

MEFV Mutations and CYP3A4 Polymorphisms Do Not Predict “Colchicine Responsiveness” in Familial Mediterranean Fever

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ABSTRACT Familial Mediterranean Fever (FMF) is an auto-inflammatory disease caused by mutations in the MEFV gene. Colchicine is the mainstay of FMF treatment. It is metabolised by cytochrome P450-3A4 (CYP3A4) enzyme. About 10-15% of FMF patients do not respond to treatment with colchicine. In this study, the researchers aimed to investigate association of colchicine non-responsiveness with MEFV mutations, CYP3A4*1B, *2, and *17 polymorphisms, and some demographic features of FMF patients. One hundred and ninety-six consecutive FMF patients (170 colchicine responders and 26 non-responders) were included in the study. CYP3A4 polymorphisms were detected using polymerase chain reaction and TaqMan probes. CYP3A4*1B and *17 were not detected in responders or non-responders. CYP3A4*2 was detected in eight responders, all of which were in heterozygous state. However, the difference was not statistically significant. Most patients (165 responders and 25 non-responders) had MEFV gene analysis available prior to participation in the study. Frequencies for M694V, M680I, V726A, and E148Q mutations and M694V/M694V genotype were similar in two groups. Mean body mass index of responders was not significantly different from that of non-responders. Attack frequency and proteinuria level were significantly higher in non-responders than in responders. Earlier age at disease onset was found to be associated with colchicine non-responsiveness. However, neither MEFV mutations nor CYP3A4 mutations were associated with colchicine non-responsiveness.

INTRODUCTION

Familial Mediterranean Fever (FMF) is a hereditary auto-inflammatory disease characterized by recurrent attacks of fever, peritonitis, pleuritis, and/or arthritis, lasting for 1-3 days on average. Mutations in MEFV gene are responsible for the disease; to date, more than 150 mutations have been linked to FMF phenotype (Touitou 2014). MEFV allelic heterogeneity is related to disease phenotype; while homozygous state for M694V mutation is associated with high penetrance (Touitou 2001) and more severe disease (Gershoni-Baruch et al. 2002; Tunca et al. 2005), E148Q is associated with reduced penetrance and mild phenotype (Touitou 2001).

Colchicine, an alkaloid derived from *colchicum autumnale*, is the mainstay of FMF treatment; it reduces frequency of FMF attacks (Goldfinger 1972) and prevents amyloidosis (Ze-

mer et al. 1986). It is metabolised by cytochrome P450-3A4 (CYP3A4) enzyme, which is expressed mainly in the liver and small intestine, and metabolizes colchicine to 2-demethyl colchicine, 3-demethyl colchicine, and 10-demethyl colchicine (Tateishi et al. 1997). CYP3A4 enzyme activity shows great inter-individual variations (Tateishi et al. 1997; Guengerich 1999; Zanger and Schwab 2013). Ozdemir et al. (2000) have reported that genetic factors are responsible for ~85% of inter-individual variability in the catalytic activity of CYP3A4. More than 40 single nucleotide polymorphisms (SNP) have been identified in the 5'-flanking region, introns, and exons of the CYP3A4 gene. CYP3A4*1B (-392A>G), CYP3A4*2 (15713T>C), and CYP3A4*17 (15615T>C) are the most common SNPs in Caucasians (Keshava et al. 2004). CYP3A4*2 (S222P) and *17 (F189S) are non-synonymous mutations in exon 7 (Sata et al. 2000; Dai et al. 2001); CYP3A4*1B is -392A to G nucleotide substitution in the 5'-regulatory region of the gene (Rebbeck et al. 1998). Whether these polymorphisms alter the gene expression and enzyme activity is still not clear.

About 10-15% of FMF patients do not respond to treatment with colchicine (Cerquaglia

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et al. 2005). Why some FMF patients do not respond to colchicine treatment still remains obscure. Limited numbers of studies dealing with “colchicine responsiveness” in patients with FMF had conflicting results (Lidar et al. 2004; Tufan et al. 2007; Bezalel et al. 2009; Soylemezoglu et al. 2010). Theoretically, MEFV mutation type and altered colchicine pharmacokinetics may cause non-responsiveness to colchicine.

Aims

The aim of this paper is to investigate association of “colchicine responsiveness” with MEFV mutations, CYP3A4 polymorphisms, and some demographic features of FMF patients.

MATERIAL AND METHODS

Patients

The study was approved by the local ethics committee of Gazi University, and all study participants gave written informed consent. Two hundred and three consecutive Caucasian patients diagnosed with FMF according to the Tel-Hashomer criteria (Livneh et al. 1997) were included in the study. Patients were recruited from the outpatient clinic of Rheumatology Department at Gazi University Hospital. Most patients had MEFV gene analysis available prior to participation in the study. Inclusion criteria for the study were as follows; to give written informed consent, age ≥ 18 , fulfilling Tel-Hashomer criteria for the diagnosis of FMF, and taking colchicine regularly for the last 6 months or longer. More than 1 attack at typical sites in 3 months, while taking colchicine ≥ 2 mg/day, was defined as “non-responsiveness to colchicine” (Lidar et al. 2004). The control group consisted of 50 healthy Caucasian volunteers.

Genotyping

Venous blood samples (5 ml in tubes containing EDTA) were stored at -20°C until studied. Genomic DNA was extracted and CYP3A4*1B (-392A>G), *2 (15713T>C) and *17 (15615T>C) targets were PCR amplified and detected by TaqMan probes using an ABI real-time PCR instrument. Allelic discrimination was facilitated by software analysis of the fluorescence data. Homozygous or heterozygous presence of three

common CYP3A4 genotype variants (*1B, *2, *17) was reported.

Statistical Analysis

Chi-square test or Fischer’s exact test was used, where appropriate, to compare allelic frequencies and genotype distributions of the study subjects. Student’s t-test was used for continuous variables. All reported p values were two-sided, and $p < 0.05$ was considered statistically significant.

Frequencies for aforementioned CYP3A4 polymorphisms in our general population are unknown. However, if the researchers assume them to be 5%, 27 patients per group are needed to detect a 30% difference between groups (two-tailed alpha 0.05, beta 0.20).

RESULTS

Demographic and clinical features of the patients are shown in Table 1. Of the 203 patients, 170 were colchicine-responders and 26 non-responders. Seven patients could not be assessed for colchicine-responsiveness, hence were not included in the study. Clinical characteristics of colchicine responders and non-responders are shown in Table 2. The two groups were not significantly different with respect to age, gender, or body mass index (BMI). However, non-responders were significantly younger at disease onset, had higher frequency of attacks and more proteinuria. None of the non-responders could tolerate colchicine doses of > 2 mg a day because of gastrointestinal side effects.

Table 1: Demographic and clinical features of patients* (n= 203)

Features	
Male/Female (no.)	69 / 134
Age (yrs)	33.5 $\pm$ 10.7 (18-60)
Age at disease onset (yrs)	17.3 $\pm$ 9.5 (1-41)
Disease duration (months)	195.7 $\pm$ 135.5 (4-600)
Delay in diagnosis (months)	133 $\pm$ 122.6 (0-576)
Colchicine non-responders (no.) (%)	26 (13.2)

* Results are given as mean $\pm$ SD (range) unless otherwise stated.

Allele frequencies of four common MEFV mutations in responders were not significantly different from those in non-responders (Table

Table 2: Characteristics of colchicine responders and nonresponders

Characteristics	Responders (n=170)	Non-responders(n=26)	P Value
Age (yrs)	34.0 ± 0.8	31.9 ± 1.9	NS
Male/Female (no.)	61/109	7/19	NS
Body mass index (kg/m ²)	24.8 ± 0.4	24.3 ± 1.1	NS
Age at disease onset (yrs)	18.2 ± 0.7	10.9 ± 1.3	0.000
FMF duration (months)	187.9 ± 10.3	253.8 ± 25.9	NS**
Attack frequency (per year)	0.9 ± 0.1	14.9 ± 2.0	0.000
Attacks (%)			
Fever	19	65	0.000
Abdominal attacks	35	92	0.000
Chest attacks	15	61	0.000
Joint attacks	4	58	0.000
Erysipelas-like erythema	4	31	0.000
Proteinuria, mg/24h	137.9 ± 8.9	330.6 ± 158.5	0.000
Daily colchicine dose, mg	1.26 ± 0.02	2 ± 0	0.000

* Results are given as mean ± SEM unless otherwise stated.

** When adjusted by "age at disease onset".

Table 3: MEFV allele and genotype frequencies in responders and nonresponders*

Genetic feature	Responders (n=165)	Nonresponders (n=25)
M694V allele	155 (47.0)	26 (52.0)
M680I allele	40 (12.1)	8 (16.0)
V726A allele	25 (7.6)	1 (2.0)
E148Q allele	12 (3.6)	3 (6.0)
M694V/M694V genotype	41 (24.8)	9 (36.0)

NOTE. No significant differences were observed.

*Results are given as no. (%).

3). M694V/M694V genotype frequencies were also similar. CYP3A4*1B and *17 polymorphism were not detected in responders or non-responders. Eight patients had CYP3A4*2 polymorphism; all of them were colchicine responders and in heterozygous state (Table 4). CYP3A4*1B, *2, and *17 was not detected in healthy controls.

Table 4: CYP3A4*2 allele and genotype frequencies*

Genetic feature	Responders (n=170)	Nonresponders (n=26)
T allele	332 (97.6)	52 (100)
C allele	8 (2.4)	0 (0)
TT genotype (wild-type)	162 (95.3)	26 (100)
TC genotype	8 (4.7)	0 (0)
CC genotype	0 (0)	0 (0)

NOTE. No significant differences were observed.

*Results are given as no. (%).

DISCUSSION

In this study, the researchers did not observe an association between colchicine responsiveness and a particular MEFV mutation. CYP3A4*1B and *17 were not detected in patients or healthy controls. CYP3A4*2 polymorphism was detected only in responders; however, the difference between responders and non-responders was not statistically significant. Non-responders were significantly younger at disease onset, had more proteinuria and higher frequency of attacks than responders, suggesting an association between disease burden and colchicine non-responsiveness.

There is no agreement on definition of "colchicine resistance" (La Regina et al. 2013). Having FMF attacks more than once in 3 months, while taking colchicine ≥2 mg/day, was defined as "non-responsiveness to colchicine" (Lidar et al. 2004). In "Interleukin-1 Trap to Treat Auto-inflammatory Disease" study, subjects were considered to be non-responsive to colchicine (up to 2 milligrams per day) on the basis of continued symptoms or flares (greater than or equal to one per month) or elevated acute phase reactants (ESR, CRP or SAA greater than or equal to 1.5 times the upper limit of normal between attacks) despite treatment with maximally tolerated doses of colchicine (U.S. National Institutes of Health 2014). Recently, it was stated that a fully compliant FMF patient suffering from more than 6 typical attacks per year or more than 3 typical attacks within 4-6 months despite taking

colchicine up to 3 mg/day should be considered resistant to colchicine (Hentgen et al. 2013). In our experience, patients non-responsive to colchicine 2 mg/day are usually also non-responsive or intolerant to higher doses of colchicine. Indeed, none of our patients could tolerate daily colchicine doses of >2 mg. Some authors have proposed that patients over 80 kg should take colchicine 2.5 mg daily before claiming treatment failure (Ben-Chetrit and Ozdogan 2008). However, mean BMIs of responders and non-responders were similar in this study, and only 4 out of 26 non-responders had BMI of >30 kg/m².

Limited numbers of studies on non-responsiveness to colchicine had contradictory results. In a study by Katz et al. (1982), mean plasma colchicine level was significantly higher in responders than in non-responders. However, plasma colchicine levels were adequate in some non-responders and low in some responders; they concluded that a study on intracellular concentration of colchicine was warranted. More than 20 years later, Lidar et al. (2004) reported that responder patients had significantly higher mean mononuclear cell (MNC) colchicine level than non-responders; however, plasma and polymorphonuclear cell (PMNC) colchicine levels were similar. They stated that further studies were warranted to define the role of various candidate genes and MNC-PMNC interactions in FMF. P-glycoprotein (P-gp) mediated efflux of intracellular colchicine plays significant role in colchicine excretion (Terkeltaub 2009). P-gp is encoded by drug transporter gene ABCB1. Two studies investigating association of colchicine responsiveness with ABCB1 3435C to T polymorphism also had contradictory results. In the first study, patients with TT genotype for the ABCB1 3435C to T variant responded better to colchicine treatment (Tufan et al. 2007). However, the other study showed that presence of one or two T alleles was associated with non-responsiveness to colchicine (Bezalel et al. 2009).

Aside from current study, to our knowledge, there are only 2 studies investigating association of MEFV mutations with colchicine responsiveness. In the first study, 59 non-responders and 51 responders were compared for MEFV mutations (Lidar et al. 2004). Distributions of the mutated MEFV alleles and frequencies of homozygote M694V genotype were similar in two groups, which are consistent with our findings. However, in a study recruiting 222 children with

FMF, homozygous state for M694V mutation was associated with unresponsiveness to colchicine treatment (Soylemezoglu et al. 2010). This result is contradictory to abovementioned studies in adult FMF patients and may be attributable to distinct physiologic conditions of children. Interestingly, in the current study, non-responders were more likely to have disease onset at a pediatric age compared to responders, which might explain these contradictory results.

Functional correlation and clinical significance of CYP3A4 polymorphisms have not been clarified yet. In some studies, CYP3A4*1B was reported to be associated with increased catalytic activity (Amirimani et al. 1999, Amirimani et al. 2003). However, other studies revealed that CYP3A4*1B had decreased or normal catalytic activity compared to wild-type and only limited effect on drug metabolism (Ball et al. 1999; Garcia-Martin et al. 2002; Magliulo et al. 2011). CYP3A4*2 and *17 have been associated with decreased or normal catalytic activity (Sata et al. 2000; Dai et al. 2001; Lee et al. 2005; Shchepotina et al. 2006; Miyazaki et al. 2008). However, whether these polymorphisms that is, CYP3A4*1B, *2, and *17 alter colchicine metabolism is not known; CYP3A4 may exhibit differential catalytic activity for different substrates (Maekawa et al. 2010), and colchicine was not used as a probe drug in abovementioned studies. In this study, the researchers did not identify CYP3A4*1B or *17, and it seems that these polymorphisms are not involved in colchicine non-responsiveness. The researchers identified CYP3A4*2 only in responders; although the difference was not significant, the researchers could not state a firm conclusion because of low statistical power of the test. In a recent study, association of daily colchicine dose with CYP3A4*1B, *2, *3, *17, and *22 polymorphisms was investigated in patients with FMF (Dogruer et al. 2013). Daily colchicine dose was not significantly different with respect to CYP3A4 polymorphisms, which is consistent with the present study.

Attack frequency, mean daily colchicine dose, and proteinuria were significantly higher in non-responders. However, interestingly, mean age at disease onset was significantly lower in non-responders than in responders (10.9 vs. 18.2, $p=0.000$), which suggests that non-responsiveness to colchicine is related to FMF itself, but not due to altered colchicine pharmacokinetics by CYP3A4 polymorphisms.

Possible polygenic inheritance might be responsible for colchicine non-responsiveness in patients with FMF. In recent years, autosomal dominant inheritance has been demonstrated in several FMF patients (Booth et al. 2000; Aldea et al. 2004; Booty et al. 2009; Stoffels et al. 2013), which raises the possibility that FMF could be a polygenic disease. Mutations in other auto-inflammatory genes or in genes encoding pyrin-interacting proteins may alter penetrance and severity of FMF as well as response to colchicine treatment.

CONCLUSION

The results suggest that FMF onset at a pediatric age might be a risk factor for colchicine unresponsiveness. However, MEFV variations and CYP3A4 polymorphisms do not seem to play a role in colchicine responsiveness. Studies investigating association of colchicine response with mutations in other FMF related genes that might also be leading to earlier disease onset or higher disease burden are warranted.

LIMITATIONS

The present study has some limitations. Plasma and cellular concentrations of colchicine could not be measured because of technical limitations. Frequencies of CYP3A4 polymorphisms were significantly lower than those the researchers expected; therefore, statistical power of the test was not high enough to state a firm conclusion.

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