Review

Loredana Serpe, Roberto Canaparo, Maria Gabriella Scordo and Edoardo Spina*

Pharmacogenetics of drug-metabolizing enzymes in Italian populations

Abstract: Drug-metabolizing enzymes play a major role in the biotransformation and subsequent elimination of most drugs and xenobiotics from the body. Both phase I and phase II enzymes are highly polymorphic. Inter-individual differences in genes coding for drug-metabolizing enzymes are important for understanding variability in drug response and for individualization of drug prescription. The prevalence of genetic polymorphisms in drug metabolism varies widely with ethnicity, and marked differences in the distribution of allelic variants of genes encoding drug-metabolizing enzymes have been documented in populations of different racial origin. This review aimed to summarize the available studies on genetic polymorphisms associated with drug metabolism conducted in Italian populations and to compare the frequency of the various metabolizer phenotypes and most common variant alleles (and resulting genotypes) with corresponding values from other populations. Notably, published data are not extensive, and most studies were performed on relatively low numbers of individuals. In general, the frequency of polymorphisms in the cytochrome P450 (*CYP*) genes as well as in the investigated phase II enzymes in the Italian population was similar to values reported for other Caucasian populations. However, the prevalence of *CYP2D6* gene duplication among Italians was found to be very high, confirming the higher frequency of *CYP2D6* ultrarapid metabolizers in the Mediterranean area compared to Northern Europe. It is worth noting that a geographic gradient in the flavin-containing monooxygenase 3 polymorphism distribution was also seen, the Italian population showing higher similarity to other Mediterranean populations than to North Europeans.

***Corresponding author: Edoardo Spina,** Department of Clinical and Experimental Medicine, University of Messina, Policlinico Universitario, Via Consolare Valeria, 98125 Messina, Italy, Phone: +39-090-2213647, Fax: +39-090-2213300, E-mail: espina@unime.it
Loredana Serpe and Roberto Canaparo: Department of Drug Science and Technology, University of Torino, Torino, Italy
Maria Gabriella Scordo: Department of Medical Sciences, Clinical Pharmacology, University of Uppsala, Uppsala, Sweden

Keywords: drug-metabolizing enzymes; Italian population; pharmacogenetics; polymorphisms.

DOI 10.1515/dmdi-2014-0028
Received September 25, 2014; accepted November 21, 2014

Introduction

Pharmacogenetics is the study of the genetic basis leading to individual variation in drug response. It is well known that therapeutic failure and severe adverse drug reactions can both be influenced by an individual genetic make-up [1]. The therapeutic outcome may be affected by genetic variation in genes coding for drug-metabolizing enzymes, drug transporters, drug targets, and biomarker genes [2]. Identification of these gene variants has become one of the main goals of modern drug therapy and is frequently described as "personalized medicine".

Metabolic processes are necessary to convert a lipophilic drug into one or more metabolites which are more water soluble, thus facilitating urinary excretion. The biochemical reactions involved in the biotransformation of drugs are catalyzed by various enzyme systems and are conventionally divided into phase I (functionalization) and phase II (conjugation) reactions, which may occur in series [3]. Phase I reactions involve the addition of a polar functional group (e.g., a hydroxyl group) or the deletion of a non-polar alkyl group (e.g., *N*-demethylation) by oxidation, reduction, or hydrolysis. In phase II reactions, the drug or the phase I metabolite is covalently attached to a water-soluble endogenous substrate (e.g., glucuronic acid, acetate, sulfate, glutathione, or glycine), usually resulting in an inactive, easily excretable compound.

Genetically determined variability in the activity of these enzymes may result in inter-individual differences in the pharmacokinetics and plasma/serum concentrations of a given drug. As most therapeutic agents undergo extensive hepatic biotransformation, differences in treatment outcome may be attributed to

Authenticated | espina@unime.it author's copy
Download Date | 1/10/15 12:07 PM

predictable variations in genes encoding drug-metabolizing enzymes. Over the past few decades, advances in pharmacogenetic research have led to the discovery of several polymorphisms associated with drug metabolism. A genetic polymorphism is defined as a stable variation in a given locus of the genetic sequence, which is detected in 1% or more of a specific population [1]. These polymorphisms reflect gene insertions and deletions, gene duplications, copy number variations, and single nucleotide polymorphisms (SNPs), and can lead to enzyme variants with higher, lower, or no activity, or occasionally the total absence of the enzyme. The resulting phenotypes associated with these genetic variants are usually classified into four groups: poor (PMs), intermediate (IMs), extensive (EMs), and ultrarapid metabolizers (UMs) [2]. Phenotyping and/or genotyping procedures may therefore allow the identification of patients at risk of inefficacy or toxicity and offer tools to individualize drug prescription [4].

Inter-ethnic differences in drug metabolism are well documented for a number of medications [5]. Racial/ethnic differences in drug metabolism may be explained by differences in the distribution of allelic variants of genes encoding drug-metabolizing enzymes in different ethnic groups. Ethnic diversity in drug response may represent an important regulatory aspect that should be considered during drug development if data on recommended doses of a drug are to be extrapolated from one ethnic group to another [6].

Given the importance of inter-ethnic pharmacogenetic differences in drug safety and efficacy, the aim of this review was to summarize the available studies on genetic polymorphisms associated with drug-metabolizing enzymes conducted in Italian populations and to compare the frequency of the various metabolizer phenotypes and most common variant alleles (and resulting genotypes) with corresponding values from other populations.

Pharmacogenetics of phase I enzymes: studies in Italian populations

The cytochrome P450 (CYP) system represents the major enzyme family responsible for the phase I oxidative reactions. Other phase I drug-metabolizing enzymes include the flavin-containing monooxygenase, alcohol dehydrogenase, monoamine oxidase, epoxide hydrolase, and esterase systems.

Cytochrome P450 enzymes

CYPs are members of a superfamily of monooxygenases that catalyze the oxidative biotransformation of many drugs, nutrients, environmental toxins, and endogenous substances (i.e., steroid hormones, prostaglandins, bile acids, fatty acids) [2, 7]. These enzymes, located in the membranes of the smooth endoplasmic reticulum as well as in the mitochondria in the liver and in many extrahepatic tissues, are classified into families and subfamilies according to similarities in their amino acid sequence [8]. The enzymes found in mitochondria are generally involved in the synthesis and metabolism of internal substances, while enzymes in the endoplasmic reticulum usually metabolize external substances, primarily medications and environmental pollutants. The human genome comprises 57 CYP genes which are grouped according to sequence homology into 18 families and 44 subfamilies. Only about a dozen enzymes belonging to the 1, 2, and 3 CYP families are involved in phase I drug metabolism [2, 8]. CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5 are the CYP isoforms which play a major role in the biotransformation of therapeutic agents [2, 7]. Other clinically relevant isoforms include CYP1A1, CYP2A6, CYP2C8, and CYP2E1. Each CYP isoform is a specific gene product and possesses a characteristic but relatively broad spectrum of substrate specificity. Genetic polymorphisms have been discovered in virtually all CYP enzymes. The most important polymorphic enzymes in relation to their clinical implications are CYP2C9, CYP2C19, and CYP2D6.

CYP2C9, CYP2C19, and CYP2D6

CYP2C9 accounts for approximately 20% of the hepatic total CYP content [9] and is responsible for the metabolism of a variety of therapeutic agents including non-steroidal anti-inflammatory drugs, oral antidiabetics, diuretics, angiotensin II receptor antagonists, anticancer drugs, oral anticoagulants (e.g., S-warfarin), and antiepileptics (e.g., phenobarbital and phenytoin) [10–12]. The *CYP2C9* gene is located at chromosome 10q24 in a multigene cluster containing the other *CYP2C* subfamily members (*CYP2C8*, *CYP2C18*, and *CYP2C19*). *CYP2C9* exhibits genetic polymorphisms and, to date, more than 35 allelic variants have been described, according to the Human Cytochrome P450 (CYP) Allele Nomenclature Database (http://www.cypalleles.ki.se). The two more common variants associated with the reduced enzyme activity are *CYP2C9*2* and *CYP2C9*3* (Table 1). There are significant inter-ethnic differences in the frequency of these two variants [2].

Authenticated | espina@unime.it author's copy
Download Date | 1/10/15 12:07 PM

Table 1 Overview of the most common and functionally relevant CYP allelic variants and their impact on metabolic activity (http://www.cypalleles.ki.se).

Gene	Allele (SNP ID)	Consequences on gene structure	Impact on enzyme activity
CYP2C9	*CYP2C9∗2* (rs1799853)	Reduced oxidoreductase affinity	Reduced activity
	CYP2C9∗3 (rs1057910)	Reduced substrate affinity	Reduced activity
CYP2C19	*CYP2C19∗2* (rs4244285)	Splicing defect	Lack of activity
	CYP2C19∗3 (rs4986893)	Premature stop codon	Lack of activity
	CYP2C19∗17 (rs12248560)	Increased transcription	Enhanced activity
CYP2D6	*CYP2D6∗3* (rs35742686)	Frameshift	Lack of activity
	CYP2D6∗4 (rs3892097)	Splicing defect	Lack of activity
	CYP2D6∗5	Gene deletion	Lack of activity
	CYP2D6∗6 (rs5030655)	Frameshift	Lack of activity
	CYP2D6∗41 (rs28371725)	Splicing defect	Reduced activity
	CYP2D6∗2xn	Gene duplication	Enhanced activity
CYP3A4	*CYP3A4∗1B* (rs2740574)	Altered 5′ regulatory element	Reduced activity
	CYP3A4∗3 (rs4986910)	Amino acid change (M445T)	Reduced activity
	CYP3A4∗4 (rs55951658)	Amino acid change (I118V)	Reduced activity
CYP3A5	*CYP3A5∗2* (rs28365083)	Amino acid change (T398N)	Lack of activity
	CYP3A5∗3 (rs776746)	Splicing defect	Lack of activity
	CYP3A5∗6 (rs10264272)	Splicing defect	Lack of activity
CYP1A1	*CYP1A1∗2C* (rs1048943)	Amino acid change (I462V)	Enhanced activity
CYP2E1	*CYP2E1∗5* (rs2031920)		Reduced activity

CYP2C9∗2 and *CYP2C9∗3* are mainly present in Caucasians at a frequency of 11% and 7%, respectively, while frequencies are lower in Africans. *CYP2C9∗2* has indeed not been detected in Asians [2].

CYP2C19 is involved in the oxidative biotransformation of many commonly used medications including proton pump inhibitors, clopidogrel, some benzodiazepines (i.e., diazepam and clobazam), and antidepressants (i.e., amitriptyline, imipramine, citalopram, and escitalopram) [2, 10, 12]. The *CYP2C19* gene is located on chromosome 10q24.1–q24.3. The *CYP2C19* gene is highly polymorphic; at least 35 allelic variants and subvariants *(∗1B* to *∗34)* have been identified [13]. *CYP2C19∗1*, the wild-type allele encoding a fully functional enzyme, is present in double or single copy in EMs (homozygotes or heterozygotes, respectively). CYP2C19 PMs are carriers of null alleles *CYP2C19∗2* and *CYP2C19∗3* (Table 1). About 2%–5% of Caucasian and Afro-Caribbean populations but up to about 25% of Asians are CYP2C19 PMs There is a marked inter-ethnic variation in the distribution of these two variant alleles [13, 14]. The allelic frequency of *CYP2C19∗2* has been shown to be 15% in Africans, 29%–35% in Asians, 12%–15% in Caucasians, and 61% in Oceanian peoples. *CYP2C19∗3* is mainly found in Asians (5%–9% in Asians, <0.5% in Caucasians). A *CYP2C19* gene variant (*CYP2C19∗17*), associated with increased gene transcription and thus a higher metabolism of CYP2C19 substrates, has been described [15]. Its frequency varies quite broadly between different ethnic groups, being 18% among Swedes and 4% in Chinese [15].

CYP2D6 is the most important polymorphic enzyme involved in drug metabolism. Although it represents <5% of the hepatic total CYP content [9], CYP2D6 plays an important role in drug metabolism, being partially or entirely responsible for the biotransformation of several commonly prescribed drugs such as antidepressants, antipsychotics, opioids, antiemetics, antiarrhythmics, beta-blockers, and anticancer drugs [12, 16, 17]. The gene encoding CYP2D6 is located in position 22q13.1 and is highly polymorphic. Currently more than 100 allelic variants and subvariants have been identified, with substantial ethnic differences in allele frequencies [7, 16]. Some variants encode an inactive enzyme or no enzyme at all, while others are characterized by gene duplication and lead to increased activity. These gene variants are associated with various drug metabolism rates – individuals being classified as PMs, IMs, EMs, or UMs according to their inherited genetic profile. PMs lack CYP2D6 activity and represent approximately 3%–10% of Caucasians, but only 1%–2% of East Asians. Among EMs, the catalytic activity varies largely, and a subgroup of subjects with extremely high enzyme activity has been classified as UMs. Four major mutated alleles, *CYP2D6∗3*, *CYP2D6∗4*, *CYP2D6∗5*, and *CYP2D6∗6*, account for 90%–95% of the PM alleles in Caucasians, while the *CYP2D6∗41* allelic variant, which causes an impaired CYP2D6 expression, is the main factor underlying the IM phenotype (Table 1). Alleles with duplication or multiduplication of a functional *CYP2D6∗2* gene are associated with increased CYP2D6 activity. The overall

Authenticated | espina@unime.it author's copy
Download Date | 1/10/15 12:07 PM

frequency of the gene duplications is between 1% and 5% in Caucasians with values up to 7%–10% in Spanish and Southern Italian populations [10]. The incidence of this gene duplication can reach 10% and even up to 50% in some Arab, Eastern African, and Pacific populations. A comprehensive worldwide study provided CYP2D6 UM frequencies of 1%–5% in Caucasians, 40% in northern Africa, and >20% in Oceania [18].

Over the past two decades, a limited number of pharmacogenetic studies have evaluated the prevalence of these three CYP polymorphisms in Italian populations. The first study on the CYP2D6-related oxidation polymorphism in an Italian population was performed by Spina et al. [19]. In this study, the frequency of the CYP2D6 phenotypes was investigated in 246 healthy volunteers from a Southern Italian population using dextromethorphan as a metabolic marker. The urinary metabolic ratios of dextromethorphan (parent drug) to dextrorphan (the oxidized metabolite of dextromethorphan) ranged from ≤0.001 to 6.6. By applying a metabolic ratio of 0.30 as cut-off to differentiate between PMs and EMs, 11 (4.5%) subjects could be classified as PMs, while the remaining 235 were classified as EMs. The frequency of the CYP2D6 PM phenotype in this population was within the range described for other Caucasian ethnic groups [16].

After a preliminary study describing the distribution of variant *CYP2C9* alleles in small samples of an Italian (n=157 subjects) and an Ethiopian (n=150) population [20], Scordo et al. [21] investigated the genotype profile of a sample of the Italian population in order to compare the *CYP2C9*, *CYP2C19*, and *CYP2D6* allele frequencies among Italians with previous findings in other Caucasian populations. Frequencies for the major *CYP2C9*, *CYP2C19*, and *CYP2D6* mutated alleles and genotypes were investigated in 360 unrelated healthy Italian volunteers (210 males and 150 females, aged 19–52 years). Most subjects were students or employees at the Medical School of the University of Messina, Sicily, Italy. The *CYP2C9*, *CYP2C19*, and *CYP2D6* allele as well as genotype frequencies in the Italian population are summarized in Tables 2 and 3, respectively. Concerning *CYP2C9* allelic variants, the frequency of *CYP2C9∗2* found in this study (0.125) was very similar to values reported in studies of different Caucasian populations [7]. In contrast, the *CYP2C9∗2* allele frequency was nil in various Oriental populations and very low in African Americans [7]. The frequency of *CYP2C9∗3* in this group of Italian volunteers (0.097) was very similar to the frequencies found in other Caucasian populations, and higher than those among African Americans and Orientals [7]. As shown in Table 3, 23 subjects (6.4%) carried two detrimental *CYP2C9* alleles, while 114 (31.7%) carried one detrimental allele. The frequency of the allelic variant *CYP2C19∗2* observed in this population (0.111) was consistent with findings in other Caucasian populations [7]. Six individuals (1.7%) were homozygous for *CYP2C19∗2*, and could therefore be classified as PM,

Table 2 Allele frequencies of *CYP2C9*, *CYP2C19*, and *CYP2D6* in 360 healthy Italian volunteers [21] and in other populations [7].

Gene	Allelic variant	Allele frequency in an Italian population (95% confidence intervals)	Allele frequencies in other populations
CYP2C9	*CYP2C9∗2*	0.125 (0.101–0.149)	0–0.02 (African American, African, Asian, Pacific)
	CYP2C9∗3	0.097 (0.075–0.119)	0.10–0.17 (Caucasian)
			0.065 (Hispanic)
			0–0.01 (African American, African)
			0.02–0.06 (Asian)
			0.06 (Caucasian)
CYP2C19	*CYP2C19∗2*	0.111 (0.088–0.134)	0.10–0.17 (African American, African)
	CYP2C19∗3	0	0.22–0.32 (Asian, Pacific)
			0.06–0.15 (Caucasian)
			0.15 (Hispanic)
			0–0.01 (African, Caucasian, Hispanic)
			0.03–0.07 (Asian, Pacific)
CYP2D6	*CYP2D6∗3*	0.007 (0.001–0.013)	Approx. 0.01 (all ethnicities)
	CYP2D6∗4	0.153 (0.127–0.179)	0.1–0.10 (African American, African, Asian, Hispanic)
	CYP2D6∗5	0.034 (0.021–0.047)	0.15–0.25 (Caucasian)
	CYP2D6∗6	0.014 (0.005–0.023)	0.03–0.06 (All ethnicities)
	CYP2D6∗2x2	0.042 (0.027–0.047)	Approx. 0.01 (All ethnicities)
			Up to 0.30 (African, Arab)
			0.01–0.09 (Caucasian)

Authenticated | espina@unime.it author's copy
Download Date | 1/10/15 12:07 PM

Table 3 Genotype frequencies of *CYP2C9*, *CYP2C19*, and *CYP2D6* in 360 healthy Italian volunteers [21].

Gene	Genotype	No. of subjects	Frequency
CYP2C9	*CYP2C9∗1/∗1*	223	61.9
	CYP2C9∗1/∗2	62	17.2
	CYP2C9∗1/∗3	52	14.5
	CYP2C9∗2/∗2	10	2.8
	CYP2C9∗2/∗3	8	2.2
	CYP2C9∗3/∗3	5	1.4
CYP2C19	*CYP2C19∗1/∗1*	286	79.4
	CYP2C19∗1/∗2	68	18.9
	CYP2C19∗2/∗2	6	1.7
CYP2D6	*CYP2D6∗1/∗1*	192	53.3
	CYP2D6∗1/∗3 (∗4, ∗5, ∗6)	126	35.0
	CYP2D6∗3/∗4, ∗4/∗4, ∗4/∗5	12	3.4
	CYP2D6∗1/∗2×2	30	8.3

while 68 subjects (18.9%) were carrying one mutated allele (*CYP2C19∗1/∗2*). On the other hand, the variant *CYP2C19∗3* was not detected among the Italian volunteers. This further illustrates the ethnic difference between Caucasian and Oriental populations, by confirming the Asian specificity of this allelic variant, the frequency of which is very low, or totally absent, in different Caucasian populations [7]. The frequency of the most common *CYP2D6* mutated alleles was similar to that described in other Caucasian populations [7]. As reported in Table 3, 12 subjects carried two *CYP2D6* detrimental alleles (*CYP2D6∗3/∗4*, n=1; *CYP2D6∗4/∗4*, n=2; *CYP2D6∗4/∗5*, n=9), and could thus be classified as PM with respect to CYP2D6. Among the rest, 126 subjects carried one detrimental allele (*CYP2D6∗1/∗3*, n=4; *CYP2D6∗1/∗4*, n=96; *CYP2D6∗1/∗5*, n=16; *CYP2D6∗1/∗6*, n=10), while in the remaining 222 volunteers, no defect alleles were identified. Of the 360 volunteers, 30 (8.3%) were found to carry a duplicated functional *CYP2D6* gene and were thus classified as UM. The results indicate that the prevalence of this allelic variant in the Southern Italian (Sicilian) population is similar to that in a Spanish population [22, 23]. This is consistent with the more frequent distribution of *CYP2D6* gene duplication in the Mediterranean area compared to Northern Europe and may be indicative of a north-south gradient of the frequency within Europe. The highest incidence of gene duplication has been found in Ethiopia in Northern Africa at 29% [24].

The preliminary findings of a study we have conducted in 150 Italian patients in therapy with warfarin to evaluate the impact of several genetic factors on the therapeutic outcome showed a 17% frequency of the *CYP2C19∗17* allele (*data not published*), which is in line with the frequencies seen in other Caucasian populations.

A recent study investigated the frequency of well-known polymorphic variants of some CYPs, namely CYP2C9, CYP2C19, and CYP2D6, in different environmental sensitivity-related illnesses including multiple chemical sensitivity, chronic fatigue syndrome, and fibromyalgia [25]. Allele and genotype distribution for different *CYP2C9*, *CYP2C19*, and *CYP2D6* variants in 113 healthy controls did not differ substantially from previously reported frequencies in the Italian general population [21].

Other CYP polymorphisms: CYP1A1, CYP2E1, and CYP3A4/3A5

Other polymorphisms in the CYP family studied in Italian populations include those related to the *CYP1A1*, *CYP2E1*, and *CYP3A4/3A5* genes.

CYP1A1 is an aryl hydrocarbon hydroxylase which catalyzes the first step in the metabolism of polycyclic aromatic hydrocarbons such as those found in cigarette smoke, transforming them into active carcinogens. The non-synonymous m2 variant (*CYP1A1∗2C*, 2454A>G, Ile-462Val) of the *CYP1A1* gene has been associated with an increased enzymatic activity [26, 27] and with an increased risk of bronchial, laryngeal, and oral cavity tumors among smokers [26]. This variant has a frequency of about 10% in the Caucasian population, but it is more common among Asian and Spanish populations [7].

CYP2E1 is a key enzyme in the metabolic activation of a variety of toxicants including nitrosamines, benzene, vinyl chloride, and halogenated solvents such as trichloroethylene [28]. This enzyme is also believed to participate in the oxidation of other compounds, such as ethanol, to produce reactive free radicals that may initiate lipid peroxidation and also contribute to carcinogenesis [28]. The variant c2 allele, recognized by *Rsa* I digestion in the 5′-flanking region of the gene, appears to be associated with decreased enzyme activity. The presence of this allele has been related to cancer susceptibility and its frequency is variable, but relatively low in different populations, being around 5% in Caucasians [28].

Bianchino et al. [29] performed a case-control study to evaluate the distribution of the major polymorphisms in the *CYP1A1* and *CYP2E1* genes in a Southern Italian population and their association with cancer susceptibility. The enrolled participants were resident of Basilicata and included 290 cancer patients (mean age of 65 years: range 34–78 years) as cases and 242 healthy individuals (mean age of 64 years: range 50–78 years) admitted to the hospital for routine blood tests and who had no personal and/or family history of cancer (controls). The *CYP1A1*

Authenticated | espina@unime.it author's copy
Download Date | 1/10/15 12:07 PM

heterozygous genotype was found in 14 (5%) of the cancer patients and in 18 (7.4%) of the controls, while the *CYP2E1* heterozygous genotype was found in 12 (4%) of the cancer patients and in 20 (8.3%) of the controls. No mutant homozygotes were identified for either of the genes in the cases or in the controls. The distribution of the studied polymorphisms did not differ from those reported for other Caucasian populations.

The human CYP3A subfamily is composed of three isoforms, 3A4, 3A5, and 3A7, with overlapping substrate specificity, encoded by genes located on chromosome 7. These enzymes play a major pharmacokinetic role, catalyzing, at least partially, the biotransformation of a large number of structurally diverse drugs, as well as endogenous compounds. CYP3A4 is the most abundant isoform in the human liver, accounting for approximately 30% of the total CYP liver content, and for the majority of CYPs in the human small bowel [9]. CYP3A4 drug-metabolizing activity has been reported to vary more than 20-fold among individuals. Three *CYP3A4* variants (*CYP3A4*1B*, *CYP3A4*3*, and *CYP3A4*4*) (Table 1) have been reported to cause a reduction in enzyme activity and to affect the *in vivo* biotransformation of some commonly used drugs including simvastatin and the immunosuppressants cyclosporine and tacrolimus [30]. On the other hand, only subjects carrying at least one *CYP3A5*1* allele (about 10% of the population) express the CYP3A5 enzyme. The absence of CYP3A5 among a majority of Caucasians is largely dependent on the *CYP3A5*3* allele [31]. *CYP3A5*6* and *CYP3A5*2* are more rare variants coding for a non-functional protein [31] (Table 1).

Magliulo et al. [32] have evaluated the distribution of *CYP3A43A/5* polymorphisms in an Italian population, and their impact on Alzheimer (AD) treatment with donepezil, in 54 AD patients and 285 Italian volunteers. Three AD patients (5.6%) and 24 (8.4%) volunteers carried one detrimental *CYP3A4* allele [AD patients: *CYP3A4*1/*1B* (n=2) and *CYP3A4*1/*3* (n=1); volunteers: *CYP3A4*1/*1B* (n=20) and *CYP3A4*1/*3* (n=4)]. Twelve AD patients (22.2%) were heterozygous for *CYP3A5*1*, while all the others (77.8%) were homozygous for the *CYP3A5*3* allele. Among the volunteers, 246 (86.3%) were homozygous for *CYP3A5*3* and 4 (1.4%) carried the *CYP3A5*3/*6* genotype. Five volunteers (1.7%) were homozygous and 30 (10.5%) were heterozygous for *CYP3A5*1*. No AD patient or volunteer carried *CYP3A4*4* or *CYP3A5*2* alleles. The frequencies of the allelic variants of CYP3A4 and CYP3A5 did not differ between patients and volunteers and were similar to those reported in other Caucasian populations [32].

Flavin-containing monooxygenases (EC 1.14.13.8)

Flavin-containing monooxygenases (FMOs) are a family of microsomal flavin adenine dinucleotide (FAD-), nicotinamide adenine dinucleotide phosphate (NADPH-), or O_2-dependent flavoprotein enzymes that catalyze the oxygenation of several nucleophilic heteroatom-containing chemicals, such as those having nitrogen, sulfur, phosphorous, and selenium as their site of oxygenation [33, 34]. FMO3 is the predominant form of the FMO family expressed in the adult human liver involved in the metabolism of xenobiotics. Substrates for FMO3 include dietary-derived tyramine, trimethylamine, and nicotine and some therapeutic agents such as the H_2-receptor antagonists cimetidine and ranitidine, the non-steroidal anti-inflammatory agents benzydamine and sulindac, the antiestrogen tamoxifen, and the antipsychotics clozapine and olanzapine [33]. E158K, V257M, and E308G are common *FMO3* polymorphisms with marked variability in frequency between major ethnicities, whereas D132H and L360P have only been identified in African Americans [35].

Mao et al. [36] investigated the frequency of these *FMO3* polymorphisms in 2152 healthy volunteers from 13 defined groups representing Caucasians, Japanese, and Africans. The Italian population consisted of 279 subjects. Significant subpopulation differences in allele frequencies were found for E158K, V257M, and E308G in Caucasians and regional differences for D132H among Africans. No carriers of *P360* were identified. Concerning the three most widespread polymorphisms, the *K158* variant was the commonest variant allele in all populations. Among Caucasians, this allele was more frequent in Swedes (44.3%) than in either Italian (33.5%) or Turkish groups (35.8%), while among Africans its frequency ranged from 33.3% to 52.0%. The Japanese had a lower frequency (22.7%) as compared to Caucasians and Africans. The frequency of the *G308* allele also varied between different populations. The frequency among Japanese (21.9%) was similar to that in Swedes (22.4%), but it was lower in both the Italian (10.8%) and Turkish population (6.0%), whilst it ranged from 0% to 1.6% in the various African groups. The frequency of the M257 variant was below 8% in Caucasians (6.3% in the Italian population) and Africans, but higher (14.5) in the Japanese group.

A recent study by D'Angelo et al. [37] investigated the distribution of the three common *FMO3* polymorphisms in an Italian population of 528 healthy subjects: 290 from Sicily and 238 from Sardinia. Variant *158K* showed the same frequency of 30% in both Sicilian and Sardinian populations, while variant *308G* showed a slightly higher frequency in the Sardinian (20%) than in the

Authenticated | espina@unime.it author's copy
Download Date | 1/10/15 12:07 PM

Sicilian sampling (10%). By contrast, the *257M* allele was present in the Sicilian sample, with a frequency of 2%, but it was not observed in the Sardinian sample. The authors hypothesize that differences can be explained by the fact that the two islands, despite their proximity, differ considerably in their ancient history [37].

Pharmacogenetics of phase II enzymes: studies in Italian populations

Phase II enzymes are involved in chemical reactions where substrates, drugs, or intermediates undergo biotransformation to generate products that are more hydrophilic than the parent compound, increasing their propensity to be readily excreted in urine and feces. They include a large number of enzyme families such as uridine diphosphate glucoronosyltransferases (UGTs), *N*-acetayltransferases (NATs), thiopurine S-methyltranferase (TPMT), catechol O-methyltransferase (COMT), sulfotransferases (SULTs), and glutathione S-transferases (GSTs) [38–40]. These enzymes also have an important role in preventing the formation of reactive intermediates and toxic metabolites generated by phase I enzymes for products such as paracetamol, polycyclic aromatic hydrocarbons, and aromatic amines [41]. The UGTs, NATs, SULTs, and GSTs conjugate these reactive species and, by rendering them more hydrophilic, minimize their toxicity and enhance their elimination. For this reason many studies have associated the presence of SNPs or allelic variants of phase II genes with the incidence of different diseases [42, 43].

Uridine diphosphate glucoronosyltransferases

The uridine diphosphate glucoronosyltransferases (UGTs) belong to a multigene family of important enzymes localized on the internal membrane of the endoplasmic reticulum and involved in the inactivation and the elimination of a wide range of nucleophilic substances, both endogenous and exogenous [44]. The UGT superfamily is classified into the UGT1, UGT2, UGT3, and UGT8 families and is further divided into subfamilies and isoforms, based on similarities between their amino acid sequences and gene organization. To date, 117 mammalian genes have been identified and some of these as *UGT1A1*, *1A3*, *1A4*, *1A6*, *1A7*, *1A8*, *1A10*, *UGT2B4*, *2B7*, *2B15*, and *2B17* with important implications in drug response.

UGT1A1 is active in the conjugation of numerous anticancer drugs including irinotecan, etoposide, and epirubicin [44]. Irinotecan undergoes bioactivation by tissue carboxylesterases to produce the active cytotoxic metabolite SN-38 which is in turn glucuronidated by the polymorphic UGT1A1. Patient response to therapy is highly variable in part because defective alleles of UGT1A1 impair glucuronidation of SN-38 leading to its accumulation and resultant toxicity, including diarrhea and leucopenia [45, 46]. Individuals who carry the variant *UGT1A1∗28* allele, which has a (TA) insertion in the *UGT1A1* promoter region, TA_7 repeats instead of more common TA_6 repeats, have a decreased capacity to glucuronidate SN-38 [47]. Up to 33% of Caucasians carry the *UGT1A1∗28* variant [48], and the US Food and Drug Administration has recommended a dose reduction for irinotecan in individuals who are homozygous for the *UGT1A1∗28* allele. Apart from irinotecan/SN-38, *UGT1A1∗28* is also associated with decreased paracetamol, lamotrigine, lorazepam, and josamycin metabolism, but the pharmacokinetic and pharmacodynamic consequences of variant alleles have not been evaluated to date [47].

The only study which investigated the frequency of *UGT1A1∗28* in an Italian population was carried out in 83 healthy Italian pediatric subjects enrolled in a case-control study aimed at investigating the genetic susceptibility to Gilbert's syndrome [49]. Among 83 unaffected controls, 9 (10.8%) were homozygous for the *UGT1A1∗28* allele and 37 (44.6%) were heterozygous (Table 4). Interestingly, the authors have also reported a rare UGT1A1 genotype (TA_7/TA_8 heterozygosity), confirming the low penetration of the TA_8 allele in Caucasian populations.

N-Acetyltransferases

N-Acetyltransferases (NATs) are cytosolic enzymes found in many tissues of various species. In humans, two forms are known, NAT1 and NAT2. Two functional human gene loci, *NAT1* and *NAT2*, were identified, described, and mapped to the short arm of human chromosome 8. The nucleotide sequences of these two genes show 85% homology and code two enzymes of different substrate specificity [61]. Typical specific substrates for human NAT1 are *p*-aminobenzoic acid, *p*-aminosalicylic acid, and *p*-aminobenzylglutamate [62]. Human NAT2 provides a major route for detoxification of drugs such as isoniazid (antituberculotic drug), hydralazine (antihypertensive drug), procainamide (antiarrhythmic drug), and sulfonamides (antibacterial drugs) [62].

Authenticated | espina@unime.it author's copy
Download Date | 1/10/15 12:07 PM

Table 4 Distribution of genes encoding phase II drug-metabolizing enzymes allele frequencies in Italian populations.

Gene	No. of subjects	Genetic polymorphisms	Allele frequencies	Genotype/ phenotype	Genotype frequencies, %	Ref.
UGT1A1	83	**28*	0.330	**1/*1*	44.6	[49]
				**1/*28*	44.6	
				**28/*28*	10.8	
NAT2	262	**5, *6, *7*	n.d.	Slow acetylators	58.9	[50]
		**4, *12, *13*		Fast acetylators	41.1	
NAT2	107	**5A, *6A, *7A*	n.d.	Slow acetylators	51.8	[51]
TPMT	103	**3A*	0.019	**1/*1*	95.5	[52]
		**3C*	0.005	**1/*3A*	3.9	
		**2*	0.002	**1/*3C*	0.1	
				**1/*2*	0.5	
TPMT	943	**3A*	0.022	**1/*1*	94.6	[53]
		**3B*	0.003	**1/*3A*	4.3	
		**3C*	0.003	**1/*3B*	0.5	
				**1/*3C*	0.5	
				**3B/*3C*	0.1	
COMT	36	472G>A	0.444	Met/Met	22.3	[54]
				Met/Val	44.5	
				Val/Val	33.2	
COMT	57	472G>A	0.509	Met/Met	21.0	[55]
				Met/Val	59.7	
				Val/Val	19.3	
SULT	107	**2*	0.220	**1/*1*	61.4	[51]
				**1/*2*	33.5	
				**2/*2*	5.1	
SULT	135	**2*	0.280	**1/*1*	52.2	[56]
				**1/*2*	40.4	
				**2/*2*	7.4	
GSTM1	120	**O*	n.d.	Null	49.2	[57]
	546				50.4	[58]
GSTT1	120	**O*	n.d.	Null	28.3	[57]
	546				16.7	[58]
GSTP1	250	**A*	0.710	**A/*A*	51.2	[59]
		**B*	0.240	**A/*B*	31.6	
		**C*	0.050	**A/*C*	8.0	
				**B/*B*	6.4	
				**B/*C*	2.8	
GSTP1	544	313A>G	0.550	Ile/Ile	23.5	[60]
			0.450	Ile/Val	62.5	
				Val/Val	14.0	

n.d., not determined; Ref., references.

Both *NAT* genes exhibit more than 26 different alleles, and based on a *NAT* genotype, individual phenotype (acetylation activity) can be divided into slow, intermediate, and rapid acetylator [38]. Acetylator status contributes to inter-individual variation in drug response with the slow acetylators more prone to dose-dependent toxicity [39]. There is a general agreement that *NAT1*4* should be considered the wild type (frequency in Caucasian approximately 75%) [38]. The most frequent variant is *NAT1*10* (allele frequency in Caucasian approximately 20%) [63]. It consists of a 1088T>A and a 1095C>A exchange in the 3′-untranslated region. Other variants are *NAT1*15* (559C>T) with no enzyme activity, due to a premature stop in codon 187, and *NAT1*14* (560G>A) with a low enzyme activity [64].

The common defective alleles of *NAT2* are 191G>A (Arg64Gln), 282C>T (Tyr94Tyr), 481C>T (Leu161Leu), 341T>C (Ile114Thr), 590G>A (Arg197Gln), 803A>G (Arg268Lys), and 857G>A (Gly286Glu). These mutations form haplotypes, the most important of which are *NAT2*5B*, *NAT2*6A*, *NAT2*7B*, *NAT2*12A*, and *NAT2*13*. These haplotypes have provided the basis for a large

Authenticated | espina@unime.it author's copy
Download Date | 1/10/15 12:07 PM

number of genotyping studies on the ethnic distribution of *NAT2* polymorphisms [65, 66]. Moreover, most *NAT2* variants are in linkage disequilibrium; therefore, in order to predict the *NAT2* phenotype from the genotype, it is sufficient to genotype only three SNPs, i.e., SNPs 282C>T, 341T>C, and 857G>A, for studies in a Caucasian or Oriental population, whereas in Africans, 191G>A should also be considered [38]. *NAT2* genotyping studies have revealed that the allelic pattern differs significantly between Caucasians, Asians, Aborigines, and Northern African populations [38]. In Caucasians, the predominant alleles code for the slow *NAT2∗5B* (40.9%) and *NAT2∗6A* (28.4%) and the rapid *NAT2∗4* (23.4%) metabolizing enzymes [38].

To the best of our knowledge, genetic variations of *NAT1* were not described in an Italian population, whereas some common allelic mutations of *NAT2* including *NAT2∗5*, *NAT2∗6*, and *NAT2∗7* were studied by Boccia et al. [51] and Betti et al. [50], who enrolled 254 and 262 healthy volunteers, respectively. In these studies, *NAT2* genotypes were investigated to find a potential association between *NAT2* haplotypes, tobacco smoke, alcohol consumption, and risk of gastric cancer [51] and between *NAT2* polymorphisms and malignant mesothelioma susceptibility [50]. In the study by Boccia et al. [51], slow acetylators, carrying *NAT2∗5A*, *NAT2∗6A*, and *NAT2∗7A* haplotypes, were present at a frequency of 51.8%, whereas in the study by Betti et al. [50], slow acetylators, carrying *NAT2∗5*, *NAT2∗6*, and *NAT2∗7* polymorphisms, had a frequency of 58.9% (Table 4). Interestingly, both studies suggested a possible correlation between *NAT2* polymorphisms and susceptibility to malignant mesothelioma and gastric cancer even though larger prospective investigations are necessary to make this correlation stronger.

Thiopurine S-methyltranferase

One of the first examples of pharmacogenetic studies applied to drug therapy is the polymorphism of the gene thiopurine S-methyltransferase (TPMT) where more than 20 TPMT SNPs have been identified and associated with a decreased level of TPMT enzyme activity inducing thiopurine drug (6-mercaptopurine, azathioprine, 6-thioguanine) toxicity [67, 68]. Therefore, some authors have suggested that patients with low TPMT activity must be treated with thiopurine drugs at a 1/10 to a 1/15 of standard dose [69].

Several population studies have identified the predominant variant alleles. *TPMT∗3A* (460 G>A and 719 A>G) and *∗3C* (719 A>G) were identified as the predominant variant alleles, with *TPMT∗2* (238 G>A) contributing to a lesser extent. These three alleles account for over 95% of cases of inherited TPMT deficiency in Caucasian subjects [70].

Rossi et al. [52] investigated the genotype-phenotype correlation for TPMT in 103 unrelated healthy Italian subjects. They found that besides the wild-type allele *TPMT∗1* (94.6%), *TPMT∗3A* was the most frequent allele (3.9%) and accounted for 72% of the mutant alleles detected in the Italian sample. *TPMT∗3C* was the second most recurrent and its frequency approached at most 1%. *TPMT∗2* occurred at a lower frequency (0.49%), whereas *TPMT∗3B* was not detected at all (Table 4) [52].

Serpe et al. [53] evaluated the impact of genotype, age, and gender on the TPMT phenotype in a large-scale healthy Italian population. In 943 unrelated healthy Italian subjects aged 0.08–68 years (623 males and 320 females), the genotype frequencies were *TPMT∗1* (94.5%), *∗3A* (4.3%), *∗3B* (0.5%), and *∗3C* (0.5%), whilst *∗2* was not detected at all (Table 4) [53]. These findings are in agreement with previous reports in Caucasian populations, confirming that the *∗3A* allele is the most common variant and also that *∗3B* allele is also present at a rather high frequency. The authors of the study [53] explained this finding by the fact that they enrolled individuals from different areas of Italy as well as Sardinia, where the frequency for *TPMT∗3B* is relatively high [71]. In conclusion, this study shows that genetic factors seem to be the major aspect in TPMT phenotype variability in adults, whereas in children other physiological factors should be taken into account when assessing the TPMT phenotype, such as age and gender.

Catechol O-methyltransferase

Catechol O-methyltransferase (COMT) is responsible for transfer of a methyl group from S-adenosylmethionine to catecholamines. COMT is presented in mammalian cells in two forms: in a cytoplasmic soluble form (S-COMT) and a membrane-bound form (MB-COMT) located in the cytosolic side of the rough endoplasmic reticulum [72]. COMT substrates include not only neurotransmitters such as norepinephrine, epinephrine, and dopamine but also drugs having a catechol structure such as levodopa and methyldopa.

COMT inhibitors are drugs commonly used in the management of patients with Parkinson's disease complicated by motor fluctuations. Among them, entacapone is the most commonly used. Both substrates and inhibitors might be affected by *COMT* gene variations, and the most well-studied polymorphism in *COMT* has been linked to

Authenticated | espina@unime.it author's copy
Download Date | 1/10/15 12:07 PM

the low enzyme activity of COMT. This polymorphism is due to a G-to-A transition at codon 158 (for MB-COMT) or codon 108 (for S-COMT) of the *COMT* gene and results in the substitution of the amino acid valine (val) for methionine (met) causing a 3- to 4-fold decrease in the activity level of the COMT enzyme [73].

The frequency of the low activity (Met158) *COMT* allele was found to be 54% in Caucasians, 49% in Southwest Asians, and 32% in Kenyans [74]. In a study aimed to evaluate the possible association between low enzyme activity *COMT* alleles and borderline personality disorder, a high frequency (44.4%) of the low-activity allele was observed in a small sample of an Italian population consisting of 36 healthy volunteers (Table 4) [54]. Another study aimed to evaluate the relationship between Met158, the closely located rs4818 SNP, and hypnotizability confirmed a high frequency (50.9%) of the low enzyme activity *COMT* allele in an Italian population (Table 4) [55]. Although larger scale studies are lacking, the observed frequency of codon 108/158 allele is consistent with findings in other Caucasian populations [74].

Sulfotransferases

Sulfotransferases (SULTs) are a gene family of enzymes that catalyze the conjugation of 3′-phosphoadenosine 5′-phosphosulfate with an O-, N-, or S-acceptor group of an appropriate molecule. Thus, they conjugate sulfate to many hormones, neurotransmitters, drugs, and xenobiotic compounds [75, 76]. Within the SULT superfamily, 10 distinct human SULT forms are known so far, differing in their tissue distribution and substrate specificities. The best described is the SULT1A family, consisting of SULT1A1, 1A2, and 1A3 in human tissue. Common functional polymorphisms of the transcribed region are known for many of them. In this respect, the most important enzyme appears to be SULT1A1 which catalyzes the sulfonylation of 4-hydroxytamoxifen and endoxifen both active metabolites of tamoxifen [77]. The gene encoding SULT1A1 is mapped to the short arm of the chromosome 16p12.1-p11.2. The *SULT1A1∗2* variant was the most common of the two common non-synonymous SNPs observed, namely *SULT1A1∗2* (638G>A, Arg213His) and *SULT1A1∗3* (667A>G, Met223Val). Individuals carrying the variant allele *SULT1A1∗2* have a diminished capacity to sulfate the substrates of SULT1A1 due to shorter protein life. Lower enzyme activity for SULT1A1 induced by *SULT1A1∗2* may theoretically lead to longer exposure of tamoxifen's active metabolites and thereby a favorable clinical outcome [78].

The frequency of *SULT1A1∗2* in Italian populations was established in two studies aimed to investigate the correlation between polymorphisms in metabolic genes such as *SULT1A1* with the risk of gastric cancer [51] or the efficacy of tamoxifen to prevent the breast cancer [56]. In the study by Boccia et al. [51], among 254 control subjects, 61.4% showed a homozygous *SULT1A1∗1/∗1* genotype, 33.5% a heterozygous *SULT1A1∗1/∗2* genotype, and 5.1% a homozygous *SULT1A1∗2/∗2* genotype with a *SULT1A1 ∗2* allele frequency of 0.22. In the study by Serrano et al. [56], among 136 subjects, 52.2% showed a homozygous *SULT1A1∗1/∗1* genotype, 40.4% a heterozygous *SULT1A1∗1/∗2* genotype, and 7.4% a homozygous *SULT1A1∗2/∗2* genotype with a *SULT1A1 ∗2* allele frequency of 0.28 (Table 4). The *SULT1A1∗2* allele frequency observed in Italian subjects was quite similar to that reported in Caucasian populations ranging from 0.25 to 0.36 [79].

Finally, gene duplication and deletion was also more common in the *SULT1A1* gene, and a correlation between the enzymatic activity and *SULT1A1* gene copy numbers has been observed [80]. In the same study, the authors reported that 5% of Caucasian subjects contained a single copy of the gene and 26% had three or more copies, while 63% of African American subjects had three or more copies [80]. To date, no data about gene duplication and deletion have been reported in Italian populations.

Glutathione S-transferases

Currently at least seven cytosolic glutathione S-transferase (GST) families have been identified in human being – α(A), μ (M), θ(T), π (P), σ (S), ω (O), and ζ (Z) – based on sequence and immunochemical relatedness and substrate specificity [81, 82]. They play a significant role in the detoxification of reactive and toxic electrophiles such as reactive oxygen species (superoxide radical and hydrogen peroxide) that arise through normal metabolic processes.

All the human GSTs are highly polymorphic, but the impact of GSTs and their variants on drug therapy has not been studied in detail. However, it is emerging that GSTs may contribute to anticancer drug resistance as they facilitate the removal of drugs from cells [83]. In fact, several anticancer drugs including etoposide, doxorubicin, cisplatin, oxaliplatin, and carboplatin are GST substrates. Several types of allelic variants have been identified in the *GST* gene families, and the most investigated are *GSTM1* and *GSTT1* deletions, which result in the null activity of their respective enzymes, and the *GSTP1* SNPs, leading to alterations in the substrate binding site and enzyme activity. Two sites in the DNA sequence are characterized

Authenticated | espina@unime.it author's copy
Download Date | 1/10/15 12:07 PM

by an A>G transition at nucleotide 313 (point mutation in exon 5) and a C>T transition at nucleotide 341 (point mutation in exon 6). The resulting codon variants encode for the amino acids Ile105 or Val105 and Ala114 or Val114 at the electrophilic "H"-site level. Four alleles have been described at the GSTP1 locus located on chromosome 11q13: *GSTP1∗A* (wild-type Ile105→Ala114), *GSTP1∗B* (Val105→Ala114), *GSTP1∗C* (Val105→Val114), and *GSTP1∗D* (Ile105→Val114) [84, 85]. Individuals carrying at least one copy of the variant *GSTP1∗B* show a greater risk of disease from cytotoxic drugs such as alkylating agents, topoisomerase inhibitors, antimetabolites, and tubulin inhibitors [86]. This finding suggests that *GSTP1∗A* is cytoprotective against the toxic effects of chemotherapy and that toxicity is greater in carriers of variant alleles that are functionally less competent. However, there have also been reports that GSTP1 variants may be associated with more favorable outcomes with certain chemotherapeutic agents, such as cyclophosphamide, adriamycin, and oxaliplatin [87], and there was a decreased risk of developing the cumulative neuropathy that is a major dose-limiting toxicity of treatment with oxaliplatin [88]. Furthermore, the conversion of azathioprine from its pro-drug form to the active metabolite mercaptopurine, an immunosuppressive compound, may be impaired in carriers of null alleles of GSTM1 reducing the production of azathioprine active metabolites [89]. Therefore, the pharmacogenetics of GSTP1 and GSTM1 may be useful in the prediction of response to such drugs, even though these findings should be confirmed by a larger number of patients in ongoing clinical studies.

In a study from a large sample of an Italian population, the frequencies of *GSTM1* and *GSTT1* null genotype were found to be 49.2% and 28.3%, respectively [57]. In another study involving a population from central Italy, the frequencies were found to be 50.4% and 16.7%, respectively (Table 3) [58]. The frequencies of the GSTM1 and GSTT1 null phenotypes in Italian population [57, 58] are in line with those observed in Caucasian populations, 40%–65% and 10%–40%, respectively [57, 90].

The *GSTP1∗A*, *GSTP1∗B*, *GSTP1∗C*, and *GSTP1∗D* allelic frequencies observed in an Italian population, mainly from the region Lazio, were the following: *f*(A)=0.71, *f*(B)=0.24, *f*(C)=0.05 and *f*(D)=0 with a genotype frequency of *GSTP1 ∗A/∗A*, *GSTP1 ∗A/∗B*, *GSTP1 ∗A/∗C*, *GSTP1 ∗B/∗B*, and *GSTP1 ∗B/∗C* genotype of 51.2%, 31.6%, 8.0%, 6.4%, and 2.8%, respectively (Table 3) [59]. Moreover, in a study aimed to investigate the possible association between GSTP1 polymorphisms with the risk of breast cancer, the authors only investigated the 313A>G non-synonymous polymorphism in exon 5 (Ile105Val) in 544 Italian women healthy volunteers. In this study, the GSTP1 frequency of genotypes Ile/Ile, Ile/Val, and Val/Val was found to be 23.5%, 62.5% and 14.0%, respectively (Table 4) [60]. To the best of our knowledge, the frequency of the *GSTP1∗A*, *GSTP1∗B*, *GSTP1∗C*, and *GSTP1∗D* genotypes has not been investigated in other Caucasian populations.

Conclusions

Marked differences have been shown in the distribution of allelic variants affecting the activity of drug-metabolizing enzymes in different populations. It is therefore of great clinical significance to define the genetic profile of different populations between and within ethnic groups. In order to characterize the Italian population, available data from different studies have been collected. In general, the frequency of polymorphisms in the *CYP* genes as well as in the investigated phase II enzymes in the Italian population was similar to values reported for other Caucasian populations. However, the prevalence of *CYP2D6* gene duplication among Italians was found to be very high, confirming the higher frequency of *CYP2D6* UMs in the Mediterranean area compared to Northern Europe. It is worth noting that a geographic gradient in the FMO3 polymorphism distribution was also seen, the Italian population showing higher similarity to other Mediterranean populations than to North Europeans.

Conflict of interest statement

Authors' conflict of interest disclosure: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission. The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.
Research funding: None declared.
Employment or leadership: None declared.
Honorarium: None declared.

References

1. Meyer UA. Pharmacogenetics – five decades of therapeutic lessons from genetic diversity. Nat Rev 2004;5:869–76.
2. Ingelman-Sundberg M, Sim SC, Gomez A, Rodriguez-Antona C. Influence of cytochrome P450 polymorphism on drug therapies: pharmacogenetic, pharmacoepigenetic and clinical aspects. Pharmacol Ther 2007;116:496–526.

Authenticated | espina@unime.it author's copy
Download Date | 1/10/15 12:07 PM

3. Cozza KL, Armstrong SC, Oesterheld JR. Concise guide to drug interaction principles for medical practice: cytochrome P450s, UGTs, P-glycoproteins, 2nd ed. Washington, DC: American Psychiatric Association, 2003.
4. Sheffield LJ, Phillimore HE. Clinical use of pharmacogenomic tests in 2009. Clin Biochem Rev 2009;30:55–65.
5. Zhou HH, Liu ZQ. Ethnic differences in drug metabolism. Clin Chem Lab Med 2000;38:899–903.
6. Yasuda SU, Zhang L, Huang SM. The role of ethnicity in variability in response to drugs: focus on clinical pharmacology studies. Clin Pharmacol Ther 2008;84:417–23.
7. Zanger UM, Schwab M. Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. Pharmacol Ther 2013;138: 103–41.
8. Nelson DR. The cytochrome p450 homepage. Hum Genomics 2009;4:59–65.
9. Shimada, T, Yamazaki H, Mimura M, Inui Y, Guengerich FP. Interindividual variations in human liver cytochrome P450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. J Pharmacol Exp Ther 1994;270:414–23.
10. Zhou SF, Liu JP, Chowbay B. Polymorphism of human cytochrome P450 enzymes and its clinical impact. Drug Metab Rev 2009;41:89–295.
11. Zhou SF, Zhou ZW, Huang M. Polymorphisms of human cytochrome P450 2C9 and the functional relevance. Toxicology 2010;278:165–88.
12. Spina E, de Leon J. CYP applications of CYP genotyping in psychiatry. J Neural Transm 2014 Sep 9. [Epub ahead of print].
13. Scott SA, Sangkuhl K, Gardner EE, Stein CM, Hulot JS, Johnson JA, et al. Clinical Pharmacogenetics Implementation Consortium guidelines for cytochrome P450-2C19 (CYP2C19) genotype and clopidogrel therapy. Clin Pharmacol Ther 2011;90:328–32.
14. Martis S. Mei H, Vijzelar R, Edelmann L, Desnick RJ, Scott SA. Multi-ethnic distribution of clinically relevant CYP2C genotypes and haplotypes. Pharmacogenomics J 2013;13: 558–66.
15. Sim SC, Risinger C, Dahl ML, Aklillu E, Christensen M, Bertilsson L, et al. A common novel CYP2C19 gene variant causes ultrarapid drug metabolism relevant for the drug response to proton pump inhibitors and antidepressants. Clin Pharmacol Ther 2006;79:103–13.
16. Zhou SF. Polymorphism of human cytochrome P450 2D6 and its clinical significance: Part I. Clin Pharmacokinet 2009;48: 689–723.
17. Zhou SF. Polymorphism of human cytochrome P450 2D6 and its clinical significance: Part II. Clin Pharmacokinet 2009;48: 761–804.
18. Sistonen J, Sajantila A, Lao O, Corander J, Barbujani G, Fuselli S. CYP2D6 worldwide genetic variation shows high frequency of altered activity variants and no continental structure. Pharmacogenet Genomics 2007;17:93–101.
19. Spina E, Campo GM, Avenoso A, Caputi AP, Zuccaro GM, Gatti G, et al. CYP2D6-related oxidation polymorphism in Italy. Pharmacol Res 1994;29:281–9.
20. Scordo MG, Aklillu E, Yasar U, Dahl ML, Spina E, Ingelman-Sundberg M. Genetic polymorphism of cytochrome P450 2C9 in a Caucasian and Black-African population. Br J Clin Pharmacol 2001;52:447–50.
21. Scordo MG, Caputi AP, D'Arrigo C, Fava G, Spina E. Allele and genotype frequencies of CYP2C9, CYP2C19 and CYP2D6 in an Italian population. Pharmacol Res 2004;50:195–200.
22. Agundez JA, Ledesma MC, Ladero JM, Benitez J. Prevalence of CYP2D6 gene duplication and its repercussion on the oxidative phenotype in a white population. Clin Pharmacol Ther 1995;57:265–9.
23. Bernal ML, Sinues B, Johansson I, McLellan RA, Wennerholm A, Dahl ML, et al. Ten percent of North Spanish individuals carry duplicated or triplicated CYP2D6 genes associated with ultrarapid metabolism of debrisoquine. Pharmacogenetics 1999;9:657–60.
24. Aklillu E, Persson I, Bertilsson L, Johansson I, Rodrigues F, Ingelman-Sundberg M. Frequent distribution of ultrarapid metabolizers of debrisoquine in an Ethiopian population carrying duplicated and multiduplicated functional CYP2D6 alleles. J Pharmacol Exp Ther 1996;278:441–6.
25. Caccamo D, Cesareo E, Mariani S, Raskovic D, Ientile R, Currò M, et al. Xenobiotic sensor- and metabolism-related gene variants in environmental sensitivity-related illnesses: a survey on the Italian population. Oxid Med Cell Longev 2013;2013:831969.
26. Landi MT, Bertazzi PA, Shields PG, Clark G, Lucier GW, Garte SJ, et al. Association between CYP1A1 genotype, mRNA expression and enzymatic activity in humans. Pharmacogenetics 1994;4:242–6.
27. Kisselev P, Schunck WH, Roots I, Schwarz D. Association of CYP1A1 polymorphisms with differential metabolic activation of 17beta-estradiol and estrone. Cancer Res 2005;65:2972–8.
28. Neafsey P, Ginsberg G, Hattis D, Johns DO, Guyton KZ, Sonawane B. Genetic polymorphisms in CYP2E1: population distribution of CYP2E1 activity. J Toxicol Environ Healt B Crit Rev 2009;12:362–88.
29. Bianchino G, Cittadini A, Grieco V, Traficante A, Zupa A, Improta G, et al. Polymorphisms of the CYP1A1, CYP2E1 and XRCC1 genes and cancer risk in a Southern Italian population: a case-control study. Anticancer Res 2011;31:1359–66.
30. Hesselink DA, van Gelder T, van Schaik RH, Balk AH, van der Heiden IP, van Dam T, et al. Population pharmacokinetics of cyclosporine in kidney and heart transplant recipients and the influence of ethnicity and genetic polymorphisms in the MDR-1, CYP3A4, and CYP3A5 genes. Clin Pharmacol Ther 2004;76: 545–56.
31. van Schaik RH, van der Heiden IP, van den Anker JN, Lindemans J. CYP3A5 variant allele frequencies in Dutch Caucasians. Clin Chem 2002;48:1668–71.
32. Magliulo L, Dahl ML, Lombardi G, Fallarini S, Villa LM, Biolcati A, et al. Do CYP3A and ABCB1 genotypes influence the plasma concentration and clinical outcome of donepezil treatment? Eur J Clin Pharmacol 2011;67:47–54.
33. Krueger SK, Williams DE. Mammalian flavin-containing monooxygenases: structure/function, genetic polymorphisms and role in drug metabolism. Pharmacol Ther 2005;106:357–87.
34. Cashman JR, Zhang J. Human flavin-containing monooxygenases. Annu Rev Pharmacol Toxicol 2006;46:65–100.
35. Cashman JR, Zhang J, Leushner J, Braun A. Population distribution of human flavin-containing monooxygenase form 3: gene polymorphisms. Drug Metab Dispos 2001;29:1629–37.
36. Mao M, Matinba A, Scordo MG, Gunes A, Zengil H, Yasui-Furukori N, et al. Flavin-containing monooxygenase 3

Authenticated | espina@unime.it author's copy
Download Date | 1/10/15 12:07 PM

polymorphisms in 13 ethnic populations from Europe, East Asia and sub-Saharan Africa: frequency and linkage analysis. Pharmacogenomics 2009;10:1447–55.
37. D'Angelo R, Esposito T, Calabrò M, Rinaldi C, Robledo R, Varriale B, et al. FMO3 allelic variants in Sicilian and Sardinian populations: trimethylaminuria and absence of fish-like body odor. Gene 2013;515:410–5.
38. Cascorbi I. Pharmacogenetics of phase II drug metabolizing enzymes. In: van der Zee A-H, Daly AK, editors. Pharmacogenetics and individualized therapy. New York: John Wiley & Sons, Inc., 2012:81–100.
39. Crettol S, Petrovic N, Murray M. Pharmacogenetics of phase I and phase II drug metabolism. Curr Pharm Des 2010;16:204–19.
40. Yiannakopoulou EC. Pharmacogenomics of phase II metabolizing enzymes and drug transporters: clinical implications. Pharmacogenomics J 2013;13:105–9.
41. Dekant W. The role of biotransformation and bioactivation in toxicity. EXS 2009;99:57–86.
42. Masood N, Yasmin A, Kayani MA. Genetic deletions of GSTM1 and GSTT1 in head and neck cancer: review of the literature from 2000 to 2012. Asian Pac J Cancer Prev 2013;14:3535–9.
43. Zhang M, An C, Gao Y, Leak RK, Chen J, Zhang F. Emerging roles of Nrf2 and phase II antioxidant enzymes in neuroprotection. Prog Neurobiol 2013;100:30–47.
44. Guillemette C. Pharmacogenomics of human UDP-glucuronosyltransferase enzymes. Pharmacogenomics J 2003;3:136–58.
45. Marcuello E, Altés A, Menoyo A, Del Rio E, Gómez-Pardo M, Baiget M. UGT1A1 gene variations and irinotecan treatment in patients with metastatic colorectal cancer. Br J Cancer 2004;91:678–82.
46. Innocenti F, Vokes EE, Ratain MJ. Irinogenetics: what is the right star? J Clin Oncol 2006;24:2221–4.
47. Kiang TK, Ensom MH, Chang TK. UDP-glucuronosyltransferases and clinical drug-drug interactions. Pharmacol Ther 2005;106:97–132.
48. Iyer L, Hall D, Das S, Mortell MA, Ramírez J, Kim S, et al. Phenotype-genotype correlation of in vitro SN-38 (active metabolite of irinotecan) and bilirubin glucuronidation in human liver tissue with UGT1A1 promoter polymorphism. Clin Pharmacol Ther 1999;65:576–82.
49. Ferraris A, D'Amato G, Nobili V, Torres B, Marcellini M, Dallapiccola B. Combined test for UGT1A1 -3279T→G and A(TA) nTAA polymorphisms best predicts Gilbert's syndrome in Italian pediatric patients. Genet Test 2006;10:121–5.
50. Betti M, Neri M, Ferrante D, Landi S, Biava A, Gemignani F, et al. Pooled analysis of NAT2 genotypes as risk factors for asbestos-related malignant mesothelioma. Int J Hyg Environ Health 2009;212:322–9.
51. Boccia S, Sayed-Tabatabaei FA, Persiani R, Gianfagna F, Rausei S, Arzani D, et al. Polymorphisms in metabolic genes, their combination and interaction with tobacco smoke and alcohol consumption and risk of gastric cancer: a case-control study in an Italian population. BMC Cancer 2007;7:206.
52. Rossi AM, Bianchi M, Guarnieri C, Barale R, Pacifici GM. Genotype-phenotype correlation for thiopurine S-methyltransferase in healthy Italian subjects. Eur J Clin Pharmacol 2001;57:51–4.
53. Serpe L, Calvo PL, Muntoni E, D'Antico S, Giaccone M, Avagnina A, et al. Thiopurine S-methyltransferase pharmacogenetics in a large-scale healthy Italian-Caucasian population: differences in enzyme activity. Pharmacogenomics 2009;10:1753–65.
54. Lazzaretti M, Fabbro D, Sala M, Del Toso K, de Vidovich G, Marraffini E, et al. Association between low-activity allele of cathecolamine-O-methyl-transferase (COMT) and borderline personality disorder in an Italian population. Behav Med 2013;39:25–8.
55. Presciuttini S, Gialluisi A, Barbuti S, Curcio M, Scatena F, Carli G, et al. Hypnotizability and catechol-O-methyltransferase (COMT) polymorphisms in Italians. Front Hum Neurosci 2014;7:929.
56. Serrano D, Lazzeroni M, Zambon C-F, Macis D, Maisonneuve P, Johansson H, et al. Efficacy of tamoxifen based on cytochrome P450 2D6, CYP2C19 and SULT1A1 genotype in the Italian Tamoxifen Prevention Trial. Pharmacogenomics J 2011;11:100–7.
57. Piacentini S, Polimanti R, Porreca F, Martínez-Labarga C, De Stefano GF, Fuciarelli M. GSTT1 and GSTM1 gene polymorphisms in European and African populations. Mol Biol Rep 2011;38:1225–30.
58. Palli D, Saieva C, Gemma S, Masala G, Gomez-Miguel MJ, Luzzi I, et al. GSTT1 and GSTM1 gene polymorphisms and gastric cancer in a high-risk Italian population. Int J Cancer 2005;115:284–9.
59. Ballerini S, Bellincampi L, Bernardini S, Iori R, Cortese C, Federici G. Analysis of GSTP1-1 polymorphism using real-time polymerase chain reaction. Clin Chim Acta 2003;329:127–32.
60. Antognelli C, Del Buono C, Ludovini V, Gori S, Talesa VN, Crinò L, et al. CYP17, GSTP1, PON1 and GLO1 gene polymorphisms as risk factors for breast cancer: an Italian case-control study. BMC Cancer 2009;9:115.
61. Hein DW, McQueen CA, Grant DM, Goodfellow GH, Kadlubar FF, Weber WW. Pharmacogenetics of the arylamine N-acetyltransferases: a symposium in honor of Wendell W. Weber. Drug Metab Dispos 2000;28:1425–32.
62. Kawamura A, Graham J, Mushtaq A, Tsiftsoglou SA, Vath GM, Hanna PE, et al. Eukaryotic arylamine N-acetyltransferase. Investigation of substrate specificity by high-throughput screening. Biochem Pharmacol 2005;69:347–59.
63. Cascorbi I, Brockmöller J, Mrozikiewicz PM, Müller A, Roots I. Arylamine N-acetyltransferase activity in man. Drug Metab Rev 1999;31:489–502.
64. Bruhn C, Brockmöller J, Cascorbi I, Roots I, Borchert HH. Correlation between genotype and phenotype of the human arylamine N-acetyltransferase type 1 (NAT1). Biochem Pharmacol 1999;58:1759–64.
65. Hein DW, Boukouvala S, Grant DM, Minchin RF, Sim E. Changes in consensus arylamine N-acetyltransferase gene nomenclature. Pharmacogenet Genomics 2008;18:367–8.
66. Umamaheswaran G, Kumar DK, Adithan C. Distribution of genetic polymorphisms of genes encoding drug metabolizing enzymes & drug transporters – a review with Indian perspective. Indian J Med Res 2014;139:27–65.
67. Weinshilboum RM, Sladek SL. Mercaptopurine pharmacogenetics: monogenic inheritance of erythrocyte thiopurine methyltransferase activity. Am J Hum Genet 1980;32:651–62.
68. Salavaggione OE, Wang L, Wiepert M, Yee VC, Weinshilboum RM. Thiopurine S-methyltransferase pharmacogenetics: variant allele functional and comparative genomics. Pharmacogenet Genomics 2005;15:801–15.
69. Krynetski E, Evans WE. Drug methylation in cancer therapy: lessons from the TPMT polymorphism. Oncogene 2003;22:7403–13.
70. Wang L, Weinshilboum R. Thiopurine S-methyltransferase pharmacogenetics: insights, challenges and future directions. Oncogene 2006;25:1629–38.

Authenticated | espina@unime.it author's copy
Download Date | 1/10/15 12:07 PM

71. Rossino R, Vincis C, Alves S, Prata MJ, Macis MD, Nucaro AL, et al. Frequency of the thiopurine S-methyltransferase alleles in the ancient genetic population isolate of Sardinia. J Clin Pharm Ther 2006;31:283–7.
72. Lundström K, Tenhunen J, Tilgmann C, Karhunen T, Panula P, Ulmanen I. Cloning, expression and structure of catechol-O-methyltransferase. Biochim Biophys Acta 1995;1251:1–10.
73. Lachman HM, Papolos DF, Saito T, Yu YM, Szumlanski CL, Weinshilboum RM. Human catechol-O-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. Pharmacogenetics 1996;6:243–50.
74. McLeod HL, Syvänen AC, Githang'a J, Indalo A, Ismail D, Dewar K, et al. Ethnic differences in catechol O-methyltransferase pharmacogenetics: frequency of the codon 108/158 low activity allele is lower in Kenyan than Caucasian or South-west Asian individuals. Pharmacogenetics 1998;8:195–9.
75. Senggunprai L, Yoshinari K, Yamazoe Y. Selective role of sulfotransferase 2A1 (SULT2A1) in the N-sulfoconjugation of quinolone drugs in humans. Drug Metab Dispos 2009;37:1711–7.
76. Gamage N, Barnett A, Hempel N, Duggleby RG, Windmill KF, Martin JL, et al. Human sulfotransferases and their role in chemical metabolism. Toxicol Sci 2006;90:5–22.
77. Brauch H, Mürdter TE, Eichelbaum M, Schwab M. Pharmacogenomics of tamoxifen therapy. Clin Chem 2009;55:1770–82.
78. Deenen MJ, Cats A, Beijnen JH, Schellens JH. Part 3: Pharmacogenetic variability in phase II anticancer drug metabolism. Oncologist 2011;16:992–1005.
79. Glatt H, Meinl W. Pharmacogenetics of soluble sulfotransferases (SULTs). Naunyn Schmiedebergs Arch Pharmacol 2004;369:55–68.
80. Hebbring SJ, Moyer AM, Weinshilboum RM. Sulfotransferase gene copy number variation: pharmacogenetics and function. Cytogenet Genome Res 2008;123:205–10.
81. Jancova P, Anzenbacher P, Anzenbacherova E. Phase II drug metabolizing enzymes. Biomed Pap Med Fac Univ Palacký Olomouc Czechoslov 2010;154:103–16.
82. Oakley A. Glutathione transferases: a structural perspective. Drug Metab Rev 2011;43:138–51.
83. Iyer L, Ratain MJ. Pharmacogenetics and cancer chemotherapy. Eur J Cancer 1998;34:1493–9.
84. Hemmingsen A, Fryer AA, Hepple M, Strange RC, Spiteri MA. Simultaneous identification of GSTP1 Ile105→Val105 and Ala114→Val114 substitutions using an amplification refractory mutation system polymerase chain reaction assay: studies in patients with asthma. Respir Res 2001;2:255–60.
85. Josephy PD. Genetic variations in human glutathione transferase enzymes: significance for pharmacology and toxicology. Hum Genomics Proteomics 2010;876940.
86. Allan JM, Wild CP, Rollinson S, Willett EV, Moorman AV, Dovey GJ, et al. Polymorphism in glutathione S-transferase P1 is associated with susceptibility to chemotherapy-induced leukemia. Proc Natl Acad Sci USA 2001;98:11592–7.
87. Stoehlmacher J, Park DJ, Zhang W, Groshen S, Tsao-Wei DD, Yu MC, et al. Association between glutathione S-transferase P1, T1, and M1 genetic polymorphism and survival of patients with metastatic colorectal cancer. J Natl Cancer Inst 2002;94:936–42.
88. Lecomte T, Landi B, Beaune P, Laurent-Puig P, Loriot M-A. Glutathione S-transferase P1 polymorphism (Ile105Val) predicts cumulative neuropathy in patients receiving oxaliplatin-based chemotherapy. Clin Cancer Res 2006;12:3050–6.
89. Stocco G, Pelin M, Franca R, De Iudicibus S, Cuzzoni E, Favretto D, et al. Pharmacogenetics of azathioprine in inflammatory bowel disease: a role for glutathione-S-transferase? World J Gastroenterol 2014;20:3534–41.
90. Spurdle AB, Webb PM, Purdie DM, Chen X, Green A, Chenevix-Trench G. Polymorphisms at the glutathione S-transferase GSTM1, GSTT1 and GSTP1 loci: risk of ovarian cancer by histological subtype. Carcinogenesis 2001;22:67–72.

Authenticated | espina@unime.it author's copy
Download Date | 1/10/15 12:07 PM