MOLECULAR ANALYSIS OF INDONESIAN LCA PATIENTS AND *IN VITRO* SPLICE CORRECTION FOR *CEP290*-ASSOCIATED LCA

ANALISIS MOLEKULER PADA PASIEN LCA *INDONESIA DAN KOREKSI PROSES SPLICING SECARA* IN VITRO *PADA LCA TERKAIT CEP290*

THESIS

Submitted to fulfill the assignment and fit-out requisite in passing Post Graduate Program

Master of Biomedical Science

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FACULTY OF MEDICINE
DIPONEGORO UNIVERSITY SEMARANG
2015

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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.

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ACKNOWLEDGEMENT

It is an honor for me to express my gratitude to everyone who have helped and supported me to finish this thesis. First of all, I would like to thank to Prof. Dr. Sultana MH Faradz, Ph.D for her advice, guidance, and support in my master program from the beginning till the end despite her own tight schedule. Thank you to dr. Kentar A Sulakso, Sp.M, MSi.Med for his support, clinical advice, and supervision in writing this thesis.

I would also like to express my gratitude to Prof. Rob WJ Collin, head of Blindness Genetic Therapy research group in the Department of Human Genetics of Radboud University Medical Centre, The Netherlands, for his kind supervision during my internship there. I am so thankful to Alex Garanto, Ph.D for his superb teaching and explanation to all that matters in my research.

It was a great experience to do research in the blindness genetic therapy lab with the help from Lonneke Duijkers as a technician, Galuh Astuti, Julio CC Serrano, Muriel Messchaert, as a Ph.D student, Manon Oud as a MSc student, and Arjen Henkes as bioinformatician.

I would also like to thank all the people from Center for Biomedical Research (CEBIOR), Semarang, Indonesia, Rita Indriati, Inthus Apriasa, Evi Nurwulan, Lusi Suwarsi, Wiwik Lestari, and Dwi Kustiani for their assistance during my master study.

I will always be grateful to be a part of Batch 7 of Masters in Biomedical Science Majoring in Genetic Counseling together with my beloved wife, Iffa Mutmainah, Mitayani, Catharina Endah W, Nura Eky V, Cinta C Rudianto, and Rendy R Pohan. Your warm companion supports me to complete my study.

My utmost gratitude and sincere thanks goes to my beloved parents, Eko Bambang Setyawan, S.E, and Dyah Adiati Warsitosiwi for all the genuine love and limitless support throughout the study. I will never be able to redeem everything you have done for me.

The opportunity to be a part of Master Student in CEBIOR and to have my research done In The Netherlands would not have been possible without the scholarship from Biro Perencanaan Kerja Sama Luar Negeri (BPKLN), Ministry of Education, Indonesia, especially DR. AB Susanto, MSc. as the coordinator of Beasiswa Unggulan. Finally, I would like to

thank all of the Master's degree and Fellowship coordinators, especially Prof. Dr. Sultana MH Faradz, Ph.D, dr. Tri Indah Winarni MSi.Med, Ph.D, and Ms. Ardina Apriliani S.E.

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CONTENTS

ABBREVIATIONS

2’-MOE	2’Methoxy-Ethoxy
2’-OMe	2’-O-Methyl
AAV/rAAV	Adeno-Associated Virus / Recombinant AAV
ABCA4	Adenosine Triphosphate-Binding Cassette, Sub-Family A, Member 4
ACTB	Actin Beta
ACHM	Achromatopsia
AIPL1	Aryl Hydrocarbon Receptor Interacting Protein-like 1
AON	Antisense Oligonucleotide
ARMS-PCR	Amplification Refractory Mutation System - PCR
BBS4	Bardet-Biedl Syndrome 4
BCA	Bicinchonic Acid
BSA	Bovine Serum Albumin
CABP4	Calcium Binding Protein 4
Cap	Capsid
CB	Chicken Beta actin
CEP290	Centrosomal Protein 290 kDa
CD	Cone Dystrophy
CEBIOR	Centre for Biomedical Research
cGMP	Cyclic Guanosine Monophosphate
CHM	Choroderemia
CK30PEG-NP	CK30 Polyethylene Glycol-Nano Particles
CORS	Cerebello-Oculo-Renal Syndrome
CRB1	Crumbs Family Member 1
CRD	Cone-Rod Dystrophy

CRX	Cone-Rod Homeobox
DAPI	4', 6-diaminidino-2-phenylindole
DMEM	Dulbecco's Modified Eagle Medium
DNA/cDNA	Deoxyribo Nucleic Acid / Complementary DNA
DTHD1	Death Domain Containing 1
EDTA	Ethylendiaminetetraacetic acid
EIAV	Equine Infectious Anemia Virus
ERG	Electroretinogram
ESE-Finder	Exonic Splicing Enhancer-Finder
GDF6	Growth Differentiation Factor 6
GT	Gene Therapy
GUCY2D	Guanylate Cyclase 2D
hRPE65	Human RPE65
IBD	Identity by Descent
IMPDH1	Inosine 5'-Monophosphate Dehydrogenase 1
iPSCs	Induced Pluripotent Stem Cells
IRD	Inherited Retinal Dystrophies
ITRs	Inverted Terminal Repeats
IQCB1	IQ Motif Containing B1
JSRD	Joubert Syndrome and Related Disorders
kDa	Kilodalton
KCNJI13	Potassium Inwardly-Rectifying Channel, Subfamily J, Member 13
LCA	Leber Congenital Amaurosis
LCA5	Leber Congenital Amaurosis 5
LINE	Long Interspersed Nuclear Elements
LP	Light Perception
LRAT	Lecithin Retinol Acyltransferase

LV	Lentivirus
MEM	Minimum Essential Media
MERTK	c-mer Proto-Oncogen Tyrosine Kinase
MIPs	Molecular Inversion Probes
MKS	Meckel-Gruber Syndrome
MYO7A	Myosin VIIA
NaCl	Natrium Chloride
NMNAT1	Nicotinamide Nucleotide Adenylyltransferase 1
NP	Nano Particles
NUB1	NEDD8 Ultimate Buster 1
OMIM	Online Mendelian Inheritance in Man
OTX2	Orthodenticle Homeobox 2
PBS	Phosphate-Buffered Saline
POD	Peptide Ocular Delivery
PPRPE	Preserved Para-arteriolar Retinal Pigment Epithelium
PVDF	Polyvinylidine Difluorida
RD3	Retinal Degeneration 3
RDH12	Retinol Dehydrogenase 12
Rep	Replication
REP1	Rab Escort Protein 1
RFLP	Restriction Fragment Length Polymorphism
RIPA	Radio Immunoprecipitation Assay
RNA/mRNA	Ribo Nucleic Acid / Messenger RNA
RP	Retiniti Pigmentosa
RPE	Retinal Pigment Epithelium
RPE65	Retinal Pigment Epithelium-Specific Protein 65 kDa
RPGRIP1	Retinitis Pigmentosa GTPaseRegulator Interacting Protein 1

RT-PCR	Reverse Transcription - PCR
sFLT01	Soluble Fms-Like Tyrosine Kinase 1 Receptor
siRNA	Small Interference RNA
SLS	Senior-Loken Syndrome
SNc	Substantia Nigra pars Compacta
SPATA7	Spermatogenesis Associated 7
SRp55	Serine-Arginine rich Protein 55 kDa
SRp40	Serine-Arginine rich Protein 40 kDa
STGD	Stargardt's Disease
TPR	Tetratricopeptide Repeat
TULP1	Tubby-like Protein 1
VMD2	Vitelliform Macular Dystrophy 2
WT	Wild Type

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GLOSSARY

PCR	Polymerase Chain Reaction. Molecular technique to amplify a single or multiple copies of a piece of DNA over several orders of magnitude.
RFLP	Restriction Fragment Length Polymorphism. A variation in the length of restriction fragments formed from a certain restriction enzyme in a sample of DNA
AON	Antisense Oligonucleotide. A synthetic polymers which are created from 15-20 chemically-modified deoxynucleotides or ribonucleotides in an antisense sequence ($3' \rightarrow 5'$), complementary to the sense sequence of an mRNA.
Centrosome	An organelle close to the nucleus of a cell that consist of the centrioles and from which the spindle fibers develop in cell division.
LCA	Leber Congenital Amaurosis. A genetic disorder that mainly affects the retina and cause severe visual impairment to blindness.

ABSTRACT

Background: Leber congenital amaurosis (LCA) is an autosomal recessive retinal disorder, characterized by an early-onset visual loss, amaurotic pupils, and retinal degeneration. Only a few studies have been described about Indonesian LCA patients. Pre-clinical studies have shown that antisense oligonucleotide (AON) restored the aberrant splicing caused by a recurrent intronic mutation in the *CEP290* gene. This study combined diagnostic study in Indonesian LCA patients and in-vitro AON-based therapy for LCA.

Methods: *CEP290*, *CRB1*, *GUCY2D*, and *AIPL1* genes were screened in 4 LCA patients from 3 unrelated families by amplification refractory mutation system (ARMS) PCR or Sanger sequencing. Meanwhile, 29 different AONs (GT1 to GT29) with two chemistries were designed and tested by RT-PCR, Western blot, and immunocytochemistry.

Results: Three variants were found in *AIPL1* gene: p.E318G, p.R324R, and p.G11G. Interestingly, p.E318G was predicted as a disease causing mutation by MutationTaster program while p.R324R and p.G11G were predicted to cause a splicing alteration by ESE-finder splicing prediction. On the other hand, from 29 AON tested in this study, 4 AONs worked efficiently in correcting the aberrant splicing, increasing the CEP290 protein, and restoring the cilium length, at low concentrations.

Conclusions: The molecular genetic analysis of Indonesian LCA patients in this study revealed three new candidate variants as a LCA causative mutation in *AIPL1*: p.E318G, p.R324R, and p.G11G. From 29 AONs tested in this study, GT2, GT3, and GT4 were the most efficient. Remarkably, these AONs shared the same chemical modification.

Keywords: Leber congenital amaurosis, splicing mutation, antisense oligonucleotide, *CEP290*

ANALISIS MOLEKULER PADA PASIEN LCA INDONESIA DAN KOREKSI PROSES *SPLICING* SECARA *IN VITRO* UNTUK LCA TERKAIT *CEP290*

WIDYA EKA NUGRAHA

ABSTRAK

Latar Belakang: Leber Congenital Amaurosis (LCA) adalah suatu penyakit genetic yang diturunkan secara autosomal resesif dan ditandai dengan hilang penglihatan sejak dini, pupil yang amaurotik, dan degenerasi retina. Penelitian tentang pasien LCA di Indonesia masih terbatas. Sementara, studi pre-klinis menunjukkan bahwa penggunaan *antisense oligonucleotide* (AON) dapat mengembalikan transkrip abnormal pada mutasi gen *CEP290* yang menjadi penyebab tersering penyakit LCA. Penelitian ini menggabungkan studi diagnostik pada pasien LCA Indonesia sekaligus koreksi proses splicing secara *in vitro* menggunakan AON.

Metode: Strategi identifikasi mutasi melibatkan gen *CEP290, CRB1*, *GUCY2D*, dan *AIPL1* pada 4 pasien dari 3 keluarga yang berbeda. Teknik molekuler yang digunakan yaitu *amplification refractory mutation system* (ARMS) PCR atau Sanger sequencing. Sementara itu, 29 molekul AON yang berbeda (GT1-GT29) diuji-coba menggunakan teknik *reverse transcriptase* (RT) PCR, *Western blot*, dan *immunocytochemistry*.

Hasil: Ditemukan tiga varian pada gen *AIPL1*: p.E318G, p.R324R, dan p.G11G. Varian p.E318G diprediksi sebagai mutasi penyebab penyakit dengan program *MutationTaster* sementara varian p.R324R dan p.G11G diprediksi menyebabkan gangguan proses *splicing* oleh program *ESE-finder splicing prediction*. Studi fungsional menunjukkan bahwa dari 29 AON yang diuji-cobakan. GT2, GT3, dan GT4 merupakan AON yang paling efisien dalam mengoreksi proses *splicing*, meningkatkan protein CEP290, dan mengembalikan panjang *cilium* pada konsentrasi minimum.

Kesimpulan: Analisis molekuler pada studi ini mengungkapkan tiga kandidat varian baru sebagai mutasi penyebab LCA untuk gen *AIPL1*: p.E318G, p.R324R, dan p.G11G. Dari 29 AON yang diuji, GT2, GT3, GT4 adalah yang paling efisien. Ketiga AON memiliki kerangka molekul yang sama.

Kata kunci: Leber congenital amaurosis, mutasi *splicing*, *antisense oligonucleotide*, *CEP290.*