APPENDIX A

Publication

Ching Chin Lee, Fatimah Harun, Muhammad Yazid Jalaludin, Choon Han Heh, Rozana Othman, and Sarni Mat Junit, A novel, homozygous c.1502T>G (p.Val501Gly) mutation in the *thyroid peroxidase* gene in Malaysian Sisters with congenital hypothyroidism and multinodular goiter, International Journal of Endocrinology, vol. 2013, Article ID 987186, 7 pages, 2013. doi:10.1155/2013/987186 (*ISI-Cited Publication*)

Ching-Chin Lee, Muhammad-Yazid Jalaludin, Fatimah Harun, Chor-Yin Lim, Khoon-Leong Ng and Sarni Mat-Junit. Functional analyses of c.2268dup in *TPO* gene associated with goitrous congenital hypothyroidism (Journal of Molecular Endocrinology: Submitted)

APPENDIX B

List of posters presented in this study

Lee Ching Chin, Fatimah Harun, Muhammad Yazid Jalaludin and Sarni Mat Junit. Nonsense-associated alternative splicing in exon 13 of *thyroid peroxidase* gene (*TPO*) as a consequence of a common c.2268dup mutation in Chinese patients with thyroid dyshormonogenesis. Proc. 36th Annual Conference of Malaysian Society for Biochemistry and Molecular Biology, Kuala Lumpur (2011), p83**.** (*Non-ISI/Non-SCOPUS Cited Publication*)

Muhammad Yazid Jalaludin, Lim Song Hai, Lee Ching Chin, Fatimah Harun, and Sarni Mat Junit. Follicular adenoma in goitrousc hypothyroidism due to *thyroid peroxidase* gene mutation in a Chinese patient. Proc. 50th Annual European Society for Paediatric Endocrinology Meeting, Glasgow (2011). (Accepted, *ISI Cited Publication*)

Lee Ching Chin, Kang In Nee, Fatimah Harun, Muhammad Yazid Jalaludin and Sarni Mat Junit. Characterisation of the human *thyroid peroxidase* gene mutation(s) in patients presented with congenital hypothyroidism, dyshormonogenesis and goiter. Proc. 35th Annual Conference of the Malaysian Society for Biochemistry and Molecular Biology, Kuala Lumpur,(2010), p80. (*Non-ISI/Non-SCOPUS Cited Publication*)

APPENDIX C

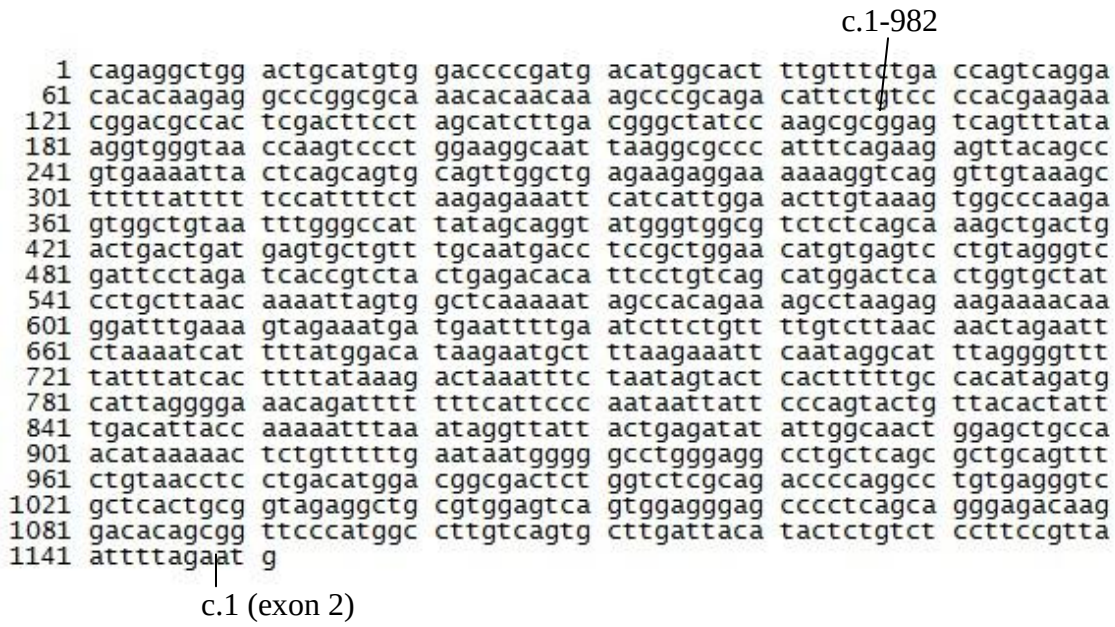
The published sequence of the:

1) Human thyroid peroxidase (TPO), transcript variant 1, mRNA (NCBI Reference Sequence: NM_000547.5)

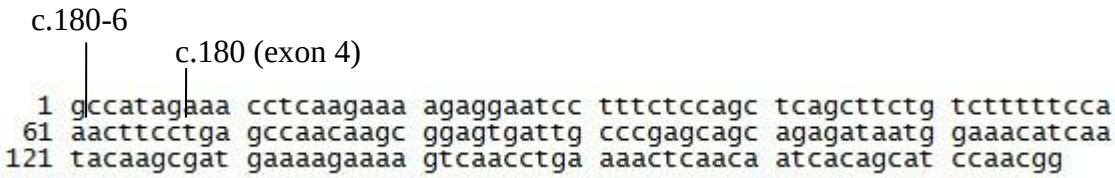
2) Human thyroid peroxidase (TPO), isoform 1, protein (Uniprot Reference Sequence: P07202-1)

```
        10         20         30         40         50         60
MRALAVLSVT LVMACTEAFF PFISRGKELL WGKPEESRVS SVLEESKRLV DTAMYATMQR

        70         80         90        100        110        120
NLKKRGILSP AQLLSFSKLP EPTSGVIARA AEIMETSIQA MKRKVNLKTQ QSQHPTDALS

       130        140        150        160        170        180
EDLLSIIANM SGCLPYMLPP KCPNTCLANK YRPITGACNN RDHPRWGASN TALARWLPPV

       190        200        210        220        230        240
YEDGFSQPRG WNPGFLYNGF PLPPVREVTR HVIQVSNEVV TDDDRYSDLL MAWGQYIDHD

       250        260        270        280        290        300
IAFTPQSTSK AAFGGGADCQ MTCENQNPCF PIQLPEEARP AAGTACLPFY RSSAACGTGD

       310        320        330        340        350        360
QGALFGNLST ANPRQQMNGL TSFLDASTVY GSSPALERQL RNWTSAEGLL RVHARLRDSG

       370        380        390        400        410        420
RAYLPFVPPR APAACAPEPG IPGETRGPCF LAGDGRASEV PSLTALHTLW LREHNRLAAA

       430        440        450        460        470        480
LKALNAHWSA DAVYQEARKV VGALHQIITL RDYIPRILGP EAFQQYVGPY EGYDSTANPT

       490        500        510        520        530        540
VSNVFSTAAF RFGHATIHPL VRRLDASFQE HPDLPGLWLH QAFFSPWTLL RGGGLDPLIR

       550        560        570        580        590        600
GLLARPAKLQ VQDQLMNEEL TERLFVLSNS STLDLASINL QRGRDHGLPG YNEWREFCGL

       610        620        630        640        650        660
PRLETPADLS TAIASRSVAD KILDLYKHPD NIDVWLGGLA ENFLPRARTG PLFACLIGKQ

       670        680        690        700        710        720
MKALRDGDWF WWENSHVFTD AQRRELEKHS LSRVICDNTG LTRVPMDAFQ VGKFPEDFES

       730        740        750        760        770        780
CDSITGMNLE AWRETFPQDD KCGFPESVEN GDFVHCEESG RRVLVYSCRH GYELQGREQL

       790        800        810        820        830        840
TCTQEGWDFQ PPLCKDVNEC ADGAHPPCHA SARCRNTKGG FQCLCADPYE LGDDGRTCVD

       850        860        870        880        890        900
SGRLPRVTWI SMSLAALLIG GFAGLTSTVI CRWTRTGTKS TLPISETGGG TPELRCGKHQ

       910        920        930
AVGTSPQRAA AQDSEQESAG MEGRDTHRLP RAL
```

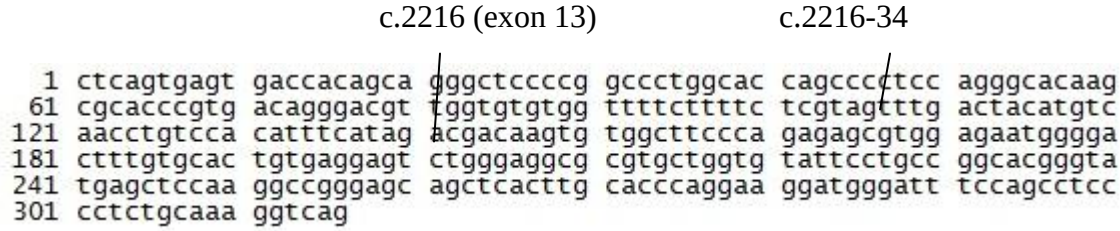
3) Human thyroid peroxidase (TPO), RefSeqGene on chromosome 2 (NCBI Reference Sequence: NG_011581.1, selected region from 4799 to 5949)



4) Human thyroid peroxidase (TPO), RefSeqGene on chromosome 2 (NCBI Reference Sequence: NG_011581.1, selected region from 24969 to 25146)



5) Human thyroid peroxidase (TPO), RefSeqGene on chromosome 2 (NCBI Reference Sequence: NG_011581.1, selected region from 87993 to 88308)



APPENDIX D

Recipe for stock solutions and general use buffers

1) Preparation of 50 X Tris-acetate-EDTA (TAE) buffer

For 1 L of 50 X TAE buffer, 40 mM tris base, 20 mM glacial acetic acid and 2 mM EDTA were mixed and the total volume was made up to 1 L with double distilled water. For agarose gel electrophoresis, the working concentration of 1X TAE buffer was used.

2) Preparation of 6 X Laemmli buffer

For 10 ml of 6 X Laemmli buffer, 1.2 g of SDS, 6 mg of bromophenol blue, 4.7 ml of glycerol, 1.2 ml of Tris 0.5 M pH 6.8 and 2.1 ml of ddH_2O were mixed and warmed until everything was dissolved. About 0.93 g of DTT was then added and completely dissolved in the buffer. The prepared buffer was aliquoted for several smaller tubes and stored at -20 ℃.

3) Preparation of 10 X SDS-PAGE running buffer

To prepare 10 X SDS-PAGE running buffer (25 mM Tris, 192 mM glycine, 0.1% SDS), 288g of glycine and 60.4 g of Tris base were dissolved in 1.8 L of ddH_2O. Twenty gram of SDS was then added and mixed into the solution. Finally, additional ddH2O was added to a final volume of 2 L.

4) Preparation of Coomassie stain

To prepare 200 ml of Coomassie blue stain (50 % MeOH, 10 % acetic acid, 0.05% Brillaint Blue R-250), 0.1 g of Brilliant Blue R-250 was first dissolved in 100 ml of MeOH. Twenty milliliter of acetic acid was then added to the solution. Finally, 80 ml of ddH2O was added to a final volume of 200 ml.

5) Preparation of 1 X Tris-glycine buffer

To prepare 2 L of 1 X Tris-glycine buffer (25 mM Tris, 192 mM glycine, 10 % methanol**)**, 28.8 g of glycine and 6.04 g of Tris base was first dissolved in 1.6 L of ddH2O. Two hundred milliliter of MeOH was then added to the solution. Finally, additional ddH2O was added to a final volume of 2 L.

APPENDIX E

Exon-exon boundary for all exons of the TPO variants

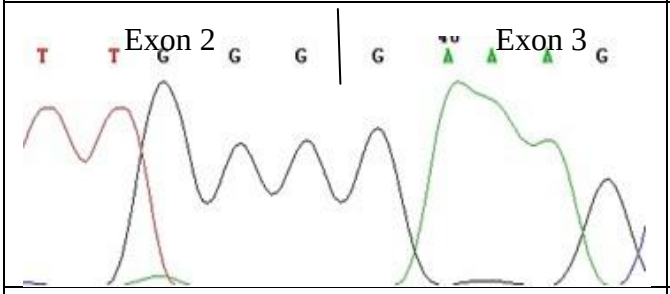
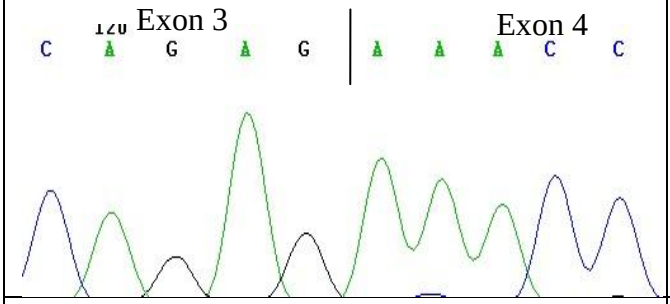
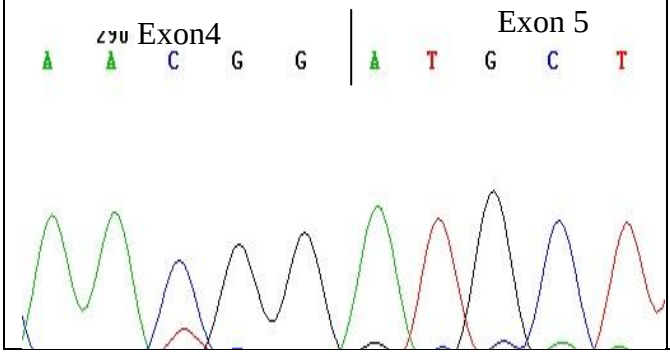
Electropherogram	Forward(F) / Reverse(R) sequencing	Exon-exon boundary	TPO
	F	2/3	1
	F	3/4	1
	F	4/5	1

Figure 1 Electropherograms showing exon-exon boundary for all exons (except exon 12 to exon 13 which had been shown in Figure 4.60) of TPO1 of the CHP33.

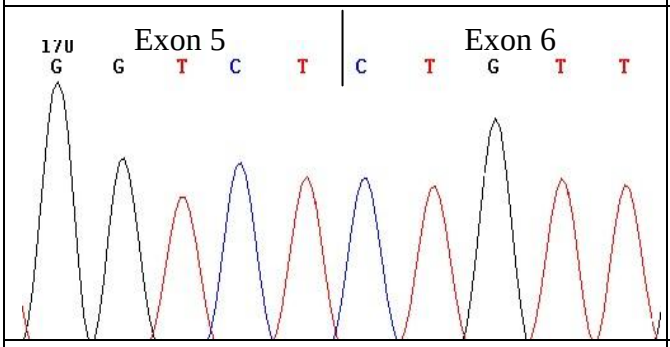
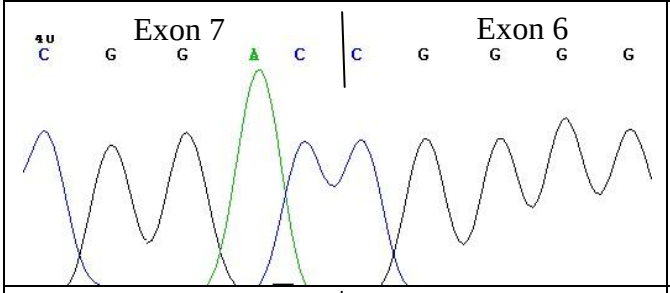
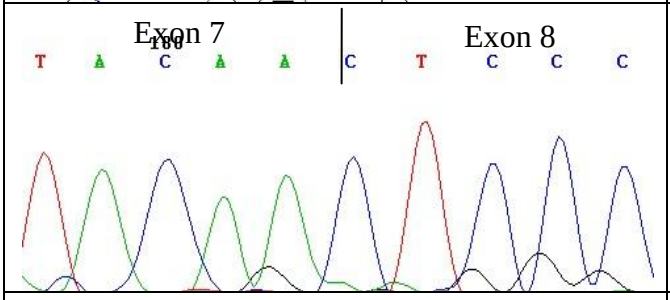
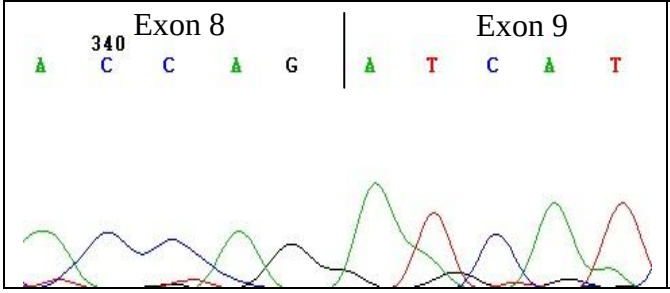
Electropherogram	Forward(F) / Reverse(R) sequencing	Exon-exon boundary	TPO
	R	6/5	1
	R	7/6	1
	F	7/8	1
	F	8/9	1

Figure 1 Continued.

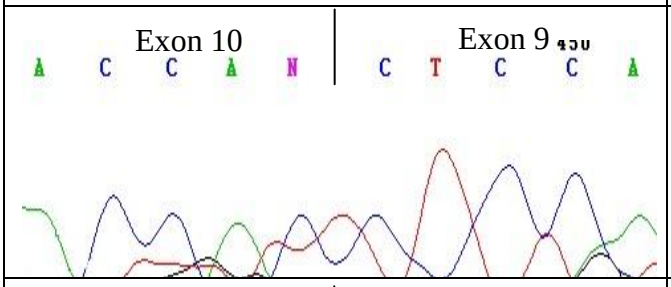
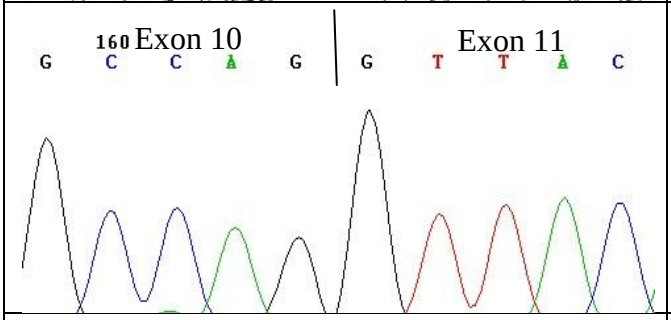
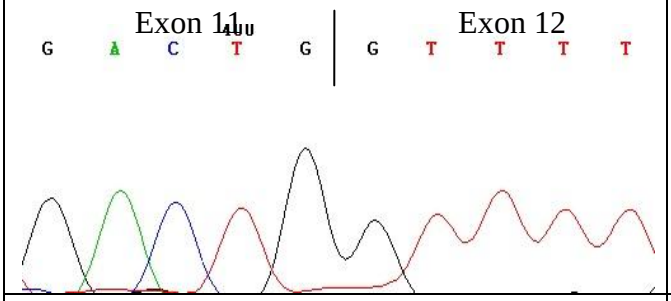
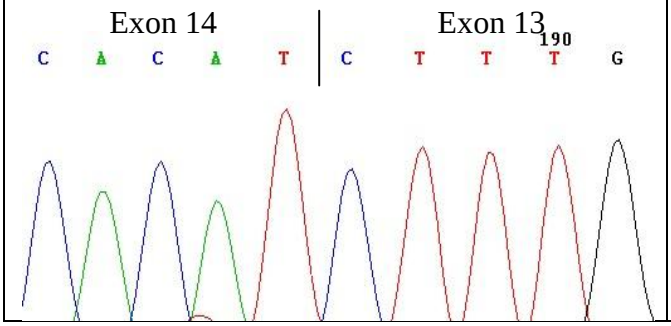
Electropherogram	Forward(F) / Reverse(R) sequencing	Exon-exon boundary	TPO
	R	10/9	1
	F	10/11	1
	F	11/12	1
	R	14/13	1

Figure 1 Continued.

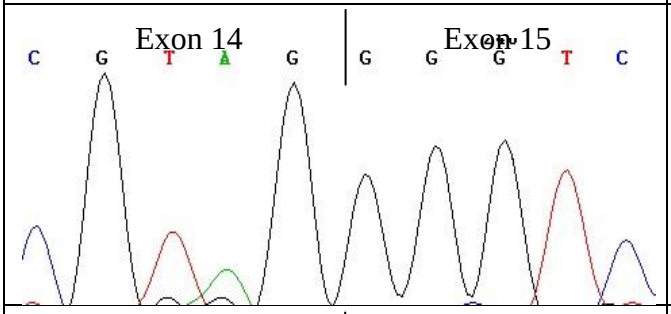
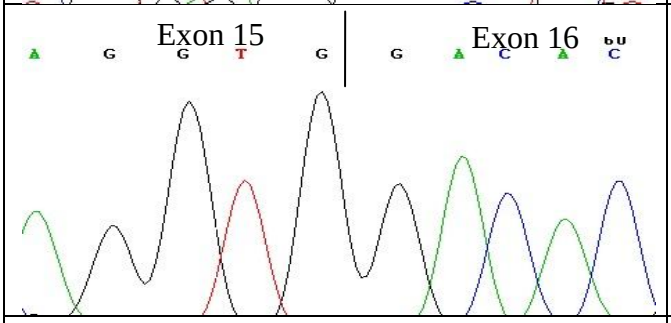
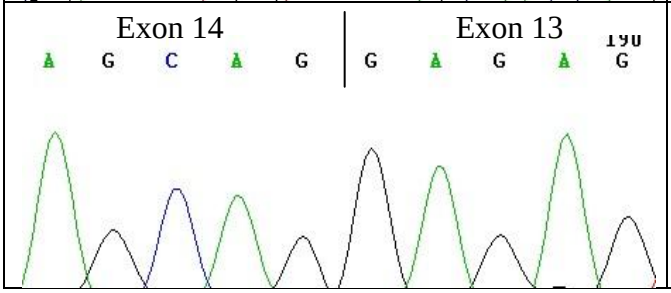
Electropherogram	Forward(F) / Reverse(R) sequencing	Exon-exon boundary	TPO
	F	14/15	1
	F	15/16	1
	F	16/17	1

Figure 1 Continued.

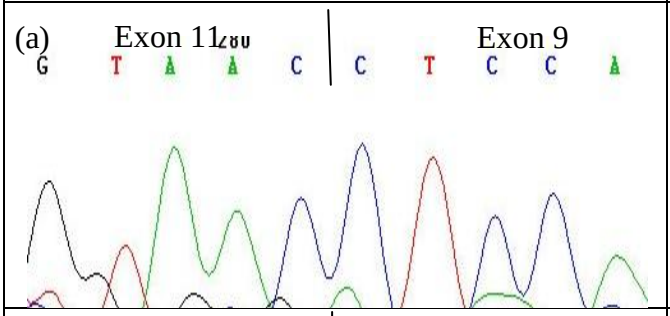
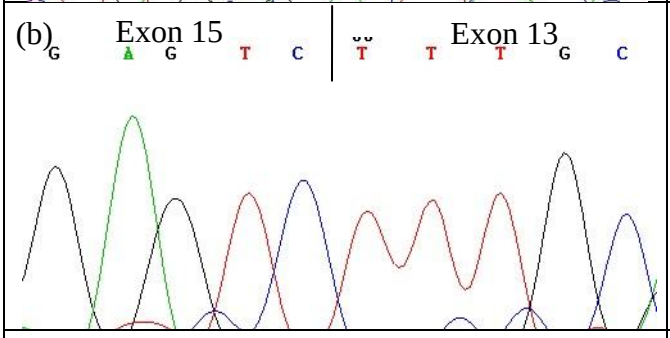
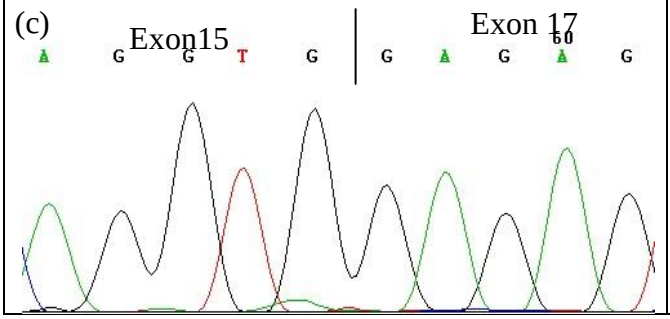
Electropherogram	Forward(F) / Reverse(R) sequencing	Exon-exon boundary	TPO
(a) Exon 11 | Exon 9 	R	11/9	2
(b) Exon 15 | Exon 13 	R	15/13	4
(c) Exon15 | Exon 17 	F	15/17	3

Figure 2 Electropherograms showing exon-exon boundary for a) exons 9/11 in TPO2, b) exon 13/15 in TPO4 and c) exons 15/17 in TPO3 of the CHP33.

APPENDIX F

BSA standard curve

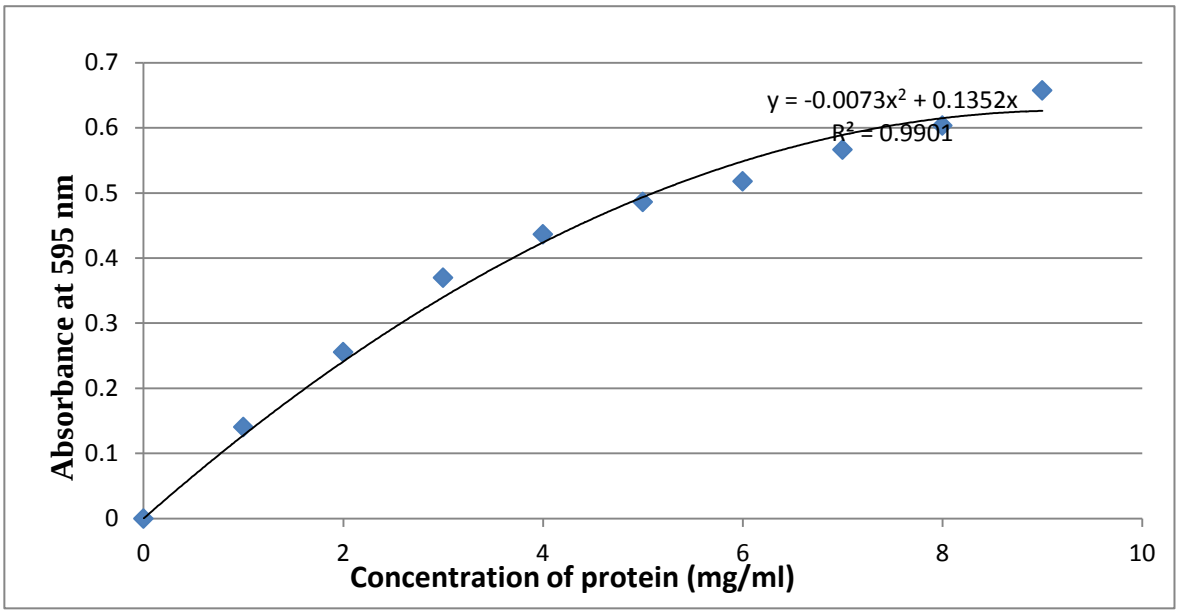
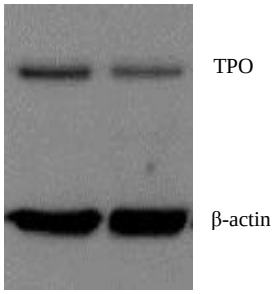
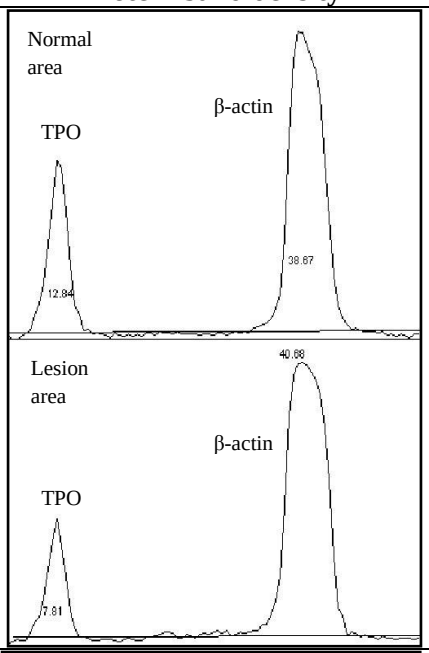
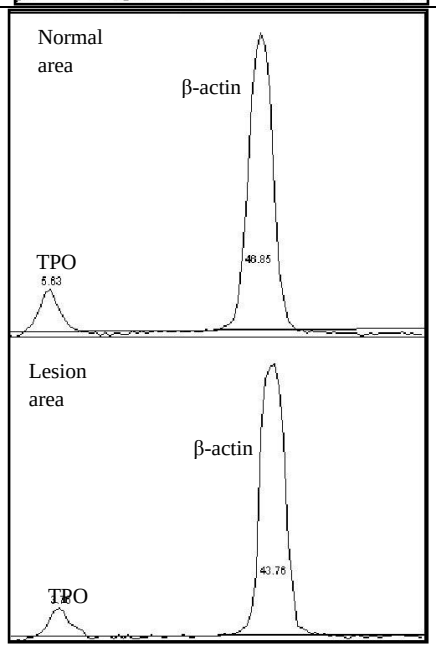


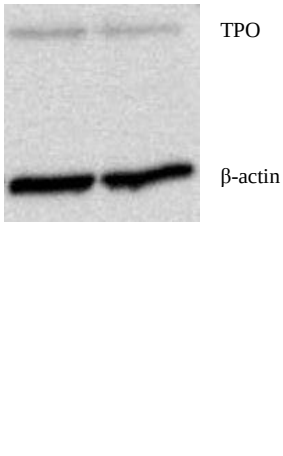
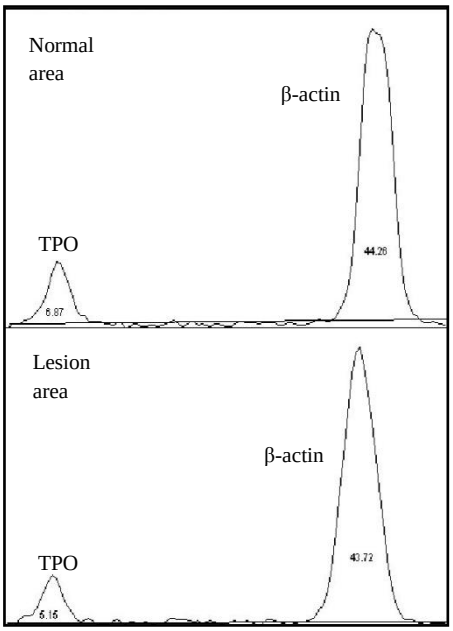
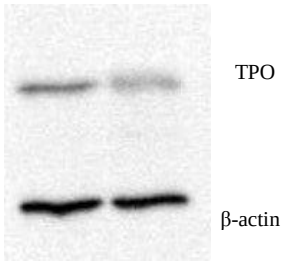
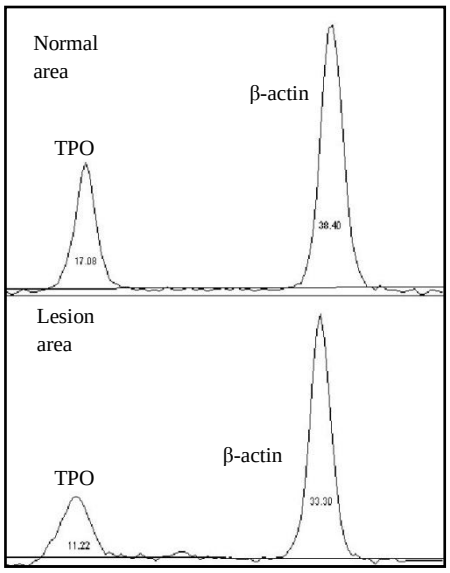
Figure 1 A standard curve of protein concentration versus absorbance at 595 nm. Bovine serum albumin (BSA) protein standard solution at a range of 0 mg/ml to 10.0 mg/ml was used. From the calibration curve, the protein concentration of the microsomal fraction extracts was to be estimated in the range of 1.12 µg/µl to 3.12 µg/µl.

APPENDIX G

The expression level of TPO protein in:

1) CHP33 (III-2)

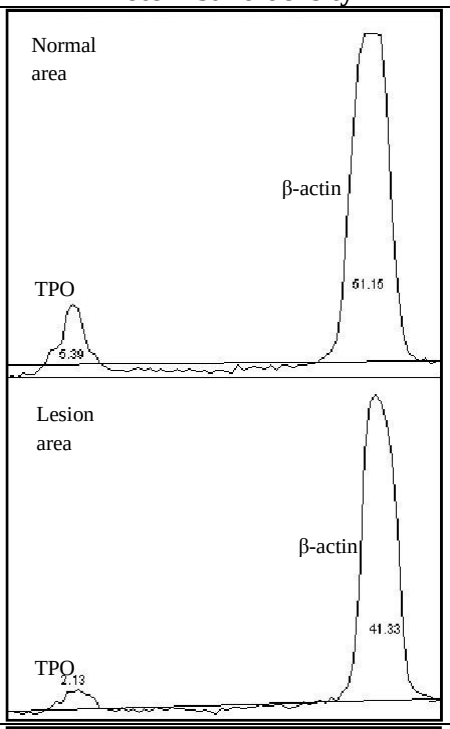
Experiment	Protein band density	Expression	
i 		**Relative Density** TPO/β-actin = 12.84/38.8 =0.33 TPO/β-actin = 7.81/40.68 =0.19	**Adjustment Density** lesion area / normal area = 0.19/0.33 X 100% = 57.58%
ii 		TPO/β-actin = 5.63/48.85 =0.12 TPO/β-actin = 3.76/43.76 =0.09	lesion area / normal area = 0.09/0.12 X 100% = 75%

Experiment	Protein band density	Expression	
		Relative Density	**Adjustment Density**
iii 		TPO/β-actin = 6.87/44.28 =0.16 TPO/β-actin = 6.16/43.72 =0.14	lesion area / normal area = 0.14/0.16 X 100% = 87.5%
iv 		TPO/β-actin = 17.08/38.4 =0.44 TPO/β-actin = 11.22/33.3 =0.34	lesion area / normal area = 0.34/0.44 X 100% = 77.27%

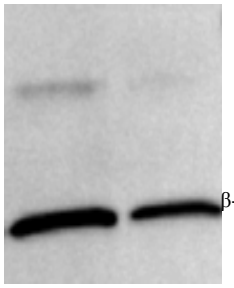
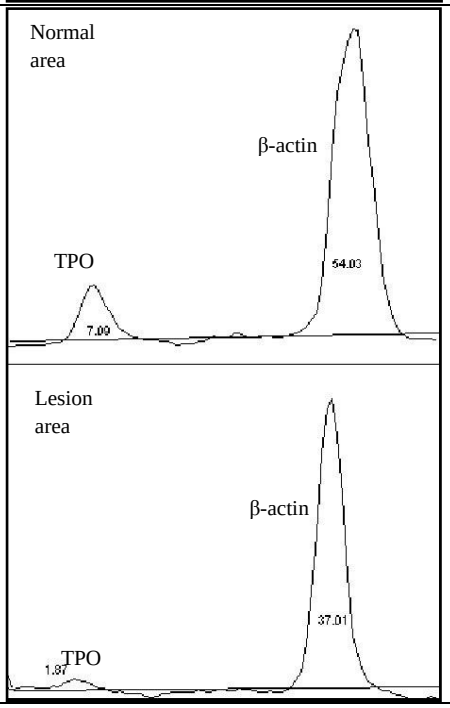
Experiment	**lesion area/normal area** Up-regulation if >100% ; Down-regulation of <100%	**Expression** (fold change)	**Average**
i	57.58%	1.74	1.36 ±0.26 fold (down regulation)
ii	75%	1.33	
iii	87.5%	1.14	
iv	77.27	1.29	

2) CHP33's sister (III-1)

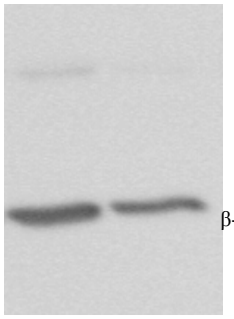
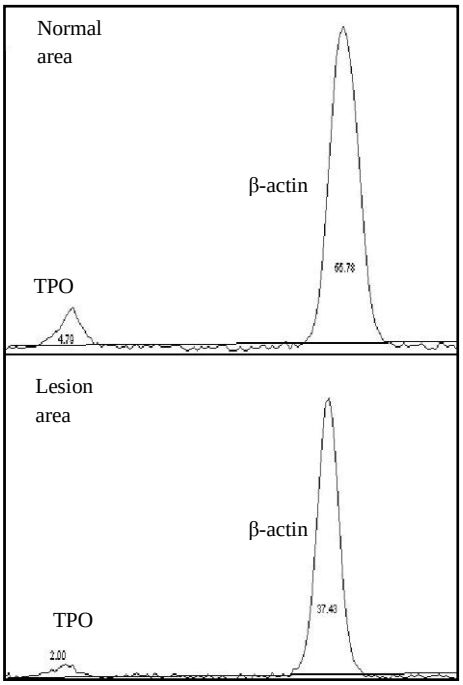
<table>
<tr><th>Experiment</th><th>Protein band density</th><th colspan="2">Expression</th></tr>
<tr><td rowspan="2">i

Normal area Lesion area
 
TPO
β-actin</td><td rowspan="2"> 
</td><td>Relative Density</td><td>Adjustment Density</td></tr>
<tr><td>TPO/β-actin = 5.39/51.15 = 0.11

TPO/β-actin = 2.13/41.33 =0.05</td><td>lesion area / normal area = 0.05/0.11 X 100% = 45.45%</td></tr>
<tr><td>ii

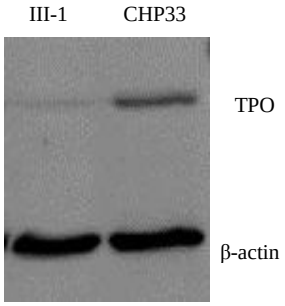
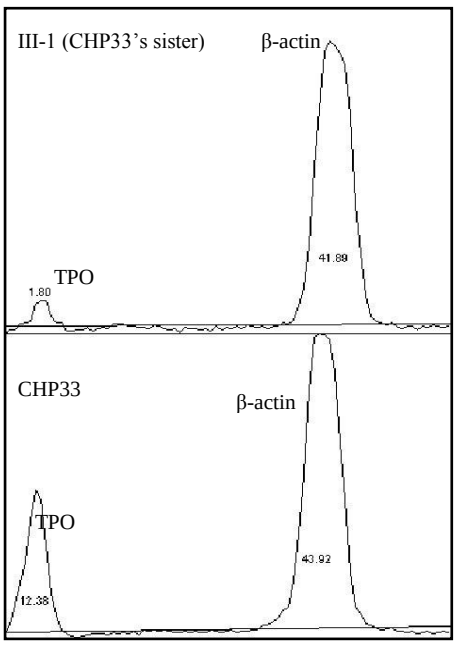
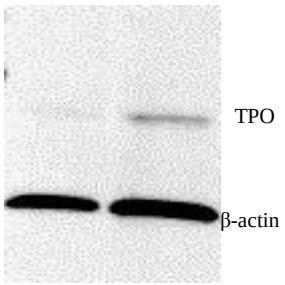
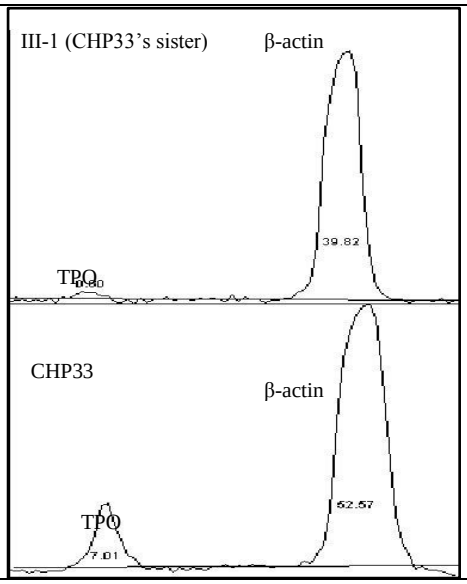
Normal area Lesion area
 
TPO
β-actin</td><td> 
</td><td>TPO/β-actin = 7.09/54.83 =0.13

TPO/β-actin = 1.87/37.01 =0.05</td><td>lesion area / normal area = 0.05/0.13 X 100% = 38.46%</td></tr>
</table>

Experiment	Protein band density	Expression	
iii		**Relative Density**	**Adjustment Density**
		TPO/β-actin = 4.79/55.78 =0.09 TPO/β-actin = 2.0/37.43 =0.05	lesion area / normal area = 0.05/0.09 X 100% = 55.56%

Experiment	**lesion area/normal area** Up-regulation if >100% ; Down-regulation of <100%	**Expression** (fold change)	**Average**
i	45.45%	2.2	2.2 ±0.4 (down regulation)
ii	38.46%	2.6	
iii	55.56%	1.8	

3) CHP33 (III-2) and CHP33's sister (III-1) (comparison)

Experiment	Protein band density	Expression	
		Relative Density	**Adjustment Density**
i 		TPO/β-actin = 1.80/41.89 =0.04 TPO/β-actin =12.38/43.92 = 0.28	III-1/CHP33 = 0.04/0.28 X 100% = 14.29%
ii 		TPO/β-actin = 0.6/39.82 =0.02 TPO/β-actin =7.01/52.57 = 0.13	III-1/CHP33 = 0.02/0.13 X 100% = 15.38%

Experiment	**Thyroid lesion/normal tissue** Up-regulation if >100% ; Down-regulation of <100%	**Expression** (fold change)	**Average**
i	14.29%	7	6.75±0.35 fold (down regulation)
ii	15.38%	6.5	

APPENDIX H

Multiple sequence alignment of amino acids in human TPO with rat TPO

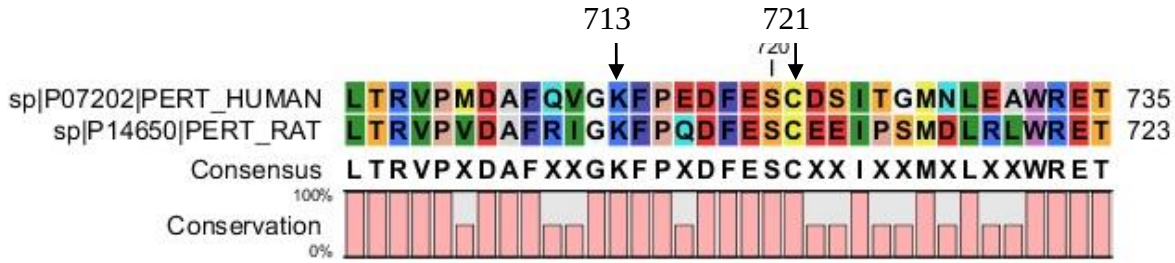


Figure 1 Multiple sequence alignment of amino acids in human TPO with rat TPO showing full conservation of all residues that located within the epitope for Moab47 (residues 713 to 721) except Glu-716.

APPENDIX I

JAWATANKUASA ETIKA PERUBATAN
PUSAT PERUBATAN UNIVERSITI MALAYA
ALAMAT: LEMBAH PANTAI, 59100 KUALA LUMPUR, MALAYSIA
TELEFON: 03-79494422 FAKSIMILI: 03-79545682

AME OF ETHICS COMMITTEE/IRB: Medical Ethics Committee, University Malaya Medical Centre ADDRESS: LEMBAH PANTAI 59100 KUALA LUMPUR	ETHICS COMMITTEE/IRB REFERENCE NUMBER: 654.16
PROTOCOL NO: TITLE: Genomic And Proteomic Analyses Of Patients With Congenital Endocrine Disorders	
PRINCIPAL INVESTIGATOR: Dr. Sarni Mat Junit TELEPHONE: KOMTEL:	SPONSOR:

The following item [✓] have been received and reviewed in connection with the above study to be conducted by the above investigator.

[✓] Borang Permohonan Penyelidikan	Ver date: 2 May 08
[ ] Study Protocol	Ver date:
[ ] Investigator Brochure	Ver date:
[✓] Patient Information Sheet	Ver date:
[✓] Consent Form	Ver date:
[ ] Questionnaire	
[✓] Investigator(s) CV's (Dr. Sarni Mat Junit)	

and have been [✓]

[✓] Approved
[ ] Conditionally approved (identify item and specify modification below or in accompanying letter)
[ ] Rejected (identify item and specify reasons below or in accompanying letter)

Comments:

i. *Investigator is required to follow instructions, guidelines and requirements of the Medical Ethics Committee.*
ii. *Investigator is required to report any protocol deviations/violations through the Clinical Investigation Centre and provide annual/closure reports to the Medical Ethics Committee.*

Date of approval: 28th May 2008

s.k Ketua
Jabatan Perubatan Molekul

Timbalan Dekan (Penyelidikan)
Fakulti Perubatan, Universiti Malaya

Setiausaha
Jawatankuasa Penyelidikan Pusat Perubatan
Fakulti Perubatan, Universiti Malaya

.....
PROF. LOOI LAI MENG
Chairman
Medical Ethics Committee

APPENDIX J

Consent by patient for clinical research University of Malaya Medical Centre, K.L.

I, ... Identity Card No.....
(Name of Patient)

of .....
(Address)

hereby agree to take part in the clinical research (clinical study/questionnaire study/drug trial) specified below:

Title of Study: .....

.....

.....

the nature and purpose of which has been explained to me by Dr. .....
(Name & Designation of Doctor)

..... and interpreted by .....
(Name & Designation of Interpreter)

..... to the best of his/her ability in ..... language/dialect.

I have been told about the nature of the clinical research in terms of methodology, possible adverse effects and complications (as per patient information sheet). After knowing and understanding all the possible advantages and disadvantages of this clinical research, I voluntarily consent of my own free will to participate in the clinical research specified above.

I understand that I can withdraw from this clinical research at any time without assigning any reason whatsoever and in such a situation shall not be denied the benefits of usual treatment by the attending doctors.

Date: ..... Signature or Thumbprint .....
(Patient)

IN THE PRESENCE OF

Name .....)
)
Identity Card No. .....) Signature .....
) *(Witness for Signature of Patient)*
Designation .....)

I confirm that I have explained to the patient the nature and purpose of the above-mentioned clinical research.

Date ..... Signature .....
(Attending Doctor)

CONSENT BY PATIENT FOR CLINICAL RESEARCH

FPU-DOF-BK-012-05-R01

R.N.
Name
Sex
Age
Unit

Consent by responsible relative for clinical research

I, ... Identity Card No.....
(Name)

of .....
(Address)

hereby agree that my relative ..... I.C. No.....
(Name)

participate in the clinical research (clinical study/questionnaire study/drug trial) specified below:-

Title of Study: .....

.....

.....

the nature and purpose of which has been explained to me by Dr. .....
(Name & Designation of Doctor)

..... and interpreted by .....
(Name & Designation of Interpreter)

..... to the best of his/her ability in ..... language/dialect.

I have been informed of the nature of this clinical research in terms of procedure, possible adverse effects and complications (as per patient information sheet). I understand the possible advantages and disadvantages of participating in this research. I voluntarily give my consent for my relative to participate in this research specified above.

I understand that I can withdraw my relative from this clinical research at any time without assigning any reason whatsoever and in such situation, my relative shall not be denied the benefits of usual treatment by the attending doctors. Should my relative regains his/her ability to consent, he/she will have the right to remain in this research or may choose to withdraw.

Date: ..... Relationship to Patient ..... Signature or Thumbprint .....

IN THE PRESENCE OF

Name .....)
)
Identity Card No. .....) Signature .....
) *(Witness)*
Designation .....)

I confirm that I have explained to the patient's relative the nature and purpose of the above-mentioned clinical research.

Date ..... Signature .....
(Attending Doctor)

CONSENT BY RESPONSIBLE RELATIVE FOR CLINICAL RESEARCH

R.N.
Name
Sex
Age
Unit