N-Acetyltransferase-2 Genotypes Among Patients with Rheumatoid Arthritis Attending Jordan University Hospital

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Aim: To determine the frequency of major N-acetyltransferase (NAT2) alleles and genotypes among Jordanian patients with rheumatoid arthritis (RA). **Methods:** The study was approved by the IRB of the Jordan University Hospital. An informed consent was signed by every patient. DNA samples from 150 healthy volunteers and 108 patients with RA were analyzed by polymerase chain reaction followed by a restriction fragment length polymorphism assay (PCR-RFLP) to determine the frequency of four major alleles: NAT2*4, NAT2*5, NAT2*6, and NAT2*7. **Results:** The most prevalent genotypes are those that encode the slow acetylation phenotype. About 59.3% of the patients with RA carried the slow, 33.3% the intermediate, and 7.4% the fast-encoding genotypes. The frequency of NAT2 alleles was 0.241 (95% confidence interval [CI] 0.184–0.298) for NAT2*4, 0.449 (95% CI 0.383–0.515) for NAT2*5, 0.273 (95% CI 0.214–0.332) for NAT2*6, and 0.037 (95% CI 0.012–0.062) for NAT2*7 allele. The overall frequency of the slow acetylation genotype in patients with RA is similar to that in healthy Jordanian volunteers. However, the NAT2*5/7 genotype was found in seven patients (6.5%) with RA and was absent in Jordanian volunteers, and the z test revealed that the difference was statistically significant. This genotype constituted 10.9% of the genotypes encoding slow acetylation. **Conclusion:** The overall acetylator genotype in RA is similar to that in healthy volunteers. The overall slow acetylator genotypes do not seem to be a genetic risk factor for RA among Jordanians. However, the NAT2*5/7 genotype seems to be related to RA. The nature of this relationship needs further clarification.

Introduction

HUMAN N-ACETYLTRANSFERASE 2 (NAT2) is a phase-two drug-metabolizing enzyme that is responsible for the acetylation of a variety of aromatic arylamine and N-arylhydroxylamines, thereby playing an important role in both detoxification and activation of numerous drugs and carcinogens (Liu *et al.*, 2007). The genetic polymorphism of NAT2 divides a human population into fast, intermediate, and slow acetylators (Brocvielle *et al.*, 2003). Slow and fast acetylators greatly differ in the occurrence of side effects of drugs that are acetylated, such as sulfasalazine (Tanaka *et al.*, 2002; Soejima *et al.*, 2008). Some studies have shown an association between NAT2 gene polymorphisms and certain autoimmune diseases (Tamer *et al.*, 2005; Pawlik *et al.*, 2009). The NAT2 slow acetylator status may be a risk factor or the development of Behcet's disease. The NAT2*5A and NAT2*6A mutant genotypes were associated with an increased risk of developing Behcet's disease with odds ratios of 66.3 and 24, respectively, whereas the NAT2*7A/B and NAT2*14A were

not (Tamer *et al.*, 2005). The slow acetylator genotypes were more prevalent in patients with atopic asthma with prevalence of the NAT2*5/NAT2*5 and NAT2*5/NAT2*6 genotypes (Pawlik *et al.*, 2009). About 91% of the children having allergic diseases had NAT2 genotypes that encode slow acetylation, while 62% of control-group children had these genotypes (Zielińska *et al.*, 1997). The risk of developing atopic diseases was five-fold greater for homozygous slow acetylators (Gawrońska-Szklarz *et al.*, 1999). The slow acetylator phenotypes were shown to be associated with certain types of cancer (Hein *et al.*, 2000). There are conflicting reports in relation to the acetylator status and systemic lupus erythematosus (SLE). One study in Germany reported that 75% of SLE patients were slow acetylators compared to 55% of healthy controls (von Schmiedeberg *et al.*, 1999), whereas a larger study did not support this association (Zschieschang *et al.*, 2002). A study in Japan demonstrated that the slow acetylator genotype was clearly associated with an increased risk of SLE (Kiyohara *et al.*, 2009). The reports concerning systemic sclerosis were also conflicting. One study showed an association between

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the slow acetylators status and the disease (80.2% of patients compared to 55% of healthy subjects were slow acetylators) (von Schmiedeberg *et al.*, 1999), while another study failed to show such an association (Skřetkiewicz *et al.*, 2005).

Rheumatoid arthritis (RA) is a chronic autoimmune disease of unknown etiology that affects 1–1.5% of the population (Nagy *et al.*, 2010). The situation concerning RA and the acetylation status or genotypes is controversial (Pawlik *et al.*, 2002, 2004; Kumagai *et al.*, 2004).

Because of the controversy that slow acetylation is a predisposing factor for RA, we designed this study to identify the acetylators genotype status among Jordanian patients with RA attending the Jordan University Hospital Rheumatology Clinic. In particular, the frequencies of the most common mutations of the *NAT2* gene, *NAT2**4, *NAT2**5, *NAT2**6, and *NAT2**7, which classify patients into slow, intermediate, and fast acetylators, were studied. The frequencies obtained were compared to those obtained from healthy unrelated Jordanian volunteers.

Methods

Sample collection

One hundred eight Jordanian patients with RA attending the Jordan University Hospital Rheumatology Clinic (87 females, 21 males) were included in the study. The control group consisted of 150 healthy Jordanian volunteers (72 females, 78 males). The study was approved by the Jordan University Hospital Institutional Review Board. An informed consent was signed by patients and volunteers to participate in the study and to donate 3–5 mL of venous blood. Juvenile RA and non-Jordanian patients were excluded from the study.

DNA extraction and amplification

DNA was extracted using the Wizard® Genomic DNA purification kit (Promega) according to the manufacturer's protocol. The three most common *NAT2* single-nucleotide polymorphisms (C481T, G590A, and G857A) associated with

low *NAT2* activity were genotyped using the polymerase chain reaction–restriction fragment length polymorphism assay (PCR-RFLP), which was described by Lemos *et al.* (1999), as follows.

An aliquot of 200 ng of genomic DNA was amplified in a 50-μL reaction volume containing 1 μL of 10 mM dNTPs, 0.5 μL of 50 mM MgCl₂, 10 μL of the green *Taq* polymerase buffer, and 1 μL of 20 pmole/μL from each of the forward (5' GCT GGG TCT GGA AGC TCC TC 3') and reverse (5' TTG GGT GAT ACA TAC ACA AGG G 3') primers (Invitrogen) and 2.5 units of *Taq* DNA polymerase. After an initial denaturation at 94°C for 10 min, 35 cycles were performed consisting of denaturation step at 94°C for 1 min, annealing step at 58°C for 1 min, and elongation step at 72°C for 1 min, completed with a final cycle of elongation at 72°C for 10 min.

RFLP and result analysis

Ten microliters of the PCR product was then subjected to a restriction enzyme analysis (Promega) by adding 10 units of *Kpn*I, 10 units of *Taq*I, and 15 units of *Bam*HI for detection of genetic variations C481T, G590A, and G857A, respectively. Each restriction reaction was done in a separate tube. The wild-type allele (*NAT2**4) was identified after complete digestion by *Kpn*I, *Taq*I, and *Bam*HI. Genetic variation in the *NAT2* gene changed the recognition sites for *Kpn*I, *Taq*I, and *Bam*HI restriction enzymes. *NAT2**5, *NAT2**6, and *NAT2**7 alleles were identified by the presence of C481T, G590A, and G857A genetic variations, respectively.

Digestion conditions were performed according to the manufacturer's instructions. Digestion products were separated on 2% agarose gels for detection of the *NAT2**5 and *NAT2**7 alleles, and 3% agarose gels for detection of the *NAT2**6 allele after gel staining with ethidium bromide.

Statistical analysis

Genotype frequencies were calculated by dividing the count of a particular genotype by the total sample number in each group (patients or volunteers). The z-test was used as a statistical tool for comparison of acetylation-encoding *NAT2*

TABLE 1. THE N-ACETYLTRANSFERASE 2 GENOTYPE FREQUENCIES AMONG JORDANIAN RHEUMATOID ARTHRITIS PATIENTS COMPARED TO HEALTHY VOLUNTEERS

Expected acetylation status	Genotype	Healthy volunteers ^a						RA patients					
		Count		Frequency		95% CI		Count		Frequency		95% CI	
Fast acetylation	<i>NAT2</i> *4/4	12	12	0.080	0.080	0.040–0.120	0.040–0.120	8	8	0.074	0.074	0.025–0.123	0.025–0.123
	<i>NAT2</i> *4/5	26	50	0.173	0.333	0.011–0.230	0.258–0.409	24	36	0.222	0.333	0.144–0.300	0.244–0.422
Intermediate acetylation	<i>NAT2</i> *4/6	18		0.120		0.070–0.170		11		0.102		0.045–0.159	
	<i>NAT2</i> *4/7	6		0.040		0.010–0.070		1		0.009		0.000–0.027	
Slow acetylation	<i>NAT2</i> *5/5	21	88	0.140	0.586	0.080–0.200	0.508–0.666	17	64	0.157	0.592	0.088–0.226	0.500–0.686
	<i>NAT2</i> *5/6	44		0.293		0.220–0.360		32		0.296		0.210–0.382	
	<i>NAT2</i> *5/7	0		0.000		0.000–0.000		7		0.065		0.019–0.111	
	<i>NAT2</i> *6/6	20		0.133		0.080–0.190		8		0.074		0.025–0.123	
	<i>NAT2</i> *6/7	2		0.013		0.005–0.030		0		0.000		0.000–0.000	
Total	<i>NAT2</i> *7/7	1		0.007		0.0006–0.02		0		0.000			
		150	150	0.999	0.999			108	108	0.999	0.999		

^aJarrar *et al.* (2010).

NAT2, N-acetyltransferase; RA, rheumatoid arthritis; CI, confidence interval.

TABLE 2. STATISTICAL ANALYSIS OF THE FREQUENCY OF N-ACETYLTRANSFERASE 2 GENOTYPES AMONG JORDANIAN PATIENTS WITH RHEUMATOID ARTHRITIS COMPARED TO HEALTHY VOLUNTEERS

Genotype	Frequency		z Statistic (statistical difference)
	Volunteers ^a (n=150)	RA patients (n=108)	
NAT2*4/4	0.080	0.074	0.22 (No)
NAT2*4/5	0.173	0.222	-0.98 (No)
NAT2*4/6	0.120	0.102	0.45 (No)
NAT2*4/7	0.040	0.009	1.51 (No)
NAT2*5/5	0.140	0.157	-0.04 (No)
NAT2*5/6	0.293	0.296	-0.05 (No)
NAT2*5/7	0.000	0.065	-3.25 (Yes)
NAT2*6/6	0.133	0.074	1.51 (No)
NAT2*6/7	0.013	0.000	1.18 (No)
NAT2*7/7	0.007	0.000	0.89 (No)

^aJarrar *et al.* (2010).

genotype frequencies between Jordanian patients with RA and healthy Jordanian volunteers. The null hypothesis will be rejected when $z > 1.96$ or $z < -1.96$ (De Muth, 2006).

Results

The most prevalent genotypes are those that encode the slow acetylation phenotype. About 59.3% of the patients with RA carried the slow, 33.3% the intermediate, and 7.4% the fast-encoding genotypes (Table 1). These values were almost identical to the values obtained from healthy Jordanian volunteers (Jarrar *et al.*, 2010), which were 58.7%, 33.3%, and 8.0%, respectively. Statistical analysis, using the z test, of the individual genotypes in patients and volunteers are shown in Table 2. The only statistically significant difference was for the NAT2*5/7 genotype, which was not found in volunteers, but was found in 6.5% of patients. The NAT2*6/7 and the NAT2*7/7 genotypes were observed at low frequencies in volunteers, but not in patients. This difference did not reach statistical significance. The frequencies of NAT2 alleles for patients and volunteers are presented in Table 3. There were no statistically significant differences between the two groups.

Discussion

The overall NAT2 genotype frequencies encoding fast, intermediate, and slow acetylation among Jordanian patients

with RA were similar to those found in healthy volunteers (Jarrar *et al.*, 2010). The heterozygote genotype, NAT2*5/6, was the most frequent genotype among patients and volunteers, where its frequency was 50% of the total slow-encoding acetylation genotypes.

However, there were some differences between patients and volunteers regarding some individual genotypes encoding the slow acetylation status: NAT2*6/7 and NAT2*7/7 were absent in this group of patients, while together they constituted only 3.4% of the genotypes encoding slow acetylation in healthy volunteers. The z test showed that this difference was not statistically significant. The NAT2*5/7 genotype, which was not seen in healthy volunteers, was seen in 6.5% of patients, and constituted 10.9% of the genotypes encoding slow acetylation. This difference was statistically significant using the z test. In Jordanian RA patients, the overall acetylation status does not seem to be a genetic risk factor for the development of RA. However, the NAT2*5/7 genotype may have some relationship to RA, the nature of which needs further clarification.

The distribution of NAT2 polymorphism among patients with RA is controversial. Lawson *et al.* (1979) reported that the frequency of the slow acetylator phenotype in a small group of RA patients ($n=25$) was 72% and in a similar group of controls was 64%, and the difference was not statistically significant. Ladero *et al.* (1993) reported on the frequency of slow acetylators in RA patients and controls in Spanish males and females. They found that 46.4% of patients and 58.3% of controls were slow acetylators. When the comparison was made according to gender, they found that 66.3% of male patients and 52.4% of controls were slow acetylators, and this difference was not significant. However, in females, 37.5% of patients and 63% of controls were found to be slow acetylators. They concluded that the rapid acetylator phenotype may be a risk factor for the development of severe RA in women, whereas Pawlik *et al.* (2002) from Poland found that the risk of development of RA was five-fold greater in slow acetylators than in fast acetylators (odds ratio 4.79). There was a statistically significant increase in the proportion of homozygous slow acetylators with two mutated alleles (84.1%) in patients with RA in comparison with healthy subjects (52.5%; $p < 0.0001$). They concluded that the NAT2 slow acetylation genotype may be a risk factor of individual susceptibility to RA. In another study, Pawlik *et al.* (2004) reported that erosive RA was diagnosed in 74.55% of the slow and 40% of the fast acetylators. The risk for the development of erosive RA was 4.39 times greater in slow acetylators than in fast acetylators, while the mean number of swollen and tender joints, as well

TABLE 3. N-ACETYLTRANSFERASE 2 ALLELE FREQUENCIES AMONG JORDANIAN RHEUMATOID ARTHRITIS PATIENTS COMPARED TO HEALTHY VOLUNTEERS

NAT2 allele	RA patients			Healthy volunteers ^a			z Statistic (statistical difference)
	Count	Frequency	95%CI	Count	Frequency	95%CI	
NAT2*4	52	0.241	0.184–0.298	74	0.247	0.198–0.296	0.16 (No)
NAT2*5	97	0.449	0.383–0.515	112	0.373	0.318–0.428	-1.74 (No)
NAT2*6	59	0.273	0.214–0.332	104	0.347	0.293–0.401	1.80 (No)
NAT2*7	8	0.037	0.012–0.062	10	0.033	0.013–0.053	-0.24 (No)
Total	216	1.0		300	1.0		

^aJarrar *et al.* (2010).

as the erythrocyte sedimentation rate and C-reactive protein values, did not differ significantly with the acetylation genotype. They concluded that NAT2 polymorphism may be a genetic risk factor for joint destruction.

Kumagai *et al.* (2004) found that there was no clear difference in the genotype frequencies between patients with RA and healthy subjects in Japan. Kuon *et al.* (2008) have reported in the American College of Rheumatology Annual Scientific Meeting that 63.7% of their group of RA patients were slow acetylators, compared to 49.4% of the controls. They concluded that the NAT2 slow acetylation genotype is associated with an increased risk of RA development or progression into established disease.

In conclusion, the overall acetylator status genotypes and alleles do not seem to be a genetic risk factor for RA among Jordanians, while an association between the NAT2*5/7 and RA among Jordanians is apparently present, the nature of which is not understood, and needs further studies for clarification.

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Author Disclosure Statement

No competing financial interests exist for any of the authors.

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