REPORT

# Autosomal-Recessive Mutations in *AP3B2*, Adaptor-Related Protein Complex 3 Beta 2 Subunit, Cause an Early-Onset Epileptic Encephalopathy with Optic Atrophy

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Early-onset epileptic encephalopathy (EOEE) represents a heterogeneous group of severe disorders characterized by seizures, interictal epileptiform activity with a disorganized electroencephalography background, developmental regression or retardation, and onset before 1 year of age. Among a cohort of 57 individuals with epileptic encephalopathy, we ascertained two unrelated affected individuals with EOEE associated with developmental impairment and autosomal-recessive variants in *AP3B2* by means of whole-exome sequencing. The targeted sequencing of *AP3B2* in 86 unrelated individuals with EOEE led to the identification of an additional family. We gathered five additional families with eight affected individuals through the Matchmaker Exchange initiative by matching autosomal-recessive mutations in *AP3B2*. Reverse phenotyping of 12 affected individuals from eight families revealed a homogeneous EOEE phenotype characterized by severe developmental delay, poor visual contact with optic atrophy, and postnatal microcephaly. No spasticity, albinism, or hematological symptoms were reported. *AP3B2* encodes the neuron-specific subunit of the AP-3 complex. Autosomal-recessive variations of *AP3B1*, the ubiquitous isoform, cause Hermansky-Pudlak syndrome type 2. The only isoform for the δ subunit of the AP-3 complex is encoded by *AP3D1*. Autosomal-recessive mutations in *AP3D1* cause a severe disorder cumulating the symptoms of the *AP3B1* and *AP3B2* defects.

Early onset epileptic encephalopathies (EOEEs) are characterized by profound cognitive, sensory, and motor impairment in the context of recurrent clinical seizures or prominent interictal epileptiform discharges during the neonatal or early infantile periods.[1] Accurate diagnosis can inform the therapeutic management of affected individuals, prognosis, and genetic counseling.[2] When no brain lesion is diagnosed, the current classification of EOEEs relies on the age at seizure onset, the presence of recognizable patterns on clinical or electroencephalographic evaluation, and the identification of the disease-causing molecular defect (International League Against Epilepsy). Approximately 100 single-gene disorders with EOEEs have been identified, and each disorder has considerable clinical and genetic heterogeneity.[1] The availability of whole-exome sequencing has dramatically accelerated

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gene identification for severe neurodevelopmental disorders.[3–5] However, the low mutational burden associated with each individual gene defect requires international collaborations to identify multiple cases, combined with careful delineation of the phenotype.[6]

In the context of clinical whole-exome sequencing for the diagnosis of EOEE (n = 57), autosomal-recessive mutations in *AP3B2* (MIM: 602166) were identified in two unrelated individuals (Table 1).[7] Whole-exome capture and sequencing were performed for individual 1 (F1-II-1 in Figure 1) and individual 2 (F2-II-1 in Figure 1) at IntegraGen from 1 μg of genomic DNA per individual with the SureSelect Human All Exon V5 51 Mb Kit (Agilent). The resulting libraries were sequenced on a HiSeq 4000 (Illumina) according to the manufacturer's recommendations for paired-end 76 bp reads. More than 4 Gb of mappable sequences per individual were generated, resulting in a depth of coverage of at least ten reads for more than 93% of RefSeq coding exons. Exome analysis was performed as previously described.[5,7] *AP3B2* was considered a candidate because (1) no variant affecting a gene previously implicated in an EOEE was deemed likely to be disease causing, and (2) according to Exome Aggregation Consortium (ExAC) Browser data, 22 truncating variants in *AP3B2* (only eight of which affect the GenBank: NM_004644.4 transcript) were detected in the 60,706 individuals. None of them was identified in the homozygous state. Thus, observing two unrelated individuals with autosomal-recessive truncating variants in *AP3B2* in a cohort of 57 individuals was highly unlikely ($p = 8.8 \times 10^{-7}$, Fisher's exact test). Individual 1 was compound heterozygous for a near-splice synonymous change (c.1182G>A [p.=] [GenBank: NM_004644.4]) in exon 10 and a splice-site change (c.1110+1G>C [GenBank: NM_004644.4]) in intron 9, each inherited from a healthy parent (Table 2; Figure S1). The splicing consequences of the mutations were assessed by RT-PCR on total RNAs extracted from lymphoblastic cell lines derived from individual 1 (Figure S2). Individual 2 was homozygous for an exon 14 deletion (chr15: g.83343184_83345634del), detected by XHMM software on whole-exome data, and both parents were heterozygous. This deletion occurred on a 106 bp repeated domain with 96.3% homology between chr15: 83,343,954–83,344,059 and chr15: 83,345,865–83,345,970 (Table 2; Figure S2). To replicate the hypothesis of the association between EOEE and autosomal-recessive variations of *AP3B2*, we sequenced the candidate gene in 86 unrelated individuals with EOEE as previously described.[7,8] Primers for exons and flanking intronic regions of *AP3B2* are listed in Table S1. For each individual, PCR products were pooled and libraries were prepared with the Nextera XT DNA Sample Preparation Kit (Illumina). Generated libraries were sequenced on a MiSeq instrument (Illumina) according to the manufacturer's recommendations for paired-end 150 bp reads. Sequencing data were processed and variants were identified as described above, except that PCR duplicates were not marked. Mean sequencing coverage of *AP3B2* coding exons (RefSeq) and splice junctions was 3.879×, and 100% of targeted bases were sequenced 100× in every subject. Autosomal-recessive *AP3B2* variations were identified in one subject (individual 3, F3-IV.3 in Figure 1). The family history of individual 3 highlighted the existence of an additional individual with a similar disorder (individual 4, F3-III-11 in Figure 1). Both individuals were diagnosed with a homozygous 4 bp deletion predicted to cause a frameshift in exon 21 (c.2522_2525delTCAC [p.Leu841Glnfs*10] [GenBank: NM_004644.4]) (Table 2; Figure S1). A search for additional individuals in the Matchmaker Exchange network identified five families with eight individuals carrying biallelic mutations in *AP3B2* (individuals 5 [F4-II.1], 6 [F4-II.2], 7 [F5-II.1], 8 [F5-II.3], 9 [F6-II.1], 10 [F7-II-1], 11 [F8-II-3], and 12 [F8-II-4]; Figure 1). Sanger sequencing confirmed the presence of all variants and the consistent familial segregation. No ethnically matched control individuals were sequenced in this project, but each variant was absent from the ExAC Browser. To assess a suspected genotype-phenotype correlation, we contacted the referring clinician of each individual (Table 1; Supplemental Note). Informed consent was obtained from the families for the diagnostic procedure and exome sequencing. All procedures were approved by the local ethics committees.

The age of onset of the epileptic disease ranged from birth to 9 months. One individual (individual 7) did not present seizures at the last follow-up (4 years of age). The epileptic manifestation included infantile spasms in 4/12 individuals, subtle myoclonic movements in 1/12 individuals, and non-specific seizures in 6/12 individuals. Initial electroencephalography (EEG) revealed hypsarrhythmia in three individuals. The 12 reported individuals presented with a severe to profound delay in gross psychomotor acquisitions anterior to epilepsy onset (Table 1). Sitting position was acquired in one individual, and another individual was able to walk with aid (at 12 years of age). Neurodevelopmental anomalies included absent speech in 11/12 individuals and sleep disturbance in 3/12. Abnormal movements were noticed and included median stereotypies (8/12), hypermobility (6/12), dystonic movements (1/12), and peripheral hypertonia (4/12). Global hypotonia was reported (12/12) with weak or absent deep tendons reflexes (8/12). At the last follow-up, nine individuals had microcephaly (ranging from –2 to −4 SDs). When available, occipitofrontal circumference measured at birth was normal in 7/8 individuals, highlighting the postnatal occurrence of the microcephaly. Brain MRI was interpreted as normal in 6/12 individuals. Two individuals had progressive cerebral and cerebellar atrophy. Poor visual contact was reported for every individual. Fundus examination was performed for six individuals and identified pigmentary changes of the retina in two individuals and optic pallor in four (ages ranged from 7 months to 4 years). Electrophysiological evaluation included electroretinography and/or visual-evoked potentials in eight individuals.

**Table 1. Clinical Description of the 12 Reported Individuals**

| | Family 1 | Family 2 | Family 3 | | Family 4 | | Family 5 | | Family 6 | Family7 | Family 8 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Individual 1 | Individual 2 | Individual 3 | Individual 4 | Individual 5 | Individual 6 | Individual 7 | Individual 8 | Individual 9 | Individual 10 | Individual 11 | Individual 12 |
| Antenatal findings | none | none | none | none | none | none | none | none | none | none | none | none |
| Geographical origin | European | Algerian | French | French | Jordanian | Jordanian | Pakistani | Pakistani | Japanese | Middle Eastern | Tunisia | Tunisia |
| Consanguinity | no | yes | yes | yes | yes | yes | yes | yes | no | yes | yes | yes |
| Gender | female | female | male | female | male | female | female | female | male | female | male | female |
| **Neonatal Period** | | | | | | | | | | | | |
| Term (weeks) | full term | full term | full term | 38 | NA | NA | 35 | 39 | 37 | full term | full term | full term |
| Birth weight (g) | 3,580 | 2,495 | 3,090 | 2,840 | 3,400 | NA | 1,945 | NA | 2,428 | 2,600 | 3,380 | 3,500 |
| Birth length (cm) | 52 | 47.5 | 50 | 48 | NA | NA | 46 | NA | 45 | NA | NA | 49.5 |
| Birth OFC (cm) | 35 | 34 | 35 | 36 | NA | NA | 31 | NA | 30.5 | NA | 34 | 36.5 |
| APGAR score (1/3/5 min) | 9/9/10 | 10/10/10 | 10/10/10 | 10/10/10 | NA | NA | required CPAP and NICU stay | NA | 8 at 5 min | needed resuscitation at birth | 10/10/10 | 10/10/10 |
| **Disease Onset** | | | | | | | | | | | | |
| Age at first symptoms | 4 months | 4 months | 15 days | NA | NA | NA | birth | birth | 9 months | 2 months | birth | 4 months |
| First symptom type | hypotonia | hypotonia | hypertonia, erratic eye movements | NA | hypotonia | hypotonia | hypotonia, feeding difficulties | hypotonia | developmental delay | seizures | hypotonia, nystagmus | seizures |
| Seizure type | tonic-clonic, psychomotor regression | infantile spasms | hypertonic | NA | NA | NA | none | subtle neonatal seizures | infantile spasms | NA | infantile spasms, generalized seizures | infantile spasms, generalized seizures |
| First status epilepticus | 6 months | none | first month of life | NA | NA | NA | none | none | none | NA | sudden death during an epileptic episode | 5 years |
| **Evolution** | | | | | | | | | | | | |
| Age at last visit | 24 years | 6 years | 32 months | 16 months | 8 months | NA | 21 months | 24 months | 11 months | 9.5 years | 8.5 months | 6.5 years |
| Weight (kg) | NA | 23 ($75^{th}$) | NA | NA | NA | NA | 8.27 ($<3^{rd}$) | 9.8 ($3^{rd}$) | 7.9 ($3^{rd}$) | 22.8 ($2.7^{th}$) | 8.830 ($25^{th}$) | 23 ($60^{th}$) |
| Height (cm) | 150 ($<5^{th}$) | NA | 88 ($10^{th}$) | 70 ($3^{rd}$) | 70 ($50^{th}$) | NA | 81 ($25^{th}$) | NA | 71.8 ($25^{th}$) | 129 ($13^{th}$) | 69 ($25^{th}$) | NA |
| OFC (cm) | 51.5 ($<3^{rd}$) | 51 ($50^{th}$) | 46.5 ($<3^{rd}$) | 43.5 ($3^{rd}$) | 42 ($3^{rd}$) | NA | 43 ($<3^{rd}$) | 44.5 ($<3^{rd}$) | 44.4 ($10^{th}$) | 45.5 ($<2^{nd}$) | 43.8 ($5^{th}$) | 47 ($3^{rd}$) |

(*Continued on next page*)

**Table 1.** ***Continued***

| | Family 1 | Family 2 | Family 3 | | Family 4 | | Family 5 | | Family 6 | Family7 | Family 8 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Individual 1** | **Individual 2** | **Individual 3** | **Individual 4** | **Individual 5** | **Individual 6** | **Individual 7** | **Individual 8** | **Individual 9** | **Individual 10** | **Individual 11** | **Individual 12** |
| **Neurodevelopmental Evolution** | | | | | | | | | | | | |
| Seizures | poorly active epilepsy | pharmaco-resistant | no more seizures | NA | NA | NA | NA | pharmaco-resistant | pharmaco-resistant | unspecified intractable seizures | pharmaco-resistant | pharmaco-resistant |
| **Motor Development** | | | | | | | | | | | | |
| Eye contact | poor | poor | poor | NA | poor | poor | poor | poor | poor | brief eye contact | poor | poor |
| Sitting position | 5 years | – | – | NA | – | – | – | – | with support | with support | no | no |
| Walking | 12 years | – | – | NA | – | – | – | – | – | – | – | – |
| Speech | – | – | – | NA | – | – | – | – | – | non-verbal | – | – |
| **Clinical Evaluation** | | | | | | | | | | | | |
| Facial dysmorphism | protruding eyes | – | – | – | – | – | – | – | – | long palpebral fissures, proptotic eyes, long lashes | – | – |
| Dermatological findings | – | hypopigmented patches | NA | NA | NA | NA | salmon macule, several moles | – | – | normal, no dyspigmentation | hyperpigmented spot on the thigh | – |
| Extremities | short | – | – | – | – | – | – | – | – | mild contractures of all toes | – | – |
| **Neurological Examination** | | | | | | | | | | | | |
| Axial hypotonia | + | + | + | + | + | + | + | + | + | + | + | + |
| Peripheral hypertonia | + | – | – | – | – | – | – | – | + | + | + | – |
| Weak deep tendon reflexes | + | – | + | + | + | + | + | + | – | + | – | NA |
| Median stereotypies | + | + | + | NA | NA | NA | + | + | + | + | NA | + |
| Dyskinesia | hyperkinesia | – | – | – | – | – | + | + | choreoathetosis | – | NA | + |
| Sleep disorders | + | + | – | – | – | – | – | – | – | + | – | – |

(*Continued on next page*)

**Table 1.** ***Continued***

| | Family 1 | Family 2 | Family 3 | | Family 4 | | Family 5 | | Family 6 | Family7 | Family 8 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Individual 1** | **Individual 2** | **Individual 3** | **Individual 4** | **Individual 5** | **Individual 6** | **Individual 7** | **Individual 8** | **Individual 9** | **Individual 10** | **Individual 11** | **Individual 12** |
| **Ophthalmological Examination** | | | | | | | | | | | | |
| Fundus | normal | normal | normal | normal | retinitis pigmentosa, mild optic disc pallor | retinitis pigmentosa, mild optic disc pallor | optic nerve pallor | optic nerve pallor | NA | normal | normal | normal |
| ERG | abnormal | normal | NA | NA | NA | NA | B-wave reduction, cortical visual loss | B-wave reduction, cortical visual loss | NA | abnormal | NA | NA |
| VEP | delayed | giant waves | NA | giant waves | NA | NA | NA | NA | NA | abnormal | normal | NA |
| **Malformative Workup** | | | | | | | | | | | | |
| Brain MRI | normal | cerebellar and cerebral atrophy | normal | NA | normal | NA | thin corpus callosum, enlarged extra-axial space | thin corpus callosum, enlarged extra-axial space | normal | cerebral and cerebellar atrophy, white-matter anomalies | normal | normal |
| Brain CT scan | – | – | – | – | – | – | NA | NA | – | – | NA | NA |
| Cardiac US | – | – | NA | NA | NA | NA | NA | NA | NA | normal | NA | – |
| Abdominal US | – | – | NA | NA | NA | NA | NA | NA | – | normal renal US | NA | – |
| Metabolic screening | – | – | – | NA | – | NA | – | – | – | – | – | – |

Abbreviations are as follows: +, present; −, absent; CPAP, continuous positive airway pressure; CT, computed tomography; ERG, electroretinography; NA, not available; NICU, neonatal intensive care unit; OFC, occipito-frontal circumference; US, ultrasound; and VEP, visual-evoked potential.

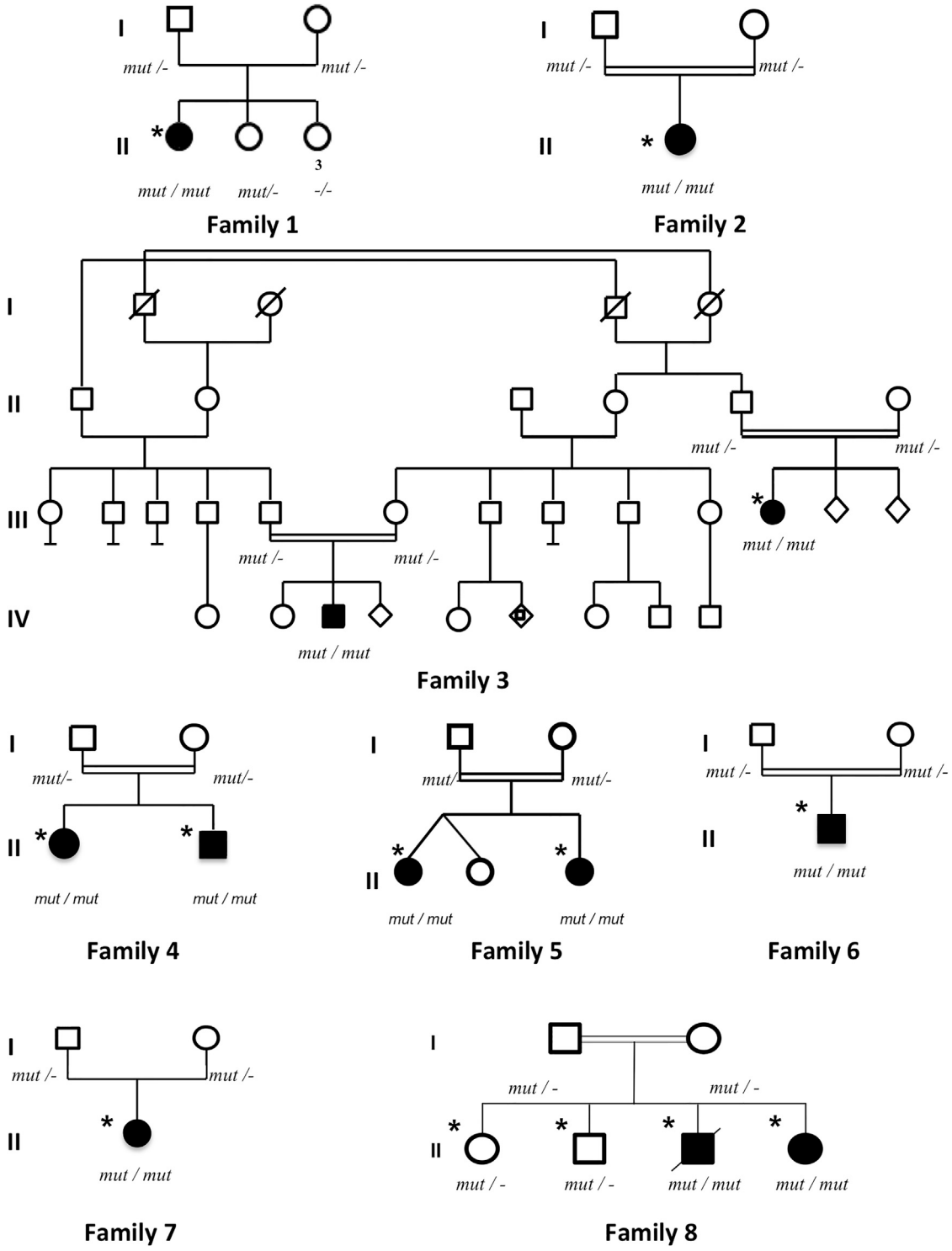


**Figure 1. Pedigrees of the Eight Families Affected by *AP3B2* Mutations**
Asterisks (*) point to the individuals who underwent whole-exome sequencing. The identified *AP3B2* variants were searched in all available relatives. "mut/mut" and "mut/−" refer to homozygous or compound-heterozygous individuals and heterozygous carriers of *AP3B2* mutations, respectively. "−/−" indicates individuals for whom no *AP3B2* mutation was identified.

Giant waves were reported in two individuals, null electroretinography was reported in three individuals, B-wave reduction was reported in two individuals (ages ranging from 4 to 21 months), and altered optic nerve conduction was reported in one individual.

*AP3B2* encodes the neuron-specific subunit of non-clathrin and clathrin-associated adaptor protein complex 3 (AP-3).[9–11] AP-3 is part of the family of heterotetrameric adaptor protein (AP) complexes (AP-1, AP-2, AP-4, and AP-5), which play a key role in signal-mediated trafficking

**Table 2. Genetic Description of the *AP3B2* Variations**

| Family | Genomic Change (hg19) | Coding Change (GenBank: NM_004644.4) | Protein Change | Inheritance | Impact on Transcript |
|---|---|---|---|---|---|
| 1 | chr15: g.83348481C>T | c.1182G>A | p.= | compound heterozygous | skipping of exon 10 skipping of exon 9 |
| | chr15: g.83348926C>G | c.1110+1G>C | splice donor | compound heterozygous | |
| 2 | chr15: g.83343184_83345634del | c:1489−245_1665+2029del | NA | homozygous | skipping of exon 14 |
| 3 | chr15: g.83331901_83331904delGTAG | c.2522_2525delTCAC | p.Leu841Glnfs*10 | homozygous | frameshift |
| 4 | chr15: g.83349863C>A | c.588+1G>T | splice donor | homozygous | not tested |
| 5 | chr15: g.83357975G>A | c.199C>T | p.Arg67* | homozygous | truncation |
| 6 | chr15: g.83330664G>A | c.2872C>T | p.Arg958* | compound heterozygous | frameshift |
| | chr15: g.83328380_83328383delCAGT | c.3178_3181delACTG | p.Thr1060Serfs*7 | compound heterozygous | frameshift |
| 7 | chr15: g.83350239G>T | c.454C>A | p.Glu152* | homozygous | truncation |
| 8 | chr15: g.83348926C>G | c.1110+1G>C | splice donor | homozygous | skipping of exon 9 |

of integral membrane proteins, such as endocytosis of plasma-membrane components, protein trafficking in the *trans*-Golgi network, or endocytosis.[12] Each complex assembles four subunits belonging to four different families: a large variable subunit (γ, α, δ, ε, or ζ subunit), a second large subunit (β subunit), a medium subunit (μ subunit), and a small subunit (σ subunit). All five AP complexes have distinct subcellular localizations and mediate different transport steps. Tissue-specific isoforms have been identified for the β, σ, and μ subunits of AP-3, whereas the δ subunit has a unique and ubiquitous isoform. *AP3B2* and *AP3M2* (MIM: 610469) are specifically expressed in neuronal cells. The ubiquitously expressed form of AP-3 is involved in vacuolar protein trafficking to organelles such as pigment granules, melanosomes, or platelet-dense granules.[11,13] Neuronal AP-3 is localized in the soma and the nerve terminals, where it mediates the sorting and transport of vesicle membrane proteins between the neuronal cell body and the nerve terminus.[14,15]

Autosomal-recessive loss-of-function mutations in genes encoding subunits of the AP complexes have been associated with several human disorders (Table 3). Some of the clinical features are recurrent across the different disorders, and some authors suggest the existence of a group of disorders named "adaptinopathies."[16] Microcephaly, mostly of postnatal onset, is a constant feature.[11,17–23] Developmental delay associated with intellectual disability is a frequent feature. Autosomal-recessive mutations affecting

**Table 3. Human Disorders Associated with Variants in Subunits of the AP Complexes**

| | AP-1 | | AP-3 | | | AP-4 | | | |
|---|---|---|---|---|---|---|---|---|---|
| Encoding gene (MIM) | *AP1S1* (603531) | *AP1S2* (300629) | *AP3B1* (603401) | *AP3B2* (602166) | *AP3D1* (607246) | *AP4M1* (602296) | *AP4S1* (607243) | *AP4B1* (607245) | *AP4E1* (607244) |
| Microcephaly | + | +/− | + | + | + | + | + | + | + |
| Severe developmental delay | − | + | − | + | + | + | + | + | + |
| Intellectual disability | + | + | − | + | + | + | + | + | + |
| Seizures | − | − | − | + | + | +/− | + | + | − |
| Immune deficiency | − | − | + | − | + | − | − | − | + |
| Hematological dysfunction | − | − | + | − | + | − | − | − | − |
| Albinism | − | − | + | − | + | − | − | − | − |
| Spasticity | − | +/− | − | − | − | + | + | + | +/− |
| Reference | Seong et al.[15] | Martinelli and Dionisi-Vici[16] | Simpson et al.[10] | this report | Hardies et al.[17] | Kong et al.[18] | Abdollahpour et al.[19] | Cacciagli et al.[20] | Martinelli et al.[21] |

Abbreviations are as follows: +, present; −, absent; and +/−, rare feature.

AP-4 seem preferentially associated with spastic paraplegia. The phenotypic spectrum of autosomal-recessive mutations in genes encoding AP-3 subunits seems highly consistent with the expression pattern of each gene. Autosomal-recessive disease-causing variations of *AP3B1* are responsible for Hermansky-Pudlak syndrome type 2 (HPS2 [MIM: 608233]), characterized by the association of oculocutaneous albinism, a bleeding disorder with platelet dysfunction, and immune deficiency. Individuals with HPS2 usually have no neurodevelopmental disorder. Here, we report on autosomal-recessive truncating variations of *AP3B2* in association with an EOEE and optic atrophy. The individuals described here have no immune deficiency, hematological disorder, or oculocutaneous albinism.[23] Strikingly, individuals with autosomal-recessive variations of *AP3D1* present with a phenotypic spectrum overlapping both *AP3B1*- and *AP3B2*-related disorders.[23] These human disorders are consistent with the phenotypes of each natural knockout mouse strain for *Ap3b1* (pearl), *Ap3b2*, and *Ap3d1* (mocha).[24–26]

Determining the precise ophthalmological phenotype associated with this disorder will require additional reports of individuals with autosomal-recessive variants in *AP3B2* and an EOEE. In the literature, EOEE was previously associated with optic atrophy in several disorders such as PEHO syndrome (progressive encephalopathy with edema, hypsarrhythmia, and optic atrophy [MIM: 260565]), mitochondrial disorders, and more recently identified genetic diseases with EOEE (EIEE3 [MIM: 609304], caused by mutations in *SLC25A22* [MIM: 609302]; EIEE36 [MIM: 300884], caused by mutations in *ALG13* [MIM: 300776]; and EIEE28 [MIM: 616211], caused by mutations in *WWOX* [MIM: 605131]). The phenotype of the individuals reported here partially overlaps features of PEHO syndrome, including an early-onset and progressive encephalopathy with a hypsarrhythmia pattern on EEG, hypotonia, developmental regression, edema of the extremities, optic atrophy, and facial dysmorphism. Recently, a PEHO-like syndrome was associated with autosomal-recessive variations of *CCDC88A* in three affected siblings.[27]

By combining pan-genomic sequencing, targeted sequencing of *AP3B2*, and international data sharing, we have identified in eight unrelated families 12 individuals who carry autosomal-recessive variants in *AP3B2* and present with EOEE and severe global developmental delay, poor eye contact with optic atrophy, and postnatal microcephaly. Consistent with tissue expression and animal models for the AP-3 subunits, individuals with autosomal-recessive variations of *AP3D1* present with a phenotypic spectrum overlapping both *AP3B1*- and *AP3B2*-related disorders.

## Supplemental Data

Supplemental Data include a Supplemental Note, two figures, and one table and can be found with this article online at http://dx.doi.org/10.1016/j.ajhg.2016.10.009.

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## Web Resources

Centre de Calcul de l'Université de Bourgogne, https://haydn2005.u-bourgogne.fr/dsi-ccub/
dbSNP, http://www.ncbi.nlm.nih.gov/projects/SNP/
ExAC Browser, http://exac.broadinstitute.org/
OMIM, http://www.omim.org
RefSeq, http://www.ncbi.nlm.nih.gov/RefSeq
UCSC Genome Browser, http://genome.ucsc.edu

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