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บทคัดย่อ

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**น. เภสัชศาสตร์อีสาน**

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ไซโตโครม พี 450 (CYP450) คือกลุ่มของเอนไซม์ที่มีหน้าที่หลักในการเปลี่ยนแปลงทางชีวภาพของสารแปลกปลอม เช่น ยา อาหาร สารเคมี และมลพิษ รวมถึงสารภายในร่างกาย สมรรถนะของเอนไซม์ดังกล่าวเป็นปัจจัยส่วนหนึ่งที่ส่งผลต่อเภสัชพันธุศาสตร์ (pharmacokinetics) ของยา โดย CYP2D6 เป็นหนึ่งในเอนไซม์ที่ส่งผลต่อสมรรถนะของยา ซึ่งมักเป็นยาที่มีช่วงการรักษาแคบ (narrow therapeutic index) อาทิ ยาต้านซึมเศร้า เช่น amitriptyline สมรรถนะของยา CYP2D6 ได้รับผลกระทบจากปัจจัยทั้งภายในและภายนอก ได้แก่ การแปรผันทางพันธุกรรมของยีนและกระบวนการเหนือระดับพันธุกรรม และยาที่ใช้ร่วมกัน การแปรผันทางพันธุกรรมของยีน CYP2D6 เกิดจากการได้รับยาที่เป็นยาพาหะแข็งของยา หรือการกลายพันธุ์ของยีนที่เกิดขึ้นภายหลังก็ได้ การแปรผันของสมรรถนะของยา CYP2D6 จากการกลายพันธุ์ของยีนที่เกิดขึ้นก็ได้ ภาวะพหุสัณฐาน (polymorphism) ซึ่งมีรายงานความสัมพันธ์ระหว่างภาวะพหุสัณฐานของ CYP2D6 กับความเสี่ยงทางการรักษาด้วยยาและความเสี่ยงของการไม่พึงประสงค์จากยา การศึกษาทางเภสัชพันธุศาสตร์ของยาเกี่ยวกับการรักษาผู้ป่วยและผลกระทบที่เกิดขึ้น

ค. ราญวิชญ์, น. เภสัชศาสตร์อีสาน, 2560; 13(1): 11-27

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คำสำคัญ : ไซโตโครม พี 450, CYP2D6, ภาวะพหุสัณฐาน, เภสัชพันธุศาสตร์
Significance of CYP2D Polymorphism

Tantai Wilaiporn¹, Kanokwan Jarukamjorn²*¹

¹ Undergraduate student (Pharm. D., International Program), Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen 40002, Thailand
² Ph.D. (Pharmaceutical Sciences), Associate Professor, Research Group for Pharmaceutical Activities of Natural Products using Pharmaceutical Biotechnology (PANPB), Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen 40002 Thailand

*Corresponding author: Kanokwan Jarukamjorn, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen 40002 Thailand.
Tel: 043-202305, Fax: 043-202379, Email: kanok_ja@kku.ac.th

Abstract

Significance of CYP2D Polymorphism

Tantai Wilaiporn¹, Kanokwan Jarukamjorn²*¹

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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 influential 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 (Anzenacker & 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 isof orm of CYP450 in human body which takes place about 30% of all isof orm, 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; Fonno-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 Asp301 and Glu216 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 Phe483, Val360, Glu216, Gly273, 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 promotor 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; Bieche et al., 2007; Duthie 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 et 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-methyltryptamine 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-demethylation, bufuralol 1'-hydroxylation, dextromethorphan O-demethylation, 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 CYP2D**

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 respond 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)

<table>
<thead>
<tr>
<th>CYP2D6 allele</th>
<th>Allele functionality on one of two chromosome</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phenotype</td>
</tr>
<tr>
<td>CYP2D6*1</td>
<td>EM</td>
</tr>
<tr>
<td>CYP2D6*1xN, N≥2</td>
<td>UM</td>
</tr>
<tr>
<td>CYP2D6*2</td>
<td>EM</td>
</tr>
<tr>
<td>CYP2D6*2 xN, N=2,3,4,5 or 13</td>
<td>UM</td>
</tr>
<tr>
<td>CYP2D6*3</td>
<td>PM</td>
</tr>
<tr>
<td>CYP2D6*4</td>
<td>PM</td>
</tr>
<tr>
<td>CYP2D6*4x2</td>
<td>PM</td>
</tr>
<tr>
<td>CYP2D6*5</td>
<td>PM</td>
</tr>
<tr>
<td>CYP2D6*6</td>
<td>PM</td>
</tr>
<tr>
<td>CYP2D6*7</td>
<td>PM</td>
</tr>
<tr>
<td>CYP2D6*8</td>
<td>PM</td>
</tr>
<tr>
<td>CYP2D6*9</td>
<td>IM</td>
</tr>
<tr>
<td>CYP2D6*10</td>
<td>IM</td>
</tr>
<tr>
<td>CYP2D6*10xN</td>
<td>IM</td>
</tr>
<tr>
<td>CYP2D6*14</td>
<td>PM</td>
</tr>
<tr>
<td>CYP2D6*17</td>
<td>IM</td>
</tr>
<tr>
<td>CYP2D6*17xN</td>
<td>EM (if N=2)</td>
</tr>
<tr>
<td>CYP2D6*18</td>
<td>PM</td>
</tr>
<tr>
<td>CYP2D6*21</td>
<td>PM</td>
</tr>
<tr>
<td>CYP2D6*29</td>
<td>IM</td>
</tr>
<tr>
<td>CYP2D6*35</td>
<td>EM</td>
</tr>
<tr>
<td>CYP2D6*35x2</td>
<td>UM</td>
</tr>
<tr>
<td>CYP2D6*36</td>
<td>IM</td>
</tr>
<tr>
<td>CYP2D6*36-10</td>
<td>IM</td>
</tr>
<tr>
<td>CYP2D6*36x2</td>
<td>IM</td>
</tr>
<tr>
<td>CYP2D6*41</td>
<td>IM</td>
</tr>
<tr>
<td>CYP2D6*41x2</td>
<td>EM</td>
</tr>
<tr>
<td>CYP2D6*44</td>
<td>PM</td>
</tr>
</tbody>
</table>

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 breastfeeding rather than plasma because the alkaline pH of breastfeeding 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 pharmacokinetic studies of tamoxifen demonstrated that the variation of CYP2D6 could explain the variation of endoxifen concentration around 30 to 50% (Mürpter et al., 2011; Teft et al., 2013; Saladores et al., 2014).
Normal, 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 et 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 et 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 ERIα 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 compiled 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 nortryptyline 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 interindividual 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 CYP2D6*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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