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AIMP1 Mutation Long-Term Follow-Up, With Decreased Brain *N*-Acetylaspartic Acid and Secondary Mitochondrial Abnormalities

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Abstract

Aminoacyl transfer RNA (tRNA) synthetase complex-interacting multifunctional protein I is a noncatalytic component of tRNA multi-synthetase complexes. Although important in joining tRNAs to their cognate amino acids, AIMP1 has several other functions including axonal growth, cytokine activity, and interactions with *N*-acetylaspartic acid in ribosomal tRNA synthetase complexes. Further, *N*-acetylaspartic acid donates an aspartate during myelination and is therefore important to axonal integrity. Mutations in *AIMP1* can disrupt these functions, as demonstrated in this clinical case study of 2 monozygotic twins, who display congenital opisthotonus, microcephaly, severe developmental delay, and seizures. Whole exome sequencing was used to identify a premature stop codon in the *AIMP1* gene (g. 107248613_c.115C>T; p.(Gln39). In the absence of whole exome sequencing, we propose that decreased *N*-acetylaspartic acid peaks on magnetic resonance spectroscopy could act as a biomarker for *AIMP1* mutations.

Keywords

AIMP1, *N*-acetylaspartic acid, mitochondrial abnormalities, seizures

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Mutations in the gene *AIMP1* (OMIM 603605 (http://omim.org/entry/603605)) have been associated with decreased *N*-acetylaspartic acid (NAA) signal on brain magnetic resonance spectroscopy, hypomyelination, microcephaly, seizures, and decreased life expectancy.[1] Aminoacyl transfer RNA (tRNA) synthetase complex-interacting multifunctional protein I (AIMP1) was first discovered to act as a noncatalytic component of a tRNA multi-synthetase complex (MSC). This MSC is composed of 3 noncatalytic proteins, joined to 9 catalytic aminoacyl-tRNA synthetases (ARS).[2] This complex has the primary role of joining tRNA's with their cognate amino acids, which is essential for translation to occur accurately. The congregation of ARS in the MSC provides an efficient trafficking channel for tRNAs and amino acids.[3] Additionally, Ray et al[4] proposed a novel function of MSCs known as the depot hypothesis: MSCs act as depots for regulatory proteins, which acquire auxiliary functions upon release from the complex. Although the MSC retains its primary function while intact, upon cleavage, the different protein subunits can engage in new activities outside of the complex and can act as a cytokine on a wide range of cells including endothelial cells, macrophages, and fibroblasts to control angiogenesis, inflammation, and dermal regeneration, respectively.[5]

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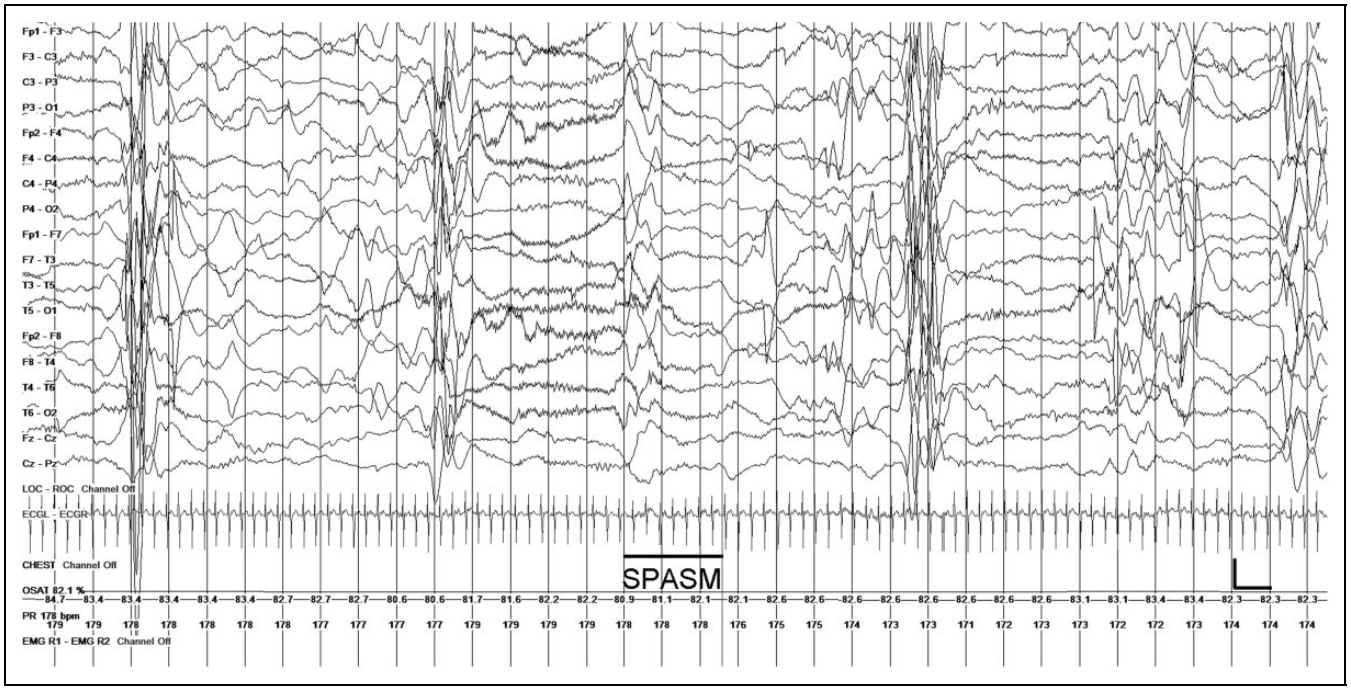

Figure 1. Electroencephalography (EEG) in one of the twins recorded at 4 months of age showing (A) an ictal electrodecremental response associated with an epileptic spasm clinically characterized by internal rotation and tonic posturing of the arms. Note that the severe slowing of the background EEG which is also disorganized and discontinuous. Bursts of generalized slowing followed by background attenuation with overriding fast activity often did not correlate with spasms as seen here. Vertical line = 400 μV, horizontal line (second) = 1 second.

AIMP1 also interacts with neurofilament light subunit, helping to maintain optimal levels of neurofilament light phosphorylation. Neurofilament light is a key component of the neuronal cytoskeleton, facilitating axonal and dendritic growth and stability.[6] A disruption in neuronal biogenesis is indicated in cases with severe mutations (deletions or protein truncation), as seen in the hypomyelination and microcephaly at birth. Decreased *N*-acetylaspartic acid signals on brain magnetic resonance spectroscopy has been presumed to be due to decreased neuronal mass or increased degradation; however, no study has yet reported the disruption of mitochondrial architecture in combination with an *AIMP1* mutation. This report provides evidence of decreased brain *N*-acetylaspartic acid in twins with a severe *AIMP1* mutation, which may serve as a biochemical indicator of the disease. Furthermore, architectural abnormalities in mitochondria indicate another mechanism of disease pathophysiology.

Case Report

Two monozygotic twin brothers were born via elective cesarean delivery to a 38-year-old G1 P0 mother. Both parents were of Filipino ancestry but nonconsanguineous. At birth, both babies were microcephalic (head circumference 30 cm; less than third centile), hypotonic, and had periodic breathing, apneas, and difficulty feeding. Additionally, they also had mild hypospadias. The patients spent ~3 weeks in the neonatal intensive care unit to manage apneas and feeding issues, which improved gradually. During this time, events of opisthotonus emerged, which were considered nonepileptic and managed with clonazepam. The magnetic resonance imaging (MRI) was performed shortly after birth was normal, while the electroencephalography (EEG) showed excessive sharp transients. At 3 months of age, the boys were readmitted to the hospital with frequent generalized tonic and focal onset seizures, the latter characterized by episodes of forced head and eye deviation. Events of limb dystonia (arm or leg posturing) and arm tremors were also observed, which were often suppressible and considered nonepileptic. The seizures were refractory to multiple medications including clobazam, phenobarbital, and keppra. The clonazepam was increased to treat the seizures and nonepileptic posturing, but this was ineffective. A trial of L-Dopa for dystonia was also unsuccessful. At 5 months of age, the patients went on to develop clear epileptic spasms associated with ictal electrodecremental responses and hypsarrhythmia on the EEG (Figures 1 and 2). Vigabatrin and the ketogenic diet were trialed with only a moderate and transient improvement in the symptoms. The ketogenic diet was discontinued after 6 months.

Brain MRIs, at that time, showed hypomyelination and a lactate doublet in both twins (Figures 3 and 4) and magnetic resonance spectroscopy showing decreased brain *N*-acetylaspartic acid levels (Figures 3 and 4). The MRI and proton magnetic resonance spectroscopy were performed on a 1.5 Tesla clinical scanner (Magnetom Avanto, Siemens, Erlangen, Germany). The MR spectra were sampled by placing a voxel at the region of left basal ganglia (TE: 135 ms, sampling voxel size: $2 \times 2 \times 2$ cm^3). A decrease in concentration of *N*-acetylaspartate was determined by subjectively comparing the height of *N*-acetylaspartic acid peak to that of creatine. For a healthy term neonate or a young

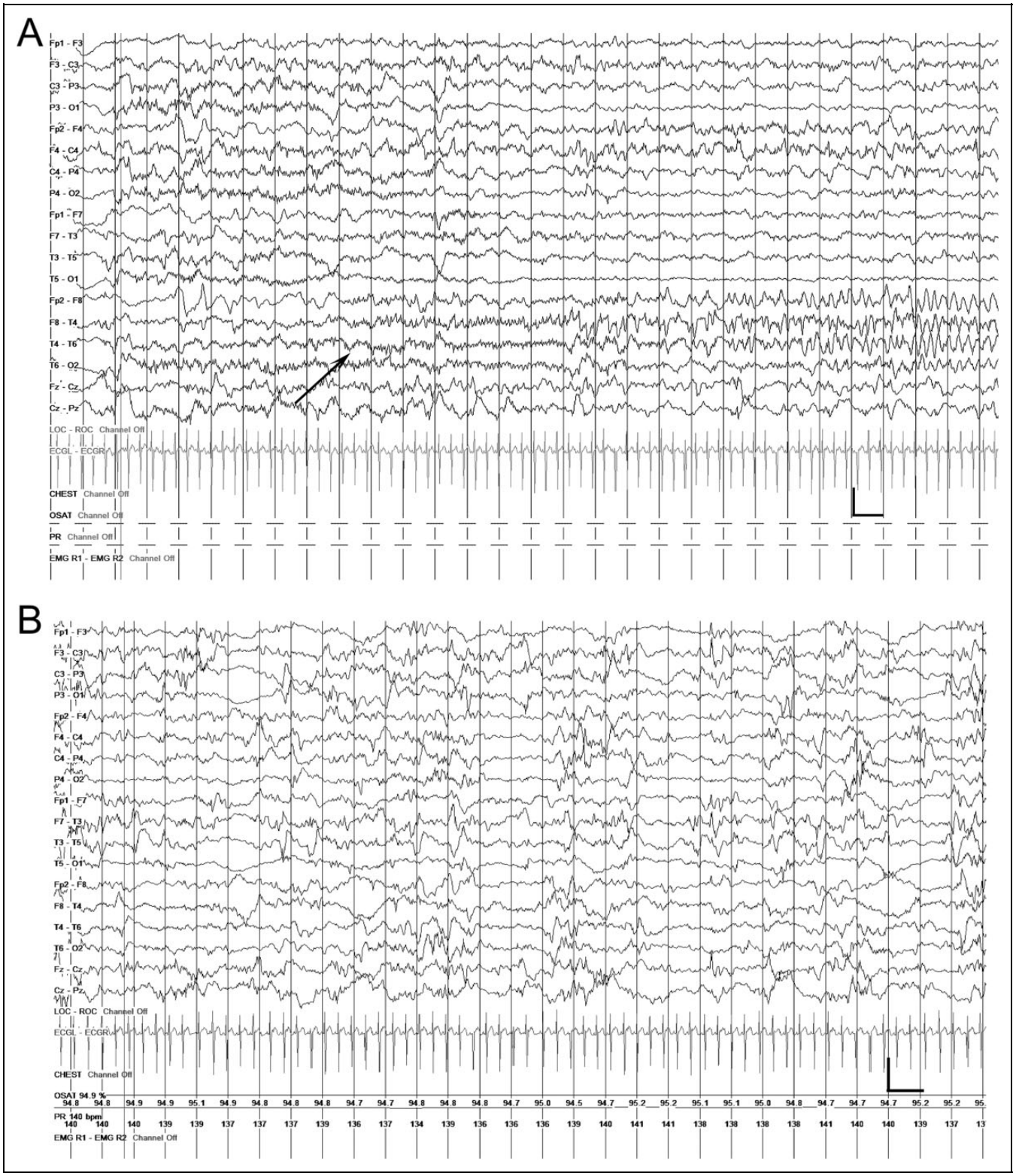

Figure 2. Electroencephalography (EEG) recorded at 5 months shows (A) right temporal onset focal seizure clinically characterized by elevation of the arms. Note the background EEG shows significant improvement following treatment with the ketogenic diet and vigabatrin. B, However, the severe developmental epileptic encephalopathy pattern characterized by multifocal spikes recorded on a disorganized background <200 μV persisted for significant portions of the EEG. Vertical line = 100 μV, horizontal line = 1 second.

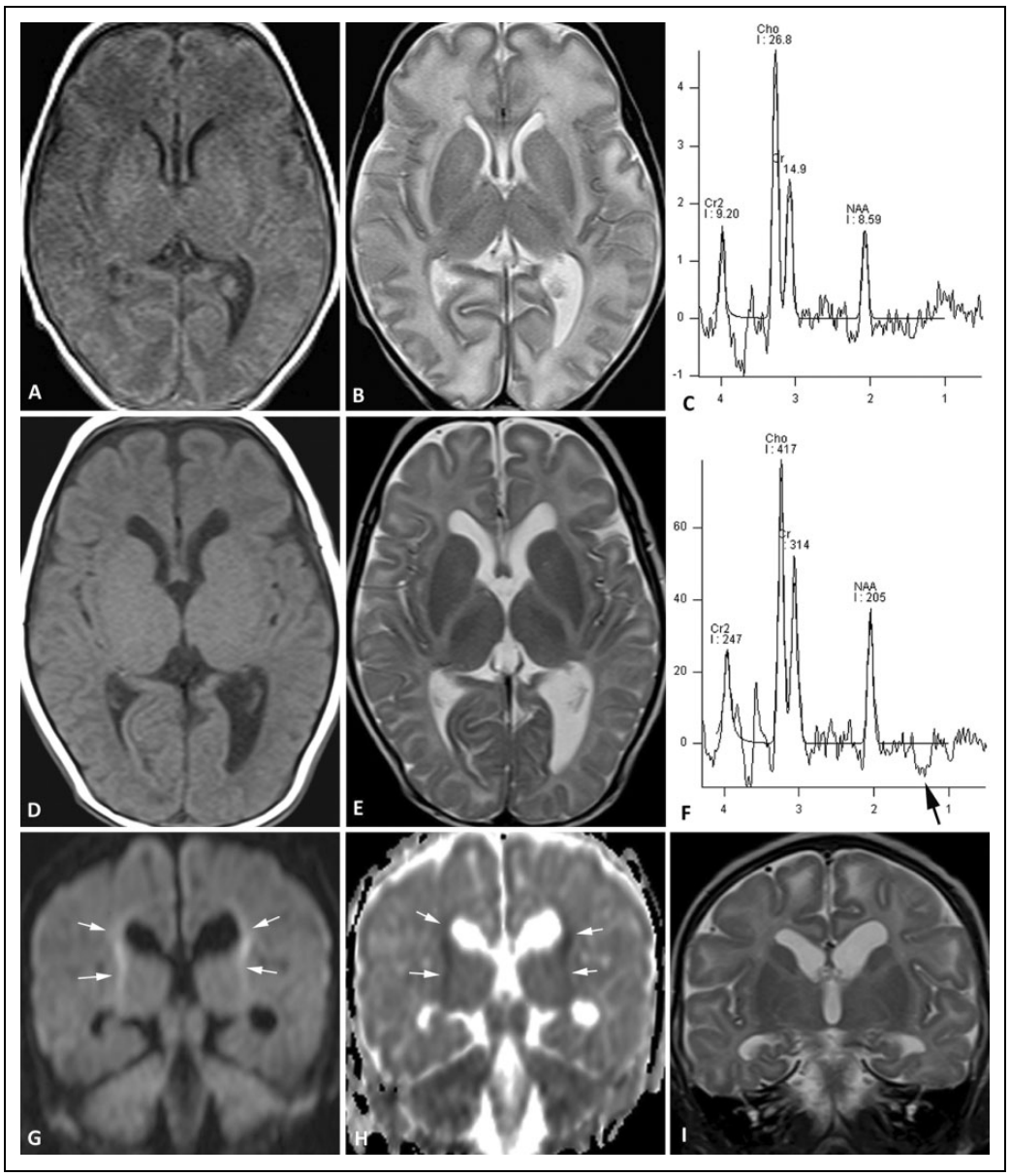

Figure 3. Brain magnetic resonance imaging (MRI) of twin A. MRI at age 19 days (corrected age of 39 weeks 3 days of gestation) including axial T1- and T2-weighted images (A, B) and magnetic resonance (MR) spectrum obtained from left basal ganglia with TE of 135 ms (C). Absence of myelination of the posterior limb of internal capsules is evident on both the T1- and T2-weighted images shown here, while absence of expected myelination at other locations of brain is also observed (not shown). Proton MR spectrum (C) revealed mildly decreased *N*-acetylaspartate (NAA) peak. On MRI at age 4 months (D-I), the T1- and T2-weighted images (D, E) again revealed absence of myelination of internal capsules, splenium of corpus callosum, and optic radiations, which are all expected to be myelinated at this age. Proton MR spectrum (F) revealed persisted decreased NAA peak, as well as a lactate doublet (arrow, F). On diffusion-weighted image (G, b = 1000) and apparent diffusion coefficient map (H), restricted diffusion is evident in bilateral corticospinal tracts (arrowheads, G and H). Abnormal hypointense T1 and hyperintense T2 signals are also seen in subcortical white matter (D, E, I). In addition, mildly enlarged lateral and third ventricles and mildly widened cerebral sulci have developed at 4 months of age (D, E, I), which was not present on the MRI at age 19 days (A, B). In the clinical setting of microcephaly, the development suggests cerebral atrophy.

infant, the *N*-acetylaspartic acid peak is expected to be at the same height or slightly higher than the creatine peak.

Additional testing, including invasive testing in one twin only, included a lumbar puncture for cerebrospinal fluid amino acids and neurotransmitters, urine organic acids, plasma acylcarnitine profile, and muscle biopsy showing nonspecific mitochondrial abnormalities, and no diagnosis was established. Urine *N*-acetylaspartic acid levels were of particular interest, as increased levels could be a potential marker of the disease. This was not seen consistently; however, as it was only increased once in one twin, both twins had serial testing for urine *N*-acetylaspartic acid.

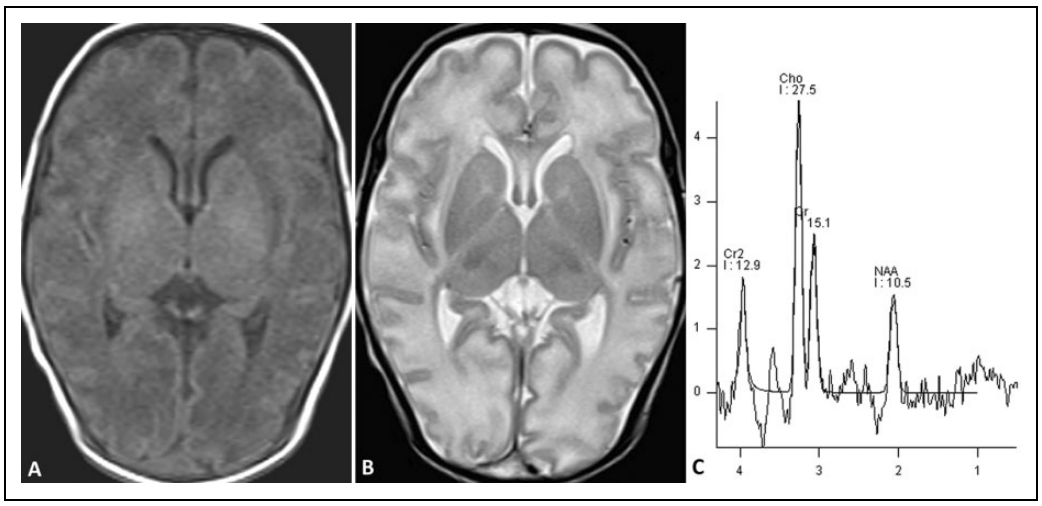

Figure 4. Brain magnetic resonance imaging (MRI) of twin B. Magnetic resonance imaging at age 19 days (corrected age of 39 weeks 3 days of gestation) including axial T1- and T2-weighted images (A, B) and magnetic resonance (MR) spectrum obtained from left basal ganglia with TE of 135 ms (C). The MRI findings are nearly identical to those of twin A (Figure 1). Absence of myelination of the posterior limb of internal capsules is evident on both the T1- and T2-weighted images shown here. Proton MR spectrum (C) revealed mildly decreased *N*-acetylaspartate (NAA) peak.

At 3½ years of age, with ongoing deterioration and a strong clinical suspicion of a mitochondrial disease, one twin had a repeat muscle biopsy to add studies of blue-native polyacrylamide gel electrophoresis and was recruited into the Mitochondrial Functional and Integrative Next Generation Diagnostics (MITO-FIND) study at the Alberta Children's Hospital to have a research exome. The second muscle biopsy showed advancement of mitochondrial cytopathy. Light microscopy revealed mild increased variation in fiber size with atrophic rounded fibers, mildly coarse lipid droplets, type 2 fiber predominance, and a lack of ragged red and COX-negative fibers. Electron microscopy revealed nonspecific findings of mitochondrial abnormalities, with mildly increased lipid droplets, swollen mitochondria, rare mitochondria with ropey cristae, and rare membranous, myelin-like whorls.

Additional markers of mitochondrial cytopathy were examined, although these were not shown to be reliable markers of this *AIMP1* mutation. Serum plasma lactate levels, measured twice at 1.6 and 2.1 mmol/L, were within the reference range of 0.5 to 2.2 mmol/L. Cerebrospinal fluid lactate levels were also normal, at 1.2 mmol/L (reference range: 1.1-2.4 mmol/L). Creatine kinase was elevated once at 225 U/L, but stabilized 6 days later and remained stable 4 months later (reference range: 0-195 U/L). Krebs cycle intermediates were normal. A lactate doublet on magnetic resonance spectroscopy in the basal ganglia was noted on 2 occasions—at 1 and 3 months of age. These results prompted consideration of a mitochondrial disease.

Whole exome sequencing was conducted using Agilent's SureSelect XT Human All Exon V5 for exome enrichment, followed by sequencing on the 5500XL SOLiD System (Life Technologies, Carlsbad, California). The sequencing data were uploaded to the Galaxy instance of University of Calgary (https://vpn.chgi.ucalgary.ca/), which applied the Genome Analysis Tool Kit and Sequence Alignment Map tools for secondary analysis.[7] The assembly used was GRCh37/hg19. The bioinformatic pipeline for tertiary analysis was developed through M.A.G.I.C. Clinic Ltd (Metabolics and Genetics in Calgary, Calgary, Alberta, Canada) Rapid Exome Analysis involved the following steps. First, a variant call file generated by the secondary pipeline was imported into VarSeq 2.0.1 (Golden Helix). The filtering workflow excluded data with read depth and genotype quality less than or equal to 10 and 20, respectively. Additionally, those variants with a minor allele frequency >1% (NHLBI, ExAC and gnomAD databases) were removed. The dbNSFP (database for nonsynonymous single-nucleotide variants) functional prediction voting, ClinVar, and combined annotation-dependent depletion (CADD) scores were utilized in the filtering process to assist in identifying the variant that is most likely the cause of the phenotype observed in the patient. The results of our analytical pipeline identified a novel (not present in queried population databases), conserved (PhyloP 7.42 & GERP++ 18.53), deleterious (CADD 12.93) homozygous variant in the *AIMP1* gene with a predicted loss of function (stop-gained) (g. 107248613_c.115C>T; p.(Gln39). The informatics indicated that the *AIMP1* gene has a low rate of benign loss of function variants as indicated by a LoF variants *z* score of 1.02. An entry in ClinVar classified this variant as pathogenic (Variant 162377). For these reasons, the variant in our software analysis was predicted to cause loss of normal protein function and was classified as pathogenic. Sorting intolerant from tolerant (SIFT) and PolyPhen evidence was not available for this variant in the dbNSFP functional prediction voting database. The variant was verified with a clinical exome trio and Sanger sequencing with the proband showing 2 *AIMP1* mutations and both parents were heterozygous and single gene Sanger sequencing identified the same variant in the affected twin (Blueprint Genetics, Helsinki, Finland).

Both patients are currently 5 years of age and still display microcephaly (less than third percentile) and severe global developmental delay. The patients are estimated to be at less than a 2-month developmental stage; specifically, they have no head control, are nonverbal, do not fix or follow, and do not smile reciprocally. They are nonambulatory and do not sit without support. Their main seizure types are focal impaired aware (staring), focal clonic (face twitching), and generalized tonic. The last EEG done at 3 years of age showed findings consistent with a severe developmental epileptic encephalopathy. The seizures, which were initially difficult to treat, are now well controlled with clonazepam, vigabatrin, topiramate, phenobarbital, and levetiracetam. They continue to have episodes of dystonic posturing (arching), which is nonepileptic, and have improved with adjustments of clonazepam.

Discussion

This specific *AIMP1* mutation has only been reported once in the Human Gene Mutation Database (HGMD). Armstrong et al[1] presented the case of a Filipino girl, born to healthy parents, who passed away at 15 months. In parallel with the case report presented here, at 3 weeks of age, she had abnormal posturing, hypotonia, vision problems, tonic seizures, decreased feeding, and aspiration pneumonia. Her MRI showed progressive cerebral atrophy and myelin deficiency. Additionally, her magnetic resonance spectroscopy showed a reduction in *N*-acetylaspartic acid. Other *AIMP1* nonsense variants that have been reported include the case of a nonfunctional protein, p.(Gln112*), and another that affected 7 individuals of a consanguineous kindred in Israel.[8,9] Of particular significance was severe microcephaly, with head circumferences measuring 2.5 to 4.5 standard deviations below the mean.

Iqbal et al[10] described the case of 2 consanguineous families in India and Pakistan that were impacted less severely by 2 missense variants, p.(Gly299Arg) and p.(Val176Gly). The families described by Iqbal et al[10] have global developmental delay, intellectual disability, and speech impairment, but notably lack neurodegeneration. Thus, there is a spectrum of deficits that can be experienced, depending on the specific *AIMP1* mutation that is present.

Mutations, as demonstrated in this report, that lead to the lack of AIMP1 protein production appear to cause more severe forms of the disease. Non-neurologic involvement has included one case of hyperglycemia, but the relationship with AIMP1 was not clear.[10] In our case, there was decreased *N*-acetylaspartic acid signal in the brain. The *N*-acetylaspartic acid is synthesized in the mitochondria through acetylation of aspartate by the membrane-bound enzyme L-aspartate N-acetyltransferase and transported out by a dicarboxylic acid transporter.[11] The *N*-acetylaspartic acid is required for myelination by donating aspartate. The most common metabolic disease associated with high *N*-acetylaspartic acid is Canavan disease, which is due to impaired degradation in *N*-acetylaspartic acid caused by cytosolic aspartoacylase deficiency. Diseases associated with low *N*-acetylaspartic acid include brain ischemia, brain injury, brain cancer, multiple sclerosis, Alzheimer disease, and other nonspecific diseases.[12] Brain *N*-acetylaspartic acid levels were determined subjectively on magnetic resonance spectroscopy by comparing the height of the *N*-acetylaspartic acid peak to that of the creatine peak. In 4 serial measurements over the lifetime of both twins, urine *N*-acetylaspartic acid was increased in one twin only once on the first measurement. While increased urine *N*-acetylaspartic acid may not be a consistent biomarker of the disease, if decreased *N*-acetylaspartic acid on magnetic resonance spectroscopy is seen commensurate with increased *N*-acetylaspartic acid in the urine, consideration should be given to *AIMP1* mutations.

The *N*-acetylaspartic acid has also been shown to form a complex with tRNA.[13] This complex is disrupted in individuals with *AIMP1* mutations. We hypothesize that AIMP1 proteins interact with *N*-acetylaspartic acid in ribosomal tRNA synthetase complexes, which, in AIMP1 deficient individuals, impairs not only protein synthesis in oligodendrocytes but may also affect proteins needed for mitochondrial integrity in the brain. The respiratory chain enzymes in the muscle biopsy did not appear to be affected on blue native polyacrylamide gel electrophoresis (BN-PAGE). Severe *AIMP1* mutations may therefore lead to reduction in *N*-acetylaspartic acid degradation through lack of production of aspartoacylase activity or decreased mitochondrial *N*-acetylaspartic acid biosynthesis. If present, in the appropriate clinical setting, decreased brain *N*-acetylaspartic acid could suggest the possibility of *AIMP1* mutations.

Although Feinstein et al[9] initially described AIMP1 deficiency as a Pelizaeus-Merzbacher-like disease, a primary demyelinating neurodystrophy, Armstrong et al[1], however, made an important distinction between them. Although AIMP1 deficiency results in hypomyelination, this is secondary to neurodegeneration. Given that AIMP1 plays a critical role in neurofilament light assembly, if axonal development is hindered, then myelination is consequently unable to proceed. Hypotonia experienced in AIMP1 deficient patients, such as the case with the twins previously described, can be partially explained by a dysfunctional neuromuscular junction (NMJ). Muscle fibers develop with little to no innervation, leading to the breakdown of the NMJ, and subsequent muscular atrophy and motor dysfunction. Zhu et al[6] demonstrated this on AIMP1 null mice, who had underdeveloped motor units, and muscle fiber density only 44% of the wild-type control mice.

Notably, there have been reports of genetic deficiencies in isolated tRNA synthetases, such as described by Sharma et al.[14] Their case describes a mutation in the gene *DARS2* (OMIM 610956(http://omim.org/entry/610956?search=610956&highlight=610956)), which encodes for mitochondrial aspartyl-tRNA synthetase. There are important similarities, as well as distinctions, between mutations in MSCs and mutations in isolated tRNA synthetases. In the Sharma et al[14] case, the patient did not experience symptoms until he was 13 years of age. He did not experience seizures, and his main clinical problem was of generalized muscle atrophy and spasticity in the distal legs. Cognitive abnormalities were not present. On magnetic resonance spectroscopy, however, a similar pattern to our patients

was observed; there was a significant decrease in *N*-acetylaspartic acid and a lactate doublet. This suggests that mutations in tRNAs impact *N*-acetylaspartic acid and lactate on magnetic resonance spectroscopy, providing further evidence that magnetic resonance spectroscopy is a useful tool in diagnosing tRNA mutations.

Conclusion

AIMP1 contributes to several essential biological processes that, when disrupted, can be extremely detrimental in nature. This is most evident in patients that inherit nonsense *AIMP1* mutations. Such mutations are inherited in an autosomal recessive manner, in which both parents are carriers. Not only is muscular development significantly hindered, but cognitive processes are impacted as a consequence of central nervous system axonal degeneration. Seizures and severe intellectual disability are just 2 of the resulting outcomes of AIMP1-deficient neurodegeneration. Treatment options mainly focus on minimizing the discomfort experienced by patients. Anti-seizure medications and the insertion of GJ-tubes for feeding are helpful in this regard.

While, in this case, exome sequencing helped confirm the *AIMP1* mutation, an initial biomarker of this disease could be seen through the pattern of decreased *N*-acetylaspartic acid levels on magnetic resonance spectroscopy and mitochondrial pathophysiology. When whole exome sequencing is not available, this pattern can be an important indicator of an underlying *AIMP1* mutation.

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Author Contributions

AK contributed to conception and design; acquisition, analysis, and interpretation. All authors drafted the manuscript, critically revised the manuscript, gave final approval, and agree to be accountable for all aspects of work ensuring integrity and accuracy.

Declaration of Conflicting Interests

The authors declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article. AK currently serves on the scientific advisory board for MitoCanada. A.K. is president of M.A.G.I.C. Clinic Ltd. (Metabolics and Genetics in Calgary).

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Ethical Approval

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