REPORT

Mutations in *MBOAT7*, Encoding Lysophosphatidylinositol Acyltransferase I, Lead to Intellectual Disability Accompanied by Epilepsy and Autistic Features

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The risk of epilepsy among individuals with intellectual disability (ID) is approximately ten times that of the general population. From a cohort of >5,000 families affected by neurodevelopmental disorders, we identified six consanguineous families harboring homozygous inactivating variants in *MBOAT7*, encoding lysophosphatidylinositol acyltransferase (LPIAT1). Subjects presented with ID frequently accompanied by epilepsy and autistic features. LPIAT1 is a membrane-bound phospholipid-remodeling enzyme that transfers arachidonic acid (AA) to lysophosphatidylinositol to produce AA-containing phosphatidylinositol. This study suggests a role for AA-containing phosphatidylinositols in the development of ID accompanied by epilepsy and autistic features.

Intellectual disability (ID) is a common neurodevelopmental disorder affecting 1 in 100 children.[1,2] The more severe forms of ID or those with additional signs or symptoms are less common and have a prevalence of roughly 1 in 200.[3,4] Diagnosis of ID is based on the impairment of general mental abilities and activities of daily living.[2] In early childhood, the diagnosis of ID is based on global developmental delays affecting speech, motor, and cognitive function in combination with an IQ below 70.[2] It has previously been reported that rare de novo or recessive mutations play a major role in severe ID.[5,6] Interestingly, more complex forms of inheritance are thought to be involved in milder cases.[2,7,8] Although ID has a strong genetic influence, the involvement of non-genetic factors, such as infections, perinatal asphyxia, or environmental exposures, might play a role in the development of other forms.[9] To date, over 1,100 genes have been either confirmed or suggested in ID etiology, yet half of ID cases still remain undiagnosed.[4,10–12]

Many individuals with ID also present with other neurological conditions, such as epilepsy[13] and autism spectrum disorder (ASD),[14] which also have a strong genetic influence. The prevalence of epilepsy is ten times higher in individuals with ID than in the general population.[13] As in ID, de novo, recessive, and dominant variants in ASD can contribute to risk; however, a genetic diagnosis can be determined only in a relatively small portion of individuals.[15] ASD is characterized by repetitive behavior and varying degrees of impairment of social interaction and communication skills.[16] Many ASD-affected individuals show evidence of heritability.[17] Genetic evidence suggests the involvement of 200–1,000 genes, including both autosomal-recessive (AR) and de novo variants, in ASD susceptibility.[18–20]

In an effort to expand our understanding of the genetic composition of neurodevelopmental disorders with AR inheritance, several centers in the US, Canada, and Germany, in cooperation with colleagues from Egypt, Pakistan, and Jordan, joined efforts to examine and recruit a large number of consanguineous families with affected children. Analyses were performed in accordance with the ethical standards of institutional review boards, and informed consent was obtained for each individual participating in this study. Exome sequencing of our database consisting of >5,000 families with neurodevelopmental disease identified three families affected by biallelic, possibly pathogenic variants in membrane-bound O-acyltransferase family member 7 (*MBOAT7* [MIM: 606048]). These three families presented with overlapping clinical signs, including ID frequently acting with epilepsy (7/8 subjects) and autistic features (7/8 subjects). On the basis of exome findings, three additional families were identified from parallel international sequencing efforts through the sharing of gene names among collaborators.

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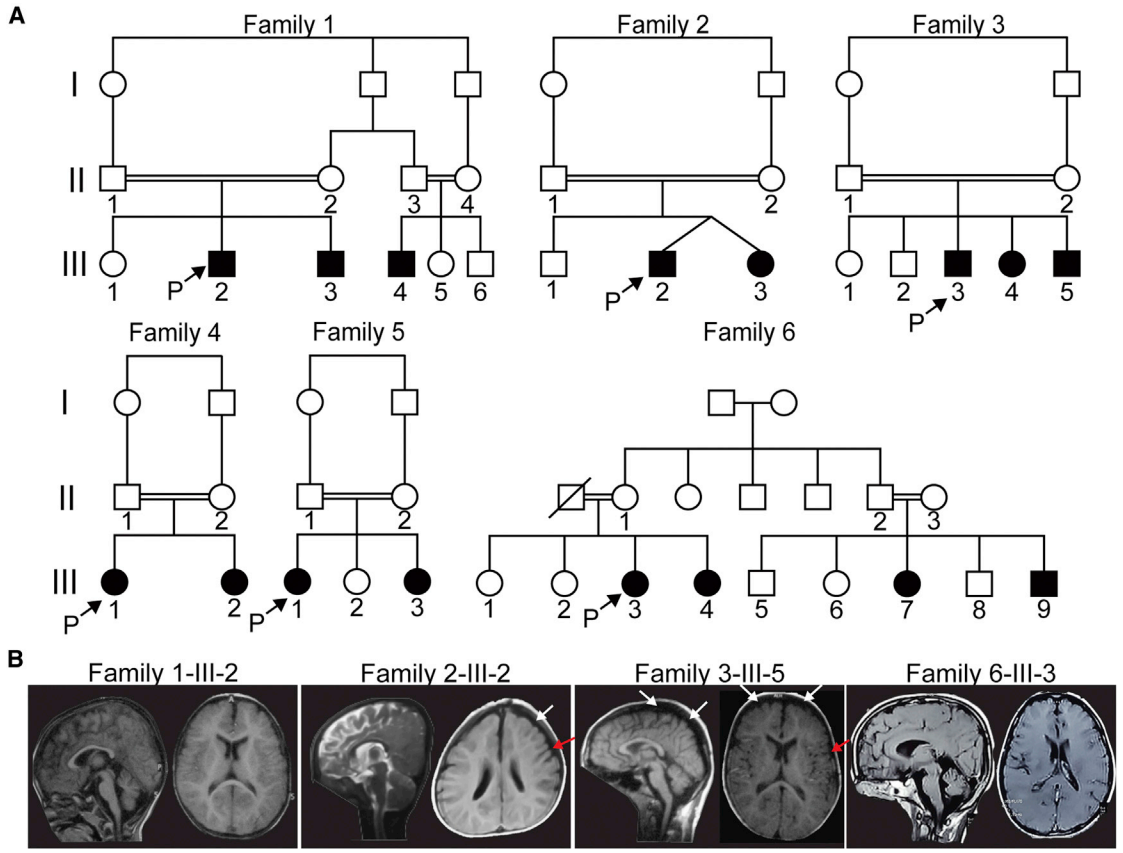

Figure 1. Consanguineous Families with Variants in *MBOAT7*, Encoding LPIAT1
(A) Pedigrees of families 1 to 6 show consanguineous marriages (double bars) with a total of 16 affected children. Probands are indicated with "P."
(B) Brain MRI for one affected individual from each of families 1–3 and 6. White arrows show cortical atrophy, and red arrows show possible polymicrogyria.

All 16 subjects were born to consanguineous parents (Figure 1A, Table S1, and Supplemental Note). The emerging clinical picture is one of moderate to severe ID given that the majority of subjects are not able to build sentences (14/16) and are non-verbal with delayed motor milestones (9/16). A few of the children (3/16) have never achieved the ability to walk, and the remaining 13 started to walk between the ages of 2 and 7 years. In 6/16 individuals, these clinical signs co-occur with infant-onset epilepsy (mostly focal and multifocal) that has been responsive to antiepileptic drugs. A further two individuals have seizures that began at 1.5 and 2.5 years, whereas another two have febrile seizures. Neurological examination showed that all children have truncal hypotonia and appendicular hypertonia. All subjects have a below-average head size, which is −2 to −3 SDs below the mean in 3/16 affected children, suggesting that microcephaly is not a consistent feature of *MBOAT7* variants. ASD was documented in only 7/16 children according to the Childhood Autism Rating Scale, and a further three showed clinical autistic features. Brain imaging was within normal limits, except in two subjects, in whom cortical atrophy was present (Figure 1B). There was some evidence of mild polymicrogyria.

Whole-exome sequencing identified a total of five distinct variants in *MBOAT7* (GenBank: NM_024298.3) from six families (Table S2). All variants were prioritized by allele frequency, conservation, blocks of homozygosity, and predicted effect on protein function. All variants were confirmed by Sanger sequencing and segregated with the disease as predicted for a fully penetrant recessive trait within all six families. Family 1, from Egypt, carries a homozygous frameshift deletion (c.126_145del [p.Leu43Hisfs*69]) in exon 3. Families 2 and 3, from Pakistan, harbor an in-frame deletion in exon 6 (c.758_778del [p.Gln253_Ala259del]). Using actual and inferred sequence data, we estimated the coalescence time of the shared founder mutation for families 2 and 3 to be 9.185 generations (SD ± 4.45 generations), or ~230 years with a generation time of 25 years. Sequencing data from family 4, from Jordan, revealed a homozygous deletion (c.423delG [p.Leu142Cysfs*8]) in exon 5. Family 5, from Iraq, carries a biallelic substitution (c.854+1G>C [p.?]) occurring at the canonical splice donor of exon 6. Family 6, from Pakistan, carries a 7 bp frameshift deletion (c.820_826del [p.Gly274Profs*47]) in exon 6. These variants were not found in dbSNP, the Greater Middle East (GME) Variome, the Exome Aggregation Consortium

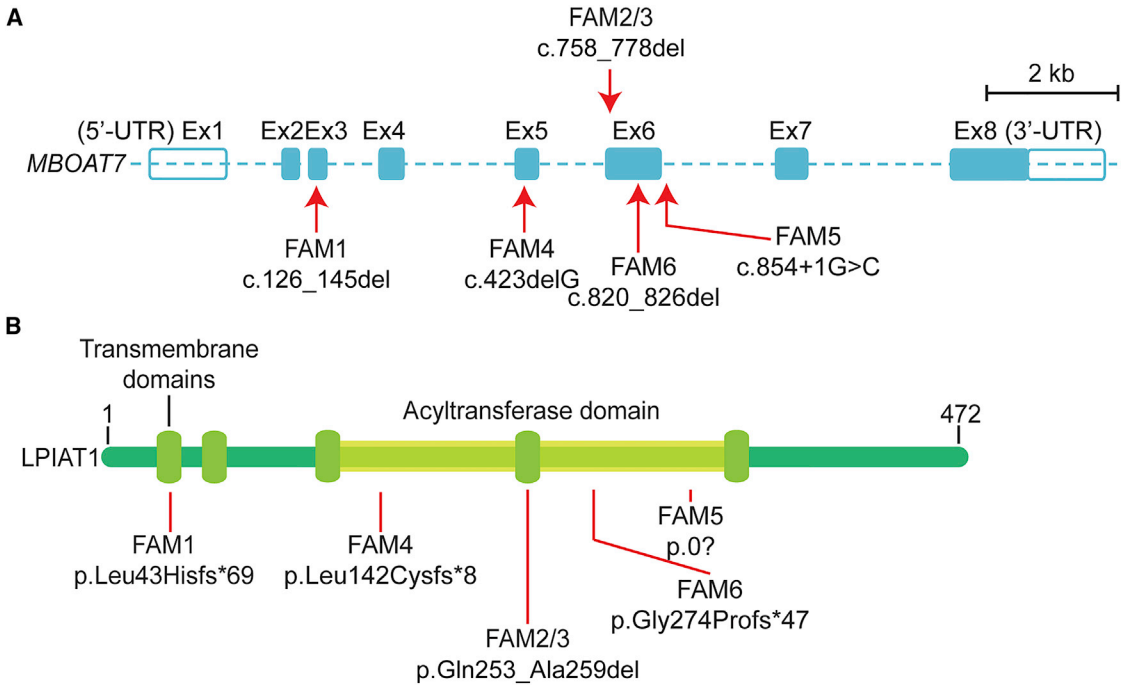

Figure 2. Location of Variants and Domains in *MBOAT7*, Encoding LPIAT1
(A) Genetic structure of *MBOAT7*. Mutations are indicated by red arrows (exons and numbers as in GenBank: NM_024298.3). In-solution exome capture was performed with the SureSelect Human All Exome 50 Mb Kit (Agilent Technologies) with 125 bp paired-end read sequences generated on a HiSeq2000 or HiSeq2500 (Illumina). Scale bar represents 2 kb.
(B) Structure of LPIAT1, which harbors five transmembrane domains and one catalytic acyltransferase domain. Variants are indicated with red lines. Amino acid numbers are provided above.

(ExAC) Browser, or 1000 Genomes and were also not present in our in-house whole-exome database (>5,000 subjects with neurodevelopmental conditions). The ExAC Browser includes over 8,000 South Asian control individuals, almost all Pakistani, from the Pakistan Risk of Myocardial Infarction study. Thus, these disease-related alleles are very rare even in ethnically similar control individuals.

MBOAT7 encodes lysophosphatidylinositol acyltransferase 1 (LPIAT1), which is a member of the MBOAT family of acyltransferases and originates from yeast Ale1p (Figure S1A).[21] The human MBOAT family has five members, each of which has a preference toward specific acyl donors and acceptors (Figure S1B).[21] LPIAT1 is the only family member that is known to primarily transfer arachidonic acid (AA) from arachidonoyl-CoA to lysophosphatidylinositol (Figure S1C),[22] suggesting an essential function. *MBOAT7* contains eight exons, resulting in four protein-coding transcripts, and three LPIAT1 isoforms. The five variants described in this study affect all protein-coding transcripts (Figure 2A), interfering with either transmembrane or catalytic domains of the protein (Figure 2B). Balanced translocation in *MBOAT1* in one subject has been linked to brachydactyly-syndactyly syndrome.[23] None of the other MBOAT genes have had genetic loss-of-function variants linked to human disease.

LPIAT1 contributes to the regulation of free AA in the cell through the remodeling of phospholipids.[24,25] Free cellular AA is under tight regulation, given that its pro-inflammatory metabolites could be harmful to cellular physiology.[26] Enzymes such as lipoxygenase (LOX) and cyclooxygenases (COX) metabolize AA into the pro-inflammatory eicosanoid lipids. The COX enzymes (1 and 2) are known targets of existing non-steroidal anti-inflammatory drugs, such as ibuprofen and aspirin.[27] There is compelling evidence linking pro-inflammatory processes to ASD, for instance, the activation of microglia and astrocytes and the overexpression of immune processes in the brains of individuals with ASD.[28–31]

A common variant in *TMC4* (rs641738), a gene adjacent to *MBOAT7*, is associated with a 20% increased risk of nonalcoholic fatty-liver disease in individuals of European descent. The variant is predicted to cause a substitution (p.Gly17Glu) early in TMC4.[32] Interestingly, this variant is just a few hundred base pairs downstream of the 3′ end of *MBOAT7*. Carriers of this allele who underwent liver biopsy were found to share reduced *MBOAT7* expression and altered phosphatidylinositol levels. None of our affected children or their parents showed evidence of clinically relevant liver disease, but no specific tests were performed. Despite a 49% carrier frequency for the minor allele in the GME Variome, none of our subjects are carriers. Therefore, the connection between this variant and the condition we describe remains uncertain.

In mice, *Mboat7* and its encoded protein, LPIAT1, are required for cortical lamination.[33] *Mboat7* knockout mice

are significantly smaller than their littermate controls and show reduced postnatal survival. In a recent study, histological analysis of embryonic day 18.5 *Mboat7*$^{-/-}$ brains showed a smaller cerebral cortex and hippocampus, abnormal cortical lamination, an increased number of apoptotic cells in the cortex, and dispersed MAP2^{+} subplate neurons.[33] The cerebral cortex showed evidence of gyral structures, whereas normally gyri are absent in the murine cortex, reminiscent of the polymicrogyria we observed in some subjects.

It is recognized that AA-containing phosphatidylinositol is a major lipid in the mammalian brain. It has been shown that *Mboat7* is required for cortical lamination is mice.[33] In this study, we have linked recessive mutations in *MBOAT7* with human neurodevelopmental disease, suggesting a critical role for AA-containing phosphatidylinositol in the developing human brain.

Supplemental Data

Supplemental Data include a Supplemental Note, one figure, and two tables and can be found with this article online at http://dx.doi.org/10.1016/j.ajhg.2016.07.019.

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Web Resources

1000 Genomes, http://www.1000genomes.org/
dbSNP, http://www.ncbi.nlm.nih.gov/SNP/
ExAC Browser, http://exac.broadinstitute.org/
GenBank, http://www.ncbi.nlm.nih.gov/genbank/
Genome Analysis Toolkit (GATK, version 2.2), http://www.broadinstitute.org/gatk/
Greater Middle Eastern (GME) Variome Project, http://igm.ucsd.edu/gme/
HGNC, http://www.genenames.org/
Mutalyzer, https://mutalyzer.nl/
NHLBI Exome Sequencing Project (ESP) Exome Variant Server, http://evs.gs.washington.edu/EVS/
OMIM, http://www.omim.org
PolyPhen-2, http://genetics.bwh.harvard.edu/pph2/
Primer3, http://biotools.umassmed.edu/bioapps/primer3
SeattleSeq (version 134), http://snp.gs.washington.edu/SeattleSeqAnnotation134/

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