

CHAPTER V

DISCUSSION

This study was carried out to investigate the relationship between erythrocyte GST activity and genetic polymorphisms of GSTT1, GSTM1 and phenotype expression of Autism Spectrum Disorder (ASD). Autism spectrum disorder is a complex and multifactorial disease. It is difficult to identify single elements that increase the risk. Autism may result from a combination of genetic susceptibility (reduced ability to remove metals including mercury or other neurotoxins from the system) and environmental exposure.^{1,2,3} Several genes have been reported associated with autism, including genes involved in methionine transmethylation and transsulfuration pathways. Polymorphism of Reduced Folate Carrier I (RFC1) gene, Methylenetetrahydrofolate Reductase (MTHFR), Transcobalamin II (TCN2), Catechol-O-methyltransferase (COMT)⁴ and Glutathione S-transferase mu 1 (GSTM1)⁵ was significantly increased among autistic children compared to control.⁴

In the present study found that frequency of male patients of ASD is higher than female (1:15). This number much more higher than Bailey A et al's study that state ASD occurs four times as frequently in males as females. The frequency of the GSTM1 null genotype in control groups was 6.7% , quite different from previous study in Jakarta, Indonesian population (55.6%)⁶, much lower than in Caucasian population (42 % - 60 %) and other Asians population, in Japan 47.9 % , Korea 52.2% and Singapore 56.2 %.⁷ The frequency of the GSTT1

null genotype was 31.1 % in our control population, similar with Japan population (35,3%), lower than previous study in Jakarta, Indonesian population (41.4 %) ⁶, in Koreans (51.5 %) and Singapore population (51.9%) ⁷, but higher than in Europeans (22%) and in African-Americans (21%) ⁸.

Steven Buyske et al (2006) concluded an association of the homozygous GSTM1 deletion genotype with autism ($p=0.028$) ⁵. This study revealed that there is no significant association between GSTM1 and GSTT1 null gene with Autism Spectrum Disorders (ASD), although the distribution of these genes among ASD higher than controls. The combination of GSTM1 null and GSTT1 null also do not contribute to ASD risk in this population. Sample size of this study ($n = 51$, for ASD) almost the same with Steven Buyske et al ($n = 54$, for ASD) meanwhile the difference result of this study compared with Steven Buyske et al because of difference race and population.

To the author knowledge, this is the first preliminary study that associate the erythrocyte Glutathione S-transferase (GST) activity among ASD patients and control group. Also the first that find out the association between GST activity and phenotype expression of ASD. In the present study showed that there is significantly decreased erythrocyte GST activity in ASD compared with controls. However, although the mean erythrocyte GST activity in severely autistic is lower than mild to moderately autistic, it was not reach significantly different.

According to previous studies, there is no association between GSTM1 null and reduced erythrocyte GST activity in Chinese, but combined GSTP1 and GSTM1 null resulted in significantly reduced GST activity ⁹. Study in India

showed patients with double deletions GSTT1 and GSTM1 genotypes have significantly low GST activity as compared to other genotypic groups.¹⁰ Both patient and control groups with double GSTT1/GSTM1 null genotypes had the lowest serum total GST activities in Caucasian. The GST activity of GSTM1 and GSTT1 null genotype was found to be lower than present genotype, although the difference was not significant.¹¹ The current study has similar result. There was no significant difference among cases with GSTM1 null genotype compared with wild type genotype for the mean erythrocyte GST activity.

The difference of result compared with others studies could be the fact that this study conducted in different race and population. Small total sample size is also the limitation of the present study, although it has fulfilled the sample size number requirement for statistical analysis. Another explanation for the differences in results is GST enzymes have a broad substrate overlap, therefore decreased in expression level of one GST may be compensated by increased expression of another. In spite of that ASD is multifactorial disease, in which there is complex environmental factors involved and multiple genes participated, results in heterogeneity. Therefore, this preliminary study needs further investigations with increased sample size, multiple genes and GST activity determination, to find out gene susceptibility of ASD and factors that contribute to the phenotype expression of ASD.

Genetic Counselling Aspect

Genetic counseling is helpful to families with ASD that are known to have a strong genetic component. It is important to do genetic testing for all

children with ASD. There are extra genes or missing genetic material, known as copy number variations, which may play a role in ASD. Recent studies have indicated that 7 to 18% of children with ASD will have positive genetic test results.¹²

The information about recurrence risk is also important for many parents. If a family has one child with ASD, the risk of having another child with ASD is 4 to 7%. Jorde et al 1991 found that the sibling recurrence risk for autism was 4.5%.¹³ Study by Ritvo et al, 1989 concluded that recurrence risk estimate of each sibling born after an autistic child will develop autism is 8.6%. If the first autistic child is a male the recurrence risk estimate is 7%, and if a female 14.5%.¹⁴

The chart lists the estimated recurrence risks based on specific factors found in a family. The recurrence risks listed are for siblings born to the same parents after identification of the affected individual.

Table 5 Recurrence Risk For Autism Spectrum Disorder¹⁴⁻¹⁶

	Recurrence Risk For Autistic Disorder	Recurrence Risk for PDD-NOS/Asperger Syndrome
Affected Proband	5 – 50 %	Not Available
Parents with Affected male child	3 – 8 %	5 %
Parents with Affected female child	3 – 8 %	7 – 8 %
Siblings of Affected Proband	Not Available	Not Available
Aunt/Uncle of Affected Proband	18 %	1 %
Cousin of Affected Proband	12 %	1 %
Parent of 2 Affected Probands	25 %	Not Available

This study can provide information about preliminary result that found lower erythrocyte GST activity as risk factor of ASD and also GSTM1 null and GSTT1 null gene that tend to be risk factor of ASD. ASD patient with low erythrocyte GST activity is more susceptible with environmental toxin exposure. This result still need further investigation with increased sample size to ensure that GSTM1 null, GSTT1 null and low erythrocyte GST activity is risk factor of ASD