

**GSTM1 Null, GSTT1 Null Gene and Low Erythrocyte GST
Activity as Risk Factor of Autism Spectrum Disorder**



Thesis

**A thesis submitted for the degree of Master of Biomedical Science majoring
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CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

Autism spectrum disorder (ASD) is neurodevelopmental disorder characterized by impairments in the development of social and communication skills, often accompanied by stereotyped and restricted patterns of interests and behavior, with onset of impairment before 3 years of age ¹.

Epidemiological studies demonstrate that the prevalence of autism has increased in recent years ². It is currently estimated that autism affects as many as 1 out of 150 children in the United States³ and occurs four times as frequently in males as females ⁴. Prevalence of ASD in Asia was 14.8 per 10,000 from 1980 to present and 10.3 per 10,000 among 2-6 years old children in China from 2000 upwards ⁵. Therefore, incidence of autism in Japan is 27.2 per 10,000 ⁶.

The definitive causes of ASD are unknown. Investigators suggested that ASD may result from an interaction between genetic and environmental factors ⁷. Both genetic and environmental factors are being studied as possible causative factors. Theories about possible causes of ASD include genetic, parental age, immune dysfunction, gut problems, early brain damage, chronic chemical exposure, heavy metal toxicity and oxidative stress. This study was focused on genetic and environmental possible causal, especially environmental toxin,

chemical and heavy metal exposure with oxidative stress as mechanism linking these risk factors ⁷.

Glutathione S-transferases (GST) are antioxidant enzymes that play important role in cellular detoxification and the excretion of environmental pollutants including many carcinogens and also in protection against oxidative stress^{8, 9}, by its ability to conjugate Glutathione (GSH) with compounds containing an electrophilic center ^{8, 10, 11}. Studies reported low plasma total GSH (tGSH) levels, elevated levels of oxidized GSH (GSSG) and low ratios of tGSH to GSSG in autism ^{7, 12, 13}, supporting the hypothesis that oxidative stress contributed to the pathology of autism ^{12, 14}.

Glutathione S-transferase mu (GSTM1) and Glutathione S-transferase theta (GSTT1) are known to be highly polymorphic. This genetic variation may change an individual's susceptibility to carcinogens, heavy metals ¹⁵, pesticides and anticancer drugs ⁸ Homozygous deletions of these genes, referred to as GSTM1 null and GSTT1 null, respectively, result in lack of enzyme activity ¹⁶. Subjects with at least one functional allele for GSTM1 are designated as GSTM1 positive ¹⁰. Two Korean case-control studies found frequencies of 53% and 56% for the GSTM1 deletion genotype ¹⁷. Indian population showed less percentage of GSTM1 null frequency (30.4–35.4%) when compared to other Asian and Caucasian population (49–53.8%) ¹⁸. Population study has reported the deletion polymorphism among U.S. Caucasians is ranging from 48%-57% ¹⁷. GSTT1 null genotype has been shown to be 11–18% in Caucasians¹⁹, 49 % in Shanghai²⁰ that similar with the Chinese population of Taiwan (47%)²¹, and the population of

Kitakyushu, Japan (44%)²², and much higher compared to the European populations of Europe and North America ($11 \pm 24\%$)²³⁻²⁵.

Association of GSTM1 null genotype with autism has been reported²⁶. Hair mercury concentrations are significantly increased in persons with the double deleted genotype (GSTT1^{-/-} and GSTM1^{-/-}) compared with the intact genotype and show that the wildtype genotype of GSTT1 and GSTM1 genes was associated with lower blood mercury levels than the deleted genotypes¹⁵. Desoto and Hitlan performed a re-analysis of data from a cross-sectional cohort study comparing hair and blood mercury (Hg) levels of ASD with a matched control group and concluded a significant relationship between Hg levels in the blood and diagnosis of an ASD²⁷. Bernard et al has hypothesized a causal connection between mercury exposure and the symptoms of autism²⁸.

Due to increased incidence of ASD and environmental toxins exposure that has been reported by previous studies, investigation based on genetic and environment factor is needed. While, antioxidant enzymes are important for detoxification and excretion of environmental pollutants. Our point of interest on GST, an antioxidant enzyme, because of its high prevalence and polymorphic. Therefore the preliminary study was conducted to investigate the role of GSTM1 and GSTT1 polymorphism as risk factor of ASD associated with GST activity and phenotype expression.

1.2 RESEARCH QUESTIONS

1. Is the GSTM1 null gene risk factor of ASD ?
2. Is the GSTT1 null gene risk factor of ASD ?
3. Is low erythrocyte GST activity risk factor of ASD?
4. Is the enzyme activity of Glutathione s-transferase in ASD lower than normal control ?
5. Is there association between GSTM1 and GSTT1 null genes with phenotype expression of ASD ?
6. Is there association between GSTM1 and GSTT1 null genes with Glutathione s-transferase activity in ASD ?
7. Is there association between Glutathione s-transferase activity with phenotype expression of ASD ?

1.3. RESEARCH OBJECTIVES

1.3.1 GENERAL OBJECTIVE

To determine GSTM1 null, GSTT1 null gene and low erythrocyte GST activity as risk factors among ASD patients and associated with Glutathione s-transferase activity and phenotype expression.

1.3.2 SPECIFIC OBJECTIVES

1. To determine GSTM1 null gene as risk factor among ASD patients.

2. To determine GSTT1 null gene as risk factor among ASD patients.
3. To determine low erythrocyte GST activity as risk factor among ASD patients.
4. To determine Glutathione s-transferase activity in ASD.
5. To analyze the association between GSTM1 and GSTT1 null genes with phenotype expression of ASD.
6. To analyze the association between GSTM1 and GSTT1 null genes with Glutathione s-transferase activity in ASD.
7. To analyze the association between Glutathione s-transferase activity with phenotype expression of ASD.

1.4 RESEARCH ADVANTAGES

1. To know that GSTM1 null, GSTT1 null gene and low erythrocyte GST activity are risk factors of ASD.
2. To know the enzyme activity of Glutathione s-transferase in ASD.
3. To provide information about association GSTM1 null, GSTT1 null gene, GST activity with phenotype expression of ASD,
4. To provide information that ASD patient with GSTM1 null and GSTT1 null gene is more susceptible with environmental pollutant.

1.5 RESEARCH ORIGINALITY

There is only one study by Steven Buyske et al, 2006 that associated GSTM1 null gene with ASD. It was case parent trios and case control study that showed significant result. According to the author knowledge, this is the first study which determine GST activity and GSTT1 null gene in ASD patients compared with control group.

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