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Association between MIF gene variation and Meniere's disease

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Summary

Several pieces of evidence support the involvement of immune system in Menière's disease (MD). Macrophage migration inhibitory factor (MIF) plays a key role in immune-mediated reactions. Several studies have shown an association between MIF gene polymorphisms and susceptibility to various inflammatory and autoimmune disorders. The aim of this study was to explore the association between MIF-173 G/C polymorphism and MD in an Iranian population. In this case–control association study, MD cases ($N = 72$) were recruited and were comprised of definitive MD ($N = 58$) and probable MD ($N = 14$) subjects. Normal healthy subjects ($N = 100$) were also included. Genotyping for MIF-173 G/C polymorphism was carried out using PCR-RFLP technique. There was a significant increase in genotype GG in patients with MD compared with the control group. (GG vs. GC + CC, $P = 0.02$, OR = 2.08, 95% CI: 1.02–4.3). This was more significant when definitive MD was stratified and compared with the controls (GG vs. GC + CC, $P = 0.009$, OR = 2.6, 95% CI = 1.19–6.18). This study's result indicates the potential role of MIF in MD of which further evaluation is required. Also, the more significant association between MIF gene polymorphism and definitive MD designates the involvement of specific pathogenic mechanisms which may be considered as a marker for diagnosis.

Introduction

Menière's disease (idiopathic endolymphatic hydrops or MD) is an inner ear condition presenting recurrent attacks of vertigo, sensorineural hearing loss and tinnitus. Despite these well-known symptoms, it is still difficult to arrive at a diagnosis and then to manage the disease. This is due to the remarkable variability of Meniere's disease, which is one of its main characteristics, to the unknown cause of underlying mechanisms, including hydrops (Thorp & James, 2005).

Menière's disease is a syndrome with clinical heterogeneity and may involve immune-mediated inner ear disorders in a subset of patients; however, there are no biological markers to clarify the exact pathogenesis yet. Although in some recent studies an increased level of circulating immune complexes and some auto antibodies attaching to the cochlea has been reported in patients with MD, no correlation has been found between abnormal laboratory findings such as antinuclear antibody (ANA) and erythrocyte sedimentation rate (ESR) and the response of the disease to corticosteroid treatment (Brookes, 1986; Suslu *et al.*, 2009).

The macrophage migration inhibitory factor (MIF) is a pro-inflammatory cytokine released from T-lymphocytes and macrophages. It plays a key role in immune-mediated reactions by its ability to inhibit the random migration of macrophages. Several studies have shown an association between MIF gene 173 G/C polymorphism and susceptibility to various inflammatory and autoimmune disorders including rheumatoid arthritis (Radstake *et al.*, 2005; Akcali *et al.*, 2010), systemic lupus erythematosus (Sanchez *et al.*, 2006; Akcali *et al.*, 2010), sarcoidosis (Amoli *et al.*, 2002), inflammatory bowel (Fei *et al.*, 2008; Akcali *et al.*, 2010) and scleroderma (Wu *et al.*, 2006; Akcali *et al.*, 2010). It has also been revealed that increased MIF concentrations have been linked to severe clinical presentations and often a poor outcome of the disease (Renner *et al.*, 2005; Akcali *et al.*, 2010). Four polymorphisms of the human MIF gene have been reported in association with disease of the immune system (Radstake *et al.*, 2005; Sanchez *et al.*, 2006; Fei *et al.*, 2008). *In vitro* studies showed that within these four loci, some allele were associated with a higher production of MIF protein, whereas some others had a lower level of basal and stimulated MIF promoter activity (Baugh *et al.*, 2002; Donn *et al.*, 2002).

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By analogy, in regard to the role of MIF in inflammatory diseases, it is rational to postulate that polymorphism in the human MIF gene would influence the susceptibility or severity of MD.

The aim of this study was to investigate the association between MIF-173 G/C polymorphism and MD in an Iranian population.

Method

Subjects

Cases ($N = 72$) were recruited into this study from patients who fulfilled the American Association of Otolaryngology, Head and Neck surgery's diagnostic criteria for definitive MD ($N = 58$) and probable MD ($N = 14$) (American Academy of Otolaryngology-Head and Neck Foundation, 1995).

Normal healthy subjects ($N = 100$) were also included as controls. In particular, they were evaluated about other vestibular disorders, MD syndrome, autoimmune diseases, corticosteroid treatment, diabetes, cardiovascular disease and history of surgery. Patients were examined in the outpatient unit of Amir-Alam hospital, an affiliated hospital of Tehran University of medical sciences.

Approval from the ethics committee of Tehran University of medical sciences was obtained. Written informed consent was obtained from all of the participating subjects.

Polymorphism genotyping

Genomic DNA was extracted from peripheral blood collected in ethylenediaminetetraacetic acid tubes as anticoagulating agent using the salting-out method. MIF gene polymorphism was genotyped by the polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) method as described previously (Donn *et al.*, 2001). The PCR reaction mixture contained 200 ng DNA, 0.3 mM of each primer, 0.2 mM dNTPs, 1 unit Taq polymerase (Fermentas), 2 mL 10 × PCR buffer (Fermentas) with 2.5 mM magnesium chloride in a total reaction volume of 25 mL. The MIF-173 forward and reverse primers were as follows: 5′-ACT-AAG-AAA-GAC-CCGAGGC-3′ and 5′-GGG-GCA-CGT-TGG-TGT-TTA-C-3′, respectively. The cycling conditions were as follows: 95°C for 10 min, followed by 35 amplification cycles at 95°C for 45 s, 60°C for 45 s and 72°C for 45 s and a final extension at 72°C for 10 min.

The PCR products were then digested using *Alu*I restriction endonuclease (Fermentas) after overnight incubation at 37°C. The digestion products were analysed on a 3% agarose gel stained with SYBR green and visualized with UV light. The PCR products for the GG genotype had a consistent restriction site resulting in 98- and 268-bp fragments, three fragments of the size 205, 98 and 63 bp for the CC genotype and four fragments of the size 268, 205, 98 and 63 bp for the GC genotype.

Statistical analysis

The strength of association between different groups and alleles or genotypes of MIF polymorphism was estimated using odds ratios (OR) and 95% confidence intervals (CI). Levels of significance were determined using contingency tables by either chi-square test or Fisher's exact analysis. All analyses were carried out employing the STATA (v8) software.

Results

Characteristics of patients are summarized in Table 1. MIF-173 G/C gene polymorphism frequency was determined in patients and controls. Allele and genotype frequencies conformed to Hardy–Weinberg equilibrium in all groups ($P > 0.05$).

Association between MIF gene polymorphism and MD

A significant increase in genotype GG was found among patients with MD when compared with the control group. (GG vs. GC + CC, $P = 0.02$, OR = 2.08, 95% CI: 1.02–4.3) Table 2. The association was independent of sex and age after adjustment using regression model.

Association between MIF gene polymorphism and patients with definite MD and probable MD

After stratifying patients into definite and probable MD, a more significant association was observed in patients with definite MD and MIF gene polymorphism when compared with the controls (GG vs. GC + CC, $P = 0.009$, OR = 2.6, 95% CI = 1.19–6.18) Table 2.

Association between MIF gene polymorphism and level of hearing loss

We examined MIF gene allele and genotype frequencies in patients stratified according to the level of hearing loss. No significant association was observed between MIF gene polymorphism and the level of hearing loss Table 3.

Table 1. Clinical characteristics of patients

Sex (M/F)	25/47
Mean age	37.7 ± 11.3
Unilateral/bilateral	21/1
Level of hearing loss	
Mild	30
Moderate	19
Severe	14
Total number of patients	72

Table 2. Allele and genotype frequencies of MIF gene polymorphisms in definite and probable MD compared with normal healthy controls

MIF −173 C/G	Controls ($N = 100$)	MD ($N = 72$)	Definite MD ($N = 58$)	*P*-value, OR, 95% CI	
				MD vs. Controls	Definite MD vs. Controls
Genotype					
GG	59 (59%)	54 (75%)	46 (79%)	$P = 0.02$, OR = 2.08	$P = 0.009$, OR = 2.6
GC	31 (31%)	16 (22%)	10 (17%)	95% CI: 1.02–4.3	OR = 2.6, 95% CI = 1.19–6.18
CC	10 (10%)	2 (2%)	2 (3%)		
Allele					
G	149 (74%)	124 (86%)	102 (88%)	$P = 0.008$, OR = 2.1	$P = 0.004$, OR = 2.49
C	51 (26%)	20 (14%)	14 (12%)	95% CI = 1.1–3.9	95% CI = 1.2–5.1

Table 3. Allele and genotype frequencies of MIF gene polymorphisms MD stratified according to the level of hearing loss

MIF −173 C/G	Mild ($N = 28$)	Moderate ($N = 19$)	Severe ($N = 10$)
Hearing Loss			
Genotype			
GG	21 (75%)	12 (63%)	10 (100%)
GC	6 (21%)	7 (37%)	0 (0%)
CC	1 (4%)	0 (0%)	0 (0%)
Allele			
G	48 (86%)	31 (81%)	20 (100%)
C	8 (14%)	7 (19%)	0 (0%)

Discussion

Menière's Disease (MD) is a chronic multifactorial disorder, whose development and pathophysiology involve environmental and genetic factors. Menière's Disease has about 10–20% familial occurrence with a probable autosomal dominant mode of inheritance proposes some genetic aetiologic factors that play a role in the pathogenesis of the disease (Morrison *et al.*, 1994).

Numerous factors have been reported to be associated with MD such as viral infections, ischaemia, trauma and autoimmune disorders (Schuknecht, 1984; Lee & Kimura, 1992; Ruckenstein, 2004) . Although these factors reflecting the unknown precise pathophysiology of the disease, also suggest that MD may have multiple aetiologic factors.

It has been shown that some major histocompatibility complexes (MHCs) and human leucocyte antigens (HLA) have been related to MD and support the autoimmune mechanism taking part in the pathogenesis of the disease (Xenellis *et al.*, 1986; Morrison & Johnson, 2002; Koo *et al.*, 2003) . A recent study by A. Greco and colleagues also upholds the hypothesis that MD is an autoimmune disorder (Greco *et al.*, 2012).

Recently, in literature, there has been considerable speculation about the potential role of MIF in the pathogenesis of autoimmune diseases and its part as a regulator in inflammatory and innate responses (Calandra *et al.*, 1994). A quickly growing body of literature has revealed the association of MIF gene polymorphisms and a vulnerability to or a severity of some inflammatory diseases. Akcali and colleagues showed that the MIF-173 CC genotype is linked to multiple sclerosis of which these patients also had an earlier onset (Akcali *et al.*, 2010).

In a study on a Japanese people, the association of ulcerative colitis (UC) with MIF polymorphism was observed. It was shown that the 5/5 CATT genotype had a protective effect especially when the disease begins after the first two decades of life. In contrast, the 7/7 CATT genotype was clearly more frequent among UC patients (Shiroeda *et al.*, 2010).

The current study indicated a significant association between MIF gene polymorphism and MD. This was more evident in the definite MD group thus indicating the pathogenic role of MIF in MD. In the study of Donn *et al.* (2001) MIF gene polymorphism was associated with susceptibility to juvenile rheumatoid arthritis (JRA). They reported a significant difference in allele and genotype frequencies of MIF-173 G/C polymorphism in JRA patients compared with their control group. The MIF-173 C allele was associated with an increased risk of JRA. Donn *et al.* (2004) also showed that MIF polymorphism plays critical role in the susceptibility to psoriasis.

Promoter sequence analysis shows that the presence of the MIF-173 C allele creates a potential activator protein 4 transcription factor binding site, which leads to significant differences in MIF expression. Donn *et al.* discovered that the promoter activity in a T-lymphoblast cell line has a direct correlation with a 7-CATT repeat and MIF-173 C allele. Also as a corollary, it seems that the MIF-173 C allele and 7-CATT repeat promote the production of MIF (Donn *et al.*, 2002).

In addition to all of these studies addressing the role of MIF in autoimmune diseases, Grevan, *et al.* discussed MIF-directed therapies as a novel therapeutic approach perhaps as an alternative or even the first choice in treatment of such diseases in the future (Greven *et al.*, 2010).

The difference in the MIF gene polymorphism association between probable and definitive MD as observed in this study may indicate the involvement of various pathogenic mechanisms which could be considered as marker for discriminating between the two

groups. However, it should be noted that small number of patients participated in the probable group presents a limitation of this study. This necessitates careful interpretation of data and requires that future studies be performed on larger sample size to confirm the results obtained in current study.

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