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Macrophage Migration Inhibitory Factor -173 G>C Gene Polymorphism Is Associated with Increased Risk of Nephrotic Syndrome in Children

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Original Paper

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Introduction. Nephrotic syndrome (NS), a common chronic pediatric kidney disease, is associated with immune system dysfunction. The exact role of MIF -137 G>C gene polymorphism on risk of NS is not clear. The current study aimed to evaluate the relationship between MIF -173 G>C (rs755622) variant and susceptibility to NS.
Methods. This case-control study conducted on 134 children with NS and 141 healthy children. Extraction of genomic DNA from whole blood was done using salting out method. Genotyping of the MIF -173 G>C polymorphism was performed using polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method.
Results. The findings showed that MIF -173 G>C variant significantly increases the risk of NS in codominant (OR = 1.82, 95%CI = 1.08-3.08, *P* = 0.026, GC vs GG), dominant (OR = 1.90, 95%CI = 1.14-3.16, *P* = 0.015, GC+CC vs GG), overdominant (OR = 1.75, 95%CI = 1.04-2.94, *P* = 0.037, GC vs GG+CC) and allele (OR = 1.76, 95%CI = 1.13-2.74, *P* = 0.014, C vs G) inheritance models. Stratified analysis performed by sex and response to treatment. The findings revealed that this variant was associated with increased risk of NS in male. No correlation between the variant and response to treatment was found.
Conclusion. In summary, the results indicated that MIF -137 G>C is significantly associated with increased risk of NS. More studies with larger sample size among different ethnicities are needed to verify our findings.

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INTRODUCTION

Nephrotic syndrome (NS) is one of the most common problem in pediatric nephrology with a prevalence of nearly 2 to 7 children per 100,000.[1] It is characterized by severe proteinuria, hyperlipidemia, hypoalbuminemia, and peripheral edema.[1,2] The common forms of idiopathic NS in children are minimal change NS (MCNS) and focal segmental glomerulosclerosis (FSGS).[3] The pathogenesis of idiopathic NS is not entirely elucidated. Though, some evidences showed that it is correlated with immune response.[4-6]

It has been proposed that macrophage migration inhibitory factor (MIF) play a key role in the regulation of innate and adaptive immunity.[7,8] Human MIF gene is mapped on chromosome 22 (22q11.2). The MIF gene comprises three exons of 205, 173 and 183 bp separated by two introns of 189 and 95 bp.[9, 10] A functional polymorphism located within the promoter region of MIF gene at

position -137 substituting G to C, affects promoter activity.[11, 12] MIF is a proinflammatory cytokine that is made by many types of cell including epithelial cells, macrophages, and lymphocytes and plays a key role in the pathogenesis of many inflammatory diseases.[10, 13]

Growing studies focus on the association between MIF -173 G>C polymorphism and INS susceptibility as well as response to treatment.[14-19] To the best of our knowledge, MIF polymorphism was not investigated in INS in the Iranian children. The current study aimed to inspect the possible association between MIF -173 G>C gene polymorphism and nephrotic syndrome in a sample of southeast Iranian children.

MATERIAL AND METHODS

This case-control study was done on 275 subjects including 134 children who suffering from NS and 141 healthy children.[20, 21] Medical history was taken from patients and healthy controls during the first visit. Patients with a positive family history of NS, patients with a syndromic form of NS who did not have any systemic illness (such as lupus nephritis or Henoch schoenlein purpura nephritis) were excluded. Study subjects were recruited from the pediatric department of Ali-ebneh Abitaleb Hospital, Zahedan, Iran. The same pediatric nephrologist inspected the participants. Table 1 shows the characteristic of the cases and controls. The study received ethical approval by the local Ethical Committee of Zahedan University of Medical Sciences.

Genomic DNA from whole blood was extracted using salting out procedure. The samples were stored at -20°C until analysis.

The MIF rs755622 (1173 G>C) polymorphism was performed by previously published PCR-RFLP method as described previously.[13] Briefly, primers used were 5`-CTCAAACA CACAAGCTCACGCATGCG-3` (forward) and 5`-ACCACTGTGGTCCCGCCTTTTGTGAC-3` (reverse). The PCR conditions were as follows: initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 30s, annealing at 65°C for 30s, extension at 72°C for 30 s with a final extension step for 10 min at 72°C. The PCR products were digested with AluI restriction enzyme (New England Biolabs, Hitchin, UK) and the fragments were resolved on 2% agarose gels. The G allele undigested (439bp fragment), while the C allele digested and produces 255bp and 184bp fragments (Figure 1.). To certify genotyping quality, approximately 10% of random samples were regenotyped, and the results showed no genotyping errors.

Table 1. Demographic and Biochemical Characteristics of Subjects Enrolled in the Study

Parameter	Case (n = 134)	Control (n = 141)	*P*
Sex (M/F)	79/55	77/64	> .05
Age, y	5.5 ± 2.8	5.4 ± 2.5	> .05
Total protein, g/dL	4.60 ± 0.85	-	-
Serum albumin, mg/dL	2.39 ± 0.55	-	-
Triglyceride, mg/dL	300.26 ± 172.56	-	-
Cholesterol, mg/dL	366.70 ± 124.49	-	-

Statistical Analysis

The statistical analysis of the data was done using the SPSS 22.0 software (SSPS Inc., Chicago, IL, USA). The continuous and the categorical data between the groups were analyzed by independent sample t-test and Chi-square test, respectively. The association between genotypes and NS were estimated by computing the odds ratio (OR) and 95% confidence interval (CI) from unconditional logistic regression analyses. Results were considered

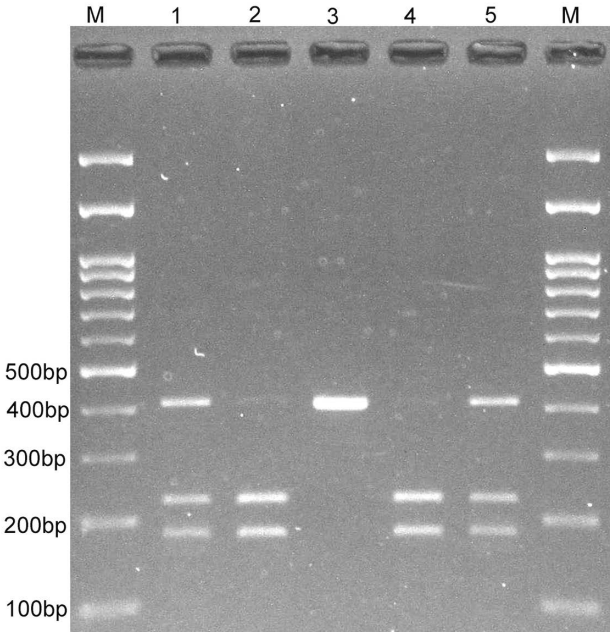

Figure 1. Photograph of electrophoresis pattern of the PCR-RFLP method for detection of MIF -173 G>C (rs755622) polymorphism. M: DNA marker; lanes 1 and 5: GC; Lanes 2 and 4: CC; Lane 3: GG.

statistically significant if the *P* values were less than .05.

RESULTS

We enrolled 134 children with NS and 141 healthy sex and age-matched children (Table 1). Frequency distribution of genotypes and alleles of *MIF* rs755622 polymorphism are shown in Table 2. The findings showed that rs755622 variant significantly increases the risk of NS in codominant (OR = 1.82, 95% CI = 1.08 - 3.08; *P* < .05, GC vs. GG), dominant (OR = 1.90, 95% CI = 1.14 - 3.16; *P* < .05, GC+CC vs. GG), overdominant (OR = 1.75, 95% CI = 1.04 - 2.94; *P* < .05, GC vs. GG+CC) and allele (OR = 1.76, 95% CI = 1.13 - 2.74; *P* < .05, C vs. G) inheritance models (Table 2).

We executed stratification analysis by sex (Table 3) and findings suggested that the GC, GC+CC genotypes and C allele significantly increase the risk of NS in male. No significant association between the variant and NS was observed in female.

We inspected the association between the MIF polymorphism and response to treatment in NS. The results did not support an association between the variant and response to treatment (Table 4).

In NS, the correlation between MIF -137 G>C polymorphism and biochemical parameters was evaluated and the results revealed that the genotype was not correlated with cholesterol, triglyceride, total protein and albumin levels (Table 5).

We assessed the Hardy–Weinberg equilibrium (HWE) and the finding showed no deviation from the HWE neither in cases ($\chi 2 = 0.565$, *P* > .05) nor controls ($\chi 2 = 0.242$, *P* > 0.05).

DISCUSSION

Childhood-onset idiopathic nephrotic syndrome is a common kidney disease. Clinical remission of primary NS can be achieved with corticosteroid

Table 2. Genotype and Allele Frequency of *MIF* -173 G>C Polymorphism in Patients with Nephrotic Syndrome and Control Subjects

MIF-173 G>C	Cases n (%)	Controls n (%)	OR (95% CI)	*P*
Codominant				
GG	80 (59.7)	104 (73.8)	1.00	-
GC	49 (36.6)	35 (24.8)	1.82 (1.08 - 3.08)	< .05
CC	5 (3.7)	2 (1.4)	3.25 (0.61 - 17.19)	> .05
Dominant				
GG	80 (59.7)	104 (73.8)	1.00	-
GC+CC	54 (40.3)	37 (26.2)	1.90 (1.14 - 3.16)	< .05
Recessive				
GG+GC	129 (96.3)	139 (98.6)	1.00	-
CC	5 (3.7)	2 (1.4)	2.69 (0.51 - 14.13)	> .05
Overdominant				
GG+CC	85 (63.4)	106 (75.2)	1.00	-
GC	49 (36.6)	35 (24.8)	1.75 (1.04 - 2.94)	< .05
Allele				
G	209 (78.0)	243 (86.2)	1.00	-
C	59 (22.0)	39 (13.8)	1.76 (1.13 - 2.74)	< .05

Table 3. Stratified Analysis of the Association of the *MIF* -173 G>C Polymorphism by Sex

MIF-173 G>C	Male				Female			
	Case n (%)	Control n (%)	OR (95% CI)	*P*	Case n (%)	Control n (%)	OR (95% CI)	*P*
Genotype								
GG	43 (54.4)	58 (75.3)	1.00	-	37 (67.3	46 (71.9)	1.00	-
GC	32 (40.5)	19 (24.7)	2.27 (1.67 - 4.42)	< .05	17 (30.9)	16 (25.0)	1.32 (0.61 - 2.89)	> .05
CC	4 (5.1)	0 (0.0)	-	-	1 (1.8)	2 (3.1)	0.62 (0.04 - 5.53)	> .05
GC+CC	36 (45.6)	19 (24.7)	2.56 (1.27 - 5.19)	< .05	18 (32.7)	18 (28.1)	1.24 (0.55 - 2.81)	> .05
Allele								
G	118 (74.8)	135 (87.7)	1.00	-	91 (82.7)	108 (85.7)	1.00	-
C	40 (25.2)	19 (12.3)	2.41 (1.30 - 4.47)	< .05	19 (17.3)	18 (14.3)	1.25 (0.61 - 2.50)	> .05

Table 4. Association of the MIF -173 G>C Polymorphism According to Response to Treatment

MIF -173 G>C,	Resistance n (%)	Responsive n (%)	OR (95% CI)	*P*
Genotype				
GG	16 (59.3)	64 (59.8)	1.00	-
GC	9 (33.3)	40 (37.4)	0.90 (0.38 - 2.26)	> .05
CC	2 (7.4)	3 (2.8)	2.67 (0.44 - 13.76)	> .05
GC+CC	11 (40.7)	43 (40.2)	1.02 (0.45 - 2.39)	> .05
Allele				
G	41 (75.9)	168 (78.5)	1.00	-
C	13 (24.1)	46 (21.5)	1.16 (0.58 - 2.35)	> .05

Table 5. Biochemical Parameters in NS Patients According to the MIF -173 G>C Genotypes

Parameters	*MIF*-173 G>C			*P*
	GG	GT	TT	
Cholesterol, mg/dL	389.1 ± 148.2	376.5 ± 117.9	385.6 ± 99.1	> .05
Triglyceride, mg/dL	277.5 ± 123.8	344.5 ± 221.8	303.4 ± 203.7	> .05
Total protein, mg/dL	4.7 ± 0.9	4.6 ± 0.9	4.4 ± 0.9	> .05
Albumin, mg/dL	2.5 ± .0.5	2.3 ± 0.5	2.4 ± 0.9	> .05

therapy. NS can be clinically classified as steroid-sensitive nephrotic syndrome (SSNS) and steroid-resistant nephrotic syndrome (SRNS) according to responsiveness to oral steroid therapy. Approximately 85–90% of NS children are SSNS, while, 10-15% are SRNS.[22] Differences in steroid response among children with NS are not entirely understood and can be attributed to genetic factors.[23] Several studies have been conducted to inspect the impact of MIF -173 gene polymorphism on NS children as well as response to treatment.[14, 16-19, 24] In the current study we tested the association between MIF -137 G>C variant on childhood NS and response to corticosteroid therapy in a sample of southeast Iranian population. Our findings showed that -173 G>C polymorphism of MIF significantly increases the risk of NS in our population. Stratification by sex revealed that this variant was correlated with increased risk of NS in male. This polymorphism was not associated with response to treatment as well as with serum levels of cholesterol, triglyceride, total protein and albumin.

Berdeli et al[14] have showed that GC genotype and C allele significantly increase the risk of NS. They found that the frequency of GG genotype was significantly higher in SRNS compared to SSNS. It has been shown that MIF -173 C allele significantly increases the risk of NS in children. Furthermore, this allele was more common in SRNS and was also associated with significantly higher risk of end stage renal disease (ESRD) within 5 years from onset.[16] Tripathi et al[15] have found that CC, CG and C allele significantly increas the risk of ESRD. However, they found no significant association between the MIF genotype and type of kidney disease in ESRD. Ramayani et al[17] reported that the allele frequency of the C allele was significantly higher in SRNS compared to SSNS patients. There was a trend toward a relationship between genotypes and serum levels of MIF. Hence, the presence of the C allele is correlated with higher levels of serum MIF. A meta-analysis performed by Tong et al[24] revealed that MIF -173 G>C polymorphism significantly increase the risk of renal disease, especially in children. Choi et al[18] have found no association between the MIF -173 G>C polymorphism and clinical parameters, renal histological findings, and steroid responsiveness. In addition, their findings did not support an association between MIF variant and NS.

There are some limitations in our study need to be pointed out. First, this study focused only on the -173 G>C polymorphism of MIF. Second, the sample size of the study is relatively small. Finally, we did not determine the serum levels of MIF.

In conclusion, the results of this study showed that MIF -173 G>C polymorphism was positively associated with NS in a sample of the southeast Iranian population. More association studies using larger sample sizes and diverse ethnicities are necessary to verify our findings.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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