MOLECULAR ANALYSIS OF SUSPECTED INDONESIAN NOONAN SYNDROME

ANALISIS MOLEKULAR PADA PASIEN INDONESIA DENGAN SUSPEK SINDROM NOONAN

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Iffa Mutmainah, MD

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Arranged by

Iffa Mutmainah, MD

22010112420048

Has been defended in front of the defense committee on July 29, 2015

And has been approved by:

The Netherlands,
Principal Supervisor,

Heiger Ytema, PhD

Indonesia,
Principal Supervisor,

Prof. Sultana MH Faradz, MD, PhD
NIP: 1952 0202 1979 012 001

Supervisor,

Willy Nillesen

Supervisor,

DR. Tri Indah Winarni, MD,MSi.Med
NIP: 1966 0510 1997 02 2001

Approved by,
Head of Master Degree Program in Biomedical Science
Faculty of Medicine Diponegoro University

Dr. Achmad Zulfa Juniarto, M.Si.Med, Sp.And, Ph.D
NIP: 19700608 1997021 001

DECLARATION

I hereby declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education, there are no elements belonging Plagiarism forth in Decree No. 17 of 2010. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of reference is given.

Semarang, May 2015

Iffa Mutmainah

PERNYATAAN

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Semarang, Mei 2015

Iffa Mutmainah

CURRICULUM VITAE

Name	: Iffa Mutmainah
Sex	: Female
Place & Date of birth	: Bogor, February 3rd, 1988
Latest Degree	: Medical Doctor
Address	: Jl KH Soleh Iskandar. Gang Saiyan Idi No.5 Rt 002/ Rw 004 Parakan Jaya, Kemang--Bogor PO.BOX 16310
Phone	: +62856 804 2165
E-mail	: iffamutmainah@gmail.com

Educational Background

2006-2010 : Diponegoro University, Medical Faculty (Bachelor degree)

2010-2012 : Diponegoro University, Medical Faculty (Medical Doctor)

2013-2015 : Master in Biomedical Science Majoring in Genetic Counseling, join program with Radboud University Medical Centre Nijmegen, The Netherlands, with one year attachment.

Participations in Trainings and Courses

2012 : One day symposium "Actual and Applicable Management of Emergency Cases in Daily Practice", Solo

2012 : World Pneumonia Day Seminar, Semarang

2013 : 11th Medical Genetic Course: From Basic to Clinic, Semarang

2013 : Cardiogenetics and Its Clinical Management, Semarang

2013 : Workshop on Writing International Research Grants and Publication, Semarang

2013 : Molecular Biology: A Recent Update, Semarang

2013 : Gene Therapy Seminar, Semarang

2013 : 3rd International Seminar on Autism and Fragile-X Syndrome, Semarang

2014 : Dysmorphology club, Leiden, The Netherlands

2014 : The Genome: more than just 4 letters, Arnhem, The Netherlands

2014 : Forty hours Breastfeeding Counseling using WHO's module, Jakarta

2015 : Hands-on workshop on cytogenetic and molecular genetics. Semarang

Scientific Paper

2010 : The difference of carcinoembryonic antigent (CEA) levels before and after therapy in patients with colorectal carcinoma. Undergraduate thesis. Medical Faculty of Diponegoro University, Semarang.

Scholarship

2013 : Excellent Scholarship Program of The Bureau of Planning and International Cooperation, Ministry of National Education, Government of Indonesia.

TOEFL score

2010 : 527

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CONTENTS

ABBREVIATIONS

A2ML1	Alpha-2-Macroglobulin-Like1
ACGS	Association of Clinical Genetic Science
ARHGEF	Rho Guanine Nucleotide Exchange Factor
ASD	Atrial Septal Defect
CBL	Casitas B-lineage Lymphoma
CDC	Centers for Disease Control and Prevention
CDC25-H	Cell Division Cycle 25-Homology
CEBIOR	Center for Biomedical Research
CFCs	Cardio Facio Cutaneous syndrome
CR	Conserved Region
DH	Dbl Homology
DNA	Deoxyribo Nucleic Acid
ECG	Electrocardiography
EEG	Electroencephalography
ENT	Ear Nose Throat
ERK	Extracellular signal Regulated Kinase
FISH	Fluorescent in situ Hybridization
FSH	Follicle Stimulating Hormone
GDP	Guanosine diphosphate
GH	Growth Hormone
GHT	Growth Hormone Therapy
Grb2	Growth factor receptor bound-protein 2

GTP	Guanosine triphosphate
HCM	Hypertrophic Cardio Myopathy
HD	Histone Domain
HL	Helicar Linker
ID	Intellectual Disability
IGF-1	Insulin-like Growth Factor-1
ISPs	Ion Sphere Particles
ISS	Ion Semiconductor Sequencing
LH	Luteinising Hormone
MAPK	Mitogen Activated Protein Kinase
MAP2K1	Mitogen-Activated Protein Kinase Kinase 1
MAP2K2	Mitogen-Activated Protein Kinase Kinase 2
MEK	MAPK/ERK Kinase
MEK1	MAPK/ERK Kinase 1
MEK2	MAPK/ERK Kinase 2
NCHS	North Country Health Services
NS	Noonan syndrome
OFC	Occipito-Frontal Circumference
PCR	Polymerase Chain Reaction
PGM	Personal Genome Machine
PH	Pleckstrin Homology
PR	Proline-rich Region
PTP	Protein Tyrosine Phosphatase

PTPN11	Protein Tyrosine Phosphatase Non-receptor type 11 gene
PVS	Pulmonary Valve Stenosis
RAF	Rapidly Accelerated Fibrosarcoma
ARAF	A-Raf proto-oncogene, serine/threonine kinase
BRAF	B-Raf proto-oncogene, serine/threonine kinase
RAF1	Raf-1 proto-oncogene, serine/threonine kinase
RAS	Rat Sarcoma viral oncogene
HRAS	Harvey-Rat Sarcoma viral oncogene
KRAS	Kirsten-Rat Sarcoma viral oncogene
NRAS	Neuroblastoma-Rat Sarcoma viral oncogene
RefSeq	Reference Sequence
REM	RAS Exchanger Motif
RIT1	RAS like without CAAX 1
RRAS	Related Rat Sarcoma viral oncogene
RTK	Receptor Tyrosine Kinase
SH2	Src-Homology 2 domain
SHOC2	Soc-2 suppressor of clear homolog (C. elegans)
SHP-2	Protein Tyrosine Phosphatase Non-receptor type 11
SIFT	Sorting Intolerant From Tolerant
SNP	Single Nucleotide Polymorphism
SOS1	Son of Sevenless homolog 1 (Drosophila)
SPRED1/2	Sprouty-related, EVH1 Domain containing 1
TS	Turner syndrome

UCSC	University of California Santa Cruz
UV	Unclassified Variant
VKGL	Vereniging Klinisch Genetische Laboratoriumdiagnostiek
VUS	Variants of Uncertain Significance
WDR59	WD Repeat domain 59
WHO	World Health Organization

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GLOSSARY

Gene	A basic physical and functional unit of heredity.
Ion semiconductor	A next generation sequencing technique which measure the PH changes induced by hydrogen release during DNA replication to determine the nucleotide order.
Mutation	A pathogenic alteration in DNA sequence with the prevalence less than 1% of general population. Germ line mutation occurs in the ovum or sperm and can be inherited to the offspring.
Noonan syndrome	An autosomal dominant disorder characterized by distinctive facial features, congenital heart defect, and short stature.
PCR	Polymerase Chain Reaction. A laboratory technique used in order to amplify the DNA sequence through the cycle of denaturation, annealing, and elongation.
Sequencing	A technique used to identify the nucleotide order within the DNA molecule.
SNP	Single Nucleotide Polymorphism. A normal genetic variation among more than 1% of general population.
Trio	A group consists of patient and the parents.
Turner syndrome	A chromosomal disorder in female, due to absence of all or part of X chromosome in female.

ABSTRACT

Background: Noonan syndrome (NS) is a genetic disorder characterized by dysmorphic facial features, congenital heart defects, and short stature. NS is previously known as pseudo Turner syndrome (TS) due to their phenotypic similarity. However, unlike TS, NS is non-chromosomal disorder and present in both males and females. Several genes involved in RAS-MAPK pathway found to be mutated in NS and sequencing was performed in order to find the mutation in those genes.

Methods: Eight Turner phenotype patients with normal karyotype were included in this study. Clinical appearances were then compared to NS scoring system. DNA was sequenced by automated ion semiconductor sequencing for the four most common NS genes; *PTPN11, SOS1, KRAS,* and *RAF1*. The data was subsequently analyzed using Sequence pilot software. In silico prediction programs were used to predict the pathogenic effect of the genetic variants and classified according to existing guideline.

Result: Three out of eight patients consider had NS face. One patient, who showed cardinal symptoms of NS, carried mutation of PTPN11 gene: an A→C transition at position 317 in exon 3 indicating an Asp106Ala substitution. Other variants found in *SOS1, KRAS,* and *RAF1* were classified either polymorphism or unlikely to be pathogenic. The remaining five patients considered as not having NS face.

Conclusion: The molecular analysis in this study revealed NS in one out of eight Turner phenotype patients with normal karyotype. No pathogenic variant found in other patients may indicate that there is either a novel unidentified gene for NS or the patients have another disorder and not NS.

Keyword: Noonan syndrome, *PTPN11, SOS1, KRAS, RAF1*, ion semiconductor sequencing

ABSTRAK

Pendahuluan: Sindrom Noonan (NS) merupakan kelainan genetik yang ditandai dengan dismorfik pada wajah, kelainan jantung bawaan, dan perawakan pendek. Pada awalnya, NS disebut sebagai pseudo sindrom Turner (TS) dikarenakan kemiripian fenotipenya. Namun demikian, berbeda dengan TS, NS tidak termasuk kedalam kelainan kromosom dan dapat terjadi pada laki-laki dan perempuan. Mutasi pada gen-gen yang berperan dalam jalur RAS-MAPK menyebabkan terjadinya NS. Sekuensing dilakukan untuk menemukan mutasi pada gen tersebut.

Metode: Delapan pasien yang memiliki fenotipe turner dan kariotipe normal diikutkan dalam penelitian ini. Tampilan klinis pasien dibandingkan dengan sistem skoring NS. DNA di sekuens dengan menggunakan *automated ion semiconductor sequencing* untuk empat gen utama penyebab NS yaitu *PTPN11*, *SOS1, KRAS*, dan *RAF1*. Data selanjutnya dianalisis menggunakan *Sequence pilot software*. Prediksi efek patogenik dari varian yang ditemukan adalah dengan menggunakan program komputer dan diklasifikasi berdasarkan pedoman yang telah ada.

Hasil: Tiga dari delapan pasien dipertimbangkan memiliki tampilan wajah NS. Satu pasien dengan tanda kardinal NS memiliki mutasi gen *PTPN11*: terjadi perubahan basa A → C di posisi ke 317 pada ekson 3 menyebabkan substitusi Asp106Ala. Varian lain yang ditemukan pada gen *SOS1, KRAS,* dan *RAF1* diklasifikasikan sebagai polimorfisme atau tidak patogenik. Lima pasien lainnya dipertimbangkan tidak memiliki tampilan wajah NS.

Kesimpulan: Analisis molekular dalam penelitian ini menemukan NS pada satu dari delapan pasien yang memiliki fenotipe turner dan kariotipe normal. Pada pasien lainnya, tidak ditemukan varian patogenik. Hal ini menunjukkan kemungkinan terdapat gen NS lain yang belum teridentifikasi atau pasien tersebut memiliki kelainan lainnya dan bukan NS.

Kata Kunci: Sindrom Noonan, *PTPN11, SOS1, KRAS, RAF1,* ion semiconductor sequencing