ABSTRACT

Congenital hypothyroidism (CH) is a public health concern affecting 1 / 3000 - 4000 newborn babies. In reference to this, *thyroid peroxidase* (*TPO*) abnormality, typically inherited as autosomal recessive traits was found to be one of the causes of dyshormonogenetic CH. Our group had previously identified a homozygous c.1159G>A mutation in exon 8 of the *TPO* gene of CHP41. In this study, the *TPO* gene of CHP41's family members was screened for the c.1159G>A mutation and the results showed that all family members carried the same mutation either in homozygous or heterozygous forms. In addition, another 20 unrelated cases of dyshormonogenetic CH were also included in this study. DNA sequence analysis of the *TPO* gene in these 20 unrelated patients revealed the presence of five *TPO* mutations: three were novel (c.670_672del in exon 7, c.1186C>T in exon 8 and c.1502T>G in exon 9) while another two had been previously reported (c.2268dup in exon 13 and c.2647C>T in exon 16). Moreover, 12 polymorphisms including two that are novel (c.1-192C>A in a GC box and c.180-6C>A at 6 bp upstream of exon 4), were also found in the 21 unrelated patients. This study shows that only individuals associated with either homozygous or compound heterozygous form of *TPO* mutation were affected with dyshormonogenetic CH whereas family members of patients with one mutant allele remained asymptomatic. *In silico* functional analyses indicated that all of the six mutations affected normal activity of TPO protein. Furthermore, the novel c.180-6C>A polymorphism is predicted to reduce the intrinsic strength of the natural splice site of exon 4 which could lead to an activation of other potential splice sites. Meanwhile, it is also believed that the novel c.1-192C>A polymorphism in the GC box might alter the expression levels of *TPO* gene in an individual. Further investigation on patients with c.2268dup mutation through biochemical and gene expression analyses confirmed the devastating effects of

the mutation. A novel TPO mRNA transcript which was believed to be associated with nonsense-associated altered splicing (NAS) mechanism was detected in patients associated with the c.2268dup mutation. In addition, lower expression of TPO protein was also detected in thyroid tissues with lesions compared to those of normal areas in the same patients with c.2268dup. In conclusion, mutations in the *TPO* gene are an underlying genetic cause of CH with dyshormonogenesis in the current cohort of patients.

ABSTRAK

Masalah hipotiroidisme kongenital (CH) merupakan penyakit kesihatan global yang menjejaskan kesihatan bayi yang baru lahir pada kadar 1 / 3000 - 4000. Ketidaknormalan gen *thyroid peroxidase* (*TPO*) yang diwarisi secara resesif autosomal telah didapati sebagai salah satu punca masalah CH yang diakibatkan oleh kelenjar tiroid yang tidak berfungsi atau berfungsi sebahagian sahaja. Kajian terdahulu yang telah kami jalankan telah mengenal pasti sejenis mutasi pada ekson 8 di gen *TPO* yang dikenali sebagai c.1159G>A pada pesakit CHP41. Dalam kajian ini, penyaringan mutasi gen yang sama telah dijalankan terhadap ahli keluarga CHP41 dan hasil kajian menunjukkan bahawa semua ahli keluarga membawa mutasi yang sama, sama ada dalam bentuk homozigus atau heterozigus. Di samping itu, kajian ini juga meneruskan usaha untuk mengenal pasti mutasi-mutasi gen *TPO* yang menyebabkan masalah CH di kalangan pesakit-pesakit lain yang mempunyai kelenjar tiroid. Analisis terhadap turutan DNA di gen *TPO* daripada 20 orang pesakit yang berasingan menunjukkan kewujudan lima jenis mutasi, di mana tiga jenis mutasi (c.670_672del di ekson 7, c.1186C>T di ekson 8 and c.1502T>G di ekson 9) adalah penemuan terbaru (novel) manakala dua jenis mutasi lagi (c.2268dup di ekson 13 and c.2647C>T di ekson 16) telah dilaporkan. Selain itu, 12 polimorfisme yang lain termasuk dua polimorfisme novel (c.1-192C>A di kotak GC dan c.180-6C>A yang terletak di tempat 6 bp sebelum ekson 4) juga ditemui dalam kajian ini. Kajian ini menunjukkan bahawa hanya individu yang dikaitkan dengan mutasi *TPO* dalam bentuk homozigus atau heterozigus ganda (compound heterozygous) mempunyai masalah CH manakala ahli keluarga mereka yang membawa satu alel mutan kekal asimptomatik. Analisis *in silico* menunjukkan bahawa semua enam mutasi menjejaskan aktiviti mRNA ataupun protein TPO. Tambahan pula, penemuan polimorfisme c.180-6C>A diramalkan akan menurunkan kadar kekuatan

intrinsik bagi tapak pemotongan (splice site) yang semulajadi pada ekson 4 dan akan mengakibatkan pengaktifan tapak pemotongan lain yang lebih berpotensi. Seterusnya, polimorfisme c.1-192C>A yang terletak di kotak GC juga dipercayai akan mempengaruhi tahap ekspresi gen *TPO*. Kajian selanjutnya terhadap mutasi c.2268dup melalui analisis biokimia dan ekspresi gen telah membuktikan kesan buruk daripada mutasi tersebut dan mendedahkan sesuatu spesies mRNA TPO novel yang dipercayai dikaitkan dengan mekanisme NAS. Selain itu, analisis terhadap kadar ekspesi protein TPO daripada pesakit-pesakit yang mempunyai mutasi c.2268dup menunjukan kadar ekspesi yang lebih rendah di kawasan tisu yang tidak normal berbanding dengan tisu yang diambil dari kawasan yang normal. Kesimpulannya, kajian ini menunjukkan bahawa mutasi dalam gen *TPO* merupakan sesuatu punca masalah CH di kalangan pesakit yang mempunyai kelenjar tiroid.

ACKNOWLEDGEMENTS

My deep gratitude goes first to Assoc. Prof. Dr. Sarni Mat Junit, who had expectedly guided me and gave the advice, support and tremendously encouragements throughout my postgraduate education. Dr. Sarni's mentoring and encouragements have been especially valuable, and her early insights contributed to the success of this project.

Heartfelt thanks to Prof. Dr. Fatimah Harun from the Department of Paediatrics, University of Malaya Medical Centre for providing the patient's blood samples and clinical consultation. Her unwavering enthusiasm for congenital hypothyroidism study kept me constantly engaged with my research, and her personal generosity helped made my time in University of Malaya. I would also like to thank Dr. Muhammad Yazid Bin Jalaludin from Department of Paediatrics for his assistance in collecting the blood samples and with the clinical data. His kindness and an inspirational style also help in sustaining a positive atmosphere in which to do research.

I am very grateful to all members of the Molecular Biology Laboratory and Lipid and Nutrition Laboratory especially Yasmin, Ursula, Christina, Chor Yin, Nani, Kong and other members from the Department of Molecular Medicine for their help and time. Also, I would like to thank Dr. Rozana and Choon Han from the Department of Pharmacy for their superb technical assistance in designing three dimensional model of TPO protein. I would also like to express my gratitude to the university of Malaya for granting me with the UM Fellowship Award.

Last but not least, I wish to extend my heartfelt gratitude to my parents for their love, patience and unconditional supports. Without them, I would not have gone this far in life.

TABLE OF CONTENTS **PAGE**

LIST OF FIGURES **PAGE**

LIST OF TABLES **PAGE**

ABBREVIATIONS

A (a)	adenine
Ala	alanine
APS	ammonium persulfate
Arg	arginine
Asn	asparagine
Asp	aspartic acid
bp	base pair
BSA	bovine serum albumin
Bis	N, N'-methylene-bis-acrylamide
C (c)	cytosine
C (cell)	calcitonin-producing parafollicular
C (product)	concentration
C (terminal)	carboxyl-terminus
CH	congenital hypothyroidism
CHP	congenital hypothyroidism patient
CCP	complement control protein
cDNA	complementary DNA
cm	centimeter
CO_2	carbon dioxide
ddH_2O	double-distilled water
dl	deciliter
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleoside triphosphates

DTT	dithiothreitol
DUOX2	dual oxidase 2
EDTA	ethylenediaminetetraacetate
EGF	epidermal growth factor
ESE(s)	exonic splicing enhancer(s)
ESSs	exonic splicing suppressors
et al.	et alia (and others)
EtBr	ethidium bromide
FNAB	fine needle aspiration biopsy
FT_4	free T_4
G (g)	guanine
g	gram
g	gravity
Gln	glutamine
Glu	glutamic acid
Gly	glycine
h	hour(s)
H_2O_2	hydrogen peroxide
HCl	hydrochloride
His	histidine
HSF	Human Splicing Finder
hTG	human thyroglobulin
I^-	iodide
I^+	iodinium
ICH-GCP	International Conference on Harmonisation-Good Clinical Practice

Ile	isoleucine
IQ	intelligence quotient
K^+	potassium ion
kbp	kilo base pair
KCl	potassium chloride
kDa	kiloDalton
K_3 EDTA	ethylenediamine tetraacetate
kg	kilogram
L	litre
Leu	leucine
LPO	lactoperoxidase
L-T_4	levothyroxine
L-T_3	liothyronine
Lys	lysine
m	mili
M	molar
Met	methionine
MeOH	Methanol
U (u)	uracil
µIU	microinternational units
µg	microgram
mg	milligram
$MgCl_2$	magnesium chloride
min	minute(s)
ml	milliliter
µl	microlitre

mcg	microgram
mM	millimolar
MNG	multinodular goitre
MPO	myeloperoxidase
mRNA	messenger ribonucleic acid
N (terminal)	amino-terminus
Na^+	sodium ions
NaCl	sodium chloride
NaOH	sodium hydroxide
NAS	nonsense-associated altered splicing
NCBI	National Center for Biotechnology Information
NIS	sodium iodide symporter
NTH	Neonatal transient hypothyroidism
nmol	nanomolar
OD_{230}	absorbance at 230 nm
OD_{260}	absorbance at 260 nm
OD_{280}	absorbance at 280 nm
OD_{470}	absorbance at 470 nm
OD_{595}	absorbance at 595 nm
PAGE	polyacrylamide gel
PAX-8	paired box gene 8
PBS	phosphate buffered saline
PCR	polymerase chain reaction
Phe	phenylalanine
PIOD	partial organification defect
pmol	picomole

Polyphen-2	Polymorphism Phenotyping-version 2
Pro	proline
ProQ	Protein Quality Predictor
PVDF	polyvinylidene fluoride
qRT-PCR	quantitative real time-polymerase chain reaction
RT-PCR	reverse transcription-polymerase chain reaction
SCN	solid cell nest
SDS	sodium dodecyl sulfate
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
sec	second(s)
Ser	serine
SIFT	Sorting Intolerant From Tolerant
SLC5A	solute carrier family 5
SNP	single nucleotide polymorphism
t	time
T (t)	thymine
T_3	3, 5, 3'-triiodothyronine
T_4	3, 5, 3', 5'-tetraiodothyronine or thyoxine
Taq	*Thermus aquaticus*
TAE	tris-acetate-EDTA
TBP	tata box binding protein
tcRNA	total cellular RNA
TDH2A	thyroid dyshormonogenesis 2A
TEMED	tetramethylethylenediamine
TFT	thyroid function test
Tg	thyroglobulin

Thr	threonine
TIOD	total iodide organification defect
TNH	transient neonatal hypothyroidism
TPO	thyroid peroxidase
TRH	thyroid releasing hormone
Trp	tryptophan
TSH	thyroid stimulating hormone
TSHB	thyroid stimulating hormone, beta
TSHR	thyroid stimulating hormone receptor
TTF-1	thyroid transcription factor-1
TTF-2	thyroid transcription factor-2
Tyr	tyrosine
UMMC	University of Malaya Medical Central
UniProt	Universal Protein Resource
UV	ultraviolet
V	volt
Val	valine
v/v	volume over volume
w/v	weight over volume
3-D	three dimensional
^{99}Tm	Technetium-99m
℃	degree centigrade
Δ	Delta

LIST OF APPENDICES **PAGE**