Original Article

Screening for Exonic Mutation L444P in Indonesian Patients with Gaucher Disease Using Exons 9–11

Rabbil Pratama Aji, Rizky Priambodo[1], Cut Nurul Hafifah[1,2], Damayanti Rusli Sjarif[1,2]

Department of Biology, Faculty of Mathematics and Natural Science, Universitas Indonesia, Depok, [1]Human Genetic Research Center, Indonesian Medical Education and Research Institute, Universitas Indonesia, [2]Department of Pediatric, Universitas Indonesia, RSUPN Dr. Cipto Mangunkusumo, Jakarta, Indonesia

Abstract

Objective: Gaucher disease (GD) is the most common lysosomal storage disorder. It is caused by a deficiency of β-glucocerebrosidase (GCase, encoded by *GBA*) and its inheritance is autosomal recessive. Analyses of common mutations in *GBA* have been performed in China, Singapore, Taiwan, and Thailand, but not previously in Indonesia. The objective of this study was to identify a common exonic mutation in exons 9–11 of *GBA* in GD patients in Indonesia. **Materials and Methods:** Genetic analysis was performed using blood samples from two GD patients and thirty non-GD patients. Peripheral leukocyte samples were collected at the Dr. Cipto Mangunkusumo Referral Hospital, Jakarta, Indonesia. The polymerase chain reaction was performed to amplify exons 9–11 of the *GBA* gene using specific primers, then the product was digested with *Nci*I restriction enzyme, and the sequence confirmed by sequence analysis. **Results:** This identified an L444P mutation located in exon 10. This missense mutation changes amino acid 483 of GCase from leucine to proline and is categorized as a pathogenic variant. **Conclusion:** This identification of the L444P mutation adds to a database for determining the prevalence of GD in Indonesia. However, further research is needed to ascertain the impact of the L444P mutation on the structure of GCase and to explore any mutations in the other exons.

Keywords: Gaucher disease, *GBA* gene, glucocerebrosidase, L444P mutation

Introduction

Gaucher disease (GD) is the most common lysosomal storage disorder. It is caused by a deficiency of β-glucocerebrosidase (GCase) and is inherited recessively on 1q21.[1] In the presence of saposin C as an activator, GCase cleaves the β-glycosidic linkage of glucosylceramide (GC)-producing ceramide and glucose.[2] A deficiency in either or both components can induce GC accumulation in the monocyte-macrophage cell lineage, which in turn leads to impairments in the liver, spleen, and even the central nervous system.[3] GD is usually caused by structural changes in the *GBA* gene.[1]

The *GBA* gene is located in chromosome 1q21 and is 7.5 kb in length. It contains 11 exons and 10 introns. Over 460 *GBA* mutations have been reported to the Human Gene Mutation Database, most of which are missense mutations.[4] Among these, there are several common mutations, including N370S, L444P, 84insG, and IVS2+1G >A. N370S and L444P are mutations in exons 9 and 10 that can be easily detected by restriction fragment length polymorphism (RFLP) analysis.[5-10]

Access this article online

Quick Response Code:

Website: www.jnsbm.org

DOI: 10.4103/jnsbm.JNSBM_38_19

Three subtypes of GD have been described. The diagnosis of all subtypes of GD is based on specific clinical manifestations and genetic analysis. In this study, screening for mutations in *GBA* was treated as part of the diagnostic procedure. N370S and L444P were the targeted mutations because both are largely localized in exons 9 and 10. Although the L444P mutation is common in Asian GD patients, the frequency of this mutation among GD patients in Indonesia is unknown. To date, there exists only one report of an Indonesian GD patients with type 2 GD evaluated using genetic analysis.[11] Here, we report the mutational profile of L444P in two GD patients in Indonesia.

Materials and Methods

Blood samples from two GD patients and two non-GD patients (control) were collected in ethylenediaminetetraacetic

Address for correspondence: Dr. Damayanti Rusli Sjarif, Komplek Depnaker RT.008/002, Jl. Empang Tiga Dalam No. 13, Pejaten Timur, Jakarta Selatan, 12510, Indonesia. E-mail: ukk.npm.idai@gmail.com

How to cite this article: Aji RP, Priambodo R, Hafifah CN, Sjarif DR. Screening for exonic mutation L444P in Indonesian patients with gaucher disease using exons 9–11. J Nat Sc Biol Med 2019;10:S59-61.

acid-containing tubes. Samples were obtained from the Pediatric Department, Dr. Cipto Mangunkusumo Referral Hospital, Jakarta and their collection was approved by the Hospital's Ethics Committee. Genomic DNA was isolated from peripheral blood leukocytes using the standard protocol for the genomic blood/cell DNA Mini Kit (GB100) (GeneAid Biotech Lt, New Taipei City, Taiwan). The concentration and purity of extracted DNA were quantified using a UV Lux Spectrophotometer. The extracted DNA was stored at −20°C until further processing.

The polymerase chain reaction (PCR) was performed on a ProFlex 96-well PCR System (Applied Biosystems, Foster City, CA, USA) using primers specific for exons 9–11 of the *GBA* gene. Primers were designed in NCBI Primer Blast and analyzed with NetPrimer Premier Biosoft to check for secondary structures. The designed primer sequences were 5′-GAACCATGATTCCCTATCTTC-3′ for the forward primer (GBA_EX9F) and 3′-CTGGGGCTTACTGATCTTTT-5′ for the reverse primer (GBA_EX9R), giving a product of 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, as suggested by the MyTaq DNA polymerase protocol.

Detection of the common mutation L444P was performed using the RFLP method. Part of the PCR product was digested for 8 h with *Nci*I restriction enzyme at 37°C. Digestion products were visualized by electrophoresis on a 1.5% agarose gel at 100 V for 60 min. To confirm the RFLP results, the remaining PCR product was sequenced and aligned against the *GBA* reference gene (NM_000157) as a control to identify mutations.

Results

The results of the screening of samples from one GD patient (P1) and non-GD controls (wt1 and wt2) are shown in Figure 1. Digestion with *Nci*I cut only the P1 sample, resulting in fragments of 865 and 469 bp. The non-GD samples were not digested, giving a single band of 1334 bp. This indicated the presence of the L444P mutation in P1 but not in the non-GD samples.

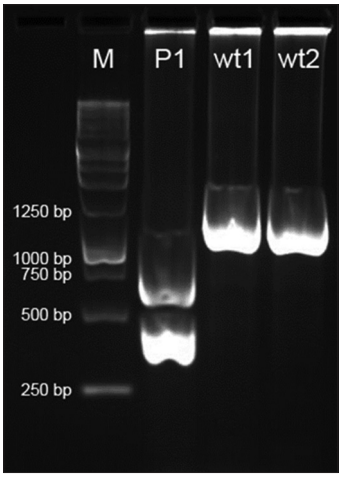

Figure 1: Results of digestion of DNA from a Gaucher disease patient (P1) and two non-Gaucher disease normal controls (wt1 and wt2) with *Nci*I restriction enzyme. Product length is indicated by the markers (M)

The alignment of the two GD patients (P1 and P2) and non-GD control with the reference sequence (NM_000157) is shown in Figure 2. At the nucleotide level, sequencing showed a thymidine (T) to cytosine (C) substitution in the exon at position 1448. This translated to a change from leucine to proline at amino acid 444 of the protein. To avoid confusion, the various notations for L444P are listed in Table 1.

Discussion

Genetic analysis using RFLP for early detection of GD patients has been performed in various subpopulations worldwide. Previous studies on non-Jewish subpopulations in the United Kingdom showed that the amplification product of the DNA of patients carrying a homozygous L444P and a carrier allele for GD could be cut using the *Nci*I restriction enzyme to yield fragments of 658 and 519 bp.[12] Our study showed that the PCR product from one GD patient in Indonesia (P1) could be digested with the same enzyme, although the length of the digested products differed. However, it is clear that P1 also has the L444P mutation that creates a new restriction site for *Nci*I, demonstrating that our results are consistent with those of previous research.

The L444P mutation in the *GBA* gene is common in Asia. A study involving Chinese GD patients reported that the frequency of L444P mutations was 42% and that all cases have splenomegaly.[13,14] Another study conducted in 14 Taiwanese GD patients (eight patients with type 1 GD and six patients with type 3 GD) showed that the frequency of L444P was 53.5%.[15]

Table 1: Notation for L444P mutation

Allele name	Exon	cDNA	gDNA	Protein	Reference
L444P	10	c. 1448T>C	g. 7319T>C	p.Leu483Pro	Hruska *et al*., 2008

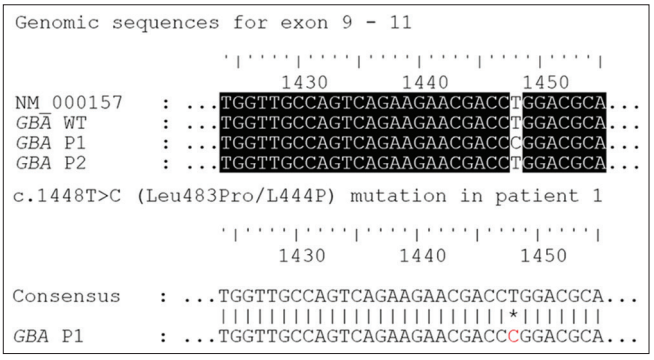

Figure 2: Alignment of samples from Gaucher disease patients (P1, P2) with non-Gaucher disease samples and reference sequence (NM_000173). The consensus sequence is comprised NM_000157, GBA WT, and GBA P2

Based on these observations alone, we suggest that L444P might be an effective marker for rapid diagnosis using RFLP.

The change of proline to leucine at amino acid 483 of the protein is often indicated as L444P. This change in amino acid residues is a missense mutation that influences the hydrophobic interaction between leucine clusters in the same domain within the structure of the GCase protein.[16] Although it does not affect the catalytic site, the mutation is still deemed pathogenic. Previous studies have shown that the L444P mutation can lead to the neuronopathic effects that are associated with type 2 and type 3 GD.[4,8-10] Our results provide a new opportunity to study the protein–pathogenicity interaction between GCase and GD in Indonesian patients.

The lack of data on the profile of the L444P mutation in Indonesia often makes the diagnosis of GD inaccurate. Even though only a few treatments for GD exist, such as enzyme replacement therapy, it would be beneficial if the disease could be recognized prenatally. Our screening and sequence analysis in this study are important because they may provide data to support the conduct of genetic counseling to allow preventive measurements against GD. We expect to see new studies of the detection and identification of L444P mutations in Indonesian GD patients in the future. In conclusion, we confirm that the L444P mutation also occurs in Indonesian GD patients. Our results are thus consistent with those of previous studies. Further research on the screening of other exons in Indonesian GD patients is anticipated.

Conclusion

We found that the L444P mutation occurred in one Indonesian patients with GD, based on the result of *_Nci_*I restriction enzyme. This might open possibilities for further research on the screening for other L444P mutation in such patients.

Acknowledgments

This work was supported by a Publikasi International Terindeks Untuk Tugas Akhir Mahasiswa UI (PITTA) Grant 2018 funded by Directorate of Research and Community Service (DRPM) Universitas Indonesia (No. 5000/UN2.R3.1/HKP. 05.00/2018).

Financial support and sponsorship

This research was supported by grants from HIBAH PITTA Universitas Indonesia 2018. The 3rd ICE on the IMERI committee supported the peer review and manuscript preparation of this article.

Conflicts of interest

There are no conflicts of interest.

References

1. Lieberman RL. Guided tour of the structural biology of Gaucher disease: Acid-(glucosidase and saposin C. Enzyme Res 2011;1:1-15. [Doi: 10.4061/2011/973231].
2. Salvioli R, Tatti M, Scarpa S, Moavero SM, Ciaffoni F, Felicetti F, *et al.* The N370S (Asn370-->Ser) mutation affects the capacity of glucosylceramidase to interact with anionic phospholipid-containing membranes and saposin C. Biochem J 2005;390:95-103.
3. Erickson AH, Ginns EI, Barranger JA. Biosynthesis of the lysosomal enzyme glucocerebrosidase. J Biol Chem 1985;260:14319-24.
4. Sidransky E, Tsuji S, Martin BM, Stubblefield B, Ginns EI. DNA mutation analysis of Gaucher patients. Am J Med Genet 1992;42:331-6.
5. Ota M, Fukushima H, Kulski JK, Inoko H. Single nucleotide polymorphism detection by polymerase chain reaction-restriction fragment length polymorphism. Nat Protoc 2007;2:2857-64.
6. Hruska KS, LaMarca ME, Scott CR, Sidransky E. Gaucher disease: Mutation and polymorphism spectrum in the glucocerebrosidase gene (GBA). Hum Mutat 2008;29:567-83.
7. Siebert M, Bock H, Michelin-Tirelli K, Coelho JC, Giugliani R, Saraiva-Pereira ML, *et al.* Novel mutations in the glucocerebrosidase gene of Brazilian patients with Gaucher disease. JIMD Rep 2013;9:7-16.
8. Wan L, Hsu CM, Tsai CH, Lee CC, Hwu WL, Tsai FJ. Mutation analysis of Gaucher disease patients in Taiwan: High prevalence of the RecNciI and L444P mutations. Blood Cells Mol Dis 2006;36:422-5.
9. Uchiyama A, Tomatsu S, Kondo N, Suzuki Y, Shimozawa N, Fukuda S, *et al.* New Gaucher disease mutations in exon 10: A novel L444R mutation produces a new NciI site the same as L444P. Hum Mol Genet 1994;3:1183-4.
10. Malini E, Grossi S, Deganuto M, Rosano C, Parini R, Dominisini S, *et al.* Functional analysis of 11 novel GBA alleles. Eur J Hum Genet 2014;22:511-6.
11. Lee YS, Poh LK, Ida H, Loke KY. Type II Gaucher disease: Compound heterozygote with RecNciI and L444P mutations. J Trop Pediatr 2001;47:115-7.
12. Walley AJ, Barth ML, Ellis I, Fensom AH, Harris A. Gaucher's disease in the United Kingdom: Screening non-Jewish patients for the two common mutations. J Med Genet 1993;30:280-3.
13. Kang L, Wang Y, Gao X, Qiu W, Ye J, Han L, *et al.* Genotypes and phenotypes in 20 Chinese patients with type 2 Gaucher disease. Brain Dev 2018;40:876-83.
14. Wang Y, Liu L, Xiong J, Zhang X, Chen Z, Yu L, *et al.* Glucocerebrosidase L444P mutation confers genetic risk for Parkinson's disease in central China. Behav Brain Funct 2012;8:57.
15. Suwannarat P, Keeratichamroen S, Wattanasirichaigoon D, Ngiwsara L, Cairns JR, Svasti J, *et al.* Molecular characterization of type 3 (neuronopathic) Gaucher disease in Thai patients. Blood Cells Mol Dis 2007;39:348-52.
16. Stone DL, Carey WF, Christodoulou J, Sillence D, Nelson P, Callahan M, *et al.* Type 2 Gaucher disease: The Collodion baby phenotype revisited. Arch Dis Child Fetal Neonatal Ed 2000;82:F163-6.