Malaria Chemotherapy, Control & Elimination

Qabaha et al., Malar Chemoth Cont Elimination 2013, 2:1
DOI: 10.4172/2090-2778.1000108

Research Article Open Access

Molecular Investigation of Clinically Suspected Familial Mediterranean Fever Patients Using ARMS-PCR

Khaled I Qabaha[1]*, Sabreen Tahayneh[1], Saisathya Thanigachalam[2] and Saleh A Naser[2]

[1]*Arab American University, Jenin, Palestine*
[2]*Burnett School of Biomedical Sciences, College of Medicine, University of Central Florida, Orlando, Florida*

Abstract

Objective: To investigate the distribution of three most common missense mutations-M680I, M694V and V726A in pyrin gene among clinically suspected Familial Mediterranean Fever (FMF) patients of northern side of the West Bank/ Palestine.

Methods: Fifty one blood samples were collected from clinically suspected FMF patients from Jenin, Nablus and RamAllah governorates and evaluated for the missense mutation in pyrin gene using ARMS-PCR technique.

Results: Among fifty one patients that were evaluated, 31(60.8%) were identified with the mutations M694V, V726A and M680I in descending order of their occurrences. The results of the other 20 were undetermined.

Conclusion: Molecular diagnosis of FMF through simple ARMS-PCR method helps to confirm the clinical diagnosis of the disease that relies on signs, symptoms, ethnicity and family history and response to colchicines.

Keywords: Familial mediterranean fever; ARMS-PCR; Pyrin gene; M694V; V726A; M680I

Abbreviations: FMF: Familial Mediterranean Fever; ARMS PCR: Amplification-refractory Mutation System Polymerase Chain Reaction; MEFV: Marevostrin Encoding Fever Gene; EDTA: Ethylene Diamine Tetra Acetic Acid: DNA: Deoxy Ribonucleic Acid

Introduction

Familial Mediterranean fever is an autosomal recessive disease that mainly affects people of Mediterranean and Middle Eastern descent that includes Jews (especially Sephardic), Arabs, Turks and Armenians [1,2].

FMF is characterized mostly by recurrent attacks of fever with inflammation of the abdominal lining (peritonitis) and of the lining surrounding the lungs and swollen and painful joints. Other symptoms may include inflammation of the testis (orchitis) and of the lining surrounding the heart (pericarditis), benign recurrent inflammation of the membrane that surrounds brain and spinal cord (meningitis) and headaches. These attacks mostly last from 1 to 3 days. One of the severe complications of FMF is Amyloidosis that may lead to renal failure [3,4].

FMF patients are identified to carry a mutation in the FMF gene that has been recently identified and cloned. The gene is called Marevostrin Encoding fever Gene (MEFV) which is located in the short arm of chromosome 16 that encodes for anti inflammatory protein, pyrin. It is composed of 781 amino acids and is expressed in leukocytes that include granulocytes, monocytes, dendritic cells and synovial, peritoneal and skin derived fibroblasts. Pyrin plays an important role in the inactivation of chemotactic factors that activates inflammation process [2,5-8].

At least 142 mutations were identified in the MEFV gene and M680I, M694V and V726A are among the most common mutations found in the gene [9-11]. This study investigated the spectrum of the above mentioned missense mutation of the pyrin gene in clinically suspected FMF patients of North end of the west bank –Palestine.

Materials and Methods

Clinical samples

In this study whole blood samples were collected in EDTA tubes from 51 clinically suspected FMF patients who were primarily diagnosed by various internists from Jenin, Nablus and Rama Allah cities.

DNA extraction

Total DNA was extracted from buffy coat using Master pure Genomic DNA purification kit (Epicenter Technologies Co) following manufacturer's instructions. The purified DNA samples were stored at -20°C until further use.

ARMS PCR

ARMS PCR was done to detect the mutations M680I, M694V and V726A. The procedure followed was according to [2] with some modifications. Each set of primers consists of three oligonucleotides as described in Table 1.

For each DNA sample, two complementary reactions were conducted. One reaction included mutant specific ARMS primers while the second included ARMS primers for the DNA sequence. The second reaction was used as an internal control for PCR amplification and to discriminate between homozygotes and heterozygote's. Negative controls were also included. The PCR reaction mixture includes total volume of 25 µl containing 0.5 U of Taq DNA polymerase.

1X PCR buffer, 1.5 mM of $MgCl_2$, 0.2 mM of deoxynucleotide 5'-triphosphate (dNTPs), 10 pmol of each primer, 100 ng of DNA template.

***Corresponding author:** Khaled I Qabaha, PhD, Arab American University, Jenin, Plestine, Tel: +05-99- 325358; E-mail: khaledqabaha@yahoo.com

Received September 26, 2013; **Accepted** October 14, 2013; **Published** October 21, 2013

Citation: Qabaha KI, Tahayneh S, Thanigachalam S, Naser SA (2013) Molecular Investigation of Clinically Suspected Familial Mediterranean Fever Patients Using ARMS-PCR. Malar Chemoth Cont Elimination 2: 108. doi: 10.4172/2090-2778.1000108

Mutation	Sequence (5'-3')	Primer Length	Primer Type
V726A	TGGAGGTTGGAGACAAGACAGCATGGATCC	30	Common
	TGGGATCTGGCTGTCACATTGTAAAAGGAGA	40	Mutant
	TGCTTCCTG		
	TGGGATCTGGCTGTCACATTGTAAAAGGAGA	40	Normal
	TGCTTCCTA		
M694V	TGACAGCTGTATCATTGTTCTGGGCTCTCCG	31	Common
	TCGGGGGAACGCTGGACGCCTGGTACTCATT	40	Mutant
	TTCCTTCCC		
	TCGGGGGAACGCTGGACGCCTGGTACTCATT	40	Normal
	TTCCTTCCT		
M680I	TTAGACTTGGAAACAAGTGGGAGAGGCTGC	30	Common
	ATTATCACCACCCAGTAGCCATTCTCTGGCG	39	Mutant
	ACAGAGCG		
	ATTATCACCACCCAGTAGCCATTCTCTGGCG	39	Normal
	ACAGAGCC		

Table 1: Primer sequences and length designed for ARMS PCR.

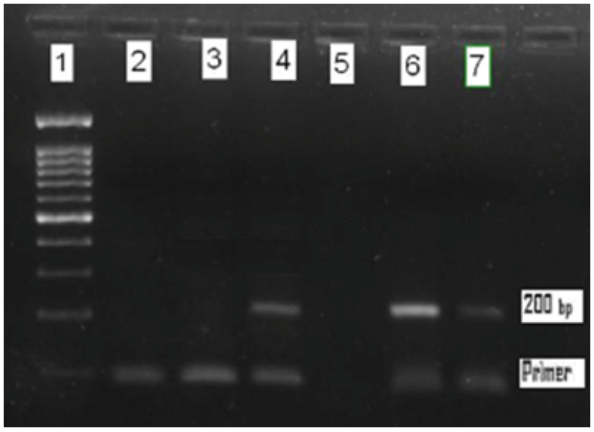

Figure 1: DNA electrophoresis of amplified parts of MEFV gene (~200 bp) using mutant and normal primers corresponding to mutations M694V (lane 3 and 4 showing only positive band for the mutant primer) and V726A (lane 6 and 7 showing positive bands for both normal and mutant primers). Lane 1 corresponds to DNA size marker, lane 2 corresponds to negative control, while lane 5 is blank.

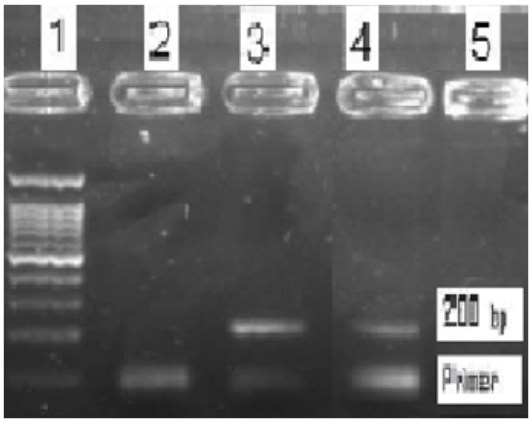

Figure 2: DNA electrophoresis of amplified parts of MEFV gene (~200 bp) using mutant and normal primers corresponding to mutation M680I (lane 3 and lane 4 showing positive bands for both normal and mutant primers). Lane 1 corresponds to DNA size marker, while lane 2 corresponds to negative control.

The cycle conditions for M680I included 94°C for 12 minutes of initial denaturation followed by 35 cycles of 94°C for 10 sec, 60°C for 30 sec, 72°C for 30 sec and final extension at 72°C for 10 min. The cycle conditions for mutations M694V and V726A are initial denaturation at 94°C for 9 minutes followed by 35 cycles with 94°C for 10 sec, 60°C for 10 sec, 72°C for 30 sec and final extension of 72°C for 10 min. PCR products were analyzed on 2% electrophoresis gel to determine the size of amplified fragment.

Results

Among 51 subjects under study, 31 (60.8%) were identified as positive for one or more mutations that is under study (Table 1). As shown in Figure 1, M694V was detected in 16 patients and V726A was detected in 15 patients whereas M680I was detected only in 11 patients (Figure 2). The most common genotype detected was V726A heterozygous (13.7%) followed by homozygous M694V (11.8%) and

Genotype	No. of Patients	%
M680I Hom	3	5.9
M680I Het	3	5.9
M694V Hom	6	11.8
M694V Het	0	0
V726A Hom	1	1.95
V726A Het	7	13.7
M680I/M694V Hom	0	0
M680I/M694V Het	4	7.8
M680I/V726A Hom	0	0
M680I/V726A Het	1	1.95
M694V/V726A Hom	0	0
M694V/V726A Het	6	11.8
Others	20	39.2

Table 2: Genotype distribution of 51 suspected FMF subjects.

compound heterozygous M694A/V726A (11.8%). The overall data is summarized in Table 2.

Discussion

Earlier FMF diagnosis was based on its signs and symptoms, family history, ethnicity and response to colchicine. Accurate clinical diagnosis for FMF still remains to be determined due to lack of pathognomonic signs. In 1997, the gene responsible for FMF was identified and isolated which paved way for diagnosis of FMF using an ARMS-PCR technique. The ARMS PCR assay used in the study enables early molecular detection of FMF cases. The assay is rapid, simple, cost effective and allows accurate detection of haplotypes with mutations involving small deletions or single base changes [2].

Definite diagnosis of FMF is not only important to avoid unnecessary colchicine therapy and genetic consultation but also to prevent lapratomies that are done due to misdiagnosis of acute abdominal pain. Also, some FMF patients develop amyloidosis at early stages of the disease, and ARMS-PCR positive result may suggest early colchicine therapy in such cases as the drug may help decrease the severity and frequency of FMF attacks when utilized on a long term basis [12,13].

As shown in Table 2, more than half of the clinically suspected FMF subjects showed positive results for the mutations under study (60.8%). The result is consistent with the findings of Aysheh et al findings, who reported that about 59% of genetically confirmed FMF patients show at least one of the three most common missense mutations (M680I, M694V, and V726A) [14].

Upon detailed analysis of the above three common mutations, the positive mutations were varying between homozygous (19.65%), simple heterozygous (19.6%), and compound heterozygous (22.55%). Among the alleles that were studied, M694V was found to be the most common allelic mutation (31.4%), followed by V726A (29.4%) and finally M680I (21%).

The highly frequent FMF mutation in this study was M694V (Table 2); which is consistent with the findings of Ayesh et al. and Ayesh et al. [4,14]. It is also the most common mutation among Arabs, Armenians, and Turks [2,15].

Such high rate of frequency could be attributed to the high rate of consanguinity, a well known habit especially among Arabs.

Studying the frequency of the most common mutations of the MEFV gene among the northern part of Palestine provides an important step toward the understanding of the pathophysiology of the FMF disease.

References

1. Settin A, El-Baz R, Abd Rasool M, El-Khalegy H, El-Sayed O, et al. (2007) Clinical and Molecular Diagnosis of Familial Mediterranean Fever in Egyptian children. JGLD 16: 141-145.
2. Eisenberg S, Aksentijevich I, Deng Z, Kastner DL, Matzner Y (1998) Diagnosis of Familial Mediterranean Fever by a Molecular Genetics Method. Ann Intern Med 129: 539-542.
3. Gershoni-Baruch R, Broza Y, Brik R (2003) Prevalence and Significance of Mutations in the Familial Mediterranean Fever Gene in Henoch-Schonlein Purpura. J Pediatr 143: 658-661.
4. Zaks N, Shinar Y, Padeh S, Lidar M, Mor A, et al. (2003) Analysis of theThree Most Common MEFV Mutations in 412 Patients with Familial Mediterranean Fever. IMAJ 5: 585-588.
5. Ritis K, Giaglis S, Spathari N, Micheli A, Zonios D, et al. (2004) Non-isotopic RNase Cleavage Assay for Mutation Detection in MEFV, the Gene Responsible for Familial Mediterranean Fever, in a Cohort of Greek Patients. Ann Rheum Dis 63: 438-443.
6. Sharkia R, Mahajnah M, Zalan A, Athamna M, Azem A, et al. (2013) Comparative Screening of FMF Mutations in Various Communities of the Israeli Society. Eur J Med Genet 56: 351-355.
7. Stoffels M, Szperl A, Simon A, Netea MG, Plantinga TS, et al. (2013) MEFV Mutations Affecting Pyrin Amino acid 577Cause Autosomal Dominant Auto Inflammatory Disease. Ann Rheum Dis.
8. French FMF Consortium (1997) A Candidate Gene for Familial Mediterranean Fever. Nat Genet 17:25-31.
9. Sharkia R, Mahajnah M, Zalan A, Athamna M, Azem A, et al. (2013) Comparative Screening of FMF Mutations in Various Communities of the Israeli Society. Eur J Med Genet 56: 351-355
10. Yepiskoposyan L, Harutyunyan A (2007) Population Genetics of Familial Mediterranean Fever: A Review. EJHG 15: 911-916.
11. Berkun Y, Karban A, Padeh S, Pras E, Shinar Y, et al. (2012) NOD2/CARD15 Gene Mutations in Patients with Familial Mediterranean Fever. Seminars Arthritis Rheum 42: 84-88.
12. Rigante D, La Torraca I, Avallone L, Pugliese AL, Gaspari S et al. (2006) The Pharmacologic Basis of Treatment with Colchicine in Children with Familial Mediterranean Fever. Eur Rev Med Pharmacol Sci 10: 173-178.
13. Livneh A, Langevitz P, Zemer D, Padeh S, Migdal A, et al. (1996) The Changing Face of Familial Mediterranean Fever. Semin Arthritis Rheum 26: 612-627.
14. Ayesh SK, Nassar SM, Al-Sharef WA, Abu-Libdeh BY, Darwish HM (2005) Genetic Screening of Familial Mediterranean Fever Mutations in the Palestinian Population. Saudi Med J 26: 732-737.
15. The International FMFC (1997) Ancient Missense Mutations in a New Member of the RoRet Gene Family are Likely to Cause Familial Mediterranean Fever. Cell 4: 797-807.