Molecular genetic analysis of *CYP2D6* and *HLA-B*15:02* in Thai autistic spectrum disorder children: Implication for pharmacogenetics testing and optimization of drug treatments

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ABSTRACT

Genetic polymorphisms of *CYP2D6* and *HLA-B* have been associated with efficacy and toxicity variation of various antipsychotic drugs including those for autistic spectrum disorders (ASD). The objective of this study is to investigate and compare allele frequency of *CYP2D6* between 79 Thai ASD patients and 154 non-ASD by using microarray technique, and of *HLA-B*15:02* between 292 Thai ASDs patients and 627 non-ASDs by using PCR-SSOP based method. The most common allele frequency of *CYP2D6*10*, **1* and **2* in ASD group were 54.43% (86/158), 22.78% (36/158) and 11.39% (18/158), respectively, and non-ASD were 44.18% (138/308), 26.30% (81/308) and 7.47% (23/308), respectively. The frequencies of *HLA-B*15:02* in ASD and non-ASD were 14.72% (43/292) and 14.19% (89/627). There was no statistical significant difference between investigated allele frequency of ASDs and on-ASDs groups. This is the first study of the *CYP2D6* and *HLA-B*15:02* gene polymorphisms in Thai autistic spectrum disorder patients. This information may be useful to determine the appropriate treatment for Thai autistic spectrum disorders.

Keywords: autistic spectrum disorders, *CYP2D6*, *HLA-B*15:02*, genetic polymorphisms, adverse drug reactions

INTRODUCTION

Autistic spectrum disorders (ASD) are among the most common neurodevelopmental disorders characterized by impairments in three major domains including socialization, communication language, and repetitive and

restricted patterns of behavior. The estimated worldwide prevalence is ranged from 0.07% to 1.8% (Fombonne, 2009). In addition, the prevalence in Thailand has been reported as 1 per 1,000 (Siriwanarangsun *et al*., 2004). Risperidone is an atypical antipsychotic used to treat autistic children and adolescents with such symptoms as aggression, temper tantrums, quickly changing moods, and deliberate self-injury with limited side effects (Aman *et al*., 2005). The most common side effects with antipsychotics include weight, dry mouth, and photosensitivity. *CYP2D6* is one of the major *CYP* genes responsible for metabolizing the majority of prescribed antipsychotics. Patients with *CYP2D6* poor metabolizer were associated with risperidone-increased BMI and waist circumference (Correia *et al*., 2010). Antiepileptic drugs (AEDs) such as valproic acid and carbamazepine are widely administered to individuals with ASD. The frequency of epilepsy in ASD reported ranges from 20% to 46% (Hughes and Melyn, 2005). Furthermore, carbamazepine (CBZ)-induced Steven–Johnson syndrome (SJS)/Toxic epidermal necrolysis (TEN) which is a dose independent adverse drug reaction has been determined by *HLA-B*15:02* alleles predominantly in Asian population (Man *et al*., 2007). In this study, we described and compared the genetic polymorphisms of *CYP2D6* and *HLA-B*1502* in ASD and non-ASD groups in Thai population.

MATERIALS AND METHODS

Patient samples

We recruited 79 Thai Autistic spectrum disorders children (ASD) between 2010 and 2012 from Yuwaprasart Waithayopathum Child Psychiatric Hospital. The genetic information of 154 subjects without ASD diagnosed (non-ASD) was collected from Laboratory for Pharmacogenetics, Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University. Genomic DNA was extracted from 1 ml of whole blood using automated MagNA Pure Compact (Roche Applied Science, Penzberg, Germany). This study was approved by the ethics committee of Ramathibodi Hospital. All patients written informed consent before enrolling.

Genotyping procedure: AmpliChip™ CYP450 analysis of the *CYP2D6*

The *CYP2D6* polymorphisms of 79 ASD and 154 non-ASD were evaluated by microarray-based assay. The AmpliChip CYP450 Test (Roche Diagnostics) was used for detection of polymorphisms in *CYP2D6* according to the manufacturer's instructions. The AmpliChip™ CYP450 Test is a microarray-based assay with five major processes: (i) PCR amplification of purified DNA, (ii) fragmenting and labeling of the amplified products, (iii) hybridization and staining, (iv) scanning of the microarray and (v) determination of the genotypes. This test identified 33 *CYP2D6*

alleles including single nucleotide poly morphisms, gene deletion, insertion, conversion and duplications.

Analysis of polymorphisms in CYP2D6

The 29 polymorphisms in *CYP2D6* had been detected in this study including: -1584C>G, 31G>A, 100C>T, 138insT, 883G>C, 1023C>T, 1039C>T, 1659G>A, 1661G>C, 1707T>del, 1758G>T, 1758G>A, 1846G>A, 1976G>A, *20 cluster, 2539-2542delAACT, 2549A>del, 2613-2615delAGA, 2850C>T, 2935A>C, 3183G>A, 3198C>G, 3277T>C, 4042G>A, *36GC, 4180G>C, 1863 Repeat Ins, Gene deletion (**5*) and Gene duplication. The AmpliChip CYP450 Data Analysis Software was used to infer the genotype, and to predict the individual's CYP2D6 enzymatic activity. We used algorithm from the AmpliChip package insert for the assignment of predicted phenotypes. There are four phenotypic categories according to alleles related enzyme activity: (1) no enzyme activity alleles (poor metabolizer; PM); **3, *4, *5, *6, *7, *8, *11, *14A, *15, *19, *20, *36, *40* and **4XN,* (2) decreased enzyme activity alleles (intermediate metabolizer; IM); **9, *10, *17, *29, *41, *10XN, *17XN* and **41XN,* normal enzyme activity alleles (extensive metabolizer; EM); **1, *2* and **35,* and increased enzyme activity alleles (ultra-rapid metabolizer; UM); **1XN, *2XN* and **35XN.*

Genotyping procedure: LABType® PCR-SSOP analysis of the *HLA-B*1502*

LABType® SSO B Locus (One Lambda, Inc., Canoga Park, CA) Test is based on five major processes: (i) PCR amplification of purified DNA, (ii) denaturation/neutralization, (iii) hybridization, (iv) labeling and (v) sample reading on the Bioplex 200 and result analysis by HLA Fusion 2.0. The *HLA-B*1502* of total 292 ASD and 627 non-ASD were carried out according to the manufacturer protocol.

Statistical analysis

Hardy-Weinberg equilibrium was conducted with Haploview 4.2. Chi-square and Fisher's exact tests were used to analyze the difference of *CYP2D6* and *HLA-B*1502* allele frequency between ASD and non-ASD. Statistical significance was set at $P<0.05$. All analysis was performed using the SPSS (v18.0).

RESULTS AND DISCUSSION

Overall, the polymorphisms observed in *CYP2D6* were in Hardy-Weinberg equilibrium. The most common allele frequency of *CYP2D6*10*, **1* and **2* in ASD were 54.43% (86/158), 22.78% (36/158) and 11.39% (18/158) whereas non-ASD were 44.18% (138/308), 26.30% (81/308) and 7.47% (23/308), respectively (Table 1). Both groups were similar in their allele frequency ($p>0.05$).

Table 1 Comparison of *CYP2D6* allele frequency between 79 ASD and 154 non-ASD samples.

Alleles	Major genetic variant	SNP ID	Enzyme activity	Predicted phenotype	ASD n=79(100%)	Non-ASD n=154(100%)	*P* value
**1*	Wild type		Normal	EM	36 (22.78)	81 (26.30)	0.622
**2*	2850C>T 4180G>C	rs16947 rs1135840	Normal	EM	18 (11.39)	23 (7.47)	0.323
**4*	1846G>A	rs3892097	None	PM	0 (0)	5 (1.62)	0.497
**5*	Gene del		None	PM	6 (3.80)	18 (5.84)	0.516
**10*	100C>T	rs1065852	Decrease	IM	86 (54.43)	138 (44.81)	0.203
**14B*	1758G>A	rs5030865	Decrease	PM	1 (0.63)	3 (0.97)	1.000
**25*	3198C>G		Unknown	Unknown	1 (0.63)	0 (0)	1.000
**35*	Wild type		Normal	EM	1 (0.63)	1 (0.32)	1.000
**36*	Gene conversion		Decrease	IM	1 (0.63)	3 (0.97)	1.000
**41*	1661G>C, 2850C>T, 4180G>C	rs1058164	Decrease	IM	6 (3.80)	6 (1.95)	0.683
**1XN*			Increase	UM	1 (0.63)	0 (0)	1.000
**2XN*			Increase	UM	1 (0.63)	0 (0)	1.000
No call					0 (0)	30 (9.74)	0.001
Total					158 (100)	308 (100)	

Abbreviations: EM, extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer; UM, ultrarapid metabolizer.

Therefore, this study found a high percentage of *CYP2D6*10* allele which is related to low enzyme activity in the population. The allele frequency of *CYP2D6*10* in this study was similar to those found in Chinese mainland (51.6%) (Ji et al., 2002), Korean (45.0%) (Lee *et al.*, 2006).

Among 79 ASD and 154 non-ASD, we found percentage of homozygosity for *CYP2D6*1/*1* genotype as 2.53% (n=2) and 8.44% (n=13), respectively. The homozygosity of *CYP2D6*10/*10* genotype was found as 22.78% (n=18/79) and 22.73% (n=35/154), whereas the heterozygosity for *CYP2D6*5/*10* genotype were 4 (5.06%) and 10 (6.49%) in ASD and non-ASD groups. Nevertheless, the most common genotype in both groups was *CYP2D6*1/*10* (ASD, 32.91%; 26/79 and non-ASD, 26.62%; 41/154) (Table 2). Unfortunately, *CYP2D6*3, *6, *7, *8, *9, *11, *14A, *15, *17, *19, *20, *26, *29, *30, *31, *40, *4XN, *10XN, *17XN, *35XN* and **41XN* were not found in our study.

The allele frequency of *HLA-B*15:02* in ASDs was 14.72% (43 of 292) and non-ASDs was 14.19% (89 of 627) (Table 3). The frequency distribution of *HLA-B*15:02* allele is slightly higher than that previously found in Thais (11.4%) (Tassaneeyakul *et al*., 2010). However, significant difference of *CYP2D6* and *HLA-B*15:02* allele frequency between ASDs and non-ASDs was not found in our study.

Table 2 *CYP2D6* genotype distribution and comparison between ASD and non-ASD.

Genotype	No. of individuals (%)		*P* value	Genotype	No. of individuals (%)		*P* value
	ASD, n(%)	**Non-ASD, n(%)**			**ASD, n(%)**	**Non-ASD, n(%)**	
**1/*1*	2(2.53)	13(8.44)	0.121	**4/*10*	0(0)	3(1.95)	1.000
**1/*2*	2(2.53)	3(1.95)	1.000	**5/*10*	4(5.06)	10(6.49)	0.756
**1/*5*	1(1.27)	6(3.90)	0.369	**5/*14B*	0(0)	1(0.65)	1.000
**1/*10*	26(32.91)	41(26.62)	0.355	**10/*10*	18(22.78)	35(22.73)	1.000
**1/*14B*	0(0)	1(0.65)	1.000	**10/*14B*	1(1.27)	1(0.65)	1.000
**1/*35*	1(1.27)	0(0)	1.000	**10/*25*	1(1.27)	0(0)	1.000
**1/*36*	1(1.27)	2(1.30)	1.000	**10/*35*	0(0)	1(0.65)	1.000
**1/*41*	0(0)	2(1.30)	1.000	**10/*36*	0(0)	1(0.65)	1.000
**2/*2*	2(2.53)	3(1.95)	1.000	**10/*41*	6(7.59)	3(1.95)	0.052
**2/*4*	0(0)	2(1.30)	1.000	**1/*2XN*	1(1.27)	0(0)	1.000
**2/*5*	1(1.27)	1(0.65)	1.000	**1XN/*10*	1(1.27)	0(0)	1.000
**2/*10*	11(13.92)	9(5.84)	0.059	No call	0(0)	15(9.74)	0.001
**2/*41*	0(0)	1(0.65)	1.000	Total	79(100)	154(100)	

Table 3 Comparison of *HLA-B*1502* frequency between ASD and non-ASD.

	Number of individuals (%)		
HLA-B allele	**ASD, n=292 n=292(100%)**	**Non-ASD, n=627 n=627(100%)**	***P* value**
1502	43 (14.72)	89 (14.19)	0.841

CONCLUSION

Our study provided essential information of the genetic variants involved in psychiatric drug response and toxicity. It might be beneficial for ASD clinical setting and for the researchers who are interested in developing pharmacogenetics test for Thai population.

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REFERENCES

Aman MG, Arnold LE, McDougle CJ, Vitiello B, Scahill L, Davies M, McCracken JT, Tierney E, Nash PL, Posey DJ *et al*. (2005) Acute and long-term safety and tolerability of risperidone in children with autism. J Child Adolesc Psychopharmacol 15: 869–884.

Correia CT, Almeida JP, Santos PE, Sequeira AF, Marques CE, Miguel TS, Abreu RL, Oliveira GG and Vicente AM (2010) Pharmacogenetics of risperidone therapy in autism: association analysis of eight candidate genes with drug efficacy and adverse drug reactions. Pharmacogenomics J 10: 418–430.

Fombonne E (2009) Epidemiology of pervasive developmental disorders. Pediatr Res 65: 591–598.

Hughes JR and Melyn M (2005) EEG and seizures in autistic children and adolescents: further findings with therapeutic implications. Clin EEG Neurosci 36: 15–20.

Ji L, Pan S, Wu J, Marti-Jaun J and Hersberger M (2002) Genetic polymorphisms of CYP2D6 in Chinese mainland. Chin Med J (Engl) 115: 1780–1784.

Lee SY, Sohn KM, Ryu JY, Yoon YR, Shin JG and Kim JW (2006) Sequence-based CYP2D6 genotyping in the Korean population. Ther Drug Monit 28: 382–327.

Man CB, Kwan P, Baum L, Yu E, Lau KM, Cheng AS and Ng MH (2007) Association between HLA-B*1502 allele and antiepileptic drug-induced cutaneous reactions in Han Chinese. Epilepsia 48:1015–1018.

Siriwanarangsun P, Kongsuk T, Arunpongpaisal S, Kittirattanapaiboon P and Charatsingha A (2004) Prevalence of mental disorders in Thailand: a national survey 2003. J Ment Health Thai 12: 177–178.

Tassaneeyakul W, Tiamkao S, Jantara roungtong T, Chen P, Lin SY, Chen WH, Konyoung P, Khunarkornsiri U, Auvichayapat N, Pavakul K *et al*. (2010) Association between HLA-B*1502 and carbamazepine-induced severe cutaneous adverse drug reactions in a Thai population. Epilepsia 51: 926–930.