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Identification of novel variation in introns three and four of the iduronate-2-sulfatase gene in patients with mucopolysaccharidosis type II

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Abstract. To identify and analyze mutations that occur in introns of the iduronate-2-sulfatase (IDS) gene of patients with mucopolysaccharidosis type II (MPS II) in Indonesia. DNA samples from 6 patients with MPS II and 49 normal individuals were analyzed with direct sequencing of introns three and four. A novel variant was found at the site c.419-132del in intron three and three variants were found at c.507+26G>A, c.507+29G>A, and c.507+63T>G in intron four of the IDS gene. These variations occur in the non-splicing site of the IDS gene in patients with MPS II and normal individuals and do not affect the structure of the IDS gene. Alterations which occur in one site of intron three and three sites of intron four are new variations of the IDS gene found in Indonesian patients. This finding suggests that the IDS gene may show variation in other sites that could be associated with MPS II. These mutations can enrich the single nucleotide polymorphism database for the IDS gene globally. Further genotype studies should be performed to obtain a full profile of the IDS gene in patients with MPS II in Indonesia.

1. Introduction
Mucopolysaccharidosis type II (MPS II, OMIM 309900), also known as Hunter syndrome, is an X-linked recessive lysosomal storage disorder caused by the deficiency of the iduronate-2-sulfatase (IDS) enzyme encoded by the IDS gene. MPS II occurs in 1 of 76,000 to 320,000 male live births [1]. However, rare cases of MPS II also occur in female patients [2]. The IDS enzyme is involved in the metabolic process of glycosaminoglycans (GAGs) by hydrolyzing heparin sulfate and dermatan sulfate.
IDS enzyme deficiency causes the accumulation of GAGs in the lysosome. This causes patients with MPS II to undergo various clinical disorders that are divided into two subtypes of phenotype: severe and attenuated (i.e., intermediate or mild). The severe phenotype is associated with patients with MPS II diagnosed approximately at 18–36 months of age. The severe phenotype also shows mental retardation seen at 4–6 years of age and is associated with a short lifespan. Patients with MPS II with the severe phenotype only survive up to 10–15 years of age. Patients with MPS II with the attenuated phenotype are diagnosed at 4–8 years of age, have no mental retardation, and have a longer life expectancy [1,3]. Nevertheless, there is no standard value for measuring the severity of the MPS II phenotype. MPS II phenotypes are assessed based on onset time, with early onset indicating the severe phenotype and late onset indicating the attenuated phenotype [4].

The IDS gene is 24 kb in length and is composed of nine exons [5]. It encodes 550 amino acids which become the structure of the active IDS enzyme. The IDS gene is located on chromosome Xq28 and is known to have a pseudogene, IDS2, 80 kb downstream of the transcribed IDS gene [6]. The existence of IDS2 causes IDS genes to be susceptible to recombination. The results of mutation analysis in IDS genes may become biased if primers become attached to the pseudogene.

To date, at least 483 mutations have been identified in the IDS gene, 9.1% of which are splicing mutations [7]. A splicing mutation is an alteration of bases that occurs in splicing sites in the gene. This type of mutation often results in significant changes in the protein. Therefore, splicing may disturb the translation process of mature mRNA into a functional protein.

In this study, we identified and analyzed mutations that occur in introns of the IDS gene from patients with MPS II in Indonesia. We found novel variations in introns three and four of these patients.

2. Materials and Methods

2.1 Sample collection and DNA extraction.

DNA was obtained from blood samples of 6 patients with MPS II and 49 normal individuals from Cipto Mangunkusumo National Referral Hospital, Indonesia. DNA isolation was performed using the Genomic DNA Mini Kit for Blood/Cultured Cell from Geneaid. The concentration and purity of the isolated DNA was quantified by spectrophotometer.

2.2 Amplification and sequence analysis

Polymerase chain reaction (PCR) primers to capture the IDS gene sequence (intron three, exon four, and intron four) were constructed using Primer3. Primer design was needed to ensure the designed primer annealed at the specific site of the sequence. PCR primers specifically amplified the target in the IDS gene, which had a length of 389 bp. PCR primer properties were verified using IDT DNA and NCBI, whereas secondary structures of PCR primers were analyzed using the NetPrimer software.

DNA samples of patients and normal individuals were amplified using the forward primer (5’TACTCAGGCTTAGGACCA3’) and reverse primer (5’CCAGCTTCACAGAACATGCAG3’). These primers were used to amplify exon four by including 200 bases of intron three and 90 bases of intron four.

The following PCR procedures were performed: pre-denaturation at 95 °C for 60 s, denaturation at 95 °C for 15 s, annealing at 59.5 °C for 15 s, and extension at 72 °C for 30 s. Processes from denaturation to extension were repeated up to 40 cycles, followed by post-extension at 72 °C for 10 min. PCR products were visualized using 2% agarose gel electrophoresis and a gel documentation system. PCR products were outsourced to First Base Sequencing Service in Singapore, and the results were analyzed. Mutations and variations in introns three and four were listed and verified using NCBI to identify the novel ones.

3. Results and Discussion

3.1 Results

The isolation process yielded DNA with concentrations ranging from 1.40 ng/μL to 2.939 ng/μL. DNA purity was measured using 260/280 ratio, with purity results ranging from 1.35 to 1.90.
DNA samples were amplified by conventional PCR to amplify the target sequences (intron three, exon four, and intron four) using specific primers. The primer showed no hairpin structure and a dimer $<10$ kcal/mol. Gradient PCR was conducted to determine the exact annealing temperature for proper amplification (Fig. 1). The DNA band was visualized at $55 \, ^\circ\text{C}$–$65 \, ^\circ\text{C}$, and it was better visualized at $59 \, ^\circ\text{C}$–$63 \, ^\circ\text{C}$. Based on PCR optimization, the annealing temperature for DNA amplification was determined as $59.5 \, ^\circ\text{C}$.

IDS gene amplification resulted in a band length of 389 bp, the same as with the primer design software, including 200 bp for intron three, 89 bp for exon four, and 90 bp for intron four (Fig. 2). The electropherogram of sequencing results was analyzed in Chromas 2.6.4 (Fig. 3). Low quality and significance values of sequence peaks were eliminated to obtain high-quality DNA sequences.

Sequences were aligned with the template of the IDS gene sequence obtained from NCBI. The alignment was performed using BioEdit (Fig. 4 and Fig. 5). The sequence coverage was 4318–4406, the coverage of exon four. Some of the sequence results covered intron three (before 4318) and intron four (after 4406). There were 16 variations and mutations in intron three and four variations and mutations in intron four. Variations and mutations in introns three and four are listed in Tables 1 and 2. Based on the alignment results, the mutations in introns three and four (Tables 1 and 2) were checked into the IDS gene database on the NCBI website.

![Figure 1. Result of the polymerase chain reaction gradient on agarose gel electrophoresis.](image1)

![Figure 2. Agarose gel electrophoresis results of the target sequence (389 bp). Wells 1–6 were loaded with DNA samples from patients with mucopolysaccharidosis type II. Wells 7–10 are normal DNA samples.](image2)
Figure 3. Electropherogram of exon four of the iduronate-2-sulfatase gene from a patient with mucopolysaccharidosis type II

Figure 4. Alignment results of intron three of the iduronate-2-sulfatase gene.

Figure 5. Alignment result of exon four and intron four of the iduronate-2-sulfatase gene.
Table 1. Summary of variation in intron three of the iduronate-2-sulfatase gene found in the samples of patients with mucopolysaccharidosis type II and normal samples.

| Number | Position | Samples |
|--------|----------|---------|
| 1      | c.419-2A>G | 35      |
| 2      | c.419-20A>C | 13, 14, 20, 28, 31, 33, 44, 46, 47, 52, 55, 57 |
| 3      | c.419-24del | 9, 13, 14, 20, 28, 31, 33, 34, 46, 47, 52, 55, 57 |
| 4      | c.419-29T>G | 9, 13, 14, 20, 28, 31, 33, 34, 46, 47, 52, 55, 57 |
| 5      | c.419-38G>A | 9, 13, 14, 20, 28, 31, 33, 34, 46, 47, 52, 55, 57 |
| 6      | c.419-46T>G | 9, 13, 14, 20, 28, 31, 33, 34, 46, 47, 52, 55, 57 |
| 7      | c.419-51T>G | 9, 13, 14, 20, 28, 31, 33, 34, 46, 47, 52, 55, 57 |
| 8      | c.419-70G>A | 9, 13, 14, 20, 24, 28, 31, 33, 35, 44, 46, 47, 52, 55, 57 |
| 9      | c.419-97G>A | 13, 14, 24, 28, 31, 33, 35, 44, 46, 47, 52, 55, 57 |
| 10     | c.419-102G>A | 9, 13, 14, 20, 24, 28, 31, 33, 35, 44, 46, 47, 52, 55, 57 |
| 11     | c.419-110G>A | 24      |
| 12     | c.419-110G>C | 13, 14, 20, 28, 31, 33, 44, 46, 47, 52, 55, 57 |
| 13     | c.419-119G>A | 24      |
| 14     | c.419-121G>A | 13, 14, 20, 28, 31, 33, 35, 44, 46, 47, 52, 55, 57 |
| 15     | c.419-124G>A | 13, 14, 20, 28, 31, 33, 35, 44, 46, 47, 52, 55, 57 |
| 16     | c.419-132del | 1, 2, 3, 4, 5, 7, 8, 9, 22, 24, 32, 36 |

Table 2. Summary of variation in intron four of the iduronate-2-sulfatase gene found in the samples of patients with mucopolysaccharidosis type II and normal samples.

| Number | Position | Samples |
|--------|----------|---------|
| 1      | c.507+2T>G | 9, 13, 14, 20, 28, 31, 33, 44, 46, 47, 52, 55, 57 |
| 2      | c.507+2T>A | 35      |
| 3      | c.507+7C>G | 9, 13, 14, 20, 28, 31, 44, 46, 47, 52, 55, 57 |
| 4      | c.507+29   | all samples |

3.2 Discussion
Mutations were found in all samples of patients with MPS II and normal samples, confirming the genetic variation of the IDS gene. Intronic variations found in both the samples of patients with MPS II and normal samples showed variation in the position of the 5’ and the 3’ splice sites. Most variations were single nucleotide polymorphisms (SNPs), whereas only two variations were intronic deletions (i.e., c.419-24del found in normal samples and c.419-132del found in samples of patients with MPS II and normal samples).

SNPs occur normally in human DNA and do not have further effects on the translation of mRNA into functional proteins. SNPs can be used as biomarkers to locate specific genes such as the IDS gene [8]. Intronic variations of c.419-2A>G in intron three (position −2 of the 3’ splice site), c.507+2T>G, and c.507+2T>A in intron four (position +2 of the 5’ splice site) showed that variation around a splice site [9] may affect the specificity and efficiency of splicing [10]. These variations could be used as
biomarkers. Further research should focus on determining whether these variations affect mature mRNA translation in terms of time and product.

4. Conclusion
Alterations which occur in introns three and four of the IDS gene are new variations of the IDS gene found in Indonesian patients. These variations can enrich the SNP database of the IDS gene globally. Intronic variation can be used as a biomarker to locate specific locations of exon four that is flanked by introns three and four. Further genotype studies should be performed to obtain full profiles of the IDS gene in patients with MPS II in Indonesia.

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