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Short Communication

Familial Mediterranean Fever: Observations from a pilot gene expression microarray analysis study

R. Mahfouz[1], K. Kreidieh[2], R. A. Khalek[1], S. Yazbek[2*]

[1] Department of Pathology and Laboratory Medicine, American University of Beirut Medical Center, Beirut, Lebanon
[2] Medical Laboratory Sciences Program, Faculty of Health Sciences, American University of Beirut, Beirut, Lebanon

Correspondence to: sy21@aub.edu.lb

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Abstract: Familial Mediterranean Fever (FMF) is an autosomal recessive autoinflammatory disease affecting people of Mediterranean ancestry. The disease is caused by mutations in the MEFV gene located on chromosome 16p13.3. The aim of this pilot study was to assess global gene expression and identify genes and pathways involved in FMF that could be downstream to MEFV mutations or could be novel involved. EDTA blood samples were collected from 14 patients showing FMF-like symptoms and age-matched to 7 controls showing healthy conditions. Microarray was used to assess global gene expression and identify genes and pathways involved in FMF. When we compared individuals with MEFV mutations (homozygous and heterozygous) to control group, probe sets of receptor proteins HLA-DQA1 and HLA-DQB1 were significantly over expressed by 5 folds among the patients group. Despite its limitations, this pilot study could strongly suggest that the role of HLA be investigated in the pathogenesis of MEFV mutation and as a potential moderator explaining penetrance and variation in symptoms among patient groups.

Key words: Familial Mediterranean Fever; FMF; Microarray; MEFV; HLA; Pilot

Introduction

Familial Mediterranean Fever (FMF) is an autosomal recessive autoinflammatory disease affecting people of Mediterranean ancestry mainly non-Ashkenazi Jews, Armenians, Turks, and Arabs (1, 2). It is distributed into two phenotypes: types 1 and 2; where FMF type 1 is described by recurrent short episodes of inflammation and serositis including fever, peritonitis, synovitis and pleuritis, and FMF type 2 is characterized by amyloidosis (3). The diagnosis of FMF is based upon three factors: typical clinical manifestations, a positive response to colchicine therapy, and genetic testing, although currently available tests do not detect all mutations associated with FMF. Tel Hashomer clinical criteria is used for FMF diagnosis based on a patient showing fever in addition to one other major symptom (abdominal pain, Systemic AA-amyloidosis, chest pain, skin eruption or joint pain and positive response to daily colchicine) and one of the following minor signs (increased erythrocyte sedimentation rate, leukocytosis or elevated fibrinogen serum concentration and family history) or showing fever plus two minor signs (4). The disease is caused by mutations in the *MEFV* gene located on chromosome 16p13.3 (5). Currently, clinical detection of the *MEFV* mutation can be detected by targeted mutation analysis or sequence analysis of select exons (6). The most prevalent mutations are V726A, M694V, M694I, M680I and E148Q with M694V being the most frequent mutation and considered the most severe one (associated with amyloidosis) and E148Q being the mildest mutation when they exist in a homozygous state (7). A recent study demonstrated distinct expression patterns of pre-activated neutrophils during attack-free period of FMF when compared to neutrophils from healthy controls; the profile consisted namely of up-regulated IL-1, TLR4, IL-8, and TNFAIP6 transcripts (8). Despite identifying over 96 variants in the MEFV gene and the strong link of sequence to phenotype, little is known about the downstream effects of the gene and its pathophysiology. The aim of this pilot study was to assess global gene expression and identify genes and pathways involved in FMF that could be downstream to *MEFV* mutations or could be novel involved. This is the first report from our country using microarrays technology to decipher a pattern of expression in genes involved in Familial Mediterranean Fever.

Materials and Methods

EDTA blood samples were collected from 14 patients showing FMF-like symptoms and presenting to the American University of Beirut Medical center and age-matched to 7 control blood samples from individuals showing healthy conditions. Whole blood samples were used since neutrophils were proven to be the predominant effector cells of acute inflammatory attacks in FMF and since the *MEFV* mutation that encodes for the pyrin protein is primarily expressed in neutrophils (9). The average age and male to female ratio of both groups were matched to reduce group to group variation. DNA was extracted from lymphocytes using reagents included in the FMF StripAssay (GenXtract DNA extraction system, ViennaLab). For the simultaneous

detection of 12 *MEFV* mutations, a reverse-hybridization assay (FMF StripAssay, ViennaLab, Vienna, Austria) was used according to the manufacturer's instructions. RNA was extracted and purified using QIAamp® RNA Blood Mini Kit and RNase-Free DNase Set from Qiagen (Manchester, United Kingdom) respectively. To increase the efficiency of the expression arrays, only samples with RNA concentration between 100-300 ng/µl were used with the ratio of 260/280 absorbance between 1.7 and 2.1. Samples were then sent to the Microarray Facility at the American University of Beirut for further analysis under manufacturer's recommendation. Hybridization procedures were carried out in accordance with *Affymetrix* protocols for single round amplifications. The GeneChip® Human Gene 1.0 ST Array was used to assess global gene expression and identify genes and pathways involved in FMF. VAMPIRE web based microarray analysis suite was used under default conditions to analyze gene expression. All participants signed an informed consent to insure their proper permission to enroll in this study that was conducted in accordance with the ethical guidelines and after approval of the Institution Review Board of the American University of Beirut.

Results

Among the 14 FMF-suspected participants, *MEFV* mutations were detected in only 5 patients and were included in the microarray analysis as patient group. In addition, 4 control samples showing neither symptoms nor any mutation and 3 samples (labeled as "special group") were included. The special group consisted of samples that unexpectedly showed a positive heterozygote mutation in the *MEFV* gene with no clinical symptoms; although they were initially recruited as controls.

The R-analysis and the non-guided clustering of gene expression patterns showed no specific clusters. Expression wise, the samples did not segregate into the three biological groups listed above. Although the sample size was small, at a 5% false positive rate and a 0.5 power, we would have been able to detect any gene expression change above 3- fold between control and patient groups . With such a small sample size, changes where the mutation might have only caused subtle downstream expression alteration would not be detected. When we compared individuals with *MEFV* mutations (homozygous and heterozygous) to control group, probe sets of receptor proteins HLA-DQA1 and HLA-DQB1 were significantly over expressed by 5 folds among the patients group (p-value<0.05).

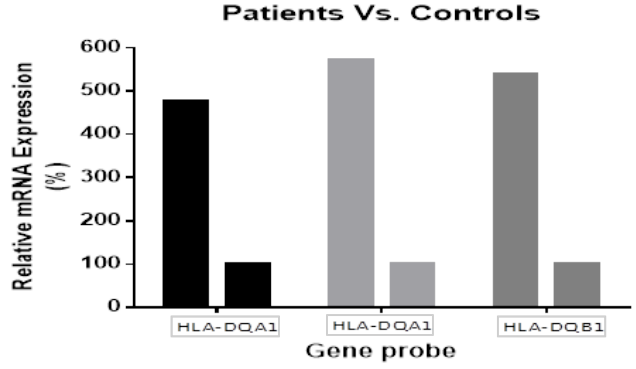

Figure 1. Relative expression of probe 213831_at and 236203_at of HLA-DQA1 and probe 209480_at of HLA-DQB1 between healthy controls and patients of MEFV. The probes display a 5 fold increase in all patients (p<0.005).

Discussion

Although no significant results were detected, this is a pilot exploratory study aimed at generation of data for a hypothesis- driven investigation of the pathogenesis of FMF. One of the pilot limitations was the difficulty to estimate the number of samples necessary to see significant changes. We recommend similar studies with a bigger sample size to identify the top 20 genes and top five FMF pathways involved in pathogenesis mechanism. One surprising observation was the identification of three heterozygote carriers among a non-symptomatic control group of 7 individuals (~40%). The above detected carrier rate is much higher than that reported in the literature for the Lebanese population (10). MEFV gene changes have been reported from healthy carriers in many countries with high rates of FMF such Turkey, Lebanon. Serbia and people of Jewish origins and do not necessarily induce an auto inflammatory disease as evident by the high frequency of MEFV variants in at-risk populations (10-13). A recent study provided, for the first time, statistical evidence that heterozygosity for a single MEFV mutation constitutes a susceptibility factor for FMF. Some individuals carrying a single MEFV mutation will develop FMF symptoms, attributed perhaps to the presence of other unidentified factors (genetic and environmental). Identification of such factors and characterization of their interaction with MEFV is an important challenge (14). Moreover, in a study conducted in Japan, more than one third of FMF patients had only one copy of *MEFV* pathological mutations (15); this observation was attributed to the presence of modifier genes (among which genes in the HLA regions were strong candidates. It would be of significant importance if this data was further investigated and individuals followed in a prospective study to monitor penetrance and effect on symptoms. Furthermore, although we acknowledge that due to lack of samples (mainly for funding reasons since the microarray technolopgy is very expensive with an average cost of 600 dollars per chip) we were not able to confirm microarray results using RT-PCR, the fold change in probe sets of HLA-DQA1 and HLA-DQB1 was profound and evident even within the small sample size. HLA DR/DQ alleles have been linked to FMF patients with specific *MEFV* mutations (16). HLA-DR expression is known to increase during symptomatic attacks in FMF patients (17). Even in the Japanese FMF patients, they were able to identify differential effects of HLA class I and II alleles and with possible moderating control of penetrance of *MEFV* mutations (18). Despite its limitations, this pilot study could strongly suggest that the role of HLA be investigated in the pathogenesis of *MEFV* mutation and as a potential moderator explaining penetrance and variation in symptoms among patient groups.

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Ethical Consideration

The study was approved by the Institution Review Board of the American University of Beirut

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