MUTATION ANALYSIS OF INDONESIAN MENTALLY RETARDED INDIVIDUALS WITH MICROCEPHALY AND MACROCEPHALY

THESIS
Submitted to fulfil the requirement in obtaining Master Degree

Biomedical Sciences Magister
Majoring in Genetic Counseling

Rahajeng N. Tunjungputri, MD
G4A009017

POST GRADUATE PROGRAM
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Defended in front of the Defence Committee
On 21 November 2011
And has been approved by the Supervising Committe

Netherlands Supervisor	Indonesian Supervisor Principal Supervisor
Helger Yntema, PhD	Prof. dr. Sultana MH Faradz, PhD NIP. 19520202 197901 2 001
	Supervisor

Approved by
dr. Tri Indah Winarni, MSi.Med
Chairman of Biomedical Science Magister Study Program
Postgraduate Program Diponegoro University

DR. dr. Winarto, Sp.MK, Sp.M (K)
NIP. 19490617 197802 1 001

TABLE OF CONTENT

DECLARATION

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma of the university or other institute of higher learning, except where due acknowledgement is made in the text

Semarang, November 2011
Rahajeng N. Tunjungputri

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LIST OF ABBREVIATION

ARX	: Aristaless related homeobox gene and protein
ASPM	: Abnormal spindle–like, microcephaly associated
BRAF	: Murine sarcoma viral oncogene homolog B1
CDC	: Center for Disease Control
CDK5RAP2	: Cyclin dependent kinase 5 regulatory associated protein 2
CENPJ	: Centromere associated protein J
CLCN7	: Chloride channel 7
DNA	: Deoxyribonucleic acid
FGFR3	: Fibroblast growth factor receptor 3
FMR1	: Fragile X mental retardation 1
HRAS	: Harvey rat sarcoma viral oncogene homolog
KRAS	: Kirsten rat sarcoma viral oncogene homolog
LGI1	: Leucine-rich, glioma inactivated 1
M-CMTC	: Macrocephaly cutis marmorata telangiectatica congenital
MCPH	: Microcephaly primary hereditary (autosomal recessive primary microcephaly)
MEK1	: Mitogen-activated protein kinase kinase 1
MEK2	: Mitogen-activated protein kinase kinase 1
MLC	: Megalencephalic leukoencephalopathy with subcortical cysts
mRNA	: Messenger Ribonucleic acid
NF1	: Neurofibromatosis, type 1
NSD1	: Nuclear receptor-binding Su-var, enhancer of zeste, and trithorax domain protein 1
OFC	: Occipito-frontal circumference
OSTM1	: Osteopetrosis associated transmembrane protein 1
PHTS	: *PTEN* (Phosphatase and tensin homologue) hamartoma tumor syndrome

PKU	: Phenylketonuria
PTEN	: Phosphatase and tensin homologue
SCN1A	: Sodium channel, voltage-gated, type I, alpha subunit
STIL	: SCL (Stem cell leukemia hematopoietic transcription factor)/ TAL1 (T-cell acute lymphoblastic leukemia) interrupting locus
STXBP1	: Syntaxin binding protein 1
TCIRG1	: T-cell immune regulator 1
TNFSF11	: Tumor necrosis factor (ligand) superfamily, member 11
WDR62	: WD (tryptophan - aspartic acid) repeat-containing protein 62
WHO	: World Health Organization

LIST OF FIGURES

LIST OF TABLES

CURRICULUM VITAE

Name : Rahajeng Nareswari Tunjungputri
Place/Date of Birth : Jakarta, 11 October 1984

Educational Background

1991 – 1993	Patra Primary School, Tanjung, South Kalimantan
1993 – 1994	Kittybrewster Primary School, Aberdeen, UK
1994 – 1996	Persit Diponegoro Primary School, Semarang, Central Java
1996 – 1999	Vidya Dahana Patra Junior High School, Bontang, East Kalimantan
1999 – 2002	Vidya Dahana Patra Senior High School, Bontang, East Kalimantan
2002 – 2009	Faculty of Medicine Diponegoro University
2009 – 2011	Master of Biomedical Science Faculty of Medicine Diponegoro University

Working experience

2003-2005	Student laboratory assistant of Medical Physics Department, Faculty of Medicine Diponegoro University
2005	Staff volunteer of Youth Action For Peace Italia (YAP-Italia), Rome, Italy
2005	Staff volunteer of Il Cantiere Giovanni Frattamagiore Immigrant Center, Napoly, Italy
2007	Medical student internship in Infectious Diseases, Department of General Internal Medicine, Radboud University Nijmegen Medical Center, The Netherlands
2009	PTT General Practitioner and Medical Emergency Team of Matak Field Hospital, Matak Island, Kepulauan Riau Province
2010	Junior lecturer, Department of Parasitology, Faculty of Medicine Diponegoro University
2010 – 2011	Research intern, DNA Diagnostics, Department of Human Genetics Radboud University Nijmegen Medical Center, The Netherland

Awards and Scholarship

2000	2nd winner of Indonesia School Debating Championship, Ministry of National Education – English Debating Society Universitas Indonesia
2000	3rd Winner in Social Science Category of National Scientific Paper Competition of Indonesian Institute of Sciences (LKIR LIPI-TVRI)
2001	Best new team, member of Team Indonesia, World Schools Debating Championship, Johannesburg, South Africa

2001	2nd Winner in Social Science Category of National Scientific Paper Competition of Indonesian Institute of Sciences (LKIR LIPI-TVRI)
2005	Recipient of Asia Europe Foundation (ASEF) Grant for Asia-Europe Volunteer Exchange, Paris, France
2006	Accomplished Student of The Year (in Dies Natalis of Faculty of Medicine Diponegoro University)
2007	Recipient of scholarship for The Course in Tropical Infectious Diseases and International Public Health, Radboud University Nijmegen, The Netherlands
2010	Recipient of Beasiswa Unggulan for Master Programme, Ministry of Education, Indonesia

Scientific Article

2000	Utilization of ultrasonic frequency for eradication of rice bugs (3rd winner of National Scientific Paper Competition of Indonesian Institute of Sciences – LKIR LIPI TVRI)
2001	The effect of BCG vaccination, stress, and combination of BCG vaccination-stress to T-Cell Proliferation in Balb/C mice (Paper for undergraduate research project)
2006	Ineffective use of words and sentences in Indonesian mass media (2rd winner of National Scientific Paper Competition of Indonesian Institute of Sciences – LKIR LIPI TVRI)

ABSTRACT

Background
Among the mentally retarded individuals, associated features are often found that may lead to specific diagnosis, such as microcephaly or macrocephaly. Mutations in the autosomal recessive primary hereditary microcephaly (MCPH) genes are known to cause mental retardation (MR) and microcephaly, while mutations in the *PTEN* gene cause MR and macrocephaly. This study aims to analyse the presence of mutations in the Indonesian individuals with MR and microcephaly or macrocephaly.

Methods
From a previous study of 527 mentally retarded individuals, 48 microcephalic and 10 macrocephalic individuals underwent DNA sequencing. Secondary data from a previous study was used to determine the head circumference based on the Nellhaus head charts. The DNA of the microcephalic subjects was analysed for mutations of the MCPH genes while *PTEN* DNA sequencing were performed on macrocephalic subjects. The likelihood of pathogenicity of the variants found was determined by comparing them to mutation database and analysis of protein prediction programs of SIFT, Align-GVGD and Polyphen-2.

Results
From 48 individuals with MR and microcephaly, 23 variants on the MCPH genes were found as likely to be pathogenic, while no pathogenic mutation was found. One subject was found to have 2 variants which were predicted as likely to be pathogenic and underwent follow up, in which a monozygotic twin with the same 2 variants was discovered to be unaffected, revealing twin discordance result. None of the 10 subjects with macrocephaly and MR had any mutations in the *PTEN* gene.

Conclusion
Microcephaly was present in 9.1% of 527 individuals with MR, while macrocephaly occured in 1.9%. This study did not find any pathogenic causative mutation in the MCPH genes as well as *PTEN* gene that underwent DNA sequencing. This might be due to the non-genetic cause of microcephaly or macrocephaly and MR or mutations that were not yet discovered in this study. Interestingly, one twins discordance result was found. The findings of MR associated with microcephaly and macrocephaly may aid in providing genetic counseling in the Indonesian setting.

Keywords : Mental retardation, microcephaly, macrocephaly, *PTEN,* MCPH

ABSTRAK

Latar Belakang

Pada individu dengan retardasi mental dapat ditemukan gejala dan tanda lain yang dapat memberi petunjuk ke arah diagnosis yang lebih spesifik, seperti mikrosefali dan makrosefali. Mutasi pada gen-gen *autosomal recessive primary hereditary microcephaly (MCPH)* diketahui dapat menyebabkan retardasi mental (RM) dan mikrosefali, sementara mutasi pada gen *PTEN* menyebabkan RM dan makrosefali. Penelitian ini bertujuan untuk menganalisis keberadaan mutasi genetik pada penderita RM dengan mikrosefali atau makrosefali.

Metode

Dari penelitian sebelumnya dengan 527 penderita RM, 48 individu dengan mikrosefali dan 10 individu dengan makrosefali menjalani *sequencing* DNA. Data sekunder dari penelitian sebelumnya digunakan untuk menentukan klasifikasi ukuran lingkar kepala berdasarkan kurva Nellhaus. Analisis gen MCPH dilakukan pada subyek mikrosefali sedangkan analisis gen PTEN dilakukan pada subyek makrosefali. Patogenitas varian genetik ditentukan dengan membandingkan dengan *database* mutasi dan program prediksi protein seperti SIFT, Align-GVGD dan Polyphen-2.

Hasil

Dari 48 individu dengan RM dan mikrosefali, 23 varian ditemukan dengan klasifikasi "*likely to be pathogenic*", dan tidak ditemukan mutasi patogenik. Ditemukan 2 varian dengan klasifikasi "*likely to be pathogenic*" pada 1 subyek yang dilanjutkan dengan pemeriksaan lanjutan di mana terdapat kembar monozigotik subyek dengan kedua varian yang sama namun tidak menderita RM maupun kelainan lingkar kepala, di mana hal ini menggambarkan adanya diskordansi kembar. Tidak ditemukan mutasi gen *PTEN* pada 10 subyek RM dengan makrosefali.

Kesimpulan

Mikrosefali ditemukan pada 9,1% dari 527 individu dengan RM, sementara makrosefali ditemukan sebanyak 1,9%. Penelitian ini tidak menemukan mutasi patogenik dengan *sequencing* gen MCPH maupun *PTEN*. Hal ini mungkin disebabkan penyebab non-genetik RM dengan mikrosefali dan makrosefali, atau terdapatnya mutasi yang tidak dapat ditemukan melalui penelitian ini. Satu hasil diskordansi kembar ditemukan dalam penelitian ini. Hasil yang ditemukan dalam penelitian RM dengan mikrosefali dan makrosefali dapat membantu layanan konseling genetika di Indonesia.

Kata kunci: retardasi mental, mikrosefali, makrosefali, MCPH, *PTEN*