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 dibiayai 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 Europe 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, Univeristas Bengkulu
2. Ketua Jurusan PMIPA, FKIP, Universitas Bengkulu
3. Ketua Prodi Pendidikan Biologi, JPMIPA, FKIP, Universitas Bengkulu

B13

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

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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.

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[#]Disajikan pada Seminar Hasil Kegiatan Peserta PAR B-C di Hotel Mellnium, Jakarta, 5 Oktober 2010.

Telah mempresentasikan
Hasil PAR di Hotel Millenium, Jakarta
pd tgl 5 oktober 2010

KEMENTERIAN PENDIDIKAN NASIONAL
DIREKTORAT JENDERAL PENDIDIKAN TINGGI

Purwanto Subroto, PhD
NIP. 132 133 925

Introduction

Spondyloepiphyseal dysplasia tarda {SEDT (MIM313400)} is an X-linked recessive osteochondrodysplasia, which has been reported to occur in about one in 500000 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).

SEDT 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 *etal.* 1973, Iceton *etal.* 1986, MacKenzie *etal.* 1996).

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

The *SEDL* gene, previously localised to Chromosome Xp22 (Szpiro-Tapia *etal.* 1988, Heuertz *etal.* 1993) was cloned and mutations identified in three Australian families (Gedeon *etal*. 1999). Gedeon *et al.* confirmed the earlier localisations (Szpiro-Tapia *etal.* 1988, Heuertz *et al* . 1993, Heuertz *etal* . 1995) and narrowed the region to Xp22.3-Xp21.3. Examination of a candidate open reading frame (ORF) revealed 3 dinucleotide deletions which were shown to cosegregate with the SEDT phenotype in these families.

SEDL 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 *etal*. 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 *etal*. 1999). It has also been shown to be widely expressed in tissues including; skeletal muscle, kidney, fetal cartilage, fibroblasts and lymphoblasts (Gedeon *etal*. 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 *SEDL* gene.

Methods

Patients

Probands of three extensive Indonesian SEDT families all from the Kedurang region, were ascertained (Figure 1). A family history of SEDT 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.

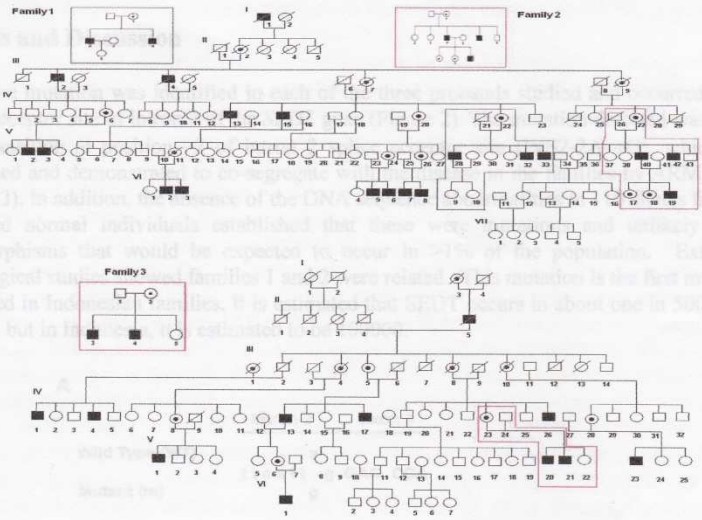

Figure 1. Family trees showing the families examined in this study. Individuals are represented as males (squares), females (circles), unaffected (open symbol), affected (filled in symbol) and unaffected carrier (dot in middle of symbol). Originally there were believed to be three families, but genealogical 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 *etal*. 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 *etal.* 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 *etal.* 1999). PCR products were gel purified and *Taq* polymerase cycle sequencing performed as described previously (Lloyd *etal.* 1996, Lloyd *etal.* 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 *etal*. 2008, Turner *etal*. 2010). These were demonstrated not to be common polymorphisms (by sequencing) in DNA obtained from 50 male and 30 female normal individuals (110 alleles). RT-PCR analysis performed as previously described (Nesbit *etal,* 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'-

CTGACATTGCGTTTCCGTTG-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.

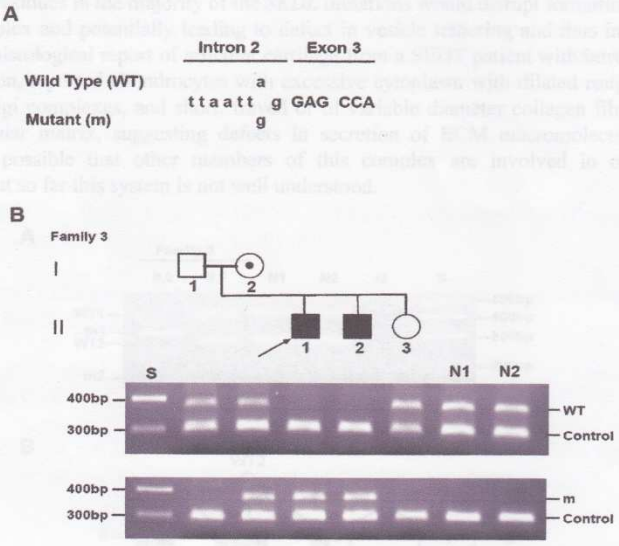

Figure 2. Detection of mutation in intron 2 of the *SEDL* gene in family 3. DNA sequence analysis of an affected male individual (II.2) revealed an a to g transition of the invariant ag dinucleotide of the acceptor splice site consensus sequence of intron 2 (A). The a to g transition did not alter a 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. Cosegregation 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 has 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, ^{111}MNPFY^{115} 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 (Gecz 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.

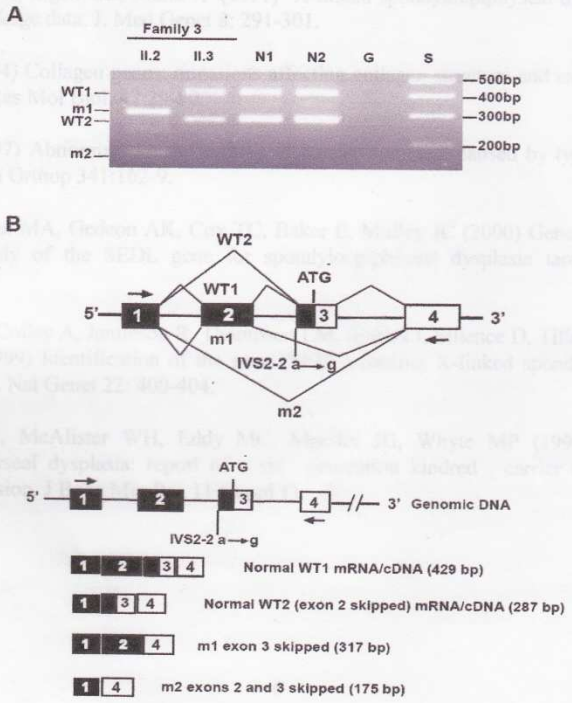

Figure 3. Exon skipping due to an intron 2 acceptor splice site consensus sequence mutation. The transcription of exons 1-4 of the *SEDL* gene was detected by RT-PCR using RNA obtained from EBV-transformed lymphoblastoids of an affected male (II.2), an unaffected female (II.3) from family 3 and two normal individuals (N1 and N2) (A). RT-PCR products were not obtained from the genomic control (G), 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 (II.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 SEDT, 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 (S; 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 *etal.* 1999; Gecz *etal.* 2000; Tiller *etal.* 2001).

Acknowledgements

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Report of The PAR-C Program (December 2009-March 2010)

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

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#Disajikan pada Seminar Hasil Kegiatan PAR II-C Tahun Anggaran 2009 di Hotel Millenium, Jakarta, 5 Oktober 2010.

Introduction

Historical facts: between Bengkulu and UK

The British East India Company established a long-running pepper-trading center and garrison at Bengkulu (Bencoolen) in 1685.

In 1714 the British built **Fort Marlborough** in the city; the fort still stands.

Sir Thomas Stamford Bingley Raffles (6 July 1781 – 5 July 1826) was an eminent British statesman

Raffles arrived in Bencoolen (Bengkulu) on 19 March 1818

It was reported that Raffles' expedition visited some areas in Bengkulu such as; Tabapenanjung, Kapahiang, and Kedurang for scientific proposes.

Furthermore, some people indicated that the expedition rested for a long time in Kedurang.

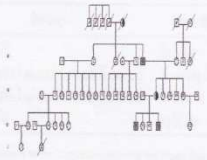
Previous studies of short stature cases in Kedurang, South Bengkulu

Morphological, anthropometrical, and pedigree studies

(Chairul Muslim, PhD, Drs. Bahakti Karyadi, M.Pd, Dr. Aceng Ruyani, 2006)

We observed 8 existing families of short stature consisting of 32 persons in Kedurang. The facts revealed that:

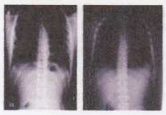
- The described as a mild dwarf (130-150 cm),
- Detected after 10 years old.
- Caused by the failure on lumber backbone growth and development.
- Only found among the men.
- Type of short stature is X-chromosomal linkage through heterozygous carrier females.

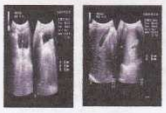


It is concluded that the short stature is SEDT

Radiological and ultrasound graph (USG) studies

(Dr. Aceng Ruyani, Chairul Muslim, PhD, Drs Bahakti Karyadi, M.Pd, dr H. Suherlan, S.Rad, 2007)





It is should be concluded that the bone morphology and the typography of liver and kidney on SEDT people from Kedurang changes to decrease as the effects of vertebrae and discus intervertebralis restriction.

Survey number and distribution of SEDT population in Kedurang

(Drs Bahakti Karyadi, M.Pd Dr. Aceng Ruyani, Chairul Muslim, PhD, 2008)

Demography of Kedurang:

Family: 3,040; People: 12,605; Male: 6,305; Female: 6,300.

The research revealed that from obtained 57 SEDT people (0.53%), it was 56 people of them could voluntary act as respondents.

SEDT population is distributed at the 20 villages in Sub district Kedurang.

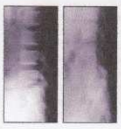
Period of age 21-30 years is majority (15 people) of the population.

Daughter and boy ratio of the SEDT families is 2:1.

This daughter is genetically predicted as the carrier which potential to increase SEDT population

Furthermore in looking for kind of compatible SEDT profession, it is necessary to generate a specific training for them.

Beside that, it should also be increased the access of SEDT people reach both formal and informal education



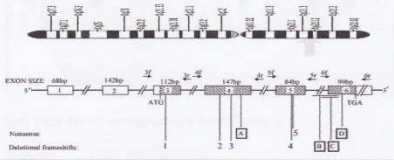
Spondyloepiphyseal dysplasia tarda {SEDT (MIM313400)} is an X-linked recessive osteochondrodysplasia, which has been reported to occur in about one in 500000 of the population in the UK (Wynne-Davies *et al.* 1985). This clinically and radiologically distinctive form of bone dysplasia was first described in a large American kindred (Jacobsen 1939).

SEDT 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 *etal.* 1973, Iceton *etal.* 1986, MacKenzie *etal.* 1996).

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

The *SEDL* gene, previously localised to Chromosome Xp22 (Szpiro-Tapia *etal.* 1988, Heuertz *etal.* 1993) was cloned and mutations identified in three Australian families (Gedeon *etal.* 1999). Gedeon *et al.* confirmed the earlier localisations (Szpiro-Tapia *etal.* 1988, Heuertz *et al* . 1993, Heuertz *etal* . 1995) and narrowed the region to Xp22.3-Xp21.3. Examination of a candidate open reading frame (ORF) revealed 3 dinucleotide deletions which were shown to cosegregate with the SEDT phenotype in these families.

SEDL 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 *etal.* 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 *etal.* 1999). It has also been shown to be widely expressed in tissues including; skeletal muscle, kidney, fetal cartilage, fibroblasts and lymphoblasts (Gedeon *etal.* 1999).



Results of mutation research from the four SEDT families were of European (UK) origin.

TABLE 1. SEDL mutations in SEDT families

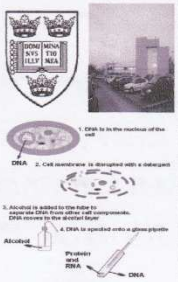
Family[a]	Location	Base change	Predicted effect[b]
A	ex4	765–776del, nt 310	Trp 78 Stop
B	in 5, ex 6	Loss of part of intron 5 and exon 6, first encodes 22 amino acids	15[th]–144th by deletion
C	in 5, ex 6	Loss of part of intron 5 and exon 6, first encodes 22 amino acids	764bp deletion
D	ex6	676–774del, nt 364	Arg 122 Stop

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 *SEDL* gene.

Methods

Patients

Probands of three extensive Indonesian SEDT families all from the Kedurang region, were ascertained (Figure 1). A family history of SEDT 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.



Mutational Analysis

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

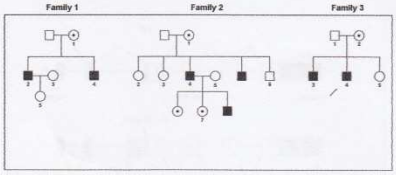
DNA sequence abnormalities were initially sought in each of the SEDT probands. Four pairs of primers (Gedeon *etal.* 1999) were used for the PCR amplification of the four coding exons of the *SEDL* gene and of their corresponding intron/exon boundaries with conditions described previously (Gedeon *etal.* 1999). PCR products were gel purified and *Taq* polymerase cycle sequencing performed as described previously (Lloyd *etal.* 1996, Lloyd *etal.* 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 *etal.* 2008, Turner *etal.* 2010). These were demonstrated not to be common polymorphisms (by sequencing) in DNA obtained from 50 male and 30 female normal individuals (110 alleles). RT-PCR analysis performed as previously described (Nesbit *etal,* 2004) to determine the splicing effect of the *SEDL* mutation.

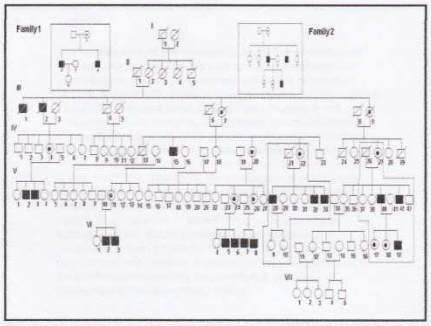
Total RNA from blood lymphocytes was extracted using Trizol reagent (Invitrogen) and was reverse transcribed using the SuperScript first-strand synthesis system (Invitrogen) and random hexamers. Subsequently, RT-PCR was performed using exon1F 5'-CTGACATTGCGTTTCCGTTG-3' and exon4R 5'-AGTGACAAATGCCGACACAA-3' primers. RT-PCR products were sequenced in both directions.

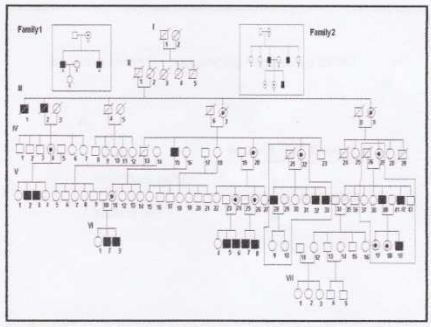
Results and Discussion

SEDT Mutational Analysis: Indonesian Family



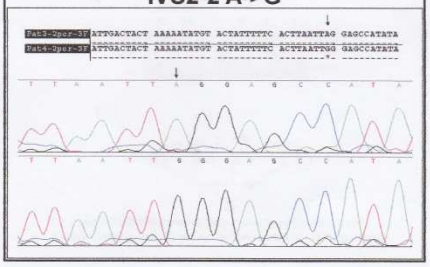
Cell lines set up on individuals from Family 3





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.

DNA sequencing results: IVS2-2 A->G



IVS2-2 A->G

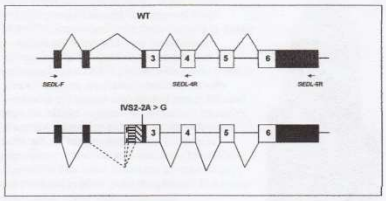
	Splice acceptor consensus strength	intron 2 / exon 3
WT	47%	ttaattag / gagccata
IVS2-2A>G	-	ttaattgg / gagccata

Cryptic splice acceptor sites upstream of mutation

-680	95%	ttttctag / aatacaaa
-514	98%	ctttctag / gtaagtga
-370	96%	ccttctag / gtgtccgt

RT-PCR and sequencing showed these cryptic splice acceptor sites are not utilized in the mutant product

SEDL Splicing -



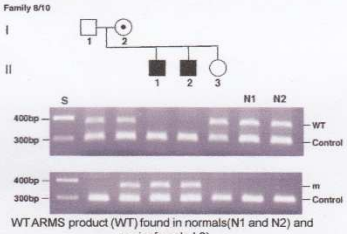
The identification of the mutation in these three families will enable presymptomatic diagnosis and the identification of asymptomatic mutant gene carriers within this population. To date, there are 47 different mutations that occur throughout the *SEDL* gene, and about 25% of these mutations are splicing 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 has 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, ^{111}MNPFY^{115} 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 (Gecz 2000).

Mutation found by sequencing of genomic DNA:

A

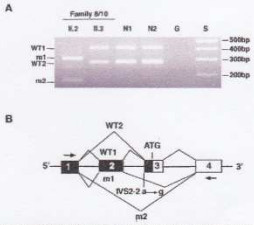
	Intron 2	Exon 3
Wild Type (WT)	a ttaatt g	GAG CCA
Mutant (m)	g	

Family tree and ARMS-PCR results



WT ARMS product (WT) found in normals(N1 and N2) and carrier female I.2)
Mutant ARMS product(m) only found in affected males (11.1 and 11.2) and carrier female I.2)

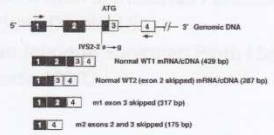
RT-PCR and diagram showing splicing



WT 1 and WT2(lacking exon 2) are normally spliced products, m1 and m2 (lacking exon 2) are mutant products both lacking exon 3

4

Diagrams showing exons and splicing



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.

Acknowledgements

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Thank you very much !

Thank you very much !!

Bengkulu, 7 Juni 2010

Kepada yth
Ibu **Istri Hardiyanti**
Ketenagaan Ditjen Dikti,
Departemen Kementrian Pendidikan Nasional,
Gedung D Lantai 5
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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 penelitan yang diperoleh pada butir a.
(c) Saat ini, Juni 2010, kami sedang berdiskusi menyiapkan 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 haturkan banyak terimakasih.

Wasselam,

Dr. Aceng Ruyani

Tembusan disampaikan Kepada Yth:
1. Pembantu Rektor I, Universitas Bengkulu
2. Dekan FKIP, Univeristas Bengkulu
3. Ketua Jurusan PMIPA, FKIP, Universitas Bengkulu
4. Ketua Prodi Pendidikan Biologi, JPMIPA, FKIP, Universitas Bengkulu

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UNIVERSITAS BENGKULU
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SURAT TUGAS
Nomor : 4002 /H 30.3/KP/2010

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

No	Nama	NIP	Unit Kerja
1	Dr. Aceng Ruyani, M.S	19600105 198603 1 006	Dosen Jurusan Pendidikan MIPA FKIP UNIB

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,

Prof. Safnil, M.A., Ph.D
NIP. 19610121 198601 1 002

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Nomor 6234 /H30/PP/2010 21 September 2010
Lampiran :
Hal : 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:

NO	NAMA/NIP	PAR	PT TUJUAN
1	Dr .Aceng Ruyani/ 196001051986031006	C	Oxford University
2	Hery Suhartoyo, Phd/ 196306251987031002	B	University of Oueensland

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

Rektor
anb
Pembantu Rektor Bidang Akademik

Dr. Ir. Fahrurozi, M.Sc
NIP 19641029 198903 1 002

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	:	S190455
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

KEDUTAAN BESAR REPUBLIK INDONESIA LONDON

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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. **Aceng 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. **Aceng 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