The American Journal of Human Genetics, Volume *99*

Supplemental Data

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

Anide Johansen, Rasim O. Rosti, Damir Musaev, Evan Sticca, Ricardo Harripaul, Maha Zaki, Ahmet Okay Çağlayan, Matloob Azam, Tipu Sultan, Tawfiq Froukh, André Reis, Bernt Popp, Iltaf Ahmed, Peter John, Muhammad Ayub, Tawfeg Ben-Omran, John B. Vincent, Joseph G. Gleeson, and Rami Abou Jamra

Supplemental Note: Case Reports

Family 1 is from Egypt with 2 affected children (III-2, III-3), and a similarly affected cousin (III-4), all born to 1st degree cousin marriages. The older affected boy (III-2) was born via normal spontaneous vaginal delivery (NSVD) following an uneventful pregnancy with normal birth centiles. He was hypotonic as an infant and did not reach any milestones until 7 months when myoclonic and focal seizures with secondary generalization were observed. The subject was started on antiepileptic drug (AED), which after trial of multiple regimens has been successful to control the seizures at age 6, and has been seizure free for the last 2 years. He started to walk at 4.5 years of age, but still speak no meaningful words. At his last examination at 8 years of age, his head circumference (HC) was 52.5 cm (-1 SD), and also his height and weight were within normal limits. He seemed hyperactive, and had repetitive behaviors such as rocking and hand flapping. Physical examination was otherwise unremarkable. In addition, he presented autistic features, and Childhood Autism Rating Scale (CARS) showed a score of 28 (>30 implies evidence of ASD).[1] Brain magnetic resonance imaging (MRI) was within normal limits.

The younger affected boy (III-3) was born via NSVD at term with normal birth centiles. Seizures, of the same character as for his older brother, began at 6 months of age and he was seizure free at 5 years of age with AED. At his last examination at age 5, his head circumference was 51 cm (-1 SD), with the height and weight also being within normal limits. He had started to walk at age 4, but he lacked any speech and did not follow any commands. He also had autistic features similar to his brother, and did not have any sphincter control. His CARS score was 30, supporting a diagnosis of ASD.

The similarly affected cousin (III-4) was born following an unremarkable pregnancy with normal birth centiles. Myoclonic seizures started at the age of 3 months and were generalized at 7 months. The seizures were under control at 3 years of age by multiple antiepileptic drugs. At his last examination at age 3, his head circumference was 48 cm (-1 SD) with other measurements being also within normal limits. He was taking a few steps on his own and had no meaningful speech. There were no dysmorphisms or any systemic malformations. Like his cousins, he displayed hyperactivity and repetitive behaviors. His CARS score was also 30. Thus Family 1 shows full expressivity of ID and epilepsy with borderline ASD features.

Family 2 is Pakistani with affected dizygotic twins (III-2, III-3) born to a 1st degree consanguineous marriage. The older affected boy (III-2) was born at term with no complications and unknown birth centiles. Hypotonia was observed during infancy, and no gross motor milestones were reached until the age of 2 years when he started having generalized tonic clonic seizures. His head control was at 2.5 years of age, and he started sitting at 3.5 years of age. His seizures were controlled at age 4 by AED. At his last examination at age 4, he presented with microcephaly (head circumference was 46 cm (-3 SD)). He was not able to walk, and there was no meaningful speech. The tone and reflexes were increased in all four extremities with bilateral positive Babinski sign. The systemic examination was otherwise normal. He displayed minimal eye contact, and had stereotypical hand movements with no social smile, and no response to his name. His CARS score was 44. Brain MRI revealed cortical atrophy and possible polymicrogyria.

The affected girl (III-3) was born without complications following her twin brother with unknown birth centiles. Hypotonia was noted during infancy, and her

developmental milestones were severely delayed. She could hold her head at 2 years of age, and started sitting without support at 3 years. At her last physical examination at 4 years, her head circumference was noted to be 45 cm (-2 SD). She had no meaningful speech and could not walk. Hypertonia and brisk reflexes were present, without dysmorphisms or any other systemic malformations. She had similar autistic features to her brother, and her CARS score was 42. There was no history of seizures so far.

Family 3 is also of Pakistani origin with 3 affected children (III-3, III-4, III-5) born to a 1st degree consanguineous marriage. The oldest affected boy (III-3) was born via NSVD at term following an uneventful pregnancy. His birth centiles are unknown. His development as an infant and toddler was slow and he was able to sit independently at 18 months of age when myoclonic seizures started. Secondarily generalized seizures started around the same age and were under control at age 5 by AED. He has been seizure free for the last 7 years and is no longer taking AED. He was able to walk at age 3.5 years and had his first words at age 5. He could build two word sentences by age 7. At his last examination at age 12, his head circumference was 53 cm (-1.1 SD), with height and weight being also appropriate for his age. He was able to build simple sentences. He was following simple orders in an occasional manner, was hyperactive with repetitive behavior, and had fleeting eye contact. Sphincter control was present. The examination was otherwise unremarkable. His CARS score was 41. Brain imaging showed cortical atrophy and possible polymicrogyria.

The affected girl (III-4) was born at term with no complications. Her birth centiles were also unknown. She also had hypotonia as an infant and an onset of seizures at the age of 4 months. The focal seizures progressed to generalized tonic-clonic and were

under control at age 9 by AED. She has been seizure free for the last 2 years. She was able to sit independently at 1.5 years of age and was walking at 2 years of age. Her first words were at 4 years of age and she was talking in 2 word sentences by 5 years of age. Her head circumference was 51 cm (-2.5 SD) at age 12. No overt dysmorphisms were observed and systemic examination was normal. She had a CARS score of 42. Brain MRI was unremarkable.

The youngest affected (III-5) was delivered at term following an uneventful pregnancy. Myoclonic seizures started at 6 months with secondary generalization. He is now seizure free at 2 years of age with AED. He was able to sit independently at 1.5 years of age. At his last examination at the age of 2, he had a head circumference of 45 cm (-2.8 SD). He could stand on his own, but no independent walking was achieved yet. He could say 'mama' and 'papa'. Like his siblings, he demonstrated moderate-severe autistic features. His CARS score was recorded as 42.

Family 4 is a 1st degree consanguineous family from Jordan, with two affected girls (III-1 and III-2). The oldest affected sibling (III-1) was born via caesarian section with a birth weight of 3 kg due to pelvic presentation. Parents noticed developmental delay at 7 months of age. Developmental milestones including head control, sitting and walking were delayed with sitting at 3 and walking at 4 years of age after extensive physiotherapy. She also had myoclonic seizures at 7 months of age. Adaptation and muscular tonus were unremarkable. Seizures were controlled by AED, and she was taken off the medication at 2 years of age. Brain MRI at 6 years of age was unremarkable. Sexual maturation at the age of 13 was unremarkable. At last examination (age 14), she could say a few words, and her HC was normal with 53 cm (-1.2 SD).

The second affected individual in family 4 (III-2) was born via NVSD following an uneventful pregnancy. Her birth weight was 2.8 kg. Following an unremarkable neonatal phase, the family sought medical consultation due to delay in developmental milestones. At the age of 1.5 years, she could sit with support, she had increased deep tendon reflexes, and appendicular hypotonia. No seizures were reported. At last examination at age 9, she could walk and speak only a few words and HC 51.1 cm (-1.5 SD). Her receptive language was better than her expressive skills.

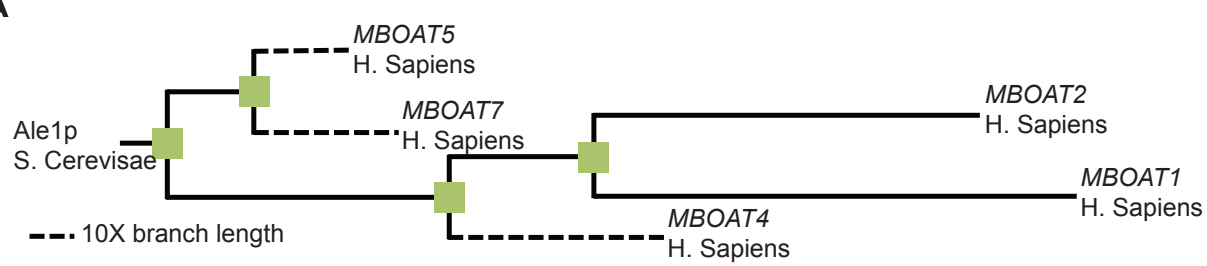
Family 5 is an Iraqi family with two affected sisters (III-1, III-3) born to a 1st degree consanguineous marriage. Both affected individuals were born at term with no complications. Their birth parameters were not available. Developmental delay, hypotonia with spasticity of the extremities in both sisters were observed at about 6 months of age. The younger sister (III-3) had one episode of febrile seizure around 2 years of age. Both could walk at about 2 years. At the last physical examination at age 11 years and 4 months, the older sister III-1 had a HC of 52 cm (-0.9 SD). Her developmental course showed ID without regression. She was able to walk with help. According to her parents she could speak 10 to 20 words. She was a friendly and hyperactive child with behavioral anomalies. The younger sister (III-3) was aged 7 years and 6 months at the last physical examination. She had a HC of 50.5 cm (-1.5 SD). She was able to walk unsteadily with help and showed muscular hypotonia. Like her older sister she spoke 10 to 20 words but mostly used signs and utterances to communicate, indicating delays in expressive skills. During examination she was conscious, hyperactive and showed behavioral problems like her sister. Both affected sisters shared only minor

unspecific dysmorphic features, the most obvious being dense eyebrows, hypertrichosis, wide palpebral fissures and dysmorphic ears.

Family 6 is a 2-branch consanguineous Pakistani family; two affected sisters were present in the first branch, and two similarly affected cousins (1 female and 1 male) were born to the second branch. All 4 subjects had ID. They started walking between ages 4 and 7. The oldest (III-3) spoke a few words, the other subjects did not exhibit any expressive language. Only III-3 had febrile seizures at age 1 which lasted a few days. None had any signs of autistic features during the examination. They were sociable and maintained eye contact. On observation their interaction with the examiner included greeting with a smile and waving hands. They engaged in play and communication with their family members. They could feed themselves and go to the restroom independently. Two individuals (III-3, III-7) became physically aggressive when frustrated. HC was between -1 and -2 SD at the latest examination. Brain imaging from the youngest affected (III-9) was within normal limits.

Figure S1

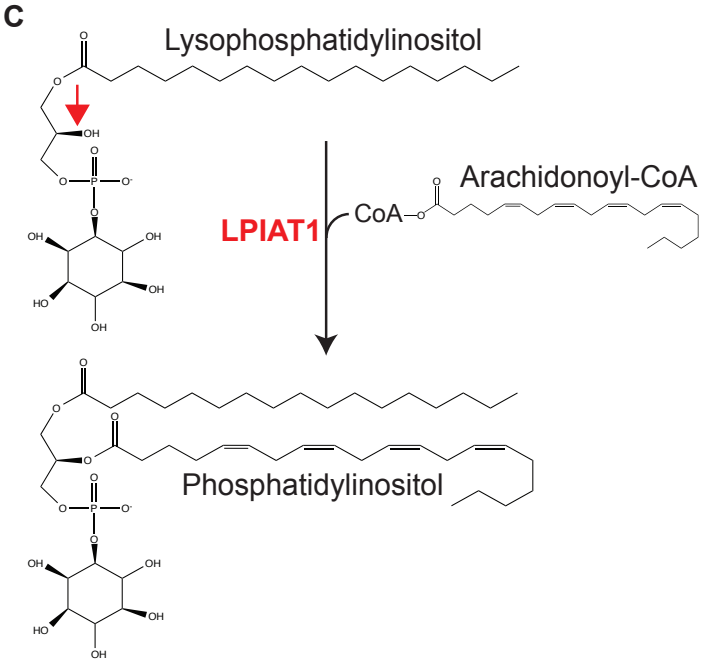
A



B

Gene	Acyl donor	Acyl acceptor
MBOAT1	Oleoyl-CoA	LysoPS
MBOAT2	Oleoyl-CoA	LysoPA, -PE
MBOAT4	Octanoic-, decanoic-, tetranoic acid	Grehlin
MBOAT5	Lineoyl-CoA, arachidonoyl-CoA	LysoPC, -PS
MBOAT7	Arachidonoyl-CoA	LysoPI

C



Supplemental figure.

Figure S1. The yeast *Ale1p* founded the 5 MBOAT paralogs in human. (A) Length of line corresponds to genetic change over time. Dashed line indicates 10X length. MBOAT1: NP_001073949. MBOAT4: NP_001094386. MBOAT7: NP_077274. HGNC provided symbols for MBOAT5: LPCAT3 (NP_005759), and MBOAT2: LPCAT4 (NP_705841). **(B)** Each of the MBOAT family members has a preference towards acyl donors and acyl acceptors. *MBOAT7*/LPIAT1 has a preference for arachidonoyl-CoA and lysophosphatidylinositol as acyl donor and acceptor, respectively. **(C)** *MBOAT7*/LPIAT1 facilitates the transfer of arachidonic acid from arachidonoyl-CoA to the sn2 position (red arrow) of lysophosphatidylinositol.

Supplemental tables.

Table S1. Clinical table.

Clinical presentation for affected subjects from families 1 to 6. HC: head circumference. AED: anti-epileptic drug. Consang: consanguinity. MRI: magnetic resonance image. CARS: child autism rating scale. PMG: polymicrogyria. DTR: deep tendon reflexes. GTC: generalized tonic-clonic.

Table S1

Family	Family 1			Family 2		Family 3			Family 4		Family 5		Family 6			
Subject	III-2	III-3	III-4	III-2	III-3	III-3	III-4	III-5	III-1	III-2	III-1	III-3	III-3	III-4	III-7	III-9
Gender	M	M	M	M	F	M	F	M	F	F	F	F	F	F	F	M
Origin	Egypt			Pakistan					Jordan		Iraq		Pakistan			
Consang.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HC at birth (SD)	0	0	0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Milestones																
Head Control	7 mos	8 mos	6 mos	2.5 yrs	2 yrs	8 mos	8 mos	1 yr	1 yr	1 yr	8 mos	9 mos	1 yr	1 yr	1 yr	1 yr
Unsupp. sitting	2 yrs	2.5 yrs	2 yrs	3.5 yrs	3 yrs	1.5 yrs	1.5 yrs	1.5 yrs	3 yrs	2 yrs	1 yr	1 yr	3 yrs	4 yrs	4 yrs	5 yrs
Unsupp. walking	4.5 yrs	4 yrs	3 yrs	-	-	3.5 yrs	2 yrs	-	4 yrs	3.5 yrs	2 yrs	2 yrs	4 yrs	6 yrs	5 yrs	7 yrs
Words	-	-	-	-	-	7 yrs	4 yrs	-	6 yrs	5 yrs	5 yrs	5 yrs	6 yrs	-	-	-
Two word sentence	-	-	-	-	-	7 yrs	5 yrs	-	-	-	-	-	-	-	-	-
Seizures																
Onset	7 mos	6 mos	7 mos	2.5 yrs	-	18 mos	4 mos	6 mos	7 mos	-	2 yrs	-	1 yr	-	-	-
Type	Myoclonic Focal		Myo-clonic	GTC	-	Myo-clonic	Focal	Myoclonic		-	Febrile (1 ep.)	-	Febrile (a few days)	-	-	-
Frequency	Under control by AED	Under control by AED	Under control by AED	Under control by AED	-	Under control by AED	Under control by AED	Under control by AED	Under control by AED	-	-	-	-	-	-	-
Autism																
CARS score	28	30	30	44	42	41	42	42	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Neurological																
HC at last exam (SD)	-1	-1	-1	-3	-2	-1.1	-2.5	-2.8	-1.2		-1.1	-0.9	-1.5	-1	-2	-2
Hypotonia (infancy)	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
Hypertonia	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brain MR imaging	Normal	N/A	Norma l	PMG & cortical atrophy	N/A	PMG & cortical atrophy	Normal	N/A	N/A							Normal

Table S2. Detailed information for all mutations detected in affected individuals from families 1-6, including gene name, transcript number, genetic (gDNA) and complementary DNA (cDNA) position, and protein position.

Family	Gene	Transcript	gDNA pos.	cDNA pos.	Protein pos.
Family 1	*MBOAT7*	NM_024298.3	g.1583_1602del	c.126_145del	p.Leu43Hisfs*69
Family 2/3	*MBOAT7*	NM_024298.3	g.9148_9168del	c.758_778del	p.Gln253_Ala259del
Family 4	*MBOAT7*	NM_024298.3	g.6260delG	c.423delG	p.Leu142Cysfs*8
Family 5	*MBOAT7*	NM_024298.3	g.9245G>C	c.854+1G>C	p.0?
Family 6	*MBOAT7*	NM_024298.3	g.9210_9216del	c.820_826del	p.Gly274Profs*47

References:

1. Schopler, E., Reichler, R.J., and Renner, B.R. (2002). The childhood autism rating scale (CARS).(Western Psychological Services Los Angeles).