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The association of endoplasmic reticulum aminopeptidase-1 (ERAP-1) with Familial Mediterranean Fever (FMF)

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Abstract

Background: The *ERAP1* gene cleaves the receptors and reduces their ability to transmit chemical signals to the cell that affect the process of inflammation and, secondly, it cleaves many types of proteins into small peptides that are recognized by the immune system.

Objective: *ERAP-1* gene mutations may create a sensitivity for Familial Mediterranean Fever (FMF).

Method: We included 15 FMF patients with the M694 (+) mutation in the study in order to exclude patients without pyrin gene mutations and create a homogeneous study group. Fifteen patients with ulcerative colitis formed the control group.

Results: There wasn't any case without *ERAP-1* gene mutations. At least one mutation at exon 3 or exon 10 was found in all cases in both groups. There were 14 *ERAP-1* gene mutations at exon 10 and 11 at exon 3 in patients with FMF. Interestingly, if there were *ERAP-1* gene mutations at exon 3, a p.Arg127 Pro (c.380 G>C) mutation always existed for three FMF patients with polymorphic mutations at this exon. There were 11 *ERAP-1* gene mutations at exon 10 and 12 gene mutations at exon 3 in patients with ulcerative colitis. Exon 3 mutations were usually single p.Arg127 Pro (c.380 G>C) mutations for 12 patients with ulcerative colitis as seen in the patients with FMF. The single mutation was always p.Ser453 Ser (c.1359T>C) for patients with ulcerative colitis at exon 10.

Conclusion: There are more *ERAP-1* mutations in the FMF group in comparison to the ulcerative colitis group. So, there may be a strong susceptibility to *ERAP-1* gene mutations in FMF patients according to our results. However, further studies with larger study and control groups are needed.

Keywords

ERAP-1, FMF, ulcerative colitis, pyrin gene mutations

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Introduction

The endoplasmic reticulum aminopeptidase-1 (ERAP-1) gene provides instructions for making a protein called endoplasmic reticulum aminopeptidase. First, it involves aminopeptidase, which is an enzyme that cleaves other proteins into peptides. Endoplasmic reticulum aminopeptidase cleaves the receptors and reduces their ability to transmit chemical signals to the cells, and thus affects the process of inflammation. Secondly, it cleaves many types of proteins into small peptides that are recognized by the immune system. These peptides are exported to the cell surface where they attach to major histocompatibility complex (MHC) class I proteins. MHC class I proteins display these peptides to the immune system. If the immune system recognizes the peptides as foreign (such as viral or bacterial peptides), it responds by triggering the self-destruction of the infected cell.[1–6]

Familial Mediterranean Fever (FMF) is an autosomal recessive disorder that frequently affects

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Turkish, North African Jewish, Arabic and Armenian populations. It is characterized by episodic attacks of inflammation in the peritoneum, synovium or pleura accompanied by fever and skin rash. However, it is usually hard to diagnose FMF because of very heterogeneous symptoms and subjective laboratory findings. The discovery of the pyrin gene mutations in patients with FMF caused huge expectations for the accurate diagnosis of the disease. However. pyrin gene mutations are not always helpful for the diagnosis of the disease.[7–15] FMF has three basic genetic forms: 1) genetically and phenotypic-positive patients, 2) genetically negative but phenotypic-positive patients, and 3) genetically positive but phenotypic-negative patients. Owing to the heterogeneity of the presentation, we still need some other markers to diagnose the disease. *ERAP-1* is essential for the maturation of a wide spectrum of proteins involved in various biological processes. Loss of *ERAP-1* function substantially alters the repertoire of peptides presented by MHC class I molecules, critically affecting recognition of both natural killer and CD8+ T cells. In addition these enzymes are involved in the modulation of inflammatory responses by promoting the shedding of several cytokine receptors and the regulation of both blood pressure and angiogenesis.[1]

Ulcerative colitis (UC) is an idiopathic, chronic inflammatory bowel disease that may present with arthritis. Non-MHC susceptibility loci such as *ERAP-1* are likely contributors to the inflammation in UC. In fact, if compared to other complex diseases such as ankylosing spondylitis (AS), fewer susceptibility loci have been identified in UC.[16] All of the physiopathogenetic mechanisms related to the *ERAP-1* gene mutations have been studied, especially in patients with UC.[1–6] UC is an auto-inflammatory disease like FMF. The association with the *ERAP-1* mutations has been shown to be very strong in this disease; therefore, we selected UC patients as a control group.

We hypothesized that *ERAP-1* gene mutations may promote pyrin gene mutations or exist together with pyrin gene mutations in FMF patients. We included only FMF patients with the M694 (+) mutation to exclude patients without pyrin gene mutations and create a homogeneous study group. We compared FMF patients with the patients with UC, which is a disease previously shown to have a relation with *ERAP-1* gene mutations.

Material and methods

This is a case-control study of *ERAP-1* gene mutations in serum from patients with FMF (study group) and from patients with UC (control group). Fifteen patients with FMF fulfilling Tel-Hashomer criteria and also known to be positive for the M694 pyrin mutation (heterozygote or homozygote) and 15 patients with UC diagnosed by clinical, laboratory, endoscopic and histopathological findings were included in the study. According to the Montreal criteria, 10 patients with UC had moderate disease and five patients with UC had severe disease.

Patients with FMF (male (M)/female (F): 4/11, mean age: 32.90 ± 8.09 years) and patients with UC (M/F: 1/14, mean age: 35.05 ± 3.33 years) were recruited from Maltepe Medical Faculty Hospital-Istanbul between November 2011 and March 2013.

Helsinki and local approval were received from the Kartal Medical Training Hospital Ethics Committee-Istanbul. Informed consent was obtained from all patients.

Detection of serum ERAP-1 gene mutations

Peripheral blood (10 ml) samples were obtained from each patient and centrifuged at 3500 rpm to obtain serum. Serum was deposited in an Eppendorf tube at –70°C for subsequent processing until assayed for *ERAP-1*. Analysis of *ERAP-1* gene mutations at exon 3 and exon 10 in both the study group and the control group were performed with polymerase chain reaction (PCR)-DNA folders. Primer pairs were used at 0.6 pico mole/ml. The other PCR components were 10 mM Tris-HCl (25°C pH: 8.8), 50 mM KCl, 0.2 mM deoxynucleotide 3′-phosphate (dATG, dGTP, dCTP, dTTP (Fermentas, Lithuania)) and 15 mM $MgCl_2$.

ERAP-1 gene primers at exon 3

5′AGT TCA ACA GCA AAG GGA ATT3′, 5′TTT TGC TTT TGT ACA TTT G3′.

ERAP-1 gene primers at exon 10

5′CTC CTC AGA GGG ATT AAC ATA, 5′TTA ACA GTG TTC CTG CAG TTG CG3′.

After denaturation and hybridization (Applied Biosystems, USA) of PCR products, amplification of the right gene region was checked by agarose gel.

After purification of the samples (Bio Basic, Canada), new PCR cycling was performed and purified again. At the end of this process samples were loaded into the DNA folders analyzer (Applied Biosystems 3130xl, USA). Obtained results were analyzed by SeqScape® Software, v 3.0.

Statistical analysis

Because of the small number of both groups, we didn't perform any statistical evaluation between the two groups.

Table 1. Results of *ERAP-1* gene mutations in patients with Familial Mediterranean Fever or ulcerative colitis.

	Patient number	Exon 3	Exon 10
FAMILIAL MEDITERRANEAN FEVER	1	p.Arg127 Pro (c.380 G>C) heterozygote	p.Ser127 Ser (c.1359T>C) heterozygote
	2	p.Arg127 Pro (c.380 G>C) Heterozygotep.Thr12Ile Pro (c.35 C>T) heterozygote	Normal
	3	p.Arg127 Pro (c.380 G>C) homozygotep.Thr12Ile Pro (c.35 C>T) Heterozygote	p.Ser127 Ser (c.1359T>C) heterozygote
	4	Normal	p.Ser127 Ser (c.1359T>C) heterozygote
	5	p.Arg127 Pro (c.380 G>C) homozygote	p.Ser453 Ser (c.1359T>C) homozygote
	6	p.Arg127 Pro (c.380 G>C) homozygote	p.Ser453 Ser (c.1359T>C) homozygote
	7	p.Arg127 Pro (c.380 G>C) heterozygote	p.Ser453 Ser (c.1359T>C) heterozygote
	8	Normal	p.Ser453 Ser (c.1359T>C) heterozygote
	9	Normal	p.Ser453 Ser (c.1359T>C) heterozygote
	10	Normal	p.Ser453 Ser (c.1359T>C) heterozygote
	11	p.Arg127 Pro (c.380 G>C) homozygotep.Thr12Ile Pro (c.35 C>T) heterozygotep. Tyr57Tyr (c.171 C>T) heterozygote	p.Ser453 Ser (c.1359T>C) heterozygote
	12	p.Arg127 Pro (c.380 G>C) heterozygote	p.Ser453 Ser (c.1359T>C) homozygote
	13	p.Arg127 Pro (c.380 G>C) homozygote	p.Ser453 Ser (c.1359T>C) homozygote
	14	p.Arg127 Pro (c.380 G>C) heterozygote	p.Ser453 Ser (c.1359T>C) homozygote
	15	p.Arg127 Pro (c.380 G>C) heterozygote	p.Arg127 Pro (c.380 G>C) heterozygote
ULCERATIVE COLITIS	16	p.Arg127 Pro (c.380 G>C) heterozygote	p.Ser453 Ser (c.1359T>C) homozygote
	17	p.Arg127 Pro (c.380 G>C) homozygote	p.Ser453 Ser (c.1359T>C) homozygote
	18	p.Arg127 Pro (c.380 G>C) homozygote	p.Ser453 Ser (c.1359T>C) homozygote
	19	p.Arg127 Pro (c.380 G>C) homozygote p.Thr12Ile Pro (c.35 C>T) heterozygotep. Tyr57Tyr (c.171 C>T) heterozygote	p.Ser453 Ser (c.1359T>C) heterozygote
	20	p.Arg127 Pro (c.380 G>C) heterozygote	p.Ser453 Ser (c.1359T>C) homozygote
	21	p.Arg127 Pro (c.380 G>C) heterozygote	p.Ser453 Ser (c.1359T>C) heterozygote
	22	p.Arg127 Pro (c.380 G>C) heterozygotep.Thr12Ile Pro (c.35 C>T) heterozygote	Normal
	23	p.Arg127 Pro (c.380 G>C) heterozygote	p.Ser453 Ser (c.1359T>C) heterozygote
	24	p.Arg127 Pro (c.380 G>C) heterozygote	Normal
	25	p.Arg127 Pro (c.380 G>C) homozygotep.Glu56Lys (c.166 G>C) heterozygote	p.Ser453 Ser (c.1359T>C) homozygote
	26	p.Arg127 Pro (c.380 G>C) heterozygote	p.Ser453 Ser (c.1359T>C) heterozygote
	27	p.Arg127 Pro (c.380 G>C) heterozygote	p.Ser453 Ser (c.1359T>C) heterozygote
	28	p.Arg127 Pro (c.380 G>C) heterozygote	p.Ser453 Ser (c.1359T>C) heterozygote
	29	p.Arg127 Pro (c.380 G>C) heterozygote	Normal
	30	p.Arg127 Pro (c.380 G>C) heterozygote	Normal

Results

There wasn't any case without *ERAP-1* gene mutations. At least one mutation at exon 3 or exon 10 was found in all cases in both groups.

There were 14 *ERAP-1* gene mutations at exon 10 and 11 *ERAP-1* gene mutations at exon 3 in patients with FMF. Exon 3 mutations were usually a single p.Arg127 Pro (c.380 G>C) mutation for eight patients with FMF. Interestingly, if there were *ERAP-1* gene

Table 2. Summary of the *ERAP-1* gene mutations.

		Diseases					
		Familial Mediterranean Fever ($n=15$)			Ulcerative colitis ($n=15$)		
MUTATIONS		Heterozygote	Homozygote	Total	Heterozygote	Homozygote	Total
Exon 10	p.Ser127 Ser (c.1359T>C)	4		4	–	–	–
	p.Ser453 Ser (c.1359T>C)	5	5	10	6	5	11
	TOTAL MUTATIONS	14			11		
Exon 3	p.Arg127 Pro (c.380 G>C)	1	7	8	10	2	12
	p.Arg127 Pro (c.380 G>C)						1
	p.Thr12Ile Pro (c.35 C>T)						
	p. Tyr57Tyr (c.171 C>T)		–	1	–	1	
	p.Arg127 Pro (c.380 G>C)						
	–p.Thr12Ile Pro (c.35 C>T)		–	2	–		1
	TOTAL MUTATIONS		11		12		

mutations at exon 3, the p.Arg127 Pro (c.380 G>C) mutation always existed for three FMF patients with polymorphic mutations at this exon. There were no mutations in four patients with FMF at exon 3. Polymorphic *ERAP-1* gene mutations were p.Thr12Ile Pro (c.35 C>T) +.Arg127 Pro (c.380 G>C) for two and p.Arg127 Pro (c.380 G>C) + p.Thr12Ile Pro (c.35 C>T) + p. Tyr57Tyr (c.171 C>T) for one patient with FMF. There were 14 single *ERAP-1* gene mutations at exon 10 in FMF patients. There were no mutations in only one patient at exon 10. The single mutation was p.Ser127 Ser (c.1359T>C) in 13 patients with FMF. Only one patient with FMF showed a p.Arg127 Pro (c.380 G>C) single gene mutation at exon 10 (Tables 1 and 2).

There were 11 *ERAP-1* gene mutations at exon 10 and 12 *ERAP-1* gene mutations at exon 3 in patients with UC. Exon 3 mutations were usually single p.Arg127 Pro (c.380 G>C) mutations for 12 patients with UC, similar to patients with FMF. There were polymorphic *ERAP-1* gene mutations in three patients: p.Arg127 Pro (c.380 G>C) + p.Thr12Ile Pro (c.35 C>T) + a p. Tyr57Tyr (c.171 C>T) mutation in one patient; p.Arg127 Pro (c.380 G>C) + p.Thr12Ile Pro (c.35 C>T) in one patient; and p.Arg127 Pro (c.380 G>C) + p.Glu56Lys (c.166 G>C) in one patient. There were 11 single *ERAP-1* gene mutations at exon 10 in UC patients. There were no mutations at exon 10 in four patients with UC. The single mutation was always p.Ser453 Ser (c.1359T>C) for patients with UC at exon 10 (Tables 1 and 2).

Discussion

We could not make any statistical evaluation between the FMF group and UC patients owing to the small number of patients but we determined that there are more *ERAP-1* mutations in the FMF group than in the UC group. So, there may be a strong susceptibility to *ERAP-1* gene mutations in FMF patients according to our results. All patients with FMF in this study had the M694 mutation, which is the most common mutation in Turkish patients with FMF. So, our FMF population was quite homogeneous. This homogeneity may also lead us to suspect an association of the *ERAP-1* gene mutation with the M694 mutation as well.

However, there are some limitations of this study. The most important one is the numbers in our study groups. Both study and control groups are very small because of financial difficulties. Second, we did not look at the *ERAP-1* mutations in a normal population. We tried to overcome these difficulties first by setting up a homogeneous FMF study group and second by making a comparison with UC, which is considered as having a similar etiopathogenesis.

FMF is a very heterogeneous disease and it has been shown before that in FMF there are pyrin gene mutations. Some other mutations can also exist. The *ERAP-1* gene controls a very important enzyme that helps to recognize proteins. Single nucleotide polymorphisms within the aminopeptidase *ERAP-1* are essential for trimming peptides before they are presented to T cells. *ERAP-1* is a highly polymorphic molecule comprising allotypes of single nucleotide polymorphisms. Therefore there is strong evidence that polymorphic *ERAP-1* alters protein function, predisposing an individual to disease via its influence on the antigen-processing pathway. This step is very important for the initiation of inflammation. On the other hand, pyrin controls the end of inflammation. Pyrin is produced in certain white blood cells that play a role in inflammation. But pyrin assists in keeping

the inflammation process under control. New research indicates that pyrin helps to regulate inflammation by interacting with the cytoskeleton. Pyrin may direct the migration of white blood cells to the sites of inflammation and stop or slow the inflammatory response when it is no longer needed. So, we can say that the *ERAP-1* gene mutation might start the inflammation without any real antigenic stimulation; however, mutant pyrin cannot control this inflammation. If this hypothesis is true, then we can explain why people with *MEFV* gene mutations do not present with the clinical findings of FMF.

The discovery of *ERAP* mutations in patients with FMF should not be a surprise. But, this is the first *ERAP-1* study in FMF patients. This is a pilot study with a very robust positive result. However, further studies with larger study and control groups are needed. We believe that the evaluation of *ERAP* mutations in FMF patients will help us to find new tools for the correct diagnosis of the disease. This study may provide a step forward in finding a new susceptibility gene in FMF.

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Conflict of interest

None declared.

Acknowledgements

Ethics approval: The protocol for this research project has been approved by our local ethics committee of the institution within which the work was undertaken, and it conforms to the provisions of the Declaration of Helsinki in 1995 (as revised in Edinburgh 2000).

Patient consent: All patients and control groups have been informed about the study and they joined the study voluntarily. Consent and patient anonymity have been preserved.

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