Original Article

Screening of Intronic Mutation IVS9+141A>G in an Indonesian Patient with Gaucher Disease

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Abstract

Objective: Gaucher disease (GD) is an autosomal recessive lysosomal storage disorder caused by the accumulation of the glycolipid glucosylceramide encoded by the *GBA* gene in certain organs. At present, more than 460 *GBA* intronic mutations have been reported in several subpopulations worldwide, but many have never been reported in Indonesia. Here, we aimed to screen for intronic mutations of *GBA* that might be present in patients with GD in Indonesia. **Materials and Methods:** Blood samples from patients with and without GD were obtained from the National Dr. Cipto Mangunkusumo Referral Hospital, Jakarta, Indonesia. Genomic DNA samples from peripheral leukocytes were extracted, purified, and amplified using the polymerase chain reaction (PCR) with specific primers. Products of PCR were visualized by gel electrophoresis and were further sequenced to analyze the presence of mutations in intron (intervening sequence [IVS]) 9 of *GBA*. **Results:** A mutant allele was identified at IVS9+141A>G, discovered at nucleotide 9335 in IVS 9. This mutation had been reported in India before and was categorized as nonpathogenic. **Conclusion:** Our study may be used as supplemental information for the GD database in Indonesia and will also open new research opportunities for predicting splicing processes in other intronic variants among patients with GD in Indonesia.

Keywords: Gaucher disease, *GBA* gene, glucocerebrosidase, IVS9+141A>G mutation

Introduction

Gaucher disease (GD) is an autosomal recessive lysosomal storage disorder caused by the accumulation of glycosphingolipid glucosylceramide (GC) in certain organs.[1] It results from the inability of the lysosomal enzyme beta-glucocerebrosidase (GCase) to catalyze GC to glucose and ceramide.[2] Clinical manifestations among patients with GD vary according to the type of disease.[1,3] Deficiencies in GCase are caused by mutations in the gene (*GBA*) that it encodes. This gene has 11 exons and 10 introns located on chromosome 1q21 with a length of 7.5 kb.[4] At present, more than 460 *GBA* mutations have been reported in several subpopulations worldwide, and some of them are intronic.[5] Such regions have important impacts on the *GBA* gene sequence. Changes in nucleotide bases in these regions disturb the protein structure of GCase by disrupting the splicing process. As a consequence, the severity of these intronic mutations might be mild or severe compared with exonic mutations.[6] Mutations in the intronic or intervening sequence (IVS) in the *GBA* gene have been widely recorded in the previous studies.[7-10] However, many of these mutations have never been reported in Indonesia. Therefore, our aim was to screen for the intronic mutations of GBA gene that might be present in patients with GD in Indonesia.

Materials and Methods

Blood samples of two patients with GD and thirty non-GD patients were collected from the National Dr. Cipto Mangunkusumo Referral Hospital, Jakarta, with approved by the hospital's ethics committee. The blood collection process was carried out by a healthcare professional, who followed the procedure based on ethical approval of the Ethical Clearance Committee. Informed consent was signed and approved by the participants whose blood samples were collected. Genomic DNA was extracted from peripheral blood leukocytes using

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Geneaid Genomic Blood/Cell DNA Mini Kits (GB100) (GeneAid Biotech Lt, New Taipei City, Taiwan). Extracted DNA was quantified for purity and concentration using a UV Lux spectrophotometer and stored at −20°C until further processing.

Polymerase chain reaction (PCR) amplification was performed using specific primers for exons 9–11 in the *GBA* gene. This area was chosen because it has been recorded to have clear intron variations. Primers used in this study were: Forward, 5′–GAACCATGATTCCCTATCTTC–3′; and reverse, 3′–CTGGGGCTTACTGATCTTTT–5′ as reverse and their product is 1334 bp. Cycling conditions were as follows: initial denaturation at 95°C for 60 s, 40 cycles of denaturation at 95°C for 15 s, annealing at 55°C for 15 s, and elongation at 72°C for 30 s, with final extension at 72°C for 10 min. The total reaction volume for PCR was 10 µL following the MyTaq DNA Polymerase protocol. Some PCR products were visualized using 1.5% agarose gel electrophoresis at 100 V for 60 min. The remaining PCR products were sequenced at 1st Base Provider in Singapore and aligned against the *GBA* reference gene sequence (NM_000157) and non-GD control sequence to identify mutations. One identified mutation was entered into the dbSNP NCBI (https://www.ncbi.nlm.nih.gov/snp) to check for its novelty, and and Human Splicing Finder version 3.1 [Aix Marseille Université] was applied to check for the impact it would have on the gene-splicing process.

Results

Screening for intronic variations in IVS 9–10 of *GBA* identified a mutation found in intron 9 in samples from both patients with GD. This mutation is located at nucleotide position 141 from IVS 9, as shown in Figure 1. The substitution of adenine (A) to guanine (G) in this position did not affect the splicing site. The IVS9+s141A>G sequence is a single-nucleotide polymorphism variant that has been found in the previous studies and is recorded in the dbSNP database (rs.28373017).

Discussion

The mutation in the intron that we found (IVS9+141A>G) is consistent with previous findings and does have any pathogenic effect.[11] This discovery could open up new opportunities for research into other intron variants in patients with GD in Indonesia. Mutations in other IVS regions could have different impacts on the splicing process.

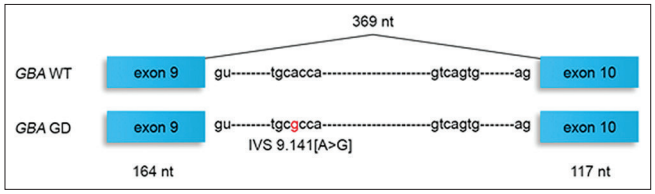

Figure 1: IVS9 sequence comparison of two patients with Gaucher disease and thirty nonGaucher disease control (WT)

Mutations in introns can trigger pseudo-exon inclusion. This process is caused by mutations that create or disrupt splicing enhancer or silencer elements. The most common mechanism is related to mutations that generate new donor sites and activate preexisting noncanonical acceptor splice sites.[12] The IVS9+141A>G mutation we found here is predicted by Human Splicing Finder to have no impact on exonic splicing silencer sites. Therefore, further research regarding the possibility of splicing processes generated in the IVS9+141A>G mutation needs to be conducted, as well as in other introns.

Conclusion

We found that the IVS9+141A>G mutation also occurred in these two Indonesian patients with GD, consistent with previous studies. This might open possibilities for further research on the screening for other *GBA* introns in such patients.

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Conflicts of interest

There are no conflicts of interest.

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