Bengkulu, 21 Oktober 2010

Kepada yth
Bapak Dr. ir. Fahrurrozi, MSc.
Pembantu Rektor Bidang Akademik
Universitas Bengkulu

Assalamualaikum wr.wb.,

Sehubungan dengan kerjasama penelitian “Molecular Study of SEDT (Spondylo-Epiphyseal Dysplasia Tarda) Cases in Kedurang, South Bengkulu to Obtain Genetics Diversity and Candidate Marker for Early Detection” antara saya, Dr. Aceng Ruyani, dan Professor R V Thakker, Academic Endocrine Unit, Nuffield Department of Clinical Medicine, University of Oxford, Inggris yang dibiyai oleh Program PAR-C Tahun 2009, perkenankan saya menyampaikan laporan sebagai berikut:

(a) Hasil Kegiatan Program PAR-C Tahun 2009 berjudul; “Characterisation of mutation of the SEDL (TRAPPC2) gene in patients with X-linked spondyloepiphyseal dysplasia tarda from a region of Indonesia” telah dipresentasikan melalui seminar yang berlangsung di Hotel Mellenium, Jakarta pada tanggal 5 Oktober 2010.
(b) Seminar pada butir a di atas ditetapkan sebagai penutup dari serangkaian Kegiatan Program PAR-C Tahun 2009.
(c) Saat ini paper “Characterisation of mutation of the SEDL (TRAPPC2) gene in patients with X-linked spondyloepiphyseal dysplasia tarda from a region of Indonesia” dalam proses untuk dipublikasikan pada European Human Genetics Journal.
(d) Kerja sama penelitian antara saya, Dr. Aceng Ruyani, dan Professor R V Thakker, secara akademik masih membuka peluang kerjasama lanjutan yang lebih luas dan akan menguntung kedua belah pihak.

Sebagai pelengkap laporan, berikut ini disampaikan satu (1) berkas dokumen sebagaimana terlampir.

Melalui surat ini saya menghaturkan banyak terimakasih atas segala dukungan dan bantuan yang telah diberikan untuk kelancaran kegiatan tersebut.

Wassalam,

Dr. Aceng Ruyani

Tembusan disampaikan Kepada Yth:
1. Dekan FKIP, Universitas Bengkulu
2. Ketua Jurusan PMIPA, FKIP, Universitas Bengkulu
3. Ketua Prodi Pendidikan Biologi, JFPMIPA, FKIP, Universitas Bengkulu
Characterisation of mutation of the SEDL (TRAPPC2) gene in patients with X-linked spondyloepiphyseal dysplasia tarda from a region of Indonesia.

A. Rayani, J. Jayashil, P. T. Christie, B. Karyadi, C. Muslimi, S. Syafii, and R. V. Thakker

1Program Studi Pendidikan Biologi, Universitas Bengkulu, Jalan Raya Kandang Limmah, Kota Bengkulu, Bengkulu, 38371, Indonesia.
2Academic Endocrine Unit, Nuffield Department of Clinical Medicine, Oxford University, Oxford Centre for Diabetes, Endocrinology and Metabolism (OCDMEM), Churchill Hospital, Headington, Oxford, OX3 7LJ, UK.
3Jasraan, Biologi, Universitas Bengkulu, Jalan Raya Kandang Limmah, Kota Bengkulu, Bengkulu, 38371, Indonesia.
4M. Yusubs Hospital, the General Public Hospital of Bengkulu Province, Jalan Hibrda Sidomulya, Kota Bengkulu, Bengkulu, 38225 Indonesia.

Summary

Spondyloepiphyseal dysplasia tarda (SEDL) is an X-linked recessive osteochondrodysplasia. It is a progressive skeletal disorder, in which affected males characteristically have a short neck and trunk, a barrel-shaped chest and hips that show degenerative disease. Changes in spine and hips become evident between 10 to 14 years of age. Obligate carrier females are generally clinically and radiographically normal. The SEDL gene (also known as the TRAPPC2 gene, located on chromosome Xp22), transcribes a 2.8kb transcript in many tissues and encodes a 140 amino acid protein called SEDLIN. SEDLIN is part of the transport protein particle (TRAPP) complex, which is involved in endoplasmic reticulum-to-Golgi vesicular transport pathway. In this study we present the results of an examination of probands from three Indonesian families, the identification and characterization of a common splice-site mutation, which results in the loss of exon 3. The identification of mutations in these families will enable presymptomatic diagnosis and the identification of asymptomatic mutant gene carriers.

Address for correspondence and reprints:
Dr. Azeng Rayani, email: rayani@lyen.com

#Disajikan pada Seminar Hasil Kajian Poserta PAR B-C di Hotel Meliussium, Jakarta, 5 Oktober 2010.
Introduction

Spondyloepiphyseal dysplasia tarda (SEDТ (MIM213400)) is an X-linked recessive osteochondrodysplasia, which has been reported to occur in about one in 500,000 of the population in the UK (Wynne-Davies et al. 1985). This clinically and radiologically distinctive form of bone dysplasia was first described in a large American kindred (Jacobsen 1939).

SEDТ is a progressive skeletal disorder, which is characterised in affected males by a short neck and trunk, a barrel-shaped chest and hips that show degenerative disease. Changes in the spine and hips become radiologically evident between 10 and 14 years of age. In adults, vertebral changes occur, especially in the lumbar region are of this osteochondrodysplasia. The distinctive radiological signs are platyspondyly with hump-shaped central and posterior portions, narrow disc spaces and moderate epiphyseal dysplasias, which can lead to premature secondary osteoarthritis (Harper et al. 1973, Iceton et al. 1986, MacKenzie et al. 1996).

Obligate carrier females are generally clinically and radiographically normal, although there have been some reports of females having arthritic complaints (Bannerman et al. 1971, MacKenzie et al. 1996, Gottesman et al. 1996, Whyte, MF et al. 1999).

The SEDЛ gene, previously localised to Chromosome Xp22 (Szpiro-Tapia et al. 1988, Heuertz et al. 1993) was cloned and mutations identified in three Australian families (Gedeon et al. 1999). Gedeon et al. confirmed the earlier localisations (Szpiro-Tapia et al. 1988, Heuertz et al. 1993, Heuertz et al. 1993) and narrowed the region to Xp22.3-Xp22.3. Examination of a candidate open reading frame (ORF) revealed 3 dimonucleotide deletions which were shown to cosegregate with the SEDТ phenotype in these families.

SEDЛ has been shown to be a novel gene that encodes a putative 140 amino acid protein named SEDLIN. A yeast orthologue has been shown to be involved in ER to golgi vesicular transport and that human SEDLIN can complement the yeast orthologue (Gedeon et al. 2003), showing that the human SEDLIN protein retains the ER-Golgi vesicular transport function. Protein homologues have been identified in yeast, Drosophila melanogaster, Caenorhabditis elegans, mouse and rat (Gedeon et al. 1999). It has also been shown to be widely expressed in tissues including: skeletal muscle, kidney, fetal cartilage, fibroblasts and lymphoblasts (Gedeon et al. 1999).

In this study we have examined three Indonesian families from the Kedurang region, members of whom show features of this characteristic bone dysplasia with X-linked inheritance, for mutations in the SEDЛ gene.

Methods

Patients

Probands of three extensive Indonesian SEDТ families all from the Kedurang region, were ascertained (Figure 1). A family history of SEDТ could be established in each case. Affected males had been diagnosed, usually in the second decade of life when they were investigated for short stature. They all had disproportionate short stature with short trunks and adult
heights of 120-140 cm as well as typical symptoms including platyspondyly with hump-shaped central and posterior portions, narrow disc spaces and moderate epiphyseal dysplasias.

Figure 1. Family trees showing the families examined in this study. Individuals are represented as males (squares), females (circles), unaffected (open symbol), affected (filled in symbol) and unaffected carrier (dot in middle of symbol). Originally there were believed to be three families, but genealogical analysis subsequently showed families 1 and 2 to be related. The three original probands are indicated by an arrow.

Mutational Analysis

DNA samples were obtained (from venous blood samples, by standard methods (Pearce et al. 1995) from the SEDT probands and other relatives, with informed consent and after approval had been obtained from the relevant ethical committees.

DNA sequence abnormalities were initially sought in each of the SEDT probands. Four pairs of primers (Gedeon et al. 1999) were used for the PCR amplification of the four coding exons of the SEDL gene and of their corresponding intron/exon boundaries with conditions described previously (Gedeon et al. 1999). PCR products were gel purified and Taq polymerase cycle sequencing performed as described previously (Lloyd et al. 1996, Lloyd et al. 1997). All coding exons and the intron/exon boundaries were sequenced in both forward and reverse directions.

In addition, DNA sequence abnormalities were confirmed by Amplification refractory mutation system (ARMS) PCR (Hannan et al. 2008, Turner et al. 2010). These were demonstrated not to be common polymorphisms (by sequencing) in DNA obtained from 50 male and 50 female normal individuals (110 alleles). RT-PCR analysis performed as previously described (Nesbit et al. 2004) to determine the splicing effect of the SEDL mutation. Total RNA from blood lymphocytes was extracted using Trizol reagent (Invitrogen) and was reverse transcribed using the SuperScript first-strand synthesis system (Invitrogen) and random hexamers. Subsequently, RT-PCR was performed using exon1F 5'-
CTGACATTGCGTTTCCGTG-3' and exon4R 5'-AGTGACAAATGCCGACACAA-3' primers. RT-PCR products were sequenced in both directions.

Results and Discussion

The same mutation was identified in each of the three probands studied and occurred at the splice acceptor site of intron 2 of the SEDL gene (Figure 2). The mutation detected was an A-to-G transition at position -2 of intron 2 splice acceptor site (IVS2-2A→G), which was confirmed and demonstrated to co-segregate with the disease in the families by ARMS PCR (Figure 3). In addition, the absence of the DNA sequence abnormalities in 110 alleles from 80 unrelated normal individuals established that these were mutations and unlikely to be polymorphisms that would be expected to occur in >1% of the population. Extensive genealogical studies showed families 1 and 2 were related. This mutation is the first mutation described in Indonesian families. It is estimated that SEDT occurs in about one in 500000 in the UK, but in Indonesia, it is estimated to be 100000.

A

<table>
<thead>
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<th>Wild Type (WT)</th>
<th>Mutant (m)</th>
</tr>
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<tbody>
<tr>
<td>a</td>
<td>ttaatt g</td>
</tr>
<tr>
<td>GAG CCA</td>
<td>g</td>
</tr>
</tbody>
</table>

B

Figure 2. Detection of mutation in intron 2 of the SEDL gene in family 3. DNA sequence analysis of an affected male individual (II.2) revealed an a to g transition of the invariant ag dinucleotide of the acceptor splice site consensus sequence of intron 2 (A). The a to g transition did not alter a restriction enzyme site, thus ARMS-PCR was used to confirm the presence of the wild-type (WT) and mutant (m) sequences in lymphoblastoid DNA (B). The affected male individuals (II.1 and II.2) were homozygous for the m sequence, whereas their mother (I.2), a carrier, was heterozygous having both the WT and m sequences. A control primer employed in both reactions confirmed that the ARMS-PCR was amplifying correctly (lower band on both gels). The positions of the size markers (S; 100-bp ladder) at 300 and 400 bp are shown. Congregation of this SEDL mutation with SEDT in the family was demonstrated and its absence from 110 alleles of 80 unrelated normal individuals, 50 males and 30 females (N1 and N2 shown) indicated that it is not a common DNA sequence polymorphism. Individuals are represented as males (squares), females (circles), unaffected (open symbol), affected (filled in symbol) and unaffected carrier (dot in middle of symbol). The proband (II.2) is indicated by an arrow.
The identification of the mutation in these three families will enable presymptomatic diagnosis and the identification of asymptomatic mutant gene carriers within this population.

To date, there are 47 different mutations that occur throughout the SEDL gene, and about 25% of these mutations are splicing mutations including the currently identified mutation. Although there is no obvious genotype-phenotype correlation, it is postulated that truncating mutations affecting the 5' end of the gene have more severe phenotype than those affecting the 3' end. (ref****). The SEDL gene encodes for a 140 amino acid widely expressed protein. Approximately 75% of the mutation would result in the loss of the highly conserved stretch of amino acids, 141MNPFY153 due to premature truncation of the SEDLIN protein. These residues are postulated to form a motif for binding to target proteins (Jang 2002) and in vitro studies of two mutations that cause loss of the motif have shown that they result in a disruption of perinuclear localization (Gez 2000). SEDLIN is a member of a large multi-protein transport protein particle (TRAPP) complex, which plays a key role in the targeting of ER-to-Golgi transport vesicles with their acceptor compartment (Kim 2006). Therefore, the loss of these residues in the majority of the SEDL mutations would disrupt formation of intact TRAPP complex and potentially leading to defect in vesicle tethering and thus intracellular transport. A histological report of articular cartilage from a SEDT patient with intron 3 splice donor mutation, reported chondrocytes with excessive cytoplasm with dilated rough ER and abundant Golgi complexes, and short, frayed or of variable diameter collagen fibrils within the extracellular matrix, suggesting defects in secretion of ECM macromolecules (Tiller 2001). It is possible that other members of this complex are involved in other bone dysplasias. But so far this system is not well understood.
Figure 3. Exon skipping due to an intron 2 acceptor splice site consensus sequence mutation. The transcription of exons 1-4 of the SEDL gene was detected by RT-PCR using RNA obtained from EBV-transformed lymphoblastoid of an affected male (Ⅱ.2), an unaffected female (Ⅲ.3) from family 3 and two normal individuals (N1 and N2) (A). RT-PCR products were not obtained from the genomic control (Ⅲ.3), thereby confirming the specificity of the primers (forward primer from exon 1 and reverse primer from exon 4). In the normal individuals (N1 and N2) and individual (Ⅲ.3), two bands were observed – a correctly spliced SEDL mRNA of 429 bp (WT1) consisting of exons 1 to 4, along with another SEDL mRNA of 287 bp (WT2), which is 142 bp less than WT1 and corresponds to the size of exon 2 (B). DNA sequence analysis of this product confirmed exon 2 skipping, with the splicing of exon 1 to exon 3. However, in the proband (Ⅱ.2) with SED1, two abnormal cDNAs were observed. The mutant bands (m1 and m2) were both smaller than the WT bands (WT1 and WT2) by 112 bp respectively, and in both cases correspond to the size of exon 3. DNA sequence analysis of this cDNA confirmed exon 3 skipping in both the m1 and m2 cDNAs (B). Thus, mutant cDNA m1 consisted of exons 1, 2 and 4, whereas mutant cDNA m2 consisted of only exons 1 and 4. The positions of the size markers (5, 100-bp ladder) at 200, 300, 400 and 500 bp are shown. Non-coding exons are filled in, and the translation start site (ATG) is indicated. The predominant SEDL transcript from several normal tissues has been reported to lack exon 2 (Gedeon et al. 1999; Geez et al. 2000; Tiller et al. 2001).

Acknowledgements

We are grateful to: The Medical Research Council (U.K.) (J. J., P.T.C and R.V.T); A Program Academic Recharging C (PAR-C) 2009, the Directorate General of Higher Education, Republic of Indonesia (A.R., B.K., C.M., Sip. and Sub.) for support. Competing Interests: The authors have declared that no competing interests exist. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References


Lloyd SE, Pearce SH, Gunther W, Kawaguchi H, Igarashi T, Jentsch TJ, Thakker RV (1997) Idiopathic low molecular weight proteinuria associated with hypercalciuric nephrocalcinosis


Characterisation of mutation of the SEDL (TRAPPC2) gene in patients with X-linked spondyloepiphyseal dysplasia tarda from a region of Indonesia

Previous studies of short stature cause in Kenduang, South Bengal

Survey number and distribution of SEDT population in Kenduang

Introduction

Historical facts between Bengkulu and UK

It should be concluded that the bone morphology and the topography of liver and kidney on SEDT people from Kenduang changes as the effect of vertebrae and skull deformities restriction.

Spondyloepiphyseal dysplasia tarda (SEDT AM07140231) is an X-linked recessive osteochondrodysplasia, which has been reported to occur in about one in 50000 of the population in the UK (Worms Davies et al 1995). This clinically and radiologically distinctive form of bone dysplasia was first described in a large American kindred (Kiddison 1939).

SEDIT is a progressive skeletal disorder, which is characterized clinically mostly by a short neck and thorax, a barrel-shaped chest and hip, with short stature. This is often referred to in a radiologically evident between 10 and 14 years of age. In adults, severity changes widely, especially in the spine. The disorder is more common in males than females. The characteristic radiological signs are platyspondyly with hour-glass vertebral and pedicular variations, narrow disc spaces and moderate epiphyseal dysplasia, which can lead to premature secondary osteoarthrosis (Chapman et al. 1973, Koff et al. 1988, Mauguen et al. 1996).
Obligate carrier females are generally healthy and radiographically normal, although there have been some reports of females having mild cranial defects. Voccali et al. (2011), Macfarlane et al. (1995), Gottstein et al. (1998), Willye, MF et al. (1992).

The SECDL gene, previously localized to Chromosome Xp22 (Spinro-Topia et al. 1998, Heurtem et al. 1993), was cloned and mutations identified in these Australian families (Gedden et al. 1998). Gedden et al confirmed the earlier observations (Spinro-Topia et al 1998, Heurtem et al. 1993, Heurtem et al. 1993) and narrowed the region to Xp22.3, Xp21.3. Examination of a candidate open reading frame (ORF) revealed 3 characteristic deletions which were shown to cosegregate with the SECDL phenotype in these families.

<table>
<thead>
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<th>FLR1</th>
<th>FLB</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2270-4812 ins</td>
<td>T</td>
<td>3rd Exon</td>
</tr>
<tr>
<td>B</td>
<td>1497-5085 del</td>
<td>T</td>
<td>3rd Exon</td>
</tr>
<tr>
<td>C</td>
<td>1600-4400 del</td>
<td>T</td>
<td>3rd Exon</td>
</tr>
<tr>
<td>D</td>
<td>(0-2270 del)</td>
<td>T</td>
<td>3rd Exon</td>
</tr>
</tbody>
</table>

In this study, we have examined three Indonesian families from the Kedung Jaya region, members of whom show features of this characteristic bone dysplasia with X-linked inheritance, for mutations in the SECDL gene.

**Methods**

Patients: Probands of three extensive Indonesian SECDL families all from the Kedung Jaya region were ascertained (Figure 1). A family history of SECDL could be established in each case. A proband and his parents were investigated for short stature, posterolateral tilted rib with short sterna, and concentric flattening of the arches with short trunks and adult heights of 125-140 cm as well as typical symptoms including pseudopudendy with scrotal and perineal skin, sexual dimorphism, and female epiphyseal dysplasia.

In addition, DNA sequence abnormalities were confirmed by Amplification refractory mutation system (ARMS) PCR (Hannan et al. 2008, Turner et al. 2010). These were demonstrated to be common polymorphism or point mutations. DNA sequence changes were determined by obtaining from 30 male and 30 female normal individuals (T0 alleles) in ARMS-PCR analysis performed as previously described (Feinberg et al. 2004) to determine the possible effect of the SECDL mutation.
The same mutation was identified in each of the three probands studied and occurred at the splice acceptor site of intron 2 of the SEST gene (Figure 2). The mutation detection was an A->G transition at position 45 of intron 2 splice acceptor site (IVS2-2A->G), which was confirmed and demonstrated to co-segregate with the disease in the families by ARMS-PCR (Figure 3). In addition, the absence of the DNA sequence abnormality in 100 alleles from 80 unrelated normal individuals established that these were mutations and unlikely to be polymorphisms that would be found at lower rates in the control population. Furthermore, population studies showed families 1 and 2 were related. This mutation is the first mutation described in Indonesian families. It is estimated that SEST occurs in about one in 100000 in the UK, but in Indonesia, it is estimated to be 100000.
The identification of the mutation in these three families will enable pre symptomatic diagnosis and the identification of at risk siblings in the entire population. To date, there are 47 different mutations that occur throughout the SEC61 gene, and about 25% of these mutations are splicing mutations including the currently identified mutation. Although there is no obvious genotype-phenotype correlation, it is predicted that truncating mutations affecting the C' end of the gene have a more severe phenotype than those affecting the N' end of the gene. The mutation described here was identified in an adult, widely expressed protein. Approximately 75% of the mutations would result in the loss of the highly conserved stretch of amino acids. GAGCCA due to premature truncation of the SEC61 protein. Those residues are postulated to form a motif for binding to target proteins (Jang 2002) and in 5' and 3' mutations that cause loss of the motif I have shown that they result in a disruption of perinuclear localization (Jang 2000).

Family tree and ARMS-PCR results

RT-PCR and diagram showing splicing

IVS2-2 A→G

SED6 Splicing -
SLEDLN is a member of a large multi-protein transport complex (TRAIPP) which plays a key role in the targeting of ER-to-Golgi transport vesicles with their acceptor compartments (Kim 2006). Therefore, the loss of these residues in the majority of the SLEDLN mutations would disrupt formation of intact TRAIPP complex and potentially leading to defects in Golgi tethering and thus intracellular transport. A histological report of muscular dystrophy in SLEDLN carrying a splice donor mutation reported the presence of extracellular matrix (ECM) and abundant rough ER and abundant dilated Golgi complexes, and short, tapered or of variable diameter collagen fibrils within the extracellular matrix, suggesting defects in secretion of ECM components (Tchernev 2001). It is possible that other members of this complex are involved in other sarcolemmas. But so far, this system is not well understood.

Acknowledgements

We are grateful to The Medical Research Council (UK) (J.J., S.L.), The University of Malaya Academic Ranking (MALAC), The Ministry of Higher Education, Republic of Indonesia (R.C.M.M., S.L.), and UniSZA for support. Conflicts of Interest: The authors have declared no competing interests exist. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.
Bengkulu, 7 Juni 2010

Kepada yth
ibu Istri Hardiyanti
Ketenaagaan Ditjen Dikti,
Departemen Kementrian Pendidikan Nasional,
Gedung D Lantai 5
Jalan Jenderal Sudirman Pintu I Senayan, Tromol Pos 190
Jakarta 10002

Assalamualaikum wr.wb.,

Sehubungan dengan kerjasama penelitian berjudul “Molecular Study of SEDT (Spondylo-
Epiphyseal Dysplasia Tarda) Cases in Kedurang, South Bengkulu to Obtain Genetics Diversity
and Candidate Marker for Early Detection” antara saya, Dr. Aceng Ruyani, dan Professor R
V Thakker, Academic Endocrine Unit, Nuffield Department of Clinical Medicine, University of
Oxford, inggris yang dibiayai oleh Program PAR-C Tahun 2009, perkenankan saya
menyampaikan laporan sebagai berikut;

(a) Program PAR-C Tahun 2009 telah berlangsung dengan baik selama periode
Desember 2009 sampai Maret 2010.

(b) Bulan April hingga Mei 2010 tim Dr. Aceng Ruyani, di Indonesia, dan Tim Professor
R V Thakker, di Inggris melakukan penelitian lanjutan di lokasi masing-masing untuk
melengkapi hasil penelitian yang diperoleh pada butir a.

(c) Saat ini, Juni 2010, kami sedang berdiskusi menyusun draft paper hasil penelitian
yang diperoleh pada butir a dan b untuk suatu publikasi internasional.

(d) Kerja sama penelitian antara saya, Dr. Aceng Ruyani, dan Professor R V Thakker,
secara akademik membuka peluang kerjasama lanjutan yang lebih luas dan akan
menguntung kedua belah pihak.

Sebagai pelengkap laporan, berikut ini disampaikan satu (1) berkas dokumen sebagaimana
terlampir.

Atas perhatian dan bantuan yang ibu berikan, saya hatukaran banyak terimakasih.

Wassalam,

Dr. Aceng Ruyani

Tembusan disampaikan Kepada Yth:
1. Pembantu Rektor I, Universitas Bengkulu
2. Dekan FKIP, Univeristitas Bengkulu
3. Ketua Jurusan PMIPA, FKIP, Universitas Bengkulu
4. Ketua Prodi Pendidikan Biologi, JPMIPA, FKIP, Universitas Bengkulu
SURAT TUGAS
Nomor: 4001/H 30.3/KP/2010

Dekan Fakultas Keguruan dan Ilmu Pendidikan Universitas Bengkulu dengan ini memberi tugas kepada:

<table>
<thead>
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<th>No</th>
<th>Nama</th>
<th>NIP</th>
<th>Unit Kerja</th>
</tr>
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<tr>
<td>1</td>
<td>Dr. Aceng Ruyani, M.S</td>
<td>19600105 198603 1 006</td>
<td>Dosen Jurusan Pendidikan MIPA FKIP UNIB</td>
</tr>
</tbody>
</table>

Untuk mengikuti Seminar Hasil Kegiatan peserta Program Academic Recharging (PAR) B-C Tahun Anggaran 2009 yang akan diadakan pada tanggal 04 s.d 07 Oktober 2010 di Hotel Millenium Jl. Fachruddin, Jakarta.

Demikianlah surat tugas ini dibuat untuk dapat dilaksanakan dengan penuh tanggung jawab.

Bengkulu, 30 September 2010
Dekan,

[Signature]

Prof. Sadjid, M.A., Ph.D
NIP. 19610121 198601 1 002
KEMENTERIAN PENDIDIKAN NASIONAL
UNIVERSITAS BENGKULU
Jalan WR Supratman Kandang Limun 21170, Fax 22105
Bengkulu 38371

Nomor Lampiran : Hal
623f H30/PP/2010 21 September 2010

: Undangan Seminar Hasil Kegiatan Peserta PAR B-C
tahun anggaran 2009

Yth. Direktur Ketenagaan Dirjen Dikti
Kementerian Pendidikan Nasional
Jln. Raya Jenderal Sudirman Pintu I Senayan
Jakarta

Memperhatikan surat nomor 2051/D4.4/2010 tanggal 06 September 2010 perihal, seperti pokok surat di atas, bersama ini diberitahukan bahwa dosen Universitas
Bengkulu yang telah mengikuti Program PAR B-C tahun 2009 akan mengikuti Seminar
hasil kegiatan di Jakarta atas nama:

<table>
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<th>NO</th>
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<th>PAR</th>
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</tr>
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<tr>
<td>1</td>
<td>Dr. Aceng Ruyani/198001051986031006</td>
<td>C</td>
<td>Oxford University</td>
</tr>
<tr>
<td>2</td>
<td>Hery Suhartoyo, Phd/196306251987031002</td>
<td>B</td>
<td>University of Queensland</td>
</tr>
</tbody>
</table>

Atas perhatian dan kerjasama yang baik, disampaikan ucapan terima kasih.

Rektor

Tembusan Yth.
Rektor
KEDUTAAN BESAR REPUBLIK INDONESIA
LONDON

SURAT KETERANGAN PENDUDUK LUAR NEGERI
Nomor: 636/VII/12/LON/2009

Kedutaan Besar Republik Indonesia di London dengan ini menerangkan bahwa:

Nama: Aceng Ruyani
Nomor Paspor: 8190455
Tanggal Tiba di UK: 15 Desember 2009
Lapor Diri di KBRI: 15 Desember 2009
Pekerjaan: Karyasiswa
Alamat Sekolah: University of Oxford
Oxford Centre for Diabetis
Oxford, OX3 7LJ
United Kingdom
Alamat Rumah: sda

Yang bersangkutan terdaftar pada Bidang Konsuler sebagai Karyasiswa Indonesia di Inggris dan akan ke Indonesia untuk kerja sama penelitian.

Demikian Surat Keterangan ini dibuat untuk Keperluan pengurusan Bebas Fiskal dan Exit Permit.

London, 22 Desember 2009
A.N. Duta Besar R.I.
Kepala Urusan Konsuler

[Signature]

LPWK.I. MIFTACH
NIP. 020004608
University of Oxford
Nuffield Department of Clinical Medicine

Academic Endocrine Unit, Oxford Centre for Diabetes, Endocrinology and Metabolism (OCDEM), Churchill Hospital, Headington, OXFORD, OX3 7LJ, UK

Professor R.V. Thakker  MD FRCP FRCPath FMedSci
May Professor of Medicine
Tel: 01865 857501  Fax: 01865 857502
E-mail: rajesh.thakker@ndm.ox.ac.uk

March 14th 2009

I stated here that Oxford Centre for Diabetes, Endocrinology and Metabolism, Churchill Hospital Academic Endocrine Unit, Nuffield Department of Clinical Medicine, University of Oxford was the established institution and has a lot experiences to study molecular aspects of SEDT (SPONDYLO-EPIPHYSEAL DYSPLASIA Tarda) cases.

I would like to invite Dr. Aceang Ruyani (Civil service data: NIP. 131 615 506; Karpeg D 438503), an educative staff of Bengkulu University, Bengkulu, Indonesia to visit the institution and research together the entitled investigation; “Molecular Study of SEDT Cases in Kedurang, South Bengkulu to Obtain Genetics Diversity and Candidate Marker for Early Detection”.

This Letter of Acceptance is required for completing Program of Academic Recharging (PAR) 2009 proposal which is applied by Dr. Aceang Ruyani to Directorate of Human Resource, Directorate General of Higher Education, Republic Indonesia Ministry of National Education, in Jakarta.

With best wishes,

Yours sincerely,

R. Thakker

Professor R V Thakker
May Professor of Medicine