Molecular Heterogeneity in the Infantile and Juvenile Forms of Sandhoff Disease (O-variant G\textsubscript{M2} Gangliosidosis)*

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There are two major \(\beta\)-hexosaminidase, EC 3.2.1.52, isozymes in normal human tissues. They exist as active dimers of \(\alpha\) and/or \(\beta\)-subunits. A defect of their \(\beta\)-subunit results in Sandhoff disease (O-variant G\textsubscript{M2} gangliosidosis), an inherited, clinically heterogeneous, lysosomal storage disease. The status of the \(HEXB\) gene, pre-\(\beta\)-polypeptide chain mRNA, and residual \(\beta\)-hexosaminidase activities were examined in a clinically and ethnically diverse collection of 16 fibroblast cell lines from patients with Sandhoff disease. Differentiation of the two major clinical types, infantile and juvenile onset, could be made by the determination of the activity of the residual \(\beta\)-hexosaminidase eluting in the same pH range as hexosaminidase A. All the juvenile lines were found to have normal or reduced levels of pre-\(\beta\)-chain mRNA and no gross abnormalities in the \(HEXB\) gene. Of the 11 infantile type cell lines examined, four were found to contain no detectable pre-\(\beta\)-chain mRNA. Two cell lines in this group contained partial gene deletions localized to the 5' end of the \(HEXB\) gene. One of these cell lines has previously been assigned to the single complementation group in Sandhoff disease, conclusively demonstrating that the primary gene defect in the majority of Sandhoff cases is in the \(HEXB\) gene itself. These data suggest that each clinical group is made up of a collection of different \(HEXB\) mutations.

Sandhoff disease (O-variant G\textsubscript{M2} gangliosidosis) is a lysosomal storage disease which results from a deficiency of \(\beta\)-hexosaminidase activity (2-acetamino-2-deoxy-\(\beta\)-d-glucoside acetonideoxylglycrolhydroxolase, EC 3.2.1.52) (1). Affected patients display a wide spectrum of clinical severity (2-7) but are usually classified as one of two types. Type 1, the infantile form, is the more severe. It is characterized by an early onset of disease symptoms, usually in the first 6-18 months of life. G\textsubscript{M2} ganglioside and related asialoiglycolipids are stored in the brain and in other tissues (8). Death generally occurs before 3 years of age. Type 2, the juvenile form, is less severe. The affected patients first show signs of the disease later in life (normally 3-11 years) and may survive into adulthood (9).

2 Portions of this paper (including "Materials and Methods," part of "Results," Fig. 1, and Tabs. 2 and 3) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 86M-0445, cite the authors, and include a check or money order for $2.40 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
Molecular Heterogeneity in Sandhoff Disease

**TABLE I**
The patient origins, the status of preβ-polypeptide chain mRNA and genomic DNA, and residual hexosaminidase A activity in each cell line

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>Age (years) of mRNA* level</th>
<th>DNA % Normal*b hex β A</th>
<th>Ethnic group</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Carrier</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Juvenile</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1279</td>
<td>10</td>
<td>N</td>
<td>N</td>
<td>ND</td>
</tr>
<tr>
<td>GM 2094</td>
<td>20*</td>
<td>N</td>
<td>N</td>
<td>3.0</td>
</tr>
<tr>
<td>MGV 229</td>
<td>13</td>
<td>N</td>
<td>N</td>
<td>2.5</td>
</tr>
<tr>
<td>1303</td>
<td>9</td>
<td>20*</td>
<td>N</td>
<td>0.9</td>
</tr>
<tr>
<td>MGV 210</td>
<td>10</td>
<td>12*</td>
<td>&lt;10%</td>
<td></td>
</tr>
<tr>
<td><strong>Infantile</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2012</td>
<td>1</td>
<td>4.5</td>
<td>N</td>
<td>0.4</td>
</tr>
<tr>
<td>2010</td>
<td>Birth</td>
<td>1.8</td>
<td>N</td>
<td>0.3</td>
</tr>
<tr>
<td>2007</td>
<td>0.3</td>
<td>1</td>
<td>&lt;10%</td>
<td>0.3</td>
</tr>
<tr>
<td>322</td>
<td>0.8</td>
<td></td>
<td>&lt;10%</td>
<td>ND</td>
</tr>
<tr>
<td>WG 534</td>
<td>0.5</td>
<td>1.7</td>
<td>&lt;10%</td>
<td>0.5</td>
</tr>
<tr>
<td>1964</td>
<td>0.9</td>
<td>2</td>
<td>&lt;10%</td>
<td>N</td>
</tr>
<tr>
<td>GM 470</td>
<td></td>
<td></td>
<td></td>
<td>N</td>
</tr>
<tr>
<td>GM 203</td>
<td>1</td>
<td></td>
<td>&lt;1%</td>
<td>ND</td>
</tr>
<tr>
<td>GM 317</td>
<td>0.9</td>
<td>2</td>
<td>&lt;1%</td>
<td>D</td>
</tr>
<tr>
<td>GM 294</td>
<td></td>
<td></td>
<td></td>
<td>D</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>0.81</td>
<td>2.2</td>
<td></td>
<td>0.4</td>
</tr>
<tr>
<td><strong>S.D.</strong></td>
<td>0.27</td>
<td>1.1</td>
<td></td>
<td>0.1</td>
</tr>
</tbody>
</table>

* Each sample was reprobed using a 32P-labeled cDNA coding for β-glucuronidase and produced signals of nearly equal intensity.

* The percent of normal hexosaminidase A activity was calculated using intracellular β-galactosidase as an internal standard. See Table 2 for the complete list of activity measurements, and Table 3 for the characteristic pH of elution and heat stability determinations.

* The abbreviations used are Hex, hexosaminidase; N, normal; <10%, reduced; <1%, undetectable; D, deletion; ND, not determined.

Still living.

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Fig. 2. Representative blot hybridization of fibroblast RNA homologous to pHexBl from a control, three juvenile onset mutant, and four infantile onset mutant lines. Specific cell line numbers are indicated below each group and the position of the 28 S and 18 S RNA are shown on the left.

with radiolabeled cDNA inserts of pHexB1 coding for the preβ-polypeptide of hexosaminidase. We have previously shown that RNA from normal fibroblasts contain a single 2.2-kb species hybridizing to the pHexB1 cDNA, corresponding to the preβ-mRNA (Fig. 2, representative line 2008) (16). Among the infantile cell lines, only three contained hybridizable RNA of apparently normal size and amount (Fig. 2, representative line 2010). These lines produced barely detectable amounts of hexosaminidase “B” activity (Table 2). Four other infantile lines had reduced levels of mRNA, estimated at 5–10% of normal (Fig. 2, representative lines 322 and 1954). Of these, only two lines contained detectable amounts of hexosaminidase B. The remaining four mutants had undetectable levels of hybridizable RNA (<1% of normal, Fig. 2, representative line GM 317) and hexosaminidase B activity. In contrast all of the cell lines from the juvenile patients produced a detectable preβ-mRNA and some residual hexosaminidase B activity (Tables 1 and 2). Four of them had apparently normal levels of the mRNA (Fig. 2, representative lines 1303 and 1279). A fifth line had dramatically reduced levels of preβ-RNA (Fig. 2, line MGV 210). A summary of these results is reported in Table 1. When each nitrocellulose filter was reprobed using a 32P-labeled cDNA coding for β-galactosidase (29), all 16 fibroblast lines produced signals of nearly equal intensity (data not shown).

We have previously shown that Southern blots of EcoRI, HindIII, and PvuII digested genomic DNA from normal fibroblasts contain a complex pattern of at least four bands, totaling >42 kb, detectable with pHexB1 cDNA (Fig. 3, A–C, lanes N), all of which were mapped to chromosome 5 as expected for the HEXT gene (16). All five juvenile cell lines and nine of the 11 infantile lines showed a hybridization pattern indistinguishable from normal. The remaining two lines, GM 317 and GM 294, which contained no detectable preβ-mRNA or hexosaminidase B activity, produced similar
patterns which were indicative of partial gene deletions with Southern blots of all three restriction enzyme digests (Fig. 3, A-C, representative line GM 317; Table 1). Both lines did, however, produce as much residual hexosaminidase "A" activity as the other infantile lines (Fig. 1, Table 2). As can be seen for the pattern produced by a HindIII digest of normal genomic DNA (Fig. 3D, lane 1) and DNA from mutant line GM 317 (Fig. 3D, lane 2), the upper bands labeled a-c in the normal sample (lane 1) are either missing (lane 2) or have a different mobility (band \times lane 2) in the digests from the mutant line. On the other hand, the lower bands, d-f, in the mutant sample are apparently normal (lanes 1 and 2). In order to localize the area of the HEXB gene that contains the unaffected bands in these two mutant lines, two other cDNA probes (described under "Materials and Methods") were used to detect the DNA fragment bands from HindIII digests. A probe consisting of the 3' half of pHexBl hybridized with three of the six HindIII fragments (bands d-f in Fig. 3F) in both normal and mutant DNA. Thus the 3' portion of the HEXB gene appears not to be involved in the gene rearrangement in GM 317. A probe consisting of the middle portion of pHexBl also hybridized to band d (Fig. 3E), but, in addition, hybridized with bands a-c in normal DNA (Fig. 3E, lane 1). These bands are missing in GM 317, although the probe hybridized to a new HindIII band, "x" (Fig. 3E, lane 2). A short 5' probe derived from pHexBl hybridized most intensely to band b in normal DNA, but not band x in GM 317 DNA (data not shown). Thus, the most 5' portion of the HEXB gene that we can examine with pHexBl appears to be absent in GM 317 DNA.

**DISCUSSION**

Using a combination of gene, mRNA, and isozyme assessment of fibroblast cultures from patients with Sandhoff disease, we have revealed considerable heterogeneity within the disease while, at the same time, established biochemical criteria for classification into the infantile or juvenile onset forms. The small amount of hexosaminidase A-like activity in all of the infantile type cell lines was shown to be caused by an independent activity by virtue of its presence in two cell lines carrying partial deletions of the HEXB gene. After subtracting this activity from mutant hexosaminidase A values, cell lines from infantile onset patients were found to have no detectable hexosaminidase A activity, while those of juvenile origin all had a detectable, residual enzyme activity. These data suggest that very little hexosaminidase A activity is required to retard onset and provide a milder progression of the disease. This may be an important consideration when evaluating the results from the proposed use of gene therapy in animal models of Sandhoff disease (30-32).

The precise evaluation of hexosaminidase A in mutant cell lines has previously been difficult because of the inability to completely separate hexosaminidase A and hexosaminidase S by conventional ion exchange procedures. We have shown chromatofocusing to be a fast and reproducible procedure for the separation of these isoenzymes. Accurate estimation of hexosaminidase S has revealed enormous variation of this isozyme in mutant cells, although in normal fibroblasts α-chains dimers are not found in significant amounts. This variation in hexosaminidase S levels extended through both infantile and juvenile-type cell lines and showed no correlation with the onset or severity of the disease. Thus while hexosaminidase S appears to be able to hydrolyze G_{M2} ganglioside in vitro it appears to be ineffectual in vivo.

One might expect the level of hexosaminidase B to represent a titration of the HEXB gene activity. However, like others, we have failed to see a correlation between residual hexosaminidase B activity and the clinical phenotype (28). On the one hand, this is not surprising since hexosaminidase B does not contribute to G_{M2} ganglioside hydrolysis, the natural substrate activity being mediated solely by hexos-
amidase A. On the other hand, it suggests that the mere presence of β-subunits is not sufficient to predict the occurrence of a hexosaminidase A activity in mutant cells, whatever the level of successfully formed hexosaminidase B, e.g. infantile line 1954 contained a larger amount of residual hexosaminidase A. On the other hand, it suggests that the mere extension of a primary gene defect to two of the other infantile lines used in this study (GM 470 and GM 203) and one juvenile line not investigated here. In addition, we have earlier examined complementation by a histochemical method (40) that had assigned juvenile line GM 2094, used in this study, to the same complementation group. We therefore conclude that all of the mutant cell lines assigned to the single complementation group defined in Sandhoff disease contain primary mutations involving the HEXB gene.

REFERENCES


Continued on next page.

* F. Quan and R. Gravel, unpublished observation.
Molecular Heterogeneity in Sandhoff Disease

Supplementary Material for: Heterogeneity in the Invasive and Intravascular Forms of Gaucher Disease (Deficiency of Glucocerebrosidase) by R. O'Brien, A. Kivela, R. Wilcox, N. Greaves, J. Lowden, and D. Mulvihill

Materials and Methods

Cell lines

Details of the fibroblast lines used in this study are presented in Table 1. Lines GM 2094, GM 439, GM 703, GM 317, and human embryonic kidney (HEK) cells were originally obtained from the American Type Culture Collection (ATCC). Line GM 2094 was from the Cell Repository of Tissue Culture, National Institutes of Health, Bethesda, Maryland. Lines GM 317, GM 439, and GM 703 were from the Cell Culture Repository, National Institutes of Health, Bethesda, Maryland. Line GM 217 was obtained from the Human Tissue Bank, National Institutes of Health, Bethesda, Maryland. All human fibroblast lines were cultured in DMEM-Ham's F12 medium supplemented with 10% fetal bovine serum. GM 317, GM 439, and GM 703 were established in our laboratory from patient's blood and grown as monolayer cultures in DMEM-Ham's F12 medium supplemented with 10% fetal bovine serum.

In vivo assay

Fibroblasts from mice and rats were harvested from the skin of the mouse and cultured for 1 day in DMEM-Ham's F12 medium supplemented with 10% fetal bovine serum. The cells were incubated with a medium of normal saline and methanol (1:1). After overnight incubation, the amount of protein was determined using the bicinchoninic acid method. The results obtained were compared with the amount of protein present in the original cell culture medium. The amount of protein present was determined using a bicinchoninic acid method. The results obtained were compared with the amount of protein present in the original cell culture medium. The amount of protein present was determined using a bicinchoninic acid method. The results obtained were compared with the amount of protein present in the original cell culture medium.
Molecular Heterogeneity in Sandhoff Disease

Table 3: Analysis of Molecular Heterogeneity in the Drug Resistance of Sandhoff Disease

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Normal</th>
<th>Control</th>
<th>1% NTS</th>
<th>10% NTS</th>
<th>100% NTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Rtes. (%)</td>
<td>5.0 ± 0.5</td>
<td>2.5 ± 0.5</td>
<td>1.0 ± 0.5</td>
<td>0.5 ± 0.5</td>
<td>0.2 ± 0.2</td>
</tr>
<tr>
<td>Median Rtes. (%)</td>
<td>3.0 ± 0.5</td>
<td>2.0 ± 0.5</td>
<td>1.0 ± 0.5</td>
<td>0.5 ± 0.5</td>
<td>0.2 ± 0.2</td>
</tr>
</tbody>
</table>

The chromosomal procedure was used to measure the distribution of the hexosaminidase A/B enzyme activity in untreated and treated cells. The enzyme profile was similar to that of normal cells, with 60% of normal levels of hexosaminidase A activity. The hexosaminidase A/B ratio was not altered by 1% or 10% NTS treatment. However, 100% NTS treatment reduced the activity to 10% of normal levels. The treatment with 1% NTS resulted in a significant increase in the activity of the hexosaminidase A/B ratio. The treatment with 10% NTS resulted in a significant increase in the activity of the hexosaminidase A/B ratio. The treatment with 100% NTS resulted in a significant increase in the activity of the hexosaminidase A/B ratio.

Note: The distribution of the hexosaminidase A/B enzyme activity in the Sandhoff disease was determined to be normal. The activity was measured using the enzyme activity assay (Table 1). The distribution of the hexosaminidase A/B enzyme activity in the Sandhoff disease was determined to be normal. The activity was measured using the enzyme activity assay (Table 1). The distribution of the hexosaminidase A/B enzyme activity in the Sandhoff disease was determined to be normal. The activity was measured using the enzyme activity assay (Table 1). The distribution of the hexosaminidase A/B enzyme activity in the Sandhoff disease was determined to be normal. The activity was measured using the enzyme activity assay (Table 1). The distribution of the hexosaminidase A/B enzyme activity in the Sandhoff disease was determined to be normal. The activity was measured using the enzyme activity assay (Table 1). 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