Single nucleotide and structural variants of CYP2D6 gene in Kinh Vietnamese population

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Abstract
CYP2D6 genetic variations could result in alteration of CYP2D6 enzyme activity, leading to dissimilarity among individuals in regard of drug metabolism.

This study aims to detect all genetic variants, allele, and genotype frequencies of CYP2D6 gene in 136 unrelated healthy Kinh Vietnamese volunteers. All single nucleotide variants (SNVs) and structural variations (SVs) of CYP2D6 gene were identified by Sanger sequencing and multiplex ligation-dependent probe amplification (MLPA) assay. Totally, 30 SNVs and 9 SVs including a whole gene deletion, 8 hybrid structures, and tandem arrangements were identified. Of the 7 novel SNVs detected, the 3157G>T (P329L) substitution was predicted to be deleterious by PROVEAN; the 3581G>A (W358X) variant resulted in a truncated protein; and the 2988G>A variant located in the intron 6 was predicted to be capable of modifying splicing motif by Human Splicing Finder. We determined 29 different genotypes of CYP2D6 from 136 individuals. The most common alleles were the CYP2D6*10 (43.75%), *1 (18.75%), and tandem arrangement *36-*10 (12.13%).

This study provides best information on CYP2D6 polymorphism comprising the newly discovered SNVs, structural variations, and their frequencies in Kinh Vietnamese. These new data would be valuable in view of precise dosing of CYP2D6 metabolized drugs and giving better treatment outcome.

Abbreviations: EM = extensive metabolizer, IM = intermediate metabolizer, LD = linkage disequilibrium, LR-PCR = Longrange PCR, MLPA = multiplex ligation-dependent probe amplification, PM = poor metabolizer, SNVs = single nucleotide variants, SVs = structural variations, UM = ultrarapid metabolizer.

Keywords: CYP2D6 gene, drug metabolism, Kinh Vietnamese, single nucleotide variants, structural variations

1. Introduction
The CYP2D6 gene encodes CYP2D6 enzyme that is involved in metabolism of approximately 25% commonly prescribed drugs on the market.[1] Polymorphism of CYP2D6 can lead to interindividual difference in clearance of many drugs such as: antidepressant, anticancer, antipsychotics, anti-arrhythmic, and opioid. According to Pharmacogene Variation Consortium (https://www.pharmvar.org), there are over 100 allelic variants of CYP2D6 gene in Kinh Vietnamese population.

CYP2D6 has been shown to have SNVs consisting of copy number variations (CNVs), conversions (structures carry a small region derived from CYP2D7), and structural rearrangements (hybrid genes and tandems) with CYP2D7 pseudogene. The phenotypes of CYP2D6 were classified according to enzyme activation: poor metabolizers (PMs), intermediate metabolizers (IMs), extensive metabolizers (EMs), and ultrarapid metabolizers (UMs). CYP2D6 enzyme activation alteration can result in variability of drug response, in which PM and UM phenotypes would be at risk of adverse effect or therapeutic failure. There is a wide variability in distribution of CYP2D6 variants among different ethnic groups all over the world. The PMs (CYP2D6*3, *4 and *5) were mainly found in Europe whereas percentages of these variants in Asia were very low (0.2%-0.7%).[2] The UM presented in North Africa and Oceania while IMs primarily located in Asia due to high frequency of CYP2D6*10.[3] Although CYP2D6 allele frequencies have been evaluated in many ethnic groups, there was limited information of SVs discrimination of this gene.[3,5] To date, CYP2D6 genetic polymorphisms in Vietnamese have been reported by 3 studies. Using pyrosequencing, Veiga et al[6] genotyped 78 Vietnamese and obtained frequencies for 4 CYP2D6 alleles *1, *4, *5, and *10. Kim et al[7] applied multiplex single-base extension to identify 12 CYP2D6 SNPs and a duplication in 758 Korean, 89 Chinese, and 122 Vietnamese. Love et al[8] used TaqMan genotyping assay to detect frequencies of 8 CYP2D6 alleles in 138 Filipino and 86 Vietnamese women for tamoxifen therapy in breast cancer. Taken together, these works reported the existence and frequencies of only 14 well-known alleles and lacked polymorphisms (SNPs) and insertions/deletions of a small number of nucleotides. In addition to single nucleotide variants (SNVs), CYP2D6 has been shown to have SNVs consisting of copy number variations (CNVs), conversions (structures carry a small region derived from CYP2D7), and structural rearrangements (hybrid genes and tandems) with CYP2D7 pseudogene. The phenotypes of CYP2D6 were classified according to enzyme activation: poor metabolizers (PMs), intermediate metabolizers (IMs), extensive metabolizers (EMs), and ultrarapid metabolizers (UMs). CYP2D6 enzyme activation alteration can result in variability of drug response, in which PM and UM phenotypes would be at risk of adverse effect or therapeutic failure. There is a wide variability in distribution of CYP2D6 variants among different ethnic groups all over the world. The PMs (CYP2D6*3, *4 and *5) were mainly found in Europe whereas percentages of these variants in Asia were very low (0.2%-0.7%).[2] The UM presented in North Africa and Oceania while IMs primarily located in Asia due to high frequency of CYP2D6*10.[3] Although CYP2D6 allele frequencies have been evaluated in many ethnic groups, there was limited information of SVs discrimination of this gene.[3,5] To date, CYP2D6 genetic polymorphisms in Vietnamese have been reported by 3 studies. Using pyrosequencing, Veiga et al[6] genotyped 78 Vietnamese and obtained frequencies for 4 CYP2D6 alleles *1, *4, *5, and *10. Kim et al[7] applied multiplex single-base extension to identify 12 CYP2D6 SNPs and a duplication in 758 Korean, 89 Chinese, and 122 Vietnamese. Love et al[8] used TaqMan genotyping assay to detect frequencies of 8 CYP2D6 alleles in 138 Filipino and 86 Vietnamese women for tamoxifen therapy in breast cancer. Taken together, these works reported the existence and frequencies of only 14 well-known alleles and lacked...
information of other known alleles, especially possible novel variants in the whole CYP2D6 gene for the Kinh Vietnamese population. Therefore, this study aimed to detect all variants of CYP2D6 gene and their frequencies in the Kinh Vietnamese by Sanger sequencing combined with multiplex ligation-dependent probe amplification (MLPA) assay. The obtained data would provide further understanding of CYP2D6 pharmacogenetics for Vietnamese population.

2. Materials and methods

2.1. Subjects and DNA extraction

One hundred and thirty six of unrelated healthy Kinh volunteers (85 women and 51 men) from Hanoi Medical University of Vietnam were recruited. All subjects were regarded as healthy according to medical history and physical examination. The study was approved by all individuals and the informed consent was obtained from each study subject before sample collection. This project was approved by the Institutional Review Board (IRB) of the Institute of Genome Research, Vietnam Academy of Science and Technology. For all subjects, 2mL of whole blood was collected and preserved in ethylenediaminetetraacetic acid (EDTA) containing tubes. Genomic DNA was extracted from the blood samples using E.Z.N.A Blood DNA Mini Kit (Omega-Biotech, Norcross, Georgia, USA) according to manufacturer’s protocol.

2.2. DNA sequencing

Sanger sequencing method was used to identify nucleotide variants in the promoter, entire 9 coding exons and their flanking regions of CYP2D6 gene. Primers provided by PHUSA Biochem Company (Can Tho, Vietnam) were listed in Supplementary Table 1, http://links.lww.com/MD/D12. Polymerase chain reaction (PCR) reaction was performed with a total volume of 20μL containing: 10 ng total genomic DNA, 1× DreamTaq Master mix (Thermo Fisher Scientific, Waltham, Massachusetts), 10 pmole each primer, 1μL DMSO (Bioworld, Dublin, Ohio), and 16.5 μL deionized water. The thermo cycle was as following: denaturation at 94°C for 5 minutes, following by 40 cycles of 95°C for 15 seconds, 58°C for 30 seconds, 72°C for 40 seconds to 2 minutes, and a final extension at 72°C for 5 minutes. For exon 5 to exon 8, the thermo cycle was as following: denaturation at 95°C for 2 minutes, following by 35 cycles of 95°C for 30 seconds, 59°C for 30 seconds, 72°C for 2 minutes, and a final extension at 72°C for 5 minutes. PCR products were purified using Multiscreen PCR 96 Filter Plate (Merck-Millipore, Burlington, Massachusetts) and were then sequenced using ABI Prism BigDye Terminator Cycle Sequencing Kit Version 3.1 (Applied Biosystems), on an ABI genetic analyzer 3500 (Applied Biosystems).

2.3. Multiplex ligation-dependent probe amplification (MLPA) assay

To identify deletions/duplications on CYP2D6 gene, MLPA technique was performed using the commercial SALSA MLPA P128-C1 Cytochrome P450 Probenmix kit (MRG-Holland, Amsterdam, Netherlands) following the manufacturer’s protocol and collected data were analyzed by Coffalyser.net software. The MLPA probes were specifically designed to amplify exons 1, 5, 6, and 9. For each genomic DNA sample, 50 ng was used for this assay.

2.4. Longrange (LR) PCR

Primers used in LR-PCR were described in previous study.[10] For detection of CYP2D6*5, a 25 μL long PCR reaction was performed. The reaction mixture consisted of: 100 ng template DNA, 1X Gotaq Long Mastermix (Promega, Fitchburg, Wisconsin), 0.16 μM of each primer DUp and Dlow, 0.32 μM of each primer DPKup and DPKlow, 10.5 μL double-distilled water. The thermo cycle was as following: denaturation at 94°C for 2 minutes, following by 35 cycles of 94°C for 20 seconds, 70°C for 5 minutes, and a final extension at 72°C for 10 minutes. All amplified fragments were subsequently analyzed on 0.8% agarose gel.

2.5. Data analysis

Biiodit software was used for initial analysis of the sequences. The CYP2D6 variants were named based on the nucleotide reference sequence (NCBI reference sequence: M33388.1) and CYP Allele Nomenclature (https://www.pharmvar.org/). Variants not reported in database of CYP Allele Nomenclature Committee were subsequently searched in NCBI SNP database (dbSNP) and 1000 Genomes Data (http://www.internationalgenome.org) were assigned as novel variants. Assessment of Linkage Disequilibrium (LD) for SNP variants was accomplished using HAPLOVIEW 4.2 (https://www.broadinstitute.org/haploview/version-42-15-september-2009).

2.6. Statistical analysis

Inter-ethnic comparison of CYP2D6 allele frequencies and difference of allele frequencies between Kinh Vietnamese and global populations were assessed by Chi-square (χ²) and Fisher exact test. P <.05 was considered as statistically significant.

2.7. In silico analysis of novel variants

The online tools as MATCH, PROVEAN (http://provean.jcvi.org/index.php/Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/), and Human Splicing Finder (http://www.umd.be/HSF3/) were used to search potential transcriptional factor binding sites in CYP2D6 promoter,[10] predict possible impact of non-synonymous SNPs in the coding regions of CYP2D6[11,12] and predict possible impact of SNPs in introns of CYP2D6.[13]

3. Results

3.1. Genetic variants and linkage disequilibrium analysis

The Sanger sequencing analysis identified totally 30 SNVs including 7 novel and 23 previously reported on the human CYP2D6 Pharmacogene Variation data (https://www.pharmvar.org/). Of the 7 novel variants, there were 3 in the promoter (−498C>A, −184A>T, −175A>T), 2 in the intron 4 and 6 (2137G>C, 2988G>A), 2 in the exon 7 and 8 (3157G>T, 3851G>A) of the CYP2D6 gene. For the 23 known SNPs, 2 were located in the promoter, 12 in introns, and 9 in exons (Table 1).

Linkage Disequilibrium analysis was performed to examine the SNPs relationship by Haplovie 4.2 software. We identified only one LD block spanning a region from 214 in intron1 (marker 214G>C) to 232 in intron 1 (marker 232G>C) and these markers showed tight correlation with LOD #2 and D’ ≤1 (Fig. 1).
To acquiring copy number information of the CYP2D6 gene, the MLPA assay measuring the dosage of the exon 1, exon 5, exon 6, and exon 9 was applied to all 136 study subjects. The dosage of the exon 1, 5, 6, and 9 based on the ratio of the peak height of the reference versus the subjects was summarized in the Table 2. There were total 12 different types of copy number chances, in which the subjects with reduced copy number of all exon 1, 5, 6, and 9 were subsequently validated by LR-PCR (Supplementary Fig 1, http://links.lww.com/MD/D12).

### 3.2. Functional assessment of CYP2D6 novel variants

To predict where the 3 novel variants in the promoter region of CYP2D6 have ability to regulate translation of gene, the promoter sequences containing each of novel variants (−498C>A, −184A>T, −175A>T) was analyzed using MATCH bioinformatic tool. However, none of them shown to be overlapped with the transcriptional factor binding sites in CYP2D6 promoter.

Of the 2 novel variants located in the coding regions, the non-synonymous mutation 3851G>A (exon 8) resulted in a premature stop codon instead of tryptophan at position 385 (W385X) and a truncated, usually reduced or no functional protein product. The other one, 3157G>T (exon 7) causing the amino acid substitution from arginine to leucine at the position 329 (R329L), was evaluated for the probable effect by using Polyphen-2 and PROVEAN online tools. As a consequence, both HumDiv and HumVar trained Polyphen-2 models predicted that this variant was benign. In contrast, PROVEAN tool indicated that the R329L mutation would be deleterious (score = −4.326) (Fig. 2A and B).

Finally, the possible impact of novel mutations detected in intron 4 and 6 was performed using Human Splicing Finder online tool. The Position Weight Matrices were developed in order to assess the strength of 5′ and 3′ splice site and branch points. As a result, the novel variant 2988G>A located in the intron 6 was predicted to affect branching points and was capable of modifying splicing motif (Fig. 2C). Meanwhile, no impact was found when examining the remaining novel variant 2137G>C in the intron 4.

### 3.3. Genotype frequency and allelic frequencies

To determine the genotypes of CYP2D6, we combined the sequencing-based genotypes and copy number alterations identified by MLPA of each individual. Consequently, 9 SVs presenting in Kinh population containing an entire gene deletion and 8 hybrid/tandem rearrangements were explored as shown in the Fig. 3. In 136 subjects, 29 different genotypes with the frequency varied from 0.7% to 22.8% were assigned (Table 2). Among these, the most abundant genotypes were the homozygous *1/∗1 (22.8%) and *1/*10 (15.4%) and *10/36-*10 (11%). Other genotypes detected in over 5% of participants were *2/∗10 (6.6%) and *1/∗1 (5.9%).

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**Table 1**

**CYP2D6 variants in Kinh Vietnamese population identified by Sanger sequencing.**

| Number | Region         | Nucleotide change | Position | SNP ID | Genbank | Effects          | Number of detected subjects |
|--------|----------------|-------------------|----------|--------|---------|------------------|----------------------------|
| 1      | Promoter       | C>T               | (−740)   | rs28624811 | Not translated | 26/136           |
| 2      | Exon 1         | G>A               | (−678)   | rs28633410 | Not translated | 32/136           |
| 3      | Intron 4       | G>C               | (−508)   | rs1080995  | Not translated | 16/136           |
| 4      | Exon 5         | A>T               | (−184)   | Novel      | Not translated | 1/136            |
| 5      | Intron 4       | A>T               | (−175)   | Novel      | Not translated | 10/136           |
| 6      | Exon 1         | C>T               | 100      | rs1065852  | Novel          | 110/136          |
| 7      | Intron 1       | T>AT              | 137–138  | rs77467100 | 47 frameshift  | 1/136            |
| 8      | Exon 1         | G>C               | 214      | rs1080995  | Not translated | 25/136           |
| 9      | Intron 3       | G>A               | 221      | rs1080996  | Not translated | 26/136           |
| 10     | Intron 4       | G>C               | 223      | rs1080997  | Not translated | 22/136           |
| 11     | Intron 4       | T>C               | 227      | Not translated | 27/136       |
| 12     | Exon 5         | G>C               | 232      | Not translated | 34/136       |
| 13     | Exon 6         | A>C               | 233      | Not translated | 25/136       |
| 14     | Exon 5         | G>C               | 245      | Not translated | 21/136       |
| 15     | Exon 7         | T>G               | 310      | Not translated | 116/136      |
| 16     | Exon 2         | C>T               | 1039     | rs1081003  | None           | 114/136          |
| 17     | Exon 3         | G>C               | 1661     | rs1058164  | None           | 125/136          |
| 18     | Intron 3       | G>A               | 1759     | rs5030865  | G169R         | 2/136            |
| 19     | Intron 3       | A>G               | 1846     | rs3892097  | Not translated | 3/136            |
| 20     | Intron 4       | A>G               | 2097     | rs2267447  | Not translated | 115/136          |
| 21     | Intron 4       | G>C               | 2137     | Novel      | Not translated | 1/136            |
| 22     | Exon 6         | A>C               | 2581     | rs16047    | Novel          | 2/136            |
| 23     | Exon 6         | G>A               | 2968     | Novel      | Not translated | 6/136            |
| 24     | Intron 7       | G>A               | 3157     | Novel      | R320L         | 1/136            |
| 25     | Exon 7         | A>C               | 3384     | rs1985842  | Not translated | 66/136           |
| 26     | Intron 7       | C>T               | 3700     | rs4987144  | Not translated | 33/136           |
| 27     | Exon 8         | G>A               | 3851     | Novel      | W358*         | 2/136            |
| 28     | Exon 9         | C>G               | 4161     | rs1135840  | S486T         | 82/136           |

The nucleotide position is according to reference sequence M33388.1 in NCBL, nucleotides were numbered according to base A in the initiation codon ATG as +1.
Except the wild type genotype, *10/*10, *36-*10/*36-*10 and *60/*60, all remaining genotypes were found as heterozygous.

For all population, there are total 9 CYP2D6 variant alleles, 8 hybrid structures or tandem arrangements, and a whole gene deletion were identified which accounted for 75.37%, 16.54%, and 8.09% of 136 individuals, respectively (Table 3). Among 9 variant alleles, the CYP2D6*10, known for decreased function, showed highest frequency (43.75%) while, the normal function alleles CYP2D6*1a and CYP2D6*2 accounted for 18.75% and 7.35%, respectively. Additionally, we identified 5 non-functional alleles, of which percentage varied from 0.37% to 8.09% (*4, *5, *14, *15, *36). Three alleles of unknown function were *60, *65, and *86 accounting for 0.74%, 2.94%, and 0.37%. Of the detected tandem arrangements, the most prominent variant was *36-*10 (12.13%) — a structure that the *36 hybrid was located upstream of *10 copy. Other tandem arrangements had frequencies ranged from 0.37% to 1.47%. The tandem types *13-*1 and *13-*2 had normal function while *36-*10 and *36-*36-*10 had reduced function. Both of *13 and *68-*4 types had no function (Table 3). We did not find any individual with homozygous deletion (*5/*5) as well as duplication (increased function) of CYP2D6 in this study.

3.4. Comparison of CYP2D6 allele frequencies among global populations

We further compared frequency of CYP2D6 alleles associated with enzyme activity alteration between Kinh Vietnamese and other populations from various geographical areas in the world (Table 4). Among nonfunctional alleles, the CYP2D6*4 frequency was significantly lower than those in other populations located in Europe and America (P < .0001). In Asian countries, the null allele CYP2D6*5 prevalence was found almost similar except Thailand (P < .05). Other alleles having no enzyme activity (*13, *14, and *15) showed no significant difference compared with populations in global. Among decreased functional alleles, it was shown that CYP2D6*10 frequency statistically higher than that found in Europe and America (P < .001) as well as in other remaining parts of the world. Concerning CYP2D6*36 percentage, this allele was shown to be of lower significance than that seen in Thai (P < .0001) but equal to those in Han Chinese, Papua New Guineans, and South Africa.

4. Discussion

To our knowledge, this is the first study applied both the Sanger sequencing and MLPA technique to identify all the CYP2D6 variants presenting in Kinh Vietnamese population. Therefore, we could newly uncovered not only all SNVs located in the 5' upstream region, 9 exons and their flanking regions but also the recurrent SVs of the CYP2D6 gene. In fact, more than half (40/76) of CYP2D6 genotypes carrying *10 allele, which were assigned by only sequencing, were reassigned to genotypes consisted of hybrid structure (*13, *36, *67) and tandem hybrids (*36-*10, *36x2-*10, *68-*4) after copy number was analyzed.
by MLPA. Furthermore, there were 22/136 individuals harboring *5, *13-*1, and *13-*2 alleles, which were only indentified after MLPA result obtained.

According to our examination, the percentage of CYP2D6*10 assigned in the previous studies possibly involved structural variants. Currently, available methodologies for CNV detection comprised of conventional methods (Long distance and multiplex PCR, Realtime PCR) and microarrays hybridization based methods (array comparative genotypic hybridization, Roche Amplichip CYP450 system, Affymetrix DMET plus microarray), but their limitations are low throughput, low reproductivity, and costly. MLPA technique has been shown to be high-throughput, cost effective, and accurate, and was widely applied in genetic testing in various inherited diseases of human.

By sequencing we found 7 novel SNVs and it was interesting to predict whether they might have impact on gene regulation or function. Among 3 new SNVs identified in the noncoding regions, only variant 2988G>A (intron 6) was shown the ability to have harmful effect on branching point thereby may cause of abnormal pre-mRNA splicing. Both novel variants found in coding regions might have effect on protein activity because the variant 3851G>A (W385X) in the exon 8 would be translated into a truncated protein while the other one, 3157G>T (R329L) in the exon 7, was predicted as deleterious by PROVEAN. In fact, the truncated protein resulted from 3851G>A would lead to a nonfunctional protein or the enzyme thereby would have no activity. However, the impact of 3157G>T mutation should be further clarified by functional analysis, in which the metabolizer status of mutant protein will be investigated in vitro and/or in vivo animal model or cell culture systems. Based on the activation of encoded proteins, we can determine whether their functions are increased/decreased or not affected. Until very recently, 2 volunteers carrying 3157G>T (R329L) and another with 3851G>A (W385X) have informed us that they have no health problem.

The prevalent of the allele CYP2D6*10 in Kinh Vietnamese from this study is in consistent with previous findings in Kinh Vietnamese as well as in other Asian populations. Statistical analysis also demonstrated significant higher frequency of CYP2D6*10 in Kinh Vietnamese compared with other worldwide regions except for Asian countries. Our work further approved the high prevalence of CYP2D6*10 retracted to Asian populations. Meanwhile, the nonfunction allele CYP2D6*4 was very rare in our study subjects as well as in other Asian populations. This was significant difference compared with figure observed in Europe, America, and Middle East, where CYP2D6*4 was reported with high frequency. This result is in accordance with previous works, in which a gradual decrease of CYP2D6*4 from Europe to Asia countries was reported. Although CYP2D6*36 frequency (0.37%) was shown significantly lower than that in Thailand (16.4%), however, similar figures were reported in various populations in the

### Table 2: Assignment of CYP2D6 genotype in Kinh Vietnamese population.

| Number | Sanger sequencing | CN (E1) | CN (E5) | CN (E6) | CN (E9) | Long range PCR | Final genotype | Frequency (%) |
|--------|-------------------|--------|--------|--------|--------|----------------|----------------|---------------|
| 1      | *1/*1             | 2      | 2      | 2      | 2      | -              | *1/*1          | 5.9           |
| 2      | *1/*2             | 2      | 2      | 2      | 2      | -              | *1/*2          | 4.4           |
| 3      | *1/*10            | 2      | 2      | 2      | 2      | -              | *1/*10         | 15.4          |
| 4      | *1/*86            | 2      | 2      | 2      | 2      | -              | *1/*86         | 0.7           |
| 5      | *1/*14            | 2      | 2      | 2      | 2      | -              | *1/*14         | 0.7           |
| 6      | *2/*10            | 2      | 2      | 2      | 2      | -              | *2/*10         | 6.6           |
| 7      | *2/*65            | 2      | 2      | 2      | 2      | -              | *2/*65         | 2.2           |
| 8      | *4/*10            | 2      | 2      | 2      | 2      | -              | *4/*10         | 0.7           |
| 9      | *60/*60           | 2      | 2      | 2      | 2      | -              | *60/*60        | 0.7           |
| 10     | *10/*10           | 2      | 2      | 2      | 2      | -              | *10/*10        | 22.8          |
| 11     | *10/*15           | 2      | 2      | 2      | 2      | -              | *10/*15        | 0.7           |
| 12     | *10/*65           | 2      | 2      | 2      | 2      | -              | *10/*65        | 2.2           |
| 13     | *1/*10            | 3      | 3      | 3      | 2      | -              | *1/*36-*10     | 1.5           |
| 14     | *10/*10           | 3      | 3      | 3      | 2      | -              | *10/*36-*10    | 11.0          |
| 15     | *10/*65           | 3      | 3      | 3      | 2      | -              | *65/*36-*10    | 0.7           |
| 16     | *10/*10           | 4      | 4      | 4      | 2      | -              | *36-*10/*36-*10| 3.7           |
| 17     | *2/*10            | 3      | 2      | 2      | 2      | -              | *2/*68-*4      | 0.7           |
| 18     | *1/*4             | 2      | 2      | 2      | 3      | -              | *4/*13-*1      | 0.7           |
| 19     | *10/*10           | 3      | 3      | 3      | 1      | Deletion       | *5/*36-*36-*10 | 0.7           |
| 20     | *10/*10           | 2      | 2      | 2      | 1      | Deletion       | *5/*36-*10     | 3.7           |
| 21     | *10/*10           | 1      | 2      | 2      | 2      | -              | *13/*10        | 1.5           |
| 22     | *2/*10            | 1      | 1      | 2      | 2      | -              | *10/*67        | 0.7           |
| 23     | *1/*2             | 1      | 1      | 1      | 2      | -              | *5/*13-*2      | 1.5           |
| 24     | *1/*1             | 1      | 1      | 1      | 2      | -              | *5/*13-*1      | 0.7           |
| 25     | *1/*1             | 1      | 1      | 1      | 1      | Deletion       | *1/*5          | 2.9           |
| 26     | *2/*2             | 1      | 1      | 1      | 1      | Deletion       | *2/*5          | 0.7           |
| 27     | *10/*10           | 1      | 1      | 1      | 1      | Deletion       | *5/*10         | 4.4           |
| 28     | *2/*10            | 1      | 1      | 1      | 1      | Deletion       | *5/*65         | 0.7           |
| 29     | *10/*10           | 1      | 1      | 1      | 1      | Deletion       | *5/*36         | 0.7           |

CN = copy number, E1 = exon 1, E5 = exon 5, E6 = exon 6, E9 = exon 9.
world containing Chinese Han, Papua New Guineans, and South African.\textsuperscript{15,32,34} Likewise, numerous studies demonstrated the infrequent existence of CYP2D6\textsuperscript{*36} in Asian populations as well as other worldwide communities.\textsuperscript{15,32,33,34,42} Because CYP2D6\textsuperscript{*10} was shown to be main variant with decreased function found in Kinh Vietnamese, then this is the major factor causing diminished enzyme activity of CYP2D6 in this population. Owing to this evidence, physicians in Vietnam should pay attention on the underlying genetic determinant in

Figure 2. Functional prediction of non-synonymous mutation 3157G>T by Polyphen-2 and Provean (A, B) and splicing effect prediction of intronic variant 2988G>A (C).

Figure 3. Graphic view of CYP2D6 structural variants determined in present study. (A) Reference gene locus of CYP2D6 (red boxes) and pseudo genes CYP2D7 (blue boxes), CYP2D8 (grey boxes), REP6 (dark purple boxes) and REP7 (black boxes) are repetitive sequences located downstream of CYP2D6 and CYP2D7, respectively. (B) Deletion of entire CYP2D6 gene. (C) Hybrid structures of CYP2D6 containing CYP2D6-2D7 (the 5’ portion is derived from CYP2D6 and the 3’ portion is derived from CYP2D7), CYP2D7-2D6 (the 5’ portion is derived from CYP2D7 and the 3’ portion is derived from CYP2D6), (D) CYP2D6 tandem arrangements including 2 or more gene copies that are different with “duplications/multiplications.” * Details of structural variants were reported in Pharmacogene Variation (PharmVar) Consortium (https://www.pharmvar.org/gene/CYP2D6). \textsuperscript{A} Detail of structural variants were reported in Andrea Gaedigk work.\textsuperscript{14}
regard to drugs response, especially CYP2D6\(^*\)10 during treatment for Vietnamese subjects having Kinh genetic background. Individuals who carry >2 copies of CYP2D6 normal alleles (classified as ultra rapid metabolizer) will metabolize CYP2D6 substrates more rapidly, leading to reduction of drug treatment efficacy. No duplication or multiplication of CYP2D6 normal function alleles were detected in present study subjects. However, the influence of CYP2D6 duplication and multiplication on corresponding phenotypes in Vietnam should be considered in future studies.

5. Conclusions

We present for the first time all genetic variants of CYP2D6 including SNVs and SVs in 136 Kinh Vietnamese subjects. Our newly obtained data provide important knowledge for determin-

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### Table 3

Allergic frequencies of CYP2D6 in the Kinh Vietnamese population.

| Variants               | Number | Allele | Frequency (%) | Function   |
|------------------------|--------|--------|---------------|------------|
| Variant alleles        |        |        |               |            |
| 1                      | *\(^1\) | 18.75  | Normal        |
| 2                      | *\(^2\) | 7.35   | Normal        |
| 3                      | *\(^4\) | 0.74   | No function   |
| 4                      | *\(^10\) | 43.75 | Decreased     |
| 5                      | *\(^14\) | 0.37   | No function   |
| 6                      | *\(^15\) | 0.37   | No function   |
| 7                      | *\(^60\) | 0.74   | Unknown       |
| 8                      | *\(^65\) | 2.94   | Unknown       |
| 9                      | *\(^66\) | 0.37   | Unknown       |
| Copy number variant    |        |        |               |            |
| 1                      | *\(^5\) | 8.09   | No function   |

### Table 4

Comparison of CYP2D6 allele frequencies responsible for functional alteration of protein between Kinh Vietnamese and populations in worldwide.

| Regions       | Population     | Number of subjects | Allele frequencies (%) |
|---------------|----------------|--------------------|------------------------|
|               |                |                    | *\(^4\) | *\(^5\) | *\(^10\) | *\(^13\) | *\(^14\) | *\(^15\) | *\(^36\) | *\(^36\)-10 | *\(^36\)-2x-10 | Reference |
| Asia          | Kinh Vietnamese| 136                | 0.74 | 0.89 | 43.75 | 1.547 | 0.37 | 0.37 | 12.13 | 0.37 | Present study |
| Vietnamese    | 74             |                    | 1.4 | 8.0 | 43.5 | – | – | – | – | – | – | [6] |
| Japanese      | 1017           |                    | 0.4 | 6.0 | 42.9 | – | 0.9 | 0.0 | 0.6 | – | – | [15] |
| Chinese Han   | 1954           |                    | 0.8 | 6.2 | 44.1 | – | 0.5 | – | – | – | – | [16] |
| Thai          | 288            |                    | 0.7 | 4.3\(^a\) | 44.6 | – | 1.0 | – | 16.4\(^d\) | – | – | [19] |
| Indian        | 881            |                    | 9.3 | – | – | – | – | – | – | – | – | [19] |
| Europe        |                |                    |                |                |                |                |                |                |                | | |
| Hungarian     | 431            |                    | 18.1\(^f\) | – | 2.4\(^f\) | – | – | – | – | – | – | [20] |
| Italian       | 218            |                    | 17.7\(^f\) | 0.0\(^d\) | – | – | – | – | – | – | – | [21] |
| Macedonian    | 184            |                    | 18.7\(^f\) | 9.1 | 2.7\(^c\) | – | – | – | – | – | – | [22] |
| Greek         | 283            |                    | 17.8\(^f\) | – | – | – | – | – | – | – | – | [23] |
| Czech         | 223            |                    | 22.0\(^d\) | 3.1\(^b\) | – | – | – | – | – | – | – | [24] |
| Danish        | 244            |                    | 19.7\(^f\) | 5.3 | 1.6\(^c\) | – | 0.0 | – | – | – | – | [24] |
| American      | 264            |                    | 10.0\(^d\) | 1.7 | 2.6\(^c\) | – | 0.0 | – | – | – | – | [25] |
| Brazilian     | 1220           |                    | 9.4\(^f\) | 4.6 | 2.1\(^c\) | – | – | – | – | – | – | [27] |
| Canadian      | 90             |                    | 8.6\(^d\) | – | 2.3\(^c\) | – | – | – | – | – | – | [28] |
| Chilean       | 321            |                    | 12.0\(^f\) | – | – | – | – | – | – | – | – | [29] |
| Mexican       | 154            |                    | 10.4\(^f\) | 0.0\(^d\) | 0.6\(^c\) | – | – | – | – | – | – | [30] |
| Oceania       | 39             |                    | 0.0 | 1.3\(^a\) | 2.6 | – | – | – | – | – | – | [31] |
| Papua New Guineas | 88   |          | 0.0 | 3.0\(^a\) | 0.0\(^d\) | – | 0.0 | 0.0 | – | – | – | [32] |
| Africa        |                |                    |                |                |                |                |                |                |                |                |                | |
| African American | 251    |          | 5.4 | 6.6\(^b\) | 3.6\(^c\) | – | 0.0 | 0.0 | – | – | – | [33] |
| South Africa  | 100            |                    | 3.1\(^a\) | 8.7 | 5.1\(^c\) | 0.0 | 0.0 | 0.0 | – | – | – | [34] |
| Middle East   |                |                    |                |                |                |                |                |                |                |                |                | |
| Iranian       | 100            |                    | 12.5\(^f\) | 3.0\(^a\) | 9.0\(^c\) | – | – | – | – | – | – | [36] |
| Saudi Arabsians | 192    |          | – | – | – | 0.3 | – | – | – | – | – | [37] |

\(^a\)\(^P<.05\) compared with data of current study.
\(^b\)\(^P<.01\) compared with data of current study.
\(^c\)\(^P<.001\) compared with data of current study.
\(^d\)\(^P<.0001\) compared with data of current study.
ing metabolic phenotypes of CYP2C6 substrates and optimizing drug therapies in Vietnam. Based on certain CYP2D6 genotype identified, individuals would receive an appropriate medication therapy and avoid the risk of side effect caused by improper dose of drugs.

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