Identification of New Alleles and the Determination of Alleles and Genotypes Frequencies at the CYP2D6 Gene in Emiratis

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Abstract

CYP2D6 belongs to the cytochrome P450 superfamily of enzymes and plays an important role in the metabolism of 20–25% of clinically used drugs including antidepressants. It displays inter-individual and inter-ethnic variability in activity ranging from complete absence to excessive activity which causes adverse drug reactions and toxicity or therapy failure even at normal drug doses. This variability is due to genetic polymorphisms which form poor, intermediate, extensive or ultrarapid metaboliser phenotypes. This study aimed to determine CYP2D6 alleles and their frequencies in the United Arab Emirates (UAE) local population. CYP2D6 alleles and genotypes were determined by direct DNA sequencing in 151 Emiratis with the majority being psychiatric patients on antidepressants. Several new alleles have been identified and in total we identified seventeen alleles and 49 genotypes. CYP2D6*4 (wild type) and CYP2D6*2 alleles (extensive metaboliser phenotype) were found with frequencies of 39.1% and 12.2%, respectively. CYP2D6*41 (intermediate metaboliser) occurred in 15.2%. Homozygous CYP2D6*4 allele (poor metaboliser) was found with a frequency of 2% while homozygous and heterozygous CYP2D6*4 occurred with a frequency of 9%. CYP2D6*2xn, caused by gene duplication (ultrarapid metaboliser) had a frequency of 4.3%. CYP2D6 gene duplication/multiduplication occurred in 16% but only 11.2% who carried more than 2 active functional alleles were considered ultrarapid metabolisers. CYP2D6 gene deletion in one copy occurred in 7.5% of the study group. In conclusion, CYP2D6 gene locus is heterogeneous in the UAE national population and no significant differences have been identified between the psychiatric patients and controls.

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Introduction

Interethnic variation in the capacity to metabolize drugs is mainly due to genetic constitution [1]. Recent advances in pharmacogenomics elucidated that some variations in DNA sequences recognized as single nucleotide polymorphisms (SNPs) may explain some of the variability in drug metabolizing enzyme activities. These contribute to drug-induced adverse reactions, toxicity, and therapeutic responses in different ethnic groups [2]. The best characterized genetically determined variations in antidepressant drug metabolism are those associated with the polymorphic N-acetyltransferase, NAT2, and two polymorphic cytochrome P450s involved with oxidation reactions, CYP2D6 and CYP2C19, all of which show marked interethnic differences in catalytic activity and allele distribution [3]. Whereas the clinical significance of the CYP2C19 polymorphism is uncertain, the clinical consequences of the CYP2D6 polymorphism are well established [4,5].

The potential importance of interethnic differences in CYP2D6 gene structure and expression lies in the large number of drugs whose elimination is catalysed by CYP2D6. These include tricyclic antidepressants and antipsychotics [6]. The CYP2D6 locus is highly polymorphic and currently more than 120 variant CYP2D6 alleles have been described (www.cypalleles.ki.se/cyp2d6.htm). These alleles can be divided into; 1) alleles resulting in no functional product (poor metabolizers, PMs); 2) alleles causing a reduced rate of metabolism (intermediate metabolizers, IMs); 3) alleles causing ultrarapid metabolism (ultra-rapid metabolizers, UMIs); and 4) alleles with no important functional consequences (extensive metabolizers, EMs) [7]. Given the relatively wide spread use of CYP2D6-metabolised medications, including antidepressants, and limited availability of data regarding the alleles and genotypes in Emiratis, this study was undertaken to determine CYP2D6 alleles and their frequencies, using direct DNA sequencing of the full coding regions and large parts of the intronic sequences, in this population.
Results
Identification of new CYP2D6 alleles
The DNA sequence analysis of all the coding regions, the splice sites and large parts of the intronic regions was carried out for 151 subjects from the UAE local population. This included 50 healthy controls and 101 subjects on antidepressants. Eight out of 151 subjects had new alleles and new genotypes (Table 1 and 2). CYP2D6*105 was the only new homozygous genotype where we found a novel mutation c.1097T>G (g.3268T>C) which causes p.F366S change in the corresponding protein. This mutation is accompanied by the two common SNPs, namely p.R296C and p.S486T [http://www.cypalleles.ki.se/cyp2d6.htm] in a homozygous state. The other new alleles were unique in their combinations. Five patients were carrying unreported combinations of known SNPs with some unknowns (Table 2). Both new SNPs g.1783 A>C and g.4028C>A were intrinsic thus their effect on the enzyme function is not clear. The ascertained haplotypes in these subjects were undetermined due to the heterozygosity of some of their SNPs. Parents were not included in this study and therefore it was not possible to assign the alleles.

CYP2D6 allele frequencies among 151 Emiratis
Within our sequenced study group (n = 151, 302 alleles) 17 different alleles were identified in which CYP2D6*4 occurred in the highest frequency of 39.1% (n = 118) (Table 3). This was followed by CYP2D6*41 with a frequency of 15.2% (n = 46), while CYP2D6*2, CYP2D6*8, CYP2D6*29, CYP2D6*39 and CYP2D6*10 had frequencies of 12.2%, 9%, 4.3%, 4% and 3.3%, respectively.

Frequencies of CYP2D6 functional alleles among 151 UAE individuals
As shown in table 4 there were only 3 (2%) individuals out of 151 who carried no functional alleles at all. Of the 151 subjects, 27 (17.9%) had only one active allele. The majority carried two active alleles with a frequency of 68.9%, whereas 17 (11.3%) individuals were carrying more than two active alleles.

Frequency of CYP2D6 Genotypes and their predicted phenotypes
CYP2D6*1, CYP2D6*2 and CYP2D6*29 encode for EMs phenotype in both homozygous and heterozygous forms. CYP2D6*10 and CYP2D6*41 encode for IMs phenotype but only in the homozygous form or when it is combined with one of the poor metaboliser alleles such as CYP2D6*4 which only encode for a PM phenotype in the homozygous form [8–10]. CYP2D6*2xn which indicates the presence of gene duplication encode for a UMs phenotype [11].

In addition to the new genotypes described in table 2, we found 44 different already known genotypes among our study group (Table 5). Twenty three out of the 44 genotypes were assigned under EMs phenotype (homozygous or heterozygous). Twelve out of the 44 were genotypes associated with duplication or multi-duplication in one of their alleles and they were assigned under UMs phenotype. Eight out of 44 genotypes were assigned as IMs phenotype. One genotype was predicted to have PM phenotype.

In the subjects studied (n = 151) the following CYP2D6 genotypes were identified with the highest occurrence:*1/*1(n = 31), *1/*2(n = 17), *1/*4(n = 14), *1/*8(n = 8), *41/*41(n = 7), *4/*41(n = 6), *2/*29(n = 4) and *1/*2xn(n = 5) (Table 5). Out of the 151 study group 2% (n = 3) were poor metabolizers carrying the *4/*4 genotype.

Discussion
The impact of CYP2D6 polymorphisms on the clinical outcome of psychoactive drugs has been extensively described in the literature [12]. CYP2D6 has more than 120 allelic variants resulting from point mutations, rearrangements, additions, deletions and duplications. Several studies have shown that the frequencies of the alternative CYP2D6 phenotypes vary significantly among different ethnic groups [2,13,14]. Examples of these ethnically specific markers are CYP2D6*17, found in Africans, and CYP2D6*10, found in Asians. Both result in slower metabolism of CYP2D6 substrates (such as risperidone and paroxetine), which result in higher plasma concentrations thereby needing lower doses for therapeutic effect [8,12,15,16].

Duplications or multiplications of the CYP2D6 gene occurs in 1% of Swedes, 5% of Spanish, 29% of Ethiopians and 19% of Arabs results in an increased number of enzymes available for metabolic processes [3,7,17]. This is called ultrarapid metabolism. Patients with these mutations will require higher doses of medications to achieve the therapeutic response. UMs were previously regarded as “noncompliant” because they did not respond to the standard medication doses administered [18]. CYP2D6 genotype frequency information had been determined for most populations around the world [12]. They displayed significant interethnic differences in CYP2D6 allele frequencies [1] resulting in variable percentages of PMs, IMs, EMs and UMs in a given population. This is the first study to genotype CYP2D6 gene in UAE local population. However, the incidence of different

| New Alleles | DNA change | Protein |
|-------------|------------|---------|
| CYP2D6*102 | Intron 1 conversion with CYP2D7 (214–245); 310G>T; 972C>T; 1661G>C; 3285C>T; 3384A>C; 3790C>T; 4180G>C; 4481G>A | A90V; R296C; S486T |
| CY2D6*103 | Intron 1 conversion with CYP2D7 (214–245); 310G>T; 972C>T; 1661G>C; 1749A>G; 2850C>T; 3384A>C; 3790C>T; 4180G>C; 4481G>A | A90V; N166D; R296C; S486T |
| CYP2D6*104 | Intron 1 conversion with CYP2D7 (214–245); 310G>T; 843T>G; 1661G>C; 1720A>T; 2850C>T; 3384A>C; 3790C>T; 4180G>C; 4481G>A | E156A; R296C; S486T |
| CYP2D6*105 | Intron 1 conversion with CYP2D7 (214–245); 310G>T; 746C>G; 843T>G; 1661G>C; 2850C>T; 3285C>T; 3384A>C; 3790C>T; 4180G>C; 4481G>A | R296C; F366S; S486T |

Nucleotide variations in bold are the major SNPs/alternatives responsible for the phenotype of the corresponding allele.
Nucleotide and amino acid variations underlined are novel and have not been reported previously in the CYP2D6 allele nomenclature database.

All nucleotide changes are based on Genbank accession number M33388* following the instructions in database http://www.cypalleles.ki.se/cyp2d6.htm.
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CYP2D6 polymorphisms in the UAE population holds a great challenge to be characterized because of the huge ethnic diversity that exists in the country.

The novel variant p.F366S found in this population has not been reported elsewhere and its clinical impact is unknown, but theoretically and based on bioinformatics tools it could be predicted to probably be protein damaging based on analysis using PolyPhen program for prediction of functional effect of human SNPs (http://genetics.bwh.harvard.edu/pph/). Unfortunately, no plasma was available for the homozygous subject, thus we were unable to determine his metabolic ratio (MR).

CYP2D6 allele frequencies in our UAE study group were compared with frequencies seen in other ethnic populations. According to Toscano et al. [19], *2 and *4 alleles are the most frequent CYP2D6 functional alleles. This is similar to our findings as the said alleles occurred in the highest frequencies namely: *1 (39.1%), *4 (13.2%) and *2 (12.2%). Unlike other populations [19], CYP2D6*41 occurred more frequently than CYP2D6*2 in UAE. In the Croatian population the frequencies of polymorphic CYP2D6*2, *3, *4, *5, and *6 alleles were 4%, 2.8%, 14%, 1%, and 1.5%, respectively. The most frequently observed null allele was CYP2D6*4, which accounted for 72% of all null alleles. Among the Croatian population studied, 60% of the subjects had EM genotype, 33% were IMs, and 3% exhibited the PM genotype. Four percent exhibited the UM genotype due to amplification of CYP2D6 gene (CYP2D6*2 allele) [20]. The allele frequency of CYP2D6*4, the most common defective allele among Caucasians (25%); was 14% in Croatians [20] and only 3.5% in the Saudi population [21] and 2% in the UAE population. Two other alleles, CYP2D6*10 (47–70% in Asians) and CYP2D6*17 (25–40% in blacks), are common in certain populations resulting in diminished enzyme activity. Both were found only at low frequencies of 3.0% each in the Saudi Arabian population [21] and 3.3% and 2.5% respectively, in the UAE population.

PM alleles (e.g.*3,*4,*5,*6,*7,*8,*11 or *45) cause absent enzymatic activity and, possibly, an increased risk of adverse drug reactions even with routine therapy with CYP2D6 substrates. CYP2D6*2,*3,*4,*5,*6 form 93–97% of the PM phenotypes in Caucasians while other inactivating alleles form less than 1% [22,23]. CYP2D6*4 is the most common defective allele in Caucasians with a frequency of 20–25% in both heterozygous and homozygous forms but only homozygous CYP2D6*4 is associated with the PM phenotype [24]. Heterozygous and homozygous CYP2D6*4 were found with a frequency of 9% in

| New Genotypes | DNA change | Protein change |
|---------------|------------|----------------|
| Genotype 1    | Homozygous SNPs: Intron 1 conversion with CYP2D7 (214–245); 310G>T; 843T>G; 4180G>C. Heterozygous SNPs: 2850C>T; 3384A>C; 3790C>T; | H94R; R296C; S486T |
| Genotype 2    | Homozygous SNPs: 310G>T. Heterozygous SNPs: 2606G>A/G; 2610T>A/T | E278K; M279K |
| Genotype 3    | Homozygous SNPs: 310G>T; 1661G>C; 3183G>A; 3834A>C; 41180G>C. Heterozygous SNPs: 100C>T/C; 1093C>T/C; 2850C>T/C; 4401C>T/C | P34S; R296C; V338M; S486T |
| Genotype 4    | Homozygous SNPs: 310G>T; 1661G>C; 2850C>T; 3384A>C; 3790C>T; | N166D; R296C; Splicing defect; S486T |
| Genotype 5    | Homozygous SNPs: 4481G>A. Heterozygous SNPs: Intron 1 conversion with CYP2D7 (214–245); 1749A>G/A; 1783A>C/A; 2988G>A/G | N166D; R296C; S486T |

**Table 2.** New genotypes found in CYP2D6 among our UAE study group.

**Table 3.** CYP2D6 allele frequencies among 151 (302 alleles) UAE nationals.
African-Americans have high frequencies of CYP2D6*17, with 25% of Asians classified as IMs [12]. In the UAE, frequencies of *1 and *2 were *10% [26,30]. In UAE, the most common duplicated alleles are *1 and *2 where CYP2D6*2xn occurred in 4.3% which is close to Palestinian’s frequency (4.9%) [27]. The number of active alleles in the sample studied (n = 151).

| Number of active alleles | N | % |
|--------------------------|---|---|
| 0 active alleles         | 3 | 2 |
| 1 active allele          | 27| 17.9 |
| 2 active alleles         | 104 | 68.9 |
| >2 active alleles        | 17 | 11.3 |

Table 5. The observed distribution of CYP2D6 known genotypes and their predicted phenotypes in the recruited individuals (n = 151).

| #   | CYP2D6 genotype | Predicted phenotype | N   | %   | No of active alleles |
|-----|------------------|----------------------|-----|-----|----------------------|
| 1   | *1/*1(wild type) | Homozygous EM       | 31  | 20.5| 2                     |
| 2   | *1/*xn           | Homozygous UM       | 1   | 0.7 | 3                     |
| 3   | *1/*2            | Heterozygous EM     | 17  | 11.3| 2                     |
| 4   | *1/*2xn          | Heterozygous UM     | 5   | 3.3 | 3                     |
| 5   | *2/*2            | Homozygous EM       | 3   | 2   | 2                     |
| 6   | *2xn/*2          | Homozygous UM       | 1   | 0.7 | 3                     |
| 7   | *2/*4            | Heterozygous EM     | 3   | 2   | 1                     |
| 8   | *2xn/*4          | Heterozygous EM     | 2   | 1.3 | 2                     |
| 9   | *2/*41           | Heterozygous EM     | 2   | 1.3 | 2                     |
| 10  | *2xn/*41         | Heterozygous UM     | 1   | 0.7 | 3                     |
| 11  | *2/*39           | Heterozygous EM     | 4   | 2.6 | 2                     |
| 12  | *2xn/*39         | Heterozygous UM     | 2   | 1.3 | 3                     |
| 13  | *2xn/*10         | Heterozygous UM     | 1   | 0.7 | 3                     |
| 14  | *2xn/*17         | Heterozygous UM     | 1   | 0.7 | 3                     |
| 15  | *2/*29           | Heterozygous EM     | 1   | 0.7 | 2                     |
| 16  | *1/*41           | Heterozygous EM     | 14  | 9.3 | 2                     |
| 17  | *1xn/*41         | Heterozygous UM     | 1   | 0.7 | 3                     |
| 18  | *1/*4            | Homozygous EM       | 8   | 5.3 | 1                     |
| 19  | *1/*35           | Heterozygous EM     | 1   | 0.7 | 2                     |
| 20  | *1/*10           | Heterozygous EM     | 2   | 1.3 | 3                     |
| 21  | *1xn/10          | Heterozygous UM     | 1   | 0.7 | 3                     |
| 22  | *1/*34           | Heterozygous EM     | 3   | 2   | 2                     |
| 23  | *1/*17           | Heterozygous EM     | 1   | 0.7 | 2                     |
| 24  | *1/*27           | Heterozygous EM     | 1   | 0.7 | 2                     |
| 25  | *1xn/*27         | Heterozygous UM     | 1   | 0.7 | 3                     |
| 26  | *1/*29           | Heterozygous EM     | 1   | 0.7 | 2                     |
| 27  | *1xn/*39         | Heterozygous UM     | 1   | 0.7 | 4                     |
| 28  | *1/*46           | Heterozygous EM     | 1   | 0.7 | 2                     |
| 29  | *2/*4            | Homozygous PM       | 3   | 2   | 0                     |
| 30  | *2/*41           | Heterozygous IM     | 6   | 4   | 1                     |
| 31  | *2/*49           | Heterozygous IM     | 1   | 0.7 | 1                     |
| 32  | *2/*43           | Heterozygous IM     | 1   | 0.7 | 1                     |
| 33  | *41/*41          | Homozygous IM       | 8   | 5.3 | 2                     |
| 34  | *35/*35          | Homozygous EM       | 1   | 0.7 | 2                     |
| 35  | *10/*10          | Heterozygous IM     | 1   | 0.7 | 2                     |
| 36  | *29/*29          | Homozygous IM       | 1   | 0.7 | 2                     |
| 37  | *17/*17          | Heterozygous IM     | 1   | 0.7 | 2                     |
| 38  | *17/*41          | Heterozygous IM     | 1   | 0.7 | 2                     |
| 39  | *17/*39          | Heterozygous EM     | 1   | 0.7 | 2                     |
| 40  | *10/*41          | Heterozygous IM     | 1   | 0.7 | 2                     |
| 41  | *10xn/*41        | Heterozygous UM     | 1   | 0.7 | 3                     |
| 42  | *10/*35          | Heterozygous EM     | 1   | 0.7 | 2                     |
| 43  | *35/*39          | Heterozygous EM     | 2   | 1.3 | 2                     |
| 44  | *39/*41          | Heterozygous EM     | 2   | 1.3 | 2                     |

EM extensive metabolizer, PM poor metabolizer, UM ultrarapid metabolizer and IM intermediate metabolizer. doi:10.1371/journal.pone.0028943.t005

the Emirati population which is similar to the Palestinians (7.8%) [25] and a bit lower than the Croatians (14%) [20] but not as high as what was reported in other Caucasians [12,24]. Three individuals (2%) were homozygous for CYP2D6*4 and were categorized as being PMs. Other defective alleles such as (*9,*5 and*6) could not be detected perhaps due to the small sample size (n = 151) we used for genotyping. Because of the fact that patients with CYP2D6*4 are PMs they are more likely to develop side effects with normal doses of CYP2D6-dependent antidepressants, particularly because most of these drugs have narrow therapeutic windows. Therefore drugs like tricyclic antidepressants (TCAs), which have a narrow therapeutic window and venlafaxine are going to pose problems in these patients. Therefore lower doses to approximately half the therapeutic doses of TCA are recommended [26,27]. The opposite is true of UMs who need higher doses of CYP2D6-dependent drugs to reach therapeutic levels.

Individuals were considered as IMs if they had one CYP2D6 allele with a decreased enzyme activity (*9,*10,*17,*29 or *41) together with a non-functional allele (e.g. *3,*4,*41n/*5,*6,*7,*8 or *11) [23] or if they had two partially functional alleles. Among the 17 variant CYP2D6 alleles that were found in the UAE population, CYP2D6*41 allele had a high frequency of 15.2% which is higher than what was reported in other Caucasians (8.5%) [1], while closer to what was reported in Palestinians (12.7%) [25]. CYP2D6*41 occurs in 22% of Ethiopians [28]. In Asia (1%) and Africa (1–2%), it occurs in a lower frequency than in Caucasians (10%) [26]. CYP2D6*41/*null form nearly 50–70% of all Caucasian IMs [29]. In this study group 4.6% had CYP2D6*41/*null (*41/*4) genotype while 8.6% had CYP2D6*IM/*IM (e.g.*41/*41, *10/*10, IM/*41 and *17/*17). CYP2D6*10 and *17 cause a decrease in the enzyme activity. The frequency of CYP2D6*10 is relatively high in Asia (51%) and therefore nearly 25% of Asians are classified as IMs [12]. Black Africans and African-Americans have high frequencies of CYP2D6*17 allele (up to 35%) and that is the reason why the majority of them are IMs [16]. On the other hand, null alleles are much rarer in Asia and Africa than in Caucasians [1]. CYP2D6*10 and CYP2D6*17 were found in UAE at a low frequencies of 3.3% and 2% respectively, and this was expected as they occurred in lower frequencies in some other Caucasians.

UMs are characterized by faster rate of drug metabolism caused by the presence of multiple active functional CYP2D6 genes (at least 3 copies) on one individual allele. Consequently these subjects do not reach the therapeutic plasma levels even at normal drug doses. Alleles with multiple gene copies were found in high frequencies only in some regions such as Saudi Arabia (21%) [21] and Ethiopia (29%) [28], while in the rest of the world the frequencies do not exceed 10% [26,30]. In UAE, the most common duplicated alleles are *1 and *2 where CYP2D6*2xn occurred in 4.3% which is close to Palestinian’s frequency (4.9%)
informed consent was obtained from all subjects.

Materials and Methods

Subjects blood sampling

Ethics Statement. This study was approved by Al-Ain District Human Research Ethics and the Faculty of Medicine and Health Sciences, UAE University Committee (07/148) and District Human Research Ethics and the Faculty of Medicine and Health Sciences, UAE University Committee (07/148) and

PCR amplification of the CYP2D gene and DNA sequencing

For each patient the produced sequences were aligned with the original CYP2D6 sequence (NM_000106.4) using ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/) to detect the presence of SNPs or mutations as well as to define each patient’s genotype. Expsa Translate tool (http://us.expasy.org/tools/dna.html) was used to translate DNA sequences into their protein sequences to examine the influence of the DNA changes at the protein level and thus the enzymatic activity.

Statistical analysis

The statistical analysis was conducted by SPSS 17.0 (for windows) to calculate frequencies. The level of significant of P value was set at less than 0.05.

Supporting Information

Table S1 Primers for PCR amplification of CYP2D6 gene (exons 1–9).

Table S2 Primers for CYP2D6 sequencing.
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Author Contributions

Conceived and designed the experiments: SMAB YMA OO. Performed the experiments: RYQ NAA OO. Analyzed the data: SMAB YMA RYQ NAA. Contributed reagents/materials/analysis tools: RYQ OO. Wrote the paper: SMAB YMA NAA RYQ. Designed the software used in the analysis: BRA NAA.

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