CHAPTER 1.

INTRODUCTION

1.1. Background

Fragile X Syndrome (FXS) is the most common inherited cause of intellectual disabilities (ID) as well as the most common single genetic cause of autism.[1, 2] The prevalence of FXS is approximately one in 2,500 to one in 4,000.[3-5] Among ID, the prevalence of FXS were estimated 2-3%.[6] Previous studies in Indonesia demonstrated FXS prevalence of 1.65-1.9% in the ID population.[7, 8]

FXS is a neurodevelopmental disorder caused by an expanded Cytosine-Guanine-Guanine (CGG) trinucleotide repeats in the 5' untranslated region (UTR) of the Fragile X Mental Retardation (*FMR1*) gene located at Xq27.3.[9] This mutation typically lead to hypermethylation of the gene because of the presence of more than 200 CGG repeats within the promoter region. Thus, that will lead silencing of the gene and result deficiency or absence of Fragile X Mental Retardation Protein (FMRP).[10] Normal individuals have approximately 5-44 CGG repeats, gray zone alleles have approximately 45 to 55 repeats, and the premutation allele is defined as 55 to 200 repeats.[11] The full mutation, the CGG repeats over 200, specific phenotype will occurs such as prominent ears, long face, hyperextensible finger joints, macroorchidism and cognitive deficits or ID.[1, 12]. FXS is also characterized by behavioral and psychological features including autistic-like behavior, anxiety, attention-deficit hyperactivity disorder (ADHD),

autism spectrum disorder (ASD), mood instability and aggression.[13-16] The level of cognitive abilities, physical features, and behavioral symptoms of FXS correlate with the level of FMRP.[17-19]

The prevalence of the fragile X premutation in the general population is estimated at one in 130 to 259 females [20, 21] and one in 250 to 813 males.[5, 22] It was thought that individual with the premutation alleles had normal FMRP production and labeled as an FXS carrier until Tassone et al demonstrated decrease production of FMRP which is associated with some clinical involvement including cognitive deficits [23] and elevation of *FMR1* messenger Ribo Nucleic Acid (mRNA) level from 2 to 8 times higher compared to normal individual, most commonly seen in the upper end of the premutation range and this leads to central nervous system (CNS) toxicity. [24-26] Although most individuals with the premutation are unaffected, a subgroup of children experience ADHD, anxiety, ASD, seizures, learning difficulties or even ID in boys but less frequently in girls.[13, 27-33] as well as psychopathology including depression, mood disorder and anxiety in adults with the premutation.[34-36] Males usually have more significant cognitive and behavioral problems than females as result of the modifying effect of a second or normal X chromosome in the females. [27, 37]

Among adulthood, expansion of CGG repeats in the premutation range is associated with Fragile X-associated Tremor Ataxia Syndrome (FXTAS), a progressive neurodegenerative disorder predominantly in older male carriers,[38] Fragile X-associated Primary Ovarian Insufficiency (FXPOI), defined as a cessation of menses before the age of 40 years [39, 40] and immune mediated

disorders including hypothyroidism, rheumatoid arthritis, fibromyalgia, and systemic lupus erythematosus in female carriers.[41]

FMRP is a Ribo Nucleic Acid (RNA) - binding protein [42-45] which controls the translation of several other genes that regulates synaptic development and plasticity and is thought to be important for synaptic plasticity, neuronal migration, and neurogenesis.[46-51] FMRP may be mildly deficient in some individuals with the premutation, particularly those with CGG repeats above 120,[23, 52, 53] however, the most important molecular abnormality in premutation carriers is the elevated level of *FMR1* mRNA.[26]

The lack of FMRP in individuals with FXS leads to excessive upregulation of protein production in the CNS,[54] upregulation of protein production of the metabotropic glutamate receptor 5 (mGluR5) pathway [55, 56] and downregulation of the Gamma-aminobutyric acid (GABA) pathways.[57, 58] The imbalance between glutamate and GABA systems can also be found in autism.[59, 60]

This study was done to identify the correlation between clinical involvement including cognitive and social deficits in males with the *FMR1* premutation and FMRP utilizing the new enzyme-linked immunosorbent assay assessment of FMRP expression levels. [61] The FMRP ELISA assay differs from the commonly used, this approach provide a quantitative measures of FMRP level, whereas the immunocytochemistry method does not measure protein level, only the proportion of cells with detectable staining. The FMRP ELISA technique is suggested as a potentially powerful tool in expanding the understanding of the relationship between FMRP levels and the various *FMR1*-associated clinical phenotypes.[62]

Thus, the utilization of FMRP measures can be helpful in understanding for which premutation patients clinical involvement is caused by dysfunction of the *FMR1* gene.

1.2. Research Questions

1.2.1. General Research Question

1.2.1.1. Is there any correlation between FMRP levels, IQ and autism spectrum disorder in males with the *FMR1* premutation?

1.2.1.2. Is there any correlation between CGG repeats, IQ and autism spectrum disorder in males with the *FMR1* premutation?

1.2.2. Specific Research Questions

1.2.2.1. Is there any correlation between FMRP levels and IQ in males with the *FMR1* premutation?

1.2.2.2. Is there any correlation between FMRP levels and autism spectrum disorders in males with the *FMR1* premutation?

1.2.2.3. Is there any correlation between CGG repeats and IQ in males with the *FMR1* premutation?

1.2.2.4. Is there any correlation between CGG repeats and autism spectrum disorders in males with the *FMR1* premutation?

1.3. Research Purposes

1.3.1. General Purposes

1.3.1.1. To identify the correlation between FMRP levels, IQ and autism spectrum disorders in males with the *FMR1* premutation.

1.3.1.2. To identify the correlation between CGG repeats, IQ and autism spectrum disorders in males with the *FMR1* premutation.

1.3.2. Spesific Purposes

1.3.2.1. To identify the correlation between FMRP levels and IQ in males with the *FMR1* premutation.

1.3.2.2. To identify the correlation between FMRP levels and autism spectrum disorders in males with the *FMR1* premutation.

1.3.2.3. To identify the correlation between CGG repeats and IQ in males with the *FMR1* premutation.

1.3.2.4. To identify the correlation between CGG repeats and autism spectrum disorders in males with the *FMR1* premutation.

1.4. Research Benefits

1.4.1. Improvement of the public awareness of genetic diseases like Fragile X Syndrome and the premutation which is apparently very common with various clinical manifestation.

1.4.2. Contribution to the development of the new treatments for Fragile X Syndrome.

1.4.3. The utilization of FMRP measures can be helpful in understanding for which premutation patients clinical involvement is caused by dysfunction of the *FMR1* gene.

1.4.4. Providing more accurate information for the premutation will enable genetic counselors to recognize the disease and the clinical manifestation.

1.4.5. Encouragement of other researchers for further studies in neurodevelopmental disorders, especially in Fragile X Syndrome and the premutation from Indonesian population.

1.5. Research Originality

The studies to identify the correlation between FMRP levels and clinical involvement in individuals with the *FMR1* premutation have been done by Tassone et al. and Loesch et al. using immunocytochemical method (see table 1). Eventhough there was a study by Hessl et al. showed decreased FMRP expression using ELISA technique that underlies amygdala dysfunction in premutation carriers, this is the first study to identify the correlation between cognitive deficits and autism in males with the *FMR1* premutation with FMRP expression levels that are measured with ELISA technique.

Table 1. The list of studies about FMRP levels in individuals with the *FMR1* premutation

Authors	Title	Design , Population	Methods of FMRP measures	Result
Tassone et al.	Clinical Involvement and Protein Expression in Individuals with the *FMR1* Premutation	Analytic study, Premutation	Immunocyto-chemical	Some individuals with the premutation have a significant FMRP deficit which appears to be associated with some clinical features. [23]
Loesch et al.	Phenotypic variation and FMRP levels in Fragile X	Analytic study Full mutation, premutation, gray zone and normal	Immunocyto-chemical	Physical phenotype and IQ scores may be primarily affected by FMRP depletion. [17]
Hessl et al.	Decreased Fragile X Mental Retardation Protein Expression Underlies Amygdala Dysfunction in Carriers of Fragile X Premutation	Analytic study, Premutation	ELISA	The reduced FMRP is the primary factor for the amygdala volume and activation in premutation carriers. [63]