HLA-B AND *CYP2D6* GENE POLYMORPHISMS IN THAI CHILDREN AND ADOLESCENTS WITH AUTISM SPECTRUM DISORDERS: A CASE-CONTROL STUDY

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ABSTRACT

Autism spectrum disorders (ASD) is a complex disorders that appears to be caused by interactions between genetic predisposition and environmental during early developmental. At present, several studies have been shown the relationship between ASD and immune genes that located in the human leukocyte antigen (HLA). The associations between HLA genotypes and ASD have established wide coverage in different ethnic background. Moreover, *CYP2D6* has also been contributed to endogenous metabolism of neuroactive substrates which can explain the hitherto observed on the relation between human behaviors and disease susceptibility. A potential influence of *CYP2D6* polymorphisms in the balanced function and physiological crosstalk of dopamine and serotonin endogenous systems

Consequently, the main aim of this retrospective case-control study was to compare the *HLA-B* (ASD 364 and Non ASD 952) and *CYP2D6* (ASD 79 and Non ASD 154) polymorphisms in 364 Thai ASD children and adolescents with 1106 control subjects in order to investigate more precise the genetic association. *HLA-B* and *CYP2D6* genotyping were performed by two platforms including sequence-specific oligonucleotide probe system (PCR-SSOP) and microarray-based technology (AmpliChip CYP450 Test), respectively.

In this study, *HLA-B*1302* (*P*=0.019, OR; 2.229), *HLA-B*4403* (*P*=0.016, OR; 1.645) and *HLA-B*5601* (*P*=1.78×10^{-4}, OR; 4.927) alleles were found significantly higher in ASD (n=364) than in controls (n=1,106). Interestingly, *HLA-B*1802* (*P*=0.016, OR; 0.375) and *HLA-B*4612* (*P*=0.008, OR; 0.147), were negatively associated with disease. For *CYP2D6* polymorphism, the most common allelic frequencies were 48.07% (**10*), 25.11% (**1*), 8.80% (**2*) and 5.15% (**5*) which related to 7.80% of intermediate metabolizer (IM), 90.37% of extensive metabolizer (EM), and 0.46% of poor metabolizer (PM), respectively. There were no statistically significant difference in *CYP2D6* genotypes and alleles frequencies between 79 ASD and 154 control subjects.

Our results demonstrated the association of *HLA-B*1302*, *HLA-B*4403* and *HLA-B*5601* with Thai ASD patients. It is our suggestion that genetic polymorphisms in the HLA region may be important in the etiology of ASD in certain subjects.

KEY WORDS: AUTISM SPECTRUM DISORDERS/*HLA-B*/*CYP2D6*/GENETIC POLYMORPHISMS

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ภาวะพหุสัณฐานของยีน *HLA-B* และ *CYP2D6* ในเด็กและวัยรุ่นไทย ซึ่งถูกวินิจฉัยเป็นกลุ่มอาการออทิซึมสเปคตรัม : การศึกษากลุ่มตัวอย่าง และกลุ่มควบคุม

HLA-B AND *CYP2D6* GENE POLYMORPHISMS IN THAI CHILDREN AND ALDOLESCENTS WITH AUTISM SPECTRUM DISORDERS: A case-control study

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บทคัดย่อ

ออทิซึมสเปคตรัม (ASD) เป็นกลุ่มอาการความผิดปกติที่มีความซับซ้อน ซึ่งมีสาเหตุมาจากการมีปฏิสัมพันธ์ร่วมกัน ระหว่าง ปัจจัยทางพันธุกรรม และปัจจัยทางสิ่งแวดล้อม ของการพัฒนาในช่วงต้น มีหลายการศึกษาที่รายงานถึงความสัมพันธ์ระหว่างผู้ป่วยออทิซึมสเปคตรัม และยีนที่เกี่ยวกับระบบภูมิคุ้มกัน (HLA) ซึ่งความสัมพันธ์ระหว่างยีน *HLA* และ *CYP2D6* มีความแตกต่างกันในแต่ละกลุ่มประชากร นอกจากนั้นยีน CYP2D6 มีความเกี่ยวข้องกับกระบวนการเมตาบอลิซึมของสารสื่อประสาทซึ่งสามารถอธิบายได้จากการสังเกตทางด้านพฤติกรรม และความไวต่อการเกิดโรค ความหลากหลายของยีน CYP2D6 มีอิทธิพลต่อสมดุล หน้าที่ และพยาธิสรีรวิทยาของสารสื่อประสามโดปามีน และเซโรโทนิน

ดังนั้นจุดมุ่งหมายหลักของการศึกษาแบบย้อนหลังครั้งนี้ เพื่อทำการเปรียบเทียบภาวะพหุสัณฐานทางพันธุกรรมของยีน *HLA-B* และ *CYP2D6* ในเด็กและวัยรุ่น 364 คนที่มีภาวะออทิซึมสเปคตรัม เทียบกับกลุ่มที่ไม่มีภาวะออทิซึมสเปคตรัม 1106 คน ด้วยเทคนิค พีซีอาร์-เอสเอสโอพี และไมโครอาเรย์ ตามลำดับ

ผลการศึกษาพบว่ายีน *HLA-B*1302* (P=0.019, OR; 2.229), *HLA-B*4403* (P=0.016, OR; 1.645) และ *HLA-B*5601* (P=1.78x10^{-4}, OR; 4.927) มีความสัมพันธ์ของความถี่อัลลีลสูงกว่ากลุ่มควบคุมอย่างมีนัยสำคัญทางสถิติต่อการเกิด ASD และยีน *HLA-B*1802* (P=0.016, OR; 0.375) และ *HLA-B*4612* (P=0.008, OR; 0.147) มีความสัมพันธ์อย่างมีนัยสำคัญทางสถิติต่อการป้องกันโรค, สำหรับยีน CYP2D6 พบความถี่สูงสุดลำดับแรกของอัลลีลเหมือนกันคือ *CYP2D6*10, *1, *2* และ **5* ซึ่งสัมพันธ์กับการทำนายลักษณะฟีโนไทป์ที่พบสูงสุดลำดับแรกเหมือนกันคือ EM, IM และ PM ในกลุ่มผู้ป่วยออทิซึมสเปคตรัม 79 ราย และผู้ป่วยโรคอื่นๆ 154 ราย ไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติระหว่างสองกลุ่มตัวอย่าง

อย่างไรก็ตาม การศึกษานี้เป็นครั้งแรกในการตรวจความหลากหลายของยีน *CYP2D6* และ*HLA-B* ในคนไทยที่มีภาวะออทิซึมสเปคตรัม ซึ่งข้อมูลนี้อาจมีประโยชน์ในการนำมาพิจารณาการรักษาที่เหมาะสมสำหรับคนไทยที่มีภาวะออทิซึมสเปคตรัม

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LIST OF ABBREVIATIONS

AD	Alzheimer's disease
ADOS-G	Autism Diagnostic Observation Schedule
ADR	Adverse drug reactions
ADI-R	Autism Diagnosis Interview-Revised
AEDs	Antiepileptic drugs
AS	Asperger's syndrome
ASD	Autism spectrum disorders
ASSQ	Autism Spectrum Screening Questionnaire
AUC	Area under the curve
BMI	Body mass index
cADRs	cutaneous Adverse drug reactions
CAST	Childhood Asperger's Syndrome Test
CBZ	Carbamazepine
CDD	Childhood Disintegrative Disorder
CDKL5	Cyclin-dependent kinase-like 5
CHAT	Checklist of Autism in Toddlers
CNV	Copy number variants
CSF	Cerebrospinal fluid
CYP	Cytochrome P450
^{o}C	Degree of Celsius
DA	Dopamine
D2	Dopamine Type 2
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders of the American Psychiatric Association 4th edition
DNA	Deoxyribonucleic acid
dbSNP	Database SNP
del	Deletion

LIST OF ABBREVIATIONS (cont.)

dNTP	Deoxynucleotide triphosphate
DRD2	D2 receptors
EDTA	Ethylene diamine tetraacetic acid
EEG	Electroencephalogram
EMs	Extensive metabolizers
ER	Endoplasmic reticulum
FDA	US Food and Drug Administration
FOXG1	Forkhead box protein G1
G	Guanine
GC	Gene conversion
g	Gram
HLA	Human Leukocyte Antigen
HR	Hazard ratio
HSS	Hypersensitivity syndrome
5-HT	5-hydrotryptamine, Serotonin
5-HT2	Serotonin Type 2
HFA	High functioning autism
HWpval	Hardy-Weinberg equilibrium p value
ICD 10	Tenth Edition of the International Classification of Diseases
IMGSA	International Molecular Genetic Study of Autism Consortium
IMs	Intermediated metabolizers
ins	Insertion
LD	Linkage disequilibrium
LTA	Lymph toxin alpha
LTG	Lamotrigine
M	Molar
MAF	Minor allele frequency
MECP2	Methyl CpG binding protein 2
M-CHAT	Modified Checklist for Autism in Toddlers

LIST OF ABBREVIATIONS (cont.)

Mg	Magnesium
MHC	Major Histocompatibility Complex
mg	Milligram
mL	Milliliter
mM	Milli molar
MPE	Maculopapular eruption
MPGN	Membranoproliferative glomerulonephritis
NAT-2	N-acetyltransferase type 2
NLGN3	Neuroligin 3
ng	Nanogram
OCD	Obsessive-compulsive disorder
OR	Odd ratio
PCR	Polymerase chain reaction
PCR-SSOP	Sequence-Specific Oligonucleotide Primed PCR
PCR-SSP	PCR using sequence-specific primers
PDDs	Pervasive developmental disorders
PDD-NOS	Pervasive developmental disorder, not otherwise specified
PD	Pharmacodynamic
PK	Pharmacokinetic
PMs	Poor metabolizers
rs	Reference SNP
RRBs	Restricted and repetitive behaviors and interests
SCQ	Social Communication Questionnaire
SJS/TEN	Stevens Johnson syndrome and toxic epidermal necrolysis
SLE	Systemic lupus erythematosus
SNPs	Single nucleotide polymorphisms
SRIs	Serotonin reuptake inhibitors
STAT	Screening Tool for Autism in Two-Year-Olds
T	Thymine

LIST OF ABBREVIATIONS (cont.)

TCRs	T-cell receptor
TdP	Torsades de pointes
TdT	Terminal deoxynucleotide transferase
TMAC	Tetramethylammonium chloride
TNF	Tumors necrosis factor alpha
U	Unit
UMs	Ultra-rapid metabolizers
US FDA	United State food and drug administration
µL	Microliter (10^{-6})

CHAPTER I
INTRODUCTION

Autism spectrum disorders (ASD) are among the most common of neurodevelopmental disorders defined behaviorally by impairments in three major domains includes reciprocal social interaction difficulties, verbal and non-verbal communication and the presence of restricted, repetitive and stereotypic behaviors and interests. However, the symptoms and their severity will vary across the three main domain, and the three main symptoms will continue to be central to the decision of a group of disorders referred to as “pervasive developmental disorders” (PDDs). Clinical features usually have developmental markers of ASD emerged during the first 3 years of life. Prevalence estimates worldwide range from 0.07% to 1.8% [1] and 0.1% in Thailand [2] with a biased male-to-female ratio of 4.2 to 1 [1]. The dramatic rise in the prevalence appears to be attributable to greater public awareness and recognition, extending ASD diagnostic criteria, younger age at diagnosis, and diagnostic improvement.

The exact causes of ASD remains largely unknown, possibly a result from a combination of environmental, neurological, immunological, genetic factors and may encompass several diseases with distinct origins interaction that lead to a general behavioral phenotype defined as ASD. The Tuberous sclerosis, Fragile X, neurofibromatosis, and chromosomal abnormalities have been shown for cases in genetic factor links and more evidences suggest that environmental factors are involves, such as exposure to toxic compounds, teratogens, anticonvulsants, paternal or maternal reproductive age, prenatal hypoxia and prenatal rubella infection may be interplay in directly or indirectly for the immune mechanisms that mediate the nervous system impairments seen in ASD. The wide phenotype variability of ASD suggests that diverse genes, gene-gene interactions, and gene-environment interactions play a vital role in this disease. It was suggested over 30 years ago by Stubbs and Magenis *et al*, 1980 [3] that the Human Leukocyte Antigen (HLA) region might be important in

autism. HLA genes are the name for the Major Histocompatibility Complex (MHC) in humans localized on the short arm of chromosome 6 (6p21; about 4×10^6 bp.). HLA genes are high levels of polymorphisms characterize and composed of Class I, II, and III molecules. The genes are directly involved in many biological processes such as inflammation, immune response, ligands for immune cell receptors, complement. The play roles of HLA-Class I will acted to inhibit connectivity and functionality also are potentially profound implications for neurodevelopmental disorders and neurological and psychiatric diseases. They were stifled axonal and dendritic growth, limit the initial establishment of cortical connections, and mediate synaptic weakening through long-term depression, as well as activity-dependent refinement of connections in the developing visual system and associated with autoimmune diseases, such as juvenile rheumatoid arthritis [4] and Hashimoto's thyroiditis [5]. Previous studies are found that *HLA-A*1, A*02, B*07, B*44, B*51, DRβ1*04 (DR4), DR11, DR13, DR14, DRB1*03, DQB1*0202, DQB1*0302, DQB1*0501* and *C4B* are associated with ASD [6]. Unfortunately, the evidence suggests possible associations between HLA alleles and ASD are wide coverage of different ethnic. Most have been reported in most Western countries but among Asian populations has less information.

Cytochrome P450 (*CYP*) 2D6 is a drug-metabolizing enzyme this takes place primarily in the liver, but metabolism can also occur in extrahepatic organs, including in neurons in the human cerebral cortex, hippocampus and cerebellum [7]. In the brain that also metabolizes endogenous neurotransmitters such as catecholamines, neurochemicals, such as serotonin (5-hydroxytrypamine or 5-HT) and dopamine (DA), carcinogen and inactivates neurotoxins such as 1-methyl-4-thenyl-1,2,3,6-tetrahydropyridine; MPTP. This takes place predominantly in the liver, but of metabolism can also occur in extrahepatic organs, such as the brain. That important for central nervous system (CNS) are acting drugs, as variation in brain CYP-mediated metabolism may be a contributing factor when plasma levels did not predict response to medication. It has been proposed that individuals with genetic variants in the *CYP2D6* gene, encoding a nonfunctional enzyme (poor *CYP2D6* metabolizers), have an increased risk for Parkinson's disease (PD) [8], human behavior and disease susceptibility (e.g., personality, neurocognition and neuropsychiatric disorders) [9] and reduced *CYP2D6* activity is inversely related to drug abuse dependence [10].

The clusters of ASD symptoms have varies exist with disparate etiologies which often remain undecided. This issue consequently makes difficult recommendations to pharmaceutical and behavioral therapy. ASD are lifelong chronic disabilities. Drugs therapy, including atypical neuroleptics, may be used. However, to treat specific disruptive emotional and behavioral problems [11, 12], Risperidone is an atypical antipsychotic approved by the Food and Drug Administration to treat autistic children and adolescents with such symptoms as anxiety, hyperactivity, inattention, aggression, temper tantrums, quickly changing moods, and deliberate self-injury with limited side effects [13]. Risperidone is improved its performance on such cognitive tasks as verbal learning and cancellation, and reducing such behaviors as irritability, disruptiveness, repetitiveness, aggression, anxiety or nervousness, depression and hyperactivity [13, 14]. Antiepileptic drugs (AEDs) is used as mood stabilizers and treating seizure disorders such as valproic acid and carbamazepine (CBZ) are commonly administered to individuals with ASD. The report of the frequency of epilepsy in ASD is ranges from 20% to 46% [15]. Almost all medications have side effects such as using of atypical antipsychotics drug children are more prone to weight gain. The most common side effects with antipsychotics include dry mouth, photosensitivity, as well as constipation. The less common side effects include diarrhea, nausea, headache, sleeplessness and fatigue. Potential risk factors that have the potential for drug efficacy or toxicity, include drug-drug interactions, the patient's age, sex, renal and liver function or other disease factors, and variables lifestyle such as nutritional status, weight, smoking and alcohol consumption. There is even more important in the determination of individual risk are inherited factors that affect the kinetics and dynamics of many medications. Thus, genetic variations in genes for drug-metabolizing enzymes, drug receptors, and drug transporters are associated with individual variability in drug efficacy and/or drug toxicity.

Additionally, in the use of CBZ, the severe hypersensitivity could be occasionally found in ASD patients. Pharmacogenetics testing could determine individual susceptibility to either dose-dependent or dose-independent adverse drug reactions (ADRs). The various from of CYP enzymes are responsible for the development of a significant number of dose-dependent ADRs. Accordingly, 59% of drugs described in ADRs studies are metabolized by polymorphic phase I enzymes, of

which 86% are P450s [16]. *CYP2D6* is one of the major *CYP* genes that metabolize the majority of prescribed antidepressants, antipsychotics, antiarrhythmics, antiemetic, beta-adrenoceptor antagonists (beta-blockers) and opioids [17]. Patients with *CYP2D6* poor metabolizer are associated with risperidone-increased BMI, waist circumference and hyperprolactinemia [18]. Furthermore, CBZ-induced Stevens–Johnson syndrome and toxic epidermal necrolysis (SJS/TEN). Which is dose independent adverse drug reaction had been determined by *HLA-B*1502* alleles predominantly in Asian population including Thai [19-21]. Due to the relationship between the genetic variation in drug efficacy and ADRs, genotyping of these genes could become a powerful tool for individualization of medical treatment.

According to the reports in Thailand, there is no association study between polymorphisms of *HLA-B* and *CYP2D6* and ASD. The aim of this study was to find the genetic association between ASD and Non-ASD in a Thai population. In this study, twelve *CYP2D6* alleles were determined by using AmpliChip^{TM} CYP450. Molecular *HLA-B* typing was performed using PCR-SSOP.

CHAPTER II

OBJECTIVES

The aims of this study were:

1. To evaluate the association between HLA-B and CYP2D6 polymorphisms in ASD in a Thai population

2. To compare allele, genotype frequencies for SNPs of *CYP2D6* and polymorphisms in *HLA-B* genes between two populations, ASD versus non-ASD and to identify significant differences.

CHAPTER III
LITERATURE REVIEW

3.1 Autism spectrum disorders (ASD)

3.1.1 Definition of Autism Spectrum Disorders

ASD was a behaviorally defined group of neurodevelopmental disorders characterized by impairs in three major areas: 1) social communication and social interaction across multiple contexts, 2) Communication difficulties, 3) and the presence of highly repetitive behavior/restricted interests (**Figure 3.1**) beginning before 3 years of age [1, 22, 23]. Other associated features include mental retardation and epilepsy, observed in ~70% and ~30% of autistic cases, respectively. Numerous studies had suggested that diagnoses of ASD made at age 2 years were stable through age 3 years and diagnoses made by age 5 years were stable up to late teens.

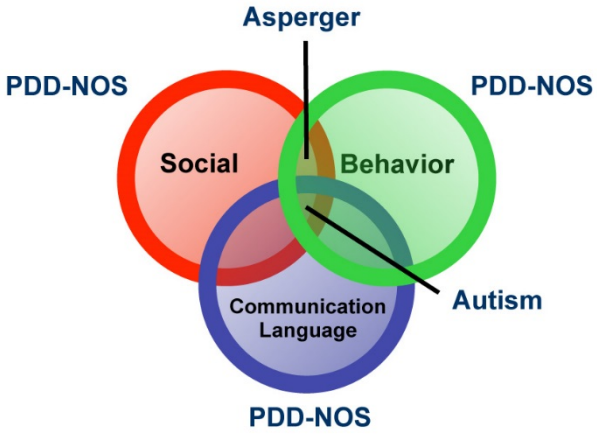


Figure 3.1: Schematic of autism as a spectrum disorder: Three circle correspond to areas of impairments, red = Social communication, green = Repetitive behavior/restricted interests and blue = Communication language, with depth of color indicating severity of impairment. Individuals with all three features (center of the

figure) meet full diagnostic criteria for autism, but those falling outside this region, who have mild or partial difficulties were candidates for a diagnosis in ASD.

3.1.2 Diagnosis of Autism Spectrum Disorders

The diagnosis and screening for ASD can be made in children between 18- and 24-month-of age, as well as adolescent using a combination of standardized instruments: a parent interview (e.g., the Autism Diagnostic Interview–Revised; ADIR), an observational scale (e.g., the Autism Diagnostic Observation Schedule; ADOS), Checklist of Autism in Toddlers (CHAT), Autism Behavior Checklist (ABC). The CHAT screening test was designed to screen autism at the age of 18 months to evaluate the player assumes. And the common interests of the telling of the parents and observation of inspectors. CHAT advantages were high positive predict value but low sensitivity. The screening test was the most widely used CARS and ABC, since both have the inter-rater reliability in screening each and CARS have lower false negative screening with ADIR and ADOS. The earliest signs recognized in infancy (≤1 year) or toddlers are nonspecific (e.g., irritability, passivity, difficulties with sleeping and eating) and impairments in three major domains (social reciprocity, communication, and restricted/repetitive interests) are typically observed. ASD was now classified under the category of Pervasive Developmental Disorders (PDD) in revising the fourth edition of Diagnostic and Statistical Manual of Mental Disorders of the American Psychiatric Association (DSM-IV-TR, fourth edition, text revision) as well as additional assessment tools such as ADOS and ADI-R when necessary.

In this study used DSM-IV-TR for diagnosis patients by technician by the person must have all four of the following characteristics below for the diagnosis of ASD; therefore, the diagnosis of ASD must also meet all of the parameters of the following six aspects in DSM-IV (Appendix A).

The studies examined the babies at risk (infant siblings of affected babies) have reported that impairment in communication and social interactions (poor eye contact and non-social smiling) can be diagnosed as early as 6 months of age [24]. These observations indicate that relevant neurochemical or neuroanatomical events may occur relatively early in the development of the CNS [25]. In conclusion, if we can understand the combination of events that cause the development of ASD and then

we can identify children at risk and developing treatments to reduce symptoms associated with this condition.

3.1.3 Prevalence of Autism Spectrum Disorders

The most recent estimate of the prevalence of ASD were at least 18.9 per 10,000 children, 9.9 per 10,000 children in Thailand [2] and the other forms of ASD may be as common as 60 per 10,000 children [1]. Current evidence does not support the hypothesis of an increase in the incidence of ASD, but the power to monitor trends seriously limited time series data available. While it is clear that estimates the prevalence has increased over time, this increase is mainly illustrates the change in the definition of availability and awareness of the ASD in both the public and private sectors to assess were whether or not the increased incidence. Perhaps the most consistent finding in the epidemiology of ASD was more common in boys than girls is ratio about 4-5:1. About 45% of individuals with ASD have intelligent disability, and 32% have regression (e.g. Loss of previously acquired skills; mean age of onset 1.8 years).

3.1.4 Etiology of Autism Spectrum Disorders

The exact causes that lead to ASD were at best poorly understood. ASD were likely to involve a combination and was likely to be of multifactorial descent encompassing of genetic, immunological, and environmental factors, and may encompass several diseases with distinct origins causes were believed to contribute to the risk for the development of this disease spectrum.

3.1.4.1 Genetic factors

According to the study, many families suggest that there are an increased prevalence of both ASD and autistic-like behaviors in the first-degree relatives of persons with ASD. Chance of autism in siblings of patients with recurrence risk of autism was 2– 8% [1, 26]. The monozygotic twins have a higher concordance rate for ASD, found 60-91% than dizygotic twins, found 0-10%. [27]. The International Molecular Genetic Study of Autism Consortium (IMGSA, 1998) narrowed the search for susceptibility loci for autism to chromosome 2q, 7q 16p and 22q [28]. Abnormalities of the long arm 15 chromosome, region 15q11–13, and long

arm 22, region 22q13 such as intrachromosomal and supernumerary inverted duplications and microdeletions, have been reported. The intrachromosomal inversion duplications were more likely to be associated with developmental delay or mental retardation and PDD especially paternally and maternally derived [29]. Some 75% of individuals with autism are male, increasing the possibility that genes on the X chromosome may be involved in susceptibility. Hallmayer et al. found modest evidence of linkage for autism on the X chromosome [30]. Skuse et al. has suggested that there may be a defensive imprinted locus on the X chromosome [31]. But these measures of ASD from the disease were just a few. ASD often do not know the course every valid cause.

3.1.4.2 Immunological factors

The immune system and brain can communicated through neurotransmitters, hormones, cytokines and chemokines by can be motivate neuronal functions and release tropic hormones such as thyroid-stimulating hormone. This system can be related to a combination firmly in control, balance and changes in one can affect others, and it is possible biological immune disorders can have a huge impact on. The nerves were function and increasing evidence that the decline of the immune system is associated with ASD [32]. In addition, abnormalities in peripheral immune cells were linked with behavioral symptoms in ASD and signs of inflammation of the nervous system had been present in the brains of postmortem individuals with ASD. Different levels of cytokine changes in brain, cerebrospinal fluid (CSF) and CNS- antibody reactions were also in individuals with ASD that were reactive to CNS proteins and have the potential for neuronal tissue destruction, with dysfunctional immunity, leading to an inappropriate or ineffective immune response to pathogen challenge. The decline of the immune system in the brain corresponding reported in children with ASD, including a reduction in the number of white blood cells, peripheral reduce the response to T cell mitogens incomplete or partial cell activation T informed by the numbers. The rise of DR + T cells without expression of the receptor IL-2 (IL-2R) dysregulated mechanism of death, and the imbalance of serum Ig Publishing addition, many genes involved in ASD such as class I; *HLA-B*44* , class II; *HLA-DRB1* alleles, class III complement *C4* alleles. HLA were polymorphic height of genes with some of the diverse allelic greatest genome gene HLA both

polygenic (containing multiple genes) and polymorphic (containing multiple variants of each gene).

3.1.4.3 Environmental

Factors that could cause environmental separated effectively into prenatal, perinatal and fetal distress factors. Implicated prenatal in first trimester the factors were including congenital rubella syndrome (CRS, secondary to rubella infection), cytomegalovirus and viral infection can activates the mother's immune response. The lifestyles of mother during pregnancy as drinking alcohol and tobacco smoking were increased the risk of ASD. Perinatal that appears to increased risk factors of ASD were associated with obstetric conditions fell into 2 categories 1) like low birth weight (LBW ≤ 2,500 g)and 2) birth asphyxia (hypoxic-ischemic insult) and others such as abnormal gestation age at birth of less than 35 weeks length, low Apgar score (≤ 7) at 5 minutes [36]. Postnatal factors involve a wide range of insults including autoimmune disease, viral infection, oxidative stress, vitamin D deficiency [37], heavy metal toxicity and controversial MMR vaccine as mentioned earlier, exposure to drugs or teratogen during pregnancy were found to increase ASD risk in the most recent meta-analyses. Prenatal exposure to valproic acid associated with an increased risk of ASD acceptable, especially in the first trimester of pregnancy. Children exposed in utero to valproic acid had 8–fold increased risk of ASD. The gestation associated with the use of psychiatric *drugs* in the mother, such as SSRI drugs were during pregnancy increased from 1.5%, 6.4% and 6.2% in 1996,2004 and 2005, respectively [38]. It was suggested that antidepressant exposure during pregnancy modestly increases the risk of ASD, especially in the first trimester [39]. Lastly, exposure in pregnancy second and third trimester were increased the risk to an pesticide as organophosphate insecticide, chlorpyrifos, thalidomide, misoprostol, was found to increase the risk of ASD [40].Moreover, the highest of parental age (father, ≥ 35 years and mother, ≥ 30 years) was found to be statistically significant an increase in risk associated with ASD.

3.2 Core Features of Autism Spectrum Disorders

3.2.1 Social and Communication Deficits

Impairments in social interaction is a major symptom of autism. The level of violence is different. Although autistic children can be tamed. By trying to close the party. But what is different from typical children by lack of feeling and attention-sharing behaviors

From the beginning, the baby is developing normally have a social life. Early in life, they turned to stare at the sound, and even a smile. In contrast, most children with ASD seem to have tremendous difficulty learning to engage in the give and take of everyday human interaction. Even in the first few months of life, many do not interact and they poor eye contact, do not like to hold or oppose the holding time and often seem to like being alone, do not be friends. Individual children, the symptoms are less visible disorder was not clear, the social interaction with others can do. However, lack of commitment and flexibility when children reach school age. Symptoms are more noticeable because social situation is more complex for children with ASD also are slower in learning to interpret and lack of understanding or a friend. Behavior patterns that do not grow as they age such as crying in class or verbal, aggression behaviors do not seem to fit with those around them. .The individual with ASD may be disruptive and physically aggressive at times, the social relationships still more difficult. They have a tendency to “lose control,” especially when they were in a strange surroundings or components, or when angry and frustrated. They may at times break thing s, attack others, or hurt themselves. In their frustration, some bang their heads, pull their hair, or bite their arms. Some infants who later show signs of ASD flap and babble during the first few months of life, but they soon stop. Others may be delayed, developing language as late as age 5 to 9 of life.

The other deficit of social and communications that occur in different developmental imaginative play such a complex player and cooperative play in a group. Many individuals with ASD are capable of verbal remained still likely to be limited gestures that are not well integrated with other modes of communication. (Such as eye contact, vocalizations) All of these deficits and delays might affect negatively the development of meaningful social relationships with friends and others.

3.2.2 Restricted, Repetitive and stereotypic patterns behavior and Interests

Another hallmark of ASD was restricted and repetitive behaviors and interests (RRBs) can occur in toddlers. Repetitive behavior is observed, help to diagnose disease as well. RRBs were including a very broad category of behaviors limited in focus, interest and intense preoccupations (e.g., children might interest and spend hours lining up their cars and trains not interest other); adherence to specific, nonfunctional routines (e.g., insisting on taking that a certain way to school sameness was resistance to change); These behaviors may be physical behaviors and movements are restricted to a few items of interest in activities such as hand flapping, head rolling, or body rocking; and preoccupation with parts of objects (e.g., peering at the wheels of toy cars while spinning them). ASD children had need, and demand, absolute stability in their environment. A slight change in any routine-in meal times, dressing, bathing, going to school at a certain time and by the same route-can be extremely disturbing.

3.2.3 Regression and Other Patterns of Onset

Some children with ASD onset to show symptoms that were specific to ASD and seen approximately 25%, typically between the ages of 18 and 24 months (mean: 20.4 months) [41]. Children were appear to be developing typically for the first year or two. In the second year of life, they lose skills and language that they had previously acquired, accompanied by the onset of ASD symptoms. The regression symptom was very specific with ASD. Some children who show loss in the domain of language but then recovered skills they were closed to typical development prior to the disruption of social continuity. However, Lord et al. [42] also argued that, for many children with loss of language, development prior to loss was rarely reported as entirely normal. In previous study, a sample from many families in which both affected family members had a history of regression showed a prevalence of linkage on chromosome 7q13.3 and 21q13.3 region, including regions containing genes expressed in the brain of the fetus [43]. In contrast, several studies have found that children with a history of regression in childhood, will later show do not differences in the severity of symptoms of ASD, work on cognitive and adaptive behaviors, seizures, and gastrointestinal problems compared to children without a history of regression.

3.2.4 Neuropathy of ASD

Postmortem studies can directly characterize the disorder of the brain, viewed as static in ASD. The few cross-sectional studies that examined age-related changes reveal a complex pattern of abnormal growth in the cerebellum, cerebrum, and amygdala and possible differences in hippocampus [44, 45]. Neurotransmitter such as DA and 5-HT were known to be involved in the immune response control. When perinatal immune change (both pre- and postnatal infection in humans) leads to neurodevelopmental dysfunction, permanent immune dysregulation and abnormal behavior, which have been shown to be accurate in translating research in neuropsychiatric disorders and neurodegenerative disorders such as schizophrenia, mood and anxiety disorders, ASD, Huntington's, PD and Alzheimer’s disease; AD. In early immune activation may be one of the environmental factors that can predispose individuals to develop schizophrenia. [46].

Neuroactive tryptophan metabolites have been degradation closely linked to the pathogenesis of several neurodegenerative diseases. Tryptophan is an essential amino acid required for protein synthesis, derived from food, is transported to the brain to make the neurotransmitter was also converted into the key neurotransmitters 5-HT and tryptamine

5-HT is similar to catecholamines and is an inhibitory neurotransmitter. It is made from the amino acid, tryptophan. Serotonin is converted to melatonin in the pineal gland. It belongs to the group of so-called biogenic amines (monoamine) neurotransmitters in the CNS. 5-HT is primarily found in the gastrointestinal tract (GI tract), platelets, and the CNS. It have been regulating cognitive and endocrine functions, hunger, thirst, mood, appetites, cardiovascular regulation, respiration, cognition, body temperature regulation, and it modulates pain sensitivity, sexual behavior, stress reactivity, circadian rhythm, and sleep-wakefulness [50]. In addition, the brain 5-HTergic system was also related to changes in behavior and mood, anxiety, aggressiveness, nervousness, depression and schizophrenia, which are known to be accompanied with immune abnormalities [51].. Among the 5-HT receptors the 5-HT_{1A}- and 5-HT_{2A}-subtypes, are of particular interest since they are known to play a crucial role in the regulation of neurotransmission, emotional and behavioral processes as well as pathophysiology of various neuropsychiatric disorders [52]. Early studies of

blood 5-HT levels in autism consistently found elevation is usually expressed as 5-HT in whole blood, and has typically been about 50% above normal levels [53]. Subsequent research has established that more than 99% of whole blood 5-HT is contained in the platelets and that platelet 5-HT accounts for the hyperserotonemia in autism [54].

DA is a neurotransmitter of the catecholamine and phenethylamine phenethylamine that is synthesized from the dietary amino acid tyrosine. Once ingested, tyrosine is hydroxylated (by tyrosine hydroxylase) removing a carboxyl group from a molecule of L-dihydroxyphenylalanine (L-DOPA), via the enzyme DOPA decarboxylase. Most DA-containing neurons lie in the midbrain; in particular, three areas substantia nigra, the ventral tegmental area and hypothalamus [47]. DA is an important neurotransmitter in the CNS involved in the control of locomotion, emotions, cognition, and neuroendocrine secretion [48] and also shown to modulate immune functions [49]. Molecular biology methods have contributed to the characterization of five subtypes of DA receptors leading to the classification of the D_1-like (D_1 and D_5) and D_2-like (D_2, D_3 and D_4) subclasses.

ASD has been recognized as a multifactorial disorder with other risk factors contributing to the phenotype. Studies have shown that 5-HT, DA and genetic differences in serotonin transport and dopamine transport could contribute to the development of ASD, as 5-HT has a vital role in stimulating cell proliferation in the developing brain during pre- and postnatal periods as well as in early infants [55].

3.3 Comorbidities of Autism Spectrum Disorders

3.3.1 Sensory and Motor Impairments

Although sensory and motor impairments were not currently part of diagnostic criteria, children with ASD often report abnormal sensory behaviors and deficits in motor skills. Both increased and decreased responsiveness to sensory stimuli have been observed in children with ASD [56]. When the perceptions of the child are correct, they can learn from what they see, feel or hear. On the other hand, if the data is faulty sensory experience of the world's children may confuse many

children with ASD are highly adaptable and even painful sensitivity to sound a certain texture, taste and smell. Some children find the feel of clothes touching their skin almost unbearable. Some sound - Vacuum, call a sudden storm, even the sound of the waves lapping shoreline- make these children to cover their ears and scream ASD in the brain seems unable to balance their feelings appropriately. Some children with ASD were oblivious to extreme cold or pain. ASD children may fall and break an arm, yet never cry. Another may bash his head against a wall and not wince, but a light touch may make the child scream with alarm. However, the severity and frequency of sensory impairments have been found to differentiate children with ASD from those with other non-ASD disorders.

Motor impairments have been also reported to be established in individuals with ASD. Up to 33% of cases of individuals with ASD show delays in motor milestones [57]. Some children with ASD, though not all, display problems with coordination and balance, gait disturbances such as tiptoeing, and significant postural abnormalities [58].

3.3.2 Intellectual Impairments

Individuals with ASD may experience other cognitive, emotional, and behavioral disorders range from severe intellectual disability to scores in the superior range on tests of cognitive functioning. In the past, it was believed that more than 50% of individuals with ASD had nonverbal IQ scores below70 (e.g., mild to severe intellectual disability). However, recent studies have showed that the proportion of children with ASD with nonverbal IQ scores below 70 is somewhere between 20 and 50% [59]. In addition, nonverbal IQ scores in most children with ASD are found to be stable in the period from age 2 to age later.

For occurrence, the results of some studies have suggested that individuals with Asperger syndrome (AS is differentiated from autism by the absence of a history of language delay) have higher verbal and/or nonverbal IQs than those with high functioning autism (HFA) [60]. Individual’s children with ASD have also varied in their IQ profiles.

3.3.3 Seizures

One-third of children with ASD will develop into seizures, risk factors for seizures and epilepsy in ASD include mental retardation, motor impairment, symptomatic etiology, and seizure onset either in early childhood (before 5 years of age) some at puberty, and some at adulthood. Seizures were caused by abnormal electrical activity in the brain (brain wave), can produce a temporary loss of consciousness (a "blackout"), structural or developmental lesions, genetic/genomic abnormalities, a body convulsion, abnormal body movements, or staring spells. Sometimes a contributing factor is a lack of sleep or a high fever. An EEG (electroencephalogram) can help confirm the seizure's presence. Seizures had been divided into two general types: 1) generalized clonic-tonic seizures were which the whole body shakes rhythmically and convulses. Cause of generalized seizure from the entire brain demonstrates abnormal electrical activity. 2) Partial seizure only one part of their body, such as an arm or leg, may demonstrate rhythmic activity, cause from the only one part of the brain experiences abnormal electrical activity. In most cases, seizures can be treated by a number of medications called "anticonvulsants or antiepilepsys". The dosage of the drug is adjusted carefully by monitored to ensure a positive response to such treatment so that the least possible and most effective.

3.4 Associated between Autistic Spectrum disorders and *HLA-B* and *CYP2D6*

3.4.1 Autism Spectrum Disorders and *HLA-B*

The MHC, (in humans these genes are called HLA) gene extends about 4×10^6 bps. In humans it contains more than 200 genes on band 6p21.3 has an essential role in the innate (non-specific) and adaptive (specific), both of which have humoral (antibody) and cellular components **(Table 3.1)** human immune system. The innate immune response was constitutively expressed and germ-line encoded and therefore does not adapt to antigen. It is available on a first line of defense and most powerful

phage time through the activation of the complement system is natural, it is very important to the antigen/protein antigen processing/presentation protein.

The MHC gene family is divided into three subgroups Class I, II and III. HLA class I has contains major *A, B* and *C* and minor *E, F* and *G*; Class II has contains *DR, DP* and *DQ,* while Class III has contains various genes which have immune related functions including the tumor necrosis factor (TNF) α and-β genes, lymph toxin alpha (LTA), heat shock proteins and many other non-immune related genes (http://hla.alleles.org/nomenclature/stats.html) **(Figure 3.4)**. The nomenclature of *HLA* alleles has been updated recently [82]. *HLA-B* was the most polymorphic gene in the human genome; it contains more than 1,600 alleles (http://www.ebi.ac.uk/imgt/hla/).

Table 3.1 Components of Adaptive and Innate Immune Systems

Component	Innate	Adaptive
Cellular	Monocytes/macrophages Gamma/delta T-lymphocytes NK cells, granulocytes	Alpha/beta T-lymphocytes B-lymphocytes
Humerol (antibody)	Complement, Acute phase- globulins, Mannose binding lectin	Antibodies-all classes

MHC I molecules were found on all nucleated cells [33]. They were trimeric proteins comprised of one transmembrane heavy chain (with three α1-3 domains), a β2-microglobulin (β2m) light chain, and a peptide bound within the groove formed by the α1 and α2 regions of the heavy chain **(Figure 3.2)** The majority of class I bound peptides were generated in the cytosol and were subsequently translocated into the lumen of the endoplasmic reticulum (ER). Peptides presented by MHC I heavy chain were derived from proteolysis of intracellular proteins and were monitored by cytotoxic CD8+ T lymphocytes (T-cells) receptor proteins (TCRs).

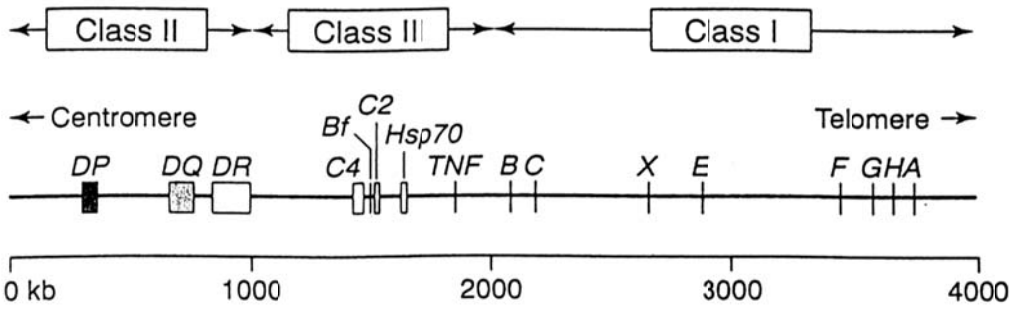
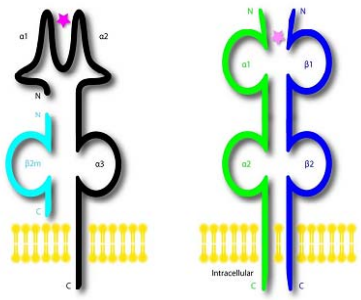


Figure 3.2 Genomic organization of the human MHC, drawn to scale. Only genes of particular interest for the immune system are indicated

MHC II molecules were found in a more restricted subset of cells in the body than MHC I. They were expressed exclusively by antigen presenting cells such as macrophages, dendritic cells and B cells, as well as some endothelial cells and the epithelium of thymus. MHC II molecules consist of two transmembrane chains (α and β); each chain contains two domains (α1-2 and β1-2) (Figure 3.2). Following endocytosis, these extracellular proteins were digested in lysosomes and then loaded onto MHC II molecules prior to trafficking to the plasma membrane. On the cell surface, MHC II presents exclusively to CD4+ helper T cells, which often either recruit phagocytes to increase local inflammation or initiate an antibody-based immune response through B cell activation. MHC II were included *HLA-DP, -DQ*, and *–DR*. Of special significance to ASD, *DR4* and *DR13/14* haplotypes bear the *DRβ1* gene that encodes the beta subunit of the *HLA-DR* which has been implicated in ASD.

MHC III molecules encode a large number of diverse proteins some with important immune functions and others with no known immune function. Those with immune functions include complement proteins (C2 and C4)-components of the classical complement pathway. MHC III also encodes the cytokines TNF-α and-β. TNF-α was a major pro-inflammatory cytokine that was responsible for early immunological responses. Additionally,which mediate cellular responses to heat, stress and viral infection. Unlike MHC I and II, MHC III molecules do not present peptides.



MHC I MHC II

Figure 3.3: Schematic representations of MHC Class I and II molecules. MHC I molecules were trimeric proteins comprised of one transmembrane heavy chain (α1-3 domains; black), a β2-microglobulin light chain (β2m; light blue), and a peptide (pink star) that was bound within the groove of α1 and α2 of the heavy chain. The MHC I peptide was generated in the cytosol. MHC II molecules consist of two transmembrane chains an α (green) and a β (dark blue); each chain contains two domains (α1-α2, and β1-β2). The peptide was bound for display in the groove of the α1 and β1 domains and was derived from extracellular pathogens.

The humoral (antibody; Ab) response was initiated by antigen presentation by HLA class II molecules to TCRs on CD4+ T cells, whereas the cell mediated response is initiated by HLA class I presentation of antigen to TCRs on CD8+ T cells. As a result of HLA class I presentation, cytotoxic T lymphocytes become activated and play important roles in the clearance and control of bacterial and viral infections. Therefore, both HLA class I and class II molecules have important functions in regulating immune responses to pathogens and have been associated with susceptibility to many autoimmune diseases. In particular, the *HLA-A2* allele has been associated with juvenile rheumatoid arthritis [61], Hashimoto's thyroiditis [62], and Lyme disease [63]. About in AD, a neurodegenerative condition with an inflammatory component, several but not all studies have found an additional of *HLA-A2* [64, 65]. The *HLA-A2* allele has been suggested to play a role as a restricting element in cytotoxic T-cell recognition in the fetomaternal relationship to male fetuses [66]. This may be of interest as ASD is four times more common in males for unknown reasons.

Ferrante [67] reported that ASD subjects consisting *HLA-A2* and -*DR11* alleles had a significant decrease in CD4+ naïve cells and an increase in CD4+ memory T cells.

Stubbs observed over 20 years ago that children with ASD often fail to build antibodies following rubella vaccination. Others have reported upper antibody titers to brain proteins in subjects with ASD. Explanations such as these were important in bringing attention to a possible connection of immune system dysfunction with ASD. It has also been shown that ASD subjects have decreased responsiveness to T-cell mitogens, reduced numbers of helper T-cells and suppressor-inducer T-cells as well as decreased natural killer cell activity. The ability to discriminate self from non-self in a highly specific manner is critical for survival**.** The HLA region is remarkable in at least 3 respects:

(a) Allelic polymorphisms;

(b) Disease associations;

(c) Gene density, sequence duplications, insertions and deletions.

There was extensive research being done into the immunological aspects of the class III region was around *C4* since many of these genes were expressed in immune cells. This laboratories had been published several documents showing HLA suggestions with ASD. These publications were important in showing that MHC genes/proteins were somehow involved or associated with ASD and should be studied to perhaps better delineate ASD pathogenesis and autoimmune characteristics. It was interesting to communication that after years of research into HLA associations, the often-overlooked class III complement components have the strongest autoimmune-associations. Increased occurrences of C4 null alleles have been associated with systemic lupus erythematosus (SLE), insulin dependent diabetes mellitus, membranoproliferative glomerulonephritis (MPGN), bacterial meningitis, scleroderma and ASD.

3.4.2 Autism Spectrum Disorders, behavior and psychopathology and *CYP2D6* polymorphisms

CYP enzymes were first discovered in drug metabolism roles, they were often thought of as restricted in locale to hepatic tissue and in function to ridding the body of unwanted hydrophobic toxins. However, we now know that P450 enzymes

have ubiquitous throughout the body and indeed throughout nature, serving to catalyze the seemingly simple. They were important in many cell types with individual isoforms expressing to varying degrees according to cellular function. They appear in intestinal tissue, where they may contribute up to 50% of the observed first-pass effect for a given drug and then appears in skin, breast, lung, and heart tissue, where we were only beginning to increase their roles in dietary and disease conditions. They were also perform in brain, where they catalyze several pathways and in a very real sense can and do contribute to who we were as human beings. We were qualified that a true pharmacogenetic polymorphism, dissimilar an inborn error of metabolism, was a genetic alteration that does not produce a discernible effect in the absence of pharmacological agent. If this enzymes in normally were pays to endogenous pathways. Consider that any drug concentrated in the brain (or other tissue) enough to be affected by local metabolism will be necessarily interact with the endogenous pathways normally catalyzed by this enzymes. *CYP2D6* was perhaps the most interesting of the xenobiotic metabolizing enzymes that are involved in the human brain because for monoamine-like molecules and its prominent role in the metabolism of many psychoactive compounds. Neuronal cells, as well as glial cells, show labeling for mRNA in brain regions such as the neocortex, caudate nucleus, putamen, globus pallidus, hippocampus, hypothalamus, thalamus, substantia nigra and cerebellum. In dissimilarity, *CYP2D6* protein has been primarily localized to large principal neurons such as pyramidal cells of the cortex and hippocampus, and Purkinje cells of the cerebellum. In glial cells, *CYP2D6* protein appeared to be absent [68]. Metabolism of drugs by *CYPs* result in the formation of hydrophilic metabolites easily excreted from the body in form biological fluids. It was possible to hypothesize that, if such metabolites were produced in the brain, it would result in the prolonged presence and lower clearance from the brain. Cerebral metabolism could modulate the pharmacological response and explain a part of the variability in response to centrally psychoactive drugs, reflecting interindividual differences in localized brain CYP-mediated metabolism. Moreover, minor metabolic pathways of drugs could potentially produce significant pharmacological or toxicological response, particularly if they occur at the site of action. Chen et al. have shown that at least the initial analgesic effects of codeine are due to morphine produced in the brain, not in the liver [69].

In accumulation to the mapping of *CYP2D6* in the brain, this enzyme has also been involved in the metabolism of tyramine to DA *in vitro* [70] and in the regeneration of 5-HT from 5-methoxytryptamine. Interestingly, *in vivo* studies have shown that UM display higher serotonin concentrations in platelets than EM and PM. A potential influence of *CYP2D6* polymorphism in the balanced functioning and physiological crosstalk of the DA and 5-HT endogenous systems has been proposed. This hypothesis was based on the results from another *in vivo* study suggesting that PM might have a higher DA tone in the pituitary, which might be in combination with lower serotonin tone since the serotonin systems exerts a tonic inhibitory control on the dopaminergic circuits [71]. 5-HT had been the role in early neural development can leave permanent alterations in brain function and behavior and may be possible etiological factor in the development with ASD and Down syndrome [70].

Moreover, *CYP2D6* polymorphisms have also been shown to contribute to endogenous metabolism of neuroactive substrates, which can explain the associations hitherto observed with human behavior and disease susceptibility (e.g., personality, neurocognition and neuropsychiatric disorders) [9].

Previous studies in healthy volunteers in Sweden [72] and Spain [73] were phenotyped with debrisoquine and evaluated with Karolinska Scales of Personality (KSP) found significant differences were again noted/replicated between PM and the EM groups in this independent study sample of Spanish subjects, admittedly from a social and environmental context different from that of Sweden, thereby lending further support for linkages between *CYP2D6* variation and personality or behavioral traits. PM was shown to report greater levels of psychic anxiety and lower socialization than EM (In PM Swedish were found to report lower levels of psychastenia than the rest the healthy volunteers or EM) [72].

Anxiety and depression, A study testing whether *CYP2D6*4/*4* homozygous carriers (two null allele) were more predisposed to anxiety and depression disorders in the elderly found no associations [74], although an increased frequency of UM has been reported among women with late pregnancy or post-partum depressive symptoms.

Eating disorders, the higher frequency of *CYP2D6* UM (evaluated by debrisoquine test) was found among the group of the population with higher scores on

a scale measuring symptoms of bulimia [63]. Similarly, *CYP2D6* allele distribution in patients with eating disorders were related to higher enzyme activity than in healthy controls [75].

Suicide, a higher frequency of *CYP2D6* UM among individuals who died by suicide than in those with a natural death has been reported [63] and also among patients with eating disorders with a history of suicidal attempts compared with those who had never attempted suicide [63].

Therefore, interindividual variability in *CYP2D6* hydroxylation capacity may have implications not only for the metabolism of several psychotropic drugs but also for explaining differences in human behaviour and psychopathology. However, considering that most *CYP2D6* endogenous neuroactive substrates show low affinity, new studies were necessary to identify the definitive role of *CYP2D6* in different human brain tissues. Furthermore, *CYP2D6* expression patterns in different brain regions and their functional implications in brain metabolism might be considered.

Besides, the evaluation of *CYP2D6* in clinical trials may be of importance for *CYP2D6* substrates given its involvement in drug metabolism, in particular for active drugs in the CNS, because local brain metabolism may be important for the interaction with neuroactive substances and their biotransformation.

3.5 Pharmacotherapy of Autistic Spectrum disorders

Lack of understanding of the origins of abnormal behaviors in autism has impeded the development of rational drug therapies. Treatments were further to complicated by a tremendous range of syndrome expression, perhaps due to the involvement of many neurotransmitters [76]. There was no specific drug to cure ASD or to treat its core symptoms of poor social relatedness, communication skills deficit and repetitive and stereotypic behaviors. Therefore, the goal of drugs treatment of ASD were, to decreased the frequencies of the maladaptive behaviors, such as hyper-activity, aggression, self-abusive behavior, and temper tantrums, liability of mood, irritability, social withdrawal, anxiety, repetitive compulsive behaviors, and stereotypies. Atypical behaviors should be examined for their antecedents and contexts

in which they occur and treatment should be considered only if the behaviors were maladaptive, interfere with programming, or improvement the quality of life.

Medical treatments for symptoms of ASD comprise a variety of pharmacologic agents including antipsychotics, psychostimulants, and serotonin reuptake inhibitors (SRIs) that were generally intended to treat common comorbidities of ASD. This was most useful when several medications were prescribed for treatments.

3.5.1 Anxiety and depression.

The SSRI were the medications most often prescribed for symptoms of anxiety, depression, and/or obsessive-compulsive disorder (OCD). Only one of the SSRI's, fluoxetine, (Prozac®) has been approved by the Food and Drug Administration (FDA/ US FDA) for both OCD and depression in children age 7 and older. Three that have been approved for OCD were fluvoxamine (Luvox®), age 8 and older; sertraline (Zoloft®), age 6 and older; and clomipramine (Anafranil®), age 10 and older. Treatment with these medications could been associated with decreased frequency of repetitive, ritualistic behavior and improvements in eye contact and social contacts.

3.5.2 Aggression and Behavioral problems

Aggression and Behavioral problems were associated symptoms of ASD that can result in significant harm to affected individuals and marked suffering for families; they were among the symptoms most likely to cause a psychopharmacological consultation or emergent psychiatric referral. Although the relationship between aggression or self-injurious behavior (SIB) and ASD remains unclear, these symptoms were treated with a broad range of pharmacological approaches. Various mechanisms have been proposed to underlie the presence of these symptoms in ASD, and numerous targets for pharmacological intervention have been suggested. These targets include the dopaminergic, serotonergic, adrenergic, and opioid systems, among others. Reviewing controlled trials to specifically target aggression using a primary outcome measure, several medications have produced significant improvement as compared to placebo, Antipsychotic medications have

including tianeptine, methylphenidate, risperidone, aripiprazole, clonidine, and naltrexone. These medications work by reducing the activity in the brain of the neurotransmitter DA**.** Among the older, typical antipsychotics, such as haloperidol (Haldol®), thioridazine, fluphenazine, and chlorpromazine, haloperidol were found in more than one study to be more effective than a placebo in treating serious behavioral problems. However, haloperidol, while helpful for reducing symptoms of aggression, can also have ADRs such as fast/irregular heartbeat, sedation, muscle stiffness, and loss of balance control. Placebo-controlled studies of the newer "atypical" antipsychotics were being conducted on children with ASD. Risperidone was the first medication to receive US FDA approval for the treatment of irritability in children with ASD. Risperidone/benzisoxazole derivative, was an atypical antipsychotic drug with high affinity for 5-hydrotryptamine (5-HT) and dopamine D2 receptors (DRD2). It was used primarily in the management of schizophrenia, inappropriate behavior in severe dementia and manic episodes associated with bipolar I disorder. Risperidone was been effective for treating the positive and negative symptoms of schizophrenia owing to its affinity for its "loose" binding affinity for DRD2 and additional 5-HT antagonism compared to first generation antipsychotics, which were strong, non-specific DRD2 antagonists. Treatment of schizophrenia in adults and in adolescents, ages 13 to 17, and for the short-term treatment of manic or mixed episodes of bipolar I disorder in children and adolescents ages 10 to 17.

3.5.3 Seizures and epilepsy

Reports of the prevalence of seizures and epilepsy in ASD ranges from 5% to 38.3% [68], most common in child with low IQ or were mute. They were treated with one or more of the anticonvulsants such medications as CBZ (Tegretol®), lamotrigine (LTG; Lamictal®), topiramate (Topamax®), and valproic acid (Depakote®). The level of the medication in the blood should be monitored carefully and adjusted so that the least amount possible was used to be effective. Although medication usually reduces the number of seizures, it cannot always eliminate them. CBZ, an anticonvulsant structurally similar to tricyclic antidepressants, was used to treat partial seizures, tonic-clonic seizures (grand mal), pain of neurologic origin such as trigeminal neuralgia and psychiatric disorders including manic-depressive illness

and aggression due to dementia. CBZ inhibits sustained repetitive firing by blocking use-dependent sodium channels. Pain relief was believed to be associated with blockade of synaptic transmission in the trigeminal nucleus and seizure control with reduction of post-tetanic potentiation of synaptic transmission in the spinal cord. CBZ also possesses anticholinergic, central antidiuretic, antiarrhythmic, muscle relaxant, antidepressant (possibly through blockade of norepinephrine release), sedative, and neuromuscular-blocking properties.

Pharmacological treatment for ASD focuses on improving core deficits in social communication, as well as addressing challenging behaviors to improve functional engagement in developmentally appropriate activities. In addition to addressing core deficits, treatments are provided for difficulties associated with the disorder (epilepsy, bipolar disorder, anxiety, attention difficulties, sensory difficulties, etc.). Individual goals for treatment vary for different children and may include combinations of therapies. The most frequently prescribed medication for patients with ASD include antipsychotics, antidepressants, anticonvulsants, mood stabilizers, and cholinesterase inhibitors [77-80]. However, the effectiveness of most of these medications among patients with ASD had been uncertain. In fact, few placebo-controlled, double-blind studies have been performed on any of these medications. As a result, much of our current knowledge of treatment of ASD has been from trials-and-errors regarding the selection of medications. Little attention has been paid to the atypical responses of individual patient to certain medications and genetic background of these individuals [79-81]. Thus, a pharmacogenomics approach is definitely needs to better the treatment of ASD.

3.6 Pharmacology of Carbamazepine and Risperidone

3.6.1 Carbamazepine

3.6.1.1 Pharmacodynamics and Pharmacokinetics of Carbamazepine

CBZ, or dibenzazepine, was approved in the US FDA used as an anti-seizure composed a tricyclic compound and indicated for the treatment of epilepsy, trigeminal neuralgia and psychiatric disorders. It was now considered the

primary drug for the treatment of partial and tonic-clonic seizures. CBZ was almost completely metabolized in the liver with only around 5% of the drug excreted unchanged. The major route of metabolism is conversion to CBZ-10, 11-epoxide (CBZ-E). This reaction is primarily catalyzed by *CYP3A4* although *CYP2C8* also plays a role, and involvement of *CYP3A5* has also been suggested **(Figure 3.3)**. Minor metabolic pathways include ring-hydroxylation to form 2-hydroxy-CBZ (2-OH-CBZ) and 3-hydroxy CBZ (3-OH CBZ).

Pharmacodynamics of CBZ, The response to CBZ was variable and may be due to its variable transport, especially across the blood-brain-barrier. The transporter that may confer drug resistance was *RALBP1*. The targets of CBZ inhibits sustained repetitive firing by blocking use-dependent sodium channels *SCN1A, SCN1B, SCN2A,* and *SCN3A* have pharmacogenomic consequences.

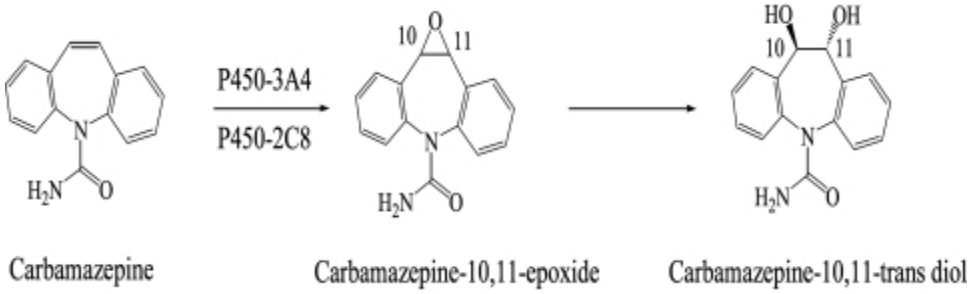


Figure 3.4: CBZ metabolism pathway. E1-monooxygenase, E2-epoxide hydrolase

The variant of *SCN1A* has been associated with high-dose requirements in patients with epilepsy. Variants in *SCN2A* and *SCN3A* may contribute to CBZ resistance to CBZ in individuals with epilepsy [75]. Pain relief was believed to be associated with blockade of synaptic transmission in the trigeminal nucleus and seizure control with reduction of post-tetanic potentiation of synaptic transmission in the spinal cord. CBZ also possesses anticholinergic, central antidiuretic, antiarrhythmic, muscle relaxant, antidepressant (possibly through blockade of norepinephrine release), sedative, and neuromuscular-blocking properties.

Pharmacokinetics of CBZ, Suspension, Conventional tablets and extended-release tablets distributed equivalent amounts of drug to the systemic circulation. However, it has been observed that the suspension was slightly faster

absorbed. Additionally, the extended-release tablet was slightly slower than the conventional tablet. The bioavailability of the extended-release tablet was 89%, compared to the suspension. Plasma levels of CBZ was variable. The time to peak concentration for the dissimilar formulations were as follows: suspension = 1.5 hours; conventional tablets = 4-5 hours; extended-release tablets = 3-12 hours. CBZ 76% were bound to plasma protein. Hepatic enzyme, *CYP3A4* was the primary isoform responsible for the formation of carbamazepine-10,11-epoxide. This metabolite was active ad shoe to be equipotent to CBZ as an anticonvulsant. CBZ was more rapidly metabolized to the aforementioned metabolite in younger patients than in adults. It also undergoes glucoronidation via *UGT2B7*, however this finding had been disputed. Elimination, 72% of the dose was in the urine while 28% was in the feces. Hydroxylated and conjugated metabolites were largely what was recovered as uncharged CBZ. Initial half-life values range from 25-65 hours, decreasing to 12-17 hours on repeated doses.

3.6.1.2 *HLA-B* and CBZ

ADRs were rated as the fifth leading cause of death among all diseases. Approximately 5-8% of all hospitalisation worldwide was due to ADRs. Severe cutaneous adverse reactions to drugs (SCARs) were the commonest ADRs (30-45%) and responsible for about 2% of hospital admissions and were considered as a major public health issue because of morbidity and even death. The clinical of drugs hypersensitivity range from mild skin rash, such as maculopapular exanthema (MPE), to potentially life-threatening reactions, including drug rash with eosinophilia and systemic symptoms (DRESS, also known as drug induced hypersensitivity syndrome (DIHS), SJS and TEN. Among AEDs, particularly drugs with aromatic structures such as CBZ, phenytoin (PHT), oxcarbazepine (OXC), phenobarbital (PB) and LTG, were the most common causes of cADRs [83-85].

CBZ was extensively metabolized in the liver, with less than 5% of an oral dose excreted unchanged in urine. It has a narrow therapeutic range between serum concentrations of 3–12 μg/mL. CBZ was the main cause of the SJS/TEN, in Southeast Asian countries. The incidence of SJS/TEN was estimated to be of around 1-2 patients per million individuals per year. SJS/TEN were classified as

the same disease with different spectrums of severity according to the magnitude of epidermal detachment. Early symptom of the abrupt onset of SJS usually start with high fever, sore throat, malaise, and a rapidly developing, blistering exanthema of macular papules and target-like lesions, accompanied by mucosal involvement. This condition was associated with a rate of death of approximately 5%. TEN has a similar clinical presentation, with even more extensive skin detachment and a death rate of 25 to 35% [86]. One mechanism that has been suggested for how CBZ hypersensitivity was CBZ or its toxic metabolites act as haptens providing antigenic stimulus. The stimulated cytotoxic CD8+T-cells clonally expand and along with the help of perforins, granzyme B, granulysins and cytokines (especially TNF-α) mediate the keratinocyte apoptosis leading to epidermal necrosis. TNF-α upregulates Fas (death receptors) on effector cells and Fas ligand (FasL) on the keratinocytes leading to their interactions: thus amplifying the apoptotic pathway. The strongest association (OR > 1,000) between *HLA-B*1502* and drug-induced hypersensitivity has been detected for CBZ- SJS/TEN in the Han Chinese population [19, 87, 88]. The association was later confirmed in several other Asian populations including Thai [21, 89], Malay [90] and Indian [20], but not in Caucasians, Japanese and Korean population [91]. Interestingly, the frequency of SJS/TEN was higher in Asian populations than in Caucasians, as was the frequency of the *B*1502* allele (http://www.allelefrequencies.net) which provides circumstantial evidence for its potential functional relevance. The FDA of US and similar regulatory agencies of other countries relabeled the drug information of CBZ, and advised physicians to screen *HLA-B*1502* before starting the treatment of CBZ in patients with Asian ancestry [92]. Apart from being ethnicity-specific, the genetic marker for CBZ induced hypersensitivity was phenotype specific. *HLA-B*1502* was only valid for SJS/TEN, but not for MPE or DRESS (drug reaction with systemic symptoms) [88]. While, MPE has been associated with *HLA-A*3101* and DRESS with the polymorphisms in the motilin gene in the Han Chinese population [88]. In Caucasians, DRESS has been associated with the ancestral *HLA* allele, *B*0801*. Patients who was developed hypersensitivity to one aromatic AEDs may also be show cross-reactivity to structurally related AEDs [93]. Several case reports have shown that the *HLA-B*1502* allele may be important in patients with CBZ, PHT, LTG and OXC-induced SJS/TEN [19, 89]. These findings however need to be validated in a larger

number of ethnically diverse populations. In Caucasians, LTG-induced DRESS has been associated with the HLA alleles, *B*5801* and *A*6801* [94], although the association was relatively weak and unlikely to be useful clinically.

3.6.2 Risperidone

3.6.2.1 Pharmacodynamics and Pharmacokinetics of Risperidone

Risperidone was 3-{2-[4-6-fluoro-1, 2-benzisoxazol-3-yl) -piperidin-1-yl] ethyl}-6H, 7H, 8H, 9H-pyrido [1, 2-*a*) pyrimidin-4-one a second-generation atypical antipsychotic medicine. It was a white crystalline powder with molecular mass and molecular formula of 410.485 g/mol and $C_{23}H_{27}FN_4O_2$, respectively. It was an approved antipsychotic drug belonging to the benzisoxazole derivative and was available as tablet, oral suspension. It was works by changing the effects of chemicals in the brain. Risperdal have been approved for patients with ASD by the US FDA, 2006 to treat symptoms of irritability.

Pharmacodynamics, Risperidone was a selective monoaminergic antagonist with high affinity for the serotonin Type 2 (5-HT2), dopamine Type 2 (D2), α1 and α2 adrenergic, and H1 histaminergic receptors. Risperidone was acted as an antagonist at other receptors, but with lower potency. The clinical effect from risperidone results from the combined concentrations of risperidone and its major metabolite, 9-hydroxyrisperidone (Paliperidone).

Pharmacokinetics, Adsorption, The absolute oral bioavailability of risperidone is 70% (CV=25%). The relative oral bioavailability of risperidone from a tablet is 94% (CV=10%) when compared to a solution. Risperidone was rapidly distributed. Plasma concentrations of risperidone, its major metabolite by *CYP2D6* (**Figure 3.5**), 9-hydroxyrisperidone, and risperidone plus 9-hydroxyrisperidone were dose proportional over the dosing range of 1 to 16 mg daily (0.5 to 8 mg twice daily). Following oral administration of solution or tablet, mean peak plasma concentrations of risperidone occurred at about 1 hour. Peak concentrations of 9-hydroxyrisperidone occurred at about 3 hours in extensive metabolizers, and 17 hours in poor metabolizers. Steady-state concentrations of risperidone were reached in 1 day in extensive metabolizers and would be expected to

reach steady-state in about 5 days in poor metabolizers. Steady-state concentrations of 9-hydroxyrisperidone were reached in 5-6 days (measured in extensive metabolizers). Distribution, The volume of distribution was 1-2 L/kg. In plasma risperidone was bound to albumin and alpha1-acid glycoprotein. The plasma protein binding of risperidone is 90%, and that of its major metabolite, 9-hydroxyrisperidone, is 77%. Metabolism, Risperidone is extensively metabolized in the liver. Excretion, Risperidone and its metabolites were eliminated via the urine and, to a much lesser extent, via the feces. As illustrated by a mass balance study of a single 1 mg oral dose of 14C-risperidone administered as solution to three healthy male volunteers, total recovery of radioactivity at 1 week was 84%, including 70% in the urine and 14% in the feces.

Risperidone was show to be well tolerated and effective in treating aggressiveness, hyperactivity, irritability, SIB, stereotipies, social withdrawal and lack of interest. Through risperidone significantly improves behavioural problems in most situations, it was also associated with mild ADRs. The most common ADRs, predictably, patients on risperidone were significantly more likely to gain weight, with a mean increase of 2.7 kg as compared to 0.8 kg with placebo. Subjects receiving risperidone were also significantly more likely to experience mild (49%) or moderate (24%) increases in appetite, fatigue (59%), drowsiness (49%), drooling (27%), and dizziness (16%). Extrapyramidal symptoms, such as muscle rigidity and dyskinesias, occurred with greater frequency in patients receiving risperidone, but differences in occurrence rates as compared to placebo were not significant. Tremor was significantly more common in the risperidone group (RUPP, 2002). Results from serum analysis of lipid profile and glucose levels were not reported, but more recent studies have suggested an increased risk of elevated triglycerides with risperidone in children and adolescents. Effective doses in children that have been studied range from a mean of 1.17 mg/day to 1.81 mg/day. Clinically, the principle of starting with the lowest possible dose (0.125–0.25 mg/day), depending on weight, and titrating slowly is always encouraged. There is a significant body of anecdotal evidence that supports efficacy at doses as low as 0.5 mg/day and side effects may be age- and dose-dependent. Weight gain, for example, has been associated with younger age and higher doses [95].

Genetic variants can alter the pharmacokinetics and pharmacodynamics of drugs, potentially affecting both drug's efficacy and toxicity. Evidence indicates that genetic factors can account for an estimated 20% to 95% of drug-metabolizing enzymes, receptors, transporters and response [96].

A significant association was found between the incidence of neurological adverse reactions such as somnolence, nervousness, dystonic reactions and tardive dyskinesia and specific polymorphisms in genetic variants of pharmacodynamics proteins such as neurotransmitter receptors/transporters (5HTT and DRD2) and risperidone [97]. However, no significant correlation was found between the variants of DRD3 gene and general symptom improvement, following an 8-week period of risperidone monotherapy in 130 schizophrenic patients from mainland China [98]. And the one hundred thirty one outpatients in stable remission meeting the DSM-IV criteria for schizophrenia spectrum disorders and receiving long-term maintenance therapy with risperidone and genetic variants of DRD1 and DRD2 no have association with extrapyramidal side effects or tardive dyskinesia [99].

Pharmacodynamics, the main metabolic pathway is through hydroxylation of risperidone to 9-OH risperidone by the enzyme, *CYP2D6*. A minor metabolic pathway is through N-dealkylation. The main metabolite, 9-OH risperidone, has similar pharmacological activity as risperidone. Consequently, the clinical effect of the drug results from the combined concentrations of risperidone plus 9-OH risperidone. *CYP2D6* is subject to genetic polymorphism (about 6%-8% of Caucasians, and a very low percentage of Asians, have little or no activity and are "PM") and to inhibition by a variety of substrates and some non-substrates, notably quinidine. EM convert risperidone rapidly into 9-OH risperidone, whereas PM convert it much more slowly. Although EM have lower risperidone and higher 9-OH risperidone concentrations than PM, the pharmacokinetics of risperidone and 9-OH risperidone combined, after single and multiple doses, were similar in EM and PM. Risperidone significantly improved psychopathology in the vast majority of study participants over the 6 week treatment period. We found increased active moiety plasma levels in patients with a longer duration of illness, without receiving significantly higher oral doses [97].

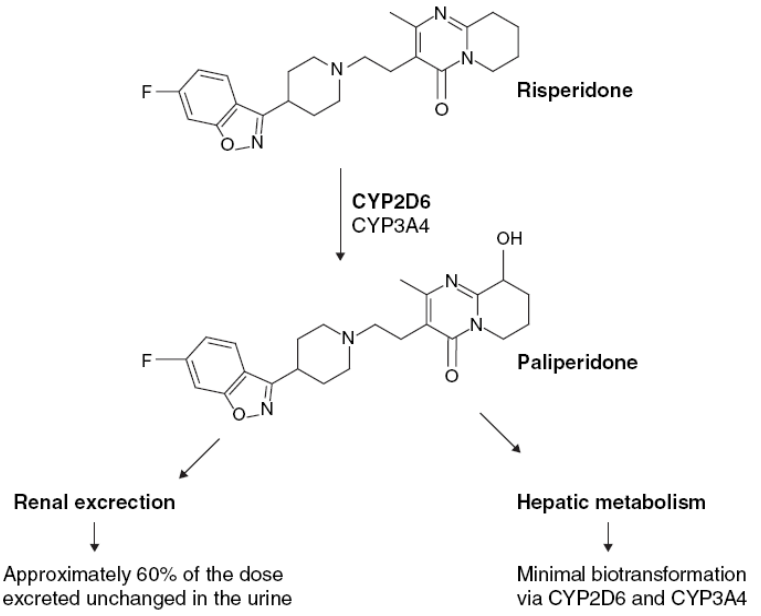


Figure 3.5 Biotransformation pathways of risperidone. Modified from Expert Opin Drug Saf .2007; Nov 6(6:(651-62

3.6.2.2 *CYP2D6* and Risperidone

The *CYP* are a major source of variability in drug pharmacokinetics and response. Of 57 putatively functional human *CYP* only about a dozen enzymes, belonging to the *CYP 1, 2,* and *3* families, are responsible for the oxidative biotransformation of most foreign substances including 70-80% of all drugs (Its drugs include antidepressants, antipsychotics, analgesics and antitussives, beta adrenergic blocking agents, antiarrythmics and anxiolytic drugs) and other lipophilic xenobiotics and are therefore of particular relevance in clinical use. The highest expressed forms in liver are *CYP 3A4, 2C9, 2C8, 2E1*, and *1A2*, while *2A6, 2D6, 2B6, 2C19* and *3A5* are less abundant and *CYP 2J2, 1A1*, and *1B1* are mainly expressed extrahepatically. While monogenic polymorphisms explain a major part of the variability for only few enzymes (such as *CYP2D6*), most enzymes are multifactorially controlled including additional polymorphisms in regulatory trans-genes and nongenetic host factors including sex, age, disease, hormonal, smoking, life-styles and diurnal influences and other factors **(Figure 3.6)** [100]. Genetic polymorphisms consequently induce an altered enzymatic activity–low activity is likely to lead to high-level drug concentrations and the potential to adverse drug reactions, and high enzymatic activity is likely to lead to reduced plasma levels and reduced drug efficacy.

If the drug is pharmacologically active, this results in increased and decreased drug effect, respectively, and potentially in drug-related toxicity due to overdosing. If the drug is metabolically activated (prodrug), the contrary is to be expected, and the pharmacological activity or toxicity of the metabolites must be considered, as for example in the case of *CYP2D6*-dependent morphine formation from codeine. The influence of genetic polymorphisms on *CYP* expression and function, as well as their clinical impact will be discussed for each *CYP* below.

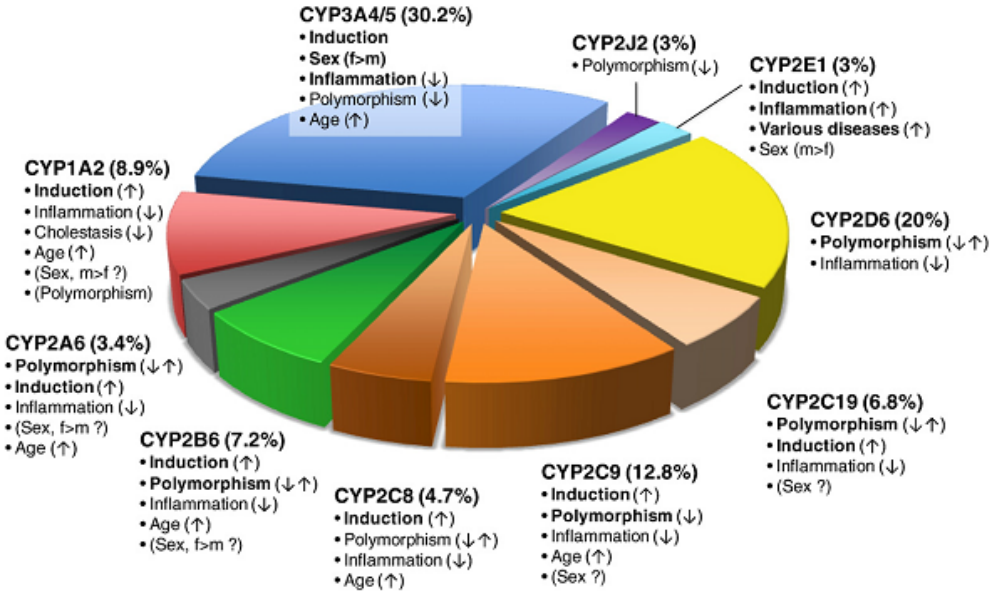
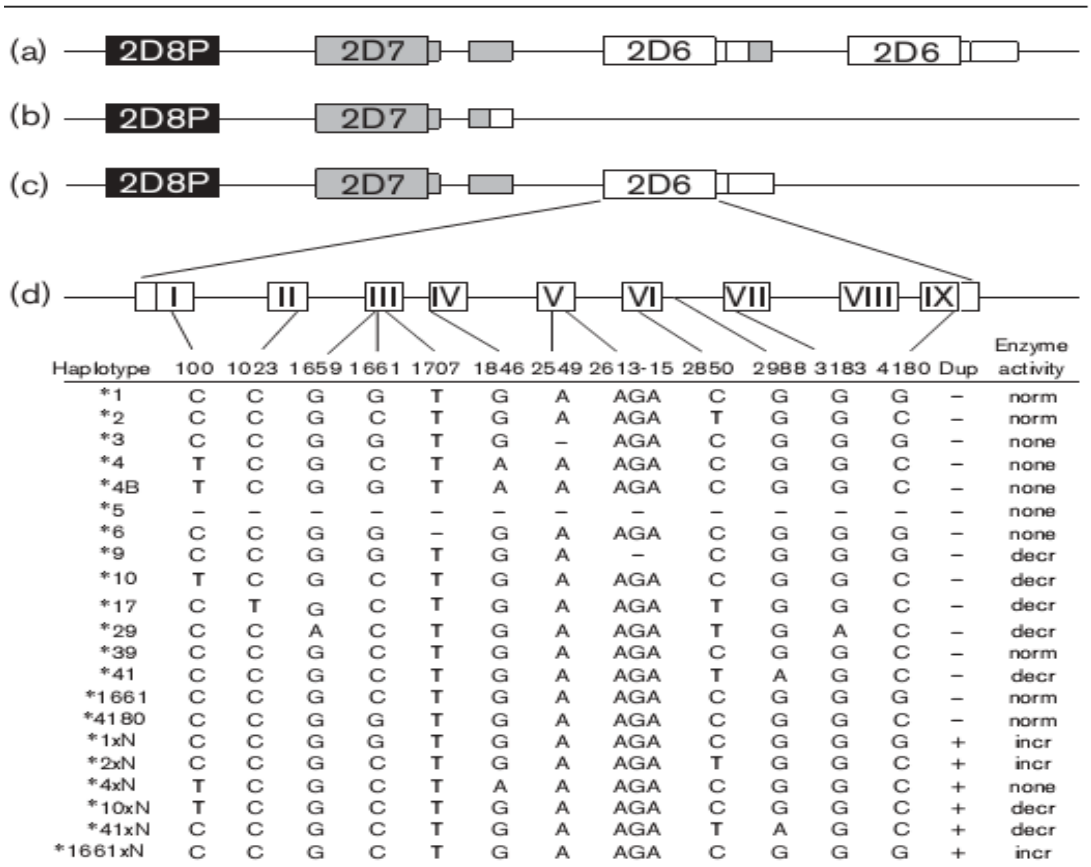


Figure 3.6 Fraction of clinically used drugs metabolized by *P450* isoforms and factors influencing variability. Important variability factors are indicated by bold type with possible directions of influence indicated (↑, increased activity; ↓, decreased activity; ↑↓, increased and decreased activity.

CYP2D6 was the protein-coding gene of the Family 2, Subfamily D, and Polypeptide 6. The *CYP2D* was located on chromosome 22(22q13.1) and is about 4408 bases (start at 42,126,499-42,130,906 bp) long with 9 exons leading to an mRNA size of 1655 bps **(Figure 3.7).** also harbors two pseudogenes, *CYP2D7* and *CYP2D8P* [101]. *CYP2D6* was highly polymorphic with more than 60 alleles and 130 variations are known (http://www.cypalleles.ki.se) as a combination of single nucleotide polymorphisms (SNPs) and copy number variations.



Haplotype	100	1023	1659	1661	1707	1846	2549	2613-15	2850	2988	3183	4180	Dup	Enzyme activity
*1	C	C	G	G	T	G	A	AGA	C	G	G	G	–	norm
*2	C	C	G	C	T	G	A	AGA	T	G	G	C	–	norm
*3	C	C	G	G	T	G	–	AGA	C	G	G	G	–	none
*4	T	C	G	C	T	A	A	AGA	C	G	G	C	–	none
*4B	T	C	G	G	T	A	A	AGA	C	G	G	C	–	none
*5	–	–	–	–	–	–	–	–	–	–	–	–	–	none
*6	C	C	G	G	–	G	A	AGA	C	G	G	G	–	none
*9	C	C	G	G	T	G	A	–	C	G	G	G	–	decr
*10	T	C	G	C	T	G	A	AGA	C	G	G	C	–	decr
*17	C	T	G	C	T	G	A	AGA	T	G	G	C	–	decr
*29	C	C	A	C	T	G	A	AGA	T	G	A	C	–	decr
*39	C	C	G	C	T	G	A	AGA	C	G	G	C	–	norm
*41	C	C	G	C	T	G	A	AGA	T	A	G	C	–	decr
*1661	C	C	G	C	T	G	A	AGA	C	G	G	G	–	norm
*4180	C	C	G	G	T	G	A	AGA	C	G	G	C	–	norm
*1xN	C	C	G	G	T	G	A	AGA	C	G	G	G	+	incr
*2xN	C	C	G	C	T	G	A	AGA	T	G	G	C	+	incr
*4xN	T	C	G	C	T	A	A	AGA	C	G	G	C	+	none
*10xN	T	C	G	C	T	G	A	AGA	C	G	G	C	+	decr
*41xN	C	C	G	C	T	G	A	AGA	T	A	G	C	+	decr
*1661xN	C	C	G	C	T	G	A	AGA	C	G	G	G	+	incr

Figure 3.7 *CYP2D* cluster on chromosome 22 and inferred haplotype.

(a) *CPP2D6* gene duplication, (b) Gene deletion, (c) normal *CYP2D6* cluster and (d) *CYP2D6* exons (white boxes). Inferred haplotypes are named as suggested by guidelines of Human Cytochrome P450 (*CYP*) Allele Nomenclature Committee.

Interestingly, decreased-of-enzyme function polymorphisms in *CYP* genes were surprisingly often affect expression and splicing variants, rather than gene transcription or protein structure [102]. Increased-of-enzyme function variants were including copy number variants (CNV) with an increased number of functional gene copies in *CYP2D6*. The polymorphisms of *CYP2D6* are affect clearly the substrate selectivity or the indelibility of drug metabolic pathways. Important polymorphisms of drug metabolizing CYPs with functional and clinical correlates are summarized in **Table 3.2**.

Table 3.2 Selected genetic polymorphisms of *CYP2D6*.

CYP allele [a]	Mutation(s)[b] rs number	Location, protein effect	Allele frequencies[c]	Functional effect	Clinical correlations
*CYP2D6*3*	2549delA (rs35742686)	Frameshift	MAF 0.009 ~0.01 all ethnicities	*None*	↓ Clearance & ↑ risk of ADRs for numerous antiarrhythmic, anti-depressants, anti-psychotics; ↓ metabolic activation & analgesic effect of opioids (codeine, dihydrocodeine, oxycodone, tramadol); ↓ metabolic activation & efficacy of tamoxifen
*CYP2D6*4*	1846G>A (rs3892097)	Splicing defect	MAF 0.106 0.01–0.10 AA, Af, As, 0.15–0.25 Ca	*None*	
*CYP2D6*5*	Recombina-tion	Deletion	0.03–0.06 all ethnicities	*None*	
*CYP2D6*6*	1707delT (rs5030655)	Frameshift	MAF 0.01 ~0.01 all ethnicities	*None*	
*CYP2D6*10*	100C>T (rs1065852)	P34S	MAF 0.26 0.08–0.12 AA, Af 0.40–0.70 As 0.02 Ca	↓ Decreased	
*CYP2D6*17*	1023C>T (rs28371706); 2850C>T (rs16947)	T107I R296C	MAF 0.049 (for 1023C>T) 0.14–0.24 Af 0.00 As, Ca	↓ Decreased	
*CYP2D6*41*	2988G>A (rs28371725)	Splicing defect	MAF 0.055 0.01–0.06 Af, As, Pc, 0.09 Ca,	↓ Decreased	
*CYP2D6*Nxn*	Recombina-tion	Copy number variations	Up to 0.30 Af, Ar 0.01–0.09 Ca	↑ Increased	↑ Toxicity of opioids

a Allowing to the CYP allele nomenclature homepage (http://www.cypalleles.ki.se).
b Genomic positions are given with corresponding rs numbers in parentheses.
c MAF, global allele frequency of the minor allele as reported in the 1000Genome phase 1 genotype data (released May 2011). Selected frequencies of individual ethnicities (AA, African American; Af African; As Asian; Ar, Arab; Ca Caucasian; In, Indian; Pc, Pacific;) were compiled from dbSNP (build 137) at http://www.ncbi.nlm.nih.gov/projects/SNP/; from the Allele Frequency Database ALFRED at http://alfred.med.yale.edu/alfred/index.asp; and from the references cited in the text.

The CYP-specific drug oxidation phenotype can be determined in *vivo* using selective model substrates. Dissimilar terms are in use for the associated pharmacokinetic in four phenotypes **(Figure 3.8)**. The usefulness of predictive testing for the UM, IM and PM phenotype has been suggested to be particularly valid in antidepressant treatment, bearing strong implications for antipsychotic treatment **(Table 3.3)** [103, 104]**.**

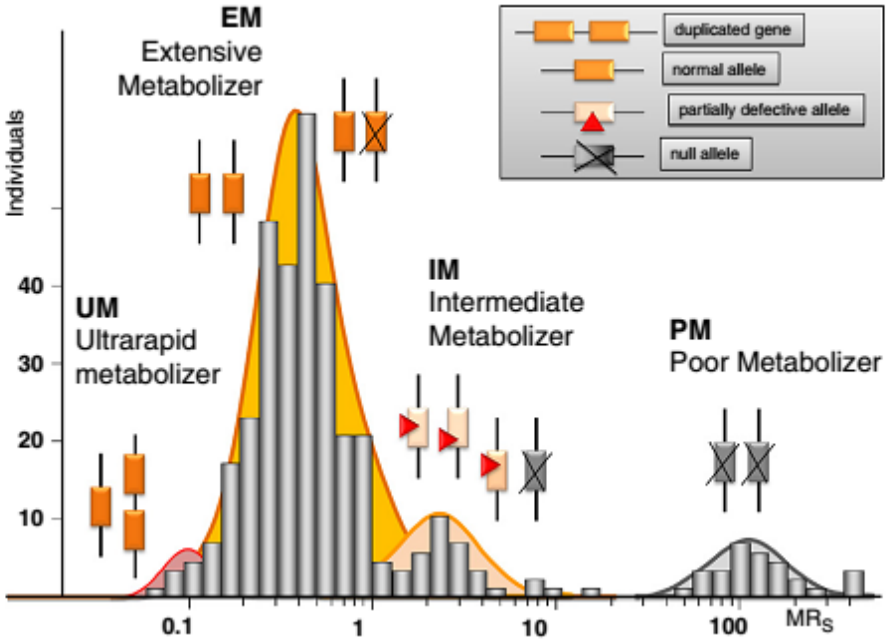


Figure 3.8 Sparteine oxidation phenotype and genotype distribution in a German population (n=308). MRS: urinary metabolic ratio for sparteine [105]. Reproduced by permission of The Royal Society of Chemistry.

Table 3.3 *CYP2D6* response phenotypes and associated antipsychotic dosing.

Phenotypes	**Suggested definition**	**Prevalent rate**	**Dosing suggestions**	**Antipsychotics metabolized by 2D6**
Poor metabolizer	No *CYP2D6* activity. Two nonfunctional alleles	7% Caucasian 1-3% other races	Phenothiazines: may be treated with approximately one half the dose. Haloperidol: maybe treated	Clozapine Haloperidol Perphenazine Quetiapine Risperidone Thioridazine

Table 3.3 *CYP2D6* response phenotypes and associated antipsychotic dosing. (cont.)

Phenotypes	Suggested definition	Prevalent rate	Dosing suggestions	Antipsychotics metabolized by 2D6
			with lower dose. Risperidone may be treated with approximately one half the dose.(can be identified by therapeutic drug monitoring)	
Ultra-rapid metabolizer	Duplication of an active gene (i.e., two or more active variants)	1% Sweden 10% South European 29% North African and Middle Eastern 1-2% USA in Caucasian and African-American among East Asians.	Haloperidol : may need increased dose. Risperidone : may need higher doses but not been well studied.	
Extensive metabolizer	At least one active copy but less than three copies	Most common among normal research subjects. Most prevalent among East Asians.		

Table 3.3 *CYP2D6* response phenotypes and associated antipsychotic dosing. (cont.)

Phenotypes	Suggested definition	Prevalent rate	Dosing suggestions	Antipsychotics metabolized by 2D6
Intermediate metabolizer	Lower than normal activity only one active copy. Less than 0 but greater than one normal active copy.			

The previous studied found that distinguished *CYP2D6* polymorphism-related with subpopulations associated with the PM of *CYP2D6*, risperidone and 9-OH risperidone concentration by lower risperidone clearance. The different proportions of individuals' phenotypes to the PM, IM or EM groups were significantly different ethnic. Among these results were differences between African–American, Asia and Caucasians. **(Table 3.4).** The ratio of risperidone to 9-OH risperidone concentrations has been reported as < 1 in EM and > 1in PM among various ethnic groups, but the sum of the active moieties was comparable in EM and PM [106].In the extreme, UM have very low ratios of risperidone to 9-OH risperidone. In previous study of 82 Korean schizophrenic patients, Roh et al. [107] found that the median concentrations of risperidone normalized for dose in the *CYP2D6*1/*1, *1/*10* and **10/*10* groups were 1.7, 2.6 and 6.7 nmol/L/mg, respectively. For 9-OH riperidone, the corresponding median concentrations were 13.1, 11.9 and 13.6 nmol/L/mg, respectively, with no significant difference between the genotypes. The medians concentration of the ratios between risperidone and 9-OH risperidone concentrations were 0.13, 0.28 and 0.46 nmol/L/mg in the *CYP2D6*1/*1,*1/*10* and**10/*10* genotypes ($p<0.05$). The adverse side effect of risperidone was to produce

a slightly decrease in blood pressure, feeling hot or cold, somnolence, headache, dizziness, hyperprolactinemia, weight gain, obesity, and related metabolic abnormalities such as hyperglycemia and dyslipidemia. *CYP2D6* polymorphisms were associated with risperidone-induced increase in BMI and waist circumference, the predicted UM phenotype was associated with 4.8 and 5.8% lower increase in BMI and waist circumference and PM phenotype was also associated with a 4% lower increase in waist circumference [18]. Risperidone produced a small decrease in blood pressure, a mild increase in QTc and a quick increase in prolactin, without significant differences between phenotypes [108]. PM *CYP2D6* showed higher risperidone C max, area under the curve (AUC), and t1/2, as well as lower clearance. Risperidone increased prolactin levels, which were higher in women than in men. The most frequent reactions were somnolence (47.1%), headache (21.4%), and dizziness (17.1%). Women had neurological effects and headache more frequently than men [109].

Table 3.4 Frequencies of the most prevalent alleles of *CYP2D6* in different ethnic group [110-112].

***CYP2D6* alleles**	**Enzyme activity**	**Caucasians (%)**	**African-Americans (%)**	**Asians (%)**
**1*	Normal	30-40	28-50	20-40
**2*	Normal	20-35	10-80	9-20
**3*	None	1-4	0-0.5	0.8-1
**4*	None	12-13	2-7	0.5-3
**5*	None	1.5-7	0.5-7	4-6
**6*	None	0.5-1	0	-
**9*	Reduced	0-3	0	-
**10*	Reduced	2-8	3-8	40-70
**17*	Reduced	0.1-0.3	10-30	0.5
**41*	Reduced	8	-	-
**1xN*	Increased	0.2-1	2-5	0.5
**2xN*	Increased	0.5-1.5	1.5-2.5	0-1
**4xN*	None	0.1-0.5	0.9	-

3.7 Technology platforms for pharmacogenomics assays, genotyping method for *HLA-B* and *CYP2D6*

3.7.1 Sequence-Specific Oligonucleotide Primed PCR (PCR-SSOP) and Use of the Luminex™ Technology

Sequence-Specific Oligonucleotide Primed PCR (PCR-SSOP) by Luminex™ Technology**.** The xMAP^{TM} technology developed by Luminex (Austin, TX) is a microsphere-based, multiplexed, flow cytometric analysis system that makes it possible to combine direct hybridization is the simplest assay with fluorescence detection. Classification of HLA alleles by Luminex system. The xMAP^{TM} suspension array technology employs synchronized applications of up to 100 probes, biotin-labeled oligonucleotide (reporter probe), which are already coated on microspheres 5.6 μm polystyrene. To be able to recognize these probes, 100 shades of two colors that are formed by the ratio of two internal fluorescent dyes are assigned for each probe. The fluoroanalyzer contains a red laser that excites the dyes in the microspheres and categorizes them based on their dye content two spectrally distinct fluorochromes. The microspheres are coated with carboxyl groups in order to achieve a covalent bridge between the oligonucleotides that contain terminal amine groups and the beads. Thus, the bound oligonucleotides also become color-coded. In addition to the 635-nm 10-mW red diode laser, the instrument contains a 532-nm, 13-mW green laser that is used to quantify fluorescently (Streptavidin-PE) labeled amplicons captured by the beads. Each probe mix contains one or more oligonucleotides that react with all alleles within the locus of interest, which also serves as an internal control system. They are used in the normalization of the values during the calculation of reaction patterns. If the minimum fluorescent intensity values defined for this control probes are not achieved, the sample test must be repeated.

3.7.2 Microarray-based technology

Microarray technology is being used in a high-throughput approach to study gene expression and sequence variation on a genomic scale. In fact, microarray has become invaluable in identifying subsets of genes that appear to have different

degrees of expression in various disease stages. Microarray-based technology has become powerful tools in the diagnosis and treatment of diseases. AmpliChip® CYP450, developed by Roche Molecular Diagnostics, is used with the GeneChip 3000Dx microarray system from Affymetrix (Santa Clara, California, USA). The chip (also referred to as 'probe microarray') contains millions of tiny DNA molecules, and the test is performed using DNA that is extracted from a patient's blood. The AmpliChip® has been approved for *in vitro* diagnostic use in the United States and Europe. The test determines the associated predictive metabolizer phenotype (PM, IM, EM, or UM) and can aid physicians in individualizing patient treatment and dosing for medications metabolized through these P450. AmpliChip® was analyses screen for gene duplications, gene deletions as well as 29 *CYP2D6* known polymorphisms and 2 major polymorphisms for the *CYP2C19* gene in one experiment. The AmpliChip® CYP450 Test is based on five major processes:(i) PCR amplification of purified DNA gene, (ii) fragmenting DNA and labeling of the amplified products, (iii) hybridization of the amplified products to the microarray and staining of the on the AmpliChip® DNA microarray, (iv) scanning of the microarray chip; and (v) determination data analysis of the *CYP2D6* and *CYP2C19* genotypes and predicted phenotype.

According to the reports in Thailand, there is no association study between polymorphisms of *HLA-B* and *CYP2D6* and ASD. In this study, twelve *CYP2D6* alleles were determined by using AmpliChip™ CYP450. Molecular *HLA-B* typing was performed using PCR-SSOP. In order to determine the association, allele and genotype frequencies of *CYP2D6* and *HLA-B* were compared between ASD and Non-ASD in a Thai population.

CHAPER IV
MATERIAL AND METHOD

4.1 Study population

The total samples of this study were 1,470 from the Thai population patients. Subjects were classified into 2 groups of patients. The first patients who had been diagnosed with ASD were identified from physicians and all participants had tested from DSM-IV. The second group consists of Non-ASD. Both of groups were studied in genotypes (genes).

Of these 233 patients, 79 ASD and 154 Non-ASD were study in allele frequencies of *CYP2D6* genotypes using microarray technique **(Figure 4.1)**.

And *HLA-B* genotyping between 364 patients who were followed up for ASD and 952 non-ASD patients by using PCR-SSOP based method **(Figure 4.2)**.

Total of the ASD patients approximately 364 (364 patients from *CYP2D6* and *HLA-B* genotyping) and were recruited between 2011 and 2012 from Yuwaprasart Waithayopathum Child Psychiatric Hospital and 1106 (154 patients from *CYP2D6* combined with 952 patients from *HLA-B* genotyping) patients between 1997 and 2012 from the Pharmacogenomics and Personalized Medicine and Virology in the Department of medicine, Ramathibodhi hospital, Mahidol University. Peripheral blood specimens were collected from EDTA tubes (lavender top) and stored at room temperature for up to 7 days, at 2-8 ^{0}C for up to 1 month or frozen at -20 ^{0}C for up to 7 weeks.

This study was approved by the ethics committee of Ramathibodi Hospital. All patients wrote informed consent document before enrolled.

4.2 Experimental design

a. Genotyping procedure Amplichip™ CYP450 analysis of the *CYP2D6*

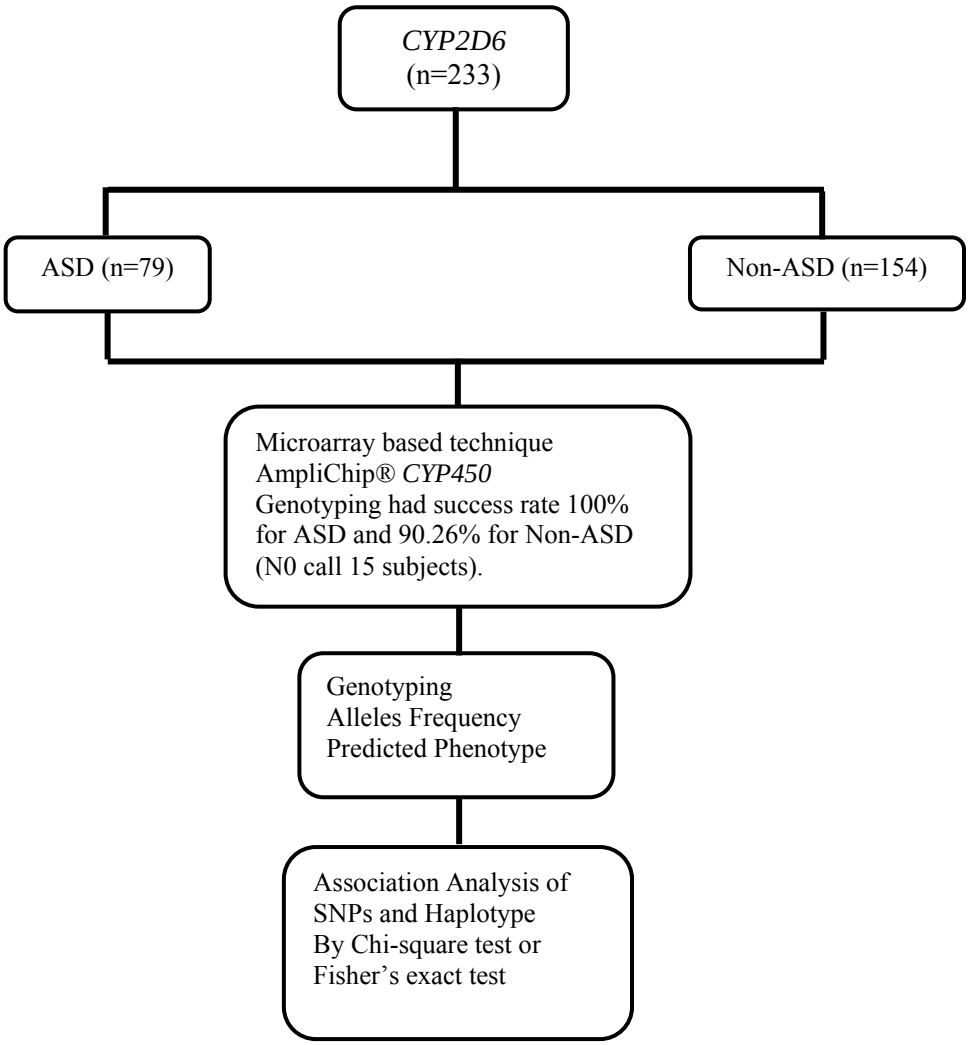


Figure 4.1 Experimental design (*CYP2D6*)

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

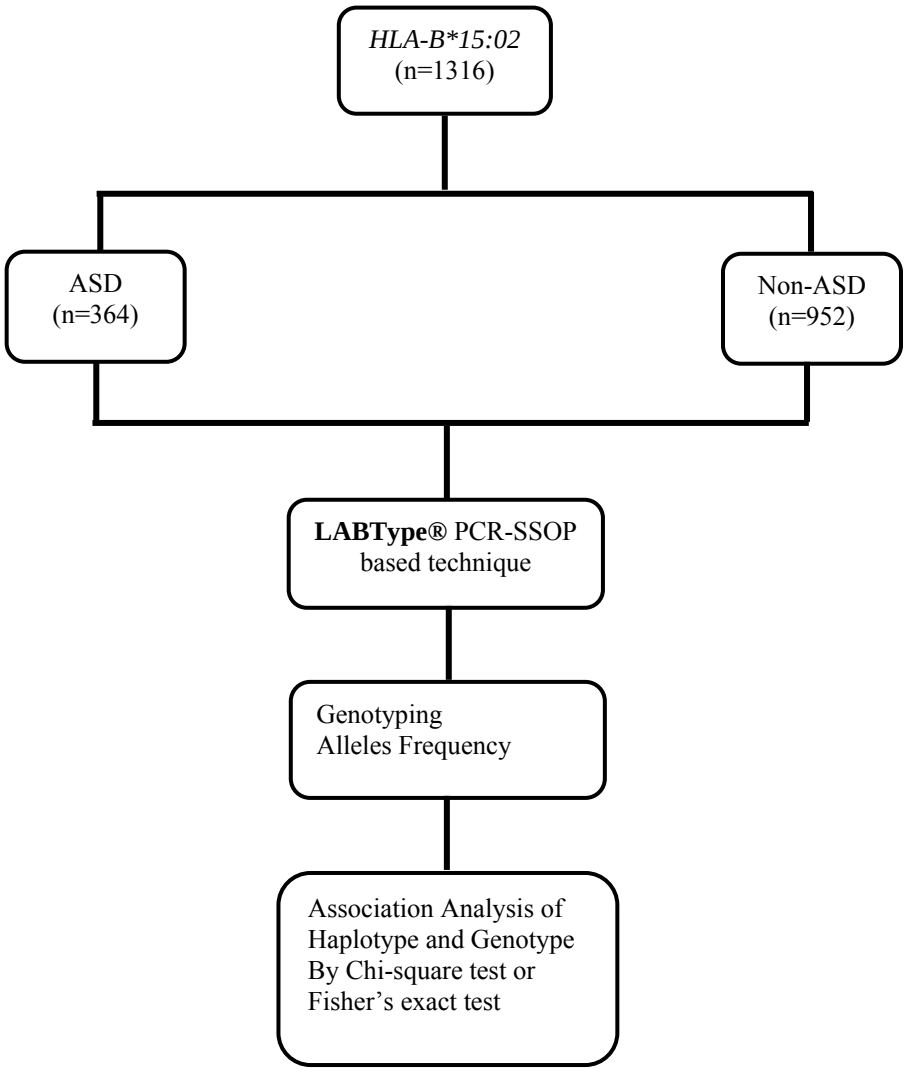


Figure 4.2 Experimental design (*HLA-B*)

4.3 Materials

4.3.1 Instrument and laboratory supplies for Extraction DNA

- MagNA Pure Compact automated)Roche Applied Science, Penzberg, Germany)
- NanoDrop 2000 Spectrophotometer (Thermo Scientific, USA)
- Autopipette 2.5/10/100/200/1000 µL : Eppendrof
- Pipette tip 10/100/200/1000 µL : Eppendrof
- Purifier Biological Safety Cabinet Class II : Labconco,
- Microcentrifuge tube (1.5 mL and 0.5 mL in size) : Treff: Switzerland
- Micro tube 2 ml with cap : Sarstedt, Germany
- Refrigerator 4°C : Zedmed, China
- Freezer (-20 °C) : Sanyo, Japan
- Freezer (-80°C) : Thermo Scientific, USA

4.3.2 Reagent for Extraction DNA

- Commercials MagNa Pure Compact Nucleic Acid Isolation Kit I-large volume (Roche applied Science)

4.3.3 Instruments and reagents for AmpliChip® CYP450

- Real time Polymerase Chain Reactions ViiA7 (ABI)
- Autopipette 2.5/10/100/200/1000 µL : Eppendorf
- Pipette tip 10/100/200/1000 µL : Eppendorf
- Deionized water
- Optical adhesive film
- Bench top microcentrifuge : Hettich, Germany
- Plate mixer for immobilization : Eppendorf
- Strip caps

4.3.4 Instrument and laboratory supplies for *HLA-B* Typing (LABType® SSOP Typing Tests)

- Bioplex 200: Bio-Rad, USA
- Biosafety Cabinet Type II: ESCO, USA
- Autopipette 10/100/200/1000 µL: Eppendorf,
- Pipette Tips 10/100/200/1000 µL: Eppendorf
- Vortex Mixer: Scientific, USA
- 1.5 mL Microcentrifuge tube: Treff, Switzerland
- Centrifuge Plates and Strips: Hettich, Germany
- Sheath fluid (OLI Cat.#LXSF20 or LSXF20X5).
- Daily maintenance reagents (70% ethanol, double-distilled [dd] H_2O).
- 20% chlorine bleach (or equivalent)

4.3.5 Reagents for PCR (LABType® SSOP Typing Tests)

- 2.25 ml Denaturation Buffer - 1 vial**.**
- 2.5 ml Neutralization Buffer - 1 vial.
- 3.4 ml Hybridization Buffer - 1 vial.
- 55 ml Wash Buffer – 1 bottle.
- 4.95 ml SAPE Buffer – 1 vial.
- 1.38 ml Primer Set D-Mix- 2 vials of 690 µL each.
- 400 µL Locus-Specific Primer Set – 1 vial.
- Bead Mixture-400 µL LABType® SSO primary-1 vial.
- Nuclease-free water.
- TE buffer pH 8.0

4.3.6 Instrument and laboratory supplies for PCR

- PCR Thermal cycler 96-well GeneAmp® PCR System 9700**:** Applied Biosystem, USA**.**
- Strip tube /96-well plate**:** Hettich, Germany
- Vortex Mixer: Scientific, USA
- Microcentrifuge tube: Treff, Switzerland

4.3.7 Instrument and laboratory supplies for Electrophoresis

- Power supply: Major Science, USA.

- Gel Electrophoresis: Major Science, USA**.**
- Gel Doc^{TM}**:** Bio-Rad, USA**.**
- Microwave : Sharp, Thailand
- Glassware : Pyrex, USA

4.3.8 Reagents for agarose gel electrophoresis

- 1 X TBE buffer: 0.89 M Tris-borate, 0.89 M Boric acid, 0.002 M EDTA**.**
- Gel loading dye solution**.**
- Gel Marker: contain linear double stranded DNA band of 1000, 300, 100 base pairs (bp.) : Bioactive, USA

4.4 Method

4.4.1 DNA Extraction

In this study, The ASD patients approximately 364 were recruited between 2011 and 2012 from Yuwaprasart Waithayopathum Child Psychiatric Hospital. The genomic DNA (gDNA) was extracted from 1 ml total 3 ml of ethylenediamine tetraacetic acid (EDTA) blood collected patient and extracted by automate MagNA Pure Compact (Roche Applied Science, Penzberg, Germany) and MagNA Pure Compact nucleic acid isolation kit I – large volume (Roche Applied Science) with the method described in the product literature, was based on magnetic-bead technology with a lysis buffer and proteinase K. Nucleic acids were bound to the surface of the magnetic glass particles. Cellular debris was removed by several washing steps and the purified nucleic acids were eluted. From the 1ml input volume of EDTA-whole blood, 200 μL output volume of extracted genomic DNA product was obtained, after the magnetic beads were separated from the solution.

1106 non-ASD patients between 1997 and 2012 including 154 and 505 subjects from DNA banking from the Virology and 447 subjects in routine Pharmacogenomics and Personalized Medicine in the Department of medicine, Ramathibodhi hospital, Mahidol University.

4.4.2 Quality and Quantitative gDNA analysis

The quality and quantity of the extract gDNA will be measured the optical density (OD) at a wave length of 230, 260 and 280 nm by spectrophotometer NanoDrop 2000 (Thermo Scientific USA). High quality of gDNA preparation, the ratio of OD 260/ OD 280 nm should be estimate 1.8 (1.7-1.9). Finally, DNA was store at 2-8 ^{0}C for up to one week or frozen at -20 ^{0}C for up to one month.

4.4.3 Detection of the *CYP2D6* Genotyping by using AmpliChip®CYP450

DNA can be purified from any human cells with validated method that meets the criteria below. Each DNA sample was to be used for PCR should be dilute in sterile water or in 1X TE buffer, pH 8.0 - 9.0 at an optimal concentration of 5 ng/ µL with the A260/A280 ratio of 1.65 - 1.80.

Reagent preparation by equilibrate working master mix components including 25 µl master mix, 25 µl of primer mix A or primer mix B and last 25 µl of magnesium chloride solution, final volume of 75 µl. Working master mix A and B must be used within 1 hour after preparation. Mix working master mix components by inverting 10-15 times and label two 2.0 mL tubes as A and B. And then pipette 75 µL of working master mix A and B into two amplification tube for each specimen and control. Do not cap the reaction tubes at this time. Fill remaining amplification tubes that will not be used for specimen or control with 100 µL of sterile water. Add 25 µL of each prepared specimen gDNA, positive and negative control to the appropriate amplification tube containing working master mix A and B. Cap the amplification tubes, amplification run must approximately time in 15 minutes. Transfer the prepared specimens and controls in the amplification tray. The remainder of the prepared specimens may be stored at 2-8 ^{o}C for 1 week or at -20 ^{o}C for later analysis.

Working master mix A and B must be used within 1 hour of preparation. Pipette 75 µL working master mix A and B into two amplification tube for each specimen and control. Do not cap the reaction tubes at this time. Fill remaining amplification tubes that will not be used for specimen or control with 100 µL water. Place the tray containing working master mix A and B in resalable plastic bag and seal

the plastic bag securely until ready to use. Working master mix A and B are stable for 1 hour at room temperature. Add 25 μL of each prepared specimen, working CYP450 positive control and negative control to the appropriate amplification tube containing working master mix A and B. Cap the amplification tubes. Once prepared specimens and controls are added to working master mix A and B, amplification must be started within 15 minutes. Transfer the prepared specimens and controls in the amplification tray. The remainder of the prepared specimens may be stored at 2-8 ^{o}C for 1 week or at -20 ^{o}C for up to 1 month, with up to three freeze-thaw cycles**.**

Test was based on five major processes:

(i) PCR amplification of purified DNA

Program the thermal cycler 9700 for the AmpliChip CYP450 Test as follows:

HOLD Program:	2 min 50 ^{o}C
HOLD Program:	10 min 95 ^{o}C
CYCLE Program (35 cycles):	20 sec 95 ^{o}C, 4 min 67 ^{o}C
HOLD Program:	7 min 72 ^{o}C
HOLD Program:	4 ^{o}C indefinitely

Place the tray or retainer assembly into the thermal cycler block and ensure that all of the tubes are tightly capped. Before start the method program. Set the ramp speed to max and reaction volume to 100 μL in the method options screen. Press start again. The program runs approximately 3 hours and 30 minutes. Upon completion of amplification including the 72 ^{o}C HOLD step, removed the tray from the thermal cycler and place in the MicroAmp base. If necessary, reaction tubes can be left in the thermal cycler at 4 ^{o}C for up to 18 hours after amplification has completed. If amplicon fragmentation will not be performed within 30 minutes of removing reaction tubes from the thermal cycler, store the amplification tray at -20 ^{o}C. The amplified products can be stored at -20 ^{o}C up to 1 week.

(ii) Fragmenting and labeling of the amplified products

Prepare a solution of 20 mM EDTA by adding 1 mL 0.5 M EDTA to 24 mL of deionized water and mix thoroughly. 20 mM EDTA is stable for 6 months from the date of preparation, stored at 2-8 ^{o}C in a clean and closed plastic container. Prepare working fragmentation mix for 24 fragmentation reactions as followed by pipetting the

finally volume 220 µL with 191.4 µL water, nuclease-free, 3.3 µL of 20 mM EDTA, 22 µL of Alkaline Phosphatase (1 U/ µL) and 3.3 µL of DNase I, RNase-free recombinant (10 U/µL). Under in the specified order into a 2.0 mL microtube that is kept on ice and briefly vortex working fragmentation mix.

Label a new MicroAmp tray/retainer assembly as fragmentation. One reaction tube is needed for each specimen and control. Place the tubes in the MicroAmp tray and lock in place with retainer, and place on ice. Remove the caps from the working master mix A and B amplification tubes. Gently mix amplicon by pipetting up and down three times before removing and transferring 8 µL of the amplicon from each working master mix A and B reaction into the appropriate tube of the fragmentation tray on ice. Add 8 µL working fragmentation mix to each of the tubes containing amplicon in the fragmentation tray on ice. Gently mix each specimen and control by pipetting up and down three times with the pipette. Cap the reaction tubes. Immediately transfer the Fragmentation tray/retainer assembly into the 9700 thermal cycler programming as follow with the DNase fragmentation thermal cycling Protocol:

HOLD Program:	20 min 25 ^{o}C
HOLD Program:	10 min 95 ^{o}C
HOLD Program:	4 ^{o}C Indefinitely

Before start the method program. Set the ramps to max and reaction volume to 24µL in the method options screen. Press start again. The program runs approximately 40 minutes. Upon completion of fragmentation, prepare the working labeling for 24 labeling reaction as follows by pipetting the finally volumes per 1 reaction 10 µL with 6.8 µL of 5X Terminal Transferase Reaction Buffer, 0.8 µL of 25 mM $CoCl_2$, 0.8 µL of AmpliChip TdT Labeling Reagent (TdT), last 1.6 µL of Terminal transferase, recombinant (400 U/ µL) listed in the below in the specified order into a 2.0 mL tube that is kept on ice:

Briefly vortex the working labeling mix. Remove the fragmentation tray from the thermal cycler and place into an amplification base. Remove the caps from the fragmentation tubes carefully to avoid creating aerosols of the fragmentation amplicon. Add 10 µL Working Labeling Mix into each of the tubes containing amplicon in the Fragmentation Tray. Gently mix each specimen or control by pipetting

up and down three times with the pipettor. Cap the reaction tubes with new caps. Immediately transfer the fragmentation tray or retainer assembly into the 9700 thermal cycler programmed as follows with the labeling thermal cycling protocol:

HOLD Program:	35 min 37 ^{o}C
HOLD Program:	5 min 95 ^{o}C
HOLD Program:	4 ^{o}C Indefinitely

Before start the method program. Set the ramp speed to max and reaction volume to 34 µL in the method options screen. Press start again. The program runs approximately 45 minutes. Upon completion of labeling of the fragmented amplicon, remove the fragmentation tray from the thermal cycler and place into an amplification base. Store the fragmentation tray at 2-8 ^{o}C for up to 18 hours until ready to perform the microarray hybridization. If not proceeding to the hybridization step within 18 hours, store the labeled fragmentation tray at -20 ^{o}C. The labeled fragmented amplified products can be stored at -20 ^{o}C for up to 1 week.

(iii) Hybridization of the amplified products to the microarray and staining of the bound products

Prepare a solution of 10% Triton X-100 by slowly pipetting 10 mL Triton X-100 into a clean empty container, then adding 90 mL water. Mix well. 10% Triton X-100 is stable for 6 months from the date of preparation, stored at 15-30 ^{o}C in a clean, closed plastic container that protects the Triton X-100 from exposure to light. Prepare hybridization buffer by adding the component 0.5 mL of total volumes with 125 of 20X SSPE, 2.5 of 10% Triton X-100, 50 of B1 Oligo, 01 of 50X Denhardt 's Solution, 9 of 5% Sodium azide and the last 303.5 of water, deionized or distilled isted at a 0.5 mL tube and mixing by inversion:

Hybridization buffer is stable for 6 months from the date of preparation, at 2-8 ^{o}C. Prepare stain solution by adding the component total 0.5 mL per 1 reaction with 140 of 20X SSPE, 25 of Acetylated Bovine Serum Albumin (20 mg/mL), 5 of Streptavidin, R-phycoerythrin conjugate (1 mg/mL), 9 of 5% Sodium azide and the last 321 of water, deionized or distilled to a 0.5 mL tube wrapped in foil and vortex for 30 seconds:

Stain Solution is stable for 6 months from the date of preparation, stored at 2-8 ^{o}C in a clean, closed plastic container and wrapped in foil that protects the stain Solution from exposure to light. Prepare Wash Buffer by combining the component final volume 1 liter with 150 mL of 20X SSPE, 0.5 mL of 10% triton X-100, 18 mL of 5% sodium azide and the last 831.5 mL of water, distilled or deionizes in a suitable container and mixing thoroughly:

Label one 1.5 mL tube for each specimen and control. Add 500 µL hybridization buffers to each tube. Mix each fragmented/labeled specimen and control amplicon with the pipette and then add 20 µL to the appropriately labeled tube. Cap the tubes and vortex each tube for 10 seconds. Incubate the tubes at 95 ^{o}C for 10 minutes in a dry heat block. Remove the tubes from the heat block and immediately place each tube in the ice water bath. Add 500 µL stain solution into the appropriate number of 1.5 mL tubes, one for each specimen and control. Label one array for each specimen and control.

Load the appropriated tube that contain hybridization buffer and denatured fragmented/labeled amplicon to position 1 and a tube containing stain solution to position 2 of each module to be used on the GeneChip fluidics station. After hybridization and staining are completed, remove the array from the GeneChip fluidics station before closing the washblock door. Visually inspect the array window for air bubbles. If air bubbles are present, reinsert the array, close the washblock door and the GeneChip fluidics station will automatically refill the array with wash buffer. Follow the module instructions to complete the protocol.

(iv) Scanning of the microarray

To prevent leaked of liquid from the array, apply one tough-spot over each of the two septa located on the back of each Array and press to ensure that the spots remain flat. Load the array into the scanner autoloader; they may be loaded in any order and scan arrays with AMDS and AmpliChip CYP450 data analysis software. Before start scanner, select *CYP2D6*, *CYP2C19* or both to define the desired report on the additional information window or batch information window within the AmpliChip CYP450 test data analysis software. Start the scanning function. The results will be automatically generated after scanning and can be reviewed by following the links in AMDS.

(v) Determination of the *CYP2D6* and *CYP2C19* genotypes and predicted phenotype. According to the manufacturer instructions**.**

The AmpliChip CYP 450 microarray contained with over 15,000 different oligonucleotide probes that was synthesized on a glass surface to analyze both sense and antisense strands of an amplified target DNA samples. The AmpliChip CYP450 microarray assay distinguished 29 known polymorphisms in the *CYP2D6* gene including gene duplication and gene deletion. The polymorphisms of *CYP2D6* were detected results in the identification of 33 alleles (**Table 4.1**).

The combination of polymorphisms allows for the prediction of the likely enzymatic activity of the *CYP2D6* allelic gene product [11]. The nucleotide changes listed in bold font define the allele.

Table 4.1 Cytochrome P450 *2D6* mutation detected 33 alleles

***CYP2D6* alleles**	**Nucleotides change**	**Effect**	**Enzyme Activity**
**1*	None		Normal
**2ABD*	-1584G, 1039C>T, 1661G>C, **2850C>T,4180G>C**	R296C, S486T	Normal
**3*	**2549Adel**	259Frameshift	None
**4ABDJK*	100C>T, 1039C>T, 1661G>C**,** **1846G>A**, 2850C>T, 4180G>C	splicing defect	None
**5*	**Gene deletion**	*CYP2D6* deleted	None
**6ABC*	**1707Tdel**, 1976G>A, 4180G>C	118Frameshift	None
**7*	**2935A>C**	H324P	None
**8*	1661G>C, **1758G>T**, 2850C>T,4180G>C	G169X	None
**9*	**2613-2615delAGA**	K281del	Reduced

Table 4.1 Cytochrome P450 *2D6* mutation detected 33 alleles (cont.)

***CYP2D6* alleles**	**Nucleotides change**	**Effect**	**Enzyme Activity**
**10AB*	**100C>T**, 1039C>T, 1661G>C, 4180G>C	P34S	Reduced
**11*	**883G>C**, 1661G>C, 2850C>T,4180G>C	Splicing defect	None
**14A*	100C>T, **1758G>A**, 2850C>T,4180G>C	G169R	None
**14B*	1661G>C,**1758G>A**, 2850C>T,4180G>C	G169R	Reduced
**15*	**138 ins T**	46Frameshift	None
**17*	**1023C>T**, 1661G>C, **2850C>T**,4180G>C	T107I, R296C	Reduced
**19*	1661G>C, **2539-2542delAACT**, 2850C>T,4180G>C	255Frameshift	None
**20*	1661G>C, **1973insG**,1978C>T, 2850C>T, 4180G>C	211Frameshift	None
**25*	**3198C>G**	R343G	Unknown
**26*	**3277T>C**	I369T	Unknown
**29*	**1659G>A**, 1661G>C, 2850C>T, **3183G>A**, 4180G>C	V136I, V338M	Reduced
**30*	1661G>C, **1855-1863ins (TTTCGCCCC)repeat**, 2850C>T,4180G>C	174_175insFRP	Unknown
**31*	1661G>C,2850C>T,**4042G>A**,4180G>C	R440H	None
**35*	-1584G, **31G>A**,1661G>C, 2850C>T,4180G>C	V11M	Normal

Table 4.1 Cytochrome P450 *2D6* mutation detected 33 alleles (cont.)

***CYP2D6* alleles**	**Nucleotides change**	**Effect**	**Enzyme Activity**
**36*	**100C>T**,1039C>T, 1661G>C,4180G>C, **gene conversion to CYP2D7 in exon 9**	P34S	Reduced
**40*	**1023C>T**, 1661G>C,**1863ins(TTT CGC CCC)2**; 2850C>T,4180G>C	T107I, 174_175insFRPx2	None
**41*	-1584C, 1661G>C, **2850C>T**, **2988G>A, 4180G>C**	R296C, splicing defect, S486T	Reduced
**1XN*	Duplicate active *1 genes (n is not determined-range 2-13)	*N* active genes	Increased
**2XN*	Duplicate active *2 genes (n is not determined-range 2-13)	*N* active genes	Increased
**4XN*	Duplicate inactive *4 genes (n is not determined)	-	None
**10XN*	Duplicate partially active *10 genes (n is not determined)	-	Reduced
**17XN*	Duplicate partially active *17 genes (n is not determined)	-	Reduced
**35XN*	Duplicate partially active *35 genes (n is not determined)	-	Increased
**41XN*	Duplicate partially active *41 genes (n is not determined)	-	Reduced

Predicted *CYP2D6* phenotypes

The combination of the activity of the enzymes encoded by the two *CYP2D6* alleles determines the overall metabolic activity for individual. There are

four phenotypic types (**Table 4.2**):

Table 4.2 Predicted phenotypes of *CYP2D6*

Predicted Phenotypes	**Description**
Poor metabolizers (PM)	No enzyme activity, increased risk for drug toxicity/side effects and little to no therapeutic effect with pro-drugs. Genotypes consistent with the poor metabolizer phenotype are those with no active *CYP2D6* alleles
Intermediate metabolizers (IM)	Reduced enzyme activity, some therapeutic effect with increased potential for toxicity/side effects. Genotypes consistent with the intermediate metabolizer phenotype are those with one active and one inactive *CYP2D6* allele, one inactive and one partially active *CYP2D*6 allele, or two partially active *CYP2D6* alleles
Extensive metabolizers (EM)	Normal enzyme activity, drug is metabolized efficiently, resulting in therapeutic effect with minimal toxicity. Genotypes consistent with the normal metabolizer phenotype include two active *CYP2D6* alleles or one active and one partially active *CYP2D6* allele. Increased caution may be appropriate for individuals having one partially active allele.
Ultra-rapid metabolizers (UM)	Excess enzyme activity, drug is extensively metabolized, resulting in increased or lack of therapeutic effect depending on pro-drug status. Genotypes consistent with ultra-metabolizer phenotype include three or more active *CYP2D6* alleles due to duplication of an active allele.

4.4.4 Detection of the *HLA-B* typing by using LABType® SSOP- PCR

Each DNA sample was to be used for PCR should be dilute in sterile water or in 1X TE buffer, pH 8.0 - 9.0 at an optimal concentration of 30 ng/ μL with the A260/A280 ratio of 1.65 - 1.80. The PCR Master Mix will be composed of 18 μL reactions containing 13.8 μL of D-Mix, 4 μL of primer and 0.2 μL of Taq polymerase

recombinant, and 2 µL of the gDNA (30 ng/µL) final volume = 20 µL, vortex and spin down. Programming the 96-Well GeneAmp® PCR System 9700, set ramp speed to 9600 and cycling conditions are as follows: hold at 96°c for 3 min, 5 cycles at 96°c for 20 sec, 60°c for 20 sec and 72°c for 20 sec, 30 cycles 96°c 10 sec, 60°c at 15 sec and 72°c 20 sec, a final extension 72°c 10 min, and a 4°c hold. And then transfer each PCR product will be checked by running on a 2 % agarose gel for 150 volt, 45 min. and stained with ethidium bromide. (2 amplicons product 1000 base pairs and 300 base pairs.)

Denaturation/Neutralization

Transfer 5 µL of PCR product into a strip tube and then add 2.5 µL. of Denaturation buffer Mix thoroughly (preferably by pipetting up and down), and incubate at room temperature (20 - 25°C) for 10 min and then pipette 5 µL. of Neutralization Buffer and mix thoroughly (preferably by pipetting up and down).

Note the color change from bright pink to pale yellow or clear. (Final volume = 12.5 µL.)

Hybridization

Note: Make sure that the thermal cycler has been turned on and the 60°C program has been started to warm the thermal block. Bead reactions will be performed in a final volume of 38 µL reactions containing 4 µL of bead mixture and 34 µL of Hybridization buffer. And then add 38 µL of Bead reactions into each strip tubes, vortex low speed and Pre-warmed thermal cycler at 60°C for 15 min, add 100 µL of wash buffer centrifuge at 2000 g for 5 min and then remove supernatant by flicking repeat wash buffer 3 times.

Labeling

SAPE stain solutions are perform in a final volume of 50 µL. The solutions mixture contain 0.5 µL of SAPE stock and SAPE buffer 49.5 µL. Add of 50 µL. SAPE solution into each strip tubes and vortex, Pre-warmed thermal cycler at 60°C for 5 min, Add 100 µL. of Wash buffer into each strip tubes and centrifuge at 2000 g for 5 min and then remove supernatant by flicking. Add 70 µL of wash buffer into each strip tubes, gently mix by pipetting. Transfer final solution into 96 well reading plate for Bioplex 200.

Note: Final volume should be at least 80 µL. Read sample on the Bioplex 200 and analysis result by HLA Fusion 2.0 (One Lambda, Inc., Canoga Park, CA).

4.5 Statistical analysis

Expected allele frequencies of *CYP2D6* polymorphism were calculated using with their Hardy-Weinberg equilibrium from the observed allelic frequencies ($p^2+2pq+q^2 = 1$) by Haploview 4.2. The differences in allele frequencies with different patients characteristic were determined using the Chi-square (χ^2) test or Fisher's exact test if the number in any cell of the 2x2 contingency tables was less than five. Odds ratio (ORs) and 95% confidence intervals (CIs) were calculated to determine levels of significances. For all test, a probability (*P*) of less than 0.05 was significant. All analysis was performed using the SPSS (v.18.0).

The Hardy-Weinberg equation is a mathematical equation that can be used to calculate the genetic variation of a population at equilibrium. In 1908, G. H. Hardy and Wilhelm Weinberg independently described a basic principle of population genetics, which is now named the Hardy-Weinberg equation. Evolution is not only the development of new species from older ones, as most people assume. It is also the minor changes within a species from generation to generation over long periods of time that can result in the gradual transition to new species, which states that the amount of genetic variation in a population will remain constant from one generation to the next in the absence of disturbing factors.

In this equation ($p^2 + 2pq + q^2 = 1$), p is defined as the frequency of the dominant allele and q as the frequency of the recessive allele for a trait controlled by a pair of alleles (A and a). In other words, p equals all of the alleles in individuals who are homozygous dominant (AA) and half of the alleles in people who are heterozygous (Aa) for this trait in a population.

CHAPTER V
RESULTS

5.1 The clinical data of ASD patients

The present study based on two genes approach using technology for genotyping to identify polymorphisms in *CYP2D6* and *HLA-B*. One thousand four hundred and sixty nine in a Thailand population were detected.

The clinicoepidemiological data of total 364 subjects who were diagnosed as autism spectrum disorders (ASD) for age, mean was 9.17 years (SD = 4.99) (range 3-47 years). There are 314 male and 50 female cases (ratio 6.28:1) with 1,106 control subjects (Non-ASD; 744 males and 362 females case; ratio 2.06:1; mean age: 45.36 years, SD = 26.15 and range 26-93 years) from Thailand.

Table 5.1 Characteristics of subjects according to two groups

Characteristics	**ASD** **Mean ± SD (range)**	**Non-ASD** **Mean ± SD (range)**
Age (years)	9.17±4.99 (3 - 47)	45.36±26.15 (26-93)
Gender		
Male	314 (86.26%)	744 (67.27%)
Female	50 (13.74%)	362 (32.73%)

5.2 Hardy-Weinberg equilibrium of ASD.

CYP2D6 genotype frequencies were tested for Hardy-Weinberg equilibrium by using Haploview 4.2. Samples were successfully genotyped 100 percent for each SNP, list in **Table 5.2** Genotype frequencies of *CYP2D6* was detected in Hardy-Weinberg equilibrium. Hardy-Weinberg equilibrium probability value

(HWpval) of *CYP2D6* included that 31G>A (1.0), 100C>T (0.654), 1039C>T (0.5071), 1846G>A (1.0), 1661G>C (0.5384), 1758G>T (1.0), 1758G>A (0.0644), 3198C>G (1.0) and 4180G>C (0.3062), respectively Minor allele frequency (MAF) of polymorphisms in *CYP2D6* including 31G>A, 100C>T, 1039C>T, 1846G>A, 1661G>C, 1758G>T, 1758G>A, , 3198C>G and 4180G>C of *CYP2D6* were 0.004, 0.451, 0.459, 0.011, 0.305, 0 , 0.013, 0.002, and 0.307, respectively and two SNPs have MAF less than 0.05 including -1584C>G (0.0261) and 2850C>T (0.0137).

Table 5.2 Minor allele frequency of polymorphisms in *CYP2D6*

No.	SNPs	Position	SNP ID	HWpval	MAF
1	4180G>C	42126611	rs1135840	0.4094	0.307
2	3198C>G	42127593	rs26708295	1.000	0.002
3	2850C>T	42523943	rs16947	0.0137	0.152
4	1846G>A	42128945	rs3892097	1.000	0.011
5	1758G>A	42129130	rs5030865	1.000	0.000
6	1758G>T	42129130	rs5030865	0.0644	0.013
7	1661G>C	42525132	rs1058164	0.5384	0.305
8	1039C>T	42525756	rs1081003	0.5071	0.459
9	100C>T	42526694	rs1065852	0.654	0.451
10	31G>A	42130761	rs769258	1.000	0.004
11	-1584C>G	42528382	rs1080985	0.0261	0.112

HWpval, Hardy-Weinberg *P* value; MAF, Minor Allele Frequency. The rs numbers in parentheses are the accession numbers in the National Center for Biotechnology information single nucleotide polymorphism (SNP) database, dbSNP. var SNP = null

5.3 Linkage disequilibrium map of *CYP2D6*

The results of linkage disequilibrium (LD) map of *CYP2D6* SNPs were shown in **Figure 5.1**. It had one LD block of *CYP2D6* that consist of four tagging SNPs including 4180G>C (rs1135840), 1661G>C, (rs1081003), 1039C>T (rs1081003) and

100C>T (rs1065852) which had length of linkage disequilibrium block approximately 4 kilo base (kb).

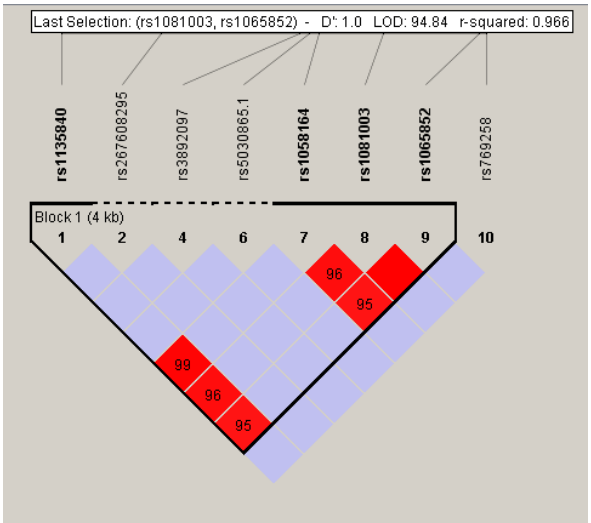


Figure 5.1 Linkage disequilibrium maps of *CYP2D6*

5.4 Haplotype analysis of *CYP2D6*

Haplotype analysis showed that *CYP2D6* four tag SNPs 100C>T, 1039C>T, 1661G>C and 4180G>C were found in linkage disequilibrium (D′= 0.99, LOD=89.22 and r-squared = 0.97) in position 1, 7, 8 and 9, respectively. Chance found that three pattern in recombinant 1. CCTT 53.4%, 2 GGCC 29.8% and CCCC 15.1%.

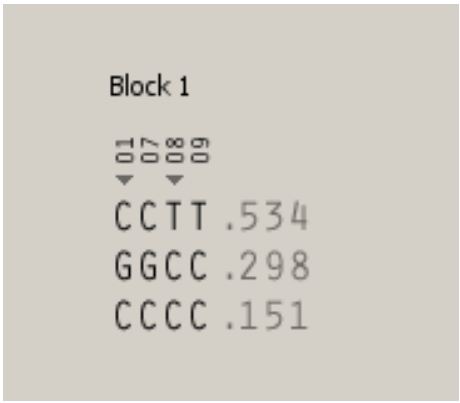


Figure 5.2 Haplotype block of *CYP2D6*

5.5 Frequency of *HLA-B* alleles

5.5.1 Allele groups (low resolution, 2-digit) frequency of *HLA-B*

This study was determined *HLA-B* typing of these 1316 patients consist of ASD (364 patients) and Non-ASD (952 patients). The results of distribution of *HLA-B* allele groups were summarized in **Table 5.3.** Twenty-eight allele groups of *HLA-B* gene were detected in this study. The five most alleles were *B*15* (403, 15.31%), **46* (333, 12.65%), **40* (295, 11.21%), **58* (230, 8.74%) and **13* (226, 8.59%), respectively.

Table 5.3 *HLA-B* allele low resolution (2-digit) frequencies distribution

Allele groups	No. of alleles, N=1316	Frequency; %
*B*15*	403	15.31
*B*46*	333	12.65
*B*40*	295	11.21
*B*58*	230	8.74
*B*13*	226	8.59
*B*18*	139	5.28
*B*35*	131	4.98
*B*51*	131	4.98
*B*44*	125	4.75
*B*38*	88	3.34
*B*27*	86	3.27
*B*07*	77	2.93
*B*39*	64	2.43
*B*52*	62	2.36
*B*55*	51	1.94
*B*54*	44	1.67
*B*57*	43	1.63
*B*56*	36	1.37
*B*08*	20	0.76
*B*48*	19	0.72
*B*37*	13	0.49
*B*14*	3	0.11
*B*41*	3	0.11
*B*50*	3	0.11
*B*53*	3	0.11

Table 5.3 *HLA-B* allele low resolution (2-digit) frequencies distribution (cont.)

Allele groups	No. of alleles, N=1316	Frequency; %
*B*36*	2	0.08
*B*67*	1	0.04
*B*73*	1	0.04
Total	2,632	100.00

5.5.2 Allele frequencies (high resolution, 4-digit) of *HLA-B* typing

One-hundred and thirty-two alleles of *HLA-B* gene were demonstrated in this study **Appendix B, Table 5.4**. The five most frequent alleles in our population were *HLA-B*4601* (295, 11.21%), **5801* (228, 8.66%), **4001* (227, 8.62%), **1502* (215, 8.17%) and **1301* (186, 7.07%), respectively.

Table 5.4 *HLA-B* allele high resolution (4-digit) frequencies distribution

Alleles	No. of alleles, N=1316	Frequency %
*B*4601*	295	11.21
*B*5801*	228	8.66
*B*4001*	227	8.62
*B*1502*	215	8.17
*B*1301*	186	7.07
*B*4403*	105	3.99
*B*5101*	92	3.50
*B*1801*	82	3.12
*B*3802*	71	2.70
*B*5201*	58	2.20
Other	1,073	40.77
Total	2,632	100.00

5.5.3 Allele frequencies HLA-B genetic markers and diseases

In this decade, several reports have shown the relationship between genes and disease. In this study (**Table 5.5**) we were shown alleles frequencies *HLA-B* in a Thai population.

Table 5.5 Genetic markers of *HLA-B* typing

Alleles	Number of individuals	Frequency; %	Adverse Drug Reaction
*B*1502*	215	8.17	Carbamazepine (CBZ),Phenytoin Stevens-Johnson syndrome (SJS) and drug-induced hypersensitivity syndrome (HSS)
*B*27*	86	3.27	Ankylosing Spondylitis
*B*1301*	186	7.07	Dapsone induced hypersensitivity reaction
*B*3505*	48	1.83	Nevirapine (NVP)-induced skin rash
*B*5701*	40	1.52	hypersensitivity reaction to Abacavir
*B*5801*	228	8.67	Allopurinol, Stevens-Johnson syndrome (SJS) and drug-induced hypersensitivity syndrome (HSS)

5.6 Frequency of *HLA-B* Genotypes

Four-hundred and seventy-nine genotypes of *HLA-B* gene were demonstrated in this study. The data was shown in **Appendix Table 5.6** The most

genotypes frequency of *HLA-B* that more than 1% were found fourteen patterns including *B*4001/*4601* (31, 2.32%), **1502/*4601* (27, 2.02%), **4001/*5801* (27, 2.02%), **4601/*4601* (23, 1.72%), **4601/*5801* (22, 1.64%), **1502/*4001* (19, 1.42%), **1301/*4601* (18, 1.34%), **1301/*1502* (17, 1.27%), **1502/*4403* (16, 1.19%), **4403/*4601* (16, 1.19%), **1301/*5801* (15, 1.12%), **3802/*4601* (15, 1.12%), **5801/*5801* (15, 1.12%) and **1301/*4001* (14, 1.05%), respectively.

Table 5.6 Frequency of *HLA-B* Genotypes

Genotypes	Number of individuals, (Total no. = 1316)	Frequency %
*B*4001/*4601*	31	2.32
*B*1502/*4601*	27	2.02
*B*4001/*5801*	27	2.02
*B*4601/*4601*	23	1.72
*B*4601/*5801*	22	1.64
*B*1502/*4001*	19	1.42
*B*1301/*4601*	18	1.34
*B*1301/*1502*	17	1.27
*B*1502/*4403*	16	1.19
*B*4403/*4601*	16	1.19
Other	1,100	83.59
Total	1,316	100.00

5.7 Frequency of the *CYP2D6* alleles, genotypes and phenotypes.

5.7.1 Alleles frequency of *CYP2D6* gene

Of these Two hundred and thirty three patients including ASD (79) and non-ASD (154) were studied in allele frequency of *CYP2D6* genotypes using microarray technique AmpliChip CYP450. The data was shown in **Table 5.7**. On the AmpliChip microarrays which failed to generate a genotype, 6.44% were “No Call”.

Therefore, 93.56% of the microarrays were successful. These study were detected 17 polymorphisms including -1584C>G, 31G>A, 100C>T, 1039C>T, 1661G>C, 1758G>T, 1846G>A, 2539-2542delACCT, 2850C>T, 2935A>C, 3198c>G, 3277T>C, **36GC*, 4180G>C, Gene Deletion and Gene Duplication from total 29 alleles were found in this study. Moreover, this study did not found many polymorphisms including 138 insT, 883G>C, 1023C>T, 1659G>A, 1707T>del, 1976G>A, *20 cluster, 2549A>del, 2613-2615delAGA, 3183G>A, 4042G>A and 1863 Repeat Ins.

Table 5.7 Allelic frequencies of *CYP2D6*

***CYP2D6* Alleles**	**Number of individuals**	**Frequency (%)**	**MAF**
	(N = 233)	(%)	
-1584C>G, rs1080985			
CC	188	80.69	
CG	38	16.31	
GG	7	3.00	G=0.112
31G>A, rs769258			
GG	231	99.14	
GA	2	0.86	A=0.004
100C>T, rs1065852			
CC	46	19.74	C=0.453
CT	23	51.07	
TT	12	8.15	
1039C>T, rs1081003			
CC	47	20.17	C=0.461
CT	121	51.93	
TT	65	27.90	
1661G>C, rs1058164			
GG	25	10.73	G=0.311
GC	95	40.77	
CC	113	48.50	
1758G>T, rs5030865			
GG	228	97.85	
No Call	5	2.15	
1758G>A, rs5030865			
GG	227	97.42	
GA	4	1.72	
AA	1	0.43	A=0.013
No Call	1	0.43	
1846G>A, rs3892097			
GG	221	94.85	

Table 5.7 Allelic frequencies of *CYP2D6* (cont.)

***CYP2D6* Alleles**	**Number of individuals**	**Frequency (%)**	**MAF**
	(N = 233)	(%)	
GA	5	2.15	A=0.016
No Call	7	3.00	
2539-2542 Del. AACT			
Negative	232	99.57	
No Call	1	0.43	
2850C>T, rs16947			
CC	175	75.11	
CT	47	20.17	
TT	11	4.72	T=0.148
2935A>C			
AA	232	99.57	
No Call	1	0.43	
3198C>G, rs267608295			
CC	232	99.57	
CG	1	0.43	G=0.002
3277T>C			
TT	232	99.57	
No Call	1	0.43	
**36GC*			
GG	229	98.28	
GC	4	1.72	C=0.008
4180G>C, rs1135840			
GG	26	11.16	G=0.313
GC	94	40.34	
CC	113	48.50	
Gene Deletion			
Negative	207	88.84	
Positive	26	11.16	
Gene Duplicaion			
Negative	227	97.42	
Positive	6	2.58	

The rs numbers in parentheses are the accession numbers in the National Center for Biotechnology information single nucleotide polymorphism (SNP) database, dbSNP

5.7.2 *CYP2D6* polymorphisms.

The results were demonstrated in **Table 5.**8. The *CYP2D6* allele frequencies were **10* = 224 (48.07%), **1* = 117 (25.11%), **2* = 41 (8.80%), **5* =24 (5.15%), **41* =12 (2.58%), **4* = 5 (1.07%), **14B, *36* = 4 (0.86%), **35* = 2 (0.43%), **25, *1xN,* *2xN = 1 (0.21%) and No calls = 30 (6.44%), respectively.

Table 5.8 Alleles frequencies of *CYP2D6* genes.

***CYP2D6* alleles**	**Nucleotides change**	**Effect**	**Enzyme activity**	**Frequency N (%)**
**1*	None		Normal	117 (25.11)
**2A, *2B, *2D*	-1584C>G, 1039C>T, 1661G>C, 2850C>T, 4180G>C	R296C, S486T	Normal	41 (8.80)
**4A,*4B, *4D,*4J, *4K*	100C>T, 1039C>T, 1661G>C, 1846G>A, 2850C>T, 4180G>C	splicing defect	None	5 (1.07)
**5*	Gene deletion	*CYP2D6* deleted	None	24 (5.15)
**10A, *10B*	100C>T, 1039C>T, 1661G>C, 4180G>C	P34S	Reduced	224 (48.07)
**14B*	1661G>C,1758G>A, 2850C>T,4180G>C	G169R	Reduced	4 (0.86)
**25*	3198C>G	R343G	Unknown	1 (0.21)
**35*	-1584G, 31G>A,1661G>C, 2850C>T,4180G>C	V11M	Normal	2 (0.43)
**36*	100C>T,1039C>T, 1661G>C,4180G>C, gene conversion to *CYP2D7* in exon 9	P34S	Reduced	4 (0.86)
**41*	-1584C, 1661G>C, 2850C>T, 2988G>A, 4180G>C	R296C, splicing defect, S486T	Reduced	12 (2.58)
**1xN*	Duplicate active *1 genes (n is not determined-range 2-13)	*N* active genes	Increased	1 (0.21)

(-) Alleles tested but not identified in this study.

Table 5.8 Alleles frequencies of *CYP2D6* genes (cont.)

CYP2D6 alleles	**Nucleotides change**	**Effect**	**Enzyme activity**	**Frequency N (%)**
**2xN*	Duplicate active *2 genes (n is not determined-range 2-13)	*N* active genes	Increased	1 (0.21)
No Calls	-		-	30 (6.44)
Total				**466**

(-) Alleles tested but not identified in this study.

A total of twelve different alleles were composed of one wild type allele (*1), one gene deletion (*5), one gene conversion (*36) and two genes duplication (*1xN, *2xN) and seven SNPs haplotypes (*2, *4, *10, *14B, *25, *35, *41) were identified **(Table 5.8)**. The most *CYP2D6* allele frequencies was *CYP2D6*10* (224/466, 48.07%). The allele frequencies in this study is consistent with previous study [113]. These study found thirty "No Calls" 30 (6.44%) signifies that automated genotype assignment was impossible due to presence of unknown alleles. (All hybridization positions were occupied and identified by the AmpliChip™ software, but based on the hybridization pattern a genotype could not be generated, no a phenotype predicted) for *CYP2D6*.

5.7.3 Frequencies of *CYP2D6* Genotypes.

The three most genotypes were *CYP2D6*1/*10* (67/218, 30.73%), *10/*10* (53/218, 24.31%) and *2/*10* (20/218, 9.17%), respectively. Twenty-four genotypes were detected in this study including *1/*1, *1/*2, *1/*5, *1/*10,*1/*14B, *1/*35, *1/*36, *1/*41, *2/*2, *2/*4, *2/*5, *2/*10,*2/*41, *4/*10, *5/*10, *5/*14B, *10/*10, *10/*14B, *10/*25, *10/*35, *10/*36, *10/*41, *1/*2xN and *1xN/*10, respectively (listed in **Table 5.9).**

Table 5.9 *CYP2D6* genotype frequencies and associated predicted phenotypes

Genotypes	Number of individuals	Frequency %
**1/*1*	15	6.88
**1/*2*	5	2.29
**1/*5*	7	3.21
**1/*10*	67	30.73
**1/*14B*	1	0.46
**1/*35*	1	0.46
**1/*36*	3	1.38
**1/*41*	2	0.92
**2/*2*	5	2.29
**2/*4*	2	0.92
**2/*5*	2	0.92
**2/*10*	20	9.17
**2/*41*	1	0.46
**4/*10*	3	1.38
**5/*10*	14	6.42
**5/*14B*	1	0.46
**10/*10*	53	24.31
**10/*14B*	2	0.92
**10/*25*	1	0.46
**10/*35*	1	0.46
**10/*36*	1	0.46
**10/*41*	9	4.13
**1/*2xN*	1	0.46
**1XN/*10*	1	0.46
Total	**218**	**100.00**

Abbreviations: EM, extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer; UM, ultra-rapid metabolizer; Unk,Unknown

5.7.4 Frequency of *CYP2D6* predicted phenotypes

The predicted phenotypes of *CYP2D6* in this study could be interpreted in

four phenotypes (**Table 5.10**) including EM, IM, EM and UM and one was Unknown predicted phenotype. The highest predicted phenotype was EM (197/218, 90.37%).

Table 5.10 *CYP2D6* predicted phenotypes and genotypes

Predicted Phenotype	Genotype	Number of individuals	Frequency (%)
UM	**1/*2xN, *1xN/*10*	2	0.92
EM	**1/*1, *1/*2, *1/*35, *2/*2, *1/*10, *1/*36, *1/*41, *2/*10, *2/*41, *10/*35, *1/*5, *1/*14B, *2/*4, *2/*5, *10/*10, *10/*36, *10/*14B, *10/*41*	197	90.37
IM	**4/*10, *5/*10*	17	7.80
PM	**5/*14B*	1	0.46
Unknown	**10/*25*	1	0.46
Total		218	100.00

Abbreviation: EM, extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer; UM, ultra-rapid metabolizer; Unk., Unknown

5.7.5. The predicted phenotypes of *CYP2D6* were classified as score of enzyme activity

In this study could classify eight activity score include ≥ 3.0, 2.5, 2.0, 1.5, 1.0, 0.5 and 0 [114]. The most frequency of predicted phenotype and activity score were EM (activity score: 1.0, 1.5, 2.0 were included) and 1.5 (94/218, 43.12%). The **Table 5.11** had been shown the relationship between predicted phenotypes frequencies and activity score.

Table 5.11 Frequency of *CYP2D6* alleles were associated between allelic variants and enzyme activity

Genotypes	Phenotype	Activity Score (AS)	Number of individuals	Frequency (%)
**1/*2xN*	UM	≥ 3.0	1	0.46
**1xN/*10*	UM	2.5	1	0.46
**1/*1, *1/*2, *1/*35, *2/*2*	EM	2.0	26	11.93

Abbreviations: EM, extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer; UM, ultra-rapid metabolizer; Unk., Unknown.

Table 5.11 Frequency of *CYP2D6* alleles were associated between allelic variants and enzyme activity (cont.)

Genotypes	Phenotype	Activity Score (AS)	Number of individuals	Frequency (%)
**1/*10, *1/*36, *1/*41, *2/*10, *2/*41, *10/*35*	EM	1.5	94	43.12
**1/*5, *1/*14B, *2/*4, *2/*5, *10/*10, *10/*36, *10/*14B, *10/*41*	EM	1.0	77	35.32
**4/*10, *5/*10*	IM	0.5	17	7.80
**5/*14B*	PM	0	1	0.46
**10/*25*	Unk.	Unk.	1	0.46
Total			218	

Abbreviations: EM, extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer; UM, ultra-rapid metabolizer; Unk., Unknown

5.8 The comparison of *HLA-B* alleles

5.8.1 *HLA-B* alleles low resolution frequency compare between ASD and Non- ASD.

In this study had been shown 25 groups of *HLA-B* alleles. The results of low resolution (2-digit) allele frequencies of *HLA-B* were summarized in **Table 5.12.** The *HLA-B* alleles were compared the difference between ASD versus Non-ASD group by Chi- square test and Fisher's exact test. There was a significant difference of *HLA-B*44* alleles frequency between ASD and non-ASD ($P = 0.011$).

Table 5.12 The association of *HLA-B* alleles between two groups.

Alleles	ASD, n= 728 (%)	Non-ASD, n=1904 (%)	OR	95% CI	*P*-value
*B*07*	22 (3.02)	55 (2.89)	1.048	0.634-1.731	0.856
*B*08*	5 (0.69)	15 (0.79)	0.871	0.315-2.405	0.789
*B*13*	56 (7.67)	170 (8.93)	0.850	0.620-1.164	0.312

Table 5.12 The association of *HLA-B* alleles between two groups (cont.)

Alleles	ASD, n= 728 (%)	Non-ASD, n=1904 (%)	OR	95% CI	*P*-value
*B*14*	2 (0.27)	1 (0.05)	5.242	0.475-57.905	0.176
*B*15*	102 (14.01)	301 (15.81)	0.868	0.681-1.106	0.252
*B*18*	31 (4.26)	108 (5.67)	0.739	0.491-1.113	0.148
*B*27*	26 (3.57)	60 (3.15)	1.138	0.713-1.818	0.588
*B*35*	31 (4.26)	100 (5.25)	0.802	0.531-1.212	0.295
*B*36*	2 (0.27)	0 (0.00)	13.107	0.628-273.362	0.096
*B*37*	4 (0.55)	9 (0.47)	1.163	0.357-3.789	0.802
*B*38*	32 (4.40)	56 (2.94)	1.517	0.974-2.363	0.065
*B*39*	16 (2.20)	48 (2.52)	0.869	0.490-1.540	0.630
*B*40*	90 (12.36)	205 (10.77)	1.169	0.898-1.522	0.246
*B*41*	0 (0.00)	3 (0.16)	0.373	0.019-7.228	0.514
B*44	**47 (6.46)**	**78 (4.10)**	**1.616**	**1.113-2.345**	**0.011**
*B*46*	89 (12.23)	244 (12.82)	0.948	0.731-1.228	0.684
*B*48*	4 (0.55)	15 (0.79)	0.696	0.230-2.103	0.520
*B*50*	1 (0.14)	2 (0.11)	1.308	0.118-14.449	0.827
*B*51*	32 (4.40)	99 (5.20)	0.838	0.557-1.261	0.397
*B*52*	12 (1.65)	50 (2.63)	0.621	0.329-1.174	0.143
*B*53*	0 (0.00)	3 (0.16)	0.373	0.019-7.228	0.514
*B*54*	15 (2.06)	29 (1.52)	1.360	0.723-2.550	0.338
*B*55*	10 (1.37)	41 (2.15)	0.633	0.315-1.270	0.198
*B*56*	15 (2.06)	21 (1.10)	1.886	0.967-3.679	0.063
*B*57*	17 (2.34)	26 (1.37)	1.727	0.932-3.202	0.083
*B*58*	66 (9.07)	164 (8.61)	1.058	0.784-1.427	0.714
*B*67*	0 (0.00)	1 (0.05)	0.871	0.035-21.406	0.933
*B*73*	1 (0.14)	0 (0.00)	7.854	0.319-193.017	0.207

5.8.2 HLA-B allele high resolution frequencies were compared according to two groups

The frequencies of *HLA-B* alleles of 364 ASD patients and 952 Non-ASD were shown in **Appendix B Table 5.13**. In *HLA-B*1302* (*P*=0.019, OR; 2.229, 95%CI; 1.099-2.463), *HLA-B*4403* (*P*=0.016, OR; 1.645, 95%CI; 1.099-2.463) and *HLA-B*5601* (*P*=1.78x10^{-4}, OR; 4.927, 95%CI; 1.958-12.399) alleles were found to be significantly associated with ASD. Two of the *HLA-B* alleles were negatively linked to ASD: *HLA-B*1802* (*P*=0.016, OR; 0.375, 95%CI; 0.169-0.836) and *HLA-B*4612* (*P*=0.008, OR; 0.147, 95%CI; 0.035-0.613).

Table 5.13 *HLA-B* allele frequencies in autism children and control

Alleles	ASD, n= 728 (%)	Non-ASD, n=1904 (%)	OR	95%CI	*P* value
B*1302	**16 (2.20)**	**19 (1.00)**	**2.229**	**1.140-4.359**	**0.019**
*B*1802*	7 (0.96)	48 (2.52)	0.375	0.169-0.836	0.016
B*4403	**40 (5.49)**	**65 (3.41)**	**1.645**	**1.099-2.463**	**0.016**
*B*4612*	2 (0.27)	35 (1.84)	0.147	0.035-0.613	0.008
B*5601	**13 (1.79)**	**7 (0.37)**	**4.927**	**1.958-12.399**	**$1.78x10^{-4}$**

* Chi-square test, OR: odds ratio; 95%CI: confidence interval. The bold font in table refers to significantly association of alleles. Bold, positively significant

5.8.3 *HLA-B* Genotype frequencies according to two groups

The frequencies of *HLA-B* genotypes of 1904 subjects were shown in **Appendix C Table 5.14.** The results showed four genotypes that there were statistically significant relationship between ASD and Non-ASD groups. In *HLA-B*3905/*5801* (*P*=0.032, OR=24.697, CI=1.326-459.879), *HLA-B*2704/*5801* (*P*=0.022, OR=6.872, CI=1.327-35.577) and *HLA-B*3501/*4403* (*P*=0.021, OR=30.269, CI=1.669-548.816), respectively.

Table 5.14 *HLA-B* Genotype frequencies according to two groups

Genotypes	ASD N=364 (%)	Non-ASD N=952 (%)	OR	95% CI	*P*-value
*B*1801/*4402*	5(1.38)	1 (0.10)	13.757	1.602-118.158	0.017
*B*2704/*5801*	5 (1.38)	2 (0.20)	6.872	1.327-35.577	0.022
*B*3501/*4403*	5 (1.38)	0 (0.00)	30.269	1.669-548.816	0.021
*B*3905/*5801*	4 (1.10)	0 (0.00)	24.697	1.326-459.879	0.032

* Chi-square test, OR: odds ratio; 95%CI: confidence interval. The bold font in table refers to significantly association of alleles.

5.9 *CYP2D6* were compared between groups

5.9.1 Compared allelic frequencies

The summarized allelic frequencies were shown in **Table 5.15**. There were seven mutant SNPs and one gene conversion of *CYP2D6* from thirty-three polymorphisms found in this study including *-1584C>G*, *100C>T*, *1039C>T, 1661G>C, 1846G>A, 2850C>T*, *4180G>C,* and one gene conversion is *CYP2D6*36GC*, and one gene deletion is *CYP2D6*5*, and two genes duplication are *CYP2D6*1xN* and *CYP2D6*2xN*. The most variant alleles is *CYP2D6*10* (*100C>T*). This results were showed significant in patients ASD with heterozygous variant (*CT*) compared those with wild type homozygous and mutant homozygous *CC and CT* at nucleotides *100C>T* (*CYP2D6*10*); *P* = 0.017, 0.110 and 0.216, respectively. Patients ASD carrying heterozygous *CT* at nucleotide *1039C>T* had significantly than patients Non-ASD with heterozygous *C/T* (*P* = 0.027). Two of the CYP2D6 alleles were negatively linked to ASD: homozygous 1661G>C (P=0.045) and 4180G>C (P=0.034).

Table 5.15 Allelic frequencies of *CYP2D6* among two groups.

***CYP2D6* Alleles**	**ASD N (%)**	**MAF**	**Non-ASD N (%)**	**MAF**	***P* value**
	N=158		N= 308		
-1584C>G, rs1080985					
CC	59 (74.68)		129 (83.76)		0.076
CG	17 (21.52)		21 (13.64)		0.121
GG	3 (3.80)	G=0.146	4 (2.60)	G=0.112	0.611
31G>A					
GG	78 (98.73)		153 (99.35)		0.629
GA	1 (1.27)	A=0.006	1 (0.65)	A=0.004	
100C>T, rs1065852					
CC	11 (13.92)	C=0.449	35 (22.73)	C=0.453	0.110
CT	49 (62.03)		70 (45.45)		0.017
TT	19 (24.05)		49 (32.82)		0.216

Chi-square test; The rs numbers in parentheses are the accession numbers in the National Center for Biotechnology information single nucleotide polymorphism (SNP) database, dbSNP.

Table 5.15 Allelic frequencies of *CYP2D6* among two groups (cont.).

***CYP2D6* Alleles**	**ASD N (%)**	**MAF**	**Non-ASD N (%)**	**MAF**	***P* value**
	N=158		N= 308		
1039C>T, rs1081003					
CC	11 (13.92)	C=0.449	36 (23.38)	C=0.461	0.088
CT	**49 (62.03)**		**72 (49.35)**		**0.027**
TT	19 (24.05)		46 (29.87)		0.348
1661G>C, rs1058164					
GG	**4 (5.06)**	**G=0.272**	**21 (13.64)**	**G=0.311**	**0.045**
GC	35 (44.30)		60 (38.96)		0.432
CC	40 (50.64)		73 (47.40)		0.640
1758G>T					
GG	79 (100.0)		149 (96.75)		0.105
No Call	0 (0)		5 (3.25)		
1758G>A					
GG	78 (98.73)		149 (96.75)		0.366
GA	1 (1.27)	A=0.006	3 (3.25)		0.696
AA	0 (0)		1 (0.65)	A=0.013	0.472
No Call	0 (0)		1 (0.65)		0.472
1758G>A					
GG	78 (98.73)		149 (96.75)		0.366
GA	1 (1.27)	A=0.006	3 (3.25)		0.696
AA	0 (0)		1 (0.65)	A=0.013	0.472
No Call	0 (0)		1 (0.65)		0.472
2539-2542 Del AACT					
Negative	79 (100.00)		153 (99.35)		0.473
No Call	0 (0)		1 (0.65)		
2850C>T, rs16947					
CC	54 (68.35)		121 (78.57)		0.088
CT	20 (25.32)		27 (17.53)		0.161
TT	5 (4.72)	T=0.285	6 (3.90)	T=0.127	0.407
2935A>C					
AA	79 (100.00)		153 (99.35)		0.473
No Call	0 (0)		1 (0.65)		0.105
3198C>G					
CC	78 (98.73)		154 (100.00)		0.162

Chi-square test; The rs numbers in parentheses are the accession numbers in the National Center for Biotechnology information single nucleotide polymorphism (SNP) database, dbSNP.

Table 5.15 Allelic frequencies of *CYP2D6* among two groups (cont.)

***CYP2D6* Alleles**	**ASD N (%)**	**MAF**	**Non-ASD N (%)**	**MAF**	***P* value**
	N=158		N= 308		
CG	1 (1.27)	G=0.006	0 (0)		
3277T>C					
TT	79 (100.00)		153 (99.35)		0.473
No Call	0 (0)		1 (0.65)		
*36GC					
GG	78 (98.73)		151 ((98.05)		0.704
GC	1 (1.27)	C=0.006	3 (1.95)	C=0.010	
4180G>C, rs1135840					
GG	**4 (5.06)**	**G=0.272**	**22 (14.29)**	**G=0.334**	**0.034**
GC	35 (44.30)		59 (38.31)		0.377
CC	40 (50.63)		73 (47.40)		0.640
Gene Deletion					
Negative	73 (92.41)		134 (87.01)		0.216
Positive	6 (7.59)		20 (12.99)		
Gene Duplication					
Negative	77 (97.47)		150 (97.40)		0.976
Positive	2 (2.53)		4 (2.60)		

Chi-square test; The rs numbers in parentheses are the accession numbers in the National Center for Biotechnology information single nucleotide polymorphism (SNP) database, dbSNP.

5.9.2 Compared alleles frequency

The results of polymorphisms of CYP26 were summarized in **Table 5.16.** Twelve alleles were detected in this study and 15 subjects in Non-ASD were No Calls. The three most alleles frequencies two group similar were *CYP2D6*10, *1* and *2* by ASD were 86 (54.43%), 36 (22.78%) and 18 (11.39%) and Non-ASD were 138 (44.81%), 81 (26.30%) and 23 (7.47%), respectively**.** No allele with significant allele frequency differences between the two groups.

Table 5.16 *CYP2D6* allele frequencies compared between groups

***CYP2D6* Alleles**	**ASD N (%)**	**Non-ASD N (%)**	***P*-value**
	N=158	N=278	
**1*	36 (22.78)	81 (29.14)	0.150
**2*	18 (11.39)	23 (8.27)	0.283
**4*	0 (0.00)	5 (1.80)	0.089
**5*	6 (3.80)	18 (6.47)	0.239
**10*	86 (54.43)	138 (49.64)	0.336
**14B*	1 (0.63)	3 (1.08)	0.638
**25*	1 (0.63)	0 (0.00)	0.184
**35*	1 (0.63)	1 (0.36)	0.684
**36*	1 (0.63)	3 (1.08)	0.638
**41*	6 (3.80)	6 (2.16)	0.315
**1XN*	1 (0.63)	0 (0.00)	0.184
**2XN*	1 (0.63)	0 (0.00)	0.184

Chi-square test, the bold font in table refers to significantly association of alleles.

5.9.3 *CYP2D6* genotype frequencies and associated predicted phenotypes compared between groups

Twenty-four genotypes were detected in this study. The two most alleles frequencies two group similar and including **1/*10 and *10/*10 were 26(32.91%), 18 (22.78%)* and 41(29.50%), 35 (22.73%), respectively listed in **Table 5.17.** Not significant allele frequency differences between the two groups. The two most predicted phenotypes frequencies two group similar and including EM and IM were 56.96% and 40.51% in ASD and 53.96% and 45.32% in Non-ASD.

Table 5.17 *CYP2D6* genotype frequencies and associated predicted phenotypes

Genotypes	**ASD N=79 (%)**	**Non-ASD N=139 (%)**	**Predicted Phenotypes**	***P*-value**
**1/*1*	2 (2.53)	13 (9.35)	EM	0.056
**1/*2*	2 (2.53)	3 (2.16)	EM	0.860
**1/*5*	1 (1.27)	6 (3.90)	EM	0.219
**1/*10*	26 (32.91)	41 (29.50)	EM	0.599
**1/*14B*	0 (0.00)	1 (0.72)	EM	0.449

Abbreviations: EM, extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer; UM, ultra-rapid metabolizer; Unk., Unknown. Chi-square test, the bold font in table refers to significantly association of alleles.

Table 5.17 *CYP2D6* genotype frequencies and associated predicted phenotypes (cont.)

Genotypes	ASD N=79 (%)	Non-ASD N=139 (%)	Predicted Phenotypes	*P*-value
**1/*35*	1 (1.27)	0 (0.00)	EM	0.184
**1/*36*	1 (1.27)	2 (1.44)	EM	0.916
**1/*41*	0 (0.00)	2 (1.44)	EM	0.284
**2/*2*	2 (2.53)	3 (2.16)	EM	0.860
**2/*4*	0 (0.00)	2 (1.44)	EM	0.284
**2/*5*	1 (1.27)	1 (0.72)	EM	0.684
**2/*10*	11 (13.92)	9 (6.47)	EM	0.067
**2/*41*	0 (0.00)	1 (0.72)	EM	0.449
**4/*10*	0 (0.00)	3 (2.16)	IM	0.188
**5/*10*	4 (5.06)	10 (7.19)	IM	0.537
**5/*14B*	0 (0.00)	1 (0.72)	PM	0.449
**10/*10*	18 (22.78)	35 (25.18)	IM	0.691
**10/*14B*	1 (1.27)	1 (0.72)	IM	0.684
**10/*25*	1 (1.27)	0 (0.00)	IM	0.184
**10/*35*	0 (0.00)	1 (0.72)	EM	0.449
**10/*36*	0 (0.00)	1 (0.72)	IM	0.449
**10/*41*	6 (7.59)	3 (2.16)	IM	0.052
**1/*2XN*	1 (1.27)	0 (0.00)	UM	0.184
**1XN/*10*	1 (1.27)	0 (0.00)	UM	0.184

Abbreviations: EM, extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer; UM, ultra-rapid metabolizer; Unk., Unknown. Chi-square test, the bold font in table refers to significantly association of alleles.

5.9.4 *CYP2D6* genotype frequencies associated predicted phenotypes and activity score (AS).

The predicted phenotypes of *CYP2D6* in this study could be interpreted in three phenotypes (**Table 5.18**) including EM (**1/*1, *1/*2, *1/*5, *1/*10, *1/*35, *1/*36, *1/*41, *2/*2, *2/*4*, **2/*10*, **2/*41*, **10/*10, *10/*14B, *10/*35, *10/*36* and **10/*41*), IM (**4/*10, *5/*10* and **5/*14B*), UM *(*1/*2xN* and **1xN/*10)* and Unknown *(*10/*25).*

In this study showed AS were nine groups including UM ≥3.0, UM 2.5, EM 2.0, EM 1.5, EM 1.0 and IM 0.5, respectively. The EM activity score 1.5 had the most frequency in both group 38 (48.10%) and 56 (36.36%) in **Table 5.18**.

Table 5.18 *CYP2D6* genotype frequencies associated predicted phenotypes and activity score [115].

Genotype	Predicted Phenotype	Activity Score (AS)	ASD N=79 (%)	Non-ASD N=139 (%)	*P*-value
**1/*2xN*	UM	≥ 3.0	1 (1.27)	0 (0.00)	0.184
**1xN/*10*	UM	2.5	1 (1.27)	0 (0.00)	0.184
**1/*1*, **1/*2*, **1/*35*, **2/*2*	EM	2.0	7 (8.86)	19 (13.67)	0.292
**1/*10*, **1/*36*, **1/*41*, **2/*10*, **2/*41*, **10/*35*	EM	1.5	38 (48.10)	56 (40.29)	0.262
**1/*5*, **1/*14B*, **2/*4*, **2/*5*, **10/*10*, **10/*36*, **10/*14B*, **10/*41*	EM	1.0	27 (34.18)	50 (35.97)	0.789
**4/*10*, **5/*10*, **5/*14B*	IM	0.5	4 (5.06)	14 (10.07)	0.130
**10/*25*	Unk.	-	1 (1.27)	0 (0.00)	0.184

Abbreviations: EM, extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer; UM, ultra-rapid metabolizer; Unk., Unknown. Chi-square test, the bold font in table refers to significantly association of alleles.

Table 5.19 *CYP2D6* genotype frequencies associated and activity score

Genotype	**Predicted Phenotype ; AS**	**ASD N=79 (%)**	**Non-ASD N=139 (%)**	***OR* *(95% CI)***	***P*-value**
**1/*2xN, *1xN/*10*	UM; ≥ 2.5	2 (1.27)	0 (0.00)	9.000 (0.427 – 189.865)	0.158
**1/*1, *1/*2, *1/*5, *1/*10,*1/*14B, *1/*35,*1/*36, *1/*41,*2/*2, *2/*4, *2/*5,*2/*10,*2/*4, *10/*10,*10/*14B, *10/*35,*10/*36, *10/*41*	EM; 1.0-2.0	72 (91.14)	125 (89.92)	1.152 (0.444 – 2.986)	0.771
**4/*10,*5/*10, *5/*14B*	IM; < 0.5	4 (5.06)	14 (10.07)	0.476 (0.151 – 1.500)	0.205
**10/*25*	Unk.	1 (1.27)	0 (0.00)	5.331 (0.215 – 132.445)	0.307

Abbreviations: EM, extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer; UM, ultra-rapid metabolizer; Unk., Unknown. Chi-square test, the bold font in table refers to significantly association of alleles.

CHAPTER VI
DISCUSSION

The overall aim of this study was to analyze the characteristic of *CYP2D6* and *HLA-B* genes and to compare the alleles frequency both genes between ASD and Non-ASD in order to develop test kits for further study to determine the genetic polymorphism that associated with the clinical drug used in Thai Autisms and also appropriate for only Thai population.

An up to date, there have been a many pharmacogenetics studied about polymorphisms of *CYP2D6* and *HLA-B* genes that were published in the database. However, no data were available on *CYP2D6* and *HLA-B* polymorphism in Thai ASD. This is the first study in Thailand to extensively examine the combined polymorphisms of two genes of different SNPs by different methods including AmpliChip™ CYP450 and LABType® PCR-SSOP assays, respectively..

Although the causes and mechanisms of ASD were unclear, the genetic and environment factors were believed to play an important role in the pathophysiology of the ASD and there was clear that no single genetic locus were the sole cause of this disorders. Recently, the genome-wide association studies revealed a number of rare mutations have been identified as causal of ASD. Many of these genes encode proteins integral to formation, refinement, maintenance, function, and/or plasticity of synapses in the CNS, others encode proteins traditionally thought to play roles exclusively in the immune system, including MHC genes [116]. In addition, increased levels of these cytokines in the plasma were found to be linked with greater impairments in characteristic ASD behavioral domains including social interaction and communication, as well as associated features such as aberrant behaviors.

Warren and his colleagues reported that the allelic products of certain genes of the MHC were associated with autism including the null allele of the *C4B* gene (located in the class III region of the MHC), and the extended haplotype *HLA-DRβ1*04* (DR4) and *HLA-DR13* in the class II and A2 and *HLA-B*44* allele in the class I region [117]. Another study from Egypt showed that there was a positive

association between *HLA-DRB1*11* allele and autism, and also a protective function assigned to *HLA-DRB1*03* [118]. Furthermore, a study from china found that *HLA-DR4*, *HLA-DR11*, and *HLA-DR14* had a different effect on intelligence and neuropsychology tests among autistic children [119].

This study revealed that only *HLA-B*44* allele group was significantly associated with ASD but the frequencies of *HLA-B*51* in ASD were not different from Non-ASD. This data was similar to the previous study by Torres *et al.* which reported a higher prevalence of *HLA-B*44* and *HLA-B*51* among ASD children than controls [120] and dissimilar to the previous finding of Al-Hakbany *et al.* which reported a higher prevalence of *HLA-B*07* and *HLA-B*51* among ASD children than controls [6]. In this study, the findings obtained have shown *HLA-B* haplotype in high resolution that *HLA-B*1802*, *HLA-B*4403*, *HLA-B*4612* and *HLA-B*5601* alleles were significantly associated with ASD. The genotypes of *HLA-B* comprise of *HLA-B*2704/*5801, HLA-B*3501/*4403* and *HLA-B*3905/*5801* were observed the statistically significant (p<0.05) difference between with ASD and non-ASD. Moreover, *HLA-B*1802*, *HLA-B*4403*, *HLA-B*4612, HLA-B*5601, HLA-B*2704/*5801, HLA-B*3501/*4403* and *HLA-B*3905/*5801* were significantly associated with ASD. These original association have not been reported before in other populations.

In the current study of HLA association with autism among Thailand children have the following positive association between *HLA-B*44, HLA-B*1802*, *HLA-B*4403*, *HLA-B*4612, HLA-B*5601, HLA-B*2704/*5801, HLA-B*3501/*4403* and *HLA-B*3905/*5801* haplotypes and genotypes demonstrate their involvement in the disease etiology possibly by playing a role in the presentation of microbial antigen within the CNS, which may interfere with the formation of synaptic plasticity and neuronal circuits in the developing brain.

Genetic studies unavoidably increase the attention to the ethnic variation in the incidence of diseases. That might be multiple causes to explain by variation in *HLA-B* genetic associations with ASD across different populations. This finding, it might serve as genetic markers for susceptibility to ASD in Thailand. However, the interaction between *HLA* allele and different infectious agents or environmental

allergen across geographical regions remains of interest for clarification of susceptible and resistance genes to ASD.

In Ten years, there were 557,978 ADR-associated admissions, representing 0.9% of total hospital admissions. Over this period the annual number of ADRs increased by 76.8% (from 42,453 to 75,076), and in-hospital mortality rate increased by 10% (from 4.3% to 4.7%) [121]. Pharmacogenetics marker in *HLA-B* gene association of the disease at the whole genome level, as shown by *HLA-B*5701* and abacavir hypersensitivity [22], *HLA-B*1502 [21, 87], HLA-A*3101* [122], HLA-B*1511 [123] and CBZ-induced SJS/TEN, *HLA-B*3505* and nevirapine (NVP)-induced skin rash [124] and *HLA-B*5801* and allopurinol-induced SJS/TEN [125]. The frequencies of *HLA-B*1502, *1511, *3505, *5701 and *5801* in this study were similar to previous study finding which reported to be 8.17%, 0.27%, 1.82%, 1.52% and 8.66%, respectively and frequencies of *HLA-B*1502* relatively high allelic in Thai (8.6%), Han Chinese (7.3%), Malaysia (12-15%), India (8.3%) and a Southeast Asian (5.5%) population, but relatively low in Japanese (0.6%) and European (0.8%) populations (dbMHC data base; http://www.ncbi.nlm.nih.gov/projects/gv/mhc/ihwg.cgi).

The results of *CYP2D6* allele frequencies in ASD group were not different from Non-ASD in Thai population. The frequency values for polymorphic alleles and genotypes corresponded to the frequencies for other previous researches in Asian population [126] and data from previous study suggest that knowing the *CYP2D6* phenotype may be clinically relevant, especially in the extremity subgroups of poor and ultra-rapid metabolizers.

The frequencies of polymorphic *CYP2D6*10, 1, *2, *5,* and ** 41* alleles were 0.481, 0.251, 0.088, 0.051, and 0.026, respectively, with the Roche Amplichip™ Assay detecting the largest number of alleles. This microarray test concluded that this technology was high-throughput, rapid and very easy perform. Of the 33 possible *CYP2D6* and 3 allelic variants in *CYP2C19* in one sample. An automatic system may help to speed up the analytical process with minor limitations in specificity, such as misinterpretation between two SNPs, one EM and the other IM for *CYP2D6* alleles included in the assays used in this study, twelve (**1, *2, *4, *5, *10, 14B, *25, *35, *36, *41, *1XN* and **2XN*) were identified among the 233 DNA samples. Laboratories did not detect alleles, twenty-*one *3, *6, *7, *8, *9, *11, *14A, *15, *17, *19, *20,*

**26, *29, *30, *31, *40, *4XN *10XN, *17XN, *35XN,* and **41XN*. We had no homozygous UM allele (*CYP2D6*1XN*, **2XN* and **35XN*), it is possible that there was small sample size.

Risperidone was a preferred treatment for children and adolescents with bipolar disorder, irritability associated with autistic disorder, disruptive behavior disorders, and schizophrenia [127, 128]. It was widely accepted that risperidone pharmacokinetic (PK) and response (desired therapeutic effect and occurrence of adverse reactions) variability were not completely predicted by dose [129]. Risperidone and 9-OH risperidone concentrations may be influenced by age, renal function, and factors affecting metabolism, including genetic polymorphisms, which vary among races/ethnicities, and drug interactions.

The metabolism of risperidone to it active metabolite 9-OH risperidone was mainly dependent on *CYP2D6*, leading to significantly higher dose-collected steady-state plasma levels of the parent in PM in than EM [130]. *CYP2D6* UM has a significantly lower propensity to waist circumference and BMI increase. The lower risperidone to 9-OH risperidone ratio that characterizes the UM phenotype may explain the lower propensity of this phenotype to induce weight gain because of the differences in the affinity of these compounds for the receptors that might be involved in weight regulation, such as 5-HT2C.

Abnormalities in serotonergic function have been linked with ASD since Schain and Freedman reported hyperserotonemia in 1961 and in previous studies confirms in 25 to 33% of individuals diagnosed with ASD, whole blood serotonin levels were found to be elevated [131]. The UM has found in ASD 2 subjects and not found in Non-ASD in contrast, PM phenotype has found in Non-ASD only, it has been hypothesized that the role of CYPs in the brain are metabolized to exogenous and endogenous compounds; and the potential impact of brain CYPs on behavior, disease pathology and treatment outcomes.

Our study has some limitations. First of all, the retrospective nature of the design is a weakness shared with all other available studies. The design study with retrospective method is lack data of correlation between polymorphisms in *CYP2D6* and plasma concentration of risperidone metabolites. In *HLA-B* genotyping, this study

analyzed the *HLA-B* only studied in other groups of HLA (*HLA-A, C, DR4* and *DR13*) which is discussed from the study before it is related to ASD.

This study has some limitations that should be taken into explanation when interpreting the results and that should be addressed in the further studies. First, the small number of patients in this study greatly limited comparisons, particularly in clinical data and the patients in the group of Non ASD has not been interviewed by the DSM-IV diagnosis for ASD. The US FDA has notified that treatment with antipsychotic medications increases the risk of adverse effect, and now several studies confirm that some drugs are riskier than others. Therefore children and adolescences with ASD in this study had required maintenance treatment with lower doses of antipsychotics and had occurred adverse effects less than adults.

Additionally, the effect of each polymorphism might have been small enough that our study design was unable to determine a statistically significant effect for some of the relations measured. However, it might be possible that the determination of these polymorphisms combined with other polymorphisms in other genes might be necessary to observe a more significant pharmacogenetics relation.

Other genetic polymorphisms which might affect prolactin concentrations, including HLA class II and III gene. And limitation in microarray Amplichip^{TM} performed poorly in terms of reliability and failed to generate a genotype, 4.0-7.0% [132] were “No Calls” (all hybridization positions were occupied and identified by the AmpliChip^{TM} software, but based on the hybridization pattern a genotype could not be generated, nor a phenotype predicted similar in this study No call 6.44% (30 of 466). It is not necessary to detect 33 alleles of *CYP2D6* by AmpliChip^{TM}, as some alleles have not been reported in Thai population and the cost of testing is very high price.

CHAPTER VII
CONCLUSION

Interestingly, our data showed that *HLA-B*44* allele had statistically significant difference between ASD and Non-ASD group. In addition, *HLA-B*4403* and *HLA-B*5601* alleles were found to be significantly associated with ASD. HLA-B haplotypes demonstrate their involvement in the disease etiology possibly by playing a role in the presentation of microbial antigen within the central nervous system, which may interfere with the formation of synaptic and neuronal circuits in the developing brain. The *CYP2D6* allele frequencies in ASD group were not different from Non-ASD.

Although, despite a relatively small sample size, this research is the first time a foreseeable risk association of *HLA-B*44* allele might serve as genetic markers for susceptibility to ASD in Thailand and should be add novel data to the current knowledge base. Thus, additional studies are warranted to examine polymorphisms of *HLA-B*44* dependent relations in other groups.

In conclusion, these results suggest that genetic polymorphisms of *CYP2D6* may be used for help physicians plan to treat patient with ASD. In our institution, as recommended in several treatment guidelines, the dosing was individualized according to the response and tolerability for children with ASD and irritability. Therefore, screening for *HLA*-genotyping before the start of drug therapy, significant impact on clinical practice.

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APPENDICES

APPENDIX A
DSM IV

DSM-IV Criteria for Autistic Disorder

(A) Total of six (or more) item from (1), (2) and (3) with at least two from (1) and one each from (2) and (3)

1. Qualitative impairment in social interaction, as manifested by at least two of the following.

- Marked impairment in the use of multiple nonverbal behaviors such as eye-to-eye gaze, facial expression, body postures, and gestures to regulate social interaction.
- Failure to develop peer relationships appropriate to developmental level.
- A lack of spontaneous seeking to share enjoyments, interest, or achievements with other people (e.g., by a lack of showing, bringing, or pointing out objects of interest).
- Lack of social or emotional reciprocity.

2. Qualitative impairments in communication as manifested by at least one of the following.

- Delay in, or total lack of, the development of spoken language (not accompanied by an attempt to compensate through alternative modes of communication such as gestures or mine).
- In individuals with adequate speech, marked impairment in the ability to initiate or sustain a conversation with others.
- Stereotyped and repetitive use of language or idiosyncratic language.
- Lack of varied, : spontaneous make-believe play or social imitative play appropriate to developmental level

3. Restricted repetitive and stereotyped patterns of behavior, interests, and activities, as manifested by at least one of the following:

- Encompassing preoccupation with one or more stereotyped patterns of interest that is abnormal either in intensity or focus.
- Apparently inflexible adherence to specific nonfunctional routine or ritual
- Stereotyped and repetitive motor mannerisms (e.g., hand or finger flapping or twisting or complex whole body movements.
- Persistent preoccupation with parts of objects.

(B) Delay or abnormal functioning in at least one of the following areas, with onset to age 3 years: (1) social interaction, (2) language as used in social communication or (3) symbolic or imaginative play.

(C) The disturbance is not better accounted for by Rett's Disorder or Childhood Disintegrative Disorder.

APPENDIX B
SUPPLIERS

Lists	Amount
1. Plate Centrifuge	1
2. Autopipette and Pipette tip	1
1000 µl	
200 µl	
100 µl	
1-10 µl	
0.1-2.5 µl	
3. Multichannel pipettes and pipette tip	1
0.5-10 µl	
10-100 µl	
30-300 µl	
4. Centrifuge Hettich MIKRO 200	1
5. Micro centrifuge	1
6.Vortex Mixture (Scientific, USA)	3
7. MagNa Pure compact (Roche Applied Science, Germany)	1
8. Deep freezer -80 ^{o}C (Thermo Scientific, USA)	2
9. Freezer -20 ^{o}C (Sanyo, Japan)	
10. Refrigerators 4-8 ^{o}C (Zedmed, China)	2
11. Biosafety Cabinet Type II (ESCO, USA)	2
12. Gel Doc (Bio-Rad, USA)	1
13. Nanodrop 2000 spectrophotometer (Thermo Scientific, USA)	1
14. Bioplex 200 (Bio-Rad, USA)	1

SUPPLIERS (cont.)

Lists	Amount
15. MicroAmp (0.2 mL) Reaction Tubes, Caps, Tray/Retainers or MicroAmp	1
16. Reaction Tubes/Tray/Retainer Assembly and Base	1
17. 2.0 mL screw cap tubes: Sarstedt	1
18. 1.5 mL microfuge tubes: VWR	1
19. Sterile polypropylene conical tubes; 15 mL	1

Solution for Electrophoresis and ethidium bromide staining

1. 10X TBE (stock solution)

Makes 1 L. Store at room temperature indefinitely.

- 1 g of NaOH
- 108 g of Tris base (m.w. 121.10)
- 55 g of boric acid (m.w. 61.83)
- 9.5 g of ethylene diamine tetraacetic acid (EDTA, disodium salt, m.w. 372.24)

Add all dry ingredients to 700 mL of deionized or distilled water in a 2 L flask. Sterile to dissolve preferably is using a magnetic stirrer until the dry was dissolved and added distilled water to bring the total solution to 1L.

Working solution (1X TBE) was prepared by adding 100 ml of 10X TBE with 900 ml of DW.

2. 50 mg/ml Ethidium bromide 100 ml

1. Ethidium bromide 0.5 g
2. Distilled water 100 ml

Dissolved ethidium bromide with distill water 100 ml (5 mg/ml) and sterile on magnetic sterer until the dry was dissolved. Store in the dark bottle and keep at room temperature.

APPENDIX C

HLA-B allele high resolution (4-digit) frequencies distribution

Alleles	Number of individuals	Frequency %
B*4601	**295**	**11.21**
B*5801	**228**	**8.66**
B*4001	**227**	**8.62**
B*1502	**215**	**8.17**
B*1301	**186**	**7.07**
*B*4403*	105	3.99
*B*5101*	92	3.50
*B*1801*	82	3.12
*B*3802*	71	2.70
*B*5201*	58	2.20
*B*1802*	55	2.09
*B*2704*	50	1.90
*B*3501*	50	1.90
*B*0705*	49	1.86
*B*3505*	48	1.82
*B*5401*	40	1.52
*B*5701*	40	1.52
*B*1525*	38	1.44
*B*4612*	37	1.41
*B*1302*	35	1.33
*B*1501*	35	1.33
*B*4002*	34	1.29
*B*5502*	34	1.29
*B*2706*	27	1.03
*B*3503*	27	1.03

HLA-B allele high resolution (4-digit) frequencies distribution (cont.)

Alleles	Number of individuals	Frequency %
*B*5102*	26	0.99
*B*1535*	25	0.95
*B*0702*	22	0.84
*B*5601*	20	0.76
*B*3909*	19	0.72
*B*3901*	18	0.68
*B*4402*	17	0.65
*B*0801*	15	0.57
*B*1513*	15	0.57
*B*3915*	14	0.53
*B*4006*	14	0.53
*B*3701*	13	0.49
*B*1517*	11	0.42
*B*1512*	10	0.38
*B*1521*	10	0.38
*B*4801*	10	0.38
*B*5501*	10	0.38
*B*1532*	9	0.34
*B*3801*	9	0.34
*B*4004*	9	0.34
*B*1511*	7	0.27
*B*1518*	7	0.27
*B*3924*	7	0.27
*B*5104*	7	0.27
*B*1504*	6	0.23
*B*2703*	6	0.23
*B*4010*	6	0.23
*B*4803*	6	0.23
*B*5604*	6	0.23

***HLA-B* allele high resolution (4-digit) frequencies distribution (cont.)**

Alleles	Number of individuals	Frequency %
*B*1507*	4	0.15
*B*1527*	4	0.15
*B*3823*	4	0.15
*B*5602*	4	0.15
*B*0713*	3	0.11
*B*3502*	3	0.11
*B*3903*	3	0.11
*B*3905*	3	0.11
*B*4821*	3	0.11
*B*5001*	3	0.11
*B*5106*	3	0.11
*B*5317*	3	0.11
*B*5616*	3	0.11
*B*5721*	3	0.11
*B*0803*	2	0.08
*B*1339*	2	0.08
*B*1402*	2	0.08
*B*1520*	2	0.08
*B*3668*	2	0.08
*B*4059*	2	0.08
*B*4101*	2	0.08
*B*5211*	2	0.08
*B*5404*	2	0.08
*B*5504*	2	0.08
*B*5603*	2	0.08
*B*0709*	1	0.04
*B*0714*	1	0.04
*B*0718*	1	0.04
*B*0802*	1	0.04

HLA-B allele high resolution (4-digit) frequencies distribution (cont.)

Alleles	Number of individuals	Frequency %
*B*0812*	1	0.04
*B*0833*	1	0.04
*B*1303*	1	0.04
*B*1309*	1	0.04
*B*1328*	1	0.04
*B*1413*	1	0.04
*B*1503*	1	0.04
*B*1506*	1	0.04
*B*1522*	1	0.04
*B*1531*	1	0.04
*B*1588*	1	0.04
*B*1809*	1	0.04
*B*1818*	1	0.04
*B*2707*	1	0.04
*B*2761*	1	0.04
*B*2786*	1	0.04
*B*3508*	1	0.04
*B*3511*	1	0.04
*B*3558*	1	0.04
*B*3813*	1	0.04
*B*3817*	1	0.04
*B*3820*	1	0.04
*B*3820*	1	0.04
*B*3822*	1	0.04
*B*4003*	1	0.04
*B*4009*	1	0.04
*B*4023*	1	0.04
*B*4110*	1	0.04
*B*4401*	1	0.04

HLA-B allele high resolution (4-digit) frequencies distribution (cont.)

Alleles	Number of individuals	Frequency %
*B*4443*	1	0.04
*B*4454*	1	0.04
*B*4616*	1	0.04
*B*5107*	1	0.04
*B*5143*	1	0.04
*B*5145*	1	0.04
*B*5207*	1	0.04
*B*5225*	1	0.04
*B*5414*	1	0.04
*B*5416*	1	0.04
*B*5510*	1	0.04
*B*5513*	1	0.04
*B*5523*	1	0.04
*B*5532*	1	0.04
*B*5544*	1	0.04
*B*5612*	1	0.04
*B*5834*	1	0.04
*B*5842*	1	0.04
*B*6701*	1	0.04
*B*7301*	1	0.04
Total	2,632	

APPENDIX D

Frequency of *HLA-B* Genotypes

Genotypes	Number of individuals, (Total no. = 1316)	Frequency %
B*4001/*4601	**31**	**2.32**
B*1502/*4601	**27**	**2.02**
B*4001/*5801	**27**	**2.02**
B*4601/*4601	**23**	**1.72**
B*4601/*5801	**22**	**1.64**
B*1502/*4001	**19**	**1.42**
B*1301/*4601	**18**	**1.34**
B*1301/*1502	**17**	**1.27**
B*1502/*4403	**16**	**1.19**
B*4403/*4601	**16**	**1.19**
B*1301/*5801	**15**	**1.12**
B*3802/*4601	**15**	**1.12**
B*5801/*5801	**15**	**1.12**
B*1301/*4001	**14**	**1.05**
*B*1502/*5801*	13	0.97
*B*4001/*5101*	11	0.82
*B*4601/*5101*	11	0.82
*B*0705/*4001*	10	0.75
*B*1502/*1502*	10	0.75
*B*1502/*1801*	10	0.75
*B*1801/*4601*	10	0.75
*B*2704/*4001*	10	0.75
*B*1301/*5101*	9	0.67
*B*1502/*5101*	9	0.67

Frequency of *HLA-B* Genotypes (cont.)

Genotypes	Number of individuals, (Total no. = 1316)	Frequency %
*B*3505/*4601*	9	0.67
*B*4403/*5801*	9	0.67
*B*1301/*1535*	8	0.6
*B*1802/*5801*	8	0.6
*B*4403/*5101*	8	0.6
*B*1301/*1301*	7	0.52
*B*1302/*4601*	7	0.52
*B*1502/*2704*	7	0.52
*B*1502/*3505*	7	0.52
*B*1801/*5801*	7	0.52
*B*2704/*4601*	7	0.52
*B*2704/*5801*	7	0.52
*B*3503/*4601*	7	0.52
*B*3802/*4403*	7	0.52
*B*3802/*5701*	7	0.52
*B*4001/*4001*	7	0.52
*B*4001/*4402*	7	0.52
*B*5101/*5502*	7	0.52
*B*1302/*4001*	6	0.45
*B*1501/*4601*	6	0.45
*B*1502/*1535*	6	0.45
*B*1502/*5201*	6	0.45
*B*1801/*4001*	6	0.45
*B*1801/*4402*	6	0.45
*B*3505/*4001*	6	0.45
*B*3802/*3802*	6	0.45
*B*4004/*4403*	6	0.45
*B*4601/*5201*	6	0.45

Appendix C Frequency of *HLA-B* Genotypes (cont.)

Genotypes	Number of individuals, (Total no. = 1316)	Frequency %
*B*4601/*5701*	6	0.45
*B*4612/*5801*	6	0.45
*B*5101/*5801*	6	0.45
*B*5201/*5801*	6	0.45
*B*0702/*4403*	5	0.37
*B*0705/*5801*	5	0.37
*B*1301/*1802*	5	0.37
*B*1301/*4403*	5	0.37
*B*1301/*5201*	5	0.37
*B*1302/*1802*	5	0.37
*B*1501/*5801*	5	0.37
*B*1502/*1802*	5	0.37
*B*1525/*5801*	5	0.37
*B*3501/*4403*	5	0.37
*B*3501/*4601*	5	0.37
*B*3909/*5801*	5	0.37
*B*4001/*4002*	5	0.37
*B*4001/*5102*	5	0.37
*B*4403/*5701*	5	0.37
*B*4601/*5401*	5	0.37
*B*1301/*1302*	4	0.3
*B*1301/*3802*	4	0.3
*B*1301/*5601*	4	0.3
*B*1501/*1801*	4	0.3
*B*1502/*1525*	4	0.3
*B*1502/*3802*	4	0.3
*B*1502/*4002*	4	0.3
*B*1512/*4601*	4	0.3

Frequency of *HLA-B* Genotypes (cont.)

Genotypes	Number of individuals, (Total no. = 1316)	Frequency %
*B*1801/*3503*	4	0.3
*B*1801/*3505*	4	0.3
*B*1802/*4403*	4	0.3
*B*1802/*5201*	4	0.3
*B*3802/*5601*	4	0.3
*B*3802/*5801*	4	0.3
*B*3901/*4001*	4	0.3
*B*3905/*5801*	4	0.3
*B*3909/*4601*	4	0.3
*B*4001/*5201*	4	0.3
*B*4001/*5401*	4	0.3
*B*4001/*5502*	4	0.3
*B*4002/*5801*	4	0.3
*B*4010/*4402*	4	0.3
*B*4403/*5201*	4	0.3
*B*5401/*5801*	4	0.3
*B*1301/*3915*	4	0.3
*B*0702/*5201*	3	0.22
*B*0705/*1301*	3	0.22
*B*0705/*1502*	3	0.22
*B*0705/*1801*	3	0.22
*B*0705/*1802*	3	0.22
*B*0705/*4601*	3	0.22
*B*0705/*5502*	3	0.22
*B*0705/*5701*	3	0.22
*B*0801/*4601*	3	0.22
*B*1301/*1501*	3	0.22
*B*1301/*3701*	3	0.22

Frequency of *HLA-B* Genotypes (cont.)

Genotypes	Number of individuals, (Total no. = 1316)	Frequency %
*B*1301/*4612*	3	0.22
*B*1301/*5501*	3	0.22
*B*1302/*1502*	3	0.22
*B*1502/*3501*	3	0.22
*B*1502/*4612*	3	0.22
*B*1502/*5401*	3	0.22
*B*1504/*5401*	3	0.22
*B*1525/*1801*	3	0.22
*B*1525/*4001*	3	0.22
*B*1525/*4403*	3	0.22
*B*1525/*5101*	3	0.22
*B*1801/*3802*	3	0.22
*B*1801/*3901*	3	0.22
*B*2704/*5101*	3	0.22
*B*2706/*5801*	3	0.22
*B*3501/*3802*	3	0.22
*B*3503/*4403*	3	0.22
*B*3505/*4403*	3	0.22
*B*3505/*5101*	3	0.22
*B*3701/*5801*	3	0.22
*B*3802/*4001*	3	0.22
*B*4001/*5701*	3	0.22
*B*4002/*4403*	3	0.22
*B*4002/*4601*	3	0.22
*B*4601/*5102*	3	0.22
*B*4601/*5601*	3	0.22
*B*5101/*5211*	3	0.22
*B*5317/*5801*	3	0.22

Frequency of *HLA-B* Genotypes (cont.)

Genotypes	Number of individuals, (Total no. = 1316)	Frequency %
*B*0702/*1801*	2	0.15
*B*0702/*4601*	2	0.15
*B*0705/*1501*	2	0.15
*B*0705/*1518*	2	0.15
*B*0705/*2706*	2	0.15
*B*0705/*3501*	2	0.15
*B*0801/*1801*	2	0.15
*B*0801/*5201*	2	0.15
*B*0801/*5801*	2	0.15
*B*0803/*1301*	2	0.15
*B*1301/*1525*	2	0.15
*B*1301/*2706*	2	0.15
*B*1301/*3503*	2	0.15
*B*1301/*3901*	2	0.15
*B*1301/*3924*	2	0.15
*B*1301/*4002*	2	0.15
*B*1301/*5401*	2	0.15
*B*1301/*5502*	2	0.15
*B*1301/*5701*	2	0.15
*B*1302/*2706*	2	0.15
*B*1302/*3503*	2	0.15
*B*1302/*3802*	2	0.15
*B*1302/*5401*	2	0.15
*B*1302/*5721*	2	0.15
*B*1302/*5801*	2	0.15
*B*1339/*3915*	2	0.15
*B*1501/*1501*	2	0.15
*B*1501/*1502*	2	0.15

Frequency of *HLA-B* Genotypes (cont.)

Genotypes	Number of individuals, (Total no. = 1316)	Frequency %
*B*1501/*1525*	2	0.15
*B*1501/*3701*	2	0.15
*B*1501/*4001*	2	0.15
*B*1502/*1513*	2	0.15
*B*1502/*1518*	2	0.15
*B*1502/*3503*	2	0.15
*B*1502/*5513*	2	0.15
*B*1502/*5601*	2	0.15
*B*1502/*5701*	2	0.15
*B*1511/*4601*	2	0.15
*B*1512/*1801*	2	0.15
*B*1518/*5401*	2	0.15
*B*1520/*5801*	2	0.15
*B*1521/*5801*	2	0.15
*B*1525/*1802*	2	0.15
*B*1525/*3924*	2	0.15
*B*1525/*4006*	2	0.15
*B*1527/*4601*	2	0.15
*B*1527/*5101*	2	0.15
*B*1535/*4001*	2	0.15
*B*1801/*3501*	2	0.15
*B*1801/*3704*	2	0.15
*B*1801/*3915*	2	0.15
*B*1801/*5101*	2	0.15
*B*1801/*5401*	2	0.15
*B*1801/*5701*	2	0.15
*B*1802/*2706*	2	0.15
*B*1802/*3802*	2	0.15

Frequency of *HLA-B* Genotypes (cont.)

Genotypes	Number of individuals, (Total no. = 1316)	Frequency %
*B*1802/*4402*	2	0.15
*B*1802/*4601*	2	0.15
*B*2704/*4403*	2	0.15
*B*2704/*5401*	2	0.15
*B*2706/*4601*	2	0.15
*B*2706/*4612*	2	0.15
*B*2706/*5101*	2	0.15
*B*2706/*5701*	2	0.15
*B*35018*4001*	2	0.15
*B*3501/*4454*	2	0.15
*B*3501/*4612*	2	0.15
*B*3502/*3901*	2	0.15
*B*3505/*4006*	2	0.15
*B*3802/*4002*	2	0.15
*B*3802/*5101*	2	0.15
*B*3802/*5102*	2	0.15
*B*3901/*4803*	2	0.15
*B*3901/*5201*	2	0.15
*B*3909/*5101*	2	0.15
*B*4001/*4006*	2	0.15
*B*4001/*4803*	2	0.15
*B*4001/*5104*	2	0.15
*B*4001/*5601*	2	0.15
*B*4006/*4801*	2	0.15
*B*4010/*4601*	2	0.15
*B*4402/*5801*	2	0.15
*B*4403/*4403*	2	0.15
*B4403/*5604*	2	0.15

Frequency of *HLA-B* Genotypes (cont.)

Genotypes	Number of individuals, (Total no. = 1316)	Frequency %
*B*4601/*3802*	2	0.15
*B*4601/*5416*	2	0.15
*B*4601/*5502*	2	0.15
*B*4801/*5801*	2	0.15
*B*4803/*5801*	2	0.15
*B*5101/*5201*	2	0.15
*B*5101/*5207*	2	0.15
*B*5101/*5401*	2	0.15
*B*5201/*5601*	2	0.15
*B*5502/*5801*	2	0.15
*B*5601/*5801*	2	0.15
*B*0702/*0705*	1	0.07
*B*0702/*1303*	1	0.07
*B*0702/*1513*	1	0.07
*B*0702/*3503*	1	0.07
*B*0702/*4401*	1	0.07
*B*0702/*5801*	1	0.07
*B*0705/*0801*	1	0.07
*B*0705/*1507*	1	0.07
*B*0705/*1525*	1	0.07
*B*0705/*1532*	1	0.07
*B*0705/*2704*	1	0.07
*B*0705/*3503*	1	0.07
*B*0705/*3823*	1	0.07
*B*0705/*4403*	1	0.07
*B*0705/*4801*	1	0.07
*B*0705/*4803*	1	0.07
*B*0705/*5101*	1	0.07

Frequency of *HLA-B* Genotypes (cont.)

Genotypes	Number of individuals, (Total no. = 1316)	Frequency %
*B*0705/*5102*	1	0.07
*B*0713/*1502*	1	0.07
*B*0714/*1522*	1	0.07
*B*0801/*1301*	1	0.07
*B*0801/*1413*	1	0.07
*B*0801/*1502*	1	0.07
*B*0801/*1802*	1	0.07
*B*0801*1802*	1	0.07
*B*0801/*4001*	1	0.07
*B*0801/*4002*	1	0.07
*B*0801/*5502*	1	0.07
*B*0802/*1801*	1	0.07
*B*0812/*4612*	1	0.07
*B*1301/*1513*	1	0.07
*B*1301/*1521*	1	0.07
*B*1301/*1532*	1	0.07
*B*1301/*1801*	1	0.07
*B*1301/*2703*	1	0.07
*B*1301/*2704*	1	0.07
*B*1301/*3501*	1	0.07
*B*1301/*3508*	1	0.07
*B*1301/*3909*	1	0.07
*B*1301/*4004*	1	0.07
*B*1301/*5102*	1	0.07
*B*1302/*1521*	1	0.07
*B*1302/*3901*	1	0.07
*B*1302/*4002*	1	0.07
*B*1302/*5201*	1	0.07

Frequency of *HLA-B* Genotypes (cont.)

Genotypes	Number of individuals, (Total no. = 1316)	Frequency %
*B*1302/*5502*	1	0.07
*B*1302/*5701*	1	0.07
*B*1309/*1809*	1	0.07
*B*1402/*4601*	1	0.07
*B*1402/*5102*	1	0.07
*B*1501/*2704*	1	0.07
*B*1501/*3501*	1	0.07
*B*1501/*3915*	1	0.07
*B*1501/*4002*	1	0.07
*B*1501/*5101*	1	0.07
*B*1501/*5701*	1	0.07
*B*1502/*1506*	1	0.07
*B*1502/*1507*	1	0.07
*B*1502/*1520*	1	0.07
*B*1502/*1521*	1	0.07
*B*1502/*1588*	1	0.07
*B*1502/*3558*	1	0.07
*B*1502/*4023*	1	0.07
*B*1502/*5001*	1	0.07
*B*1502/*5101*	1	0.07
*B*1502/*5104*	1	0.07
*B*1502/*5106*	1	0.07
*B*1502/*5502*	1	0.07
*B*1502/*5504*	1	0.07
*B*1502/*5604*	1	0.07
*B*1503/*4001*	1	0.07
*B*1504/*5201*	1	0.07
*B*1504/*5510*	1	0.07

Frequency of *HLA-B* Genotypes (cont.)

Genotypes	Number of individuals, (Total no. = 1316)	Frequency %
*B*1507/*5102*	1	0.07
*B*1507/*5106*	1	0.07
*B*1511/*3505*	1	0.07
*B*1511/*5101*	1	0.07
*B*1511/*5801*	1	0.07
*B*1512/*1525*	1	0.07
*B*1512/*3802*	1	0.07
*B*1512/*3909*	1	0.07
*B*1512/*4001*	1	0.07
*B*1512/*5101*	1	0.07
*B*1512/*5401*	1	0.07
*B*1513/*1521*	1	0.07
*B*1513/*4001*	1	0.07
*B*1513/*4002*	1	0.07
*B*1513/*4006*	1	0.07
*B*1513/*4403*	1	0.07
*B*1513/*4612*	1	0.07
*B*1513/*5101*	1	0.07
*B*1513/*5502*	1	0.07
*B*1513/*5801*	1	0.07
*B*1517/*3501*	1	0.07
*B*1517/*3505*	1	0.07
*B*1517/*5145*	1	0.07
*B*1517/*5701*	1	0.07
*B*1520/*3505*	1	0.07
*B*1521/*4001*	1	0.07
*B*1521/*4004*	1	0.07
*B*1521/*5502*	1	0.07

Frequency of *HLA-B* Genotypes (cont.)

Genotypes	Number of individuals, (Total no. = 1316)	Frequency %
*B*1525/*1532*	1	0.07
*B*1525/*1802*	1	0.07
*B*1525/*3505*	1	0.07
*B*1525/*3802*	1	0.07
*B*1525/*3823*	1	0.07
*B*1525/*3909*	1	0.07
*B*1525/*3915*	1	0.07
*B*1525/*4101*	1	0.07
*B*1525/*4601*	1	0.07
*B*1525/*5502*	1	0.07
*B*1531/*3511*	1	0.07
*B*1532/*2706*	1	0.07
*B*1532/*3802*	1	0.07
*B*1532/*4601*	1	0.07
*B*1532/*5102*	1	0.07
*B*1532/*5801*	1	0.07
*B*1535/*1535*	1	0.07
*B*1535/*1802*	1	0.07
*B*1535/*5701*	1	0.07
*B*1801/*1802*	1	0.07
*B1801/*3909*	1	0.07
*B*1801/*4006*	1	0.07
*B*1801/*5504*	1	0.07
*B*1802/*4001*	1	0.07
*B*1802/*4101*	1	0.07
*B*1802/*4612*	1	0.07
*B*1802/*5101*	1	0.07
*B*1818/*3505*	1	0.07

Frequency of *HLA-B* Genotypes (cont.)

Genotypes	Number of individuals, (Total no. = 1316)	Frequency %
*B*2703/*3802*	1	0.07
*B*2703/*4006*	1	0.07
*B*2703/*5801*	1	0.07
*B*2704/*2706*	1	0.07
*B*2704/*3802*	1	0.07
*B*2704/*4612*	1	0.07
*B*2704/*5106*	1	0.07
*B*2706/*3505*	1	0.07
*B*2706/*3508*	1	0.07
*B*2706/*3802*	1	0.07
*B*2706/*3915*	1	0.07
*B*2706/*4001*	1	0.07
*B*2706/*4403*	1	0.07
*B*2706/*5201*	1	0.07
*B*2706/*5502*	1	0.07
*B*2706/*5601*	1	0.07
*B*2707/*4001*	1	0.07
*B*2761/*5501*	1	0.07
*B*2786/*4001*	1	0.07
*B*3501/*3505*	1	0.07
*B*3501/*3924*	1	0.07
*B*3501/*4402*	1	0.07
*B*3501/*4803*	1	0.07
*B*3501/*5001*	1	0.07
*B*3501/*5401*	1	0.07
*B*3501/*5502*	1	0.07
*B*3502/*5401*	1	0.07
*B*3502/*5801*	1	0.07

Frequency of *HLA-B* Genotypes (cont.)

Genotypes	Number of individuals, (Total no. = 1316)	Frequency %
*B*3503/*4002*	1	0.07
*B*3503/*5102*	1	0.07
*B*3503/*5701*	1	0.07
*B*3505/*3505*	1	0.07
*B*3505/*3802*	1	0.07
*B*3505/*3909*	1	0.07
*B*3505/*3915*	1	0.07
*B*3505/*4002*	1	0.07
*B*3505/*4801*	1	0.07
*B*3505/*5502*	1	0.07
*B*3505/*5701*	1	0.07
*B*3505/*5801*	1	0.07
*B*3701/*4001*	1	0.07
*B*3701/*4601*	1	0.07
*B*3701/*5701*	1	0.07
*B*3801/*4009*	1	0.07
*B*3801/*4601*	1	0.07
*B*3802/*3901*	1	0.07
*B*3802/*3909*	1	0.07
*B*3802/*4006*	1	0.07
*B*3802/*4801*	1	0.07
*B*3802/*5107*	1	0.07
*B*3802/*5201*	1	0.07
*B*3813/*5801*	1	0.07
*B*3817/*4601*	1	0.07
*B*3820/*5801*	1	0.07
*B*3822/*5104*	1	0.07
*B*3823/*5701*	1	0.07

Frequency of *HLA-B* Genotypes (cont.)

Genotypes	Number of individuals, (Total no. = 1316)	Frequency %
*B*3901/*3909*	1	0.07
*B*3901/*4403*	1	0.07
*B*3901/*5604*	1	0.07
*B*3901/*5801*	1	0.07
*B*3909/*4801*	1	0.07
*B*3909/*5102*	1	0.07
*B*3909/*5401*	1	0.07
*B*3909/*5834*	1	0.07
*B*3915/*4601*	1	0.07
*B*3924/*4001*	1	0.07
*B*4001/*5107*	1	0.07
*B*4001/*5404*	1	0.07
*B*4001/*5501*	1	0.07
*B*4001/*5603*	1	0.07
*B*4002/*4402*	1	0.07
*B*4002/*5101*	1	0.07
*B*4002/*5143*	1	0.07
*B*4002/*5201*	1	0.07
*B*4002/*5502*	1	0.07
*B*4002/*5601*	1	0.07
*B*4003/*4612*	1	0.07
*B*4004/*5401*	1	0.07
*B*4004/*5601*	1	0.07
*B*4004/*5701*	1	0.07
*B*4004/*5721*	1	0.07
*B*4006/*4006*	1	0.07
*B*4006/*4601*	1	0.07
*B*4006/*5102*	1	0.07

Frequency of *HLA-B* Genotypes (cont.)

Genotypes	Number of individuals, (Total no. = 1316)	Frequency %
*B*4059/*5801*	1	0.07
*B*4110/*4612*	1	0.07
*B*4401/*5544*	1	0.07
*B*4403/*4612*	1	0.07
*B*4403/*4801*	1	0.07
*B*4403/*5102*	1	0.07
*B*4403/*5501*	1	0.07
*B*4403/*5601*	1	0.07
*B*4601/*3501*	1	0.07
*B*4601/*5001*	1	0.07
*B*4601/*5104*	1	0.07
*B*4601/*5601*	1	0.07
*B*4612/*4612*	1	0.07
*B*4612/*5102*	1	0.07
*B*4612/*5201*	1	0.07
*B*4612/*5616*	1	0.07
*B*4616/*5801*	1	0.07
*B*4801/*5201*	1	0.07
*B*4801/*5501*	1	0.07
*B*4801/*5701*	1	0.07
*B*4821/*5801*	1	0.07
*B*5101/*5102*	1	0.07
*B*5101/*5501*	1	0.07
*B*5101/*5501*	1	0.07
*B*5101/*5601*	1	0.07
*B*5101/*5701*	1	0.07
*B*5401/*5604*	1	0.07
*B*5501/*5601*	1	0.07

Frequency of *HLA-B* Genotypes (cont.)

Genotypes	Number of individuals, (Total no. = 1316)	Frequency %
*B*5601/*5701*	1	0.07
*B*5604/*5801*	1	0.07
*B*5701/*5801*	1	0.07
*B*5801/*7301*	1	0.07

APPENDIX E

HLA-B allele frequencies in autism children and control

Alleles	Number of individuals, n=728	Number of individuals, n=1904	OR	95%CI	*P* value
*B*0702*	3 (0.41)	19 (1.00)	0.411	0.121-1.391	0.153
*B*0705*	19 (2.61)	30 (1.58)	1.674	0.936-2.993	0.082
*B*0709*	0 (0.00)	1 (0.05)	0.871	0.035-21.406	0.933
*B*0713*	0 (0.00)	3 (0.16)	0.373	0.019-7.228	0.514
*B*0714*	0 (0.00)	1 (0.05)	0.871	0.035-21.406	0.933
*B*0718*	0 (0.00)	1 (0.05)	0.871	0.035-21.406	0.933
*B*0801*	4 (0.55)	11 (0.58)	0.951	0.302-2.996	0.931
*B*0802*	0 (0.00)	1 (0.05)	0.871	0.035-21.406	0.933
*B*0803*	1 (0.14)	1 (0.05)	2.618	0.164-41.906	0.496
*B*0812*	0 (0.00)	1 (0.05)	0.871	0.035-21.406	0.933
*B*0833*	0 (0.00)	1 (0.05)	0.871	0.035-21.406	0.933
*B*1301*	40 (5.49)	146 (7.67)	0.700	0.488-1.004	0.527
B*1302	**16 (2.20)**	**19 (1.00)**	**2.229**	**1.140-4.359**	**0.019**
*B*1303*	0 (0.00)	1 (0.05)	0.871	0.035-21.406	0.933
*B*1309*	0 (0.00)	1 (0.05)	0.871	0.035-21.406	0.933
*B*1328*	0 (0.00)	1 (0.05)	0.871	0.035-21.406	0.933
*B*1339*	0 (0.00)	2 (0.11)	0.522	0.025-10.893	0.675
*B*1402*	2 (0.27)	0 (0.00)	13.107	0.629-273.362	0.097
*B*1413*	0 (0.00)	1 (0.05)	0.871	0.035-21.406	0.933
*B*1501*	12 (1.65)	23 (1.21)	1.371	0.678-2.769	0.379
*B*1502*	55 (7.55)	160 (8.40)	0.891	0.648-1.226	0.477
*B*1503*	1 (0.14)	0 (0.00)	7.854	0.319-193.017	0.207
*B*1504*	1 (0.14)	5 (0.26)	0.522	0.061-4.479	0.554
*B*1506*	0 (0.00)	1 (0.05)	0.871	0.035-21.406	0.933
*B*1507*	1 (0.14)	3 (0.16)	0.872	0.091-8.393	0.905
*B*1511*	0 (0.00)	7 (0.37)	0.174	0.010-3.044	0.231
*B*1512*	3 (0.41)	7 (0.37)	1.121	0.289-4.348	0.868

Chi-square test, OR: odds ratio; 95%CI: confidence interval. The bold font in table refers to significantly association of alleles.

HLA-B allele frequencies in autism children and control (cont.)

Alleles	Number of individuals, n=728	Number of individuals, n=1904	OR	95%CI	*P* value
*B*1513*	2 (0.27)	13 (0.68)	0.401	0.090-1.780	0.229
*B*1517*	1 (0.14)	10 (0.53)	0.261	.034-2.039	0.200
*B*1518*	3 (0.41)	4 (0.21)	1.965	0.439-8.804	0.377
*B*1520*	2 (0.27)	0 (0.00)	13.107	0.629-273.362	0.097
*B*1521*	4 (0.55)	6 (0.32)	1.748	0.492-6.211	0.388
*B*1522*	0 (0.00)	1 (0.05)	0.871	0.035-21.406	0.933
*B*1525*	11 (1.51)	27 (1.42)	1.067	0.526-2.161	0.858
*B*1527*	2 (0.27)	2 (0.11)	2.619	0.368-18.634	0.336
*B*1531*	0 (0.00)	1 (0.05)	0.871	0.035-21.406	0.933
*B*1532*	1 (0.14)	8 (0.42)	0.326	0.041-2.601	0.291
*B*1535*	3 (0.41)	22 (1.16)	0.354	0.106-1.186	*0.092*
*B*1588*	0 (0.00)	1 (0.05)	0.871	0.035-21.406	*0.933*
*B*1801*	24 (3.30)	58 (3.05)	1.085	0.669-1.759	*0.741*
*B*1802*	7 (0.96)	48 (2.52)	0.375	0.169-0.836	*0.016*
*B*1809*	0 (0.00)	1 (0.05)	0.871	0.035-21.406	*0.933*
*B*1818*	0 (0.00)	1 (0.05)	0.871	0.035-21.406	*0.933*
*B*2703*	1 (0.14)	5 (0.26)	0.522	0.061-4.479	*0.554*
*B*2704*	13 (1.79)	37 (1.94)	0.917	0.485-1.736	*0.791*
*B*2706*	12 (1.65)	15 (0.79)	2.111	0.983-4.531	*0.055*
*B*2707*	0 (0.00)	1 (0.05)	0.871	0.035-21.406	*0.933*
*B*2761*	0 (0.00)	1 (0.05)	0.871	0.035-21.406	*0.933*
*B*2786*	0 (0.00)	1 (0.05)	0.871	0.035-21.406	*0.933*
*B*3501*	14 (1.92)	36 (1.89)	1.017	0.546-1.898	*0.957*
*B*3502*	1 (0.14)	2 (0.11)	1.308	0.118-14.449	*0.826*
*B*3503*	6 (0.82)	21 (1.10)	0.745	0.299-1.854	*0.527*
*B*3505*	9 (1.24)	39 (2.05)	0.599	0.288-1.242	*0.168*
*B*3508*	1 (0.14)	0 (0.00)	7.854	0.319-193.017	*0.207*
*B*3511*	0 (0.00)	1 (0.05)	0.871	0.035-21.406	*0.933*
*B*3558*	0 (0.00)	1 (0.05)	0.871	0.035-21.406	*0.933*
*B*3668*	2 (0.27)	0 (0.00)	13.107	0.629-273.362	*0.097*

Chi-square test, OR: odds ratio; 95%CI: confidence interval. The bold font in table refers to significantly association of alleles.

HLA-B allele frequencies in autism children and control (cont.)

Alleles	Number of individuals, n=728	Number of individuals, n=1904	OR	95%CI	*P* value
*B*3701*	4 (0.55)	9 (0.47)	1.163	0.357-3.789	*0.802*
*B*3801*	5 (0.69)	4 (0.21)	3.285	0.879-12.267	*0.077*
B*3802	**27 (3.71)**	**44 (2.31)**	**1.628**	**1.001-2.649**	***0.049***
*B*3813*	0 (0.00)	1 (0.05)	0.871	0.035-21.406	*0.933*
*B*3817*	0 (0.00)	1 (0.05)	0.871	0.035-21.406	*0.933*
*B*3820*	0 (0.00)	1 (0.05)	0.871	0.035-21.406	*0.933*
*B*3822*	0 (0.00)	1 (0.05)	0.871	0.035-21.406	*0.933*
*B*3823*	0 (0.00)	4 (0.21)	0.289	0.016-5.391	*0.406*
*B*3901*	7 (0.96)	11 (0.58)	1.412	0.561-3.554	*0.463*
*B*3903*	0 (0.00)	3 (0.16)	0.373	0.019-7.228	*0.514*
*B*3905*	1 (0.14)	2 (0.11)	1.308	0.118-14.449	*0.826*
*B*3909*	6 (0.82)	13 (0.68)	1.209	0.458-3.192	*0.702*
*B*3915*	1 (0.14)	13 (0.68)	0.200	0.026-1.532	*0.121*
*B*3924*	1 (0.14)	6 (0.32)	0.435	0.052-3.621	*0.441*
*B*4001*	72 (9.89)	155 (8.14)	1.239	0.923-1.661	*0.153*
*B*4002*	10 (1.37)	24 (1.26)	1.091	0.519-2.293	*0.818*
*B*4003*	0 (0.00)	1 (0.05)	0.871	0.035-21.406	*0.933*
*B*4004*	5 (0.69)	4 (0.21)	3.285	0.879-12.267	*0.077*
*B*4006*	3 (0.41)	11 (0.58)	0.712	0.198-2.559	*0.603*
*B*4009*	0 (0.00)	1 (0.05)	0.871	0.035-21.406	*0.933*
*B*4010*	0 (0.00)	6 (0.32)	0.200	0.011-3.563	*0.274*
*B*4023*	0 (0.00)	1 (0.05)	0.871	0.035-21.406	*0.933*
*B*4059*	0 (0.00)	2 (0.11)	0.522	0.025-10.893	*0.675*
*B*4101*	0 (0.00)	2 (0.11)	0.522	0.025-10.893	*0.675*
*B*4110*	0 (0.00)	1 (0.05)	0.871	0.035-21.406	*0.933*
*B*4401*	0 (0.00)	1 (0.05)	0.871	0.035-21.406	*0.933*
*B*4402*	6 (0.82)	11 (0.58)	1.430	0.527-3.881	*0.482*
*B*4403*	40 (5.49)	65 (3.41)	1.645	1.099-2.463	*0.016*
*B*4443*	0 (0.00)	1 (0.05)	0.871	0.035-21.406	*0.933*
*B*4454*	1 (0.14)	0 (0.00)	7.854	0.319-193.017	*0.207*
*B*4601*	86 (11.81)	209 (10.98)	1.086	0.832-1.419	*0.543*

* Chi-square test, OR: odds ratio; 95%CI: confidence interval. The bold font in table refers to significantly association of alleles.

HLA-B allele frequencies in autism children and control (cont.)

Alleles	Number of individuals, n=728	Number of individuals, n=1904	OR	95%CI	*P* value
*B*4612*	2 (0.27)	35 (1.84)	0.147	0.035-0.613	*0.008*
*B*4616*	1 (0.14)	0 (0.00)	7.854	0.319-193.017	*0.207*
*B*4801*	3 (0.41)	7 (0.37)	1.121	0.289-4.348	*0.868*
*B*4803*	1 (0.14)	5 (0.26)	0.522	0.061-4.479	*0.554*
*B*4821*	0 (0.00)	3 (0.16)	0.373	0.019-7.228	*0.514*
*B*5001*	1 (0.14)	2 (0.11)	1.308	0.118-14.449	*0.826*
*B*5101*	24 (3.30)	68 (3.57)	0.921	0.573-1.478	*0.732*
*B*5102*	6 (0.82)	20 (1.05)	0.783	0.313-1.957	*0.600*
*B*5104*	1 (0.14)	6 (0.32)	0.435	0.052-3.621	*0.441*
*B*5106*	0 (0.00)	3 (0.16)	0.373	0.019-7.228	*0.514*
*B*5107*	1 (0.14)	0 (0.00)	7.854	0.319-193.017	*0.207*
*B*5143*	0 (0.00)	1 (0.05)	0.871	0.035-21.406	*0.933*
*B*5145*	0 (0.00)	1 (0.05)	0.871	0.035-21.406	*0.933*
*B*5201*	10 (1.37)	48 (2.52)	0.538	0.271-1.070	*0.077*
*B*5207*	1 (0.14)	0 (0.00)	7.854	0.319-193.017	*0.207*
*B*5211*	1 (0.14)	1 (0.05)	2.618	0.164-41.906	*0.496*
*B*5225*	0 (0.00)	1 (0.05)	0.871	0.035-21.406	*0.933*
*B*5317*	0 (0.00)	3 (0.16)	0.373	0.019-7.228	*0.514*
*B*5401*	14 (1.92)	26 (1.37)	1.416	0.735-2.728	*0.298*
*B*5404*	0 (0.00)	2 (0.11)	0.522	0.025-10.893	*0.675*
*B*5414*	0 (0.00)	1 (0.05)	0.871	0.035-21.406	*0.933*
*B*5416*	1 (0.14)	0 (0.00)	7.854	0.319-193.017	*0.207*
*B*5501*	1 (0.14)	9 (0.47)	0.289	0.037-2.290	*0.240*
*B*5502*	8 (1.10)	26 (1.37)	0.803	0.362-1.781	0.588
*B*5504*	0 (0.00)	2 (0.11)	0.522	0.025-10.893	0.675
*B*5510*	0 (0.00)	1 (0.05)	0.871	0.035-21.406	0.933
*B*5513*	1 (0.14)	0 (0.00)	7.854	0.319-193.017	0.207
*B*5523*	0 (0.00)	1 (0.05)	0.871	0.035-21.406	0.933
*B*5532*	0 (0.00)	1 (0.05)	0.871	0.035-21.406	0.933
*B*5544*	0 (0.00)	1 (0.05)	0.871	0.035-21.406	0.933
B*5601	**13 (1.79)**	**7 (0.37)**	**4.927**	**1.958-12.399**	**1.78x10-4**

* Chi-square test, OR: odds ratio; 95%CI: confidence interval. The bold font in table refers to significantly association of alleles.

HLA-B allele frequencies in autism children and control (cont.)

Alleles	Number of individuals, n=728	Number of individuals, n=1904	OR	95%CI	*P* value
*B*5602*	0 (0.00)	4 (0.21)	0.289	0.016-5.391	0.406
*B*5603*	0 (0.00)	2 (0.11)	0.522	0.025-10.893	0.675
*B*5604*	0 (0.00)	6 (0.32)	0.200	0.011-3.563	0.274
*B*5612*	0 (0.00)	1 (0.05)	0.871	0.035-21.406	0.933
*B*5616*	2 (0.27)	1 (0.05)	5.242	0.474-57.905	0.176
*B*5701*	16 (2.20)	24 (1.26)	1.760	0.929-3.333	0.083
*B*5721*	1 (0.14)	2 (0.11)	1.308	0.118-14.449	0.826
*B*5801*	65 (8.93)	163 (8.56)	1.047	0.775-1.415	0.764
*B*5834*	1 (0.14)	0 (0.00)	7.854	0.319-193.017	0.207
*B*5842*	0 (0.00)	1 (0.05)	0.871	0.035-21.406	0.933
*B*6701*	0 (0.00)	1 (0.05)	0.871	0.035-21.406	0.933
*B*7301*	1 (0.14)	0 (0.00)	7.854	0.319-193.017	0.207

* Chi-square test, OR: odds ratio; 95%CI: confidence interval. The bold font in table refers to significantly association of alleles.

APPENDIX F

HLA-B Genotype frequencies according to two groups

Genotypes	ASDs N=364 (%)	Non-ASDs N=952 (%)	OR	95% CI	*P*-value
*B*0702/*0705*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*0702/*1303*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*0702/*1513*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*0702/*1801*	0 (0.00)	2 (0.20)	0.542	0.259-11.310	0.693
*B*0702/*3503*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*0702/*4401*	1 (0.28)	0 (0.00)	8.164	0.332-200.875	0.199
*B*0702/*4403*	0 (0.00)	5 (0.51)	0.246	0.014-4.451	0.342
*B*0702/*4601*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*0702/*5201*	1 (0.28)	2 (0.20)	1.359	0.123-15.035	0.802
*B*0702/*5801*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*0705/*0801*	1 (0.28)	0 (0.00)	8.164	0.332-200.875	0.199
*B*0705/*1301*	2 (0.55)	1 (0.10)	5.457	0.493-60.367	0.166
*B*0705/*1501*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*0705/*1502*	0 (0.00)	3 (0.30)	0.387	0.020-7.502	0.530
*B*0705/*1507*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*0705/*1518*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*0705/*1525*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*0705/*1532*	1 (0.28)	0 (0.00)	8.164	0.332-200.875	0.199
*B*0705/*1801*	0 (0.00)	3 (0.30)	0.387	0.020-7.502	0.530
*B*0705/*1802*	0 (0.00)	3 (0.30)	0.387	0.020-7.502	0.530
*B*0705/*2704*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*0705/*2706*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*0705/*3501*	0 (0.00)	2 (0.20)	0.542	0.259-11.310	0.693
*B*0705/*3503*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*0705/*3823*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*0705/*4001*	4 (1.1)	6 (0.61)	1.820	0.511-6.486	0.356
*B*0705/*4403*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951

* Chi-square test, OR: odds ratio; 95%CI: confidence interval. The bold font in table refers to significantly association of alleles.

HLA-B Genotype frequencies according to two groups. (cont.)

Genotypes	ASDs N=364 (%)	Non-ASDs N=952 (%)	OR	95% CI	*P*-value
*B*0705/*4601*	1 (0.28)	2 (0.20)	1.359	0.123-15.035	0.802
*B*0705/*4801*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*0705/*4803*	1 (0.28)	0 (0.00)	8.164	0.332-200.875	0.199
*B*0705/*5101*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*0705/*5102*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*0705/*5502*	2 (0.55)	1 (0.10)	5.457	0.493-60.367	0.166
*B*0705/*5701*	2 (0.55)	1 (0.10)	5.457	0.493-60.367	0.166
*B*0705/*5801*	2 (0.55)	3 (0.30)	2.511	0.417-15.105	0.315
*B*0713/*1502*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*0714/*1522*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*0801/*1301*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*0801/*1413*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*0801/*1502*	1 (0.28)	0 (0.00)	8.164	0.332-200.875	0.199
*B*0801/*1801*	0 (0.00)	2 (0.20)	0.542	0.259-11.310	0.693
*B*0801/*1802*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*0801/*4001*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*0801/*4002*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*0801/*4601*	1 (0.28)	2 (0.20)	1.359	0.123-15.035	0.802
*B*0801/*5201*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*0801/*5502*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*0801/*5801*	0 (0.00)	2 (0.20)	0.542	0.259-11.310	0.693
*B*0802/*1801*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*0803/*1301*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*0812/*4612*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1301/*1301*	1 (0.28)	6 (0.60)	0.451	0.541-3.761	0.462
*B*1301/*1302*	2 (0.55)	2 (0.20)	2.726	0.383-19.423	0.317
*B*1301/*1501*	0 (0.00)	3 (0.30)	0.387	0.020-7.502	0.530
*B*1301/*1502*	4 (1.10)	13 (1.32)	0.829	0.269-2.560	0.745
*B*1301/*1513*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1301/*1521*	1 (0.28)	0 (0.00)	8.164	0.332-200.875	0.199
*B*1301/*1525*	0 (0.00)	2 (0.20)	0.542	0.259-11.310	0.693
*B*1301/*1532*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951

* Chi-square test, OR: odds ratio; 95%CI: confidence interval. The bold font in table refers to significantly association of alleles.

HLA-B Genotype frequencies according to two groups. (cont.)

Genotypes	ASDs N=364 (%)	Non-ASDs N=952 (%)	OR	95% CI	*P*-value
*B*1301/*1535*	2 (0.55)	6 (0.61)	0.905	0.182-4.504	0.903
*B*1301/*1801*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1301/*1802*	0 (0.00)	5 (0.51)	0.246	0.014-4.451	0.342
*B*1301/*2703*	1 (0.28)	0 (0.00)	8.164	0.332-200.875	0.199
*B*1301/*2704*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1301/*2706*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*1301/*3501*	1 (0.28)	0 (0.00)	8.164	0.332-200.875	0.199
*B*1301/*3503*	0 (0.00)	2 (0.20)	0.542	0.259-11.310	0.693
*B*1301/*3505*	0 (0.00)	2 (0.20)	0.542	0.259-11.310	0.693
*B*1301/*3508*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1301/*3701*	2 (0.55)	1 (0.10)	5.457	0.493-60.367	0.166
*B*1301/*3802*	2 (0.55)	2 (0.20)	2.726	0.383-19.423	0.317
*B*1301/*3901*	0 (0.00)	2 (0.20)	0.542	0.259-11.310	0.693
*B*1301/*3909*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1301/*3915*	0 (0.00)	3 (0.30)	0.386	0.019-7.502	0.529
*B*1301/*3924*	0 (0.00)	2 (0.20)	0.542	0.259-11.310	0.693
*B*1301/*4001*	3 (0.83)	10 (1.01)	0.813	0.223-2.972	0.755
*B*1301/*4002*	0 (0.00)	2 (0.20)	0.542	0.259-11.310	0.693
*B*1301/*4004*	1 (0.28)	0 (0.00)	8.164	0.332-200.875	0.199
*B*1301/*4403*	2 (0.55)	3 (0.30)	1.815	0.302-10.909	0.515
*B*1301/*4601*	7 (1.93)	11 (1.12)	1.743	0.670-4.531	0.254
*B*1301/*4612*	0 (0.00)	3 (0.30)	0.386	0.019-7.502	0.529
*B*1301/*5101*	1 (0.28)	8 (0.81)	0.338	0.042-2.710	0.310
*B*1301/*5102*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1301/*5201*	0 (0.00)	5 (0.50)	0.246	0.014-4.451	0.903
*B*1301/*5401*	0 (0.00)	2 (0.20)	0.542	0.259-11.310	0.693
*B*1301/*5501*	1 (0.28)	2 (0.20)	1.359	0.123-15.035	0.802
*B*1301/*5502*	0 (0.00)	2 (0.20)	0.542	0.259-11.310	0.693
*B*1301/*5601*	2 (0.55)	2 (0.20)	2.726	0.383-19.423	0.317
*B*1301/*5701*	0 (0.00)	2 (0.20)	0.542	0.259-11.310	0.693
*B*1301/*5801*	2 (0.55)	13 (1.32)	0.415	0.093-1.847	0.248
*B*1302/*1502*	1 (0.28)	2 (0.20)	1.359	0.123-15.035	0.802

* Chi-square test, OR: odds ratio; 95%CI: confidence interval. The bold font in table refers to significantly association of alleles.

HLA-B Genotype frequencies according to two groups. (cont.)

Genotypes	ASDs N=364 (%)	Non-ASDs N=952 (%)	OR	95% CI	*P*-value
*B*1302/*1521*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1302/*1802*	1 (0.28)	4 (0.41)	0.678	0.756-6.088	0.729
*B*1302/*2706*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*1302/*3503*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*1302/*3802*	0 (0.00)	2 (0.20)	0.542	0.259-11.310	0.693
*B*1302/*3901*	1 (0.28)	0 (0.00)	8.164	0.332-200.875	0.199
*B*1302/*4001*	3 (0.83)	3 (0.30)	2.731	0.549-13.591	0.220
*B*1302/*4002*	1 (0.28)	0 (0.00)	8.164	0.332-200.875	0.199
*B*1302/*4601*	2 (0.55)	5 (0.51)	0.544	0.063-4.688	0.578
*B*1302/*5201*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1302/*5401*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*1302/*5502*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1302/*5701*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1302/*5721*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*1302/*5801*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*1309/*1809*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1339/*3915*	0 (0.00)	2 (0.20)	0.542	0.259-11.310	0.693
*B*1402/*4601*	1 (0.28)	0 (0.00)	8.164	0.332-200.875	0.199
*B*1402/*5102*	1 (0.28)	0 (0.00)	8.164	0.332-200.875	0.199
*B*1501/*1501*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*1501/*1502*	0 (0.00)	2 (0.20)	0.542	0.259-11.310	0.693
*B*1501/*1525*	0 (0.00)	2 (0.20)	0.542	0.259-11.310	0.693
*B*1501/*1801*	2 (0.55)	2 (0.20)	2.726	0.383-19.423	0.317
*B*1501/*2704*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1501/*3501*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1501/*3701*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*1501/*3915*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1501/*4001*	2 (0.55)	0 (0.00)	13.644	0.654-284.903	0.092
*B*1501/*4002*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1501/*4601*	1 (0.28)	5 (0.51)	0.542	0.063-4.655	0.577
*B*1501/*5101*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1501/*5701*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951

* Chi-square test, OR: odds ratio; 95%CI: confidence interval. The bold font in table refers to significantly association of alleles.

HLA-B Genotype frequencies according to two groups. (cont.)

Genotypes	ASDs N=364 (%)	Non-ASDs N=952 (%)	OR	95% CI	*P*-value
*B*1501/*5801*	3 (0.83)	2 (0.20)	4.100	0.682-24.638	0.123
*B*1502/*1502*	2 (0.55)	8 (0.81)	0.677	0.143-3.204	0.623
*B*1502/*1506*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1502/*1507*	1 (0.28)	0 (0.00)	8.164	0.332-200.875	0.199
*B*1502/*1513*	0 (0.00)	2 (0.20)	0.542	0.259-11.310	0.693
*B*1502/*1517*	1 (0.28)	0 (0.00)	8.164	0.332-200.875	0.199
*B*1502/*1518*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*1502/*1520*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1502/*1521*	1 (0.28)	0 (0.00)	8.164	0.332-200.875	0.199
*B*1502/*1525*	1 (0.28)	3 (0.30)	0.905	0.094-8.730	0.931
*B*1502/*1535*	0 (0.00)	6 (0.61)	0.208	0.012-3.693	0.284
*B*1502/*1588*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1502/*1801*	3 (0.83)	7 (0.71)	1.166	0.299-4.532	0.825
*B*1502/*1802*	1 (0.28)	4 (0.41)	0.678	0.076-6.088	0.729
*B*1502/*2704*	1 (0.28)	6 (0.61)	0.451	0.054-3.761	0.462
*B*1502/*3501*	0 (0.00)	3 (0.30)	0.387	0.020-7.502	0.530
*B*1502/*3503*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*1502/*3505*	2 (0.55)	5 (0.51)	1.087	0.210-5.628	0.921
*B*1502/*3558*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1502/*3802*	1 (0.28)	3 (0.30)	0.905	0.094-8.730	0.931
*B*1502/*4001*	4 (1.10)	15 (1.52)	0.721	0.238-2.188	0.564
*B*1502/*4002*	1 (0.28)	3 (0.30)	0.905	0.094-8.730	0.931
*B*1502/*4023*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1502/*4403*	5 (1.38)	11 (1.12)	1.238	0.428-3.588	0.694
*B*1502/*4601*	8 (2.20)	19 (1.93)	1.147	0.498-2.643	0.748
*B*1502/*4612*	0 (0.00)	3 (0.30)	0.387	0.020-7.502	0.530
*B*1502/*5001*	1 (0.28)	0 (0.00)	8.164	0.332-200.875	0.199
*B*1502/*5101*	3 (0.83)	6 (0.61)	1.361	0.399-5.471	0.664
*B*1502/*5102*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1502/*5104*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1502/*5106*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1502/*5201*	0 (0.00)	6 (0.61)	0.208	0.012-3.693	0.284

* Chi-square test, OR: odds ratio; 95%CI: confidence interval. The bold font in table refers to significantly association of alleles.

HLA-B Genotype frequencies according to two groups. (cont.)

Genotypes	ASDs N=364 (%)	Non-ASDs N=952 (%)	OR	95% CI	*P*-value
*B*1502/*5401*	2 (0.55)	1 (0.10)	5.457	0.493-60.367	0.166
*B*1502/*5502*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1502/*5504*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1502/*5513*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*1502/*5601*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*1502/*5604*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1502/*5701*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*1502/*5801*	4 (1.10)	9 (0.91)	1.209	0.370-3.952	0.753
*B*1503/*4001*	1 (0.28)	0 (0.00)	8.164	0.332-200.875	0.199
*B*1504/*5201*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1504/*5510*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1507/*5102*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1507/*5106*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1511/*3505*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1511/*4601*	0 (0.00)	2 (0.20)	0.542	0.259-11.310	0.693
*B*1511/*5101*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1511/*5801*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1512/*1525*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1512/*1801*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*1512/*3802*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1512/*3909*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1512/*4001*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1512/*4601*	2 (0.55)	2 (0.20)	2.726	0.383-19.423	0.317
*B*1512/*5101*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1512/*5401*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1513/*1521*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1513/*4001*	1 (0.28)	0 (0.00)	8.164	0.332-200.875	0.199
*B*1513/*4002*	1 (0.28)	0 (0.00)	8.164	0.332-200.875	0.199
*B*1513/*4006*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1513/*4403*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1513/*4612*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1513/*5101*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951

* Chi-square test, OR: odds ratio; 95%CI: confidence interval. The bold font in table refers to significantly association of alleles.

HLA-B Genotype frequencies according to two groups. (cont.)

Genotypes	ASDs N=364 (%)	Non-ASDs N=952 (%)	OR	95% CI	*P*-value
*B*1513/*5502*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1513/*5801*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1517/*3501*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1517/*3505*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1517/*5145*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1517/*5701*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1518/*5401*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*1520/*3505*	1 (0.28)	0 (0.00)	8.164	0.332-200.875	0.199
*B*1520/*5801*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*1521/*4001*	1 (0.28)	0 (0.00)	8.164	0.332-200.875	0.199
*B*1521/*4004*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1521/*5502*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1801/*3915*	0 (0.00)	2 (0.20)	0.542	0.259-11.310	0.693
*B*1801/*4001*	1 (0.28)	5 (0.51)	0.542	0.063-4.655	0.577
*B*1801/*4006*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1801/*4402*	5 (1.38)	1 (0.10)	13.757	1.602-118.158	0.017
*B*1801/*4601*	0 (0.00)	10 (1.01)	0.128	0.008-2.189	0.156
*B*1801/*5101*	0 (0.00)	2 (0.20)	0.542	0.259-11.310	0.693
*B*1801/*5401*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*1801/*5504*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1801/*5701*	0 (0.00)	2 (0.20)	0.542	0.259-11.310	0.693
*B*1801/*5801*	2 (0.55)	5 (0.51)	1.087	0.210-5.628	0.921
*B*1802/*2706*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*1802/*3802*	0 (0.00)	2 (0.20)	0.542	0.259-11.310	0.693
*B*1802/*4001*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1802/*4101*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1802/*4402*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*1802/*4403*	1 (0.28)	3 (0.30)	0.905	0.094-8.730	0.931
*B*1802/*4601*	0 (0.00)	2 (0.20)	0.542	0.259-11.310	0.693
*B*1802/*4612*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1802/*5101*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1802/*5201*	1 (0.28)	3 (0.30)	0.905	0.094-8.730	0.931

* Chi-square test, OR: odds ratio; 95%CI: confidence interval. The bold font in table refers to significantly association of alleles.

HLA-B Genotype frequencies according to two groups. (cont.)

Genotypes	ASDs N=364 (%)	Non-ASDs N=952 (%)	OR	95% CI	*P*-value
*B*1802/*5801*	1 (0.28)	7 (0.71)	0.386	0.047-3.151	0.374
*B*1818/*3505*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*2703/*3802*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*2703/4006*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*2703/*5801*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*2704/*2706*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*2704/*3802*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*2704/*4001*	3 (0.83)	7 (0.71)	1.166	0.230-4.532	0.825
*B*2704/*4403*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*2704/*4601*	1 (0.28)	6 (0.61)	0.451	0.541-3.761	0.462
*B*2704/*4612*	1 (0.28)	0 (0.00)	8.164	0.332-200.875	0.199
*B*2704/*5101*	0 (0.00)	3 (0.31)	0.387	0.020-7.502	0.530
*B*2704/*5106*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*2704/*5401*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
B*2704/*5801	**5 (1.38)**	**2 (0.20)**	**6.872**	**1.327-35.577**	**0.022**
*B*2706/*3505*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*2706/*3508*	1 (0.28)	0 (0.00)	8.164	0.332-200.875	0.199
*B*2706/*3802*	1 (0.28)	0 (0.00)	8.164	0.332-200.875	0.199
*B*2706/*3915*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*2706/*4001*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*2706/*4403*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*2706/*4601*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*2706/*4612*	0 (0.00)	2 (0.20)	0.542	0.259-11.310	0.693
*B*2706/*5101*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*2706/*5201*	1 (0.28)	0 (0.00)	8.164	0.332-200.875	0.199
*B*2706/*5502*	1 (0.28)	0 (0.00)	8.164	0.332-200.875	0.199
*B*2706/*5601*	1 (0.28)	0 (0.00)	8.164	0.332-200.875	0.199
*B*2706/*5701*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*2706/*5801*	1 (0.28)	2 (0.20)	1.359	0.123-15.035	0.802
*B*2707/*4001*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*2761/*5501*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*2786/*4001*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951

* Chi-square test, OR: odds ratio; 95%CI: confidence interval. The bold font in table refers to significantly association of alleles.

HLA-B Genotype frequencies according to two groups. (cont.)

Genotypes	ASDs N=364 (%)	Non-ASDs N=952 (%)	OR	95% CI	*P*-value
*B*3501/*3505*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*3501/*3802*	0 (0.00)	3 (0.30)	0.387	0.020-7.502	0.530
*B*3501/3924*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*3501/*4001*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*3501/*4002*	1 (0.28)	3 (0.30)	0.905	0.094-8.730	0.931
*B*3501/*4402*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
B*3501/*4403	**5 (1.38)**	**0 (0.00)**	**30.269**	**1.669-548.816**	**0.021**
*B*3501/*4454*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*3501/*4601*	3 (0.83)	2 (0.20)	4.100	0.682-24.638	0.123
*B*3501/*4612*	0 (0.00)	2 (0.20)	0.542	0.259-11.310	0.693
*B*3501/*4803*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*3501/*5001*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*3501/*5401*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*3501/*5502*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*3501/*5801*	1 (0.28)	3 (0.30)	0.905	0.094-8.730	0.931
*B*3502/*3901*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*3502/*5401*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*3502/*5801*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1521/*5801*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*1525/*1532*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1525/*1801*	2 (0.55)	1 (0.10)	5.457	0.493-60.367	0.166
*B*1525/*1802*	0 (0.00)	3 (0.30)	0.387	0.020-7.502	0.530
*B*1525/*3505*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1525/*3802*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1525/*3823*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1525/*3909*	1 (0.28)	0 (0.00)	8.164	0.332-200.875	0.199
*B*1525/*3915*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1525/*3924*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*1525/*4001*	0 (0.00)	3 (0.30)	0.387	0.020-7.502	0.530
*B*1525/*4006*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*1525/*4101*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1525/*4403*	2 (0.55)	1 (0.10)	5.457	0.493-60.367	0.166

* Chi-square test, OR: odds ratio; 95%CI: confidence interval. The bold font in table refers to significantly association of alleles.

HLA-B Genotype frequencies according to two groups. (cont.)

Genotypes	ASDs N=364 (%)	Non-ASDs N=952 (%)	OR	95% CI	*P*-value
*B*1525/*4601*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1525/*5101*	0 (0.00)	3 (0.30)	0.387	0.020-7.502	0.530
*B*1525/*5502*	1 (0.28)	0 (0.00)	8.164	0.332-200.875	0.199
*B*1525/*5801*	2 (0.55)	3 (0.30)	2.511	0.417-15.105	0.315
*B*1527/*4601*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*1527/*5101*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*1531/*3511*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1532/*2706*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1532/*3802*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1532/*4601*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1532/*5102*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1532/*5801*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1535/*1802*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1535/*4001*	0 (0.00)	2 (0.20)	0.542	0.259-11.310	0.693
*B*1535/*5701*	1 (0.28)	0 (0.00)	8.164	0.332-200.875	0.199
*B*1801/*1802*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*1801/*3501*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*1801/*3503*	2 (0.55)	2 (0.20)	2.726	0.383-19.423	0.317
*B*1801/*3505*	1 (0.28)	3 (0.30)	0.905	0.094-8.730	0.931
*B*1801/*3701*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*1801/*3802*	1 (0.28)	2 (0.20)	1.359	0.123-15.035	0.802
*B*1801/*3901*	1 (0.28)	2 (0.20)	1.359	0.123-15.035	0.802
*B*1801/*3909*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*3802/*5201*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*3802/*5601*	2 (0.55)	2 (0.20)	2.726	0.383-19.423	0.317
*B*3802/*5701*	4 (1.10)	3 (0.30)	3.651	0.813-16.392	0.091
*B*3802/*5801*	2 (0.55)	1 (0.10)	5.457	0.493-60.367	0.166
*B*3813/*5801*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*3817/*4601*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*3820/*5801*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*3822/*5104*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*3823/*5701*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951

* Chi-square test, OR: odds ratio; 95%CI: confidence interval. The bold font in table refers to significantly association of alleles.

HLA-B Genotype frequencies according to two groups. (cont.)

Genotypes	ASDs N=364 (%)	Non-ASDs N=952 (%)	OR	95% CI	*P*-value
*B*3901/*3909*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*3901/*4001*	2 (0.55)	1 (0.10)	5.457	0.493-60.367	0.166
*B*3901/*4403*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*3901/*4803*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*3901/*5201*	0 (0.00)	2 (0.20)	0.542	0.259-11.310	0.693
*B*3901/*5604*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*3901/*5801*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
B*3905/*5801	**4 (1.10)**	**0 (0.00)**	**24.697**	**1.326-459.879**	**0.032**
*B*3909/*4601*	1 (0.28)	3 (0.30)	0.905	0.094-8.730	0.931
*B*3909/*4801*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*3909/*5101*	0 (0.00)	2 (0.20)	0.542	0.259-11.310	0.693
*B*3909/*5102*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*3909/*5401*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*3909/*5801*	1 (0.28)	4 (0.41)	0.678	0.756-6.088	0.729
*B*3909/*5834*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*3915/*1301*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*3915/*4601*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*3924/*4001*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*4001/*1301*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*4001/*4001*	1 (0.28)	6 (0.61)	0.451	0.541-3.761	0.462
*B*4001/*4002*	2 (0.55)	3 (0.30)	2.511	0.417-15.105	0.315
*B*4001/*4006*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*4001/*4402*	3 (0.83)	4 (0.41)	2.046	0.456-9.186	0.350
*B*4001/*4403*	0 (0.00)	3 (0.30)	0.387	0.020-7.502	0.530
*B*4001/*4601*	10 (2.75)	21 (2.13)	1.302	0.607-2.792	0.498
*B*4001/*4803*	0 (0.00)	2 (0.20)	0.542	0.259-11.310	0.693
*B*4001/*5101*	4 (1.10)	7 (0.71)	1.558	0.454-5.355	0.481
*B*4001/*5102*	3 (0.83)	2 (0.20)	4.100	0.682-24.635	0.123
*B*4001/*5104*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*4001/*5107*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*4001/*5201*	1 (0.28)	3 (0.30)	0.905	0.094-8.730	0.931
*B*4001/*5401*	1 (0.28)	3 (0.30)	0.905	0.094-8.730	0.931

* Chi-square test, OR: odds ratio; 95%CI: confidence interval. The bold font in table refers to significantly association of alleles.

HLA-B Genotype frequencies according to two groups. (cont.)

Genotypes	ASDs N=364 (%)	Non-ASDs N=952 (%)	OR	95% CI	*P*-value
*B*4001/*5404*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*4001/*5501*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*4001/*5502*	2 (0.55)	2 (0.20)	2.726	0.383-19.423	0.317
*B*4001/*5601*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*4001/*5603*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*4001/*5701*	1 (0.28)	2 (0.20)	1.359	0.123-15.035	0.802
*B*4001/*5801*	7 (1.93)	20 (2.03)	0.950	0.398-2.265	0.707
*B*4002/*4402*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*4002/*4403*	0 (0.00)	3 (0.30)	0.387	0.020-7.502	0.530
*B*4002/*4601*	1 (0.28)	2 (0.20)	1.359	0.123-15.035	0.802
*B*4002/*5101*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*4002/*5143*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*4002/*5201*	1 (0.28)	0 (0.00)	8.164	0.332-200.875	0.199
*B*4402/*5502*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*4002/*5601*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*4002/*5801*	1 (0.28)	3 (0.30)	0.905	0.094-8.730	0.931
*B*4003/*4612*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*4004/*4403*	3 (0.83)	3 (0.30)	2.731	0.549-13.591	0.220
*B*4004/*5401*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*4004/*5601*	1 (0.28)	0 (0.00)	8.164	0.332-200.875	0.199
*B*4004/*5701*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*4004/*5721*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*4006/*4006*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*4006/*4601*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*4006/*4801*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*4006/*5102*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*4010/*4402*	0 (0.00)	4 (0.40)	0.300	0.016-5.592	0.420
*B*4010/*4601*	0 (0.00)	2 (0.20)	0.542	0.259-11.310	0.693
*B*4059/*5801*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*4110/*4612*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*4401/*5544*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*4402/*5602*	2 (0.55)	1 (0.10)	5.457	0.493-60.367	0.166

* Chi-square test, OR: odds ratio; 95%CI: confidence interval. The bold font in table refers to significantly association of alleles.

HLA-B Genotype frequencies according to two groups. (cont.)

Genotypes	ASDs N=364 (%)	Non-ASDs N=952 (%)	OR	95% CI	*P*-value
*B*4402/*5801*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*4403/*4403*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*4403/*4601*	7 (1.93)	9 (0.91)	2.135	0.789-5.774	0.135
*B*4403/*4612*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*4403/*4801*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*4403/*5101*	3 (0.83)	5 (0.51)	1.635	0.389-6.877	0.502
*B*4403/*5102*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*4403/*5201*	1 (0.28)	3 (0.30)	0.905	0.094-8.730	0.931
*B*4403/*5501*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*4403/*5601*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*4403/*5604*	0 (0.00)	2 (0.20)	0.542	0.259-11.310	0.693
*B*4403/*5701*	1 (0.28)	4 (0.41)	0.678	0.076-6.088	0.729
*B*4403/*5801*	4 (1.10)	5 (0.51)	2.186	0.584-8.186	0.246
*B*4601/*3501*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*4601/*3802*	0 (0.00)	2 (0.20)	0.542	0.259-11.310	0.693
*B*4601/*4601*	3 (0.83)	20 (2.03)	0.403	0.119-1.363	0.144
*B*4601/*5001*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*4601/*5101*	3 (0.83)	8 (0.81)	1.019	0.269-3.861	0.978
*B*4601/*5102*	1 (0.28)	2 (0.20)	1.359	0.123-15.035	0.802
*B*4601/*5104*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*4601/*5201*	2 (0.55)	4 (0.41)	1.360	0.248-7.458	0.723
*B*4601/*5401*	3 (0.83)	2 (0.20)	4.100	0.682-24.638	0.123
*B*4601/*5416*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*4601/*5502*	0 (0.00)	2 (0.20)	0.542	0.259-11.310	0.693
*B*4601/*5601*	0 (0.00)	3 (0.30)	0.387	0.020-7.502	0.530
*B*4601/*5602*	0 (0.00)	2 (0.20)	0.542	0.259-11.310	0.693
*B*4601/*5701*	3 (0.83)	3 (0.30)	2.731	0.549-13.591	0.220
*B*4601/*5801*	3 (0.83)	19 (1.93)	0.424	0.125-1.442	0.169
*B*4612/*4612*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*4612/*5102*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*4612/*5201*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*4612/*5616*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951

* Chi-square test, OR: odds ratio; 95%CI: confidence interval. The bold font in table refers to significantly association of alleles.

HLA-B Genotype frequencies according to two groups. (cont.)

Genotypes	ASDs N=364 (%)	Non-ASDs N=952 (%)	OR	95% CI	*P*-value
*B*4612/*5801*	1 (0.28)	5 (0.51)	0.542	0.063-4.655	0.577
*B*4616/*5801*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*4801/*5201*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*4801/*5501*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*4801/*5701*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*4801/*5801*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*4803/*5801*	0 (0.00)	2 (0.20)	0.542	0.259-11.310	0.693
*B*4821/*5801*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*5101/*5102*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*5101/*5201*	0 (0.00)	2 (0.20)	0.542	0.259-11.310	0.693
*B*5101/*5207*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*5101/*5211*	1 (0.28)	2 (0.20)	1.359	0.123-15.035	0.802
*B*5101/*5401*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*5101/*5501*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*5101/*5502*	2 (0.55)	5 (0.51)	1.087	0.210-5.623	0.921
*B*5101/*5601*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*5101/*5701*	1 (0.28)	0 (0.00)	8.164	0.332-200.875	0.199
*B*5101/*5801*	1 (0.28)	5 (0.51)	0.542	0.063-4.655	0.577
*B*5201/*5601*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*5201/*5801*	0 (0.00)	6 (0.61)	0.208	0.012-3.693	1.071
*B*5317/*5801*	0 (0.00)	3 (0.30)	0.387	0.020-7.502	0.530
*B*5401/*5604*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*5401/*5801*	2 (0.55)	2 (0.20)	2.726	0.383-19.423	0.317
*B*5501/*5601*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*5502/*1301*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*5502/*5801*	0 (0.00)	2 (0.20)	0.542	0.259-11.310	0.693
*B*5601/*5701*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*5604/*5801*	1 (0.28)	1 (0.10)	2.721	0.170-43.618	0.480
*B*5701/*5801*	0 (0.00)	1 (0.10)	0.904	0.037-22.235	0.951
*B*5801/*5801*	6 (1.65)	9 (0.91)	1.824	0.645-5.162	0.257
*B*5801/*7301*	1 (0.28)	0 (0.00)	8.164	0.332-200.875	0.199

Chi-square test, OR: odds ratio; 95%CI: confidence interval. The bold font in table refers to significantly association of alleles.

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