APPENDIX A

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


APPENDIX C

The published sequence of the:

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

Reference Sequence: NM_000547.5

```
1  ggaaggcaac taaagccccc ctttcatca gctgcacag tctgctctct ctaaagttta cctctgatcg
26  caagcacttc cccttcttttt ttgcattttag tctgctctct ctgctctctt ctgctctctct ctaaagttta
c31  gcaagtttag tttgtttag gccgattatt tctgctctct tctgctctct tctgctctctct ctaaagttta
c36  aatcattagt tctgctctct tctgctctct ctaaagttta cctctgatcg
41  gccgattatt cctgctctct tctgctctct tctgctctct ctaaagttta cctctgatcg
46  gccaagtttag tttgtttag gccgattatt tctgctctct tctgctctct tctgctctctct ctaaagttta
c51  aatcattagt tctgctctct tctgctctct tctgctctct ctaaagttta cctctgatcg
56  gccgattatt cctgctctct tctgctctct tctgctctct ctaaagttta cctctgatcg
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71  gccgattatt cctgctctct tctgctctct tctgctctct ctaaagttta cctctgatcg
76  gccaagtttag tttgtttag gccgattatt tctgctctct tctgctctct tctgctctctct ctaaagttta
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86  gccgattatt cctgctctct tctgctctct tctgctctct ctaaagttta cctctgatcg
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c96  aatcattagt tctgctctct tctgctctct tctgctctct ctaaagttta cctctgatcg
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136  gccaagtttag tttgtttag gccgattatt tctgctctct tctgctctct tctgctctctct ctaaagttta
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146  gccgattatt cctgctctct tctgctctct tctgctctct ctaaagttta cctctgatcg
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241  gccaagtttag tttgtttag gccgattatt tctgctctct tctgctctct tctgctctctct ctaaagttta
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251  gccgattatt cctgctctct tctgctctct tctgctctct ctaaagttta cctctgatcg
256  gccaagtttag tttgtttag gccgattatt tctgctctct tctgctctct tctgctctctct ctaaagttta
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266  gccgattatt cctgctctct tctgctctct tctgctctct ctaaagttta cctctgatcg
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301  gccaagtttag tttgtttag gccgattatt tctgctctct tctgctctct tctgctctctct ctaaagttta
c306  aatcattagt tctgctctct tctgctctct tctgctctct ctaaagttta cctctgatcg
311  gccaagtttag tttgtttag gccgattatt tctgctctct tctgctctct tctgctctctct ctaaagttta
```

stop codon (ending with c.2802)
2) Human thyroid peroxidase (TPO), isoform 1, protein (Uniprot Reference Sequence: P07202-1)

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70   80   90  100  110  120  
NLKKRGILSP AQLLSFSKLP EPTSGVIAARA AEIMETSIQAA MKKVRNLKTQ Q52HPTDALS
130  140  150  160  170  180  
EDLSSIMUM SGCLPVMLPP KCPNTCLANK YRPITGACNN RDHPWRGASN TALARWLPVPV
190  200  210  220  230  240  
YEDGFSQPRLG WNPFDLYNGFH PLPPVREVT HVIQVSNEVG TDRODSEILM MAWQOQYLDHD
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IAFTPQSTSK AAFFGGGACQD NTCENQQNCF PQLPPEEARAP AAGTACLPFY RSSAAGTTGD
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RAYLPFVPPR APAACAEPEPG IPGETRGPCF LAGDSRASEV PSMTLHNLW LREHNRLLAAA
430  440  450  460  470  480  
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490  500  510  520  530  540  
VSNVFSTAAF RFGHAFIPHL VRLRDLDFQIE HDPDLGLWHL QAFFSPWPTT RGGGLDPLIR
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PRLTPSADLS TAIASRSVAD KILDLYKHPD NIVWLVGLLA ENFLPRARTG PLFACLIGKQ
670  680  690  700  710  720  
MKALRDGDWF WWENSHVFDT AQRRELEKHS LRSCIDTNG LTRVPMQAFQ VGKFCADEFS
730  740  750  760  770  780  
CDSITGMNLE AWRETPFQOED KCFGEPESVEN GDFVHCEESG RRVLVYSCRH QRIESQREQL
790  800  810  820  830  840  
TCTQEHWDFQ PPLCDPNVNE ADGAHPPLCA SARCRNKGG FQGCLADAPY LGDGDGCVD
850  860  870  880  890  900  
SGRLPRVVTI SMSLAALLIG GFAGLSTTVI CRWTRGTTKS TLPSESTGGG TPELRCCGHQ
910  920  930  
AVGTSQPARAA AQDSEQUESG MEGRDTHRLP RAL
```
3) Human thyroid peroxidase (TPO), RefSeqGene on chromosome 2 (NCBI Reference Sequence: NG_011581.1, selected region from 4799 to 5949)

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</tr>
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<td>271 tatcatatgg gtcagatcag tctccagctg tcaagtgtgga cagccagcga</td>
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303 c.1 (exon 2)

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

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

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303 cgcctctcgc cgcctctcgc cgcctctcgc cgcctctcgc cgcctctcgc
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 ddH₂O 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 °C.

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 ddH₂O. 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% Brilliant 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

<table>
<thead>
<tr>
<th>Electropherogram</th>
<th>Forward(F) / Reverse(R) sequencing</th>
<th>Exon-exon boundary</th>
<th>TPO</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Exon 2 to Exon 3" /></td>
<td>F</td>
<td>2/3</td>
<td>1</td>
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<tr>
<td><img src="image" alt="Exon 3 to Exon 4" /></td>
<td>F</td>
<td>3/4</td>
<td>1</td>
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<td>F</td>
<td>4/5</td>
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**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.
<table>
<thead>
<tr>
<th>Electropherogram</th>
<th>Forward(F) / Reverse(R) sequencing</th>
<th>Exon-exon boundary</th>
<th>TPO</th>
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</thead>
<tbody>
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<tr>
<td><img src="image3" alt="Exon 7 to Exon 8" /></td>
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<tr>
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**Figure 1** Continued.
Electropherogram

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<table>
<thead>
<tr>
<th>Forward(F) / Reverse(R) sequencing</th>
<th>Exon-exon boundary</th>
<th>TPO</th>
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<tr>
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<td>F</td>
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<td>R</td>
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**Figure 1** Continued.
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<th>Exon-exon boundary</th>
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<td>F</td>
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<td>F</td>
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**Figure 1** Continued.
**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**

![BSA standard curve graph](image)

**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)

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Protein band density</th>
<th>Expression</th>
</tr>
</thead>
</table>
| i          | ![Image](image1.png) | TPO/β-actin = 12.84/38.8 = 0.33
          |                      | lesion area / normal area = 0.19/0.33 X 100% = 57.58% |
| ii         | ![Image](image2.png)  | TPO/β-actin = 7.81/40.68 = 0.19
          |                      | lesion area / normal area = 0.09/0.12 X 100% = 75% |

Normal area
Lesion area

β-actin

TPO
### Experiment

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<tr>
<th>Experiment</th>
<th>Protein band density</th>
<th>Expression</th>
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<tbody>
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<td>iii</td>
<td>Normal area Lesion area</td>
<td>TPO/β-actin = 6.87/44.28 =0.16</td>
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<tr>
<td></td>
<td></td>
<td>lesion area / normal area = 0.14/0.16 X 100% = 87.5%</td>
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<tr>
<td>iv</td>
<td>Normal area Lesion area</td>
<td>TPO/β-actin = 17.08/38.4 =0.44</td>
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<tr>
<td></td>
<td></td>
<td>lesion area / normal area = 0.34/0.44 X 100% = 77.27%</td>
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### Expression

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<th>Expression (fold change)</th>
<th>Average</th>
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<td>1.74</td>
<td>1.36±0.26 fold (down regulation)</td>
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<td>ii</td>
<td>75%</td>
<td>1.33</td>
<td></td>
</tr>
<tr>
<td>iii</td>
<td>87.5%</td>
<td>1.14</td>
<td></td>
</tr>
<tr>
<td>iv</td>
<td>77.27%</td>
<td>1.29</td>
<td></td>
</tr>
</tbody>
</table>
2) CHP33’s sister (III-1)

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Protein band density</th>
<th>Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Relative Density</td>
</tr>
<tr>
<td>i</td>
<td><img src="image1.png" alt="image" /></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Normal area</td>
<td>β-actin TPO</td>
</tr>
<tr>
<td></td>
<td>Lesion area</td>
<td>β-actin TPO</td>
</tr>
<tr>
<td></td>
<td></td>
<td>β-actin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ii</td>
<td><img src="image2.png" alt="image" /></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Normal area</td>
<td>β-actin</td>
</tr>
<tr>
<td></td>
<td>Lesion area</td>
<td>β-actin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment</td>
<td>Protein band density</td>
<td>Expression</td>
</tr>
<tr>
<td>------------</td>
<td>---------------------</td>
<td>------------</td>
</tr>
<tr>
<td>iii</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Relative Density</th>
<th>Adjustment Density</th>
</tr>
</thead>
</table>

TPO/β-actin = 4.79/55.78 = 0.09

Lesion area / normal area = 0.05/0.09 X 100% = 55.56%

<table>
<thead>
<tr>
<th>Experiment</th>
<th>lesion area/normal area</th>
<th>Expression (fold change)</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>i</td>
<td>45.45%</td>
<td>2.2</td>
<td>2.2 ± 0.4 (down regulation)</td>
</tr>
<tr>
<td>ii</td>
<td>38.46%</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>iii</td>
<td>55.56%</td>
<td>1.8</td>
<td></td>
</tr>
</tbody>
</table>
3) CHP33 (III-2) and CHP33’s sister (III-1) (comparison)

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Protein band density</th>
<th>Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Relative Density</td>
</tr>
<tr>
<td>i</td>
<td><img src="image" alt="III-1 CHP33" /></td>
<td>TPO/β-actin = 1.80/41.89 =0.04</td>
</tr>
<tr>
<td></td>
<td><img src="image" alt="III-1 (CHP33’s sister)" /></td>
<td>TPO/β-actin = 12.38/43.92 =0.28</td>
</tr>
<tr>
<td>ii</td>
<td><img src="image" alt="II-1 CHP33" /></td>
<td>TPO/β-actin = 0.6/39.82 =0.02</td>
</tr>
<tr>
<td></td>
<td><img src="image" alt="III-1 (CHP33’s sister)" /></td>
<td>TPO/β-actin = 7.01/52.5 7 = 0.13</td>
</tr>
</tbody>
</table>

### Thyroid lesion/normal tissue

**Up-regulation if >100% ; Down-regulation of <100%**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Expression (fold change)</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>i</td>
<td>7</td>
<td>6.75±0.35 fold (down regulation)</td>
</tr>
<tr>
<td>ii</td>
<td>6.5</td>
<td></td>
</tr>
</tbody>
</table>
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**

**ADDRESS:** LEMBAH PANTAI, 59100 KUALA LUMPUR

**PROTOCOL NO.:**

**TITLE:** Genomic And Proteomic Analyses Of Patients With Congenital Endocrine Disorders

**PRINCIPAL INVESTIGATOR:** Dr. Sarni Mat Junit

**TELEPHONE:**

**ETHICS COMMITTEE/IRB REFERENCE NUMBER:** 654.16

**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
- [ ] Study Protocol
- [ ] Investigator Brochure
- [✓] Patient Information Sheet
- [✓] Consent Form
- [ ] 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:**

---

1. Investigator is required to follow instructions, guidelines and requirements of the Medical Ethics Committee.
2. 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.

<table>
<thead>
<tr>
<th>I, .............................................</th>
<th>Identity Card No .........................................................</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Name of Patient)</td>
</tr>
<tr>
<td></td>
<td>Address</td>
</tr>
<tr>
<td></td>
<td>(Address)</td>
</tr>
<tr>
<td>hereby agree to take part in the clinical</td>
<td>(Name &amp; Designation of Doctor)</td>
</tr>
<tr>
<td>research (clinical study/questionnaire</td>
<td>..........................................................................................</td>
</tr>
<tr>
<td>study/drug trial) specified below:</td>
<td>(Name &amp; Designation of Doctor)</td>
</tr>
<tr>
<td></td>
<td>..........................................................................................</td>
</tr>
<tr>
<td>Title of Study: ..................................</td>
<td>..........................................................................................</td>
</tr>
<tr>
<td></td>
<td>..........................................................................................</td>
</tr>
<tr>
<td>the nature and purpose of which has been</td>
<td>..........................................................................................</td>
</tr>
<tr>
<td>explained to me by Dr. .......................</td>
<td>(Name &amp; Designation of Doctor)</td>
</tr>
<tr>
<td></td>
<td>..........................................................................................</td>
</tr>
<tr>
<td>................................................................</td>
<td>(Name &amp; Designation of Interpreter)</td>
</tr>
<tr>
<td>................................................................</td>
<td>..........................................................................................</td>
</tr>
<tr>
<td>................................................................</td>
<td>..........................................................................................</td>
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<td>..........................................................................................</td>
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<td>..........................................................................................</td>
</tr>
<tr>
<td>................................................................</td>
<td>..........................................................................................</td>
</tr>
<tr>
<td>I have been told about the nature of the</td>
<td>..........................................................................................</td>
</tr>
<tr>
<td>clinical research in terms of methodology,</td>
<td>..........................................................................................</td>
</tr>
<tr>
<td>possible adverse effects and complications</td>
<td>..........................................................................................</td>
</tr>
<tr>
<td>(as per patient information sheet).</td>
<td>..........................................................................................</td>
</tr>
<tr>
<td>After knowing and understanding all the</td>
<td>..........................................................................................</td>
</tr>
<tr>
<td>possible advantages and disadvantages of</td>
<td>..........................................................................................</td>
</tr>
<tr>
<td>this clinical research, I voluntarily</td>
<td>..........................................................................................</td>
</tr>
<tr>
<td>consent of my own free will to participate</td>
<td>..........................................................................................</td>
</tr>
<tr>
<td>in the clinical research specified above.</td>
<td>..........................................................................................</td>
</tr>
<tr>
<td>I understand that I can withdraw from</td>
<td>..........................................................................................</td>
</tr>
<tr>
<td>this clinical research at any time</td>
<td>..........................................................................................</td>
</tr>
<tr>
<td>without assigning any reason whatsoever</td>
<td>..........................................................................................</td>
</tr>
<tr>
<td>and in such a situation shall not be</td>
<td>..........................................................................................</td>
</tr>
<tr>
<td>denied the benefits of usual treatment by</td>
<td>..........................................................................................</td>
</tr>
<tr>
<td>the attending doctors.</td>
<td>..........................................................................................</td>
</tr>
<tr>
<td>Date ............................................</td>
<td>Signature or Thumbprint ..................................................</td>
</tr>
<tr>
<td>(Patient)</td>
<td>..........................................................................................</td>
</tr>
<tr>
<td>IN THE PRESENCE OF ................................</td>
<td>..........................................................................................</td>
</tr>
<tr>
<td>Name ............................................</td>
<td>Signature .............................................................................</td>
</tr>
<tr>
<td>Identity Card No. ................................</td>
<td>(Witness for Signature of Patient)</td>
</tr>
<tr>
<td>Designation ....................................</td>
<td>..........................................................................................</td>
</tr>
<tr>
<td>I confirm that I have explained to the</td>
<td>..........................................................................................</td>
</tr>
<tr>
<td>patient the nature and purpose of the</td>
<td>..........................................................................................</td>
</tr>
<tr>
<td>above-mentioned clinical research.</td>
<td>..........................................................................................</td>
</tr>
<tr>
<td>Date ............................................</td>
<td>Signature .............................................................................</td>
</tr>
<tr>
<td>(Attending Doctor)</td>
<td>..........................................................................................</td>
</tr>
</tbody>
</table>
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 ........................................................ (Witness)
     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