MEFV Common mutations detection in Iranian Azeri Turk patients with Ulcerative colitis: a case –control study

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Abstract: Background Ulcerative colitis and Crohn's disease constitute the two major forms of chronic inflammatory bowel diseases with unknown etiology. It is suggested that genetic background and environmental factors are important factors in the diseases susceptibility and patient's response to therapy. Familial Mediterranean fever is an auto-inflammatory disease mainly inherited as an autosomal recessive condition. Since there are similarities between FMF and IBD, the responsible gene for FMF (MEFV) has been introduced as a modifier gene for IBD. **Study:** This case –control study was conducted in a setting of 139 unrelated patients referred with UC compatible symptoms and 200 matched controls without any positive familial history of Behcet syndrome, FMF or IBD in Azerbaijan during 2008-2010.They were screened for the five most common MEFV mutations (E148Q, V726A, M680I, M694I, and M694V) applying PCR-RFLP and ARMS-PCR techniques. All cases, being of Azeri Turk origin were matched in terms of age, sex and race. **Results** Thirty one (22.3%) patients and 52 (26%) control individuals carried one of the studied mutations. All studied types of MEFV mutations except for M694I and V726A could be detected in the patients. However, E148Q and V726A mutations were only observed in the control group. E148Q was the most frequent mutation observed in this cohort. There was a significant difference between the two groups regarding M694V mutation (p=0.002). **Conclusion** Although the correlation between M694V mutation and UC seems to be significant among the Iranian Azeri Turk patients, studies on a large number of UC and control series and also on further MEFV mutations are required to determine the role of MEFV mutations in UC.
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1. Introduction

Inflammatory bowel disease (IBD) is an immune-mediated chronic intestinal condition. Being a polygenic disorder, IBD gives rise to multiple clinical subgroups within Ulcerative colitis (UC) and Crohn's disease that are thought to result from a dysregulated mucosal immune response to gut lumen bacterial antigens in a genetically susceptible host(Podolsky, 2002).

Eleven genome scans have been undertaken in IBD, resulting in detection of a number of Susceptibility loci on chromosomes 1, 3, 4, 5, 6, 7, 10, 12, 14, 16, 19 and X. According to their initial report and independent confirmations, the locations on chromosomes 16q, 12, 6, 14, 5, 19, 1, 16p and 10 have been renamed IBD 1 to IBD 9, respectively (Vermeire, 2006). While new genes being reported recently, genome-wide searches have shown disease-associated loci on many chromosomes like 16p(Barrett, 2008).

Familial Mediterranean fever (FMF) affecting primarily the people of Mediterranean ancestry, is common among Turks (Touitou, 2001). It is an autosomal recessive genetic disorder which is characterized by recurrent self-limited attacks of fever with serositis, synovitis, or erysipelas-like skin lesions (Lidar and Livneh, 2007). One of the main complications of the disease is amyloidosis, which mainly affects the kidneys; it however may be accumulated in other organs and tissues, including the heart and intestines.

The familial Mediterranean fever (FMF) gene (MEFV) that has been mapped to chromosome 16p13.3 consists of 10 exons and encodes a protein of 781 amino acids called Pyrin, a major regulator of the inflammasome platform controlling caspase-1 activation and IL-1b processing. Pyrin has been shown to interact with the gene product of NLRP3, NALP3/cryopyrin, also an important active member of the inflammasome(Nuray, 2010). The NLRP3 region has recently been reported to be associated with Crohn's disease (CD) susceptibility. Therefore we sought to evaluate MEFV as an IBD susceptibility

gene (Podolsky, 2002; The French FMF Consortium, 1997; Kinikli, 2005).

FMF and IBD concordance has been investigated in a few studies suggesting a relation between IBD and FMF, and also a disease modifying effect of MEFV mutation on IBD (Nuray, 2010; Cattan, 2000; Sari, 2008).

Recently, Sari et al. reported the concurrent manifestation of IBD and FMF in three infants(less than 6 month of age) in whom infantile UC was associated with the MEFV mutation. Later, they suggested that the onset of UC in infants should prompt a search for MEFV mutations as this association may influence the management of the disease(Sari, 2008).

Furthermore Cattan et al, Fidder et al. and other several studies confirm this theory (Nuray, 2010; Cattan, 2000; Giaglis, 2006; Fidder, 2002).

As FMF is no longer a rare disease in Azerbaijan[13] and both IBD and FMF are characterized by uncontrolled immune response, we hypothesized that Azeri Turks with IBD may have high prevalence of MEFV mutation(Esmaeili, 2008).

The aim of the present study was to determine the presence of common MEFV mutations in Azeri Turk patient's whit UC.

2. Material and Methods

The present randomized analytical case – control study was conducted in Azerbaijan, in a setting of 139 consecutive patients diagnosed with UC compatible symptoms and histological diagnosis during 2008-2010 and also a clinical healthy control group consisting of 200 individuals without any positive familial history of Behcet syndrome, FMF or IBD. The control group was matched in terms of age and sex.

Ethical approval was provided by Ethics Committee of Tabriz University of medical science. Furthermore every patient and control group was informed about the study and a written consent was signed by the patient and control group for blood sampling.

All subjects were of Azeri Turkish origin. Informed written consents were obtained from all patients after their being provided with the comprehensive explanation about the study. After bloods samples were taken, genomic DNA was extracted from peripheral blood leukocytes using standard protocols. Each sample was initially analyzed for the presence of the five most common MEFV mutations by using amplification refractory mutation system–polymerase chain reaction (ARMS-PCR) and PCR–restriction fragment length polymorphism (PCR-RFLP) methods as follow.

The mutations analyzed by ARMS-PCR were M694I (c.2082G>A)(The French FMF Consortium, 1997) ,V726A (c.2177T>C)(Eisenberg, 1998) and the mutations analyzed by PCR-RFLP were E148Q (c.442G>C)(Livneh, 1999), M680I (c.2040G>C & c.2040G>A)(Medlej-Hashim, 2000) M694V (c.2080A>G)(Medlej-Hashim, 2000).

The appropriate positive and negative controls were employed for each test. The positive results were repeated to ensure reproducibility.

To determine any differences between groups, data were analyzed by Chi-Square or Fisher's test; the odds ratio and 95% confidence interval were calculated to determine the relationships between the examined variables. Descriptive and analytical statistics were used throughout data analysis in a number of ways using SPSS version 17. $P < 0.05$ was considered to be statistically significant.

3. Results

In the present study, the 139 consecutive patients were diagnosed with UC based on the established clinical criteria and histological diagnosis. The patient group ranged in age from 16 to 72 years (mean 32.85 ± 12.112 years) and included males72 (51.4%) and females 67 (48.6%).

The main symptoms of patients during presentation were rectorhagia (45%), weight loss (44%), anorexia (34.2%), and dysentery (33.3%). Positive familial history was reported in 19 (13.5%). The allele frequency mutation in patients and the matched group is presented in Table1. V726A and E148Q mutations in the matched control group were detected as well.

Table1. The result of 5 common MEFV mutations in patients with ulcerative colitis and the matched control group

Genotype	Ulcerative colitis patients(139)	Control group(200)	P
M694V	7(5%)	0(0%)	
Heterozygous	7	0	0.002
Homozygous	0	0	
V726A	0(0%)	7(3.5%)	
Heterozygous	0	7	0.02
Homozygous	0	0	
E148Q	23(16.5%)	45(22.5%)	
Heterozygous	20	44	0.21
Homozygous	3	1	
M680I	1(0.72%)	0(0%)	
Heterozygous	1	0	0.41
Homozygous	0	0	
M694I	0(0%)	0(0%)	
Heterozygous	0	0	….
Homozygous	0	0	
Total subjects with mutation	31(22.3%)	52(26%)	
Heterozygous	28	51	0.52
Homozygous	3	1	
Subjects with no identified mutations	108(77.7%)	148(74%)	0.52
Total	139(100%)	200(100%)	….

4. Discussions

Crohn's disease (CD) and ulcerative colitis (UC) are known as the two main clinical subtypes of inflammatory bowel disease (IBD) sharing some common clinical features such as abdominal pain, diarrhea, arthralgia, arthritis, and fever. Both IBD and

FMF are characterized by uncontrolled immune response resulting in recurrent febrile attacks involving more than two systems. As FMF is prevalent among the Turkish, Iranian Azeri Turks are believed to be a high-risk population for developing FMF (Esmaeili, 2008).

In recent years, the possible association between FMF and IBD has been of interest. Therefore, we hypothesized that Iranian Azeri Turks with IBD may have high prevalence of MEFV mutations.

The present study is the first assessment to have been performed on the common MEFV mutations in Iranian Azeri Turk IBD patients; however, due to financial issues we were not able to study effect of nutrition and other modifying gen which can be considered as a limitation of the present study. Since the cloning of the MEFV gene in 1997(The French FMF Consortium, 1997; Kinikli, 2005), approximately 40 disease-associated mutations have been identified (Mor, 2005). Five founding mutations, E148Q, M680I, M694V, M694I, and V726A, have been reported for more than 70% of deleterious alleles (Touitou, 2003).

Esmaeili et al. analyzed latter five most common MEFV mutations in 190 Iranian Azeri Turk FMF patients detecting none of these misses mutations in 182(48%) of the alleles studied and therefore, they determined that FMF is prevalent among Iranian Azeri Turks(Eisenberg, 1998).

In the literature review, Cattan et al. reported that IBD appears to be more prevalent and severe in non-Ashkenazi Jewish patients with FMF (Cattan, 2000). Fidder et al. found seven patients with concomitant CD and FMF among a cohort of 4,978 FMF patients showing a higher attack frequency than the compared with an ethnically matched population (Fidder, 2002). Giaglis et al. identified seven patients carrying MEFV mutations heterozygously among 25 UC patients (Giaglis, 2006).

Although Nuray Uslua et al. study was a small cohort, disease causing MEFV mutations and FMF disease rate were increased among patients with IBD (Nuray, 2010).

However, other studies like Karban et al. and Yurtcu et al. did not find any association between FMF gene mutations and IBD phenotypic characteristics. Karban's study (an Israeli cohort of 209 CD patients), investigating mutations in the MEFV gene, does not implicate MEFV mutations as major modifiers in CD. Likewise, Yurtcuet al. in a similar study carried out on 47 patients with IBD and 25 healthy individuals did not find any association either (Karban, 2005; Yurtcu, 2009).

In our study all studied types of MEFV mutations except for M694I and V726A were found in the 22.3% of patients. However, E148Q and V726A mutations were only observed in the 26% of control group and V726A is reported in just 3.5% of healthy controls not in patient (Table 1). It maybe plays a protective role in controls but we did not study that. These data could offer additional incentive to us to plan for the more study in future. E148Q was the most frequent mutation in the two groups. There was a significant difference between the two groups regarding M694V mutation (p=0.002). We did not detect any of the 5 mutations in 108(77.7%) of the Studied patient. This could possibly be due to many factors, including the presence of other rare or unknown mutations, genetic heterogeneity(Akarsu, 1997; Cazeneuve, 2000), the presence of modifier genes (Touitou, 2001), and unknown environmental factors. The possible modifying role of MEFV mutations on the clinical expression of UC patient with mutation is not supported by our observations because we just find mutation in just 22.3% of patient so we did not study the correlation between mutation and disease course.

In conclusion, we found that some MEFV mutations (M694V,E148Q and M680I) could be an additional genetic susceptibility factor in UC, although they are only found in a minority of patients with UC (31 out of 139). This finding could be confirmed by sequencing coding regions of MEFV in a larger study. Thanks to the explosive growth of research involving the human genome, knowledge of molecular basis of IBD has advanced immensely within the past decades. Expanding conducted experiments in terms of their number and also investigating more MEFV mutations may result in having more reliable findings. This can contribute to determining the fact that these mutations could be involved in causing the illnesses. Furthermore, the fact that E148Q mutation even in homozygote cases has less severe symptoms, it can persuade us to think that the same rule could apply to ulcerative colitis (Gershoni-Baruch, 2002).

In conclusion, in Azerbaijan where FMF is frequent, patients with IBD should be screened for FMF.

Abbreviations:

Inflammatory bowel disease: IBD, Crohn's Disease: CD, Familial Mediterranean fever: FMF.

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Conflicts of Interest and Source of Funding:

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