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RESEARCH

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Explore the distribution of (rs35742686, rs3892097 and rs1065852) genetic polymorphisms of cytochrome P4502D6 gene in the Moroccan population

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Abstract

Background: The *CYP2D6* gene encodes a crucial enzyme involved in the metabolic pathways of many commonly used drugs. It is a highly polymorphic gene inducing an interethnic and interindividual variability in disease susceptibility and treatment response. The aim of this study is to evaluate the frequency of the three *CYP2D6* most investigated alleles (*CYP2D6*3*, *CYP2D6*4,* and *CYP2D6*10* alleles) in Morocco compared to other populations.

This study enrolled 321 healthy Moroccan subjects. *CYP2D6* genotypes and allele frequencies were assessed using a restriction fragment length polymorphism–polymerase chain reaction genotyping method. The Principal Component Analysis (PCA) and dendrogram were conducted to evaluate genetic proximity between Moroccans and other populations depending on *CYP2D6* allele frequencies.

Results: According to the current study, the results observed the homozygous wild type of the three studied SNPs were predominant among the Moroccan population, while 1.4% of Moroccans carried the *CYP2D6*4* allele responsible for a Poor Metabolizer phenotype and associated with low enzyme activity which may induce a treatment failure. The PCA and cluster dendrogram tools revealed genetic proximity between Moroccans and Mediterranean, European and African populations, versus a distancing from Asian populations.

Conclusion: The distribution of *CYP2D6* polymorphisms within Morocco follows the patterns generally found among the Mediterranean, European and African populations. Furthermore, these results will help to lay a basis for clinical studies, aimed to introduce and optimize a personalized therapy in the Moroccan population.

Keywords: *CYP2D6* gene, Polymorphism, Moroccan population, PCA

Introduction

Cytochrome P450 2D6 (CYP2D6) enzyme is a key member of the Cytochrome P450 superfamily implicated in detoxification and metabolism of a wide range of endogenous and exogenous compounds, as well as in hormone synthesis and breakdown [1, 2]. CYP2D6 is one of the most extensively investigated enzymes owing to its role in the metabolism of 25% of all clinically used drugs, including various antidepressants, β-blockers, several opioid analgesics and anticancer drugs [3]. *CYP2D6* is a highly polymorphic gene located on chromosome 22q13.1 [4, 5]. So far, more than a hundred *CYP2D6* allelic variants were reported in the Pharmacogene Variation Consortium (https://www.pharmvar.org/). Genetic polymorphisms are responsible for null, decreased, normal or increased functions, altering enzyme activity among individuals

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and populations and consequently affecting pharmacological therapy outcomes [6–8]. Several metabolic phenotypes have been reported depending on *CYP2D6* genotypes. Actually, individuals with two non-functional alleles are considered poor metabolizers (PM) unable to metabolize drugs, while carrying at least one increased function allele confers an ultrarapid metabolizer phenotype (URM) with increased CYP2D6 activity [9]. Otherwise, individuals with at least one decreased function allele are supplying reduced CYP2D6 activity and are considered as intermediate metabolizers (IM). Normal metabolizers (NM) are individuals with wild type alleles and normal CYP2D6 enzyme activity. Clinical studies reported an association between abnormal metabolizer phenotypes and risk of undergoing dose-related adverse events or lack of treatment efficiency [10]. Furthermore, *CYP2D6* genetic polymorphisms have been associated with many treatment failures in several diseases [11–13]. CYP2D6 is implicated in tamoxifen activation, a drug administered in hormonal breast cancer therapy, inducing an association between *CYP2D6* genotype and treatment response in breast cancer disease [8, 14, 15].

Due to its implication in the susceptibility and treatment of several diseases, and the fact that polymorphic expression of CYP2D6 affects its activity, analysis of CYP2D6 genetic variability is required to develop appropriate therapy for successful treatment. Therefore, CYP2D6 has been investigated in many populations, and the finding showed a substantial variation in allele frequencies among populations [3, 16–18]. Indeed, to evaluate *CYP2D6* genetic polymorphisms, three alleles were most studied among various populations considering their association with several disease outcomes and treatments [19–21]. *CYP2D6*3* (rs35742686) is a single-base deletion at exon 5 (A2549del) causing null activity [22, 23]. *CYP2D6*4* (rs3892097), a splice site mutation (1846G > A), yields an absence or defective protein in the liver (Sachse et al. 1997). The CYP2D6*10 (rs1065852) 100C/T polymorphism leads to substitution of proline to serine and causes a mRNA splicing defect which produces an IM phenotype [22]. The aim of the present study is to evaluate, for the first time, the distribution of the *CYP2D6* gene *(CYP2D6*3/*4/*10)* in the Moroccan population.

Methods

Subjects and blood sample

The current population study enrolled 321 unrelated healthy volunteers recruited during a blood donation campaign. All volunteers from southern Morocco, whom were Arabic, Amazigh, or sub-Saharan Moroccans. All patients received a medical examination during the blood donation campaign and donors with any disease suspicion (diabetes, high arterial blood pressure, etc.) or cancer history were excluded from the study. Peripheral blood was collected based on the World Health Organization criteria (Blood Donor Selection Guidelines, 2012), and written informed consent was obtained from all individuals enrolled in the study. This study was performed under the approval of the Ethics Commission of Cadi Ayyad University Hospital Center (CHU) Mohammed VI, in Marrakech, Morocco.

CYP2D6 genotyping

Genomic DNA was extracted from whole blood using the conventional salting out procedure [24] with phenol chloroform purification. Extracted genomic DNA was quantified by Qubit Fluorometers (Invitrogen) and the measured values vary 50–74 ng/µl. After PCR amplifications, restriction fragment-length polymorphism analysis (RFLP) was used to genotype CYP2D6 allele analysis. Primers for DNA amplification are given in Table 1. All PCRs were performed in a total volume of 25 µl containing approximately 100 ng of DNA template, 1U of MyTaq of DNA Polymerase enzyme (Bioline, USA), MyTaq Reaction Buffer (0.5 mM NTPs, 1.5 mM MgCl2 with stabilizers and enhancers), along with 200 nM of each appropriate primer [25, 26]. Amplified PCR products were digested with *Bsa*I, *Bst*NI and *Hph*I enzymes (New England Biolabs, USA) for *CYP2D6*3*, *CYP2D6*4* and *CYP2D6*10* alleles, respectively. Amplification sizes

Table 1 Primers and restriction enzymes used for genotyping

SNPs *CYP2D6*	Primers (Tm)	Enzyme	Size (Wild-type)	Size (Heterozygote)	Size (Mutant)
*3 (2549delA)	F/5′-GCTGGGGCCTGAGACTT-3′ R/5′-GGCTGGGTCCCAGGTCATAC-3′	BsaAI	201 pb	201-180-20 pb	180-20 pb
*4 (G1934A)	F/5′-CCTGGGCAAGAAGTCGCTGGACCAG-3′ R/5′-GAGACTCCTCGGTCTCTCG-3′	BstNI	190-163 pb	353-190-163 pb	353 pb
*10 (C100T)	F/5′-GTGCTGAGAGTGTCCTGCC-3′ R/5′-CACCCACCATCCATGTTTGC-3′	HphI	282-62 pb	282-182-100-62 pb	182-100-62 pb

details and expected digestion results for each SNP are also shown in Table 1.

Statistical analysis

Allele and genotype frequencies, as well as Hardy–Weinberg equilibrium (HWE), were assessed using SNPStats software [27]. Results are considering significant when *P*-value is less than 0.05. Pairwise linkage disequilibrium (LD) within the three SNP was performed in terms of Lewontin's (*D'*) defined based on normalizing coefficient of linkage disequilibrium D which measures the deviation of haplotype frequencies from expected values based on gene frequencies and informing if alleles are inherited together, and Pearson's coefficient of correlation (r^2) defined as D2 normalized by the product of all allele frequencies. Linkage disequilibrium was assessed using Haploview software [28]. Haplotypic frequencies were assessed using SNPStats software [27]. To compare *CYP2D6* allele frequencies in Moroccans with other populations from different ethnic origins, we carried out a qui-square test, a Principal Component Analysis (PCA) and a dendrogram clustering, using R environment [29].

Results

The allele and genotype frequency distributions of the *CYP2D6*3* (A2549del), **4* (G1846A) and **10* (C100T) variants were analyzed in the blood samples of 321 healthy volunteers. Figure 1 presents the alleles in the pattern of the fragments digested for the detection of *CYP2D6*3* (A), *CYP2D6*4* (B), *CYP2D6*10* (C).

According to HW equilibrium analysis (Table 2), all the studied SNPs were in HW equilibrium (*P*-value > 0.05).

Concerning *CYP2D6* genotypes (Table 3), carriers of homozygous wild type for *CYP2D6*3* were 76.4%, while 23.6% were heterozygous. The carriers of the wild homozygous genotype of the CYP2D6*4 variant were the most predominant with 80.3%, and the heterozygous genotype represented 18.3%, while the homozygous mutant genotype was 1.4%, while the wild homozygous and heterozygous of the CYP2D6*10 variant were 84.7% and 15.3%, respectively. Minor allele frequencies for *CYP2D6*3, CYP2D6*4* and *CYP2D6*10* were 11.8%, 10.5% and 7.7%, respectively.

According to our results, the AGC haplotype was the most predominant within our population (77.19%) (Table 4). A strong LD ($D'=0.69$; $r^2=0.33$; $P=0.000$)

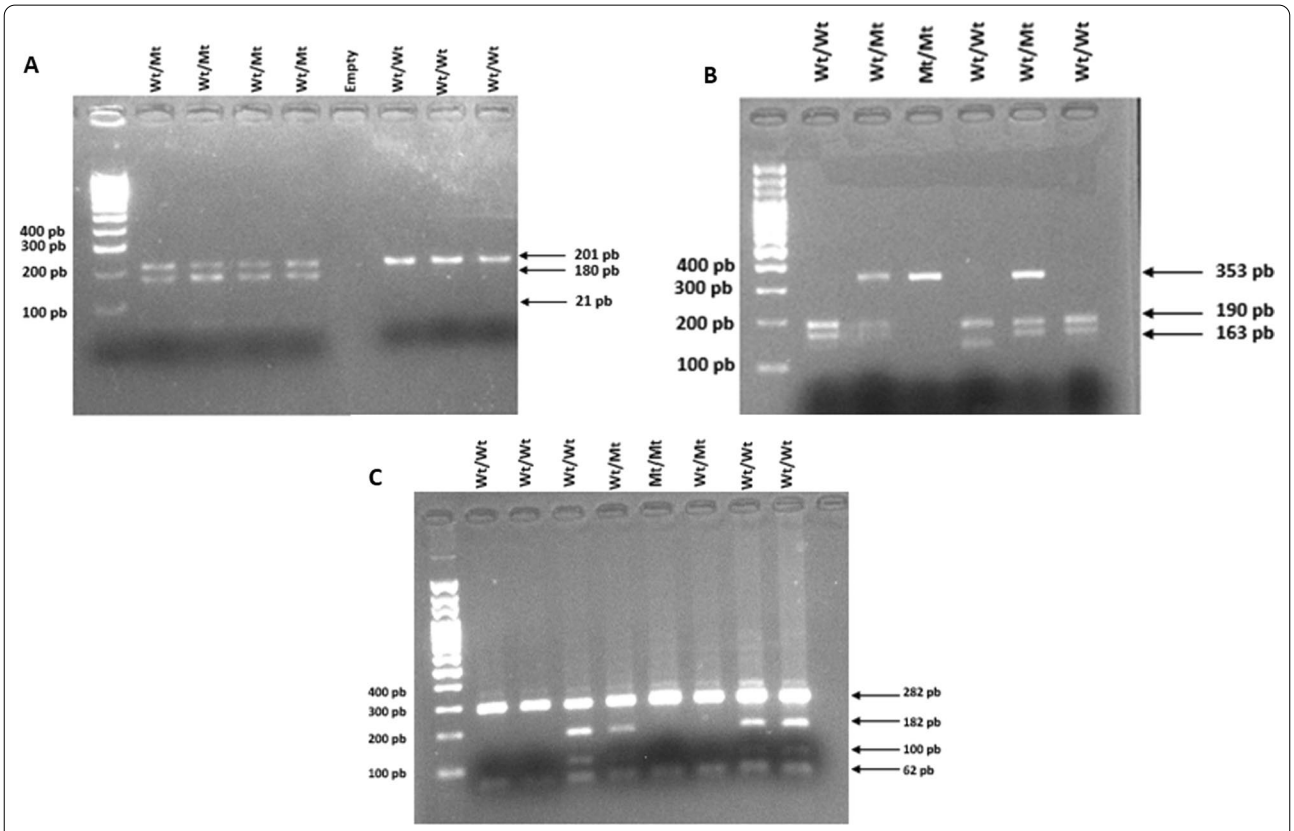

Fig. 1 Pattern of the fragments digested **A** Electrophoretic separation of cytochrome P450 *2D6*3* digested segments. **B** Electrophoretic separation of cytochrome P450 *2D6*4* digested segments. **C** Electrophoretic separation of cytochrome P450 *2D6*10* digested segments

Table 2 Hardy–Weinberg Equilibrium for *CYP2D6* polymorphisms in the Moroccan population

	Observed genotypes %	Expected genotypes %	Chi-squared	HWE. P-value	HWE deviation
*CYP2D6*3 (rs35742686)*					
A/A	76.4	77.79	5.46	0.065	Not deviated
A/Del	23.6	20.81			
Del/Del	0	1.4			
*CYP2D6*4 (rs3892097)*					
G/G (NM)	80.3	80.1	0.244	0.884	Not deviated
G/A (IM)	18.3	18.80			
A/A (PM)	1.4	1.10			
*CYP2D6*10 (rs106585)*					
C/C (NM)	84.7	85.19	2.15	0.339	Not deviated
C/T (NM)	15.3	14.21			
T /T (IM)	0	0.6			

Table 3 Genotypic and allelic frequencies of *CYP2D6* polymorphisms in the Moroccan population

	Allelic and Genotypic Frequencies
	Genotype/ Allele n = 321, No (%)
*CYP2D6*3 rs35742686*	
A/A (NM)	233 (76.4)
A/del (IM)	72 (23.6)
Del/del (PM)	0
A	538 (88.2)
delA	72 (11.8)
*CYP2D6*4 rs3892097*	
G/G (NM)	233 (80.3)
G/A (IM)	53 (18.3)
A/A (PM)	4 (1.4)
G	519 (89.5)
A	61 (10.5)
*CYP2D6*10 rs1065852*	
C/C (NM)	265 (84.7)
C/T (NM)	48 (15.3)
T /T (IM)	0
C	578 (92.3)
T	48 (7.7)

was found between *CYP2D6*4* and *CYP2D6*10* SNP, as shown in Fig. 2.

Allele frequencies of the three *CYP2D6* polymorphisms were compared with different populations including Mediterraneans, Europeans, Africans, South Americans and Asians (Table 5). The considered studies

Table 4 Haplotypes frequencies of the *CYP2D6* (*CYP2D6*3/CYP2D6*4/CYP2D6*10*) polymorphisms

*CYP2D6*3*	*CYP2D6*4*	*CYP2D6*10*	Frequency	*n* = 320
A	G	C	0.7719	247
DelA	G	C	0.1016	32
A	A	T	0.0495	16
A	A	C	0.0421	13
A	G	T	0.0184	6
DelA	A	C	0.0081	3
DelA	A	T	0.006	2
DelA	G	T	0.0024	1

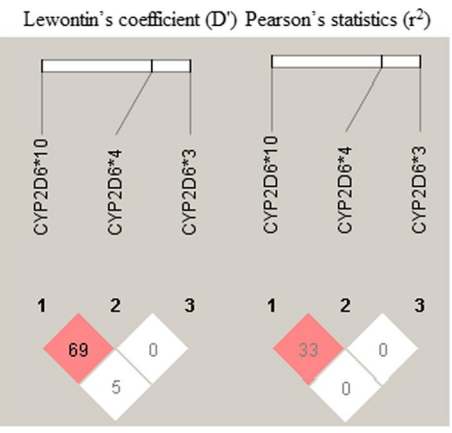

Fig. 2 Haploview figure showing Linkage disequilibrium (LD) plots characterizing haplotype blocks in *CYP2D6* gene polymorphisms, in terms of Lewontin's coefficient (*D'*) and Pearson's (r^2) statistics

Table 5 Distribution of *CYP2D6* allele frequencies in the Moroccan population compared to other worldwide populations

Population	No of Individuals	Non-functional				Reduced		References
		*3	*P*-value	*4	*P*-value	*10	*P*-value	
Moroccan	321	11.8	–	10.5	–	7.7	–	Present study
Tunisian	230	–	–	15.2	0.04	–	–	[30]
Egyptian	29	–	–	13.8	0.34	–	–	[31]
Syrian	51	0	0.00	9.8	0.93	2.94	0.11	[32]
South African	99	0	0.00	7.07	0.33	2.53	0.00	[33]
Ghanaian	193	0	0.00	7	0.20	3.1	0.006	[34]
Ethiopian	69	0	0.00	5.9	0.19	8	0.85	[35]
Venda	81	0	0.00	3	0.01	12	0.11	[36]
Spanish	133	0.75	0.00	11.65	0.37	0.38	0.00	[37]
Portuguese	1138	1.4	0.00	15.6	0.00	2.5	0.00	[38]
French	672	1.8	0.00	17.2	0.00	1.5	0.00	[39]
Italian	360	0.7	0.00	15.3	0.00	–	–	[40]
German	589	2.04	0.00	21	0.00	1.53	0.00	[41]
Polish	145	2.1	0.00	23	0.00	–	–	[42]
Finnish	857	2.6	0.00	10.7	0.43	12.7	0.00	[18]
British	94	3.3	0.00	24.2	0.00	0.5	0.007	[43]
Greek	283	2.3	0.00	17.8	0.00	–	–	[44]
Turkish	200	6	0.002	10	0.793	26	0.00	[45]
Iranian	100	0.5	0.00	9	0.539	–	–	[17]
	100	–	–	12.5	0.24	9	0.79	[46]
Brazilian	95	9.4	0.12	13.1	0.57	–	–	[47]
	179	1.5	0.00	6.32	0.12	4.02	4.40	[48]
Indian	83	0	0.00	8	0.30	15	0.00	[49]
Chinese	223	0	0.00	0.2	0.00	51.3	0.00	[50]
Japanese	98	0	0.00	0.5	0.00	40.8	0.00	[51]

consisted in the evaluation of *CYP2D6* genetic frequencies in a healthy subject (population studies), or investigation the impact of *CYP2D6* genotypes on therapy outcomes, and exploring risk susceptibility of *CYP2D6* (Association studies/Case–control studies). Results of the three SNPs frequencies comparison showed a significant difference in *CYP2D6*3* allele frequency between Moroccans and other ethnic groups, while no difference was observed in *CYP2D6*4* and *CYP2D6*10* allelic frequencies within the Moroccan population and the majority of European, Mediterranean and African populations (Table 5).

We also performed a PCA and cluster dendrogram to compare *CYP2D6* allele frequencies between Moroccans and other populations. However, *CYP2D6*3* allele was excluded from PCA analyses due to the significant difference in this allele frequency between Moroccans and other ethnic groups which affect substantially the PCA results. The PCA including data of *CYP2D6*4* and *CYP2D6*10* alleles revealed genetic proximity between the Moroccan population and Europeans as well as Africans (Fig. 3B). The cluster dendrogram also showed genetic proximity between Moroccans and Africans as well as European populations (Fig. 3A).

Discussion

The CYP2D6 enzyme is implicated in the metabolism of a wide range of clinically used drugs, such as tamoxifen. Many investigations reported an association of *CYP2D6* genetic polymorphisms with susceptibility and response to treatment in many diseases such as autoimmune conditions, cardiovascular diseases and several cancers [52–57]. Furthermore, clinical studies reported that *CYP2D6* genetic polymorphisms are associated with adverse responses to many drugs such as opioids including codeine, antiarrhythmic drugs and anti-cancer drugs [11–13]. Indeed, *CYP2D6* is implicated in tamoxifen transformation, a drug usually administrated in breast cancer hormonal therapy [58]. Furthermore, numerous studies reported an association between the *CYP2D6* poor metabolizer genotype and recurrences of breast cancer disease with worse event-free survival rates [59]. The role of CYP2D6 includes a large number of medical specialties; indeed, the pharmacogenomics

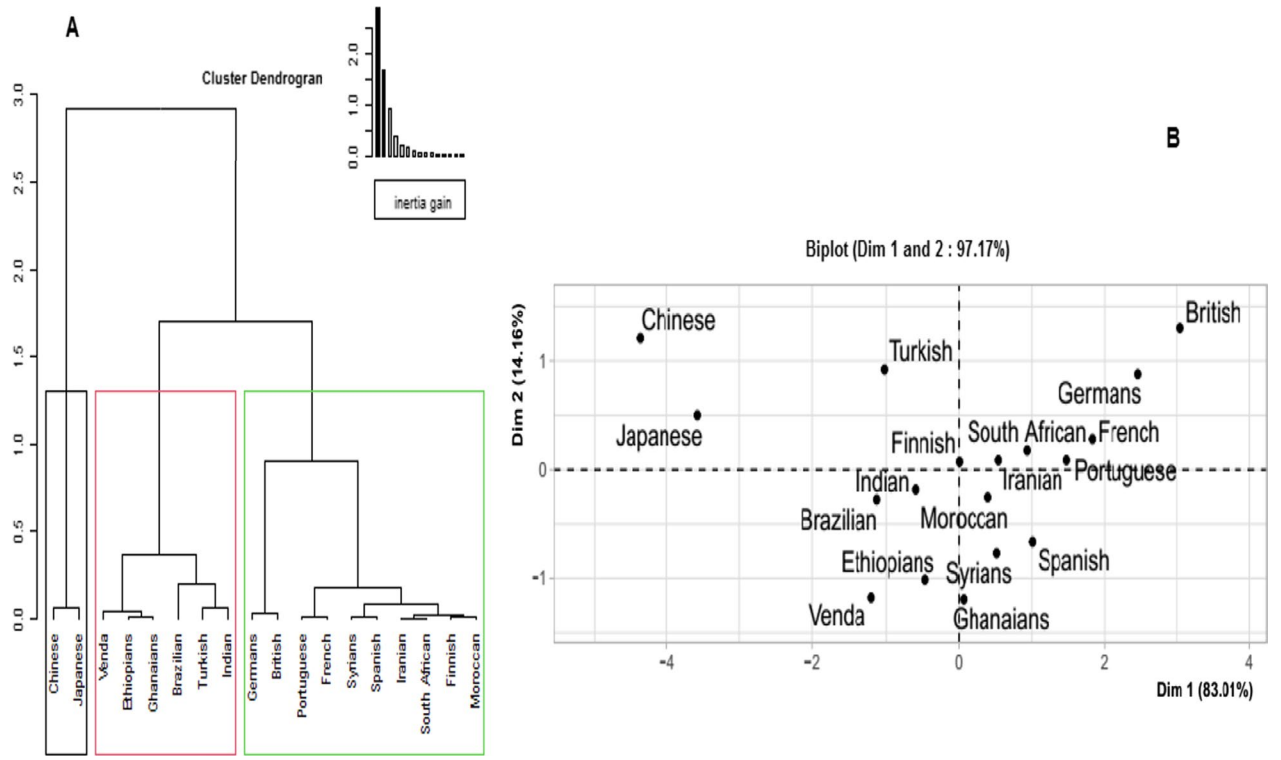

Fig. 3 **A** Dendrogram cluster showing relatedness between Moroccan population and Mediterranean, African and Asian populations for *CYP2D6*4* and *CYP2D6*10* alleles. **B** Principal component analysis of the *CYP2D6* allele frequencies distribution on the Mediterranean, South African and Asian populations

guideline committees have reviewed clinical relevance of CYP2D6, and compiled therapeutic recommendations for more than 48 drugs and developed recommendations based on CYP2D6 genotype/phenotype drugs combinations for 26 drugs (PharmGKB Clinical Guideline Annotations (https://www.pharmgkb.org/guidelineAnnotations). Thus, having information on *CYP2D6* genotypes is crucial in deciding the most appropriate therapy for each patient.

Therefore, our study contributes to the determination of the genetic profile of *CYP2D6* within the Moroccan population. Overall, the Moroccan population showed a predominance of the wild-type genotype (*CYP2D6*3* AA (76.4%); *CYP2D6*4* GG (80.3%); *CYP2D6*10* CC (84.7%)). These genotypes are responsible for the NM phenotype. For each SNP, about 19% of individuals were carrying an IM phenotype. However, 1.4% of Moroccans had PM phenotype for *CYP2D6*4.* This latter was investigated in many populations and the results attested to its association with breast cancer susceptibility and treatment [60–62]. Haplotype analysis revealed a predominance of AGC haplotype in the Moroccan population (77.19% of cases).

According to our results, an increased *CYP2D6*3* (Null allele metabolizer) allelic frequency was observed in Moroccan population compared to others. This allele was found to be associated with acute lymphoblastic leukemia and breast cancer disease [53, 57]. A similar *CYP2D6*3* allele frequency was also observed within Brazilians. This proximity might be a consequence of numerous factors, as the fact that Brazilian population have received significant immigration from descendants of original north-African groups, including Berbers [63], previously stablished in Iberic peninsula (Spain and Portugal) during the large Arabic occupation for about 700 years (711–1492) [64]. Some of them moved directly to Brazil, when settled by Portuguese in 1500 and especially after its independence from Portugal in 1822 [65], while others made previous migration to Holland and Azoras islands [66].

The *CYP2D6*4* was the most studied null allele within populations. Concerning *CYP2D6*4* allelic frequencies, results revealed a significant difference between Moroccan and both Asian and African populations (Ghanaians, Ethiopians), versus a similarity with European and Mediterranean populations

(Turkish, Iranian, Finnish, Spanish and Egyptians) (Table 5). This similarity is probably resulting from their closer geographical distance, inducing a certain admixture of populations which boosts a degree of genetic similarity [67]. For *CYP2D6*10,* a reduced-function allele, frequencies showed a great variability within populations. Indeed, *CYP2D6*10* frequencies in Moroccans showed a disparity with European populations, versus a similarity with African, Iranian and Syrian populations. PCA results for *CYP2D6*4* and *CYP2D6*10* alleles clearly showed genetic proximity between Moroccans and both European and African populations. The dendrogram clustering of populations with such diverse ethnic backgrounds revealed three clusters, the first one represented by the Asians. This cluster is characterized by a high allelic frequency of the *CYP2D6*10,* and low frequency for *CYP2D6*4,* and it is clearly distinct from other populations. The second and the third clusters are composed of European and African populations, which are characterized by an average value for the two allele frequencies for the second cluster, and a *CYP2D6*4* high allelic frequency and *CYP2D6*10* low frequency for the third one. This latter includes Moroccans and other European and African populations (Spanish, Finnish, French, South African).

This study suggests that the Moroccans genetic profile was impacted by historical and demographic events, along the Mediterranean, European, and sub-African populations, leading to its current genetic diversity. Actually, this relevant genetic pool could be interpreted by demographic events, for instance, the influx of Arab populations from the Middle East, Sub-Saharans as well as populations around the Mediterranean area. All these groups contributed to the genetic patrimony of the present-day Moroccan population. [68]. Indeed, the present work and other studies of North African genetic variation, which devote attention to the history of North African and Mediterranean populations, presumed that demographic events contributed to the genetic homogeneity with the nearby regions [68, 69].

Overall, our study revealed that 1.4% of our population carried a PM phenotype for *CYP2D6*4* polymorphism. Since CYP2D6 is implicated in the metabolism pathway of many drugs used in several disease treatments, carrying out of *CYP2D6*4* polymorphism may affect treatment response. Therefore, pharmacogenetic screening for this gene before any therapy is crucial to avoid treatment failure, hence reduce cost-related issues [70]. Indeed, in many countries, patients take advantage of *CYP2D6* screening before treatments [71]. Moroccan population should also consider this recommendation.

Conclusion

The present study attested to the genetic proximity between Moroccan, African and European populations. Furthermore, these results will help to lay a basis for clinical studies, aimed to introduce and optimize a personalized therapy for the Moroccan population.

Abbreviations

CYP: Cytochrome P450; CYP2D6: Cytochrome P450 2D6; dNTP: Deoxynucleotide; DNA: Deoxyribonucleic acid; NM: Normal metabolizer; HWE: Hardy–Weinberg equilibrium; IM: Intermediate metabolizer; LD: Linkage disequilibrium; PCA: Principal component analysis; PCR: Polymerase chain reaction; PM: Poor metabolizer; RFLP: Restriction fragment length polymorphism; SNP: Single-nucleotide polymorphism; UM: Ultrarapid metabolizer phenotype.

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Author contributions

The first two authors contributed equally to this work; EE and SEA contributed to the manuscript writing, data collection and software. EHI contributed to the manuscript editing and literature review and analysis. NI contributed to the manuscript editing and analysis. All the authors have read and approved the final manuscript.

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Availability of data and materials

The datasets supporting the results are included within the article. The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available because of privacy or ethical restrictions.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethical Committee of Cadi Ayyad University Hospital Center (CHU) Mohammed VI, Marrakech, Morocco. (The patient provided written consent).

Consent for publication

Written informed consent was obtained from all patients and controls.

Competing interests

The authors declare that they have no competing interests.

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