Gaucher disease: molecular screening of the glucocerebrosidase 1601G and 1601A alleles in Victoria, British Columbia, Canada

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Abstract

Gaucher disease is the most prevalent lysosomal storage disease and it results from inherited deficient glucocerebrosidase activity. The glucocerebrosidase gene from normal people was sequenced by several laboratories and it was noted that a G or A nucleotide may be present at cDNA position 1601, resulting in ⁴⁹⁵arginine or ⁴⁹⁵histidine in the glucocerebrosidase polypeptide. In order to rule out the possibility of cloning error and to elucidate the genetic status of the two genotypes and their distribution in the population, we have developed a convenient and reliable method for the molecular screening of the 1601G and 1601A genotypes in the population. This method uses PCR amplification of glucocerebrosidase genomic DNA in blood samples, followed by *Bsa*HI restriction fragment length polymorphism analysis. Out of the 256 subjects without Gaucher disease and 15 Gaucher patients surveyed, the 1601G genotype was present in the homozygous form in all of the asymptomatic subjects and 14 Gaucher patients. In one Gaucher patient who was diagnosed as having type 1 (non-neuropathic) Gaucher disease with the A1226G/T1366G mutations, the heterozygous 1601G/A genotype was detected. These findings indicate that the 1601G genotype which encodes ⁴⁹⁵arginine of the glucocerebrosidase polypeptide is not a cloning error. Instead, it constitutes the normal as well as predominant genotype in the population in the municipality of Greater Victoria, British Columbia. The 1601A genotype, on the other hand, appears to be quite infrequent in this population. The availability of our restriction enzyme based method has allowed the screening and frequency determination of these two alleles in other populations.

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Gaucher disease is the most prevalent inherited lysosomal storage disease.¹ It results from deficient glucocerebrosidase activity and is transmitted as an autosomal recessive trait.² Three clinical forms of Gaucher disease have been described: type 1, non-neuronopathic; type 2,

acute neuronopathic; and type 3, subacute neuronopathic.¹

Recently, we reported the identification of a novel mutation in a non-Jewish, white type 1 Gaucher disease patient.³ It is a T→G transversion at cDNA nucleotide 1366 that results in ⁴¹⁷Phe→⁴¹⁷Val of glucocerebrosidase. Sequence analysis of the entire coding region of the glucocerebrosidase gene from the patient also showed the presence of a heterozygous A→G transition at cDNA nucleotide position 1601 that results in ⁴⁹⁵His→⁴⁹⁵Arg.³ This finding is of interest since both the 1601A and 1601G genotypes were identified in normal subjects^{4,5} and may represent polymorphism of the glucocerebrosidase gene in the population. It was also suggested that the 1601G genotype may be a cloning error,⁶ since this sequence was identified from a glucocerebrosidase genomic clone.⁵ In order to elucidate the exact genetic status and physiological role of the 1601A and 1601G alleles in Gaucher disease, it is important to develop an accurate and convenient method for molecular screening of the two genotypes in the population. In this paper, we describe a relatively simple method that uses selective PCR amplification of glucocerebrosidase genomic DNA from blood samples and restriction endonuclease analysis for the identification of the 1601A and 1601G genotypes. We also present the findings of our molecular screening of these two genotypes in 256 randomly selected subjects asymptomatic for Gaucher disease in the municipality of Greater Victoria, British Columbia, Canada.

Anonymous human venous blood samples were provided by the Department of Laboratory Medicine of the Greater Victoria Hospital Society. These samples had been drawn for routine clinical chemistry or haematology testing and were donated to this research laboratory rather than being discarded. All of the donors were asymptomatic for Gaucher disease. The blood was stored in EDTA vacutainers at 4°C for up to 72 hours.

A three stage method was devised in order to amplify selectively from whole blood the glucocerebrosidase functional gene, and not the pseudogene which shares more than 96% sequence similarity with the functional gene.⁵ In the first stage, the blood samples were frozen at -20°C overnight and thawed at room temperature for the lysis of the blood cells. Then 2.5 µl of each of the blood samples was added to a reaction mixture of 38 µl of distilled water and 4.5 µl of PCR buffer (50 mmol/l tricine, pH 8.8, 15 mmol/l MgCl₂, 0.5% Tween

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Figure 1 Selective PCR amplification of glucocerebrosidase exon 11 for *Bsa*HI restriction endonuclease analysis. In the first amplification, primers A and B were used to amplify glucocerebrosidase exons 9–11 of the functional gene and not the pseudogene. The DNA fragment amplified was then used as template in the second and nested PCR amplification of glucocerebrosidase exon 11, where the G or A nucleotide is located at cDNA position 1601.^{4,5,9} Please refer to the text for details of the PCR amplification and table 1 for the nucleotide sequence of the primers.

Table 1 Nucleotide sequence of primers used in the PCR amplification

Primer	Nucleotide sequence (5' to 3')	Orientation	Position* (genomic nucleotide)
A	ACTTTGTCGACAGTCCCATC	Sense	5919–5938
B	CTTTAATGCCAGGCTGAGC	Antisense	6737–6718
C	GATCCTGCTGTGGGCTTCCT	Sense	6616–6635

* The genomic nucleotide position of glucocerebrosidase functional gene is numbered according to Horowitz *et al.*⁵ The cDNA nucleotide position of glucocerebrosidase gene is numbered according to Sorge *et al.*⁹

20, and 500 mmol/l KCl). To ensure complete lysis of the blood cells and denaturation of the haemoglobin, the sample was subjected to 20 thermal cycles at 90°C and 50°C for one minute, respectively. The heat treated blood samples were then used as genomic template. In the second stage, a nested PCR procedure was used for the selective amplification of glucocerebrosidase functional gene. As shown in fig 1, the nucleotide sequences in primer A (table 1) which flanks glucocerebrosidase exon 9 are absent in the pseudogene.⁵ Subsequently, when primer A and primer B (which flanks glucocerebrosidase exon 11) are used to amplify glucocerebrosidase genomic DNA, only the functional gene will be amplified.^{5,7} The PCR amplifications were performed as previously described⁸ with one minor modification. The denaturation of genomic DNA at 94°C during the first cycle was lengthened to 10 minutes to ensure complete separation of the two strands. *Taq* polymerase was then added when the temperature was lowered to 58°C for primer annealing. As shown in fig 1, this PCR amplification yielded a DNA fragment of 819 bp, bracketing glucocerebrosidase exons 9–11, where the A or G nucleotide at cDNA position 1601 is located. If a G is present, it will create a new cleavage site for the restriction endonuclease *Bsa*HI. However, this fragment is still unsuitable for *Bsa*HI analysis, as there are too many *Bsa*HI cleavage sites within the fragment that complicate the interpretation of results. To this end, an additional PCR amplification nested on exon 11 was performed before *Bsa*HI digestion. In this PCR, the 819 bp DNA band was used as template to amplify a 122 bp region flanked by primers B and C (fig 1, table 1). The conditions of the amplification were similar to the previous one, except that the initial 10 minute heat denaturation was shortened to

Figure 2 *Bsa*HI restriction fragment length polymorphism analysis of PCR amplified glucocerebrosidase exon 11. The DNA fragment was amplified by a nested PCR method as described in the text. After amplification, the fragment was digested by *Bsa*HI restriction endonuclease and analysed by polyacrylamide gel electrophoresis. From left, lane 1: digested genomic DNA of a homozygote for the 1601G allele. Lane 2: a DNA sample containing the 122, 64, and 58 bp bands indicative of either the heterozygous 1601A/1601G genotype or, occasionally, homozygous 1601G genotype that was incompletely digested. Lane 3: 1 kb ladder DNA standards from *Hin*I digested DNA fragments (New England BioLabs) of 134, 154, 201, 220, 298, 344, 396, 506, and 517 bp. Lane 4: undigested sample shown as a negative control.

30 seconds (since the 819 bp band instead of genomic DNA was being used as a template).

The conditions for *Bsa*HI restriction endonuclease digestion and polyacrylamide gel electrophoresis were as previously described.⁸ Sequence analysis was performed using the dideoxynucleotide chain termination method of Sanger *et al.*¹⁰ and the Thermal Cycle™ Kit (Promega Corporation, Madison, WI, USA) as described previously.^{3,11}

Our three stage nested PCR method has selectively amplified glucocerebrosidase exon 11 from genomic DNA of minute blood samples, and the result of a typical *Bsa*HI restriction digest is shown in fig 2. A person who is homozygous for the the 1601G genotype possesses this *Bsa*HI cleavage site in both alleles. Upon complete digestion, two bands of 58 bp and 64 bp from the original 122 bp band are detected, as shown in lane 1 of fig 2. Conversely, a person homozygous for the 1601A genotype will exhibit only the undigested 122 bp band. A partially digested sample (lane 2, fig 2) indicates the presence of a heterozygote with the 1601A/1601G genotype.

Using the above *Bsa*HI restriction analysis, we have screened for the presence of the 1601G and 1601A genotypes in 256 people, as well as 15 patients with the three different clinical forms of Gaucher disease,⁷ for a total of 542 alleles. The results obtained from our molecular screening showed that all of the controls are homozygous for the 1601G genotype. Of the 15 Gaucher patients, 14 are homozygous

for the 1601G genotype, while one type 1 patient is heterozygous for the 1601G/A genotype.³

It is unlikely that the high frequency of the 1601G genotype as shown by our *Bsa*HI restriction endonuclease analysis is because of experimental error such as incomplete enzyme digestion. As discussed earlier, the 1601G genotype creates a new *Bsa*HI cleavage site and results in the formation of two smaller digested DNA bands. If there had been experimental error resulting from incomplete digestion, the partial digestion should result in a 122 bp as well as the smaller 64 and 58 bp bands. This will produce a false positive result that indicates the presence of the heterozygous 1601A/1601G genotype. Indeed, five out of our 256 samples apparently contained some unknown trace contaminants that inhibited complete digestion by *Bsa*HI endonuclease. This has resulted in false positive heterozygous 1601A genotypes. However, DNA sequence analysis of these samples showed that this is not the case, as the G nucleotide was found in the homozygous form at position 1601 in all samples. In view of this finding, the 1601G genotype is not a cloning error as previously suggested.⁶ On the contrary, it appears to constitute not only the normal but also the predominant genotype in the population. Out of the 256 controls or 512 normal alleles surveyed in this study, the 1601G genotype is present in the homozygous form in all of them. This finding also eliminates the possibility that the 1601G genotype is a deleterious mutation hidden in an asymptomatic carrier who was misidentified as normal.

The 1601A genotype was originally identified by Sorge *et al.*⁴ in a cDNA clone from a normal person. While the possibility of cloning error exists, it is unlikely since this genotype was also identified, in the heterozygous form as 1601A/1601G, in a type 1 Gaucher disease patient by direct sequence analysis of PCR amplified cDNA without cloning.³ Grabowski *et al.*¹² reported that the 1601A genotype is a neutral mutation, since site directed mutagenesis that resulted in ⁴⁹⁵Arg→⁴⁹⁵His did not alter the catalytic properties of the enzyme.¹³ In view of this finding and the results of our molecular screening, the 1601A genotype is a

normal glucocerebrosidase allele that occurs at a low frequency in the municipality of Greater Victoria, British Columbia. As its frequency is less than 1%, it cannot be defined as a polymorphism in this population. However, the distribution of the two genotypes in other populations may be quite different. The availability of our restriction enzyme based method has allowed accurate molecular screening of these alleles and frequency determination in other populations.

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