Genotype and allele frequencies of *TPMT, NAT2, GST, SULT1A1* and *MDR-1* in the Egyptian population

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Aims The goal of this study was to determine the frequencies of important allelic variants in the *TPMT, NAT2, GST, SULT1A1* and *MDR-1* genes in the Egyptian population and compare them with the frequencies in other ethnic populations.

Methods Genotyping was carried out in a total of 200 unrelated Egyptian subjects. *TPMT★2* was detected using an allele-specific polymerase chain reaction (PCR) assay. *TPMT★3C* and *NAT2* variants (★5, ★6 and ★7) were detected using an allele-specific real-time PCR assay. Detection of *GSTM1* and *GSTT1* null alleles was performed simultaneously using a multiplex PCR assay. Finally, a PCR–restriction fragment length polymorphism assay was applied for the determination of *TPMT★3A* (★3B), *SULT1A1★2* and *MDR-1* (*3435T*) variants.

Results Genotyping of *TPMT* revealed frequencies of 0.003 and 0.013 for *TPMT★3A* and *TPMT★3C*, respectively. No *TPMT★2* or ★3B was detected in the analysed samples. The frequencies of specific *NAT2* alleles were 0.215, 0.497, 0.260 and 0.028 for ★4 (wild-type), ★5 (341C), ★6 (590A) and ★7 (857A), respectively. *GSTM1* and *GSTT1* null alleles were detected in 55.5% and 29.5% of the subjects, respectively. *SULT1A1★2* was detected at a frequency of 0.135. Finally, the frequencies of the wild-type allele (*3435C*) and the *3435T* variant in the *MDR-1* gene were found to be 0.6 and 0.4, respectively.

Conclusions We found that Egyptians resemble other Caucasians with regard to allelic frequencies of the tested variants of *NAT2, GST* and *MDR-1*. By contrast, this Egyptian population more closely resemble Africans with respect to the *TPMT★3C* allele, and shows a distinctly different frequency with regard to the *SULT1A1★2* variant. The predominance of the slow acetylator genotype in the present study (60.50%) could not confirm a previously reported higher frequency of the slow acetylator phenotype in Egyptians (92.00%), indicating the possibility of the presence of other mutations not detectable as T341C, G590A and G857A. The purpose of our future studies is to investigate for new polymorphisms, which could be relatively unique to the Egyptian population.

Keywords: drug-metabolizing enzymes, Egyptians, MDR–1, pharmacogenetics

Introduction

Cross-ethnic differences in response to drugs have been related to genotypic variants (polymorphisms) of key enzymes and proteins that affect the safety and efficacy of a drug in the individual patient [1]. For thiopurine S–

methyltransferase (TPMT), four alleles have been associated with low or absent enzyme activity. The four prevalent alleles, namely *TPMT★2* (G238C), *TPMT★3A* (G460A and A719G), *TPMT★3B* (only G460A) and *TPMT★3C* (only A719G), account for more than 80% of the defective phenotypes [2]. TPMT deficiency is associated with severe haematopoietic toxicity when patients are treated with standard doses of thiopurine drugs [2]. In contrast, TPMT-deficient patients with acute lymphocytic leukaemia could be successfully treated with 5–15% of the conventional dose of mercap-

topurine [3]. Recently, the genetic basis for this inherited trait has been elucidated in different ethnic populations; approximately 10% of the population have intermediate activity due to heterozygosity at the *TPMT* locus, and about 0.33% are TPMT deficient [4].

Similarly, *N*-acetyltransferase 2 (NAT2) activity is controlled by genetic polymorphisms, and the individual phenotypes can be classified as rapid, intermediate, or slow acetylators according to their acetylation activity [5]. NAT2 metabolizes a range of drugs including isoniazid, procainamide, and some polycyclic amines such as sulphonamides and hydralazines [6]. The relationship between polymorphic acetylation of isoniazid and procainamide by NAT2 and idiosyncratic drug toxicity has been well documented [7]. Polymorphisms at positions 341, 580, and 857 of the *NAT2* gene have been observed in several human populations and characterize the major *NAT2* defective alleles (*NAT2*★*5, NAT2*★*6 and NAT2*★*7*, respectively). The impact of ethnicity is well known, with more than 90% being slow acetylators in some Mediterranean populations, with only 10% in Japan (reviewed in [8]).

Large differences between racial groups are also seen for the glutathione S-transferases (GSTs). GSTs are a superfamily of enzymes that catalyse the conjugation of xenobiotics and endogenous substrates with glutathione, and thereby play a significant role in the inactivation and occasionally the activation of many drugs and xenobiotics [9]. In humans, one member of the mu class gene family (*GSTM1*) has been shown to be polymorphic and is absent (null) in 35–60% of individuals. Similarly, *GSTT1*, a member of the theta class gene family, is also polymorphic and absent in 10–65% of human populations (reviewed in [10]).

Similarly, human sulfotransferases (SULTs) are a superfamily of multifunctional enzymes. Six cytosolic SULTs have been identified in human tissues and are involved in the metabolism of many drugs, xenobiotics, neurotransmitters and hormones [11]. Previous biochemical pharmacogenetic studies have demonstrated a large individual variation (about 50-fold in some studies) in the activity of platelet SULTs in humans [11–14]. It was reported recently that a large portion of this variability could be explained by a newly identified common polymorphism (a G to A transition) in the coding region of the *SULT1A1* gene (*SULT1A1*★*2*) [14]. This base change results in an arginine to histidine substitution at codon 213, and individuals homozygous for the *His* allele had only about 15% of the SULT activity in platelets compared with those with other genotypes [14].

P-glycoprotein (PGP), the product of the multidrug resistance (*MDR-1*) gene also plays an important role in the bioavailability of a wide variety of drugs, including the cardiac glycoside digoxin and anthracycline antibiot-

ics, vinblastine, daunomycin, and cyclosporin A [15]. Recently, a C to T transition (C3435T) has been described in exon 26 of the *MDR-1* gene [16]. Individuals who are homozygous for the *3435T* variant have a significant decrease in intestinal PGP expression, and increased plasma digoxin levels after oral administration [16].

The frequencies of the important allelic variants in the *TPMT*, *NAT2*, *GST*, *SULT1A1* and *MDR-1* genes have been extensively studied in many ethnic groups, and the accumulated data show striking interethnic variation in the distribution of these variants. However, no information is available for the Egyptian population (except for *GST* [17, 18]). In the present study, we have therefore investigated the frequencies of the *TPMT* (★*2*, ★*3A,* ★*3B* and ★*3C*), *NAT2* (★*5,* ★*6 and* ★*7*), *GST* (*M1* and *T1* null alleles), *SULT1A1* (*SULT1A1*★*2*) and *MDR-1* (C3435T) polymorphisms in Egyptian individuals (200 subjects), providing a basis for future clinical studies concerning variability in the response and/or toxicity to drugs known to be substrates for TPMT, NAT2, GST, SULT1A1 and MDR-1.

Methods

Subjects

Two hundred unrelated Egyptian subjects participated in this study. The Egyptian population is divided into several cultural groups: Bedouins, Nubians, Berbers, Peasants and Urbanites. The subjects who participated in our study were students and staff at Cairo University, thereby considered as Urbanites living in Cairo or other surrounding cities. Each subject gave a sample of approximately 1 mL saliva after detailed explanation of the purpose of the study; a signed written consent was also obtained from each subject. Genomic DNA was isolated from the saliva using a QIAamp DNA Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations. Sample collection and DNA isolation were performed under the supervision and approval of the Dean of the Faculty of Pharmacy of Cairo University. The isolated DNA samples were sent to our laboratory in Japan, and the genotyping protocol was approved by the institutional ethics committee of Tohoku University School of Medicine, Sendai, Japan, and Faculty of Pharmacy, Cairo University, Cairo, Egypt.

Oligonucleotides

The primers were synthesized by Nihon Gene Research Laboratories, Inc. (Sendai, Japan). The TaqMan probes were synthesized by Applied Biosystems (Foster City, California, USA) and contained 6-carboxyfluorescein

(FAM) at the 5′ end and 6–carboxytetramethylrhodamine (TAMRA) with a phosphate molecule at the 3′ end.

Genotyping

TPMT★2 (G238C) The genotyping of each individual for the G238C mutation was performed using an allele-specific polymerase chain reaction (PCR) assay as previously described by Yates *et al.* [19].

TPMT★3A (★3B) (G460A) The genotyping of each individual for the G460A mutation was performed using a previously described PCR-restriction fragment length polymorphism assay (RFLP) [19]. In brief, a 694-bp fragment containing nucleotide 460 was amplified and digested with the restriction enzyme *Mwo*I. The wild-type allele, but not the mutant allele, contains a *Mwo*I restriction site allowing RFLP analysis of the digested products after their separation on a 3% agarose gel. Digestion of the 694-bp fragment at the *Mwo*I site yielded fragments of 443 and 251 bp.

TPMT★3C (A719G), NAT2 variants (★5 (T341C), ★6 (G590A) and ★7 (G857A)) TPMT★3C (A719G), *NAT2★6* (G590A) and *NAT2★7* (G857A) were detected using an allele-specific real-time PCR assay as previously described by Hiratsuka *et al.* [20]. However, in that report the assay was designed for the determination of *NAT2★5B* (C481T mutation). In the present study and in order to give a collective frequency of the *NAT2★5* variants (★5A, ★5B and ★5C), we have developed another allele-specific real-time PCR assay and have applied it for the determination of the T341C mutation, which is common in all *NAT2★5* variants. Details concerning the primers and the probes are given in Table 1.

GST (M1 and T1 null alleles) The determination of *GSTM1*, *GSTT1* null alleles was done simultaneously in a single assay using a multiplex PCR approach as described by Abdel-Rahman *et al.* [17]. The presence or absence of *GSTM1* and *GSTT1* genes was detected by the presence or absence of a band at 215 bp (corresponding to *GSTM1*) and a band at 480 bp (corresponding to *GSTT1*). A band at 312 bp (corresponding to *CYP1A1* gene) was always present and was used as an internal control to document successful PCR amplification.

SULT1A1 (G638A) The *SULT1A1* gene is one of three very closely related sulfotransferase genes located on chromosome 16; the open reading frames derived from *SULT1A1* and its neighbours *SULT1A2* and *SULT1A3* share more than 94% identity. Thus the primer pair was chosen specifically to amplify the appropriate region from

Table 1 Summary of primer pairs [wild-type (WT) or mutant (MT) and common (CM) primers] and TaqMan probe (TM) sequences (5′–3′).

TPMT★3C *(A719G)*	
WT:	TATGTCTCATTTACTTTTCTGTAAGTAG*T*T
MT:	TATGTCTCATTTACTTTTCTGTAAGTAG*T*C
TM:	AGACAGTCAATTCCCCAACTTTTATGTCGTTCTT
CM:	TTAACATGTTACTCTTTCTTGTTTCA
NAT2★5 *(T341C)*	
WT:	CCTTCTCCTGCAGGTGACC*T*T
MT:	CCTTCTCCTGCAGGTGACC*T*C
TM:	TTACATTGTCGATGCTGGGTCTGGAAGCTC
CM:	AGGCTGCCACATCTGGGAG
NAT2★6 *(G590A)*	
WT:	GTATTCATAGACTCAAAATCTTCAATTGT*C*C
MT:	GTATTCATAGACTCAAAATCTTCAATTGT*C*T
TM:	TTCTGTCAAGCAGAAAATGCAAGGCACCTG
CM:	CCAGATGTGGCAGCCTCTAGAA
NAT2★7 *(G857A)*	
WT:	TTTTGTTCCTTATTCTAAATAGTAAGGGA*C*C
MT:	TTTTGTTCCTTATTCTAAATAGTAAGGGA*C*T
TM:	TCACCAGGTTTGGGCACGAGATTTCT
CM:	AACTCTCACTGAGGAAGAGGTTGAAG

The mismatched nucleotide sites at position −2 are underlined.

SULT1A1 using intron sequences flanking exon VII where sequence identity was lower, as described in the method of Engelke *et al.* [21]. After specific amplification of a 281-bp fragment of DNA including exon VII of the *SULT1A1* gene, PCR products were digested with *Hae*II. Fragments were then resolved on 3% agarose gel. Digestion of the amplified sequence resulted in the formation of two fragments with lengths of 104 and 177 bp. Complete and partial digestion indicated the genotypes *SULT1A1★1/★1* and *SULT1A1★1/★2*, respectively. Lack of digestion indicated the genotype *SULT1A1★2/★2*.

MDR-1 (C3435T) In the present study, we have designed a PCR-RFLP assay and successfully applied it for the analysis of the C3435T mutation of the *MDR-1* gene. A 231-bp fragment containing nucleotide 3435 was amplified with 0.67 μM MDR-1F (5′-ACTCT TGTTTTCAGCTGCTTG-3′) and MDR-1R (5′-AGAGACTTACATTAGGCAGTGACTC-3′). PCR amplification consisted of an initial denaturation step at 94 °C for 5 min followed by 33 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 20 s, and extension at 72 °C for 30 s. The final extension step was performed at 72 °C for 5 min. All these steps were carried out using a Perkin-Elmer (Shelton, Connecticut, USA) thermocycler. The PCR products were digested with the restriction enzyme *Sau*3AI at 37 °C overnight. The digested products were then separated on a 3% agarose

gel. The wild-type allele, but not the mutant allele contains a *Sau*3AI restriction site allowing RFLP analysis of the digested products. Digestion of the 231-bp fragment at the *Sau*3AI site yielded two fragments of 163 and 68 bp.

Throughout the genotyping assays, positive controls (samples with known genotypes) were not included. Instead, all the subjects were genotyped twice for all the tested mutations, which makes the possibility of genotyping error less likely.

Statistical analysis

Data were compiled according to the genotype and allele frequencies estimated from the observed numbers of each specific allele. The frequency of each allele in our subjects is given together with the 95% confidence interval (CI). Differences in allele frequencies between Egyptians and other ethnic populations were measured using the χ^2 test and Fisher's exact test. A *P*-value <0.05 was considered to be statistically significant throughout the population comparisons.

Results

Tpmt

The presence of the three inactivating mutations (G238C, G460A and A719G) that characterize the most common defective alleles of the *TPMT* gene was examined in our subjects. A total of 194 subjects (97.00%) was apparently homozygous for the wild-type allele (*TPMT*★1), i.e. they did not carry any of the tested mutations. Six individuals (3.00%) were heterozygous for one of the mutant alleles. One subject was *TPMT*★1/ *TPMT*★3A and five subjects were *TPMT*★1/*TPMT*★3C, giving allele frequencies of 0.003 (95% CI 0, 0.007) and 0.013 (95% CI, 0.003, 0.023) for *TPMT*★3A and *TPMT*★3C, respectively (Table 2). Neither G238C (*TPMT*★2), G460A alone (*TPMT*★3B) nor the homozygous form of any mutant allele was detected in Egyptian individuals. In genotype assignment, when both the mutations G460A and A719G were detected in the same DNA sample, a heterozygous genotype *TPMT*★1/ *TPMT*★3A was assumed. In fact, the possible alternative genotype, i.e. a compound heterozygous genotype *TPMT*★3B/*TPMT*★3C, was reasonably excluded, since the *TPMT*★3B allele (only G460A mutation) was not detected in any of our subjects.

Nat2

The allele-specific real-time PCR assay used in this study provided a simple and efficient method for the analysis of the three major nucleotide substitutions in the *NAT2*

Table 2 The observed and expected genotype distribution of the tested variants among the 200 Egyptian subjects according to Hardy–Weinberg equilibrium.

Tested genes	n	Observed frequency (%)	Predicted frequency (%) by Hardy–Weinberg law
TPMT			
★1/★1	194	97.00	96.82
★1/★2	0	0	0
★1/★3A	1	0.50	0.59
★1/★3C	5	2.50	2.56
Mut/mut†	0	0	0.03
NAT2‡			
★4/★4	7	3.50	4.62
★4/★5	49	24.50	21.37
★4/★6	21	10.50	11.18
★4/★7	2	1.00	1.20
★5/★5	45	22.50	24.70
★5/★6	56	28.00	25.84
★5/★7	4	2.00	2.78
★6/★6	11	5.50	6.76
★6/★7	5	2.50	1.46
★7/★7	0	0	0.09
SULT1A1			
★1/★1	149	74.50	74.84
★1/★2	48	24.00	23.36
★2/★2	3	1.50	1.80
MDR-1			
C/C	68	34.00	36.00
C/T	103	51.50	48.00
T/T	29	14.50	16.00

N, Number of subjects. †Include *TPMT*★2, ★3A (★3B) and ★3C. ‡Because of failure of the polymerase chain reaction amplification of one sample, only 199 subjects could be analysed.

gene (T341C, G590A and G857A) amongst the Egyptian individuals. Throughout the study and in population comparisons, since *NAT2*★5A, ★5B and ★5C all contain the T341C substitution, these alleles comprise the group *NAT2*★5. Similarly, *NAT2*★6 and *NAT2*★7 represent the sum of *NAT2*★6A and ★6B and *NAT2*★7A and ★7B, respectively. By contrast, *NAT2*★4 was assigned to the allele which did not carry any of the three mutations analysed in our study.

The genotype frequencies obtained from all individuals are summarized in Table 2. Because of failure of PCR amplification of one sample, only 199 subjects could be analysed. A total of four fast acetylator genotypes, amounting to 39.50%, were observed. Slow acetylators accounted for 60.50% and were distributed among the five different genotypes. Calculation of the allele frequencies revealed that the most common allele was *NAT2*★5 (0.497, 95% CI 0.451, 0.549), followed by *NAT2*★6 (0.260, 95% CI 0.218, 0.304) and the wild-type allele *NAT2*★4 (0.215, 95% CI 0.175, 0.256).

NAT2★7 was found to be the least common variant with a frequency of 0.028 (95% CI 0.011, 0.043).

Gst

A multiplex PCR approach was applied in a total of 200 Egyptian subjects. This method cannot differentiate between the wild type and heterozygous state. It determines only the percentages of the homozygous deletion of both *GSTM1* and *GSTT1* genes. The numbers of individuals homozygous for the null alleles of the *GSTM1* and *GSTT1* were 111 (55.50%) and 59 subjects (29.50%), respectively.

Sult1a1

Among the 200 subjects who were genotyped for *SULT1A1*, the genotype distribution was (*SULT1A1★1/★1*, *★1/★2*, *★2/★2*: 74.50%, 24.00%, 1.50%) (Table 2), giving allele frequencies of 0.865 (95% CI 0.831, 0.898) and 0.135 (95% CI 0.101, 0.168) for *SULT1A1★1 and SULT1A1★2*, respectively. It should be noted that our study was not designed to detect the rare alleles *SULT1A1★3* and *★4,* so carriers of these alleles may be scored as carriers of *SULT1A1★1* or *SULT1A1★2*.

Mdr-1

Genotyping of our subjects for the C3435T mutation revealed a genotype distribution of (*C/C, C/T, T/T*: 34.00%, 51.50%, 14.50%) (Table 2). The frequencies of

the wild-type allele (*3435C*) and the *3435T* variant were found to be 0.600 (95% CI 0.549, 0.645) and 0.400 (95% CI 0.355, 0.450), respectively.

Our results (Table 2) were in good accordance with the expected genotype distributions of the tested genes, calculated by the Hardy–Weinberg law. The frequencies of the tested alleles in our Egyptian subjects are given in Tables 3–6, with comparison with other data reported in various other ethnic populations.

Discussion

Generally, human populations are divided into three major races, namely, the Caucasoid, Mongoloid and Negroid groups. However, some anthropologists regarded Arabs, Jews, Latinos and Spaniards as 'distinct races', probably because of the considerable genetic admixture in these populations. Among the Arabs, the Egyptian population was the point of our interest. Egypt is unique geographically, as it is located centrally to the three continents of Africa, Europe and Asia, so its population is highly affected by the rapid pace of intercontinental transportation and large-scale immigration. Throughout history, the Greeks, Romans, Arabs, Turks, French and British have all ruled Egypt and mixed with its people, so that modern Egypt now is an amalgam of all these legacies. In the present study, we thought it worthwhile to attempt to investigate the prevalence of important allelic variants of *TPMT, NAT2, GST, SULT1A1* and *MDR-1* genes in the Egyptian population, and compare our results with other data previously reported for various ethnic groups.

Table 3 Allele frequencies of *TPMT* in various ethnic groups.

Population	n	TPMT★2	TPMT★3A	TPMT★3C	Reference
Egyptians	200	0	0.003	0.013	Present study
Caucasians					
American	282	0.002	0.032	0.002	[22]
British	199	0.005	0.045	0.003	[23]
French	191	0.005	0.057	0.008	[23]
Italian	206	0.005	0.039	0.010	[24]
Saami-Norwegian	194	0	0	0.033	[25]
Norwegian	66	0	0.034	0.003	[25]
Asians					
Japanese	192	0	0	0.015	[26]
Chinese	192	0	0	0.023	[27]
South West Asians†	99	0	0.01	0	[27]
Africans					
Kenyan	101	0	0	0.054	[23]
Ghanaian	217	0	0	0.076	[28]
African-Americans	248	0.004	0.008	0.024	[22]

n, Number of subjects. †South West Asians included people from India, Pakistan, Sri Lanka and Nepal.

Table 4 Allele frequencies of *NAT2* in various ethnic groups.

Population	n	NAT2*4	Allele frequency† NAT2*5	NAT2*6	NAT2*7	Reference
Egyptians	199	0.215	0.497	0.260	0.028	Present study
Caucasians						
American	266	0.220	0.470	0.280	0.003	[30, 31]
German	844	0.244	0.465	0.278	0.013	[32]
Portuguese	128	0.212	0.433	0.328	0.027	[33]
Danish	242	0.254	0.473	0.250	0.023	[34]
Scottish	96	0.203	0.490	0.271	0.036	[35]
Swedish	70	0.194	0.507	0.278	0.021	[35]
Asians						
Japanese	79	0.641	0.019	0.230	0.110	[31]
Hong Kong Chinese	70	0.473	0.057	0.310	0.160	[31]
Korean	85	0.692	0.018	0.180	0.110	[31]
Filipino	100	0.395	0.065	0.360	0.180	[31]
Taiwanese	100	0.515	0.025	0.310	0.150	[31]
Indians	61	0.257	0.330	0.380	0.033	[31]
Polynesians	25	0.600	0.040	0.340	0.020	[31]
Native Africans						
Gabonese	52	0.356	0.404	0.221	0.019	[36]
Dogons	65	0.300	0.300	0.370	0.030	
Hispanic-Americans	65	0.390	0.320	0.190	0.100	[31]
African-Americans	214	0.430	0.295	0.230	0.045	[30, 31]

n, Number of subjects. †NAT2*4 represent the allele that did not contain any of the mutations analysed in our study, while NAT2*5, NAT2*6 and NAT2*7 represent the alleles that contained 341C, 590A and 857A mutations, respectively.

Table 5 Allele frequencies of *SULT1A1* in various ethnic groups.

Population	n	Allele frequency *Arg	*His	P *vs.* Egyptian	Reference
Egyptians†	200	0.865	0.135		Present study
Caucasians					
American‡	150	0.690	0.310	<0.0001	[14]
American‡	245	0.670	0.330	<0.0001	[41]
German†	300	0.630	0.370	<0.0001	[21]
British†	106	0.680	0.320	<0.0001	[40]
Scottish†	187	0.680	0.320	<0.0001	[40]
Asians					
Chinese‡	290	0.914	0.080	0.0018	[41]
Africans					
Nigerian†	52	0.730	0.270	0.0031	[40]
African-Americans‡	70	0.710	0.290	<0.0001	[41]

n, Number of subjects. (Differences in allele frequencies were measured using Fisher's exact test.) †These studies were designed to detect only *SULT1A1*2*, so carriers of *3 or *4 alleles may be scored as carriers of *SULT1A1*1* or *2. ‡These studies were designed to detect either *2 and *3 or *2*, *3 **and** *4. For this table, the common allele *SULT1A1*1 and* the rare alleles *3 and *4 were combined with the allele *SULT1A1*1 and* assigned as *Arg.

Tpmt

Population studies [22–28] have demonstrated significant ethnic differences in the distribution of TPMT variant alleles among various ethnic groups (Table 3). *TPMT*3A* accounts for more than 80% of the variant alleles in Caucasians, but for only approximately 17% of the variant alleles in African-Americans. However, *TPMT*3C* accounts for only 5% of the variant alleles in Caucasians, 70% in African-Americans and 100% in the Japanese and

Table 6 Genotype percentages and allele frequencies of *MDR-1* (C3435T) polymorphism in various ethnic groups.

Population	n	Genotype % C/C	C/T	T/T	Allele frequency C	T	P (vs. Egyptians)	Reference
Egyptians	200	34.00	51.50	14.50	0.600	0.400		Present study
Caucasians								
British	190	24.00	48.00	28.00	0.480	0.520	0.0005	[42]
Portuguese	100	22.00	42.00	36.00	0.430	0.570	<0.0001	[42]
German	188	28.00	48.00	24.00	0.520	0.480	<0.0001	[16]
Saudi	96	37.00	38.00	26.00	0.550	0.450	NS	[42]
Asians								
Japanese	50	34.00	46.00	20.00	0.570	0.430	NS	[43]
Chinese	132	32.00	42.00	26.00	0.530	0.470	NS	[42]
Filipino	60	38.00	42.00	20.00	0.590	0.410	NS	[42]
South-west Asian	89	15.00	38.00	47.00	0.340	0.660	<0.0001	[42]
Africans								
Sudanese	51	52.00	43.00	6.00	0.730	0.270	<0.0001	[42]
Kenyan	80	70.00	26.00	4.00	0.830	0.170	<0.0001	[42]
Ghanaian	206	66.00	34.00	0	0.830	0.170	<0.0001	[42]
African-Americans	88	68.00	31.00	1.00	0.840	0.160	<0.0001	[42]

n, Number of subjects. Differences in allele frequencies were measured using Fisher's exact test. NS, There were no significant differences ($P > 0.05$).

Chinese. In the present study, we found that *TPMT⋆3C* is the ancestral *TPMT* variant allele in the Egyptian population, accounting for 86% of the variant alleles detected. *TPMT⋆3A* is the second most recurrent variant and accounts for the remaining 14% of the *TPMT* variant alleles in Egyptian individuals.

The frequency of *TPMT⋆3C* found in the present study (0.013) is similar ($P > 0.05$) to the frequencies reported for Caucasians (0.002–0.010) [22–25], Saami-Norwegian (0.033) [25] and Asian populations (0.015–0.023) [26, 27], but significantly lower than the frequencies reported for Kenyan (0.054) [23] ($P < 0.005$) and Ghanaian (0.076) [28] ($P < 0.0001$) populations. *In vivo* studies [19] indicate that homozygosity for *TPMT⋆3C* impairs metabolism of thiopurine drugs, possibly due to the intrinsic instability of the enzyme compared with that of *TPMT⋆1* [29]. Therefore, the six *TPMT⋆3C* heterozygote individuals found in the present study may have a decreased capacity to metabolize thiopurine drugs.

TPMT⋆3A, which causes the largest decrease in enzyme activity [29], was detected in only one Egyptian individual (heterozygote of *TPMT⋆1/TPMT ⋆3A*) with an allele frequency of 0.003. Similar low frequencies of *TPMT⋆3A* ($P > 0.05$) were reported for South-west Asians (0.010) [27] and African-Americans (0.008) [22]. However, *TPMT⋆3A* was found in significantly higher frequencies ($P < 0.0001$) in Caucasian populations, including Americans (0.032) [22], British (0.057) [23], French (0.057) [23], Italians (0.039) [24] and Norwegians (0.034) [25].

In conclusion, the present study showed that the Egyptians have a relatively low frequency of *TPMT* mutant alleles. Only six out of 200 Egyptian individuals (3.00%) carried a variant TPMT allele (*TPMT⋆3A* or *TPMT⋆3C*). The low frequency of variant *TPMT* alleles in the Egyptian population infers that the Egyptians may have higher TPMT activity than other Caucasian or African-American populations. This may contribute to both reduced response and also low incidence of toxicity following thiopurine treatment.

Nat2

According to the rich literature regarding the ethnic variation in the distribution of defective *NAT2* alleles all over the world [30–36], two major groups could clearly be distinguished according to the incidence of the *NAT2⋆5 and NAT2⋆7* variants. Caucasians, Indians and Africans are very similar (> 0.290 and <0.050, respectively). Asiatic and Pacific groups also appear to make up a homogeneous group (< 0.070 and >0.090, respectively). However, the frequency of the *NAT2⋆6* variant is only slightly different among various populations.

In the present study, we found that *NAT2* allele frequencies among the Egyptians were similar to those reported for other Caucasian populations (Table 4). In Egyptians, as in Caucasians, the majority of *NAT2* alleles are composed of *NAT2⋆4* (wild type), *NAT2⋆5* and *NAT2⋆6*, which were found in our study in frequencies of 0.215, 0.497 and 0.260, respectively. We also found the *NAT2⋆7* variant had a frequency of 0.028. This

frequency is in the range of that of the Caucasian-African group (0.003–0.045) and significantly lower ($P < 0.0001$) than that of the Asiatic group (0.095–0.180) (Table 4).

The predominance of the slow acetylator genotype in Egyptians (60.50%) conforms to findings amongst other populations: 52.60% in Caucasian-Americans, 60.00% in Germans [37], and 63.40% among Polish children [38]. In contrast, in the Japanese, Chinese, Koreans and Filipinos, the fast genotype is largely over-represented (up to 90.00%) [31]. It was reported previously that Egyptians have the highest incidence on record of the slow-acetylator phenotype (92.00%) [39]. However, our results could not confirm this finding, indicating either a difference in the populations studied or the presence of other mutations not detectable as T341C, G590A and G857A.

Gst

The frequency of the *GSTM1* null genotype was previously reported in the Egyptian population (44.00% among 34 subjects [17] and 48.00% among 21 subjects [18]). The frequency of the *GSTT1* null genotype was also previously reported in the Egyptian population (15.00% among 34 subjects [17]). In the present study we report relatively higher frequencies of both null genotypes (55.50% and 29.50%) for *GSTM1* and *GSTT1*, respectively. This difference may be explained by the larger number of subjects examined in our study (200) compared with the small number of subjects analysed in the other two studies (34 [17] and 21 [18]).

According to our results, the frequency of the *GSTM1* null genotype (55.50%) was in the range of Europeans (39.00–62.00%) and White Americans (35.00–62.00%), higher than that of African-Americans (23.00–41.00%), and Japanese (41.00–51.00%). Although the frequency of the *GSTT1* null genotype (29.50%) was a little higher than the range of Europeans (10.00–21.00%) and Africans (15.00–26.00%), it was within the range of Caucasian-Americans (10.00–36.00%), and considerably lower than the frequency reported for the Japanese (44.00%). Our data confirmed that Egyptians are similar to other Caucasian populations in the frequency distribution of both *GSTM1 and GSTT1* null alleles.

Sult1a1

Fifteen different alleles have been described for the *SULT1A1* gene, including four that resulted in alterations in encoded amino acids (the wild-type allele (*SULT1A1*1*) and *SULT1A1*2–*4* variants) [14]. A comparison of the distribution of *SULT1A1* alleles between various ethnic groups has been reported previously [40, 41]. The results of those studies showed striking ethnic differences in allele frequencies, with a *SULT1A1*1* frequency that was highest in Chinese (0.914) and lowest in African-American subjects (0.477). Conversely, the frequency of the *SULT1A1*3* allele was highest in African-Americans (0.299) and lowest in Chinese subjects (0.006). The frequency of the *SULT1A1*2* allele did not differ significantly between Caucasians (0.332) and African-Americans (0.294), but it was much lower in Chinese (0.080) than in the other two ethnic groups [41]. The rare allele *SULT1A1*4* was only detected in a sample of 150 Caucasian Americans with a frequency of 0.003 [14].

According to our results, the frequency of *SULT1A1*2* was 0.135 in Egyptian individuals. Interestingly, this frequency is significantly lower than other frequencies reported in other Caucasian (0.310–0.370) [14, 21, 40, 41], African (0.270) [40] and African-American (0.290) [41] subjects. However, this frequency is significantly higher than that in the Chinese group (0.080) [41] (P-values are listed in Table 5). Our data support the need for further investigation about this different pattern in the frequencies of the *SULT1A1*2* variant in the Egyptian population.

Mdr-1

Although PGP expression is highly variable between subjects, the molecular basis for this variation was not clear until recently, when a functional single nucleotide polymorphism (SNP) resulting in a C to T transition (C3435T) was described in exon 26 of the *MDR-1* gene [16]. Strikingly, there was a significant relationship of this silent SNP with intestinal PGP expression levels and oral bioavailability of digoxin. Individuals who were homozygous for the *3435T* variant had significantly decreased intestinal PGP expression and increased digoxin plasma levels after oral administration [16].

Our result from genotyping of 200 subjects for the C3435T mutation showed genotype frequencies of 34.00%, 51.50%, and 14.50% for *C/C*, *C/T* and *T/T*, respectively. The frequencies of the *C*-allele (0.600) and the wild-type *C/C* (34.00%) in Egyptian subjects were similar ($P > 0.05$) to Saudi Arabians (0.550 and 32.00%, respectively) [42], possibly due to the common Arabic origin of the two populations. However, the frequency of the *C*-allele in Egyptian subjects (0.600) was significantly different from in Caucasian populations, including the British (0.480), Portuguese (0.430) and Germans (0.520) [42]. It was also different from the South Asian population (0.340), Africans (0.170–0.270) and African-Americans (0.160) [42] (P-values are listed in Table 6). Interestingly, the frequency of the *C*-allele in Egyptian subjects (0.600) did not differ from that reported in Oriental Asian populations including Japanese (0.570) [43], Chinese (0.530) and Filipinos (0.590) [42].

In conclusion, our results of genotyping Egyptian individuals for important allelic variants of *TPMT*, *NAT2*, *GST*, *SULT1A1* and *MDR-1* provide further evidence for racial heterogeneity in drug metabolism and disposition. It is hoped that our results will aid in understanding the ethnic diversity of the Egyptian population, and offer a preliminary basis for more rational use of drugs that are substrates for TPMT, NAT2, GST, SULT1A1 and MDR-1 in this population.

It should be noted that in the present study we have focused on genotyping Egyptian individuals for important allelic variants previously reported in other ethnic groups, providing the first description of these alleles in the Egyptian population. This does not exclude the possibility of the presence of new SNPs relatively unique to the Egyptian population, which is exemplified by the discordance between NAT2 phenotype and genotype in this population, and which will be the main focus of our future studies.

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