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OTOLOGY

Sex-specific association of RANTES gene −403 variant in Meniere's disease

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Abstract Several studies have shown the correlation between RANTES gene and inflammatory disorders; the aim of the present study was to investigate the association between RANTES promoter gene polymorphism and Meniere's disease (MD) in an Iranian population. In this study patients with MD comprising definite MD ($N = 56$) and probable MD ($N = 15$) were selected according to diagnostic criteria of AAO-HNS. The control group ($N = 101$) were healthy normal subjects who did not have a history of ear disease and vertigo. PCR–RFLP for RANTES −403G>A has been performed. We found a protective role for RANTES −403A allele in male group in our population. None of the male patients with MD were carrier of allele A which was significantly different from the presence of allele A in the male control group (AA+GA vs. GG: $p = 0.0004$, OR 0.05, 95 % CI 0.001–0.39). This difference was not significant in female group. There was no significant association between RANTES gene polymorphism and the level of hearing loss. our results showed a sex-specific association between RANTES gene polymorphism and MD but more studies are necessary to further assess this association.

Keyword RANTES · Gene · Variation · Meniere's

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Introduction

RANTES (regulation upon activation normal T cell expressed and secreted) gene is localized on 17q11.2–q12 and previous studies have reported two functional polymorphisms within RANTES promoter region including −28C>G and −403G>A [1–3]. RANTES gene encodes a 8 KD protein which is a subfamily of chemotactic cytokines [4] and it codes for a chemotactic protein attracting inflammatory cells such as memory T cells (CD4+, CD45RO+), stimulated T cells (CD8+, CD4+), natural killer cells [5], basophils, eosinophils [6], dendritic cells, mast cells [7], monocytes [8], and microglia [9]. RANTES gene expression has been determined in many inflammatory diseases [10, 11]. Several studies have suggested an association between −403G>A polymorphism in the promoter region of RANTES gene with some inflammatory disease such as asthma, sarcoidosis and atherosclerosis [12].

Meniere's disease (MD) is an autoimmune inner ear disorder characterized by sensorineural hearing loss, recurrent tinnitus and vertigo. MD is a complex trait in which both genetic and environmental factors contribute to the etiology of the disease [13, 14]. Some etiologies have been mentioned such as trauma, viral infections, Syphilis at end stage, Cogan's syndrome [15–17].

The aim of this study was to assess the association between RANTES gene −403G>A polymorphism and MD in an Iranian population.

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Methods

Subjects

In this study, the case group ($N = 71$) consisting of definite MD ($N = 56$) and probable MD ($N = 15$) were selected according to diagnostic criteria of American Academy of Otolaryngology-Head and Neck Surgery (AAO-HNS). Inclusion criteria were: at least two attacks of vertigo that has lasted at least 20 min, sensorineural hearing loss-oscillation which is confirmed by audiometry, tinnitus and earfullness. The control group ($N = 101$) were healthy normal subjects who did not have a history of ear disease and vertigo. The controls subjects were collected from the same area as patients group and were examined and selected for the study by an expert clinician. MD patients were selected from Amir-Alam Hospital. Written consent forms were obtained from all patients and controls. The study was approved by Ethics Committee of Tehran University of Medical Sciences.

DNA extraction and genotyping

Genomic DNA from two groups of participants, case and control, was extracted from anticoagulated blood collected in EDTA tubes using salting out method followed by polymorphism assessment by PCR–RFLP method as previously reported [18]. In brief PCR reaction was performed in 20 µl final volume using pre-mix from Amplicon Company, 0.5 µM of each primers and 50 ng DNA. Amplification performed using following primers: Forward: 5′-GCCTCAATTTACAGTGTG-3′ and reverse: 5′-TGCTTATTCATTACAGATGTT-3′. PCR cycles optimized as follows: 95 °C for 2 min, followed by 40 cycles of 95 °C for 40 s, 40 s at 50 °C, 40 s at 72 °C and 5 min at 72 °C as the final step.

7.35 µl of PCR product was digested with 2 unit/µl restriction enzyme *Mae*III after overnight incubation at 55 °C in 20 µl final volume. Digested product was visualized on 4 % agarose gel.

The enzyme does not cut the PCR product carrying "G" allele resulting in a single 135 bp band while the mutant type "A" yields two fragments of 112 and 23b after digestion.

Statistical analysis

Odds ratios (OR) and 95 % confidence intervals (CI) were used to assess strength of association between different groups and alleles or genotypes of RANTES polymorphism. Levels of significance were determined using contingency tables by either Chi-square test or Fisher's exact analysis. All analyses were performed using the STATA (v8) software.

Table 1 Clinical features of patients

Mean age (years)	39.7 ± 11.5
Sex (M/F)	25/46
Unilateral/bilateral	65/6
Level of hearing loss	
Mild	31
Moderate	20
Severe	13
Profound	0
Total number	71

Results

Patient's clinical features are given in Table 1. The mean age ± standard deviation (SD) in control group was 40.5 ± 11.93 and 53 females (52.5 %) and 48 males (47.5 %) were included in this group.

Association between RANTES gene −403G>A polymorphism and MD patients

There was no significant association between RNATES gene polymorphism and MD patients in our population (Table 2). We also stratified MD patients according to the level of hearing loss and evaluated RANTES genotype frequencies in different groups. There was no statistically significant differences between RANTES gene polymorphism and various groups (Table 2).

Association between RANTES gene polymorphism and MD patients stratified according to gender

After stratifying MD patients according to gender, it was observed that the frequency of carrying either AA or GA genotype in male MD patients was 0 % which was significantly different from male control group conferring a protective effect (AA+GA vs GG: $P = 0.0004$, OR 0.05, 95 % CI 0.001–0.39). The frequency of RANTES gene polymorphism was not statistically different in female case group compared to female control group (Table 3).

Discussion

Meniere's disease (MD) is a multi-factorial disorder with the influence of many genetic and environmental factors [19]. 5–13 % of MD cases have a positive family history for this disease and a study by Klar et al. has shown that an MD gene was linked to chromosome 12p12.3 [20]. Numerous anatomic and physiologic etiologies have been proposed for this disease such as ischemia, trauma and autoimmune disorders [21].

Table 2 Genotype frequencies of RANTES gene in three groups, definite MD, probable MD and level of hearing loss compared with normal healthy controls

RANTES −403G/A	Controls ($N = 100$)	MD ($N = 71$)	Definite MD ($N = 56$)	Probable MD ($N = 15$)	Level of hearing loss			*P* value, OR, 95 % CI		
					Mild ($N = 31$)	moderate ($N = 20$)	Severe ($N = 13$)	MD vs. Controls	Definite MD vs. Controls	Probable MD vs. Controls
Genotype										
GG	56 (56 %)	43 (60 %)	33 (59 %)	10 (66 %)	19 (61 %)	10 (50 %)	9 (70 %)	$P = 0.3$, OR 1.2 95 % CI 0.6–2.3	$P = 0.7$, OR 1.1 95 % CI 0.5–2.3	$P = 0.4$, OR 1.5 95 % CI 0.4–2.6
GA	35 (35 %)	23 (32 %)	18 (32 %)	5 (33 %)	10 (32 %)	9 (45 %)	2 (15 %)			
AA	9 (9 %)	5 (7 %)	5 (9 %)	0 (0 %)	2 (6 %)	1 (5 %)	2 (15 %)			

Table 3 RANTES gene polymorphism in patients with MD stratified according to gender in cases and control groups

	AA (%)	GA (%)	GG (%)
Control			
Female	2 (3)	23 (41)	31 (56)
Male	7 (16)	12 (27)	25 (57)[a]
Case			
Female	5 (12)	16 (37)	22 (51)
Male	0 (0)	0 (0)	25 (100)[a]

[a] AA+GA vs. GG: $P = 0.0004$, OR 0.05, 95 % CI 0.001–0.39

RANTES plays an important role in the development of inflammatory and autoimmune disorders such as multiple sclerosis, asthma, rheumatoid arthritis, atopy and systemic lupus erythematosus (SLE) [22]. Previous studies have demonstrated the correlation between RANTES gene polymorphism and susceptibility to many autoimmune and inflammatory conditions. A study on 201 Caucasian subjects has shown an association between −403G>A RANTES polymorphism with atopy and asthma. In this study, Freyer and his colleagues represented that −403A variant of RANTES is associated with increased risk of atopy and asthma. This variant is related to high level of RANTES transcription. RANTES is a chemoattractant for eosinophils, lymphocytes, monocytes and basophils so increase in RANTES production results in enhanced local inflammatory reactions [6, 23, 24]. In addition to asthma, some other inflammatory disorders such as rheumatoid arthritis and multiple sclerosis have also shown RANTES up regulation [25, 26]. Takada et al. identified the A allele as a genetic risk factor for sarcoidosis. They suggested it may result from increased RANTES expression in AA genotype patients compared with those with other genotypes [27].

According to the Simeoni et al., RANTES A−403 was strongly associated with coronary artery disease (CAD). Platelet secretary vesicles stored RANTES and it is secreted as a result of platelet activation on the inflamed endothelium surface. Therefore carriers of the A−403 allele may increase RANTES production and release [28–31].

In the study of Makki et al. [32], RANTES−403 gene polymorphism was examined in polymyalgia rheumatic (PMR), giant cell arteritis (GCA) and rheumatoid arthritis (RA). A significant increase in allele A frequency in PMR patients compared to healthy subjects and a slight increase in allele frequency was observed in RA but not in GCA group [32].

In a study of Iranian population, no significant difference in allele and genotype frequencies of RANTES−403 polymorphism was observed between coronary atherosclerosis (CAD) patients and healthy controls [11]. In current

study we have found a significant difference for distribution of genotype AA+GA between males MD patients compared to the controls indicating a gender specific role for RANTES−403A allele in MD.

Some studies investigated the sex effect and RANTES gene polymorphism in different autoimmune disorders; Wojciech and colleagues have reported an association between two functional polymorphisms in RANTES gene receptor (CCR5) and elevated risk of diabetic nephropathy in type 1 diabetic men. This study was performed on 794 type 1 diabetic patients in which 496 subjects had proteinuria or end-stage renal disease (ESRD) and 298 control subjects had normo-albuminuria. It was determined that male carriers of G allele of CCR5 gene and male carriers of the 32-bp deletion in the same gene had higher risk of developing diabetic nephropathy than non-carriers separately [33]. Another study by Chen et al. showed the gender association with RANTES among patients with uveitis [34]. Moreover, Laplana et al. found a sex-specific association with two functional polymorphisms in RANTES. They observed that RANTES −403A and RANTES Int1.1T alleles acted as pro-inflammatory markers which were over expressed in elderly males while up-regulation of RANTES anti-inflammatory haplotype −403G-Int1.1C was observed in elderly females [35]. The present study showed that RANTES−403A allele has a protective role in MD in male group and RANTES−403 allele G has been observed as susceptibility allele. However, this effect was not found in female patients. The discrepancies in rate of autoimmune and inflammatory disorders between male and female may partly be due to sex hormones effect. Some studies have suggested anti-inflammatory effects for estrogen by up-regulation of antioxidant enzymes [35–37]. In addition to the effect of difference in sex hormones between male and female our data support the fact that genetic makeup in the context of hormonal milieu might also contribute to diverse tendency towards the inflammatory disease in male and female which requires further investigation. This might help us in understanding the mechanism of inflammatory conditions. Some studies in Japanese population showed that −403A variant of RANTES was associated with slower rate of HIV progression; individuals with A allele had lower rates of $CD4^+$ T cells depletion [2, 38, 39]. In the present study, we observed that allele A had a protective role in men but not in women against MD. Therefore it could be speculated that the function of RANTES is variably regulated as a result of various hormonal production and their anti-inflammatory effect in women vs. men and this might be regarded as a contributing factor for distinction between male and female susceptibility to MD.

One of the main limitations in our study was small sample size. Additional studies with larger sample size and in different populations are required to confirm the results we have obtained in this study. Also further studies looking at the CCR5 gene polymorphisms as RANTES receptor would be useful in understanding the role of RANTES in pathogenesis of MD.

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