The 3435T polymorphism in the ABCB1 gene and colchicine unresponsiveness in familial Mediterranean fever

Sirs,

Familial Mediterranean fever (FMF), an autosomal recessive disease caused by mutations in MEFV, is characterized by recurrent episodes of fever, sterile peritonitis, arthritis and/or pleuritis. Amyloidosis, leading to renal failure, is the most severe manifestation (1). Colchicine, the mainstay of FMF treatment, reduces attack frequency and duration and prevents renal amyloidosis. Approximately 5-10% of FMF patients are resistant to colchicine (2).

The mechanism by which colchicine prevents FMF attacks remains at large unknown. Current opinion deems that it accumulates in neutrophils, inhibits neutrophil chemotaxis, impedes synthesis and secretion of cytokines and thereby decreases the inflammatory process (3).

The ATP-binding Cassette B1 (ABCB1) (formerly MDR1) gene product P-glycoprotein (PGP) is a transmembrane efflux transporter that contributes to the elimination of therapeutic agents and xenobiotics (4). Initially identified in cancer cells, PGP mediates resistance to many drugs. It is expressed in normal tissues and in various leukocyte lineages with highest expression in CD56+ natural killer cells (5).

Multiple mutations have been identified in ABCB1. Most of them are intronic or silent. It could be expected that persons carrying different allelic variants of ABCB1 exhibit different pharmacokinetic properties affecting the efficacy and toxicity of drugs (6). One silent single nucleotide polymorphism (SNP) in exon 26 (C3435T) of ABCB1 is of special interest. Although silent, it is associated with lower PGP expression in various tissues including lymphocytes (7). Individuals homozygous for the T allele have on average a 2-fold lower intestinal PGP expression compared with those with the 3435CC genotype. In persons with the CC genotype, higher PGP-mediated rhodamine efflux from $CD56^+$ natural killer cells has been observed (6). The G2677T/A polymorphism in exon 21 has also been correlated with PGP expression and function (8).

Using RFLPs analysis, we determined the distribution of the C3435T and the G2677T/A polymorphisms in FMF patients classified according to their response to colchicine. The study included 105 FMF patients sub-grouped as responders (n=47) or non-responders (n=58) to a daily dose of 2mg colchicine, comparable with respect to gender, age, duration and onset of disease, association with other diseases, compliance with colchicine therapy and mutations in the MEFV gene. Patients were enrolled at the Sheba Medical Center, as part of another study (3).

Table I. Distribution of ABCB1 genotypes and intracellular colchicine concentration in lymphocytes of non-responders and responders.

Parameter	Non responders	Responders	Significance χ^2- test *p*-value
Genotype (n=105)	58 (55.2%)	47 (44.8%)	
C3435T			
CC (n=28)	11 (39.3)	17 (60.7)	
TT (n=21)	14 (66.7)	7 (33.3)	
CT (n=56)	33 (58.9)	23 (41.1)	* p=0.047
G2677T/A			
GG (n=29)	15 (51.7)	14 (48.3)	
TT (n=17)	10 (58.8)	7 (41.2)	
TG (n=59)	33 (55.9)	26 (44.1)	NS
Colchicine concentration in lymphocytes (ng/10^9 cell) Mean±SD			*t*-test *p*-value
C3435T MDR1 genotype (total; n=105)	100.69 ± 69.0	240.63 ± 183.15	p<0.001
CC (n=28)	89.545 ± 31.49	194.59 ± 111.26	p=0.002
TT (n=21)	108.86 ± 70.27	168.0 ± 65.11	p=0.128
CT (n=56)	99.86 ± 80.17	308.72 ± 230.96	p<0.001
One way ANOVA *p*-values	p=0.796	p=0.174	

*p-value addresses TT and CT compared to CC genotypes.

ABCB1 genotypes and intracellular colchicine levels are depicted in Table I. The overall odds of being non-responders in the presence of one or two T alleles was more than 2-fold (p=0.047; OR – 2.4; 95% – CI 1.0-5.88). Levels of colchicine in lymphocytes of responders, although being twice the concentration found in non-responders' lymphocytes, were not associated with either the T or C allele. G2677T/A genotypes were similarly distributed in responders and non-responders.

The C3435T polymorphism is expected to affect tissue distribution of numerous ABCB1 substrates including colchicine (4). We have previously shown that non-responsiveness to colchicine, in FMF patients, was coupled with lower levels of colchicine in lymphocytes of non-responders compared to responders (3). As such, excessive activity of the PGP efflux pump in mononuclear cells of non-responders was suggested. However, our results ally non-responsiveness with the 3435T allele, a polymorphism linked with low PGP activity and no association between the C3435T polymorphism and colchicine in patients' lymphocytes was noted. Responders and non-responders had higher and lower colchicine levels in lymphocytes irrespective of their ABCB1 C3435T status. Paradoxically, Tufan *et al.* (9) reported that the ABCB1 3435TT genotype is related to colchicine responsiveness arguing that their findings negate those reported by us (10), subject to differences in confounding factors such as ethnic variability and the representative nature of study samples.

Population discrepancies between ABCB1 expression studies, combined with the fact that the C3435T polymorphism is not within a known regulatory expression element, may suggest that the C3435T polymorphism is in linkage disequilibrium with unknown regulatory polymorphism(s) directly influencing ABCB1 expression. The correlation between plasma drug levels and PGP activities reflects complex pharmacokinetics of absorption, distribution, and elimination for individual drugs suggesting that the known functional ABCB1 gene SNPs are not the ultimate determinants of PGP function.

The role of ABCB1 in resistance to colchicine in patients with FMF remains divisive and needs to be further addressed.

Y. BEZALEL[1]
R. GERSHONI-BARUCH[1,2]
E. DAGAN[1,3]
M. LIDAR[4]
A. LIVNEH[4,5]

[1]Institute of Human Genetics, Rambam Medical Center, Haifa, Israel; [2]The Bruce Rappaport School of Medicine, Technion-Israel Institute of Technology, Haifa, Israel; [3]Department of Nursing, Faculty of Welfare and Health Sciences, University of Haifa, Haifa, Israel; [4]Heller Institute of Medical Research, Sheba Medical Center, Tel-Hashomer, Israel; [5]Sackler School of Medicine, Tel-Aviv University, Israel.

Address correspondence and reprint requests to: Ruth Gershoni-Baruch, Department of Human Genetics, Rambam Medical Center, Haifa, 31096, Israel.
E-mail: rgershoni@rambam.health.gov.il

Competing interests: none declared.

References

1. SOHAR E, GAFNI J, PRAS M, HELLER H: Familial Mediterranean fever. A survey of 470 cases and review of the literature. *Am J Med* 1967; 43: 227-53.
2. ZEMER D, PRAS M, SOHAR E, MODAN M, CABILI S, GAFNI J: Colchicine in the prevention and treatment of the amyloidosis of familial Mediterranean fever. *N Engl J Med* 1986; 314: 1001-5.
3. LIDAR M, SCHERRMANN JM, SHINAR Y *et al.*: Colchicine nonresponsiveness in familial Mediterranean fever: clinical, genetic, pharmacokinetic, and socioeconomic characterization. *Semin Arthritis Rheum* 2004; 33: 273-82.
4. UEDA K, CORNWELL MM, GOTTESMAN MM *et al.*: The mdr1 gene, responsible for multidrug-resistance, codes for P-glycoprotein. *Biochem Biophys Res Commun* 1986; 141: 956-62.

5. KLIMECKI WT, TAYLOR CW, DALTON WS: Inhibition of cell-mediated cytolysis and P-glycoprotein function in natural killer cells by verapamil isomers and cyclosporine A analogs. *J Clin Immunol* 1995; 15: 152-8.
6. HITZL M, DRESCHER S, VAN DER KUIP H *et al*.: The C3435T mutation in the human MDR1 gene is associated with altered efflux of the P-glycoprotein substrate rhodamine 123 from CD56+ natural killer cells. *Pharmacogenetics* 2001; 11: 293-8.
7. KIMCHY-SARFATY C, OH JM, KIM IW *et al*.: A "silent" polymorphism in the MDR1 gene changes substrate specificity. *Science* 2007; 315: 525-8.
8. EICHELBAUM M, FROMM MF, SCHWAB M: Clinical aspects of the MDR1 (ABCB1) gene polymorphism. *Ther Drug Monit* 2004; 26: 180-5.
9. TUFAN A, BABAOGLU MO, AKDOGAN A *et al*.: Association of drug transporter gene ABCB1 (MDR1) 3435C to T polymorphism with colchicine response in familial Mediterranean fever. *J Rheumatol* 2007; 34: 1540-4.
10. GERSHONI-BARUCH R, PERETZ Y, LIDAR M, DAGAN E, SCHERRMANN JM, LIVNEH A: The influence of polymorphisms in MDR1 on colchicines unresponsiveness in familial Mediterranean fever [abstract]. 4th International Congress on Systemic Autoinflammatory Diseases 2005; 25.