Distribution and characterization of a Sandhoff disease-associated 50-kb deletion in the gene encoding the human \( \beta \)-hexosaminidase \( \beta \)-chain

Hennie Bikker\(^1\), Frank M. van den Berg\(^2\), Ruud A. Wolterman\(^1\), Wim J. Kleijer\(^3\), Jan J. M. de Vijlder\(^1\), and Piet A. Bolhuis\(^1\)

\(^1\)Department of Experimental Medicine (G-2-108) and \(^2\)Department of Pathology, Academic Medical Centre, NL-1105 AZ Amsterdam, The Netherlands
\(^3\)Department of Clinical Genetics, Erasmus University, NL-3000 DR Rotterdam, The Netherlands

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Summary. A 50-kb deletion was demonstrated in the gene encoding for the \( \beta \)-subunit of human hexosaminidase (HEXB), using field inversion gel electrophoresis (FIGE) of \( SfiI \)-digested chromosomal DNA from patients with Sandhoff disease. We investigated 14 patients from different parts of Europe and found no deletion in 5 patients, 2 patients homozygous for the deletion, and 7 patients with the deletion in one allele. The distribution of the 50-kb deletion was approximately in agreement with the Hardy-Weinberg equilibrium. The deletion was characterized using chromosomal DNA from one of the two homozygous patients. Restriction fragments were hybridized with a 1.6-kb (almost complete) and a 0.4-kb (5') HEXB cDNA clone. It appeared that the deletion started in intron 5, extending in the 5' direction and causing the loss of exon 1–5 and the promoter area of the HEXB gene.

Introduction

Lysosomal beta-hexosaminidase occurs in two major forms, hexosaminidase A and hexosaminidase B. Hexosaminidase A is composed of \( \alpha \)- and \( \beta \)-subunits, whereas hexosaminidase B is composed of \( \beta \)-subunits only. The \( \alpha \)-subunit is made up of a single polypeptide that is encoded by the HEXA gene residing on chromosome 15 (Chern et al. 1977). The \( \beta \)-subunit consists of three polypeptides joined by disulfide bonds, derived from the cleavage of a pre-\( \beta \)-polypeptide (Quon et al. 1989; Mahuran et al. 1988; Stirling et al. 1988). It is encoded by the HEXB gene localized on chromosome 5 (Gilbert et al. 1975).

Mutations in the HEXA or HEXB gene lead to Tay-Sachs or Sandhoff disease, respectively (O'Brien 1983). Genetic deficiency of the \( \beta \)-subunit abolishes catalytic activity of both the iso-enzymes, resulting in GM2 gangliosidosis. In the classic form of Sandhoff disease, no hexosaminidase activity is found (Sandhoff and Christomanou 1979), whereas in the juvenile and adult forms residual activity is present (Bolhuis et al. 1987).

The cDNA encoding the beta-subunit has been cloned (O'Dowd et al. 1985) and used to investigate the structure of the HEXB gene (Neote et al. 1988) and the molecular defects associated with Sandhoff disease (O'Dowd et al. 1986). Neufeld (1989) has recently reviewed the cell biology and the molecular genetics of \( \beta \)-hexosaminidase. The HEXA gene has been studied in more detail than the HEXB gene.

A specific genetic abnormality in a juvenile form of Sandhoff disease was described by Nakano and Suzuki (1989). They observed a 24-base insertion between exon 12 and 13 of the HEXB gene. We demonstrated a 50-kb deletion in the 5' part of one allele of the HEXB gene of a classic Sandhoff patient (Bikker et al. 1989). In this paper, we show that 9 out of 14 Sandhoff patients possess this deletion (7 heterozygous and 2 homozygous). The deletion was characterized further by restriction mapping of the HEXB gene of a Sandhoff patient homozygous for the deletion.

Materials and methods

To prepare high molecular weight DNA, intact fibroblasts were suspended in low-temperature-gelling agarose inserts (Smith et al. 1986). The DNA was purified and digested in situ with \( SfiI \) (New England Biolabs, Beverly, Mass.). Field inversion gel electrophoresis (FIGE) was performed as described earlier (Bikker et al. 1989). Southern blots of FIGE gels were hybridized with a 1.6-kb cDNA probe encoding a large part (exons 1–13) of the human hexosaminidase gene (HEXB) (O'Dowd et al. 1985).

For restriction mapping of smaller fragments, DNA was isolated from fibroblasts of patients with classic and adult Sandhoff disease. Control DNA was isolated from fibroblasts of healthy people. The DNA was digested with EcoRI, BamHI, HindIII, XhoI, and \( SfiI \) (New England Biolabs), electrophoresed, blotted,
Fig. 1. FIGE analysis of human genomic DNA, digested with SfiI and hybridized with a HEXB 1.6-kb cDNA probe. Lanes 1–8 (out of 14) classic Sandhoff patients. Lane 9 a normal control. Lanes 1, 3, 4, 6 show apparently undeleted Sandhoff alleles; lane 5 contains DNA of a Sandhoff patient homozygous for the 50-kb deletion; lanes 2, 7, 8 DNA from heterozygous Sandhoff patients hybridized and autoradiographed as described by Baas et al. (1984). The probe used was the 1.6-kb HEXB cDNA fragment described above, and a 0.4-kb 5'EcoRI-fragment of HEXB, containing exon 1 and part of exon 2.

Results and discussion

Chromosomal DNA was obtained from 14 Sandhoff patients from different European countries, viz., Scotland, England, Denmark, Finland, Czechoslovakia, German Democratic Republic, Federal Republic of Germany, Switzerland and the Netherlands. DNA of 13 classic Sandhoff patients and one adult Sandhoff patient was digested with SfiI, and analyzed with field inversion gel electrophoresis. The HEXB cDNA probe hybridized with 110-kb and 150-kb bands in 2 normal controls, 4 classic Sandhoff patients, and the adult Sandhoff patient. Two classic Sandhoff patients had 2 deleted alleles resulting in a single hybridizing 210-kb band. This 210-kb band arises from the loss of a SfiI-site caused by a 50-kb deletion in the 5' region of the HEXB gene (Bikker et al. 1989). The other 7 classic Sandhoff patients had one deleted HEXB allele, resulting in a 210-kb SfiI-band, and one allele of normal length, restricted by SfiI into 110-kb and 150-kb bands (Fig. 1). In these cases a compound mutation was involved.

The position of the 50-kb deletion was established by comparing the restriction maps of Sandhoff patients homozygous and heterozygous for the deletion, with a normal control. We used restriction endonucleases EcoRI, BamHI, HindIII, XhoI, and SstI. From a comparison of our data with the restriction map of Neote et al. (1988), we conclude that the deletion starts in intron 5 and stretches 50 kb in the 5' direction, causing the deletion of exons 1–5 and the HEXB gene promoter (Fig. 2). Consequently, no HEXB mRNA production will be possible from this allele. Since we could not detect HEXB mRNA in two heterozygous classic Sandhoff patients (H. Bikker and P.A. Bolhuis, unpublished observations), the other allele must also carry a mutation that prevents transcription, although the 5' restriction-sites are present (Fig. 2). We were not able to show differences in

Table 1. Distribution of the 50-kb deletion in not-directly related Sandhoff patients. C, Classic Sandhoff patients; A, adult Sandhoff patient; ▲, deleted allele resulting in a 210-kb SfiI-band with a frequency of 0.39; –, undeleted allele resulting in 110-kb and 150-kb SfiI-bands with a frequency of 0.61. The predicted distribution is calculated from the Hardy-Weinberg equation.

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<tr>
<th>Genotype</th>
<th>Phenotype</th>
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<td>–/–</td>
<td>C</td>
<td>4</td>
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