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Genetic variations of complement factor H and C3 in patients with thrombotic thrombocytopenic purpura (TTP) in North-West of Iran

Sepideh Zununi Vahed[1], Bahram Niknafs[1], Mahmoud Shekari Khaniani[2], Mohammadreza Ardalan[1*]

[1]Kidney Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[2]Department of Medical Genetics, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran

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ABSTRACT

Background: Thrombotic thrombocytopenic purpura (TTP) is a common form of thrombotic microangiopathy. These patients have renal insufficiency as well as thrombocytopenia and microangiopathic hemolysis.

Objectives: The present study was aimed to assess if TTP patients with renal failure have prompting polymorphisms in the complement system genes as seen in patients with the atypical hemolytic uremic syndrome (aHUS).

Patients and Methods: Twenty TTP patients and 30 healthy individuals were included. Two single-nucleotide polymorphisms rs3753394 and rs2230199 respectively in the complement factor H (*CFH*) and complement component 3 (*C3*) genes were determined using the PCR-restriction fragment length polymorphism (RFLP) method. To evaluate the power of the associations between the polymorphisms and TTP development, odds ratios (ORs) and 95% confidence intervals (CIs) were employed.

Results: In rs2230199 polymorphism, the frequency of the C and G alleles and genotype were not significantly different in case and control groups. Moreover, the frequency of T allele and CC, CT, and TT genotypes of the rs3753394 polymorphism in TTP patients were not significantly different from those in the controls, the OR of 0.77 [CI: 0.33 to 1.79] and 0.76 [CI: 0.24 to 2.38], respectively ($P > 0.05$).

Conclusions: Based on our results, there was no significant association between the incidence of TTP and polymorphisms of the *CFH* and *C3* genes, neither at the allele nor at the genotypic levels ($P > 0.05$). This finding can be affected by the limited sample size or the genetic context of the studied population.

Implication for health policy/practice/research/medical education:
In the present study we aimed to examine the impact of the complement factor genes polymorphisms (*CFH* and *C3* genes) in TTP patients. No significant association was found between the incidence of TTP and polymorphisms of the *CFH* and *C3* genes, neither at the allele nor at the genotypic levels.

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1. Background

Thrombotic thrombocytopenic purpura (TTP) is a life-threatening blood disorder and a common form of thrombotic microangiopathy that is described by fever, microangiopathic hemolytic anemia, impaired renal function, thrombocytopenia, and neurological dysfunction (1). The cause of TTP is still unknown, but it has been attributed to the spontaneous accumulation of ultra-large von Willebrand factor (UL-VWF) multimers and platelets in the vessel followed by the development

*Corresponding author: Prof. Mohammadreza Ardalan, Email: ardalan34@yahoo.com

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of microangiopathic hemolytic anemia due to deficiency of ADAMTS13 metalloprotease, which plays a central role in UL-VWF cleavage (2). The complement system and its related gene variants may be also involved in the pathology of the TTP.

The complement system is a key component of the innate immune system, which has an important role in the removal of microbes and apoptotic cells, management of immune complex, and regulation of acquired immune responses. The complement system includes plasma and membrane-associated proteins and it can be stimulated by three independent pathways (e.g., classical, lectin, and alternative). This system is an aggressive self-reinforcing cascade that requires precise regulation by inhibitors to avoid damage to the host tissue. The inadequate activation of the complement alternative pathway with uncontrolled inhibition is linked to pathologic processes of a number of autoimmune and inflammatory diseases (3). There are reports suggesting that gene deficiency, mutation or polymorphic variations in complement factor H (CFH) and complement component 3 (C3) play a vital role in the susceptibility to the renal diseases and development of the diseases, specifically glomerulonephritis with C3 deposition (like membranoproliferative glomerulonephritis (MPGN) and the atypical hemolytic uremic syndrome (aHUS) (1, 3-5). So far, researchers have identified over 80 mutations and polymorphisms in the *CFH* gene (6).

Like TTP, aHUS belongs to the category of thrombotic microangiopathies. In view of the above, gene mutations and polymorphisms in *CFH* and *C3* genes can be expected to also appear in TTP patients.

2. Objectives

The present study designed to evaluate the prevalence of *CFH* and *C3* polymorphisms in patients with TTP. Establishing a relationship between TTP and CFH/C3 polymorphisms may contribute to the identification of patients who respond well to a particular treatment based on the observed mutations.

3. Patients and Methods

3.1. Subjects

This cross-sectional study was conducted on 20 patients with clinical diagnosis of TTP and 30 control individuals from the Azari ethnic group of the northwestern of Iran from 2014-2016. Although the low ADAMTS13 level is more discriminatory for TTP, we tried to distinguish TTP and HUS patients based on the presence or absence of neurologic abnormalities and diarrhea. TTP was diagnosed by an expert nephrologist based on the international TTP criteria. We tried to match the controls with our cases. All subject in both groups were in the same age range. The control group consisted of healthy individuals with no history of TTP or other diseases of thrombotic microangiopathy group in themselves or their family. Informed consent was taken from all participants after explaining the purpose of our study.

3.2. Genetic analysis

DNA extraction: Genomic DNA from peripheral blood leukocytes was extracted according to the Samadi Shams et al, protocol (7). Quality and quantity of the extracted DNA were tested by NanoDrop 1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The 260/230 and 260/280 nm absorbance ratios were used to determine the DNA purity and DNA and protein contamination of samples.

PCR; two comment polymorphisms rs3753394 and rs2230199 in *CFH* and *C3* genes, respectively, were studied using PCR and PCR-restriction fragment length polymorphism (RFLP) approaches (Table 1). PCR conditions were initial denaturation at 95°C for 7 minutes followed by 95°C for 1 minute, 64 and 51°C (respectively for *C3* and *CFH* polymorphisms) for 30 seconds and 72°C for 30 seconds (35 cycles). The products were digested with restriction enzymes listed in Table 1 and analyzed by electrophoresis in 2% agarose gel.

3.3. Ethical issues

The research followed the tenets of the Declaration of Helsinki. Our study protocol was approved by the ethics committee of Tabriz University of Medical Sciences (ethics code# IR.tbzmed.REC.1393.218). This study was conducted as the nephrology fellowship of Bahram Niknafs. The patients' medical records were used for data gathering and this process was secret.

3.4. Statistical analysis

Comparisons were made between groups by χ^2 test. Odds ratios (ORs) and 95% confidence intervals (CIs) for relative risks were calculated. Analyses were performed with SPSS software version 17. The *P* value <0.05 was considered significant.

4. Results

In the present study, we compared two rs3753394 and rs2230199 polymorphisms respectively in the *CFH* and *C3* genes in TTP patients and healthy individuals. Patients included 6 males and 14 females with a mean age of 35.25 ± 12.65 years. Statistical analysis of the age, gender, diseases (diabetes and CKD) between the studied groups showed no differences ($P > 0.05$). The detailed demographic data is presented in Table 2.

The frequency of the polymorphism rs2230199 in the *C3* gene was determined, Arg80Gly; C>G. Nine cases

(45 %) were homozygous for C allele (CC or SS), six patients (30 %) were heterozygote GC (FS), and five patients were homozygous for G allele (GG or FF) (3%). The allele frequency was 24 % for C3S and 16 % for C3F. However, there was no significant association between cases genotype and TTP, (OR of 0.69 [CI: 0.30 to 1.60, $P=0.39$] and 0.55 [CI: 0.17 to 1.71, $P=0.29$], Table 3). Moreover, the frequency of T and C alleles and CC, CT, and TT genotypes of the rs3753394 polymorphism in TTP patients were not significantly different from those in the controls, (OR of 0.77 [0.33 to 1.79] and 0.76 [0.24 to 2.38], respectively) ($P > 0.05$; Table 4). The minor allele (C3G) frequencies for rs2230199 was 0.40% while the minor allele (CFHT) for rs3753394 was 0.37% (Tables 3 and 4).

5. Discussion

It has been suggested that TTP, HUS, and MPGN have the same pathological basis and are associated with similar complement activity. Although TTP is identified by the severe lack of ADAMTS13, aHUS is characterized by hyperactivation of the alternative complement pathway as a result of mutations in an effector gene (such as C3) and a regulatory gene (like CFH) (8-11). Therefore, there is a possibility of interference of the complement components gene mutations and polymorphisms in the development of TTP. With this hypothesis, we investigated the *CFH* and *C3* gene polymorphisms in our study but found no significant difference in term of allele and genotype between TTP patients and healthy subjects. In other words, we found no association between the observed polymorphisms and TTP disease. The clinical significance of the rs2230199 has been underlined by different studies. The rs2230199 allele of *C3* represents a substitution (C>G) in nucleotide 304 at exon 3 of C3, which provides two allotypic variants with different stimulation rates in gel electrophoresis (C3 slow [C3S] and C3 fast [C3F]) that may have effect on the occurrence of inflammatory disease (12). The C3S allele is the most common type in human. Numerous studies have found significant associations between the presence of C3F and various diseases, for instance, IgA

Table 1. Primer sequences and restriction enzymes

Gene	Primers	Restriction Enzymes	Alleles	Fragment length (bp)
C3 c.304C>G polymorphism (rs2230199)	F: CTCACCTGTGGAGCCAGGGGTGTA R:CCAAAACGGCCACCTCGGAAGACC	*Hin6I*	CC CG GG	300 133-167-300 133-167
CFH −257C > T polymorphism (rs3753394)	F: CATTGTCTGGGTGCTGATTG R: TAGGGAAATTCTCCGTTGGA	*EcoR-v*	TT CT CC	216 61-155-216 61-155

Table 2. Demographic data

Characteristics	TTP patients	Control group	*P* value
Age	35.25 ± 12.65	38.56 ± 9.29	0.29
Sex			
Male	6 (30%)	12 (40%)	
Female	14 (70%)	18 (60%)	0.47
Diabetes	4 (20%)	5 (16.7%)	0.76
Chronic kidney disease	6 (30%)	6 (20%)	0.41
Disease duration	3.12 ±1.15	-	-

Table 3. Allele and genotypic frequency of the rs2230199 polymorphism

	TTP group	Control group	OR (95% CI)	*P* value
Allele frequency				
C	24 (60%)	41 (68%)	0.69 (0.30 to 1.60)	0.39
G	16 (40%)	19 (32%)		
Total	40 (100%)	60 (100%)		
MAF	0.40	0.31		
Genotypic frequency				
CC or SS	9 (45%)	18 (60%)	0.55 (0.17 to 1.71)	0.29
CG or FS	6 (30)	5 (17%)		
GG or FF	5 (25%)	7 (23%)		
Total	20 (100%)	30 (100%)		

Abbreviation: MAF: minor allele frequency.

Table 4. Allele and genotypic frequency of the rs3753394 polymorphism

	TTP group	Control group	OR (95% CI)	*P* value
Allele frequency				
C	25 (62.5%)	41 (68%)	0.77 (0.33 to 1.79)	0.54
G	15 (37.5%)	19 (32%)		
Total	40 (100%)	60 (100%)		
MAF	0.37	0.31		
Genotypic frequency				
CC or SS	10 (50%)	17 (57%)	0.76 (0.24 to 2.38)	0.64
CG or FS	5 (25%)	7 (23%)		
GG or FF	5 (25%)	6 (20%)		
Total	20 (100%)	30 (100%)		

Abbreviation: MAF: minor allele frequency.

Nephropathy (13), age-related macular degeneration (AMD) (14), systemic lupus erythematosus (SLE) (15), and renal allograft survival (12), and rejection (16). A meta-analysis study on the role of the polymorphism indicated that the rs2230199 G allele was a risk factor for AMD in Caucasians, but not in Asians (17). In a study carried out by Bazyar et al, on patients with renal allograft rejection, investigation of the *C3* gene polymorphisms showed C3S in 79% and C3F in 21% of patients. They also found all varieties of FF, FS, and SS genotypes in patients, and concluded that there is no relationship between this polymorphism and renal transplantation rejection (16). In a study conducted on congenital TTP patients (N = 32), Fan et al could not find any association between this polymorphism and congenital TTP (18). Likewise, in the present study, the presence of both *C3* alleles and three FF, FS, and SS genotypes were observed that could be the cause of finding no association between TTP and the genetic polymorphism. However, this possibility cannot be definitely confirmed or rejected without further multicenter and multiethnic studies.

CFH is an inhibitor of the alternative complement cascade. Impaired CFH expression or function may pose risk to the development of some diseases such as HUS, MPGN, and AMD. Studies have provided valuable information on the implication of genetic variants of *CFH* gene with the diseases susceptibility, especially rs3753394. This SNP is located at -257 upstream in the *CFH* promoter. Locating between glucocorticoid and nuclear factor-kappa B (NF-κB) binding sites makes it possible for the *CFH* gene SNP to respond to signals via the NF-κB pathway (19). The link between the T variant of the polymorphism rs3753394 and susceptibility to developing the aHUS (5, 20), dengue fever (19), and AMD (21) have been reported. In a comprehensive analysis by Caprioli et al, on the gene polymorphism in 101 HUS patients, 32 TTP patients, and 106 healthy subjects, TTP group showed no mutation in *CFHR1* or *CFH* (5). In the present study, we also could not find any significant association between *CFH* rs3753394 polymorphism and TTP.

6. Conclusions

Based on our result, there is no significant association between the incidence of TTP and the rs3753394 and rs2230199 polymorphisms, neither at the allele nor at the genotypic level ($P > 0.05$). The results indicated that the majority of the TTP patients with renal deficiency did not carry these gene polymorphisms when compared to controls. This finding can be affected by the limited sample size or the genetic context of the studied population.

Limitations of the study

There are definite limitations in the present study. One of the limitation is small sample size due to the low incidence of TTP. The second limitation of this study is that we did not examine the possible connotation between the studied polymorphisms and response to treatment in patients with TTP.

Authors' contribution

MRA designed the study and selected the patients. BN collected the samples. MS performed experimental analysis and interpretation of the data. SZV wrote the manuscript. All authors reviewed and approved the final manuscript.

Conflicts of interest

The authors declare no conflict of interest.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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