GM2 Activator Deficiency Caused by a Homozygous Exon 2 Deletion in *GM2A*

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Abstract GM2 activator (GM2A) deficiency (OMIM 613109) is a rare lysosomal storage disorder, with onset typically in infancy or early childhood. Clinically, it is almost indistinguishable from Tay-Sachs disease (OMIM 272800) or Sandhoff disease (OMIM 268800); however, traditionally available biochemical screening tests will most likely reveal normal results. We report a 2-year-old male with initially normal development until the age of 9 months, when he presented with developmental delay and regression. Workup at that time was unrevealing; at 15

months, he had abnormal brain MRI findings and a cherry red spot on ophthalmological examination. Family history and all laboratory studies were uninformative. The combination of a cherry red spot and developmental regression was strongly suggestive of a lysosomal storage disorder. Sequence analysis of *GM2A* did not reveal any pathogenic variants; however, exon 2 of *GM2A* could not be amplified by PCR, raising suspicion for a large, homozygous deletion. Subsequent copy number analysis confirmed a homozygous deletion of exon 2 in *GM2A*. This is the first reported case of GM2A deficiency being caused by a whole exon deletion. We describe previously unreported electron microscopy findings in this disease, thus expanding the clinical and variant spectrum for GM2 activator deficiency. These findings demonstrate the increased degree of suspicion required for diagnosis of this rare disorder.

Brief Summary: This case of GM2 activator deficiency was caused by a homozygous deletion in *GM2A*, demonstrating the need to include exon level copy number analysis in any workup to fully exclude this disorder.

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Introduction

Complete degradation of gangliosides in human cells requires proper function of three gene products: *HEXA*, *HEXB*, and *GM2A*. Pathogenic variants in *HEXA* and *HEXB* cause Tay-Sachs and Sandhoff disease, respectively, conditions which can be easily identified by a variety of biochemical assays, including enzyme analysis for both and urine oligosaccharide screening for Sandhoff disease (Xia et al. 2013; Hall et al. 2014). Tay-Sachs disease and Sandhoff disease are caused by defective subunits of the hexosamini-

dase enzyme. Variants in *GM2A* cause a clinical phenotype indistinguishable from infantile Tay-Sachs or Sandhoff disease; however, there is no readily available biochemical assay for the identification of this condition. Identification and diagnosis requires a high degree of suspicion and persistence, despite consistently normal or uninformative results. At present, only a small number of patients have been reported and all of these are of infantile onset (Schroder et al. 1993; Schepers et al. 1996; Sakuraba et al. 1999; Smith et al. 2012; Renaud and Brodsky 2015). A 2015 summary of available literature identified fewer than ten published cases of molecularly confirmed *GM2A* deficiency (Renaud and Brodsky 2015).

Case Report

A 2-year-old male was evaluated in our clinic for loss of milestones and a cherry red spot on ophthalmological exam. Pregnancy and delivery were uncomplicated. Both parents are Chinese, with no known consanguinity. Earliest milestones were achieved at a normal time including rolling over and sitting. The first concerns were around 8 or 9 months of age, when the child was not advancing and had lost the ability to babble and reach for toys. At the time, the workup included EMG, EEG, and pelvic CT, all of which were unremarkable. Initial MRI at 15 months of age showed abnormal T2 hyper-intensities in the basal ganglia and also periventricular white matter changes. An ophthalmology exam revealed a cherry red spot. At this time, a karyotype was performed which was normal and lysosomal enzyme assay results were all within normal limits. Family history was noncontributory for any similarly affected individuals. The patient underwent extensive genetic testing

in China, including a sequencing panel for metabolic disorders which included several disorders associated with the clinical finding of a cherry red spot, including Tay-Sachs disease and Sandhoff disease, but not *GM2* activator deficiency.

At 2 years of age, he was unable to crawl or sit independently, and could no longer roll over. He was nondysmorphic, normocephalic without organomegaly, had excessive startle reflex, was irritable, non-verbal, made no eye contact, and did not track objects. He had axial hypotonia and profound weakness, brisk deep tendon reflexes, but no Babinski sign. The cherry red spot was confirmed at this time and repeat lysosomal enzyme testing was normal. Further metabolic workup including lactic acid, acylcarnitine, amino acids, urine organic acid, urine oligosaccharide screening and pterins showed all results within normal limits.

Although the repeat lysosomal enzyme analysis was normal, the suspicion was still high for a lysosomal storage disorder largely because of the combined history of a cherry red spot and developmental regression.

Electron microscopy of a skin biopsy (Fig. 1) showed a variety of abnormal inclusions, granular membrane bound vesicles, membrane cytoplasmic bodies (MCB), and zebra bodies. In some cases these were clearly in Schwann cells; in other cases the cell type was unclear. Axons also showed retraction, and debris indicated subacute degeneration. Some axons showed MCB.

Based on this combination of findings, molecular analysis for *GM2A* was considered the next appropriate step. Sequence analysis of *GM2A* did not detect any pathogenic variants; however, despite attempts with multiple primer sets, exon 2 could not be amplified by PCR which suggested a homozygous deletion involving this region. Copy number

Fig. 1 (a) Electron microscopy from a skin biopsy of our patient. Axons with degenerative changes containing membrane bound debris (solid white arrows), areas of axonal retraction, and Schwann cell cytoplasm also filled with membrane bound debris (white outline arrows). Original magnification 6000x. (b) Electron microscopy from

a skin biopsy of our patient. Cell with “zebra bodies” (solid white arrows), myelinosomes (white star), and granular membrane bound vesicles (outline arrow). This may represent a Schwann cell but definite myelin is not seen, so identification is uncertain. Original magnification 6000x

analysis was performed using a custom designed comparative genomic hybridization array, and the child was confirmed to have a homozygous deletion encompassing exon 2 of *GM2A*. Subsequently, breakpoint mapping analysis was performed using PCR amplification and Sanger sequencing, and the genomic breakpoints of the deletion were refined to nucleotide positions g.150,636,648 in intron 1 and g.150,642,789 in intron 2 (Fig. 2). This 6,142 bp deletion has not been previously reported in affected individuals or in the general population. Of note, a 7 bp microhomology was detected at the breakpoint junction.

Discussion

Diagnosis of GM2A deficiency requires the clinician to maintain a high degree of suspicion. In the context of developmental regression, and often a cherry red spot, commonly available biochemical testing (enzyme analysis for both Tay-Sachs disease and Sandhoff disease; urine

oligosaccharide screening) will most likely return normal results. Sequence analysis of *HEXA* and *HEXB* may not detect any pathogenic variants. At present, only a small number of cases of GM2A deficiency have been reported worldwide and all reported pathogenic variants are sequence variants that include single nucleotide variants and small (1–3 base pair) deletions that are detectable by sequence analysis (Stenson et al. 2014). The case described herein highlights the need to follow-up in the context of negative biochemical testing, and negative sequence analysis. While our case was homozygous for a whole exon deletion and strongly suspected to be so because of failure of amplification by PCR, an individual with a heterozygous deletion would not have provided the same clue. Moreover, given that only a handful of variants have been associated with disease in the literature, most variants when detected by clinical sequencing would likely be classified as variants of unknown significance (VOUS). As a result, even in a case where a high degree of suspicion leads to the pursuit of sequence

Fig. 2 Exon 2 deletion in *GM2A*. **(a)** Schematic representation of the deletion detected in the *GM2A* gene. The deleted region is represented with dotted lines whereas the intact region is represented with solid lines. The downward arrows indicate the breakpoints of the deletion. Also indicated are the intact (Black) and deleted (Gray) bases flanking each breakpoint. **(b)** Sequence alignment showing microhomology at the breakpoint junction. Patient sequence across the deletion breakpoint, as obtained by Sanger sequencing, was aligned to the reference

sequences from both the 5' breakpoint and the 3' breakpoint. As shown in the figure, a 7 bp sequence, “AGCTGGG” was detected on either sides of the deletion. The deletion has been refined to be 6,142 bp long extending from and including chr5:g.150,636,648 to chr5:g.150,642,789. These results, together with the failure of PCR amplification of exon 2, confirm a homozygous deletion of exon 2 of the *GM2A* gene in this individual. Ref. reference, Seq sequence, Bkpt breakpoint

analysis of *GM2A*, a negative result or one in which a single VOUS was detected may end the pursuit without further analysis for larger deletions or duplications.

Our report of the first whole exon deletion expands the spectrum of pathogenic variants identified in the *GM2A* gene and highlights the need for aCGH analysis in the molecular evaluation of GM2A deficiency. In addition, the 7 bp microhomology detected at the breakpoint junction suggests that the deletion may have been mediated by either of the various microhomology-mediated mechanisms that include break-induced serial replication slippage, microhomology mediated break-induced replication, and fork stalling and template switching (Lee et al. 2007; Chen et al. 2010). We also present the first description of electron microscopy findings in GM2 activator deficiency. MCB, lysosomes with granular material, zebra bodies, and myelinosomes are found in the GM2 gangliosidoses and other conditions, including Fabry disease and chloroquine exposure. Myelinosomes have been reported in peripheral ganglia and peripheral nerves in Tay-Sachs disease (Schmitt et al. 1979).

Identification of the precise genetic defect in the proband of any family has several benefits, including putting an end to the diagnostic odyssey, knowing that a treatable disorder was not missed, and enabling the family to pursue advanced reproductive options, such as prenatal diagnosis or preimplantation genetic diagnosis, for future pregnancies. Thorough investigation of the variant spectrum for all diseases will shed light on the need to follow-up negative, or inconclusive sequence results with copy number analysis. In the case of *GM2A*, with the small number of identified cases, and the high degree of clinical suspicion required for the diagnosis, copy number analysis may allow confirmation of additional cases of this rare condition.

Compliance with Ethics Guidelines

Conflict of Interest

Patricia Hall is employed by Emory University and is a laboratory director at EGL Genetic Diagnostics, LLC, a clinical genetics laboratory which performs testing described in this paper.

John Alexander is employed by Emory University and is a laboratory director at EGL Genetic Diagnostics, LLC, a clinical genetics laboratory which performs testing described in this paper.

Arun Ankala is employed by Emory University and is a laboratory director at EGL Genetic Diagnostics, LLC, a clinical genetics laboratory which performs testing described in this paper.

Regina Laine, Hart Lidov, Lisa Teot, and Irina Anselm declare that they have no conflict of interest.

Ethics Approval/Patient Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5). This study was approved by the institutional review boards of Boston Children's Hospital and Emory University where relevant.

Author Contributions

All authors have reviewed and approved the manuscript as submitted.

PLH drafted the manuscript, incorporated edit, reviewed, and interpreted biochemical genetic testing.

RL drafted the clinical synopsis and saw the patient in clinic.

AA and JJA reviewed and interpreted molecular genetic testing.

LAT and HGWL reviewed and interpreted electron microscopy results.

IA saw the patient in clinic and edited the manuscript.

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Animal Usage

None.

References

- Chen JM, Cooper DN, Ferec C, Kehrer-Sawatzki H, Patrinos GP (2010) Genomic rearrangements in inherited disease and cancer. *Semin Cancer Biol* 20:222–233
- Hall P, Minnich S, Teigen C, Raymond K (2014) Diagnosing lysosomal storage disorders: the GM2 gangliosidoses. *Curr Protoc Hum Genet* 83:17.16.11–17.16.18

- Lee JA, Carvalho CM, Lupski JR (2007) A DNA replication mechanism for generating nonrecurrent rearrangements associated with genomic disorders. *Cell* 131:1235–1247
- Renaud D, Brodsky M (2015) GM2-gangliosidosis, AB variant: clinical, ophthalmological, MRI, and molecular findings. *JIMD Rep* 25:83–86
- Sakuraba H, Itoh K, Shimmoto M et al (1999) GM2 gangliosidosis AB variant: clinical and biochemical studies of a Japanese patient. *Neurology* 52:372–377
- Schepers U, Glombitza G, Lemm T et al (1996) Molecular analysis of a GM2-activator deficiency in two patients with GM2-gangliosidosis AB variant. *Am J Hum Genet* 59:1048–1056
- Schmitt HP, Berlet H, Volk B (1979) Peripheral intraaxonal storage in Tay-Sachs' disease (GM2-gangliosidosis type 1). *J Neurol Sci* 44:115–124
- Schroder M, Schnabel D, Hurwitz R, Young E, Suzuki K, Sandhoff K (1993) Molecular genetics of GM2-gangliosidosis AB variant: a novel mutation and expression in BHK cells. *Hum Genet* 92:437–440
- Smith NJ, Winstone AM, Stellitano L, Cox TM, Verity CM (2012) GM2 gangliosidosis in a UK study of children with progressive neurodegeneration: 73 cases reviewed. *Dev Med Child Neurol* 54:176–182
- Stenson PD, Mort M, Ball EV, Shaw K, Phillips A, Cooper DN (2014) The human gene mutation database: building a comprehensive mutation repository for clinical and molecular genetics, diagnostic testing and personalized genomic medicine. *Hum Genet* 133:1–9
- Xia B, Asif G, Arthur L et al (2013) Oligosaccharide analysis in urine by maldi-tof mass spectrometry for the diagnosis of lysosomal storage diseases. *Clin Chem* 59:1357–1368