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New AP4B1 mutation in an African-American child associated with intellectual disability

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Abstract. Prevalence of intellectual disability (ID) varies from 1–3%. Genetic causes of ID are being increasingly recognized. Although multiple mutations have been identified as a cause of syndromic ID, the genetic etiology of non-syndromic ID is poorly understood. However, more than 100 loci have been mapped that are associated with non-syndromic ID. There have been a couple of reports of *AP4B1* gene mutation causing severe intellectual disability, absent speech, shy character, stereotypic laughter, muscular hypotonia that progressed to spastic paraplegia, microcephaly, foot deformity, decreased muscle mass of the lower limbs, inability to walk, and growth retardation. They had structural brain abnormalities and seizures. The reported cases were from Arab families where consanguineous marriage is common. We encountered an African-American child who presented first at the age of 24 mo with language difficulties and was subsequently found to have moderate to severe intellectual disability by standardized tests. Shortly, he started to have seizures and problems with ambulation. Although he was hypotonic at the time of presentation, legs slowly became spastic at the age of 4 yr. After a thorough work up, he was found to have heterozygous mutation in the *AP4B1* gene along with another missense mutation in the same gene. There has been no report of mutation in this gene in the North American population. Although *AP4B1* typically is said to be an autosomal recessive disease-causing gene, our case is different in the sense that there are two mutations in the same gene one of which has never been reported before and co-exists with a known disease causing mutation. Yet, the phenotype of the case closely resembles those published previously.

Keywords: Intellectual disability, *AP4B1*, hereditary spastic paraplegia

1. Introduction

American association of intellectual and developmental disabilities defines intellectual disability (ID) in the following way: 'Intellectual disability is a disability characterized by significant limitations in both intellectual functioning and in adaptive behavior, which covers many everyday social and practical skills. This disability originates before the age of 18 yr [1]. Intellectual functioning-also called intelligence-refers to general mental capacity, such as learning, reasoning, problem solving, and so on. Adaptive behavior is the collection of conceptional, social, and practical skills that are learned and performed by people in their everyday lives. By definition, this is a congenital handicap. Syndromic ID is associated with specific morphologic and behavioral abnormalities. Intellectual disability is the only problem in non-syndromic ID. The distinction between syndromic and non-syndromic ID is not precise. Conditions previously regarded as non-syndromic forms of ID may have additional clinical findings that were not initially recognized or emphasized. New technology and recent advances in the understanding of genetic disorders have allowed us to identify genetic underpinning of an increasing number of syndromic or non-syndromic

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causes of ID. One of the most recent genetic mutation identified is an autosomal recessive (AR) frame-shift mutation in *AP4B1* gene in Arabic families that had what is collectively called '*AP4B1* complex syndrome' [2,3]. Mutations in other subunits of AP-4 complex like *AP4E1*, *AP4M1*, and *AP4S1* have been reported to cause ID and spastic paraparesis. AP-4 complex is a heterotetramer composed of four subunits encoded by different genes: *AP4E1, AP4B1, AP4M1*, and *AP4S1* [3–5]. It is ubiquitously expressed in neurons throughout the embryologic and postnatal developmental stages and interacts with delta2 and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) glutamate receptors to selectively transport them from the trans-Golgi network to the postsynaptic somatodendritic domain [6]. Non-functional AP-4 complex leads to abnormal brain growth and development. Here, we report a new mutation in *AP4B1* concurrent with another genetic sequence change in an African-American child born from a non-consanguineous relationship.

2. Case report

We report a 4-year-old African-American boy born after uncomplicated pregnancy out of a non-consanguineous relationship. He initially presented with delayed motor milestones and hypotonia at the age of 13 mo. He started having generalized myoclonic seizures at about the same age that have been well controlled with levetiracetam. At age 4, neuropsychological testing revealed moderate intellectual disability with full scale IQ<50 and intellectual measures <24 mo. At this time, he was spastic bilaterally in his legs making his ambulation difficult. His height and head circumference were consistently below 5th percentile (standard center for disease control charts) adjusted for the age, sex and parental height. The only word at the age of four was 'mom', and no sign language.

A thorough work up including brain magnetic resonance imaging (MRI), endocrinology evaluation, metabolic studies, karyotyping, chromosomal microarray and analysis of *FMR-1* gene (for fragile X syndrome) was done and they were all negative. The patient did not have any other features suggestive of neurocutaneous syndromes. Family did not report a past history of infection or head trauma. There were no members in the family reported to have developmental delay or birth defects. Comprehensive non-syndromic ID panel test was obtained, which identified two sequence changes in the *AP4B1* gene. The first change is a base pair deletion in exon 3, c.311delC.The second sequence change is c.577G>A in exon 5, which results in amino acid change, p. Val193Ile. Patient was heterozygous for both the sequence changes. Unfortunately, the parents could not be tested because of their unwillingness.

Deoxyribonucleic acid (DNA) sequence analysis was carried out in a commercial laboratory as a part of the clinical evaluation, which did not require a review by Institute of Research Board approval. This test included complete sequence analysis of the coding region of the included genes. The target region included all coding exons of the transcript, plus 8 bp of flanking intronic sequence. In addition, exon 4 of MeCP2 was also amplified and gel analyzed for the common large deletions of that exon. Targets of interest are amplified using highly parallelized and multiplexed polymerase chain reaction. Reactions assembled with the Raindance systems [7]. DNA was sequenced using Illumina technology. Sequence reads are aligned to the reference sequence and variants are identified and evaluated using the validated custom bioinformatics pipeline. Gaps or regions of the poor coverage in the next generation data set are filled by Sanger sequencing. All novel and/or potentially pathogenic sequences are confirmed by Sanger sequencing. Only the coding and immediate flanking regions of the included genes were analyzed. Changes in the promoter region or non-coding region will not be detected by this testing. Technical sensitivity of this test is estimated to be more than 99% for single nucleotide changes and insertion and deletion of less than 200 bp.

Verbal consent was obtained from the patient's mother to publish the final content of this manuscript. I have tried my best to keep the anonymity of the case without revealing any personal identifiers.

3. Discussion

We describe an African-American child from a non-consanguineous marriage who presented with spastic paraparesis, ID and myoclonic seizures. Brain MRI was normal with no structural abnormalities. Genetic testing was positive for two heterozygous sequence changes in the *AP4B1* gene. Cis/trans- nature of these changes could not be established because of the unavailability of the parents' genetic tests. There have been two reports of similar cases with intellectual disability and spastic paraparesis. First report had two siblings of consanguineous Arabic origin. They had

thin corpus callosum and periventricular white matter changes in MRI and suffered from febrile convulsions and partial seizures unlike ours. It was actually proposed that this family had phenotypic expression of hereditary spastic paraplegia type 47 [2]. The second family reported in the same year was similar in many ways to the first case but they did not have seizures [3]. Our case is clinically similar to them in regards to ID, seizures and spastic paraparesis. The involved gene is also the same with different mutations.

Here, the genetic change is a novel frameshift mutation, c.311delC, which creates a premature stop codon at downstream amino acid 52. This causes abnormal truncated protein likely to be involved in the pathogenesis. Two of the cases described before had different frameshift mutation [2,3]. The second *AP4B1* sequence change in our case, c.577G>A in exon 5, results in amino acid change, p.Val193Ile. This sequence variant has not been previously described in patients with *AP4B1*-related disorders. The sequence change has been described in the National Heart, Lung, and Blood Institute exome sequencing project at a very low frequency (0.01%) [8]. The p.Val193Ile change affects a highly conserved amino acid residue located to be deleterious in a domain of the *AP4B1* protein that is known to be functional. The p.Val193Ile substitution appears to be deleterious using sorting intolerant from tolerant and align GV-GD in-silico prediction tools. However, its contribution to this patient's disease phenotype cannot be definitely determined. Here, we have identified a c.311delC sequence variant in one of the patient's *AP4B1* alleles that is likely to be deleterious (Table 1). As AP-4 complex-related disorders are autosomal recessive (AR) conditions, testing of this patient's parents for both sequence changes would have helped to determine the cis/tran- nature of these sequence changes and may provide further evidence to the pathogenicity of the c.577G>A (p.Val193Ile) sequence change. However, it may be theorized that the second mutation could be pathogenic in the presence of the already known pathogenic sequence change in an AR disease. The frequency of *AP4B1* gene mutations in non-Arabic population is unknown as there have been no other reports till this date. Unique features of our case are that this genetic mutation occurs in non-Arabic patient, from a non-consanguineous family (remember

Table 1
Reference and coding sequences

Mutations	Coding sequence	Reference protein	Predicted variant	Net effect
NM_006594. 2:c.311del	Exon 3 Reference coding sequence …….GACCCCAATC CAATGGTGCGAGG GC310T311G312G CGTTACGGAGCA TGTGTAGCCTCAG338 Predicted coding sequence …GACCCCAATCCAA TGGTGCGAGGGC3 10 -G312GCGTTAC GGAGCATGTGT AGCCTC338	……..DIVQKKLVYL YMCTYAPLKP DLALLAINTL CKDCSDPNPM VRG103L114 ALRSMC SLRMPGVQEY IQQPILNGLR DKASYVRRVA VLGCAKMHNL HGDSEVDGAL VNELYSLLRD QDPIVVV NCL180……..	……DIVQKKLVYL YMCTYAPLKP DLALLAINTL CKDCSDPNPM VRG113R114 RYGACV ASGCLVCRSI YNSLFSMVCG IRLHMSGEWQ SLDVPRCIIF METLK52*	Premature termination of translation
NM_006594. 2:c.577G>A	Exon 5 Reference coding sequence ……GGAAA TTCTGAAACAG GAAGGAGGCGT TGTCATCAATA AGCCCATTGCTCA…. Predicted coding sequence …GGAAATTCTGAAA CAGGAAGGAGGC A TTGTCATCAATA AGCCCATTGCTCA….	…..RSLEEILKQE GGVVINKPIA HHLLNRMSKL DQWGQAEVLN FLLRYQPRSE EELFDILNLL….	….RSLEEILKQE GG I VINKPIA HHLLNRMSKL DQWGQAEVLN FLLRYQPRSE EELFDILNLL….	Substitution of amino acid

*Predicted coding sequence and variant protein were obtained using an online tool [9]. Nomenclature is according to the Human Genome Variation Society guidelines.

each mutation is heterozygous in itself), normal MRI with no structural changes, different seizure semiology.

Adaptor protein complexes, AP-1, AP-2, AP-3, and AP-4, have been shown to control vesicular trafficking of membrane proteins in the biosynthetic and endocytic pathways [10]. It has been said that the defect in any one of the subunits of AP-4 complex can cause abnormal cycling of the glutamate receptors and lead to AP4-complex deficiency syndrome although there would be some phenotypic variability. Misrouting of GluRdelta2 and AMPA receptors in brain of knockout mice lacking the AP-4 beta-subunit has recently been shown [11]. It has also been proposed that motor disturbances observed in patients with mutations in the AP-4 complex might be because of cerebellar dysfunction caused by mislocalization of glutamate receptors. Similarly, defective AMPA receptor sorting might impact synaptic plasticity in hippocampal neurons and cause ID [2]. Additionally, AP-4 mediates the transport of amyloid precursor protein (APP) from the trans-Golgi network to endosomes. Disruption of the APP-AP-4 interaction enhances gamma-secretase-catalyzed cleavage of APP to amyloid-beta peptide, making AP-4 deficiency a potential risk factor for Alzheimer's disease [12].

In conclusion, *AP4B1* has been established as one of the causes of intellectual disability. Although previous two case reports claimed the prevalence of the mutation only in the middle-eastern population with consanguinity, our case is very different in that this happens to be the first reported to be present in an African-American patient. The frameshift mutation, which we think is the disease causing mutation, also results from unique amino acid substitution. Even more interestingly, this mutation is accompanied by another mutation that can potentially cause disease but has never been reported before or linked to the disease.

Supplementary material

Genes tested included the following under the nonspecific intellectual disability panel by a commercial laboratory:

- X-linked: ACSL4, BRWD3, FLNA, HUWE1, MECP2, OFD1 PRPS1, SMC1A, ZDHHC9, AFF2, CASK, FMR1 IGBP1, MED12, OPHN1, PTCHD1, SMS, ZNF711AP1S2, CCDC22, FRMPD4, IL1RAPL1, MID1, PAK3, RAB39B, SRPX2, ZNF81, ARHGEF6, CDKL5, FTSJ1, IQSEC2, NAA10, PCDH19, RPL10 SYN1, ARHGEF9, CLIC2, GDI1, KDM5C, NHS, PDHA1, RPS6KA3, SYP, ARX, CUL4B, GRIA3 KIAA2022, NLGN3, PHF6, SHROOM4, TSPAN7ATP6AP2, DCX, HCFC1, KLF8, NLGN4X, PHF8 SLC16A2, UBE2A, ATRX, DLG3, HPRT1, L1CAM NSDHL, PLP1, SLC6A8, UPF3B, BCOR, EIF2S3 HSD17B10, MAOA, OCRL, PQBP1, SLC9A6 ZDHHC15
- Autosomal recessive: ALG6, AP4S1, CC2D1A, DDHD2, KCNJ10, MAN1B1 PCNT, ST3GAL3, VLDLR, AP4B1, ARFGEF2 CNTNAP2, ERLIN2, L2HGDH, MED23, PRSS12 TECR, VPS13B, AP4E1, C12orf57, CRBN GRIK2 LINS, NRXN1, SLC25A1, TRAPPC9, ZC3H14, AP4M1, CA8, D2HGDH, IDH2, LRP2 NSUN2, SOBP, TUSC3, ZNF526
- Autosomal dominant: ANK3, DYRK1A, GRIN2A, PACS1, SYNGAP1 ARID1B, EHMT1, GRIN2B, RAI1, TCF4 CACNG2, EPB41L1, KIF1A SCN2A, TUBA1A CDH15, FOXG1, KIRREL3, SHANK2, UBE3A CTCF, FOXP1, MBD5, SHANK3, ZEB2 CTNNB1, GATAD2B, MEF2C, SMARCA4, ZNF407

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