

## Assesment of Common Exon 10 MEFV Gene Mutations in Patients with Refractory Rheumatoid Arthritis

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### Abstract

**Objective:** Rheumatoid arthritis (RA) is a common chronic inflammatory arthritis with many flare ups in some patients. New studies showed the importance of Mediterranean fever gene mutations in certain inflammatory conditions. We investigated molecular mutation in exon 10 of MEFV gene in patients with refractory RA and healthy individuals as a control group in North-West of Iran.

**Methods:** We conducted thirty four patients with refractory RA and fifty healthy controls in this study. Their DNA samples were analyzed with specific primers, standard PCR and Sequencing techniques. We studied the most common exon 10 variations on MEFV gene which include; M680I, M694V, M694I and V726A.

**Results:** The mean ages of patients with refractory RA were  $43.8 \pm 7.7$  years and disease duration was  $49.8 \pm 7.36$  months. The mean of DAS 28 (Disease Activity score) of patients with refractory RA was about  $4.2 \pm 0.51$ . The mean ages of healthy control cases were  $45.4 \pm 8.27$  years. The result of DNA sequencing showed no mutations in common exon 10 variations of MEFV gene in both groups.

**Conclusion:** According to the result, we found no association between severity of RA and common exon 10 MEFV gene mutations.

**Keywords:** Refractory Rheumatoid arthritis, MEFV gene, Mutation, Exon 10.

### INTRODUCTION

Rheumatoid arthritis (RA) is a chronic symmetric inflammatory polyarthritis with unknown etiology. It is the most common form of chronic inflammatory arthritis and often results in joint destruction and severe disability [1]. RA frequency varies between population around the world based on the geographic region and ethnicity and its incidence in rural and urban area of Iran is about 0.19 and 0.51 percent, respectively [2, 3]. The risk of developing RA has been linked to genetic factors for many years. Also, it has been shown that immune system genes have additional role in development of RA [4].

This has regularly been demonstrated in many populations that the main genetic risk factor associated with RA is the HLA DRB1 alleles. Tumor necrosis factor

(TNF) alleles also play some roles in the developing of RA [5, 6]. A number of other non-MHC genes have thus been accredited to be part of RA [5].

Drugs such as methotrexate and biologic DMARDs are mainstay of treatment which inhibits the inflammation and autoimmune activation. In spite of this effective therapies, the refractory RA exists and affects patient's quality of lives [7, 8].

One important genetic factor that may have some roles in severity and resistance of rheumatoid arthritis to drugs is recently recognized MEFV (Mediterranean fever) gene mutation [9]. MEFV gene is located on chromosome 16p13 and comprises 10 exons and 781 codons and produces a protein named pyrin or Marenostin. It proposed that, the protein has inhibitory effects on inflammation through leucocyte

cytoskeletal organization on polymorphonuclear cells and monocytes [9-13]. The carrier rate of MEFV gene in Mediterranean and middle eastern populations is about one in three to one in five [14].

Almost thirty six mutations have been introduced in exons 1, 2, 3, 5, 9 and 10 in the MEFV gene. Two apparent mutational 'hot spots' in MEFV gene exist mostly in exon 10 and exons 2. Four of five common mutations M694V, V726A, M680I, and M694I have been located in exon 10 and one E148Q in identified in exon 2 [11, 13].

An association between MEFV mutations and arthritic diseases was introduced by Booth et al., in 2001 who outlined that one of the most common mutations (E148Q) is highly tractable in white and Indian ethnic groups presenting amyloidosis and inflammatory arthritis [15-18]. In the study published in 2008 they found that MEFV mutations prevalence is meaningfully high in Ankylosing spondylitis (AS) patients in which the clinical features of Familial Mediterranean Fever is lacking; nevertheless, this is less likely to influence the prognosis of disease [16]. In the Study of disease severity in patients with rheumatoid arthritis carrying a mutation in the MEFV gene which has been done by Rabinovich et al., in 2005, the results were similar and they reported the possibility of the modifying effect of the MEFV gene on the expression of certain inflammatory diseases including RA [9]. The study also showed that the disease severity was much more in patients who were carriers of mutated gene compared with non-carrier group and most of mutations were in exon 2 [9]. There are some other studies that showed the modifying effect of MEFV mutations on inflammatory arthritis [12, 15, 19].

According to the literature MEFV mutations were linked to rheumatoid arthritis and also MEFV mutations may have roles in the disease severity, therefore it is important to investigate the impact of this genotype on patients with refractory RA. We adopted a case-control design, to compare the MEFV mutation frequency between patients with severe refractory RA and healthy peoples.

### MATERIAL AND METHODS

This study was carried out in the Emam Reza hospital, Faculty of Medicine, Tabriz University of Medical Sciences. Experimental procedures were carried out under Tabriz University of medical science Ethics

Committee guide-lines, department of internal medicine (number 92/3 - 8/30), and conducted according to the WMA Declaration of Helsinki for medical Research. Patients invited voluntarily without any cost to take part in the study. Consent forms were completed either by patients or medical staff.

### PATIENT SELECTION

Thirty eight consecutive patients with severe refractory RA which were diagnosed according to Rheumatology/ European League Against Rheumatism Collaborative criteria participated in the study. The severity of disease was evaluated based on DAS 28(Disease Activity score in 28 joint) criteria [20]. These patients had not been in remission despite treatment with Methotrexate 25 mg weekly, Prednisolone 7.5 mg daily, Hydroxychloroquine 400 mg daily and sulfasalazine 1500 mg daily and had DAS 28 more than 2.6. For comparison, fifty healthy peoples were also included in the study as control group. Four patients with severe refractory RA were excluded because of poor quality of their samples.

### SAMPLE PREPARATION AND DNA ISOLATION

For this aim, 500 µl of blood sample was collected from each participant in a tube under sterile conditions. Samples were collected in tubs containing 1000 µl cell lysis buffer (Tris HCL, 10 mm/l, sucrose 11% w/v, MgCl<sub>2</sub> 5 mm/l and Triton X-100 (1% v/v: PH=8) and then total solution were centrifuged at 6000 rpm for 2 min, then supernatant was removed and this step was repeated 1-3 more times. In the next step, 300 µl of nuclei lysis buffer (Tris HCL 10mm/l, SDS 1% w/v, EDTA 10mm/l, Sodium Citrate 10 mm/l; PH=8) was added and incubated in room temperature for 2 min, followed by addition of 100 µl of saturated NaCl and 600 µl of Chloroform. Tubes were centrifuged for 2 min at 6000 rpm. Then 300-500 µl of supernatant was transferred to a new 1.5 ml microfuge tube and 600 µl of cold Isopropanol was added and centrifuged for 1 min at 13000 rpm. Finally some of supernatant was removed and tube was dried on room temperature and 50-100 µl of TE was added. The quality and quantity of extracted DNA was measured by Nano-drop (Thermo Scientific). Extracted genomic DNA was stored in 20°C below zero for analyzing.

Specific primers were designed for DNA amplification of MEFV gene exon 10, protein coding region (Table 3); four common MEFV gene mutations M680I, M694V, M694I, and V726A were covered by primers. The

PCR amplification contained 10 µl of Sina Gen PCR Master Mix (Cat no. PR8250C), 1.5 µl extracted DNA and 1 µl each of MEFV primers in 20 µl total volume was performed. The reaction was conducted in the following settings: hold at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 56°C for 30 sec and extension at 72°C for 30 sec. The last cycle was followed by an extra extension step of holding at 72°C for 5 min. In order to identify the quality and fragment size of PCR products, one-fifth of samples was electrophoresed on a 2% agarose gel.

**SEQUENCING**

MEFV (exon 2) Sequencing was undertaken in both forward and reverse directions using the sequencing primers. Sequencing reactions were prepared for DNA fragments and products were sent for sequencing.

MEFV forward sequence 5'-TTACTGGGAGGTGGAGGTTG-3' and MEFV reverse sequence 5'-GAGGAGCTGTGTTCTTCCTC-3' and the result were analyzed by SnapGene software and online alignment using clustal Omega.

**STATISTICAL ANALYSIS**

We used a chi-square test, when appropriate, to analysis the difference in the prevalence of exon 10 MEFV variants between refractory RA patients and control group. All P values were 2-tailed, and P values less than 0.05 were weighted significant. Descriptive findings were reported as mean ± SD.

**RESULTS**

Genotyping of exon 10 MEFV gene failed for 4 refractory RA patients. Consequently, the gene analysis was performed on the remaining 84 subjects with complete data for exon 10 of 34 refractory RA patients (1 men and 33 women with a mean ± SD age of 43.8 ± 7.7 years), and 50 healthy control subjects (50 women with a mean ± SD age of 45.4 ± 8.27 years). Mean DAS score in refractory RA patients were about 4.2 ± 0.52. The demographic features of study subjects are summarized in table 1.

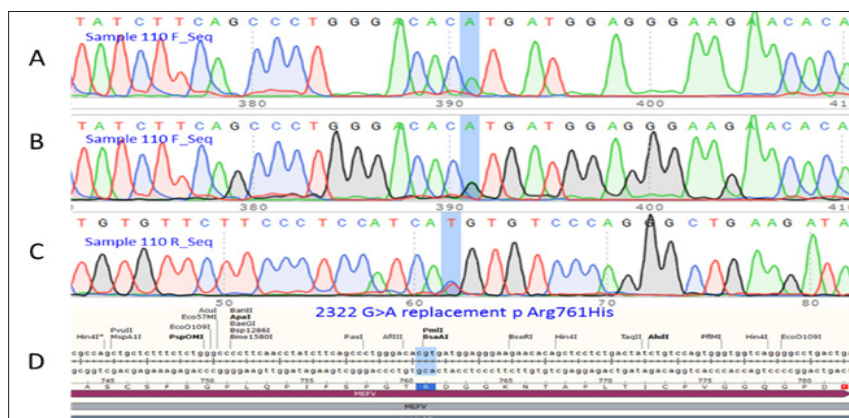
**Table 1.** Clinical and demographic description of refractory RA patients and healthy subjects

	Refractory RA patients	healthy subjects
Number	34	50
Median age(year), mean ±SD	43.8±7.7	45.4±8.27
BMI(kg/m2)	26.3	25.4
ESR	34.6	8.5
Women, No%	33(97)	50(100)

According to the result,there were no common exon 10 MEFV gene mutations in both groups.

We found one possibly uncommon homozygous variation according to reverse and forward sequence matching in refractory RA group at the position of 2322 G>A replacement substituting arginine to

histidine at the position of R761H (figure 1). Because the mutation frequency of exon 10 MEFV variants in both groups were not seen, No statistically analyze was performed. According to the result, we found no association between severity of rheumatoid arthritis and common exon 10 MEFV gene mutations.



**Fig 1.** Possible homozygous variation in one patient with refractory RA. A) In forward sequence there is overlap of chromatogram which is separated by omitting G from the chromatogram in forward sequence. B) Forward sequence. C) Reverse sequence presenting double nucleotides overlapping together (T & C). D) Position of change and CDS area of exon 10 with protein translation. In case of mutation Arg 761 will be substituted to His.

### DISCUSSION

In this study, we investigated the presence of mutations in the exon 10 of MEFV gene in patients with a clinical diagnosis of refractory RA and healthy peoples as a control group.

Former studies have shown two aspects of MEFV gene mutations in patients with RA: the prevalence of MEFV mutations in patients with treatment-responsive RA and healthy individuals [16-18, 21] and Association between RA severity and the presence of mutations in the MEFV gene in patients with RA [9, 10, 16].

These studies demonstrated that the rate of MEFV gene mutations increased in certain inflammatory diseases including rheumatoid arthritis and these mutations are linked to the disease severity [10, 21, 22].

In study undertaken in 2005, among 98 patients with RA, the rate of MEFV gene mutations were increased in patients with RA and healthy controls but the difference was not significant. The joint deformities of mutation carrier patients were more severe than non-carrier patients [9]B. There are some other studies which presented similar results [10, 12, 21]. This Studies which just mentioned, shown that MEFV gene mutations may are attributed with severe RA and all of these studies conducted on normal RA populations. But, less information is available regarding genetic studies on MEFV gene mutations on the patients with severe refractory RA so from this aspect our study holds of particular importance.

In our study, we found no known mutations in the exon 10 of MEFV gene variations, including M680I, M694V, M694I and V726A, in both groups. Subsequently we found no association between severity of rheumatoid arthritis and common exon 10 MEFV gene mutations; however, review of other studies showed that the rate of MEFV gene mutations have been increased in patients with RA [21, 23, 24].

The difference between this study and former studies which found association between RA severity and MEFV gene mutations could be due to the exon differences. They showed that most of MEFV gene mutations exist on exon 2 [9, 21, 24]. In addition, ethnicity of study population could be another reason in which these mutations are rare or less prevalent in Iranian population mainly in North West which our study carried out.

Despite studies which showed the increase frequencies of exon 10 of MEFV gene mutations in patients with RA [9, 18, 22], There is other studies which support the low incidence of exon 10 of MEFV gene mutations in RA patients [21,24]. In the study which carried out by Migita and colleagues in 2008, they found no Mutations in exon 10 (M694I) in any of the patients with rheumatoid arthritis and healthy controls [24]. Similarly in the study carried out by Kolahi and his group,2014, they also found no mutations in exon 10 of patients with RA [21].

The results of our study is also in correlation with the results of previous studies and show there is no significant difference in MEFV gene mutations between patient with RA and control subjects [10, 12, 21] which depicted possibly no role for FMF mutations in developing of RA.

We found one probably uncommon homozygous variation at the position of R761H, which need more studies for confirmation.

### CONCLUSION

the results of our present study, along with above mentioned published literature, indicate that the prevalence of exon 10 MEFV gene mutations in patients with rheumatoid arthritis is low and possibly there is no association between severity of rheumatoid arthritis and common exon 10 MEFV gene mutations. Further studies with larger populations is required to confirm or reject these findings.

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## Assesment of Common Exon 10 MEFV Gene Mutations in Patients with Refractory Rheumatoid Arthritis

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