A Novel mutation in iduronate-2-sulfatase gene Exon 6 of an Indonesian patient with Mucopolysaccharidosis type II

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Abstract. Mucopolysaccharidosis type II (MPS II or Hunter syndrome) is a rare X-linked recessive disease caused by mutation of the gene encoding the lysosomal enzyme iduronate-2-sulfatase (IDS). Deficient lysosomal degradation of the glycosaminoglycans dermatan sulfate and heparan sulfate by mutant IDS leads to their accumulation in multiple tissues, causing progressive tissue hypertrophy (e.g., hepatosplenomegaly and macroglossia), various deformities, and narrowing of the respiratory tract among other symptoms. Here, we searched for additional IDS mutations in Indonesian patients to provide information for establishing associations with disease traits. Exon-specific PCR amplification and sequencing of IDS gene exon 6 were conducted on DNA samples from MPS II patients and healthy controls at Cipto Mangunkusumo National Referral Hospital, Jakarta, Indonesia. A novel mutation in an Indonesian patient with MPS II was identified, a 14 bp insertion mutation (c.792_793insCCCCTGTGGCCTAC) in IDS gene exon 6 resulting in the amino acid changes p.Tyr264_X269insProLeuTrpPro and X269Thr. This novel mutation likely alters the structure and function of IDS. We are currently analyzing other IDS gene exons to identify additional mutations linked to IDS. Such studies will help clarify MPS II pathophysiology and may lead to novel treatment strategies.

1. Introduction
Iduronate-2-sulfatase (IDS) is a lysosomal enzyme important for the degradation of the glycosaminoglycans (GAGs) dermatan sulfate and heparan sulfate. Mucopolysaccharidosis type II [1-3] (MPS II or Hunter syndrome) is a rare X-linked recessive disease with male predominance resulting from IDS mutation, ensuing enzyme deficiency, and concomitant lysosomal GAG accumulation. The disease is divided into two main classes, “severe” and “mild,” based on time of onset and severity of symptoms. “Severe” patients can be diagnosed at a young age (early onset), whereas the “mild” group patients will start showing symptoms around age 20 years (late onset) [4]. The symptom profile of MPS II is
believed to reflect specific mutations in the IDS gene that affect the stability and function of the protein product [5].

The IDS gene is located at band Xq28 on the X chromosome. It is 28 kb in length and consists of nine exons [6]. Mutations will cause various alterations in IDS protein structure and enzymatic function. Numerous mutations in the IDS gene have been identified in several exons and may be missense, nonsense, insertion, deletion, or inversion. Here, we genotyped exon 6 among MPS II patients to identify additional mutations and establish possible associations with disease characteristics.

2. Materials and Methods

This study included six Indonesian MPS II patients treated at Cipto Mangunkusumo National Referral Hospital and 48 healthy controls. DNA samples were isolated from blood using The Geneaid™ DNA Isolation Kit. Concentration and purity were measured using a spectrophotometer. The IDS gene was amplified by PCR with exon 6-specific primers designed using NCBI Primer Blast. The IDS gene exon 6 transcript was characterized by agarose gel electrophoresis and sequencing (1st Base Sequencing Service, Singapore). Sequencing results were analyzed using BioEdit.

3. Results and Discussion

3.1 Results

The DNA concentrations in the samples ranged from 1.40 to 2,939 ng/μL and the 260 to 280 nm optical density ratio (OD260/280) from 1.35 to 1.90. The concentration and purity of the usable patient samples are shown in Table 1. In these five samples, all OD260/280 values were around 1.8 and the OD 260/230 values were around 2.4, indicating high purity for subsequent amplification and sequencing.

| No | Concentration (ng/μl) | Purity 260/280 | Purity 260/230 |
|----|-----------------------|----------------|---------------|
| 1  | 1428.00               | 1.88           | 2.41          |
| 2  | 2384.00               | 1.89           | 2.41          |
| 3  | 898.90                | 1.84           | 2.42          |
| 4  | 963.90                | 1.85           | 2.44          |
| 5  | 757.40                | 1.86           | 2.46          |
| 6  | 1120.00               | 1.87           | 2.43          |

Gradient PCR was performed to find the optimal annealing temperature for amplification (Figure 1). On the basis of these data, annealing for exon 6 amplification was performed at 54 °C. The PCR results for MPS II patients and healthy controls are shown in Figure 2. Specific DNA band length was 227 bp, similar to that predicted on the basis of the primer design. Sequencing results are shown in Figure 3 as an electropherogram. Sequences were aligned using BioEdit and the IDS gene sequence from NCBI as a template. This analysis revealed a novel 14 bp insertion mutation (c.792_793insCCCCTGTGGCCTAC) (Figure 4) in one MPS II patient with severe symptoms but not in the other 5 MPS II patient or 48 healthy control samples. To the best of our knowledge, this is the first documented instance of this specific mutation. The mutation is predicted to confer the following amino acid alterations (Figure 5): p.Tyr264_X265insProLeuTrpPro and p.X265Thr.
Figure 1. Optimization of primers for exon 6 by gradient PCR

Figure 2. Electrophoresis of DNA samples from MPS II patients and healthy controls

Figure 3. Electropherogram of a healthy sequence to be aligned
3.2 Discussion
Reliable measurement of DNA concentration and purity is critical for subsequent applications such as PCR amplification and sequencing, and impurities in DNA samples reduce the accuracy of DNA concentration measurements. The concentration and purity of DNA vary markedly among samples, which may affect the visibility of bands by electrophoresis. In this study, we focused on one exon and designed exon-specific primers. Further, we conducted gradient PCR to find the optimal annealing temperature (54 °C) for PCR. Using the determined PCR temperature, a “running” process was conducted with samples from MPS II patients. The lack of a band from patient 2 resulted from the low DNA concentration of the sample. In addition, other control samples (14 in total) did not yield bands at the expected position. For this reason, we did not include some samples in the sequencing analysis. Nonetheless, the overall results of the running process were adequate to show that the primers are exon specific, as the band appeared at the predicted target area based on the length of the amplicon (227 bp).

Sequencing was conducted by a commercial DNA sequencing service (1st BASE, Singapore). Sequences were first checked and cleaned for alignment using BioEdit. The alignment process was achieved for the 5 MPS II patient and 34 control samples deemed suitable for sequence analysis using the IDS gene consensus coding sequence as the base template. As a result, a novel mutation in exon 6 of the IDS gene was identified in one MPS II patient, a 14 bp insertion (c.792_793insCCCCTGTTGGCCTAC). This mutation was not observed in the other 4 MPS II patients or the 34 control samples. The mutation leads to the amino acid alterations p.Tyr264_X265insProLeuTrpPro and p.X265Thr. Of the 14 inserted nucleotides, 12 encode 4 additional amino acids (p.Tyr264_X265insProLeuTrpPro) and 2 nucleotides merge with the following nucleotide to encode another amino acid (p.X265Thr). This causes an error in reading the rest of the nucleotides and formation of an amino acid sequence entirely different from the wild-type IDS protein. These alterations presumably change the structure and impair the function of the IDS enzyme, which may lead to lysosomal GAG accumulation and the observed symptoms in this MPS II patient.

4. Conclusion
A novel mutation was identified in IDS gene exon 6 of an Indonesian patient with MPS II. Given the severe phenotype of the patient, this mutation likely reduces the activity of the enzyme. This finding also suggests that the gene pool in Indonesia harbors unique mutations in the IDS gene. Therefore,
further analyses of exon 6 and other IDS gene exons may reveal additional novel mutations. Establishing associations of specific mutations with disease phenotypes may provide clues to the pathophysiological mechanism underlying MPS II.

Acknowledgement
This research was funded by the PITTA (Publikasi Internasional Terindeks untuk Tugas Akhir) Grant from DRPM (Direktorat Riset dan Pengabdian Masyarakat or Directorate of Research and Community Service), Universitas Indonesia. This research was conducted at the Human Genetics Research Center, Indonesian Medical Research Institute, Faculty of Medicine, Universitas Indonesia.

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