Juvenile-onset motor neuron disease caused by novel mutations in β -hexosaminidase

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Abstract

A 12 year-old female presented with a seven-year history of progressive muscle weakness, atrophy, tremor and fasciculations. Cognition was normal. Rectal biopsy revealed intracellular storage material and biochemical testing indicated low hexosaminidase activity consistent with juvenile-onset G_{M2} -gangliosidosis. Genetic evaluation revealed compound heterozygosity with two novel mutations in the hexosaminidase β -subunit (c.512-3 C>A and c.1613+15_1613+18dup). Protein analysis was consistent with biochemical findings and indicated only a small portion of β -subunits were properly processed. These results provide additional insight into juvenile-onset G_{M2} -gangliosidoses and further expand the number of β -hexosaminidase mutations associated with motor neuron disease.

Keywords

Motor neuron disease; Sandhoff disease; β -Hexosaminidase; Neurodegeneration; G_{M2} -gangliosidosis; Lysosomal storage

1. Introduction

Tay–Sachs disease (TSD; MIM# 272800) and Sandhoff disease (SD; MIM# 268800) are lysosomal storage diseases resulting from decreased lysosomal β -hexosaminidase A activity

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(Hex A). Hex A deficiency leads to the accumulation of G_{M2}-ganglioside in toxic concentrations within the central, peripheral and enteric nervous systems and causes progressive neurodegenerative disease [1]. In humans, β -hexosaminidase exists as two major isoforms: Hex A is a heterodimer of α -(encoded by the *HEXA* gene) and β -(encoded by the *HEXB* gene) subunits and Hex B is a homodimer of β -subunits. Hex S, a minor isoform, is a homodimer of α -subunits. Only Hex A has a unique physiological function, since Hex B and Hex S cannot hydrolyze G_{M2} ganglioside in vivo [2]. TSD is caused by the absence of functional α -subunits with resultant loss of Hex A activity. SD is caused by the absence of functional β -subunits with a resultant loss of both Hex A and Hex B activities, and the appearance of a small amount of the very labile Hex S isozyme [3]. Hex A and S are heat-labile and catabolize 4-methylumbelliferyl-2-acetamido-2-deoxy-6-sulfo- β -d-glucopyranoside (MUGS), while Hex B is heat-stable and has a low affinity for MUGS. All three isozymes hydrolyze 4-methylumbelliferyl-2-acetamido-2-deoxy-6- β -d-glucopyranoside (MUG), with ~MUG/MUGS ratios of 1/300 for Hex B, 1/4 for Hex A and 1/1 for Hex S [4]. The diagnosis of TSD or SD, in individuals with low total Hex activity, can be made by using these characteristics to perform comparative biochemical evaluation [1,3].

Numerous mutations have been identified in *HEXA* and *HEXB*, with disease severity and age of onset often correlating with the level of residual Hex A activity specific for each mutation [1]. Null mutations have no Hex A activity, while some mutations (e.g. missense and partial splice site mutations) can result in reduced Hex A activity [5]. Infantile-onset G_{M2}-gangliosidosis present with a relatively homogenous clinical phenotype (a severe global neurodegenerative disorder), resulting from two null alleles of a specific subunit. In contrast, juvenile and especially adult-onset G_{M2}-gangliosidosis patients, even with the same genotype (e.g. P417L; [6]) can present with extremely variable clinical phenotypes [7,8], which can include dementia, cerebellar ataxia, and/or motor neuron disease [9–12]. These individuals possess one or two alleles that can produce residual Hex A activity that is below the critical threshold of 5–10% of normal levels needed to prevent the accumulation of G_{M2} ganglioside [13].

In disease-associated mutations where some protein is formed, the majority of mutations cause some adverse effect on the folding of the mutant subunit. Misfolded subunits are identified in the endoplasmic reticulum quality control pathway (ERQC) and subsequently directed to and degraded by the ER-associated degradation pathway (ERAD) [14]. In the case of the Hex subunits, their folding, dimerization and lysosomal trafficking can be indirectly assessed by western blot analysis. Both the α - and β -subunits are synthesized as a single ~65 kDa poly-peptide, which undergo proteolytic processing once they enter the lysosome. One of two α -polypeptides making up the mature α -subunit can be observed at ~56 kDa and one of three mature β -polypeptides can be detected at ~28 kDa [15]. Misfolded proteins can be detected as aberrations to these profiles.

This report documents a young girl with motor neuron disease due to decreased Hex A activity. The patient had novel compound heterozygous mutations in her *HEXB* genes. One mutation affected the third intron splice acceptor site, resulting in the skipping of exon 4 and the production of a truncated protein. The other mutation created a cryptic splice donor site

in the thirteenth intron, which resulted in the expression of a mutant misfolded protein and a small amount of wild-type β -subunit protein. These variants represented a novel null mutation and another associated with residual enzyme activity, respectively.

2. Material and methods

University ethics committees approved this work and informed consent was obtained from the family. DNA and RNA were obtained from blood and cultured fibroblasts using standardized methods. Reverse transcription of mRNA and direct sequencing of the β -subunit cDNA and genomic DNA were performed as previously described [12,16]. Rectal and skin biopsies were analyzed by standard histology and histochemical analysis. Western blot analysis of cultured fibroblast lysates from our proband, normal controls, infantile-onset TSD patients, and infantile-onset SD patients was performed and incubated with rabbit anti-human Hex A antibody, as previously described [17]. Bands were visualized, recorded, and their optical density quantitated using ImageJ software (<http://rsbweb.nih.gov/ij/>).

During miglustat treatment, therapeutic efficacy was monitored at numerous intervals over the next two years with serial neurological exams, left knee extension myometry, and motor unit number estimates (MUNEs).

3. Results

3.1. Clinical and laboratory evaluations and findings

The proband was a 12 year-old female of mixed European descent who presented to our clinic with a seven-year history of undiagnosed progressive muscle weakness, atrophy, ataxia and fasciculations. The product of a 37-week gestation without complications, she had normal development until five years when she developed hand tremors and bilateral foot drop. Over the next few years she was also noted to develop exercise intolerance, ataxia, lower extremity muscle weakness and atrophy.

Family history was negative for motor neuron disease. Her mother had a history of strabismus, mild ptosis, weakness, and tremor. Her father had a history of sarcoidosis and splenectomy due to “abnormal blood cells”. The proband’s older brother and younger half-sister were unaffected.

Our exam revealed normal cognition and language. She had prominent saccadic pursuit eye movements. Mild dysarthria was present especially with lingual sounds, and there was no dysphagia. She had diffuse muscle atrophy and weakness, with ankle dorsiflexion and hip flexion being most affected. She had a waddling gait and could not heel or toe-walk. Fasciculations were obvious in numerous muscle groups, including her face and tongue. A 3–5 Hz resting tremor was present in her hands, which increased in amplitude with intention. She was dysmetric on finger-to-nose testing and could not stand with her eyes closed. She was hyperreflexic throughout, but became areflexic in her lower extremities over the next several years. Sensation was normal. Brain imaging, retinal, and neuropsychological evaluations were within normal limits. There was no hepatosplenomegaly.

Nerve conduction studies and electromyography indicated an axonal motor neuropathy. Rectal biopsy revealed the presence of “foamy” cells between the mucosa and submucosal layers, which were likely storage-filled ganglion cells (Fig. 1A). Transmission electron microscopy of this tissue revealed similar cells with enlarged lysosomes filled with storage material (Fig. 1B) [18]. Total Hex activity was somewhat low with a dramatic skewing of isoform activity with 90% being derived from the Hex A and/or Hex S isoforms (Table 1). This skewing along with the low total activity was suspicious for a diagnosis of juvenile-onset Sandhoff disease, prompting specific genetic evaluation and characterization of mutations in *HEXB*.

At the time of diagnosis, the patient was started on miglustat (Actelion, Allschwil, Switzerland), an inhibitor of UDP-glucose:ceramide glucosyltransferase (glucosylceramide synthase), the initial step in the biosynthesis of the globo-, lacto- and ganglio-series of glycosphingolipids [19]. During miglustat treatment, serial neurological exams revealed a progressive clinical decline with further weight loss, muscle atrophy, tremor, weakness, and loss of ambulation. Left knee extension myometry and MUNE also showed progressive decline (Fig. 2). In addition, she became incontinent of bladder function and then experienced a bowel perforation requiring partial colectomy. Her condition worsened over the next years with further testing being suspended due to parental request. She succumbed to her illness at age 16 without autopsy. There were no seizures or loss of vision. She always demonstrated normal cognitive function by receiving above average grades in regular high school classes.

3.2. Genetic and protein evaluation

RT-PCR of the proband's lymphocytic mRNA resulted in the isolation of three separate cDNA species. One species was the predicted size and sequence for the wild-type cDNA; while the other two species were abnormal in size, with one shorter and the other longer than predicted. Sequencing of the shorter cDNA revealed a loss of 47 bp consistent with the exclusion of exon 4. This exclusion produced a translational frameshift and alteration of amino acids 171–174 followed directly by early termination. Sequencing of the longer cDNA species revealed the addition of 16 bp of sequence from intron 13. This addition also resulted in a frameshift at amino acid 538 with immediate premature stop codon at amino acid 539 resulting in the loss of the terminal 17 amino acids. Genomic DNA sequencing revealed compound heterozygous mutations. The shorter cDNA species was the result of a paternally-derived C-to-A mutation at the -3 position of intron 3 (c.512-3 C>A), which disrupted this intron's splice acceptor site and resulted in the exclusion of exon 4. The longer cDNA species was the result of a maternally-derived 4 bp (AAGT) duplication at the +18 site of intron 13 (c.1613+15_1613+18dup), producing a cryptic splice donor site. The presence of normal cDNA products indicates mRNA likely produced from this allele underwent proper splicing to some degree. Neither of these mutations has been previously seen in any Hex mutation databases or in healthy controls.

Protein assays of the proband's cultured fibroblasts revealed the presence of a large amount of unmodified or misfolded β -subunit in its precursor form, as well as a small amount of

fully processed, mature wild-type protein (Fig. 3). When compared to wild-type patients, the proband was thought to produce ~5% of normal β -subunit levels.

4. Discussion

Juvenile-onset motor neuron disease is a rare disorder with etiological heterogeneity due to acquired or genetic causes. The differential diagnoses of acquired causes include autoimmune or paraneoplastic syndromes (e.g. anti-MuSK antibodies), vitamin deficiencies (e.g. B₁₂), heavy metal toxicity (e.g. lead), and infectious agents (including poliomyelitis, West Nile virus, HIV, and HTLV-II). Genetic etiologies include spinal muscular atrophy (MIM# 253400), juvenile ALS (e.g. ALS2 MIM# 205100 and ALS4, MIM# 602433), distal hereditary motor neuropathies (e.g. HMN2A, MIM# 158590), hereditary spastic paraplegias (e.g. SPG3A, MIM# 182600; SPG10, MIM# 604187; and SPG31, MIM# 610250), and the G_{M2}-gangliosidoses.

G_{M2}-gangliosidoses are a rare cause of upper and lower motor neuron diseases [7,8]. Further complicating the diagnosis of motor neuron disease due to Hex A deficiency is the heterogeneous way the disorder can present, with additional phenotypic features that include spinocerebellar ataxia, dementia and/or psychosis [9–12]. Therefore the complete evaluation of children with any form of motor neuron disease should include the evaluation of β -hexosaminidase activity.

The proband's Hex deficiency was caused by novel compound heterozygous mutations in her *HEXB* genes. These alleles consisted of one null mutation and another associated with residual activity. The latter contained a cryptic splice site that predominantly produced a misfolded mutant protein. This misfolded protein likely remained as an inactive precursor in the endoplasmic reticulum; however, this allele also produced small amounts of the wild-type transcript and protein. Previously, a unique asymptomatic set of individuals were described as having no detectable Hex B and ~10% residual Hex A. This novel isozyme pattern was termed “hexosaminidase Paris” and was caused by a similar cryptic splice site mutation in IVS-13 [20]. It was later hypothesized that the novel isozyme pattern was caused by the low levels of normal β -subunits, produced by the small fraction of properly spliced β -mRNA. These normal β -subunits were completely absorbed by the pool of normal α -subunit monomers, which are retained in the ER in order to promote heterodimer formation, and resulted in residual Hex A with no detectable Hex B. Thus the residual amount (~5%) of the normal mature β -subunit protein found in our patient's cell lysates (Fig. 3) was likely primarily associated with α -subunits in vivo and reflects the presence of a similar amount of normal functional Hex A. This explains the patient's later-onset phenotype as compared to the more fulminant infantile variant of Sandhoff disease. The higher levels of “Hex A” measured as MUGS activity, in the patient's lysate likely reflect the presence of Hex S, which can account for 2–5% of residual Hex A-like activity levels in Sandhoff cells [21].

As potential treatments for these disorders are becoming more available [19,22], it is important to rapidly determine the diagnosis before further neurodegeneration can occur. Her response to miglustat treatment was minimal; however, as treatment was started late in the course of her disease, this result may have been predictable. In addition, other treatments

such as chaperone therapy can potentially stabilize the misfolded mutant protein as it is trafficked through the endoplasmic reticulum and lead to increased lysosomal enzyme activity [22]. This approach may be used as an adjunct to substrate reduction (miglustat) therapy, and with earlier treatment, it may be more likely to lead to a better outcome, but this requires careful investigation, ideally in a clinical trial setting. A challenge with rare disorders, particularly when diagnosis is delayed, lies with initiation of potential therapeutic agents after the onset of neurological problems that may not be fully reversibly.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.ymgme.2012.10.023>.

Fig. 1. Rectal biopsy. (A) “Foamy” cells between the mucosa and submucosal layers of rectal biopsy. 40× magnification. (B–D) Transmission electron microscopy revealed cells with increased number of lysosomes filled with storage material (3500×, 5600×, and 8900×, respectively).

Fig. 2.

Late-onset of miglustat treatment does not rescue muscle weakness or motor unit number estimate values. Left knee extension myometry and MUNE values showed progressive decline for over 60 weeks after initiation of miglustat treatment.

Fig. 3.

Western blot analysis of cultured fibroblast lysates incubated with rabbit anti-human Hex A antibody as previously described [17]. Lanes 1–3: normal controls. Lane 4: infantile TSD patient. Lanes 5–7: infantile-onset SD patients and lane 8: our proband. The proband produced an estimated 5% of normal β -m levels and had a noticeable increase in α/β -precursors. α -m = mature α subunit; β -m = mature β subunit; α/β -p = α and β precursors. Micrograms of protein per lane is as noted. MUG (nm/hr/mg)=nanomoles MUG catalyzed per hour per milligram lysate. MUGS (nm/h/mg)=nanomoles MUGS catalyzed per hour per milligram lysate. E2= $\times 10^2$, E3= $\times 10^3$, E4= $\times 10^4$.

Table 1

Hexosaminidase enzyme testing performed on leukocytes.

	Total Hex ^a	Hex B ^a	Hex A % ^b	Hex B %	Interpretation
Proband	936	89	90%	10%	Affected
Father	1740	420	76%	24%	Obligate carrier
Mother	1404	612	56%	44%	Obligate carrier
Control	1872	583	70%	30%	Normal

Normal ranges^a

Total hexosaminidase	1860–2780
Hexosaminidase B	500–1027
Leukocytes % Hex A	>59

^aActivity units=nmol/mg/h.

^bDerived after heat exposure.