A novel silent mutation at exon 9 of iduronate-2-sulfatase gene in an Indonesian patient with mucopolysaccharidosis type II

R Priambodo¹, Y Ariani¹,²,³, Y Pangestika¹,⁴, C N Hafifah¹,², A Bowolaksono⁵ and D R Sjarif¹,²

¹Human Genetic Research Center, Indonesian Medical Education and Research Institute (IMERI), Universitas Indonesia, Jakarta, Indonesia
²Department of Pediatric, University of Indonesia, RSUPN Dr. Cipto Mangunkusumo, Jakarta, Indonesia
³Department of Medical Biology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
⁴Master Program in Biomedical Sciences, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
*E-mail: ukk.npm.idai@gmail.com

Abstract. This study aimed to assess the data available on mucopolysaccharidosis type II (MPS II) from the Pediatric Department of Cipto Mangunkusumo National Hospital and to investigate unrelated Indonesian patients with MPS II in order to characterize their genotypes. Mutational analysis was performed on DNA extracted from whole blood samples of 6 patients with MPS II after designing primers, amplifying exon 9, visualizing the amplification product; sequencing the product, and analyzing the exon 9 sequence. Silent mutation was detected at c.1404G>A at exon 9 of the iduronate-2-sulfatase (IDS) gene in only 1 of the 6 patients. The mutation did not change the amino acid in the IDS enzyme. The silent mutation detected in this study is a novel mutation not reported earlier. The reported data is expected to contribute to the formation of new database of mutations for patients with MPS II, especially in Indonesia.

1. Introduction
Mucopolysaccharidosis type II (MPS II; OMIM 309900), also known as the Hunter Syndrome, is an X-linked recessively inherited lysosomal disorder caused by the deficiency of iduronate-2-sulfatase activity (IDS; EC 3.1.6.13), which is involved in the degradation of glycosaminoglycan (GAGs) dermatan sulfate and heparan sulfate (DS-HS). Failure to hydrolyze the terminal iduronate-2-sulfate esters in these GAGs results in the progressive accumulation of non-degraded substrates within the lysosomes and in the clinical manifestations related to MPS II. Deficiency in the lysosomal enzyme IDS leads to the accumulation of GAGs and DS-HS. The IDS gene is located in Xq28, spanning 24-kb size and containing 9 exons. An IDS-like pseudogene comprising of copies of exons 2 and 3 and intron 7 is located at about 20-kb distance away from the active gene [1]. Approximately 10%–20% of patients with MPS II present with large gene alterations, including rearrangements and total IDS gene deletions, whereas 80%–90% of them present with only small gene alterations [2,3]. According to the Human Gene Mutation Database, 483 different mutations have been described in the IDS gene until November 2017, which includes missense/nonsense (246 mutations), splicing (44 mutations), small deletions (90
mutations), small insertions (39 mutations), small indels (9 mutations), gross deletion (37 mutations), gross insertions/duplications (4 mutations), and complex rearrangements (14 mutations) [4].

Patients with MPS II are classified as having its severe, intermediate, or attenuated forms, depending on the degree of mental retardation presented. The severe form of this disease becomes apparent between 2 and 4 years of age and is characterized by progressive neurological and somatic involvement. A milder form of the Hunter syndrome is characterized by the preservation of intelligence and survival into the adulthood, albeit with obvious somatic involvement [1]. However, considering that there is no standardized scoring index of severity for MPS II, this classification is not always reliable, especially in the case of patients diagnosed with MPS II during their childhood [3].

Genotype identification of mutations in Indonesian patients with MPS II is important because of the varying clinical symptoms associated with mutation in the IDS gene. In addition, there is no research and reports on MPS II in Indonesia. In the present study, we have focused on MPS II based on the data from the Pediatric Department of Cipto Mangunkusumo National Hospital; MPS II is the most common type of MPS in Indonesia. This study investigated 6 unrelated Indonesian patients with MPS II in order to characterize their genotypes.

2. Materials and Methods
DNA were obtained from the whole blood samples of 6 patients with MPS II presenting at the Cipto Mangunkusumo National Referral Hospital, Jakarta. The DNA was extracted by following the salting out method. The extracted DNA was quantified to determine its concentration and purity with NanoDrop spectrophotometer. The primer of exon 9 was designed with reference to the NCBI primer BLAST listed in Table 1. Briefly, initial denaturation was performed at 95 °C for 60 s, followed by denaturation at 95 °C for 15 s and annealing with gradient to optimize the amplification process (50°, 52°, 54°, 56°, 58°, and 60 °C) for 15 s, and extension at 72 °C for 30 s in 40 cycles. Next, a 10 min of final extension was performed at 72 °C. After reaching the appropriate annealing temperature from the gradient PCR process, the PCR mix was used at a top-up volume for sequencing. PCR products was visualized by using 1.8% agarose gel electrophoresis with the aid of 1-kb DNA ladder Gene aid and stained with Gel Red. The PCR product for each sample was sent to the 1st Base Sequencing Services at Singapore to obtain the sequence data using the Sanger method. All sequences data were verified with the database on the NCBI gene bank, followed by processing with the Bio Edit software to locate the mutation.

Table 1. Primer and conditions for PCR amplification of IDS exon 9.

| Sequence (5'-3') | Tm | GC% | Product length | Secondary structure |
|-----------------|----|-----|----------------|--------------------|
| Forward primer  | 59.97 | 55 | 780bp | 0 | 0 |
| Reverse primer  | 56.65 | 47.37 | -9.28 | 0 | 0 |

3. Results
3.1 DNA isolation
DNA isolation produced two main data sets: DNA concentration and DNA purity (Table 2). The DNA concentrations value ranged from 757.40 ng/µL to 2.3894 ng/µL, whereas DNA purity ranged from 1.84 to 1.89 for the 260/280 ratio. Our results cumulatively indicate the absence of contamination of RNA or other proteins in the DNA sample and ascertains the purity of the sample for use in the amplification process.
Table 2. Concentration and purity of extracted DNA.

| Sample Code | Status     | Sex  | Concentration (ng/μL) | Purity 260/280 | Purity 260/230 |
|-------------|------------|------|-----------------------|----------------|----------------|
| Ex9_1       | MPS type II| Male | 1428.00               | 1.88           | 2.41           |
| Ex9_2       | MPS type II| Male | 2384.00               | 1.89           | 2.41           |
| Ex9_3       | MPS type II| Male | 898.90                | 1.84           | 2.42           |
| Ex9_4       | MPS type II| Male | 963.90                | 1.85           | 2.44           |
| Ex9_5       | MPS type II| Male | 757.40                | 1.86           | 2.46           |
| Ex9_51      | MPS type II| Male | 1120.00               | 1.87           | 2.43           |

3.2 Amplification
Optimization for amplification was performed by the PCR gradient process involving six different temperatures ranging from 50° to 60 °C (Fig 1). Single DNA bands appeared at all temperature conditions. This process indicated that the specific annealing temperature for exon 9 could be 60 °C because the DNA bands continued to appear as the maximum gradient. Another indication is that both the primers were constructed before they could anneal specifically on the gene target. Amplification of exon 9 was of size 780 bp (Fig 2).

![Figure 1. Gel image of PCR optimization for exon 9 with six different temperatures. Bands thickly appeared from 50 °C to 60 °C with no multibands. Temperature at 60 °C could be selected as the annealing temperature (Note: M = 1-KB DNA ladder Geneaid).](image1)

![Figure 2. Gel image of amplification after optimization process for each sample at exon 9. The size of DNA estimated at 780 bp (Note: M = 1 KB DNA ladder Geneaid; 1 = Ex9_1; 2 = Ex9_2; 3 = Ex9_3; 4 = Ex9_4; 5 = Ex9_5; 6 = Ex9_51).](image2)
3.3 Mutation analysis
A mutation occurred only at c.1404G>A in exon 9 in 1 patient; this mutation has not been reported earlier (Table 3). This mutation changed one nucleotide base from guanine to adenine. Based on the amino acid alignment (Fig 3), no change in the amino acid sequence was noted.

Table 3. Mutation analysis

| Sample Code | Mutation | Consequence | Mutation | Reference               |
|-------------|----------|-------------|----------|-------------------------|
| -           | c.1402C>T| p.R468W     | Missense | Crotty et al., 1992 [5]  |
|             | c.1403G>A| p.R468Q     | Missense | Whitley et al. 1993 [6]  |
|             | c.1265>T | p.C422F     | Missense | Chiong et al. 2017 [7]   |
| 1461_1462insN[710] | -       | Insertion   |          | Chiong et al. 2017 [7]   |
| c.1226C>G   | p.T409R  | Missense    |          | Chiong et al. 2017 [7]   |
| Ex9_3      | c.1404G>A| p.468Pro    | Silent   | This Study              |

Figure 3. DNA sequence alignment analysis result using Bioedit software, identifying a silent mutation at c.1404G>A on Ex9_3 (upper panel). Codon alignment result using Bioedit software showing the same amino acid at p.468Pro on Ex9_3 (lower panel).

4. Discussion
Only one mutation was identified at exon 9 of IDS gene among 6 patients with MPS II. This mutation has not been reportedly earlier although some mutations at exon 9 have been reported in the literature [5-7]. The mutation site c.1404G>A showed a change in the codon from CCG to CCA at p.468Pro. Both these codons translate to the same amino acid proline. This novel mutation is a silent mutation. Silent mutations do not have any effect on the enzyme structure. In this novel study, no effect of codon alteration was noted on the IDS enzyme, which plays an important role in MPS II.

However, some studies in the literature have reported impacts of silent mutations. In recent years, the long-held dogma has been refuted by evidence that even a single synonymous codon substitution (silent mutation) can have a significant impact on the gene expression levels, protein folding, and protein cellular function [8-11]. Some experimental evidence have been provided regarding the role of synonymous codon substitutions, particularly in slow-translating regions of the mRNA, and their impact on the protein structure or function have also been summarized [12]. Interestingly, we adopted a bioinformatics approach in this study, which may facilitate the comprehension of secondary structure and implications of functional alteration.

5. Conclusion
A novel silent mutation c.1404G>A was identified at exon 9 in Indonesian patients with MPS II. The discovery of this novel mutation is expected to contribute significantly to the new database of MPS II globally. Further studies on the secondary structure is warranted for deeper understanding.
Acknowledgement
This research was funded by PITTA Grant 2017 from DRPM Universitas Indonesia. We thank the team of Human Genetic Research Center, Indonesian Medical Education and Research Institute (IMERI), Universitas Indonesia for the support and cooperation.

References
[1] Neufeld E F and Muenzer J 2001 The Mucopolysaccharidoses vol 12, ed C R Scriver, A L Beaudet, W S Sly and D Valle (New York: McGraw-Hill)
[2] Mirella F, Bonucelli G, Corsolini F, Mazzotti R, Cusano R and Gatti R 2001 Molecular analysis of 40 Italian patients with mucopolysaccharidosis type II: new mutations in the iduronate-2-sulfatase (IDS) gene Hum. Mutat. 18 164-5.
[3] Froissart R, Moreira da Silva I, Guffon N, Bozon D and Maire I 2002 Mucopolysaccharidosis type II genotype/phenotype aspects Acta Paediatr. 91 82-7.
[4] HGMD 2017 The Human Gene Mutation Database, http://www.hgmd.cf.ac.uk/ac/index.php search 2017.
[5] Crotty P L, Braun S, Anderson R and Whitley C 1992 Mutation R468W of the iduronate-2-sulfatase gene in mild Hunter syndrome (mucopolysaccharidosis type II) confirmed by in vitro mutagenesis and expression Hum. Mol. Genet. 9 755-7.
[6] Whitley C R, Anderson R A, Aronovich E L, Crotty P L, Anyane-Yeboa K, Russo D and Warburton D 1993 Caveat to genotype-phenotype correlation in mucopolysaccharidosis type II: discordant clinical severity of R468W and R468Q mutations of the iduronate-2-sulfatase gene Hum. Mutat. 2 235-7.
[7] Chiong M A D, Canson D M, Abacan M A R, Baluyot M M P, Cordero C P and Silao C L T 2017 Clinical, biochemical and molecular characteristics of Filipino patients with mucopolysaccharidosis type II - Hunter syndrome Orphanet. J. Rare. Dis. 2 1-11.
[8] Chamary J V, Parmley J L and Hurst L D 2006 Hearing silence: Non-neutral evolution at synonymous sites in mammals Nat. Rev. Genet. 7 98.
[9] Marin M 2008 Folding at the rhythm of the rare codon beat Biotechnol. J. 3 1047-57.
[10] Saunders R and Deane C M 2010 Synonymous codon usage influences the local protein structure observed Nucleic Acids Res. 38 6719-28.
[11] Plotkin J B and Kudla G 2011 Synonymous but not the same: The causes and consequences of codon bias Nat. Rev. Genet. 12 32-42.
[12] Evelina A 2011 Codon usage: Nature’s roadmap to expression and folding of proteins Biotechnol. J. 6 650-9.