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Characterization of Unusual Hexosaminidase A (HEX A) Deficient Human Mutants

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INTRODUCTION

Recently several unusual hexosaminidase A (HEX A) human mutants have been discovered. One patient described by Sandhoff et al. [1], termed variant AB(+), had the Tay-Sachs disease (TSD) phenotype with massive cerebral accumulation of ganglioside G_{M2} but normal HEX A and B activities when tested with synthetic substrates. With ganglioside G_{M2} as substrate, no activity was detected. A second patient presented with the TSD phenotype but with 35% normal HEX A activity in fibroblasts and serum. With ganglioside G_{M2} as substrate, 6% of normal activity was present [2].

Other mutants have been described in which a striking deficiency of HEX A (measured with synthetic substrates) occurred in normal, healthy adults [3–5]. Using ganglioside G_{M2} as substrate, activity measured about half normal in fibroblasts [2] and leukocytes [6]. It has been proposed that clinically normal HEX A deficient adults are allelic compounds consisting of the Tay-Sachs allele and a second allele whose product lacks activity for synthetic substrates but retains activity for ganglioside G_{M2} [2–5]. Heterozygotes for the anomalous mutation should have reduced HEX A activity using synthetic substrates but normal activity with G_{M2}.

Another report describes absent HEX A and B (synthetic substrates) in a normal adult male [7] who fathered two children with the TSD phenotype. G_{A2} cleavage was measured in the heterozygous range. It was postulated that he is an allelic compound for the Sandhoff disease mutation and a second mutation which impairs activity against the artificial but not the natural substrate.

In this paper, we report studies of two families in which two different mutations appeared to be present. The first leads to a reduction of G_{M2} cleaving activity but not of HEX A activity measured with synthetic substrates, whereas the converse is true of the second. This report is the first to do studies of HEX A activity using both synthetic substrates and ganglioside G_{M2} in parents of probands with unusual HEX A mutations.

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CASE REPORTS

M Family (Seattle)

The proband (C. M.) was a female who presented at 17 months with symptoms of a degenerative central nervous system disease. She developed normally until 4 months when motor weakness was first noted. This weakness persisted and was accompanied by progressive symptoms of muscular wasting, dulling of the sensorium, decrease in spontaneous movements, hyperacusis, and eventually seizures. At 17 months, she was hypertonic, hyper-reflexive, with decerebrate posturing, a dull facies, and bilateral optic atrophy with cherry-red spots in the fundi. She died at 3 years of bronchial pneumonia. An autopsy was refused.

The parents were unrelated and of English and northern European descent. Serum samples and skin biopsies were obtained from the patient and both parents.

S Family (Houston)

The proband (D. S.) is a 28-year-old, healthy male of above average intelligence without neurological symptoms. His mother (Mrs. S.) is of Ashkenazi Jewish origin and his father (Mr. S.) of Syrian Sephardic Jewish origin. D. S.'s very low serum HEX A activity was discovered when he volunteered for a TSD community screening program. Skin biopsies were obtained from D. S., Mr. S., and Mrs. S.

MATERIALS AND METHODS

Total hexosaminidase activities in fibroblasts [8] and serum were estimated as described previously [9]. HEX A in serum was estimated by a modification of the heat denaturation method [9]. HEX A in cultured fibroblast strains was estimated by the batchwise isolation method of Dance et al. [10]. This involves selective absorption of HEX A to DEAE-cellulose (DE-52, Whatman, Maidstone, Kent, England) and after washing off HEX B, preferential elution of HEX A with 0.2 M NaCl. The accuracy of this method was assessed by isolating HEX A and B in this manner and examining their purity by electrophoresis and by isoelectric focusing (IEF). The latter was carried out in 40 ml glass columns of ampholytes (LKB Instruments, Rockville, Md.) of pH 4–8 using 600–800 V and 2–3 milliamps. IEF was carried out 24 hr at 5°C after applying 1 ml of serum, HEX A or B preparations as above, or 150 μl fibroblast supernatants. The latter were prepared by harvesting fibroblasts with a rubber policeman from one 75 ml falcon flask, after rinsing the media with two washes of isotonic sodium chloride (5 ml), sonicating cell pellets for 30 seconds in 200 μl water, and centrifuging at 48,000 g for 30 min. The supernatant was applied to the isoelectric focusing column, and an aliquot taken for electrophoresis and DEAE batchwise estimation of HEX A. After focusing, 5-drop fractions were collected, and an aliquot taken for assay of hexosaminidase activity using 4-methylumbelliferyl-β-D-*N*-acetyl-glucosaminide as substrate (1.0 mM in 0.1 M citrate-phosphate buffer, pH 4.3, containing 1 mg/ml human serum albumin, Sigma, St. Louis, Mo., Fraction IV). After incubation, the reaction was stopped by adding 1 ml 0.085 M glycine-carbonate buffer pH 10, and the fluorescence read on a fluorometer [9]. A digital pH meter measured the pH of peak tubes. Km's were estimated according to Lineweaver and Burk [11] employing 4-methylumbelliferyl-β-D-*N*-acetyl-glucosaminide as substrate on HEX A preparations obtained by the DEAE batch isolation method.

Cellogel electrophoresis of hexosaminidases was performed on cellulose acetate gel (Cellogel, Chematron, Milan, Italy) in 0.05 M potassium-phosphate buffer, pH 7.0, for 1 hr at room temperature and stained according to Okada and O'Brien [12].

Assays of ganglioside G_{M2} activity in cultured fibroblast strains were done according to O'Brien et al. [2]. Activities of HEX A, HEX B, and ganglioside G_{M2} *N*-acetyl-β-D-galactosaminidase were measured in previously subcultured fibroblasts. Measurements were made in triplicate on at least three falcon flasks from each subject. Protein determinations were by the method of Lowry et al. [13]. Conditions for culturing skin fibroblasts have been previously described [8].

Control specimens from healthy individuals of both sexes (2–50 years) were assayed identically. TSD heterozygotes were parents of patients with TSD.

RESULTS

M Family

The activity of ganglioside G_{M2} *N*-acetyl-β-D-galactosaminidase in C. M. averaged 6% of the normal mean (table 1). This is similar to patients with Tay-Sachs or Sandhoff disease [2]. Both of C. M.'s parents had G_{M2} cleaving activities less than half the normal mean of controls and close to values for TSD heterozygotes. When HEX A was measured in fibroblasts from Mr. M., by either the DEAE or the IEF method, values fell in the range for heterozygotes for TSD. In serum, his values fell between controls and TSD heterozygotes. Mrs. M., both in serum and fibroblasts, had HEX A percentages in the normal range. Total hexosaminidase activities (not reported) were in the normal range for all three subjects in fibroblasts and serum.

S Family

The activity of G_{M2} *N*-acetyl-β-D-galactosaminidase in fibroblasts from D. S. was 41% and 51% of the normal mean in Mr. S., but the activity in fibroblasts from Mrs. S. fell in the normal range (135% of the normal mean). When HEX A was measured in fibroblasts from D. S., 8% was found by the DEAE method, but none was detected after IEF or electrophoresis. Fibroblasts from both parents had levels of HEX A at one-half normal (the range for TSD heterozygotes). Total hexosaminidase activities were normal in all three subjects.

Examiniation of IEF profiles of HEX A in all six individuals revealed no qualitative abnormalities. The isoelectric pH of HEX A was normal (table 1) in all five subjects where it was present. The HEX A peak was also normal in all subjects even in C. M. When HEX A was isolated from fibroblasts of C. M. and substrate saturation kinetics performed with the synthetic substrate, a normal Km was found. A Km for the G_{M2} substrate could not be measured due to low activity. C. M.'s fibroblast HEX A isolated by the DEAE batch method also had a normal heat denaturation curve.

Cellogel electrophoresis of fibroblast supernatants revealed normal migration of HEX A in C. M., absent HEX A activity in D. S., a reduced ratio of HEX A relative to B in Mr. S. and Mr. M., and normal proportions of HEX A in Mrs. M.

DISCUSSION

There is general agreement that HEX A cleaves ganglioside G_{M2} and Hex B does not [14–18]. Both cleave globoside, G_{A2}, and synthetic β-D-hexosaminides. HEX A appears to be a tetramer of two nonidentical pairs of subunits ($\alpha_2\beta_2$), while HEX B is a homopolymeric tetramer ($\beta_2\beta_2$) [19]. The mutation in TSD leads to an equal reduction of activity of HEX A for ganglioside G_{M2} and synthetic substrates [2], accounting for the successful use of the latter for genotype ascertainment [12].

We propose the following models to explain the results in the two families giving the designations for HEX A mutants as suggested in the News and Comments section of this issue [20]. Mr. M. is heterozygous for the TSD (or similar) mutation (HEX A2), Mrs. M. is heterozygous for a new mutation (HEX A 4), and C. M. is an allelic

TABLE 1

HEXODAMINIDASE A AND G_{M2} β-D-*N*=ACETYLGALACTOSAMINIDASE ACTIVITIES

SUBJECTS	SKIN FIBROBLASTS				SERUM	
		% HEX A			% HEX A	
	G_{M2} *N*-Acetyl-β-D-Galactosaminidase	DEAE	IEF	HEX A Isoelectric pH	DEAE	Heat
Control:						
Mean	3.7	70.6	82	5.5	60	70
Range	2.5–7.0	61–79	78–84	5.4–5.6	53–66	62–78
N.	11	23	6	6	9	500
S Family:						
D. S.	1.5 (41%)	8	0	...	...	...
Mr. S.	1.9 (51%)	56	42	5.5	...	...
Mrs. S.	5.0 (135%)	53	40	5.5	...	...
M Family:						
C. M.	0.2 (6%)	20	12	5.4	26	17
Mr. M	1.4 (37%)	54	57	5.4	48	59
Mrs. M	1.3 (36%)	70	75	5.5	63	64
TSD Heterozygotes	1.0–2.0	40–57	43–60	5.5	35–45	43–52

NOTE.—Values for G_{M2} *N*-acetyl-β-D-galactosaminidase are in nM/mg protein/hr. Nos. in parentheses = percentages of control mean. HEX A values are percentage of total hexosaminidase using 4MU-β-D-*N*=acetylglucosamine estimated by DEAE batch separation, integrating peak areas after IEF or heat denaturation. HEX A isoelectric pH's were determined by IEF.

compound (HEX A 2-4). The HEX A 4 mutation leads to normal activity of HEX A using the synthetic substrate but reduced activity using ganglioside G_{M2}. One would expect three HEX A tetramers to be generated in C. M.—HEX A 2-4, HEX A 4, and HEX A 2. Since the latter, the Tay-Sachs mutation, leads to absent HEX A activity, only the first two HEX A species should give activity. We carefully examined C. M.'s HEX A isoelectric focusing peak for a doublet or broadening and found none. Perhaps the HEX A 4 mutation does not lead to a charge change. Possibly some patients with the Tay-Sachs phenotype and normal HEX A (AB + mutants) may be homozygous for the HEX A 4 mutation. This hypothesis needs to be tested by measuring G_{M2} cleaving activities in such individuals and their parents.

D. S. is proposed to be an allelic compound (HEX A 2-5), heterozygous for the TSD (or similar) mutation (HEX A 2) and a second mutation (HEX A 5). Mr. S. is heterozygous for the TSD (or similar) mutation and Mrs. S. is heterozygous for mutation HEX A 5. It is proposed that the HEX A 5 mutation leads to an alteration of HEX A, such that the enzyme cleaves G_{M2} but not the synthetic substrate.

The disparity between G_{M2} and synthetic substrate cleavage by HEX A is crucial to this proposal. We believe the results are reliable and sufficiently accurate to substantiate the argument. The results can be explained by a "one gene-one enzyme–many substrates" model similar to that proposed for β-galactosidase [21]. Lysosomal enzymes are unique in their broad substrate specificity, and HEX A is known to cleave *N*-acetyl-glucosamine and *N*-acetyl-galactosamine moieties from a variety of substrates including G_{M2}, its asialo derivative G_{A2}, globoside, steroid hexosaminides [22], and oligosaccharides [23]. A structural mutation could lead to a differential effect upon two different substrates especially those with such different physical properties as ganglioside G_{M2} and 4-methylumbelliferyl-hexosaminides. The results emphasize the importance of employing the G_{M2} assay for genotype assessment in unusual HEX A variants. Both false-positive and false-negative heterozygote ascertainment and in utero diagnosis will result when synthetic substrates alone are used for HEX A assay.

SUMMARY

Two families with unusual hexosaminidase A (HEX A) mutations are described. In one, the proband had the Tay-Sachs disease phenotype with considerable HEX A activity. In the second, the proband was phenotypically normal with absent HEX A activity. Activities using ganglioside G_{M2} as substrate demonstrate markedly reduced activities in the first case and half-normal activities in the second. Pedigree analyses indicate the presence of two different mutations. In the first, the proband appears to be an allelic compound HEX A 2-4 where mutation HEX A 4 leads to a diminution of HEX A activity against G_{M2} but not for the synthetic substrate, 4MU-β-D-*N*-acetyl-glucosaminide, with HEX A 2 being the Tay-Sachs disease (or similar) mutation. In the second family, the proband is an allelic compound HEX A 2-5 where mutation HEX A 5 leads to a diminution of HEX A activity against the synthetic substrate, 4MU-β-D-*N*-acetyl-glucosaminide, but not for G_{M2}. The presence of either mutation will lead to false-negative (HEX A 4) or false-positive (HEX A 5) assignments of heterozygosity or homozygosity for G_{M2} gangliosidosis when synthetic substrates are

employed. In both families, G_{M2} *N*-acetyl-β-D-galactosaminidase activity in fibroblasts was an accurate determinant of phenotype.

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