CHAPTER 1

INTRODUCTION

1.1 Background

Intellectual disability (ID) is a common neurodevelopmental disorder that is characterized by an intelligence quotient (IQ) of 70 or below, and deficits in at least two behaviors related to adaptive functioning diagnosed by 18 years of age.[1] The prevalence of ID is between 1% and 3% in the world wide population and is present in every social class and culture.[2] In 2009, the prevalence of ID was about 20.71% among people with disability in Indonesia.[3] Establishment of the etiology of ID is very important for the management and genetic counseling of affected individual and their families.

The etiology of ID can be classified as genetic and non-genetic factors. The genetic factors include chromosomal abnormalities, a mutation in a single gene, and multifactorial disorders with a polygenic predisposition. The non-genetic factors are, for example, maternal malnutrition, pollutants and chemicals, prematurity, fetal infections, as well as peri- and postnatal trauma[4,5] It has been estimated that at least 60% of ID cases have an underlying genetic etiology.[6,7] Chromosomal abnormalities are considered to be the most common genetic cause of ID. Types of chromosomal abnormalities range from whole chromosomal aneuploidies and multiploidies, chromosomal rearrangements such as

translocations, supernumerary marker chromosome, and large (>5 Mb) deletions and duplications to submicroscopic chromosomal abnormalities which nowadays known as copy number variations.[7,8,9,10] A copy number variation (CNV) is a genomic structural variation ranging from kilobases (kb) to megabases (Mb) that are not identifiable by conventional chromosomal banding. CNVs may result from deletions, duplications, triplications, insertions and unbalanced translocations.[11]

Up to now, routine cytogenetic analysis remained the major diagnostic test for ID patients in Indonesia. It only provides the detection of chromosomal aberrations with a resolution of up to 5 Mb. This lead to the identification of several chromosomal abnormalities like unbalanced translocations, supernumerary marker chromosomes and large deletions and duplications.[10] The rate of microscopically detectable chromosome abnormalities varies greatly among different studies. In a large meta-analysis, a median rate of 9.5% was found.[12]

During the last decades, molecular techniques became available to detect submicroscopic chromosomal abnormalities or CNVs. Fluorescent in situ hybridization (FISH), targeted qualitative Polymerase Chain Reaction (qPCR), Multiplex Ligation-dependent Probe Amplification (MLPA) allowed the identification of CNVs.[13,14] Adding these mentioned techniques to the diagnostic tool for ID resulted in an increase of the diagnostic yield from 5-10% for karyotyping alone, to 10-15% if combined with FISH and subtelomeric MLPA

analysis.[15,16,17] However, these techniques are limited to a specific region, e.g. the subtelomeric region, and cannot identify submicroscopic imbalances elsewhere.

In recent years, a new technique has been introduced based on comparative genomic hybridization (CGH), where an array contains thousands or even millions of different probes. Such a microarray platform allows the detection of CNVs at a resolution much higher than 5 Mb and enables genome wide screening as well.[18,19] The genome wide array technology has proven successfully to define new submicroscopic aberration syndromes. This new technology has increased the detection rate in ID with an additional 10%.[20] The use of genome wide array analysis has a greater diagnostic yield than routine G-banded karyotyping. Therefore, the International Standard Cytogenetic Array (ISCA) Consortium as well as American Society of Human Genetics (ASHG) and European Society of Human Genetics (ESHG) suggested in 2009 the use of genome wide array as the first-tier cytogenetic diagnostic test for patients with developmental delay / ID, autism and multiple congenital anomalies (MCA), while the G-banded karyotyping and FISH should be kept for patients with obvious chromosomal syndromes (i.e Down syndrome), a history of multiple miscarriages and a family history of a chromosomal rearrangement. [20,21]

The genetic etiology of ID studies by Indonesian researchers is still lacking. The first screening study in a large cohort of Indonesian individuals by Hussein and Faradz et al in 1998 and 1999 was carried out using conventional cytogenetic and FMR1 gene analyses with the main focus on males.[22,23] In 2012, a genetic-

diagnosis survey in ID individuals carried out by Mundhofir involved 527 ID individuals from institutes and special schools in Java who were tested for microscopically cytogenetic abnormalities and by some additional analyses such as FISH, MLPA, and analysis of the *FMR1* promoter CGG(n) repeat.[24] In this present study, high resolution genome wide array was employed for the detection of copy number variations in intellectually disabled individuals who had normal results after karyotyping, CGG(n) repeat analysis in the *FMR1* gene and sub-telomeric MLPA testing.

1.2 Research Questions

What is the molecular diagnostic of undiagnosed ID patients with common molecular testing from Indonesia using high resolution genome wide array analysis?

1.3 Research Purposes

1.3.1 General Research Purposes

To identify copy number variations responsible for undiagnosed ID patients with common molecular testing from Indonesia using high resolution genome wide array analysis.

1.3.2 Specific Research Purposes

1. To learn the practical workflow of high resolution genome wide array analysis for detecting, analyzing and interpreting copy number variations.

2. To identify genotype-phenotype associations after finding an abnormal array result.

1.4 Research benefits

1. To introduce and implement the high resolution genome wide array technique in genetic diagnostics for ID individuals in Indonesia.

2. To lay down a basis for genetic counseling.

1.5 Research Originality

This is the first study for ID patients in Indonesia who had normal karyotyping, CGG(n) repeat in *FMR1* gene and sub-telomeric deletion and duplication screening to be investigated for copy number variations using high resolution genome wide array analysis.