CHAPTER V
DISCUSSION

This study found 48 (23 female and 25 male) microcephalic and 10 macrocephalic individuals from a previous study of 527 unrelated individuals with mental retardation. This counted for 9.1% of microcephaly and 1.9% of macrocephaly finding in the study. The only available comparison is a previous study by Watemberg (2002) that described the microcephaly prevalence in children referred for developmental disabilities in Tel Aviv Child Development Center; where 34.2% of the 240 children with mental retardation were microcephalic.

Measurement of head circumference in this study was done using the Nellhaus chart. Although important, the choice of cut-off point of defining microcephaly is subjective (Nicholas, 2009). Microcephaly definition has been described to be either below -2SD or -3SD by several different authors (Woods 2005; Kaindl 2010; Abuelo 2007; Nicholas, 2009). The different opinions must be taken into account when encountering patients at the lower range of the normal curve.

Comparisons of head circumference charts from different countries show that head growth differs in population of one country to another (Karabiber, 2001; Ayatollahi, 2006), and that the smaller head circumference of some ethnicities reflects the smaller body stature (Tsuzaki, 1990). The closest available head chart to Indonesian stature is the Singaporean chart, however, it is only for children age 0-6 years (National Healthcare Group Polyclinics, 2000).

A recent preliminary study conducted in Semarang, Central Java, indicated that the mean value of head circumference of Indonesian normal schoolchildren age 7-12 years is smaller compared to the normal range of the Nellhaus charts. The boys mean values are between the 3rd and under 50th percentile, while the

girls are between the 10th and under 50th percentile (Mundhofir, unpublished). The interpretation of head circumference must be considered using the specific head charts appropriate for application in the different countries. It is therefore reasonable that this study chose the Nellhaus charts with <-2SD as microcephaly threshold and >2SD as macrocephaly threshold, with the possibility of underestimation of macrocephalic individuals due to the smaller average head circumference of the Indonesian population.

In this study, a total of 186 DNA sequencing and analysis was performed, which consisted of *ASPM* and *WDR62* gene sequencing on 48 samples; *MCPH1, CDKRAP2, CENPJ* and *STIL* gene sequencing on 20 samples; and *PTEN* gene sequencing on 10 samples. From this analysis, we detected 25 unknown variants classified as UV2 ("unlikely to be pathogenic") and UV3 ("likely to be pathogenic") in *ASPM* and *WDR62* genes of 15 individuals.

Single nucleotide polymorphisms (SNP) were found in all of the genes analysed and may provide reference of the polymorphisms in the Indonesian population. This SNP data may also provide reference for possible association studies in the future.

Follow up was only done in the family of one microcephalic individual because of the presence of 2 unknown variants classified as UV3 in subject DNA 11-02676. The follow up concluded that autosomal recessive inheritance of microcephaly and mental retardation in this family is unlikely due to the presence of the 2 variants in the unaffected father and sister.

As a result of finding the exact same variants on the twin sister of subject DNA 11-02676, and the possibility of a hidden mutation on the maternal allele if the twins were dizygotic, monozygosity status of the twins were examined using identifiler test. Surprisingly, the result was that the twin sisters were monozygotic, showing a phenomenon referred to as "twin discordance" in the literature. Discordant twins are monozygotic twins which show different phenotypes from each other. This may be explained by mechanisms that alter the gene structure and

expression at the time or meiosis and mitosis (Machin, 2009). Epigenetic changes are thought to contribute to the etiology of twin discordance (Javierre, 2010).

No causative pathogenic mutations was detected in this study. This negative finding may be explained by several things, such as the unknown nature of the microcephaly and macrocephaly in the included individuals and the possibility of de novo causative mutation.

The first explanation for the negative finding is that the nature of microcephaly in these individuals is not genetic. The exact types of the microcephaly were unknown, whether they were congenital or acquired microcephaly, as growth chart and precise history were not obtainable from the parents. In these individuals, it was unknown whether birth OFC was ever recorded. A study by Baxter (2009) revealed that acquired or progressive microcephaly is a relatively common form of microcephaly besides congenital or primary microcephaly. In acquired microcephaly, a child's head circumference is within normal range at birth but does not increase as a normal child and later measurement falls below it. It is therefore essential to record head circumference at birth, followed by regular measurement using consistent head chart in order to know exactly when a child's head growth falter off the normal range of head circumference. Three perinatal risk factors were significantly found to be more common in microcephalic children compared to normocephalic children: prematurity, perinatal asphyxia and small for gestational age (Watemberg, 2002). These risk factors might be present but could not be accurately obtained from the parents during history-taking. Furthermore, OFC measurement and plotting on head chart were not routinely done in the Indonesian population nor properly recorded.

The second probable explanation is that the cause of mental retardation with microcephaly or macrocephaly is genetic, but there is failure in detecting the causative mutations. This may be due to to the unknown presence of mutations in

parts of the genes that have not been tested or deletions/duplications that cannot be tested with the methods applied in this study.

The third reason is that it may still be caused by a genetic defect present in a gene that has not yet been discovered. Another possible explanation for the negative finding might be that de novo mutations may be causing the phenotype of mental retardation with either microcephaly or macrocephaly, while MCPH in this research is an autosomal recessive disease. Recently, a study by Vissers *et. al* (2011) suggested that the major cause of unexplained mental retardation is de novo mutations. The study using exome sequencing showed that the de novo mutations found in the genes with known functional link with mental retardation strongly supports their pathogenicity.

Based on these findings, future studies may be directed at performing MLPA in testing of MCPH genes as well as *PTEN* gene in order to find the possible deletion or duplication that could not be found with sequencing method, also by finding a new gene that may explain the pathology by performing whole exome sequencing, epigenetic studies and follow up studies on the family of subject DNA 11-02676 and the twin sister and comparing them.

Limitations of Study

The limitation of this study is the unavailability of MLPA kit for MCPH genes until the time of the data analysis period of this study, and therefore MLPA technique could not be included in this study. This results in possibly missed detection of exonic or whole gene deletions of the MCPH genes, which counts for less than 10% of all pathogenic mutations of MCPH genes. For future studies, it is recommended that MLPA for MCPH genes as well as *PTEN* gene is also performed.Another limitation in this study is the use of secondary data of OFC from the previous study in the same population. Kappa reliability test was not performed, and therefore the possibility of inter-observer bias in measuring the head circumference could not be assessed.