Review Article

นัยสำคัญของภาวะพหุสัณฐานของ *CYP2D*

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บทคัดย่อ

นัยสำคัญของภาวะพหุสัณฐานของ *CYP2D*
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ว. เภสัชศาสตร์อีสาน 2560; 13(1) : 11-27
รับบทความ : 5 มกราคม 2560 **ตอบรับ :** 3 มีนาคม 2560

ไซโตโครม พี450 (CYP450) คือกลุ่มของเอนไซม์ที่มีหน้าที่หลักในกระบวนการเปลี่ยนแปลงทางชีวภาพของสารแปลกปลอม เช่น ยา อาหาร สารเคมี และมลพิษ รวมถึงสารภายในร่างกาย สมรรถนะของเอนไซม์ไซโตโครม พี450 เป็นปัจจัยสำคัญหนึ่งที่ส่งผลต่อเภสัชจลนศาสตร์ (pharmacokinetics) ของยาโดย CYP2D6 เป็นหนึ่งไอโซฟอร์ม ของ CYP450 ที่รับผิดชอบต่อการเมแทบอลิซึมยาส่วนใหญ่ที่ใช้ในทางคลินิก ซึ่งมักเป็นยาที่มีช่วงการรักษาแคบ (narrow therapeutic index) อาทิ ยาต้านซึมเศร้า เช่น amitriptyline สมรรถนะของเอนไซม์ CYP2D6 ได้รับผลกระทบจากปัจจัยทั้งภายในและภายนอกร่างกาย ได้แก่ การแปรผันทางพันธุกรรมของยีนและกระบวนการเหนือระดับพันธุกรรม และยาที่ใช้ร่วมกัน การแปรผันทางพันธุกรรมของยีน *CYP2D6* เกิดจากการได้รับยีนที่กลายพันธุ์จากบรรพบุรุษหรือการกลายพันธุ์ที่เกิดขึ้นภายหลังก็ได้ การแปรผันของสมรรถนะของเอนไซม์ CYP2D6 จากการกลายพันธุ์ของยีนเรียกว่า ภาวะพหุสัณฐาน (polymorphism) ซึ่งมีรายงานความสัมพันธ์ระหว่างภาวะพหุสัณฐานของ *CYP2D6* กับความล้มเหลวทางการรักษาด้วยยาและความเสี่ยงของอาการไม่พึงประสงค์จากยา การศึกษาทางเภสัชจลนศาสตร์ของยามักศึกษาในสัตว์ฟันแทะขนาดเล็กโดยกำหนดใช้ไอโซฟอร์มที่มีลำดับนิวคลิโอไทด์ที่คล้ายคลึงกับ *CYP2D6* ของมนุษย์ (human orthologue) อย่างไรก็ตามยังพบความแตกต่างอย่างชัดเจนระหว่าง *CYP2D6* ของมนุษย์และ *Cyp2d* ของสัตว์ฟันแทะขนาดเล็ก ดังนั้นนิพนธ์ปริทรรศน์นี้จึงมุ่งเน้นทบทวนนัยสำคัญของเอนไซม์ CYP2D6 ในมนุษย์และไอโซฟอร์มของสัตว์ฟันแทะที่คล้ายคลึงกับ CYP2D ของมนุษย์ การแปรผันทางพันธุกรรมและพหุสัณฐานของ *CYP2D6* และผลกระทบทางคลินิกจากพหุสัณฐานของ *CYP2D6*

คำสำคัญ : ไซโตโครม พี450, CYP2D6, ภาวะพหุสัณฐาน, เภสัชพันธุศาสตร์

Significance of *CYP2D* Polymorphism

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Abstract

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IJPS, 2017; 13(1) : 11-27

Received : 5 January 2017 **Accepted :** 3 March 2017

Cytochrome P450 (CYP450) is a superfamily of metabolizing enzymes which play a major role in the biotransformation of xenobiotics, i.e. foods, drug, chemicals, pollutants, and endogenous compounds. The activity of CYP450 is one of the most influent factors on the drug-pharmacokinetics. CYP2D6 is one of CYP450 isoforms which takes responsibility in the metabolism of varieties clinical drugs that usually have narrow therapeutic index such as antidepressants i.e. amitriptyline. The activity of CYP2D6 is affected by both exogenous and endogenous influences, including genetic variation, epigenetic variation, and concurrently used drugs. Genetic variation of *CYP2D6* genes is either hereditary or acquired mutation. The variation of CYP2D6 activity due to genetic mutation is referred to polymorphism. Polymorphism of *CYP2D6* has been reported to associate with failure of pharmacotherapy and risk of adverse effects. Small rodents are often employed for the pharmacokinetic study by using the human orthologue as the determinant, however, the difference of human *CYP2D6* and small rodent *Cyp2d* are remarkable. Hence, this review focuses on significance of human *CYP2D*, its rodent human orthologue, *CYP2D6* genetic variation and polymorphism, and some clinical impacts of *CYP2D6* polymorphism.

Keyword : cytochrome P450, CYP2D6, polymorphism, pharmacogenetics

Introduction

Cytochrome P450 (CYP450) is a group of mixed function oxidation enzymes which metabolize xenobiotics and biosynthesize endogenous compounds such as steroid hormone, bile acid, and fat-soluble vitamin (A, D, E, and K). In 1956, Williams was the first scientist who noticed an unusual peak with a wavelength maximum near 450 nm (Anzencacher & Zanger, 2012). Omura and Sato later characterized this pigment and named it as 'CYP450' which referred to its absorption at 450 nm (Omura & Sato, 1962). There are more than 50 human CYP450 isoforms identified up to now (Martignoni *et al.*, 2006). To group and name CYP450 enzymes, nucleotide sequence similarity is applied (Nelson, 2004). If two sequences are at least 40 percent identical, they will be grouped in the same family. If two

sequence are at least 55 percent similar, they will be belonged in the same subfamily. The name of CYP450 represents the family, subfamily, and gene number. For example, CYP2D6 is an enzyme in family 2, subfamily D, and gene number 6. CYP3A4 is the major isoform of CYP450 in human body which takes place about 30% of all isoform, followed by CYP2D6 (20%), and CYP2C9 (13%) (Zanger *et al*., 2013).

The biotransformation of xenobiotics via CYP450 shows an inter-individual variability due to polymorphism. Polymorphism of the enzyme can result in undesirable outcome and sometimes could be fatal. In many studies of CYP450 activity, rodents are the most frequent selected animal models. Different species of rodents have different CYP450 expression. For example in rodents, such as mice and rats, the isoforms of *Cyp2d* are different from those of human. Mice have 9 functional *Cyp2d* genes which are *Cyp2d9-2d13, Cyp2d22, Cyp2d26, Cyp2d34,* and *Cyp2d40* where humans have only one active *CYP2D6* gene. Although humans and mice have totally different functional genes, yet some isoforms of *Cyp2d* of mice have sequential identity to human *CYP2D6*. For instance mouse *Cyp2d22* have 87% to 90% similarity to human *CYP2D6* DNA sequence. Hence, mouse *Cyp2d22* is considered to be an orthologue of human *CYP2D6*.

CYP2D and its significance

CYP2D is a cluster of genes located on chromosome 22q13.1. The cluster is comprised of one active gene and two pseudogenes which are *CYP2D6*, *CYP2D7P*, and *CYP2D8P*, respectively (Kimura *et al*., 1989; Heim & Meyer, 1992). *CYP2D7P* is a true pseudogene because it has numbers of insertion and deletion within nucleotide sequences where *CYP2D8P* is inactive due to the insertion of the first exon that causes a change in reading frame and a premature stop codon (Kimura *et al*., 1989). *CYP2D6* gene consists of 9 exons and responses for coding of the 497-amino acid protein with molecular weight of 55.8 kDa (Zhou, 2009). Although CYP2D6 is found in low level in the liver, it is responsible for metabolism of about 150 drugs or 20-25% clinical drugs (Kirchheimer *et al*., 2004; Zanger *et al*, 2004; Ingelman-Sundberg, 2005; Bernard, 2006). The common structure of CYP2D6 substrate is basic nitrogen which is 5 to 7 Å away from the site of oxidation as well as alkaloid structures (Wolff *et al*., 1985; Fonne-Pfister & Meyer, 1988). The assumption of mechanism based on protein homology modeling was the formation of an ion pair between the basic nitrogen and acidic amino acid (Koymans *et al*., 1992; Ellis *et al*., 1995). The studies of binding sites of CYP2D6 suggested that Asp^{301} and Glu^{216} play critical roles in the substrate-binding (Paine, 2002; Zanger *et al*., 2004). In addition, the study of Nagy and Oostenbrink (2012) demonstrated the binding model of propanolol, a substrate of CYP2D6, with CYP2D6 enzyme and informed that Phe^{483}, Val^{360}, Glu^{216}, Gly^{373}, and heme of CYP2D6 had functions to bind with its substrates. CYP2D6 were presented to be inhibited by numbers of drugs and chemicals, yet it is not significantly inducible by smoking or alcohol (Bock *et al*., 1994; Glaeser *et al*., 2005; Zhou *et al*., 2009). The substrates and inhibitors of CYP2D6 are including anti-arrhythmia, anti-depressants, anti-psychotics, beta-blockers, opioid analgesics, and anti-neoplastic drugs (Zanger & Hofmann, 2008; Stingl *et al*., 2012), in which most of them have narrow therapeutic window, therefore it is necessary to concern about alteration of the blood concentration from the interaction between CYP2D6 substrates and inhibitors.

The study of Cairns *et al*. (1996) reported that the proximal part of promotor of CYP2D6 displays a positive DR-1 element which is crucial in binding and responding to the presentation of hepatocyte nuclear factor 4-alpha (HNF4α) and chicken ovalbumin upstream promoter transcription factor-1 (COUP-TF-1). The study in mouse of Corchero, *et al*. (2001) revealed that the inactivation of HFN4α leads the reduction of debrisoquine 4-hydroxylase activity, therefore, the balance of HNF4α and COUP-TF-1 should have an influence on expression of CYP2D6. The distribution of CYP2D6 found to be prominent in the liver, but CYP2D6 is also found at lower level in extra-hepatic

tissues, including lung, kidney, intestine, ovary, placenta, keratinocyte, testis, and brain (Siegle *et al*., 2001; Miksys *et al*., 2002; Ding & Kaminsky, 2003; Du *et al*., 2006; Paine *et al*., 2006; Bièche *et al*., 2007; Dutheil *et al*., 2009; Thelen & Dressman, 2009). In gastrointestinal tract, CYP2D6 shows relatively low abundance and less activity compared to that of the liver. So, it is suggested to have minimal effect on drugs first-pass metabolism (Madani *et al*., 1999). Furthermore, CYP2D6 was reported to have a role in a generation of endogenous neuroactive amine, including dopamine and serotonin. Dopamine, a neurotransmitter found mostly in nigrostriatal pathway which has a major role in control voluntary movement, is originally derived from phenylalanine, which then phenylalanine will be transformed to tyrosine via phenylalanine hydroxylase (Haduch *et al*., 2013a). Tyrosine will be further oxidized by tyrosine hydroxylase into dihydroxyphenylalanine (L-dopa). Finally, dopamine will be formed by aromatic-amino acid decarboxylation of L-dopa. In addition to tyrosine hydroxylase pathway, CYP2D6 is an alternative pathway of dopamine formation in the brain (Bromek *et al*., 2010). Nevertheless, the reaction with CYP2D6 directly converts tyrosine to dopamine by skipping the formation of L-dopa. *In vitro* study, CYP2D6 showed the capability of dopamine synthesis by the direct aromatic hydroxylation to tyrosine (Bromek *et al*., 2010). Quinidine was additionally reported to decrease the activity of aromatic hydroxylation of tyramine (Bromek *el al*., 2010). *In vivo* study, the rat model with completely blocked dopamine synthesis pathway was administered exogenous tyramine into the striatum area and then the observational level of extracellular dopamine was markedly increased (Bromek *et al*., 2011). Therefore, these observations indicated that CYP2D6 plays an important role in dopamine formation. A meta-analysis reported that poor metabolizers of CYP2D6 were significantly more susceptible to develop Parkinsonism (Lu *et al*., 2014). CYP2D6 has a relevance to the biosynthesis of serotonin. *In vitro* studies, 5-methyltryptamine was converted into serotonin via *O*-demethylation by CYP2D6 and occurred principally at cerebellum in rat brain microsome (Bromek *et al*., 2010; Haduch *et al*., 2013b). The *in vivo* study on serotonin formation by using microdialysis probe showed that the level of serotonin increased after the administration of 5-methytryptamine and the addition of quinidine demonstrated the inhibition of *O*-demethylation of 5-methyltryptamine (Bromek *et al*., 2013). Hence, the catalysis of serotonin biosynthesis by CYP2D6 may occur in rat brain (Haduch, *et al*., 2013a). According to the information about association of CYP2D6 with dopamine and serotonin biosynthesis, CYP2D6 has been suggested to have an involvement with substance addiction and psychiatric disorders (Haduch *et al*., 2013a). Interestingly, CYP2D6 mRNA was found in human breast and breast tumor tissues (Huang *et al*., 1997) and suggested the benefit for tamoxifen treatment in breast cancer in term of the production of its potent metabolite. Tamoxifen, a selective estrogen receptor modulator used in the treatment of metastasis breast cancer, is mainly metabolized by CYP2D6 into potent anti-estrogenic metabolites, endoxifen and 4-hydroxytamoxifen. Therefore, the level of CYP2D6 expression might affect on tamoxifen therapy (Ter Heine *et al*., 2014). *CYP2D6* phenotype has been investigated by debrisoquine *O*-demethlylation, bufraralol 1'-hydroxylation, dextromethorphan *O*-demethy-lation, and spartine 4-hydroxylation, but only debrisoquine and spartine are solely selective toward CYP2D6 (Kahn *et al*., 1982; Broly *et al*., 1989; Newton *et al*., 1995; Pearce *et al*., 1996; Marcucci *et al*., 2002; Zanger *et al*., 2013).

Orthologues of human CYP2D to small rodents

In the pharmacokinetics study of the drugs, animal models are often employed as the representation of human body. CYP450 is the main metabolizing enzymes in phase I metabolism, hence to identification the pattern of CYP450 expression in animals is important for the selection of an appropriate animal model. *CYP2D6* is the only active *CYP2D* gene in humans. Other species such as rodents, birds, frogs, and non-human primate are reported to express *CYP2D* gene as well. However, the property of encoded

protein, number of isoforms, and amino acid sequences are varied among species. Rodents are the most popular animal models used in drug discovery and biomedical study (Wartha *et al*., 2014). Therefore the identification of the orthologue of human *CYP2D6* and the pattern of *Cyp2d* expression in rodents are essential in the study of these fields.

According to GRCm38.p4 mouse genome assembly (http://www.ncbi.nlm.nih.gov/genome), mouse *Cyp2d* genes locate on chromosome 7q34. For mouse (*Mus musculus*) *Cyp2d*, at least 9 active genes are identified, namely *Cyp2d9-13*, *Cyp2d22*, *Cyp2d26*, *Cyp2d34*, and *Cyp2d40*, and 8 pseudogenes which are *Cyp2d32p*, *Cyp2d33p*, *Cyp2d35-39p*, and *Cyp2d41p*. Mouse *Cyp2d* isoforms share high similarity of amino acid sequence with human *CYP2D6*. Nonetheless, only *Cyp2d22* is declared to be the orthologue of human *CYP2D6*. The overall distribution of mouse *Cyp2d* is similar to which of human *CYP2D6*. The level of human *CYP2D6* and mouse *Cyp2d* were found prominently in the liver with the lesser level in extra hepatic tissues such as kidney and brain (Miksys *et al*., 2005). In intestine, there was a significant different pattern of expression between two species (Emoto *et al*., 2000). For human *CYP2D6*, it was found in the highest level at duodenum and decreased distally until the colon where mouse expression of *Cyp2d* was in colon and decrease proximally (Emoto *et al*., 2000). Despite of different pattern of expression, either human *CYP2D6* or mouse *Cyp2d* expression were in the enterocytes of the intestinal mucosa and concentrated at the tips of villi. Thus, these findings implied that CYP2D6 in the duodenum may have a role in the first pass metabolism in human and is the barrier for harmful xenobiotics. Anyhow, if the metabolite of the certain xenobiotic is toxic, it will become harmful (Zhang *et al*., 1999; Ding & Kaminsky, 2003). In the kidney, both human *CYP2D6* and mouse *Cyp2d* were detected in the highest amount in the proximal tubule (Manns *et al*., 1989; Duclos-Vallee *et al*., 2000). While the expression of mouse *Cyp2d* in the brain was found in the similar pattern to human *CYP2D6* in most of the regions. The pyramidal neurons of frontal cortex layers II and VI expressed the high level of mouse *Cyp2d* and human *CYP2D6* (Miksys *et al*., 2000) whereas Purkinje cells expressed human *CYP2D6* at the very low level and mouse *Cyp2d* at the moderate-to-high level (Miksys *et al*., 2002). In the comparison of catalytic capacity between human *CYP2D6* and mouse *Cyp2d* by using dextromethorphan, mouse *Cyp2d22* were found to be weaker in *O*-demethylation and gave less active metabolite (Yu & Haining, 2006), but mouse *Cyp2d22* tended to project more *N*-demethylation similarly to human CYP3A4 in dextromethorphan metabolism. In addition to dextromethorphan, the metabolism of codeine by mouse *Cyp2d22* appeared to be similar to human CYP3A4 since they metabolized codeine via *N*-demethylation where CYP2D6 metabolize codeine through *O*-demethylation (Yu & Haining, 2006). Interestingly, the addition of quinidine (CYP2D6 inhibitor) and ketoconazole (CYP3A4 inhibitor) did not show the strong inhibition of *O*-demethylation of dextromethorphan via mouse CYP2D22 enzyme. Therefore even mouse *Cyp2d22* is suggested to be identical to human *CYP2D6*, the properties of enzymes were remarkably different (McLaughlin *et al*., 2008; Zhou *et al*., 2015).

In rat (*Rattus norvegicus*), particularly for *Cyp2d*, there are 6 isoforms which are *Cyp2d1-5* and *Cyp2d18* which is the *Cyp2d4* variant (Nelsons *et al*., 2004). Of 6 isoforms, *Cyp2d3* was identified as the human orthologue. The distribution of rat *Cyp2d* mainly found in the liver, and lower abundance was found in kidney and brain. The human orthologue, *Cyp2d3*, was detected prominently in liver, small intestine, and kidney. In rat brain, rat *Cyp2d* expression pattern is similar to which of human in most regions. *Cyp2d4* and *Cyp2d18* are the most abundant isoform of rat *Cyp2d* in the brain. The high level of *Cyp2d* was found in pyramidal neuron of frontal cortex similarly to human (Miksys *et al*., 2000; Miksys *et al*., 2005). However, the moderate-to-high level of rat *Cyp2d* was identified at Purkinje cells whereas it was found in the very low level in human Purkinje neurons (Miksys *et al*., 2000). It was even found in rat adrenal glands,

ovary, testis, and breast (Miksys *et al*., 2002; Zhou *et al*., 2015). Hence *Cyp2d4* and *Cyp2d18* were implied to be important in the neuroactive substances in rat. The comparison studies on catalytic capacity between rat *Cyp2d* and human *CYP2D* revealed that rat microsome derived from liver and brain were capable for metabolism of dextromethorphan (*O*-dextromethorphan) (Zhou *et al*., 2015). Surprisingly, all rat CYP2D enzymes, except CYP2D1, can metabolize debrisoquine and bufuralol which are well-established substrates for human CYP2D6 (Hiroi *et al*., 2002). Although *Cyp2d4* was reported to be the richest in rat brain, it only showed the capability of metabolizing dextromethorphan, not imipramine and desipramine which are human-CYP2D6-substrate anti-depressants (Zhou *et al*., 2015). Effects of the addition of quinine to rat and human 4-hydroxylation of debrisoquine were different; human CYP2D6 and rat CYP2D metabolism of debrisoquine were inhibited at 1.7 and 0.6 μM, respectively. The same manner to quinidine, human CYP2D6 and rat CYP2D activity on debrisoquine metabolism were subsequently inhibited at 13 μM and 50 μM, respectively (Zhou, *et al*., 2015). This information indicates there might be a difference of *CYP2D* property between these two species.

Guinea pig (*Cavia porcellus*) is one among rodents that were often used in laboratory study. There are 4 functional genes and 1 pseudogene identified for guinea pig *Cyp2d*. The functional genes are including *Cyp2d6*, *Cyp2d16*, *Cyp2d17*, and *Cyp2d27* where *Cyp2d3p* is the only pseudogene. Despite the 4 *Cyp2d* genes are stated as functional genes in guinea pig, only *Cyp2d16* is the orthologue to human *CYP2D6* (Zhou *et al*., 2015). Despite being the human *CYP2D6* orthologue, guinea pig *Cyp2d16* produces different metabolite from bufuralol metabolism compared to human *CYP2D6*. Guinea pig *Cyp2d16* gave 1'-hydroxybufuralol as the major metabolite where human *CYP2D6* produced 6'-hydroxybufuralol as the main product (Colby *et al*., 2001). Guinea pig *Cyp2d16* is expressed in adrenal cortex, liver, and kidney. Its expression level in the inner site of adrenal cortex is substantially high compared to which of liver, kidney, and even outer site of adrenal cortex (Zhou *et al*., 2015). Yuan *et al*. (2001) reported that expression of *Cyp2d16* was non gender- and age-dependent. Anyhow, the rate of bufuralol metabolism by guinea pig adrenal microsome was largely influenced by the amount of zona reticularis. Many studies reputed that the expression level of *Cyp2d16* was increasing with age and gender. Usually female guinea pigs exhibit higher level of *Cyp2d16* than males. Although female express more *Cyp2d16*, the estrogen seems to have the inhibitory effect on bufuralol metabolism rate. Mature female guinea pig (14 weeks) exhibited significantly lower rate than prepubertal females (7 weeks) and then the rate of metabolism was significantly increased in the retired breeders (30 weeks) (Yuan *et al*., 2001; Zhou *et al*., 2015).

The overall information of rodent *Cyp2d* distribution in comparison to human *CYP2D6* indicates that human *CYP2D6* and rodent *Cyp2d* have some different in their properties such as catalytic capacity. The study of rodent model and the extrapolation of result from rodent models should be done with caution due to the variation between two species.

Polymorphism of *CYP2D6*

Drug treatment failure is one among major causes of mortality nowadays. The factors contribute to the drug treatment failure are the potency and efficacy of drugs, drug-drug interaction, dosage forms of drugs, and patient factors, i.e., genetic, age, lifestyle, gender, and state of disease (Israeli & Dayton, 2001; Grahame-Smith & Aronson, 2003). Genetic variation of drug metabolizing enzymes is the most important uncontrollable factor in drug metabolism. This variation reflects on the inter-individual difference of pharmacokinetic or referred to 'Polymorphism' of metabolizing enzyme. Polymorphism of CYP450 is a phenomenon of variation of CYP450 metabolizing enzymes that cause alteration of metabolism rate and pharmacological activity. Polymorphism of enzyme function leads to unpredictable clinical outcomes of drug treatment,

including drug toxicity, and failure or success of therapeutic drug use. The variation of the enzyme can be contributed from genetic and epigenetic (Ingelman-Sundberg *et al*., 2008). Epigenetic is referred to the regulation of gene expression without an involvement with DNA, for example gene methylation regulation and microRNA (miRNA). Gene methylation was found to occur at CpG-site particularly at carbon number 5 of cytosine around the promoter and 5'-UTR site of DNA sequence. When methylated cytosine at CpG site is found in high level, it will be called 'CpG island' (Gardiner-Garden & Frommer, 1987). The consequences of gene methylation are the blockade of binding of transcription factors with promoter site, and structural change of chromatin, which consequently lead to the prevention of gene expression (Tate & Bird, 1993; Rountree *et al*., 2001). The recently found epigenetic regulation of gene expression is miRNA. miRNA is translated from non-protein-coding gene and its role in regulation of gene expression is in the post-translation step through RNA-induced silencing complex (RISC). RISC is the complex of miRNA and ribonucleoprotein complex which mainly binds to 3'-UTR site and some other sites of mRNA sequence and eventually causes mRNA cleavage (Wienholds & Plasterk, 2005). Single nucleotide polymorphism (SNP) and copy number variation (CNV) are two most common genetic mutation found in CYP450 polymorphism (Redon *et al*., 2006). SNP is a mutation of one single nucleotide which consists of nucleotide insertion and deletion which possibly lead to missense mutation or translation of a new amino acid, nonsense mutation or the alteration of codon into stop codon, silent mutation or a mutation that cause no alteration in amino acid, and frameshift mutation or an alteration of reading frame of nucleotide sequence. Even silent mutation causes no change of amino acid synthesis, it possibly affect the primary structure of an encoded protein which perhaps alter the stability of mRNA. Another importance of SNP is a mutation of intron. Intron is non-coding region of DNA, however, this kind of mutation can generate an abnormality of splicing process of exon. Copy number variation (CNV) reflects on numbers of genes especially in active genes which consequently lead to higher protein activity (Kukongviriyapan, 2012; Zhou *et al*., 2015). CYP450 that were reported to have high degree of polymorphism are *CYP2C* and *CYP2D* (Ingelman-Sundberg *et al*., 2008). Nowadays more than 100 polymorphisms of *CYP2D6* alleles are identified; also CYP2D6 was the first enzyme that was identified to have CNV (Johansson *et al*., 1996; Bertilsson *et al*., 1993). *CYP2D6* polymorphism in term of function is largely described by genetic variation since this enzyme is not inducible (Ingelman-Sundberg, 2005). The variations in molecular level of CYP2D6 are explained by alleles on chromosomes, including null alleles, reduced-function alleles, and increased-function alleles. Null alleles indicates alleles that do not demonstrate enzymatic activity such as *CYP2D6*4* and *CYP2D6*5*. Reduced-function alleles contribute to the phenotype of less expression of *CYP2D6* compared to wild type allele such as *CYP2D6*9* and *CYP2D6*10*. Increased-function alleles describe the multiple copies of functional *CYP2D6* that fused in head-to-tail orientation at *CYP2D6* locus as a result of unequal crossover events and other mechanisms (Bertilsson *et al*., 1993; Johansson *et al*., 1994; Lundqvist *et al*., 1999). According to the phenotype of *CYP2D6* in several alleles, CYP2D6 metabolizers are grouped into poor metabolizer (PM), intermediate metabolizer (IM), extensive metabolizer (EM), and ultrarapid metabolizer (UM) (Zanger *et al*., 2004). PM referred to people who contain homologous null alleles which lead to the absence of enzyme. IM is the term defines people who contain one null allele or decreased-function allele and one wild type allele. Therefore, IM is still able to encode the CYP2D6 enzyme but the activity is relatively decreased whereas EM describes a normal expression of the CYP2D6 enzyme activity. In the opposite, UM are people who express more level of CYP2D6, and this type of metabolizer is a result of CNV. The variations of CYP2D6 alleles are described in Table 1. For example, *CYP2D6*5* is null allele which has CYP2D6 gene deletion. So if people who contain *CYP2D6*5* homologous on their chromosome,

they will be classified as a poor metabolizer (PM) or if those people contain only one, they will be called intermediate metabolizer. IM will response to drug metabolism slower than those who are EM, consequently the drug tends to be accumulated in IM more. By the accumulation, the drug can cause the toxicity or prolong the therapeutic effect, however, the benefit and the risk of polymorphism is individual.

Table 1. The polymorphism of CYP2D6 alleles and phenotype (Adapted from Ingelman-Sundberg *et al*., 2008)

CYP2D6 allele	Allele functionality on one of two chromosome	
	Phenotype	numeric value *
*CYP2D6*1*	EM	1
*CYP2D6*1xN, N≥2*	UM	1xN
*CYP2D6*2*	EM	1
*CYP2D6*2 xN, N=2,3,4,5 or 13*	UM	1xN
*CYP2D6*3*	PM	0
*CYP2D6*4*	PM	0
*CYP2D6*4x2*	PM	0
*CYP2D6*5*	PM	0
*CYP2D6*6*	PM	0
*CYP2D6*7*	PM	0
*CYP2D6*8*	PM	0
*CYP2D6*9*	IM	0.7
*CYP2D6*10*	IM	0.2
*CYP2D6*10xN*	IM	0.2xN
*CYP2D6*14*	PM	0
*CYP2D6*17*	IM	0.5
*CYP2D6*17xN*	EM (if N=2)	0.5xN
*CYP2D6*18*	PM	0
*CYP2D6*21*	PM	0
*CYP2D6*29*	IM	0.7
*CYP2D6*35*	EM	1
*CYP2D6*35x2*	UM	2
*CYP2D6*36*	IM	0.05
*CYP2D6*36_*10*	IM	0.25
*CYP2D6*36x2*	IM	0.1
*CYP2D6*41*	IM	0.5
*CYP2D6*41x2*	EM	1
*CYP2D6*44*	PM	0

Note. * indicates the fraction of CYP2D6 alleles function in 1 unit. EM, extensive metabolizer; UM, ultrarapid metabolizer; PM, poor metabolizer; IM, intermediate metabolizer

Not only inter-individual variation affected by polymorphism, but also inter-ethnic variation was reported in some studies. This inter-ethnic *CYP2D6* polymorphism could be beneficial in predictions of drug treatments in different population. For example, normally null alleles are nearly absent in all population, but *CYP2D6*4* was appeared to be common in Caucasian with frequency around 20-25%. So this could assume the lower rate of PM in Asians and Africans compared to Caucasians (Wang *et al*., 1993; Johansson *et al*., 1994; Dahl *et al*., 1995a; Dahl *et al*.,1995b; Zanger *et al*., 2004). In addition, *CYP2D6*10* has high prevalence among Asian population rather than Caucasian and African population. Thus, the term 'personalized therapy' was introduced as the new option of treatment to maximize the treatment outcome for individuals.

The clinical impact of *CYP2D* polymorphism

Ultrarapid metabolizer of *CYP2D6*2* and morphine toxicity in an infant

A 7- day- old infant developed difficulty of breastfeeding and increasing of lethargy. On the day 11, the infant was brought to the pediatrician with concerns of skin color and a decrease in milk intake. Finally, on the day 13 the infant with cyanosis skin and no vital signs was taken to the emergency unit. The blood sample was investigated and found morphine at 70 ng/ mL and paracetamol at 0. 59 μg/ mL. The medication history of the mother revealed that she was prescribed Tylenol® 3 (codeine 30 mg plus paracetamol 500 mg) twice a day for her postnatal pain. Due to the development of difficulty in breastfeeding, the mother collected and stored milk in the freeze. The frozen milk was investigated by enzyme- linked immunosorbent assay method, and found the concentration of morphine at 87 ng/ mL. The genotype analysis of CYP2D6 via the enzyme responsible for *O*- demethylation of codeine to morphine, of the mother showed heterozygous *CYP2D6*2* and *CYP2D6*2x2* alleles. These results indicated that the mother has more than one functional *CYP2D6* gene which is characterized as the ultrarapid metabolizer (UM) phenotype. In the UM, codeine is metabolized to morphine in a higher rate compared to the wild type, leading to morphine toxicity even a small dose in adults. Besides the rate of metabolism, morphine prefers to be in breastmilk rather than plasma because the alkaline pH of breastmilk favors morphine rather than neutral pH of plasma.This case report raised the awareness of morphine administration for postnatal pain since it could be life- threatening to infants, and presently the pain management after laboring is strongly recommended the use of non- steroidal anti- inflammatory drugs (NSAIDs) (Feilberg *et al*., 1989; Madadi *et al*., 2007).

Genotype of *CYP2D6* and ERα in tamoxifen therapy

Breast cancer is one among cancer types that cause high mortality rate. Tamoxifen is a drug of choice for the treatment of breast cancer in a patient who presents estrogen receptor-alpha (ERα)-positive. Tamoxifen is a selective estrogen receptor modulator (SERM) used in either the treatment or the recurrent prevention of breast cancer which effectively reduces relapse and mortality rate (Early Breast Cancer Trialists' Collaborative Group, 2005). Although estrogen receptors exist in 2 subtypes, only ERα has been confirmed the role in the breast cancer treatment (Thomas & Gustafsson, 2011). Tamoxifen is the time-dependent ERα antagonist (Wu *et al*., 2009) formulated as a prodrug which can be metabolized into 2 major metabolites, *N*-desmethyl-tamoxifen and 4-hydroxy-tamoxifen. The metabolism of tamoxifen was associated with CYP2D6 and CYP3A4/5. However, *N*-desmethyl-tamoxifen or endoxifen, a high potent anti-estrogenic receptor, was substantially generated by CYP2D6 compared to 4-hydroxy-tamoxifen which was generated by CYP3A4/5 (Desta *et al*., 2004). Endoxifen demonstrated affinity toward ERα 100-fold more potent than tamoxifen, hence it showed the stronger effect against cell proliferation. The pharmaco-kinetic studies of tamoxifen demonstrated that the variation of *CYP2D6* could explain the variation of endoxifen concentration around 30 to 50 % (Mürdter *et al*., 2011; Teft *et al*., 2013; Saladores *et al*., 2014).

Normally, the tamoxifen dosage regimen is 20 mg once daily, however, the responses were dissimilar among CYP2D6 metabolizers. Poor metabolizers (PM) tend to have more relapse and mortality of breast cancer than extensive metabolizers (EM) due to the lesser exposed level of endoxifen. Surprisingly, an increase in the dose of tamoxifen 20 mg to 40 mg in the PM gave the comparable level of endoxifen to that of the EM, but the adverse effects were correlated with the increasing of tamoxifen dose (Irvin *el al*., 2011). The *CYP2D6* pharmacogenetic study in Caucasians revealed that those who contained homologous *CYP2D6*4* alleles had significant lower disease-free survival (DFS) compared to the wild type and heterozygous *CYP2D6*4* (Bonanni *el al*., 2006). Similarly to the study in Chinese women *CYP2D6*10*, those who had homozygous *CYP2D6*10* tended to have shorter DFS than the wild type and heterozygous *CYP2D6*10* (Xu *et al*., 2008). The study of *CYP2D6*10* polymorphism on tamoxifen in Thai breast cancer women of Sirachainan *et al*. (2012) reported that Thai women who had homozygous *CYP2D6*10* significantly had lower DFS than the heterozygous *CYP2D6*10*. The significant difference of DFS between the wild type and the heterozygous *CYP2D6*10* was also reported. However, there was no significant different among the wild type, the heterozygous *CYP2D6*10*, and the homozygous *CYP2D6*10* due to the small number of subjects. Nonetheless, the meta-analysis noted that benefit of *CYP2D6* polymorphism in the tamoxifen therapy is still controversial (Aurelia et al., 2015).

The US food and drug administration (US FDA) recommends the *CYP2D6* genotyping prior to the treatment in the label of tamoxifen (Phan & Venitz, 2006). Thus the genotype of *CYP2D6* along with ERα screening should be examined in order to personalize the tamoxifen treatment for individuals.

Polypharmacy and genetic variation of *CYP2D6* in clinical outcome

Venlafaxine is an antidepressant in a group of serotonin- norepinephrine reuptake inhibitor (SNRI) . Venlafaxine is dominantly biotransformed by CYP2D6 to its active metabolite, *O*- desethylvenlafaxine (ODV) , and is slightly metabolized by CYP3A4 to the inactive product, *N*- desmethylvenlafaxine. ODV inhibits serotonin-norepinephrine reuptake. The adverse effects of venlafaxine are tachycardia, fatigue, agitation, and hypertension.

Wijnen *et al*. (2009) reported that a 42- year- old woman came to the outpatient hematology clinic due to the 6- week progression of fatigue, dyspnea, paresthesia of the fingers, hands and legs. Her heart rate at rest was 110 beats per minute. She informed about her two-months-ago suicidal thinking, and the psychiatrist prescribed her venlafaxine 75 mg, however, there was no mental status improvement. The doctor persisted the use of venlafaxine with the elevated dose as 225 mg, still there was no improvement. Her mental status got worsened at the presentation. Her concurrent medications are ursodeoxycholic acid, furosemide, metoprolol, simvastatin, zopiclone, rosiglitazone, and pantoprazole. Her clinical presentation was complied with the adverse effects of venlafaxine. Her blood was drawn for clinical analysis, and the results turned out that the venlafaxine and ODV concentration in her serum was 1300 μg/ L and <100 μg/ L, respectively. The detected concentration was enormously greater than the recommended therapeutic range, 195- 400 μg/ L (Veefkind *et al*., 2000).

According to the high level of venlafaxine and lesser level of ODV, *CYP2D6* active was suspected to be absent in this patient, hence the doctor then analyzed her genotype of *CYP450* gene. The finding was that she had homozygous *CYP2D6*4/*4*. The findings proposed the confirmation of the clinical association between the accumulation of venlafaxine and the failure of treatment and *CYP2D6* PM (Michalets, 1998; Wijnen *et al*., 2007). In addition to venlafaxine, metoprolol is also the substrate of CYP2D6, yet she has been using metoprolol years before the onset of depression, but there was no observed adverse effect from the usage of metoprolol. Later the doctor stopped venlafaxine and metoprolol in the patient and considered the new treatment

for her. On the other hand, the inhibition of venlafaxine metabolism was contributed from the drug-drug interaction as well, in case of the co-administration of CYP2D6 inhibition. In conclusion, this case report pointed the significance of genetic variation of *CYP2D6* in clinical outcome and raised the awareness of genetic screening prior to the prescription of drugs those likely cause adverse effects as the results of *CYP450* polymorphism.

***CYP2D6* polymorphism associated with the failure of nortryptyline treatment**

A 52-year-old Korean man was admitted to hospital according to his suicidal attempt. He had suffered from depression for the last 2 months. The physician performed the physical examination and report that his blood pressure, heart rate, and body temperature were normal. His electrocardiogram (EKG) was unremarkable. Upon the admission, he was diagnosed for major depression with psychotic symptoms. Nortryptyline (50 mg/day), lorazepam (1 mg/day), and risperidone (2 mg/day) were initiated for the treatment. Then the dose of nortryptyline was elevated to 100 mg/day. His blood was drown to be analyzed for nortryptyline serum concentration by high performance liquid chromatography (HPLC), and the result showed that the level of serum nortryptyline was 181.4 ng/mL after 6 days of the initiation (the recommended range is 50-150 ng/mL). However, the physician still persisted to increase the dose of nortryptyline to 150 mg/day to achieve the clinical improvement. After the increasing of nortryptyline dose, his serum concentration of nortryptyline after 6 days of the administration was revealed at 470.6 mg/day. He appeared to develop anticholinergic symptoms including dry mouth, constipation, and dizziness due to the remarkably high concentration of nortryptyline. The physician then reduced nortryptyline dose to 100 mg/day and his clinical symptoms were improved, yet his serum concentration was still over the range (198.7-222.7 ng/mL). According to the unusual serum concentration of nortryptyline, the genotype of *CYP2D6* analysis was performed in order to detect the polymorphism. The man was found to have *CYP2D6*5/*10* alleles which results in low activity of CYP2D6 (Lee *et al*., 2008). The *CYP2D6*5* allele indicates the loss of CYP2D6 function where *CYP2D6*10* allele has decreased function of CYP2D6, hence the heterogenous *CYP2D6*5/*10* results in the relatively low activity of CYP2D6. Since nortyptyline is metabolized through CYP2D6 and its therapeutic and toxic effect mainly depend on the clearance of the drug (Preskorn, 1993), nortryptyline tended to accumulate in this patient's body and resulted in adverse drug reactions. The recommended dose of nortryptyline for ordinary patients is 50 mg/day and gradually increase to the average dose of 150 mg/day or until the optimum serum concentration is achieved. *CYP2D6* poor metabolizers can get toxicity from notryptyline at the standard dose compared to the extensive metabolizer (Dahl *et al*., 1996; Dalen *et al*., 1998). Thus, the analysis of genotype in prior to the administration of narrow therapeutic drugs, for instance nortryptyline, is essential for the most benefit of the patients.

Conclusion

CYP2D6 is the CYP450 metabolizing enzyme which involves in both xenobiotic and endogenous compound metabolism. Despite sharing small proportion in the total number of CYP450, CYP2D6 responds for approximately 30% of total clinical drugs. There are 3 *CYP2D* genes found in human chromosome 22q13.1, yet only *CYP2D6* is the active gene and responsible for protein encoding. The level of *CYP2D6* is prominent in the liver, and substantially lesser in the extra-hepatic tissues. Interestingly, *CYP2D6* was also detected in the human brain. The finding was proposed to be associated with the biosynthesis amine neurotransmitter, including dopamine and serotonin. Moreover, *CYP2D6* was found in breast tissue and breast cancer tissue, and this finding led to the implementation that breast *CYP2D6* was a benefit to tamoxifen therapy in breast cancer women. The drugs in several classes belong to be CYP2D6 substrates, for example beta-adrenergic blocker and antipsychotics. Most of CYP2D6 substrates are narrow therapeutic index drugs which can easily get to toxic and sub-therapeutic levels, thus they may require therapeutic drug monitoring program in

order to closely observe the serum concentration level. To study the pharmacology or toxicology of the drugs, animal models, for example small rodents, are often used. However, the difference between human and animal *CYP2D* isoforms has to be concerned because even the homology of DNA sequence is high, but catalytic activities between species are markedly different. *CYP2D6* was the first report in *CYP450* polymorphism which largely contributed to inter-individual clinical outcomes. *CYP2D6* phenotype was characterized based on *CYP2D6* alleles on chromosome. *CYP2D6* phenotype includes poor metabolizer (PM), intermediate metabolizer (IM), extensive metabolizer (EM), and ultrarapid metabolizer (UM). The particular metabolizer responds to CYP2D6 substrates differently. Furthermore, *CYP2D6* polymorphism is happened among ethnics as well. For example, about 20% of Caucasians contain *CYP2D4*6* null allele whereas Asians have very low prevalent of *CYP2D6*4*. This *CYP2D6* polymorphism phenomenon has the clinical relevance, yet benefit or downsides depend on the type of compounds. Hence, the further study on the clinical relevance of the *CYP2D6* is of interest in order to optimize and personalize drug use for an individual.

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