GENOMICS

Identification of novel loss of function variants in *MBOAT7* resulting in intellectual disability

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ABSTRACT

The membrane bound O-acyltransferase domain-containing 7 (*MBOAT7*) gene codes for an enzyme involved in regulating arachidonic acid incorporation in lysophosphatidylinositol. Patients with homozygous nonsense mutations in *MBOAT7* have intellectual disability (ID) accompanied with seizure and autism. Accumulating evidences obtained from human genetic studies have shown that *MBOAT7* is also involved in fatty liver disease. Here we identified two novel homozygous variants in *MBOAT7*, NM_024298.5: c.1062C > A; p.(Tyr354*) and c.1135del; p.(Leu379Trpfs*9), in two unrelated Iranian families by means of whole exome sequencing. Sanger sequencing was performed to confirm the identified variants and also to investigate whether they co-segregate with the patients' phenotypes. To understand the functional consequences of these changes, we overexpressed recombinant wild type *MBOAT7* and mutants *in vitro* and showed these mutations resulted in abolished protein synthesis and expression, indicating a complete loss of function. Albeit, we did not trace any liver diseases in our patients, but presence of globus pallidus signal changes in Magnetic Resonance Images might be indicative of metabolic changes as a result of loss of *MBOAT7* expression in hepatic cells. These signal changes could also help as an important marker of *MBOAT7* deficiency while analyzing the genomic data of patients with similar phenotypes.

1. Introduction

Mammalian Membrane bound O-acyltransferase domain-containing (MBOAT) superfamily (MBOAT1, MBOAT2, MBOAT4, MBOAT5, MBOAT7) has been evolved from their *Ale1p* (acyltransferase for lyso-PtdEtn) orthologue in yeast [1]. These proteins act through transferring acyls from specific donors to their unique acceptors. This phospholipid remolding pathway, called the Lands` cycle, regulates the asymmetry of glycerphospholipids and the properties of cellular membranes [2,3]. Among the members of this family, MBOAT5 and MBOAT7 share more sequence homology and have similar affinity for Arachidonoyl-CoA as acyl donor [1,4]. Arachidonic acid is of paramount importance as it is a precursor for thromboxanes, leukotrienes and prostaglandins (collectively termed eicosanoids), molecules involved in inflammation pathways. Thus, these enzymes control the amount of arachidonic acid pool within inflammation pathways [4,5].

MBOAT7 gene is located on chromosome 19 and encodes for a 472 amino acids-long enzyme with six transmembrane domains attached to endomembranes of cells [6]. This O-acyltransferase, also named as Lysophosphatidylinositol acyltransferase 1 (LPIAT1), was the first enzyme discovered using arachidonyl-CoA as acyl donor to convert lyso-phosphatidylinositol into phosphatidylinositol, and therefore regulating the content of arachidonic acid in cells [7].

MBOAT7 is involved in mammalian brain development. Mice possessing deficiency in *MBOAT7* have been shown to present disordered cortical lamination and delayed neuronal migration [7]. Regard to

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human pathophysiology, individuals harboring mutations in *MBOAT7* represented a wide range of clinical features, including intellectual disability (ID), epilepsy and autistic features, which demonstrates the importance of inflammation pathways in brain function [1,8–11]. Recently, the role of the MBOAT7 protein has been posited in liver homeostasis due to the association of a common genetic variant (*rs641738*) with increaseed risk of fatty liver disease (FLD) and alcoholic liver cirrhosis [12,13]. Liver specific depletion of *Mboat7* results in a primary increase in liver fat content due to higher triglyceride synthesis fueled by a non canonical pathway, namely elevated phosphatidyl inositol turnover [14,15].

In the current study, we report two novel nonsense variants in *MBOAT7* gene in two Iranian families by means of Whole Exome Sequencing (WES) resulting in a complete loss of function of the MBOAT7 protein.

2. Materials and methods

2.1. Ethical considerations

This research was performed in accordance with approval of the local medical ethics committee of Tarbiat Modares University, Tehran, Iran (Ethical approval code: IR.MODARES.REC.1398.166). Parents of both enrolled families provided the informed, written consent prior to initiating research and data collection. Participants were also informed that all derived data would be used only for scientific not for commercial purposes.

2.2. Genetic analysis

Baiting the exonic regions and flanking exon-intron boundary regions of the genome was performed according to the Agilent's Sure Select Human All Exon V6 kit (Agilent, SantaClara, CA, USA), using > 250 ng of genomic DNA extracted from blood of the patients. Paired-end sequencing was performed on generated library utilizing Illumina's HiSeq4000 instrument in accordance to the manufacturer's protocols (Illumina Inc., USA). The Raw sequences were mapped to the human genome reference (NCBI build37/hg19 version) with BWA-MEM after excluding possible artifacts and low quality reads. SNP and INDEL variants were jointly called through GATK 4.1 (http://www.broadinstitute.org/gatk/). Variants prioritization applied according to custom scheme and after annotating the variants using Annovar [16]. Then, opting variants with less than 1% Minor allele frequency was done using available public databases, including dbSNP132 (https://www.ncbi.nlm.nih.gov/projects/SNP), 1000 Genomes Project (http://www.1000genomes.org), Exome Sequencing Project (ESP) (http://evs.gs.washington.edu/EVS), Exome Aggregation Consortium (ExAC) database and Iranome (http://www.iranome.ir/). Assuming that intragenic, UTRs regions, deep intronic and synonymous variants could not be linked to observed phenotypes, they were excluded from later analysis. Remained variants were ranked according to pathogenicity evaluator tools (FATHMM: http://fathmm.biocompute.org.uk, MutationTaster: http://www.mutationtaster.org, SIFT: https://sift.bii.a-star.edu.sg) and plausible pathogenic variants were checked out in HGMD (http://www.hgmd.cf.ac.uk) and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar) for their previous clinical reports.

To validate or eliminate suspected variants, bidirectional Sanger sequencing and segregation of the variants inspected into the members of the families was carried out. Primer sequences could be found in Supplementary table S2.

2.3. Generation of human MBOAT7 mutant constructs

The *MBOAT7* wild type (WT), *MBOAT7 p.Tyr354*,* and *MBOAT7 p.Leu379Trpfs*9* cDNAs were synthesized and cloned into pcDNA3.1 having a V5 epitope at the C-terminus, by GeneArt Synthesis (Thermo

Fisher Scientific, Rockfors, IL, USA). The lysophospholipid acyltransferase 7 isoform 1 [*Homo sapiens*] (NCBI Reference Sequence: NP_077274.3) was used for the synthesis of all the MBOAT7 forms.

2.4. Expression of MBOAT7 constructs

HepG2 cells were purchased from American Tissue Culture Collection (Manassas, VA, USA). HepG2 cells were cultured in Minimum Essential Medium Eagle (MEM) containing 10% Fetal Bovine Serum (FBS) and 5% penicillin-streptomycin (GE Healthcare Life Sciences, Pittsburgh, PA, USA). Cells were transfected with plasmids containing the *MBOAT7 WT*, *MBOAT7 p.Tyr354*,* or *MBOAT7 p.Leu379Trpfs*9* plasmid, using the Lipofectamine3000 transfection reagent (Thermo Fisher Scientific), according to the manufacturer's instructions.

2.5. Western blotting analysis

After 48 h, HepG2 cells were collected and proteins were extracted. Protein samples were mixed with Laemmli buffer containing 2-mercaptoethanol, and boiled at 95 °C for 5 min. Proteins were size-separated by SDS-PAGE and transferred onto nitrocellulose membranes. Membranes were incubated with anti-V5 (Invitrogen, cat. #P/N46-0705, Carlsbad, CA, USA), or anti-calnexin (Sigma-Aldrich, cat. #C4731, St. Louis, MO, USA), washed with 0.2% TBS-T twice, and incubated with anti-mouse (GE-Healthcare, cat. #NA931V), or anti-rabbit (GE-Healthcare, cat. #NA934V). Membranes were washed with 0.2% TBS-T twice. Membranes were incubated for 5 min with chemiluminescent HRP substrate (Millipore Corporation, Billerica, MA), bands were visualized by Chemidoc XRS System (Bio-Rad Laboratories, Hercules, CA, USA) and quantified using Image Lab Software (Bio-Rad).

2.6. Gene expression analysis by qPCR

After 48 h, HepG2 were collected, RNA was extracted by RNeasy Plus kit (Qiagen, Hilden, Germany), and retro-transcribed by high-capacity cDNA reverse transcription kit (ThermoFisher Scientific). *MBOAT7* gene expression was detected using TaqMan probes (*MBOAT7* cat. #hs00383302, *β-actin* cat. #hs01060665, Thermo Fisher Scientific) by real-time qPCR (Bio-Rad). *MBOAT7* gene expression levels were compared to *β-actin* gene expression level, and all were related to the one with the highest expression.

3. Results

3.1. Identification of MBOAT7 variants

Whole exome sequencing (WES) was performed on one affected proband per family. Same procedure for analyzing raw data was used for each generated data from sequencing machine. Potentially pathogenic variants were found across untranslated regions, flanking ± 20 intronic bases, splice junctions and protein-encoding exons. In-depth data analysis using a custom scheme (Fig. 1A) revealed two variants within exon 8 of *MBOAT7* in two under study families (NM_024298.5:c.1135del and c.1062C > A) (Table 1). We also detected a reported homozygous variant in *CYP1B1* (c.1169G > A:p.(Arg390His)) patient A1 as a cause of congenital glaucoma which was heterozygous in his sibiling (no presentation of ophatamological issue) [17]. Sanger sequencing validated the results of WES and variants cosegregated in all family members (Fig. 1B).

3.2. Clinical features of individuals with MBOAT7 variants

In the following study, three patients and their unaffected parents from two consanguineous pedigrees were clinically evaluated (Table 2). A detailed summary of clinical characterization of our cases and
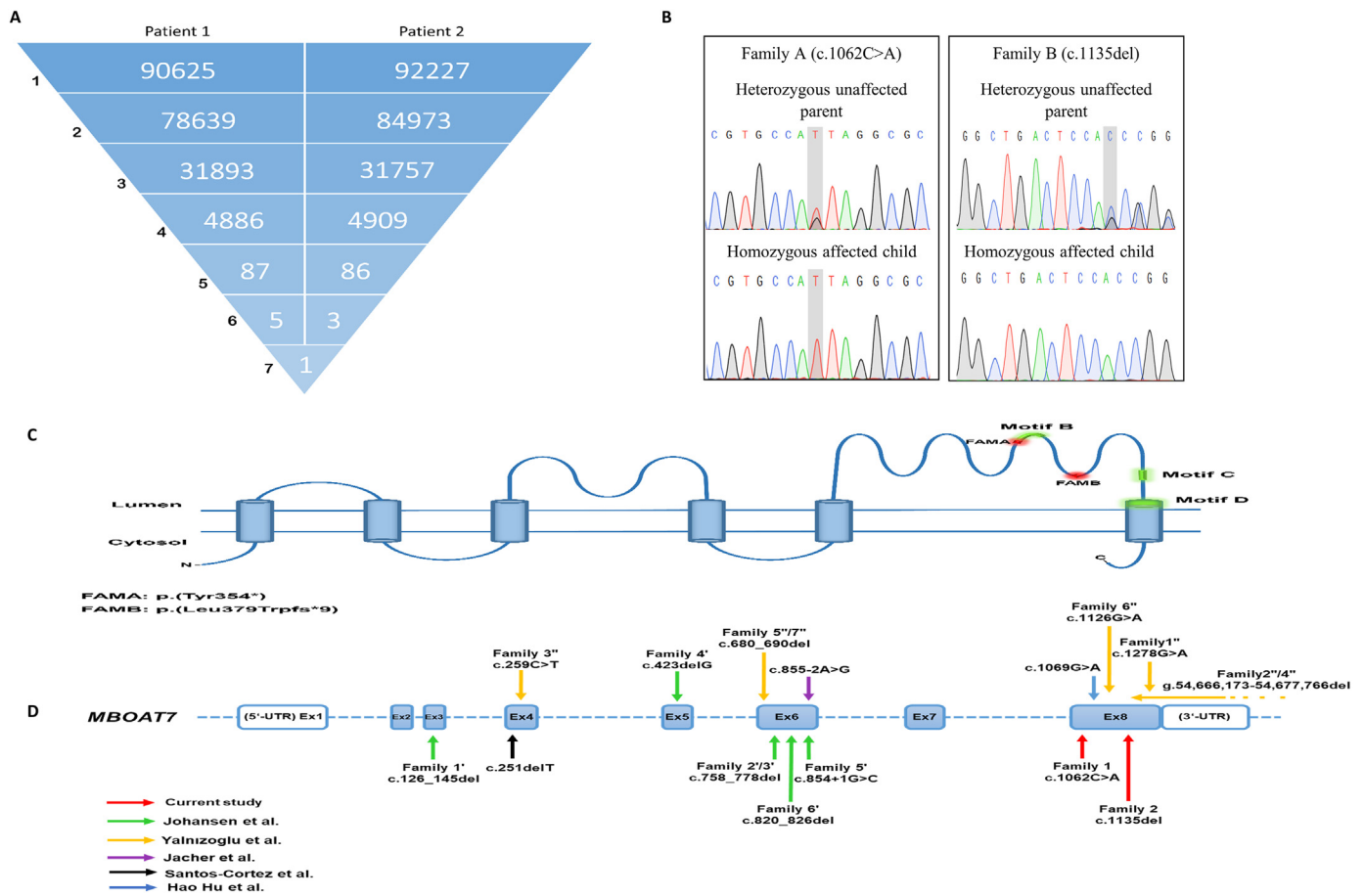
Fig. 1. (A) Filtering process applied on annotated variants, led to identification of *MBOAT7* variants in both patients: 1. Total variants 2. Variants after base-quality filtering 3. Homozygous or Heterozygous variants 4. Non-synonymous/indel/splice site variants 5. Low frequent variants (ESP/1000GP/GnomeAD) 6. Genes with plausible disease association 7. *MBOAT7* variant confirmed by Sanger sequencing (B) Exemplary results of Sanger sequencing of *MBOAT7* exon eight in affected individuals and their carrier parents. (C) Schematic illustration of MBOAT7 protein structure, highly conserved motifs (shown in green) and location of identified mutations (shown in red) represented in protein. (D) Localizations of the mutations previously reported and described in this study (indicated by red arrow). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

previously reported patients harboring *MBOAT7* mutation is provided in Supplementary table S1. In all affected individuals, the age of onset was almost the same. As expected, in all three affected individuals the prominent manifestations were ID and seizure, without any prominent minor abnormalities.

First two affected patients were siblings (brother and sister) born to healthy consanguineous parents. They were born *via* cesarean section without any pri/post-natal insults with good apgar score and normal birth weight and head circumference.

The older offspring was 18.5 years old boy (1A) who was referred to our clinic with hypotonia, ID, seizure, and ophthalmic problem. Since birth, he has shown delay in reaching neurodevelopmental milestones,

as he just gained the ability to walk independently yet clumsy in his 7 years old with occupational therapy help, could speak only a few words. His seizures started around nine months old, presenting itself as focal motor seizures without an altered level of consciousness. Antiepileptic drugs (phenobarbital and sodium valproates), have been used mostly to control of seizures over years.Also, he had congenital bilateral corneal opacity since birth and developed optic atrophy and complete blindness in the later years, which has made him entirely dependent.

Brain MRI without contrast at age of one year old showed increased extraaxial fluid, mild supratentorial atrophy with mild thinning of corpous collosum without any significant white matter signal changes

Table 1
MBOAT7 variants identified in both families and evaluation of their pathogenicity using online prediction tools.

Patient	Variant	Gene/genomic location	Allele Frequencies	Effect
1A & 2A (family A)	NM_024298.5:c.1062C > A p.(Tyr354*)	MBOAT7: g.54678095G > T	gnomAD: NR 1000 Genome: NR ESP: NR Iranome: NR	Mutation Taster: Disease causing FATHMM-MKL: Damaging
1B (family B)	NM_024298.5:c.1135del p.(Leu379Trpfs*9)	MBOAT7: g.54678022del	gnomAD: NR 1000 Genome: NR ESP: NR Iranome: NR	Mutation Taster: N/A FATHMM-MKL: N/A

Abbreviations: Het, Heterozygote; Hom, Homozygote; N.R, Not Reported. N/A, Not Available.

Table 2
Clinical signs and laboratory test findings in the three patients with *MBOAT7* variants.

Patients		1A	2A	1B
Gender		M	F	M
Age at last examination		18.6 yr	8.2 yr	3 yr
Cognitive delay/ Intellectual disability		+	+	+
Speech and motor delay /impairment		A few words	A few words	A few words
Age of walking (y)		7	3	2
Age at first seizure (onset/type)		4 m/focal	3 m/focal,myoclonic	6 m/clonic
Autistic behaviors		+	+	−
Aggressive behaviors		−	−	−
Hyperactivity		−	+	+
Microcephally/macrocephally		−	−	−
Appendicular hypertonicity		−	−	−
Axial hypotonicity		+	+	+
Strabismus		−	−	+
Metabolic profile	ALT (Nl: < 39 U/l)	32	15	15
	AST (Nl: < 37 U/l)	26	21	36
	Triglyceride (mg/dl)	88	52	65
	Total cholesterol (mg/dl)	128	147	98
	Alkaline phosphatase (142–335 U/l)	185	202	352
MRI findings		Bilateral symmetric globous pallidous signal changes and mild supratentorial atrophy, thining of corpous collosum	Brain atrophy, globous pallidous (GP) abnormal signal changes in T2-W images	Globus pallidi signal changes and dentates nuclei hyperintensity

Abbreviations: ALT, Alanine transaminase; AST, Aspartate transaminase.
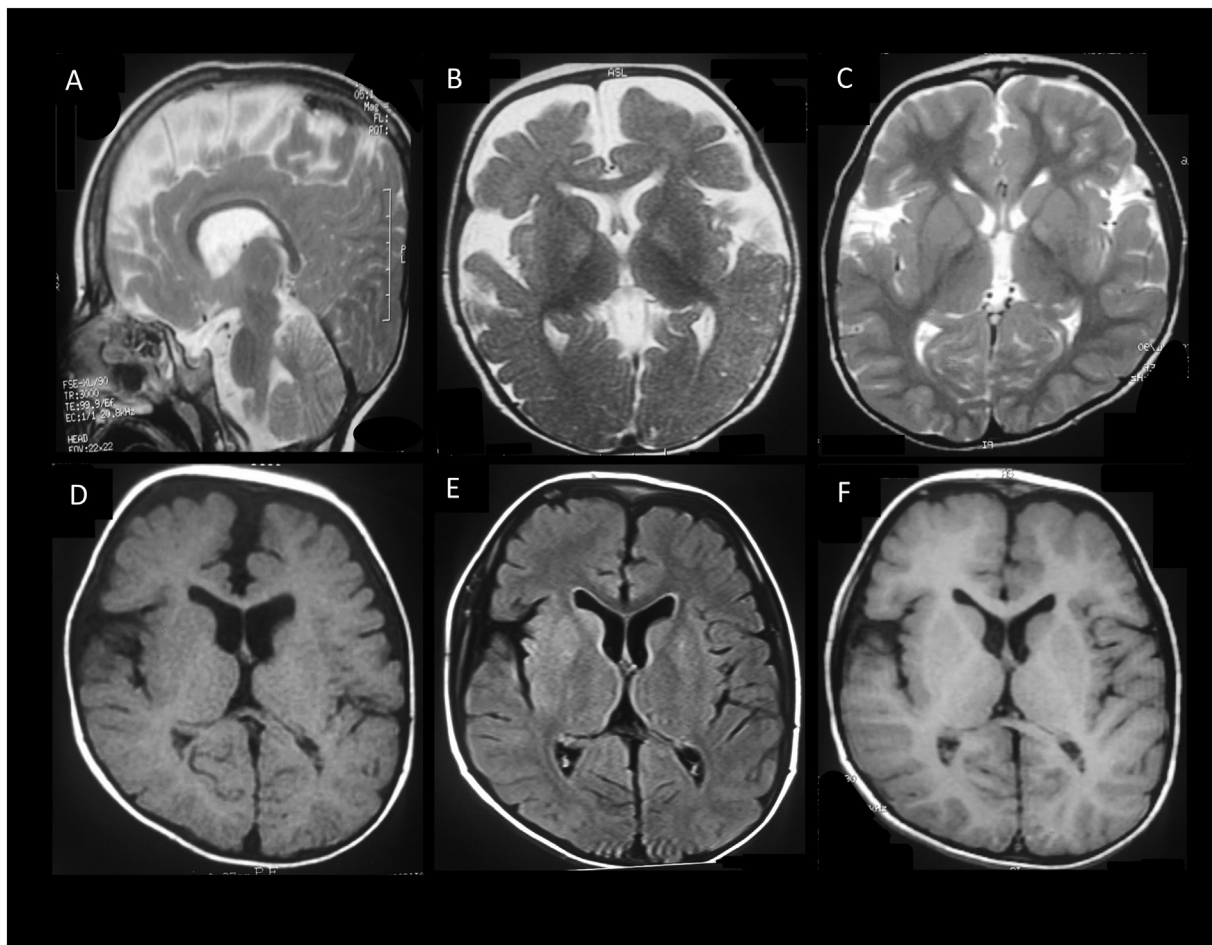

Fig. 2. Serial brain MRI images of patients 1-A at age 1 year and 1-B at age 5 years including T1-W, T2-W and FLAIR sequences shows mild supratentorial atrophy and increased extra-axial fluids in frontal area (A,B,D,F) and bilateral symmetric Globous Pallidus signal changes in T2-W and FLAIR sequences images (C,E).

according to his chronological age (Fig. A,B). The second brain MRI at age of 5 years revealed fine bilateral symmetric globous pallidous (GP) abnormal signal changes in T2-W sequences images in addition of unchanged previous findings (Fig. 2 C,D-compare T1-W and T2-W images). The third brain MRI with and without contrast at age of eleven years remained unchanged compare to the second brain MRI.

The younger sister (2A) was eight years old at the time of the last clinical examination and, unlike his brother, she was otherwise normal until her first seizure in her 3-month-old. She gained head control and social smile at that age. She could walk normally in her 3-year-old with occupational therapy assistance and show a higher level of cognition compared to her brother. However, she could only be able to create just one or two words. Although her seizure frequency was lower than his brother, she experienced both focal and myoclonic seizures. She has been seizure-free since her 4-year-old with phenobarbital maintenance mono-therapy. Regarding behavior, she is hyperactive, has low concentration and some stereotypical behaviors. The findings of first brain MRI at age of 12 months were very similar to her brother, including bilateral symmetric globous pallidous signal changes and mild supratentorial atrophy in association with thinning of corpous callosum (Fig. 2 E,F- FLAIR and T1-W images). The second brain MRI at age of 2 years confirmed the above mentioned findings without any progression in GP signal change intensity or brain atrophy. Spiral brain CT-Scan of this patient at age of 6 years did not show calcification in GP or other anatomic areas of the brain.

The third patient was a 3-year-old boy (1B) from different consanguineous parents with normal development and growth indices until his six-month-old when he had experienced a developmental regression episode following colonic seizure episodes. He developed intermittent strabismus, dystonic posturing in hands and lost his head control ability, which had gradually improved during the following several months. With regular occupational therapy, he obtained the ability to walk in his two-year-old as in his last follow up session, could walk clumsily, only speak one or two words, yet, his cognition was better. His neuropsychological assessment showed some degree of ID and hyperactivity without any particular autistic or self-injurious behavior. Since his nine-month-old with monotherapy with phenobarbital, he has been seizure-free. His brain MRIs demonstrated diffuse cereberal atrophy and hyperintensity in globus pallidus and dentate nucleus of the cerebellumin T2-W images. In the following longitudinal MRIs, the brain atrophy worsened while the GP and dentate hyperintensity seemed to be fixed over time (MRI is not shown). Paraclinical studies in all our three patients, including all basic metabolic screening tests and EDx EMG-NCV examinations, were in the normal range and liver sonography showed no sign of fatty liver or other liver malfunction.

3.3. Expression and functional analysis

To test whether the *MBOAT7 p.Tyr354** and *MBOAT7 p.Leu379Trpfs*9* mutations cause a reduction in the synthesis of the protein in human hepatocytes, we transiently transfected HepG2 cells with a plasmid containing the *MBOAT7 wild type*, the *MBOAT7 p.Tyr354**, or the *MBOAT7 p.Leu379Trpfs*9*. Cells transfected with the *MBOAT7 p.Tyr354** or *MBOAT7 p.Leu379Trpfs*9* mutants showed a complete absence of protein synthesis compared to cells transfected with the *MBOAT7* wild type plasmid (Fig. 3B).

To investigate whether the *MBOAT7 p.Tyr354** and *MBOAT7 p.Leu379Trpfs*9* mutations lead to a reduction in the *MBOAT7* gene expression, HepG2 cells were transiently transfected with a plasmid containing the *MBOAT7 wild type*, the *MBOAT7 p.Tyr354**, or the *MBOAT7 p.Leu379Trpfs*9*. Cells transfected with the *MBOAT7 p.Tyr354** or *MBOAT7 p.Leu379Trpfs*9* mutants showed a strong mRNA downregulation compared to cells transfected with the *MBOAT7 wild type* plasmid (Fig. 3A).

4. Discussion

In the current study, we found two novel mutations in *MBOAT7* gene as a cause of intellectual disability. We also detected a previously reported mutation in *CYP1B1* gene in patient 1A which eliminated the possibility of any relation of *MBOAT7* variants to congenital glaucoma. Therefore, the co-occurrence of two pathogenic variants in one patient is rare, but this observation underscores the importance of considering previously reported variants while narrowing down the candidate disease causing genes in the process of data analysis. Moreover, detection of signal changes of globus palidus in magnetic resonance images of all three patients of this study is of no coincidence and indicates that these alteration could be used as a marker of *MBOAT7* deficiency in the analysis of patients with similar phenotypes.

There are at least three putative motifs (B, C, D) previously described as functional sites for lysophosphatidylinositol acyltrasferase [18]. In the present study, we identified two stop codon variants at upstream of these motifs, leading to truncated proteins (Fig. 1C). The *c.1062C > A* variant resulted in a premature translation-termination codon (PTC) in position 354 of the encoded protein. This variant is juxtaposed with motif B, leading to the elimination of all three motifs of the MBOAT7 protein, including the His356 which has been reported as one of the two amino acids composing the putative catalytic dyads of this protein [6]. Hence, aforementioned variant will probably cause a massive mutilation in the protein structure. The other variant, *c.1135del*, alters the frame after Leu379 and results in the loss of motif C and D suggesting a potential loss of protein function.

Only one missense mutation has been described as a cause of disease among the 15 mutations identified (Fig. 1D). This observation is consistent with the idea that complete loss of function of MBOAT7 is needed to result in this disease. However, no functional analysis study were done to see to what extent these mutations affected the protein expression, synthesis and function. In our work, to understand the consequences of the newly identified mutations, we overexpressed *MBOAT7 wild type* and mutant proteins in HepG2 cells and showed an abolished synthesis. The reduction in the protein synthesis was driven by a robust reduction of *MBOAT7* mRNA expression from the mutant proteins, and it is probably attributable to nonsense mRNA decay. Nonsense-mediated mRNA decay (NMD) is an evolutionary conserved mechanism to protect cells from any detrimental effect of truncated proteins by eliminating mRNAs harboring nonsense mutations, [19].

Nonsense mutations in *MBOAT7* result in ID, epilepsy and autistic features [1]. Consistently, mice knock out for *Mboat7* have a very high mortality due to cerebral cortex and hippocampus atrophy, disordered cortical lamination and delayed neuronal migration in the cortex [20]. A common genetic variation, *MBOAT7* rs641738, was also shown associated with fatty liver disease, alcoholic liver disease and cancer [12,13,21]. Functional studies in mice with specific downregulation of the *Mboat7* in the liver showed normal cognitive function and higher fat in the liver [14,22]. Nevertheless, no specific sign of fatty liver disease was found in our study using liver sonography and liver circulating enzymes. This inconsistency can be due to several reasons: first, all patients in this study are in their early childhood and might manifest the liver disease later in life. Second, ultrasonography and liver function tests have low sensitivity in detecting fatty liver. Third, carriers of *MBOAT7* mutations had low adiposity and low body weight that might have camouflaged the absence of *MBOAT7* product in liver.

In summary, we report two novel genetic variants in *MBOAT7* gene resulting in a complete loss of function of MBOAT7 protein and causing intellectual disability in humans.

Availability of data and material

Human variant and pertinent phenotypes have been reported to ClinVar (Accession number: SCV001156245 & SCV001156236) and LOVD (Individual IDs: 00274323 & 00274324). The Whole exome
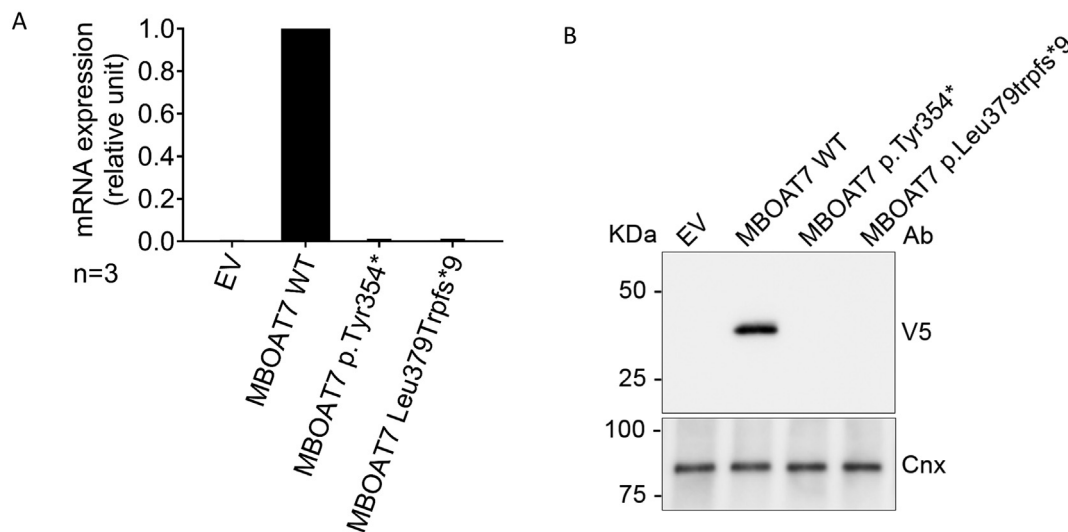
Fig. 3. HepG2 cells were transiently transfected with EV, MBOAT7 WT, MBOAT7 p.Tyr354*or MBOAT7 p.Leu379Trpfs*9 and were subjected to Western blotting and gene expression analysis by qPCR. (A) The expression of MBOAT7 with pathogenic variants went under the none sense mediated decay.(B) Protein expression of both variants decreased rigorously comparing to the wild type MBOAT7.

sequencing dataset used and analyzed in the current study is available from the corresponding author on reasonable request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygeno.2020.07.008.

References

[1] A. Johansen, R.O. Rosti, D. Musaev, E. Sticca, R. Harripaul, M. Zaki, ... T. Froukh, Mutations in MBOAT7, encoding lysophosphatidylinositol acyltransferase I, lead to intellectual disability accompanied by epilepsy and autistic features, Am. J. Hum. Genet. 99 (4) (2016) 912–916.

[2] W.E. Lands, Metabolism of glycerolipides: a comparison of lecithin and triglyceride synthesis, J. Biol. Chem. 231 (2) (1958) 883–888.

[3] H. Shindou, D. Hishikawa, T. Harayama, M. Eto, T. Shimizu, Generation of membrane diversity by lysophospholipid acyltransferases, J. Biochem. 154 (1) (2013) 21–28.

[4] W.R. Riekhof, J. Wu, M.A. Gijón, S. Zarini, R.C. Murphy, D.R. Voelker, Lysophosphatidylcholine metabolism in *Saccharomyces cerevisiae* the role of P-type ATPases in transport and a broad specificity acyltransferase in acylation, J. Biol. Chem. 282 (51) (2007) 36853–36861.

[5] S.P. Khanapure, D.S. Garvey, D.R. Janero, L. Gordon Letts, Eicosanoids in inflammation: biosynthesis, pharmacology, and therapeutic frontiers, Curr. Top. Med. Chem. 7 (3) (2007) 311–340.

[6] A. Caddeo, O. Jamialahmadi, G. Solinas, A. Pujia, R.M. Mancina, P. Pingitore, S.J. Romeo, MBOAT7 is anchored to endomembranes by six transmembrane domains, J. Struct. Biol. 206 (3) (2019) 349–360.

[7] H.-C. Lee, T. Inoue, J. Sasaki, T. Kubo, S. Matsuda, Y. Nakasaki, ... N Kono, LPIAT1 regulates arachidonic acid content in phosphatidylinositol and is required for cortical lamination in mice, Mol. Biol. Cell 23 (24) (2012) 4689–4700.

[8] H. Hu, K. Kahrizi, L. Musante, Z. Fattahi, R. Herwig, M. Hosseini, ... F. Larti, Genetics of intellectual disability in consanguineous families, Mol. Psychiatry 24 (7) (2019) 1027–1039.

[9] J.E. Jacher, N. Roy, M. Ghaziuddin, J. Innis, Expanding the Phenotypic Spectrum of MBOAT7-related Intellectual Disability, (2019).

[10] S. Khan, L.E. Rawlins, G.V. Harlalka, M. Umair, A. Ullah, S. Shahzad, ... W.J.B.M.G. Ahmad, Homozygous variants in the HEXB and MBOAT7 genes underlie neurological diseases in consanguineous families, BMC Med. Genet. 20 (1) (2019) 199.

[11] D. Yalnızoğlu, R.K. Özgül, K.K. Oğuz, B. Özer, D. Yücel-Yılmaz, B. Gürbüz, ... A. Dursun, Expanding the phenotype of phospholipid remodelling disease due to MBOAT7 gene defect, 42 (2) (2019) 381–388.

[12] S. Buch, F. Stickel, E. Trépo, M. Way, A. Herrmann, H.D. Nischalke, ... M. Ridinger, A genome-wide association study confirms PNPLA3 and identifies TM6SF2 and MBOAT7 as risk loci for alcohol-related cirrhosis, Comp. Stud. *47* (12) (2015) 1443.

[13] R.M. Mancina, P. Dongiovanni, S. Petta, P. Pingitore, M. Meroni, R. Rametta, ... O. Wiklund, The MBOAT7-TMC4 variant rs641738 increases risk of nonalcoholic fatty liver disease in individuals of European descent, Gastroenterology 150 (5) (2016) 1219–1230. e1216.

[14] M. Meroni, P. Dongiovanni, M. Longo, F. Carli, G. Baselli, R. Rametta, ... M. Gaggini, Mboat7 down-regulation by hyper-insulinemia induces fat accumulation in hepatocytes, Ebiomedicine 52 (2020) 102658.

[15] Y. Tanaka, Y. Shimanaka, A. Caddeo, T. Kubo, Y. Mao, T. Kubota, ... N. Kono, LPIAT1/MBOAT7 depletion increases triglyceride synthesis fueled by high phosphatidylinositol turnover, Gut (2020), https://doi.org/10.1136/gutjnl-2020-320646.

[16] K. Wang, M. Li, H. Hakonarson, ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data, Nucleic Acids Res. 38 (16) (2010) e164.

[17] I. Stoilov, A.N. Akarsu, I. Alozie, A. Child, M. Barsoum-Homsy, M.E. Turacli, G. Brice, et al., Sequence analysis and homology modeling suggest that primary congenital glaucoma on 2p21 results from mutations disrupting either the hinge region or the conserved core structures of cytochrome P4501B1, Am. J. Hum. Genet. 62 (3) (1998) 573–584.

[18] H. Shindou, M. Eto, R. Morimoto, T. Shimizu, Identification of membrane O-acyltransferase family motifs, Biochem. Biophys. Res. Commun. 383 (3) (2009) 320–325.

[19] S. Brogna, J. Wen, Nonsense-mediated mRNA decay (NMD) mechanisms, Nat. Struct. Mol. Biol. 16 (2) (2009) 107.

[20] H.C. Lee, T. Inoue, J. Sasaki, T. Kubo, S. Matsuda, Y. Nakasaki, ... H. Arai, LPIAT1 regulates arachidonic acid content in phosphatidylinositol and is required for cortical lamination in mice, Mol Biol Cell 23 (24) (2012) 4689–4700, https://doi.org/10.1091/mbc.E12-09-0673.

[21] B. Donati, P. Dongiovanni, S. Romeo, M. Meroni, M. McCain, L. Miele, ... L. Valenti, MBOAT7 rs641738 variant and hepatocellular carcinoma in non-cirrhotic individuals, Sci Rep 7 (1) (2017) 4492, , https://doi.org/10.1038/s41598-017-04991-0.

[22] R.N. Helsley, V. Varadharajan, A.L. Brown, A.D. Gromovsky, R.C. Schugar, I Ramachandiran, ... C.K. Neumann, Obesity-linked suppression of membrane-bound O-acyltransferase 7 (MBOAT7) drives non-alcoholic fatty liver disease, Elife 8 (2019).