ (antisense).

TABLE 1 Clinical characteristics of CD group and control group

| Characteristic         | Patients (n = 86) | Controls (n = 142) |
|------------------------|------------------|--------------------|
| Age at diagnosis       |                  |                    |
| A1a, n (%)             | 22 (25.6)        | 39 (27.5)          |
| A1b, n (%)             | 64 (74.4)        | 103 (72.5)         |
| Mean (±SD)             | 10.2 (±3.6)      | 9.4 (±2.5)         |
| Gender, n (%)          |                  |                    |
| Females                | 27 (31.4)        | 49 (34.5)          |
| Males                  | 59 (68.6)        | 93 (65.5)          |
| Disease behavior, n (%)|                  |                    |
| B1                     | 81 (94.2)        |                    |
| B2 + B3                | 5 (5.8)          |                    |
| Disease location, n (%)|                  |                    |
| L1 ± L4                | 9 (10.5)         |                    |
| L2 ± L4                | 6 (7.0)          |                    |
| L3 ± L4                | 53 (61.6)        |                    |
| L4 only                | 11 (12.8)        |                    |
| Unknown                | 7 (8.1)          |                    |
| Perianal lesion, n (%) |                  |                    |
| 23 (26.7)              |                  |                    |

Abbreviations: A1a, diagnosed with Crohn’s disease <10 years old; A1b, diagnosed with Crohn’s disease 10–17 years old; B1, uncomplicated inflammatory disease; B2, occurrence of constant luminal narrowing; B3, occurrence of bowel preformation; CD, Crohn’s disease; L1, ileal; L2, colonic; L3, ileocolonic; L4, upper gastrointestinal; Perianal lesion, 23 of 86 CD patients were also diagnosed with the perianal lesion.

2.3 Statistical analysis

Pearson’s chi-square (χ²) test (or Fisher’s exact test) was used to compare categorical variables, such as genotype and allele frequencies between patients and controls. The frequencies of allele and genotype were also compared between patients with and without clinical features of CD. The odds ratio (OR) and 95% confidence interval (CI) were calculated for every explanatory variable. Each SNP was tested for agreement with the Hardy-Weinberg equilibrium (HWE) using a goodness-of-fit chi-square (χ²) test before analysis. Linkage-disequilibrium (LD), and haplotypes were performed by using Haploview version 4.2 software. p < 0.05 was considered as a significant difference. SPSS software package (revision 22.0) was used for statistical analysis.

3 RESULTS

3.1 Gene polymorphisms in CD patients and healthy children

All three studied SNPs were in HWE in both patient and control groups (p > 0.05). Then polymorphisms of three SNPs located in the IL-10 promoter region were detected, and allelic association
tests of the three SNPs with CD were examined by using $\chi^2$ test (Table 2). Among the three SNPs, rs1800872, associated with CD, was identified. T allele in rs1800872 showed a high risk for pediatric CD (Pearson $\chi^2 p = 0.030$). However, the other two SNPs, rs3790622 and rs1800896, did not archive statistical significance ($p$-value 0.512 and 0.239, respectively). And then, the genotype association was also analyzed for the SNP of rs1800872. Compared with the frequency of TG+GG genotypes in rs1800872, the frequency of TT genotype in the patient group was significantly higher (OR 1.986, 95% CI 1.146–3.442, $p = 0.020*$). In another word, TT genotype of rs1800872 had a higher risk of developing pediatric CD in the recessive inheritance model. The frequency of each genotype in the other two SNPs (rs3790622 and rs1800896) showed no statistical difference between the patient and control groups.

Then, LD analysis was performed in CD patients and healthy controls using the halpoview 4.2 software. The three polymorphic sites of IL-10 gene were in a state of linkage disequilibrium with one another in all participants (for rs1800872 and rs1800896, $D' = 1.0$, $r^2 = 0.173$; for rs1800872 and rs3790622, $D' = 1.0$, $r^2 = 0.039$; for rs1800896 and rs3790622, $D' = 1.0$, $r^2 = 0.007$). As the haplotype analysis results showed in Table 3, the most frequent haplotypes for rs3790622, rs1800872, and rs1800896 were GTT in both CD and control groups. And the GTT haplotype frequency in the CD group was 68.6%, higher than the figure of 58.2% in the control group (OR 1.570, 95% CI 1.054–2.341, $p = 0.028$). However, the other two SNPs haplotype frequencies showed no statistical difference between CD and control groups.

### 3.2 | Gene polymorphisms in A1a group and A1b group

According to the age diagnosed with CD. We divided our patients into two groups. A1a group diagnosed with CD <10 years. A1b group diagnosed with CD at 10–17 years old. As shown in Table S1, there were no significant differences in genotype between A1a and A1b groups in all the three SNPs (rs3790622, rs1800872, and rs1800896) ($p > 0.05$). As shown in Table S2, the haplotype analysis results of the IL-10 gene of the three SNPs (rs3790622, rs1800872, and rs1800896) indicate no significant differences between A1a and A1b groups. Our results suggest that age at diagnosis is not associated with CD risk.

### 3.3 | Gene polymorphisms in CD patients with or without perianal lesion

Depending on the CD patients with or without perianal lesions, we divided the cases into two groups. Three IL-10 polymorphisms (rs3790622, rs1800872, and rs1800896) in CD patients with perianal lesions and CD patients without perianal lesions are shown in Tables S3 and S4. There were no significant differences in genotype
Control, fiber leads to a lower risk for CD. Here the genetic association example, recent studies figured out that a higher intake of dietary immune regulation of intestinal mucosa, and abnormal intestinal studies have shown that IL-10 plays an important role in maintaining thus directly damaging the intestinal mucosal barrier. More and more gene BCL3 and upregulation of expression of IL-17, IFN-γ, and TNFα, thought to inhibit the overreaction of inflammatory cells effectively. IL-10 is considered as an important anti-inflammatory cytokine in IBD. Jarry et al. revealed that the loss of IL-10 in intestinal epithelial cells leads to the downregulation of immunosuppressive gene BCL3 and upregulation of expression of IL-17, IFN-γ, and TNFα, thus directly damaging the intestinal mucosal barrier. More and more studies have shown that IL-10 plays an important role in maintaining the immune regulation of intestinal mucosa, and abnormal intestinal mucosal barrier function is one of the key factors in the pathogenesis of IBD. IL-10 can antagonize the production of inflammatory factors and maintain the integrity of the intestinal mucosal barrier, thus reducing the occurrence and progression of IBD. Moreover, studies have shown that enhancing the expression of IL-10 in vivo or supplementing IL-10 can prevent the occurrence of enteritis. It is thought that SNPs (rs18000872 and rs1800896) in the IL-10 promoter region influence the expression of IL-10 and are strongly associated with altered levels of circulating IL-10, thereby influencing the organism’s immune response. Fowler et al. reported that a relationship with disease severity showed a significant association of higher producing IL10-1082G and TNFα-857C alleles with stricturing behavior. In our study, we did not detect a correlation between rs1800896 SNP polymorphism and pediatric CD or CD severity, which may be related to differences in the population. In conclusion, it is logically possible that the abnormality of the IL-10 gene or its polymorphism affects the occurrence and progression of CD. However, for CD, genetic risk prediction is still in the exploratory stage, and no highly correlated loci have been identified so far, which is of low clinical efficacy. For example, in a meta-analysis based on 1890 IBD patients and 2929 controls, polymorphism of rs3021097 (C-819T) in IL-10 was significantly associated with CD, but not statistically associated with IBD. In a study of CD patients aged from 5 to 20 in Montreal, Canada, the genotype frequency of SNPs rs1800896, rs3021097, and rs1800872 in IL-10 were not associated with CD, but the GCC haplotype was associated with the colonic location, and ACC haplotypes were associated with terminal ileum location in CD. In addition to IL-10, SNPs rs3810936, rs6478108, and rs6478109 of TNFSF15 gene were extremely significantly correlated with CD in Korean children, and their OR values were all over 5. Therefore, many researchers believe that CD susceptibility is the result of the interaction of many minor genes. As more and more related genes are identified, there is an opportunity to improve CD risk prediction. This study demonstrates that the incidence of childhood CD is statistically correlated with the SNP rs18000872 of IL-10 in Chinese Han children, which can support the risk prediction of CD. However, the low occurrence of CD adds difficulty to predicting the CD risk. Therefore, there is still a huge gap between rs1800872 polymorphism mentioned in the article and the risk prediction of CD, which can only be limited before dietary habits and CD surveys of family members and other factors are taken into consideration. The goal of this study is to collect more information about genetic association with CD, and we will continue to do so in the future. Thus, the genetic regulatory network of CD can be constructed.

### TABLE 3 Haplotype frequencies of IL-10 in CD and control groups

| Haplotypes (rs3790622, rs18000872, rs1800896) | CD, n = 86 | Control, n = 142 | OR (95% CI) | p-value |
|---|---|---|---|---|
| GTT | 118 (68.6) | 165.25 (58.2) | 1.570 (1.054–2.341) | 0.028* |
| GGT | 31 (18.0) | 71.25 (25.1) | 0.656 (0.409–1.053) | 0.104 |
| ATT | 14.5 (8.4) | 23.75 (8.4) | 1.009 (0.510–1.996) | 1.000 |

Abbreviations: CI, 95% confidence interval; OR, odds ratio.

*Statistically significant (p < 0.05).
to reveal the possible drug targets and finally achieve a better treatment effect of CD.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION
Additional supporting information can be found online in the Supporting Information section at the end of this article.

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