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# A partially inactivating mutation in the sodium-dependent lysophosphatidylcholine transporter *MFSD2A* causes a non-lethal microcephaly syndrome

Vafa Alakbarzade[1,2,12], Abdul Hameed[3,12], Debra Q Y Quek[4,12], Barry A Chioza[1,12], Emma L Baple[1,5,6,12], Amaury Cazenave-Gassiot[7], Long N Nguyen[4], Markus R Wenk[7], Arshia Q Ahmad[8,9], Ajith Sreekantan-Nair[1], Michael N Weedon[1], Phil Rich[10], Michael A Patton[1,11], Thomas T Warner[2], David L Silver[4] & Andrew H Crosby[1]



**The major pathway by which the brain obtains essential omega-3 fatty acids from the circulation is through a sodium-dependent lysophosphatidylcholine (LPC) transporter (MFSD2A), expressed in the endothelium of the blood-brain barrier. Here we show that a homozygous mutation affecting a highly conserved MFSD2A residue (p.Ser339Leu) is associated with a progressive microcephaly syndrome characterized by intellectual disability, spasticity and absent speech. We show that the p.Ser339Leu alteration does not affect protein or cell surface expression but rather significantly reduces, although not completely abolishes, transporter activity. Notably, affected individuals displayed significantly increased plasma concentrations of LPCs containing mono- and polyunsaturated fatty acyl chains, indicative of reduced brain uptake, confirming the specificity of MFSD2A for LPCs having mono- and polyunsaturated fatty acyl chains. Together, these findings indicate an essential role for LPCs in human brain development and function and provide the first description of disease associated with aberrant brain LPC transport in humans.**

We investigated an extensive Pakistani pedigree (**Fig. 1a**) comprising individuals with an autosomal recessively inherited progressive neurological condition in which the cardinal features included microcephaly, spastic quadriparesis and intellectual disability with absent speech. Neuroimaging was available for two affected individuals and showed a consistent radiological phenotype with microcephaly and a profound lack of posterior white matter (**Fig. 1b** and **Supplementary Table 1**). To map the disease-associated locus, we used DNA samples from affected and unaffected family members to perform genome-wide SNP genotyping, assuming that a founder mutation was responsible. This mapping identified a single notable homozygous region of 19.9 Mb on chromosome 1p34 (maximum logarithm of odds (LOD) = 7.3) shared by all affected individuals (**Supplementary Fig. 1**). The region, considered likely to correspond to the disease locus, is delimited by the recombinant SNP markers rs3767088 and rs1033729 and contains 395 genes. To identify the causative mutation, we performed whole-exome sequence analysis of a single affected individual (II:3) to identify potential disease-causing variants. After filtering, we identified only one likely deleterious variant within the critical region, in exon 10 of the *MFSD2A* gene (encoding major facilitator superfamily domain–containing 2a; c.1016C>T, NC_000001.11: g.39967632C>T; p.Ser339Leu). We did not detect any likely deleterious sequence variants in other genes responsible for microcephaly, including the microcephalin genes, all of which are located outside of the homozygous critical interval, nor in *SLC2A1* (*GLUT1*), also located on chromosome 1p, which was further excluded by dideoxy sequence analysis. The *MFSD2A* sequence variant affected a stringently conserved amino acid residue and cosegregated with the disease phenotype (**Fig. 1**), was predicted to be highly damaging using standard programs (PROVEAN score < −2.5), and was absent from the online genomic dbSNP 141, 1000 Genomes Project and National Heart, Lung, and Blood Institute (NHLBI) ESP6500 databases.

We previously identified MFSD2A as the major transporter for uptake of the omega-3 fatty acid docosahexaenoic acid (DHA) into the brain, which is selectively expressed in the blood-brain epithelium[1]. MFSD2A is a plasma membrane protein that belongs to the major facilitator superfamily of secondary transporters, which have 12 membrane-spanning domains[2]. We recently showed that MFSD2A transports DHA and other long-chain fatty acids into the brain in the chemical form of LPC, defining MFSD2A as the first characterized sodium-dependent facilitative transporter of LPCs[1]. *Mfsd2a*-knockout mice are deficient for DHA in the brain and present with severe

[1]Institute of Biomedical and Clinical Science, University of Exeter Medical School, RILD Wellcome Wolfson Centre, Exeter, UK. [2]Reta Lila Weston Institute of Neurological Studies, Department of Molecular Neurosciences, University College London Institute of Neurology, London, UK. [3]Institute of Biomedical and Genetic Engineering (IBGE), Islamabad, Pakistan. [4]Signature Research Program in Cardiovascular and Metabolic Disorders, Duke–National University of Singapore Graduate Medical School, Singapore. [5]Human Genetics and Genomic Medicine, Faculty of Medicine, University of Southampton, Southampton, UK. [6]Wessex Clinical Genetics Service, Princess Anne Hospital, Southampton, UK. [7]Life Sciences Institute, National University of Singapore, Singapore. [8]Department of Physical Medicine and Rehabilitation, Indiana University–Purdue University Indianapolis (IUPUI), Indianapolis, Indiana, USA. [9]Rehabilitation Hospital Indiana, Indianapolis, Indiana, USA. [10]Department of Neuroradiology, St. George's Hospital, London, UK. [11]Southwest Thames Regional Genetics Service, St George's Healthcare National Health Service (NHS) Trust, London, UK. [12]These authors contributed equally to this work. Correspondence should be addressed to A.H.C. (a.h.crosby@exeter.ac.uk) or D.L.S. (david.silver@duke-nus.edu.sg).

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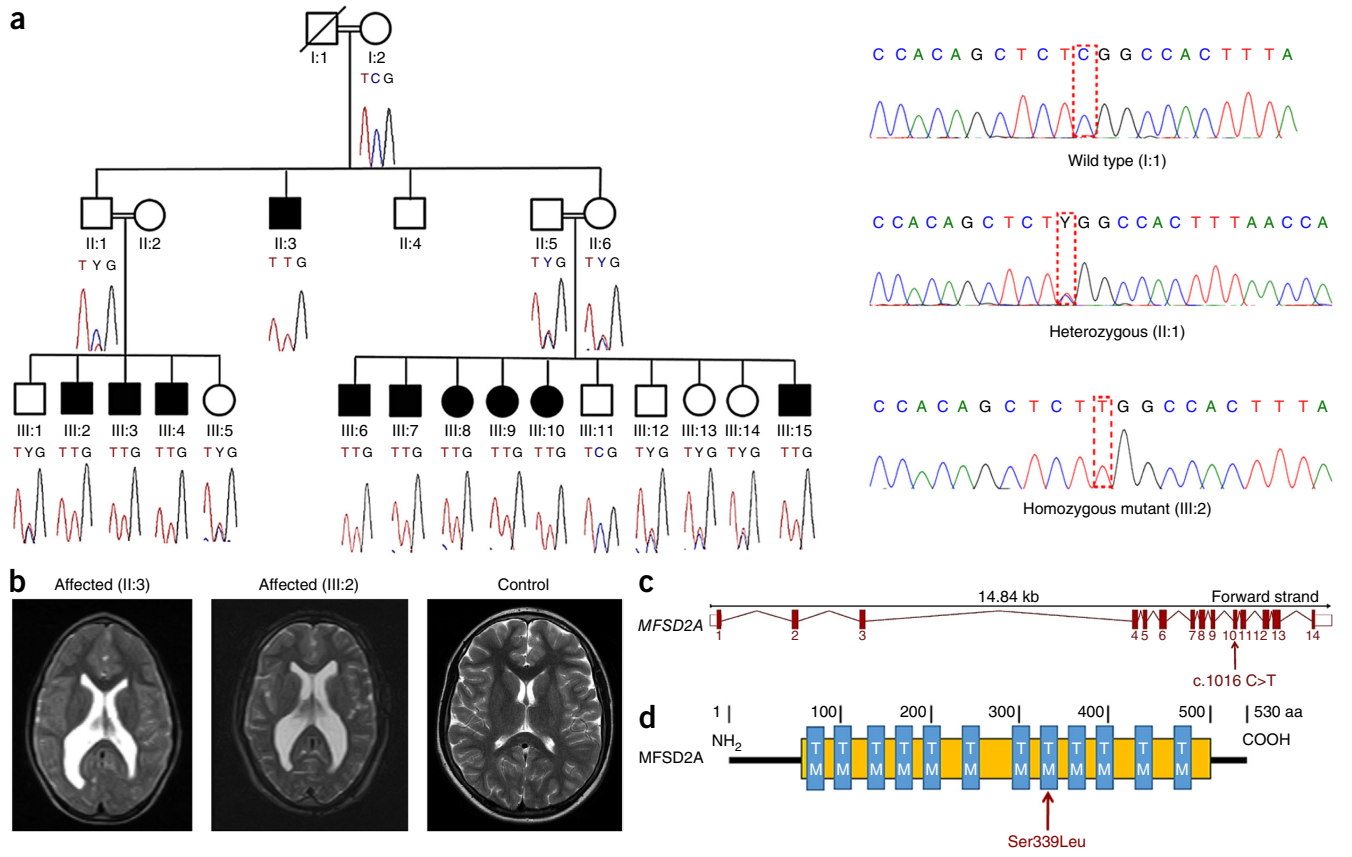


**Figure 1** Mutation of *MFSD2A* causes non-lethal neurodevelopmental delay and microcephaly. (**a**) Pedigree of the extended Pakistani family investigated, showing cosegregation of the c.1016C>T variant with disease and extended 20-bp sequence electropherograms for wild-type, heterozygous and homozygous mutant individuals. (**b**) In affected individuals (II:3 and III:2), axial T2-weighted scans at the level of the lateral ventricle demonstrate a paucity of cerebral white matter volume, particularly posteriorly around the lateral ventricles, causing scalloping of the ventricular walls. Myelination appears complete, and there is no signal abnormality to indicate an acquired injury. Cortical thickness and folding appear normal. The pattern is identical in the two subjects. One has trigonocephaly (left); the other has a normal frontal convexity in the axial plane (middle). A control image taken at the same level is shown for comparison (right). (**c**,**d**) Organization of the *MFSD2A* gene (**c**) and MFSD2A protein (**d**) showing the position of the c.1016C>T (p.Ser339Leu) sequence variant. TM, transmembrane domain. (**e**) Amino acid alignment showing high conservation of the Ser339 residue in vertebrates.

microcephaly, ataxia, and behavioral and cognitive impairment, consistent with the disabilities seen in affected humans homozygous for the variant encoding p.Ser339Leu. To determine the consequences of the p.Ser339Leu alteration on MFSD2A function, we introduced the variant into human *MFSD2A* cDNA, which we expressed in HEK293 cells. Overexpression of MFSD2A in HEK293 cells resulted in the presence of three main protein species detected by immunoblotting of ~55, ~68 and ~100 kDa due to glycosylation[3], as indicated by the downward shift in the molecular weights of the major glycosylated species after treatment with the N-glycosylase PNGase F (**Fig. 2a**). In addition, we observed multiple bands on the immunoblot that likely represent MFSD2A aggregates, as mock-transfected cells completely lacked these protein bands. In HEK293 cells overexpressing the Ser339Leu mutant, the levels of the glycosylated ~68-kDa species were reduced and the total levels of the Ser339Leu mutant were slightly but non-significantly reduced in comparison to cells overexpressing the wild-type protein (**Fig. 2b**). These relative changes in the levels of glycosylated species of the Ser339Leu mutant might be indicative of structural changes in the mutant protein. The small, non-significant reduction in the total amount of MFSD2A Ser339Leu expressed in HEK293 cells does not likely account for the disease in the affected family members, as the reduction of MFSD2A protein levels by ~50% in mice heterozygous for a *Mfsd2a*-knockout allele did not result in microcephaly or DHA deficiency in the brain[1,3] and the heterozygous parents of the affected individuals did not present with disease. Furthermore, we found that the cell surface localization of the Ser339Leu mutant was similar to that for wild-type MFSD2A (**Fig. 2c**). Taken together, the data indicate that expression of the Ser339Leu mutant is not greatly affected and that the mutant is localized to the plasma membrane, similarly to wild-type MFSD2A, suggesting that the transport function of the Ser339Leu mutant might be impaired.

To assess transport activity in this cell-based system, we transfected HEK293 cells to express either wild-type or mutant (Ser339Leu) MFSD2A or mock transfected them as a negative control, and we then incubated the cells with increasing concentrations of LPC-[$^{14}$C]DHA, LPC-[$^{14}$C]oleate or LPC-[$^{3}$H]palmitate, which we previously characterized as physiological LPC substrates for MFSD2A[1]. The amounts of LPC-[$^{14}$C]DHA and LPC-[$^{14}$C]oleate transported were significantly reduced relative to those observed with wild-type MFSD2A, whereas the transport of LPC-[$^{3}$H]palmitate was completely abrogated and similar to that observed in mock-transfected cells (**Fig. 2d**). The lack

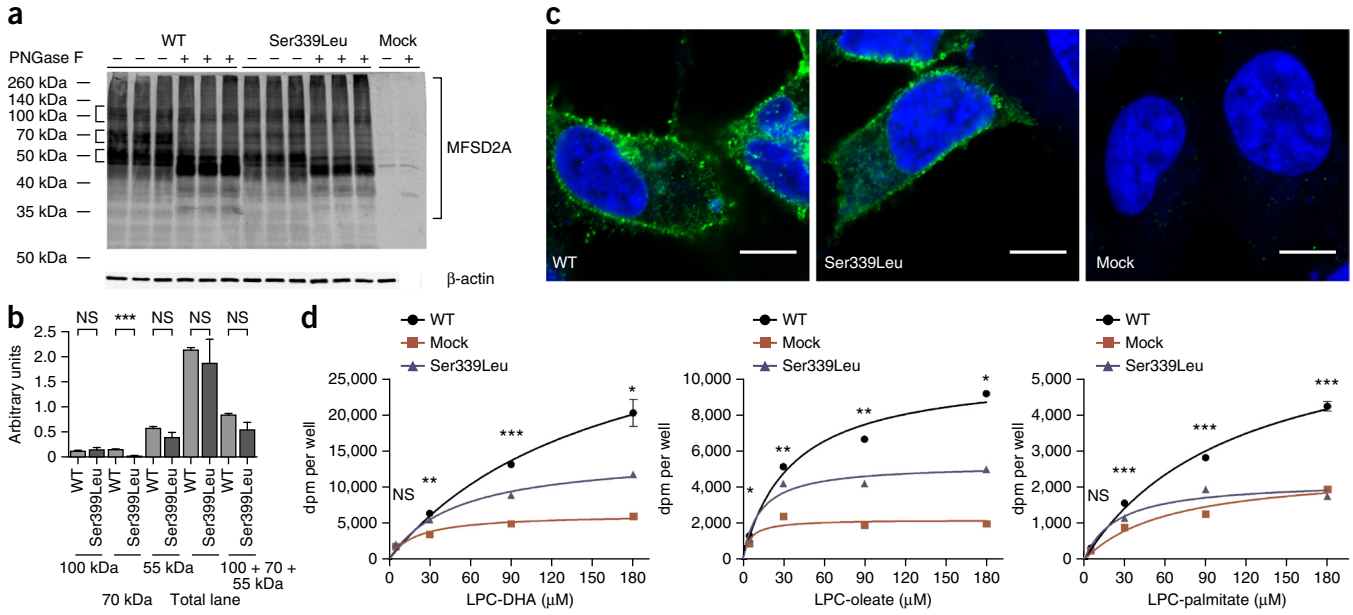


**Figure 2** The MFSD2A p.Ser339Leu alteration results in partial loss of function. (**a**) Immunoblot of wild-type (WT) and Ser339Leu MFSD2A proteins expressed in HEK293 cells (the small brackets on the left highlight distinct glycosylated species of MFSD2A). The immunoblot shows three biological replicates for each construct. PNGase F was used to remove glycans from MFSD2A. In the mock condition, cells were transfected with empty vector. (**b**) Quantification of the band intensities in **a** for three distinct glycosylated species of MFSD2A (represented by molecular weight) and total MFSD2A (the total MFSD2A in each lane or the sum of the amounts of the three glycosylated species), normalized to the band intensity for β-actin. Values are represented as means ± s.e.m. (**c**) Immunofluorescence localization of wild-type and Ser339Leu proteins expressed in HEK293 cells. MFSD2A is shown in green. Nuclei were stained with Hoechst 33342 (blue). Scale bars, 10 μm. (**d**) Concentration-dependent transport of LPC-[$^{14}$C]DHA, LPC-[$^{14}$C]oleate and LPC-[$^{3}$H]palmitate after 30 min in HEK293 cells expressing wild-type or Ser339Leu MFSD2A protein (as measured by disintegrations per minute (dpm) per well). Experiments were performed twice with three biological replicates. Values are represented as means ± s.e.m. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; NS, not significant.

of transport of LPC-[$^{3}$H]palmitate by the mutant protein likely occurs because LPC-palmitate is a low-affinity LPC ligand for MFSD2A, as previously shown[1]. Although the mechanistic explanation for the partial inactivity arising from the p.Ser339Leu alteration remains uncertain, the Ser339 residue is located along a transmembrane domain that may face inward into the substrate-binding pocket, and the mutant leucine residue may interfere with substrate binding. When considered together, these data are consistent with the p.Ser339Leu alteration causing the microcephaly syndrome in individuals homozygous for this variant (**Supplementary Fig. 2**). Notably, the phenotype of these individuals is substantially milder than the phenotype described in an accompanying study characterizing completely inactivating mutations of *MFSD2A*[4], in which affected individuals with complete MFSD2A transporter inactivation present with a severe, lethal microcephaly early in life with profound intellectual disability, absence of speech, spastic quadriparesis, central hypotonia, dysphagia and seizures. Although the affected individuals described here have overlapping features, the severity of microcephaly and rate of progression of the condition are notably reduced and the condition is associated with less severe motor, cognitive and behavioral impairment and an absence of seizures. Relative to the clinical features of individuals with complete loss of MFSD2A function, the milder clinical findings described in the current study define a unique clinical spectrum associated with partial loss of function of MFSD2A and closely align with the phenotype reported for *Mfsd2a*-knockout mice.

As cerebral spinal fluid was not available and it was not feasible to measure brain DHA levels or LPC brain uptake in the affected individuals, we sought to identify a surrogate measurement for defective LPC transport. We hypothesized that plasma LPC levels would be increased as a consequence of partial inactivation of MFSD2A at the blood-brain barrier, the major site for MFSD2A expression in the body. LPCs are produced by the liver and circulate on albumin at physiological concentrations similar to those found for unesterified fatty acids in human blood[5,6]. We used a targeted mass spectrometry approach to measure plasma LPC concentrations in several of the patients, their unaffected siblings and random age-matched controls living in the same geographical region as the affected family members. We found that total plasma LPC levels were increased in the individuals homozygous for the variant encoding p.Ser339Leu variant relative to all controls (increase of 54%, $P = 0.015$; **Fig. 3a**), consistent with defective transport of LPCs at the blood-brain barrier in these individuals. Remarkably, we found that the increase in total plasma LPC concentrations in these individuals was due to specific increases in the levels of LPCs containing monounsaturated (LPC 18:1: percent increase of 92%, $P = 0.004$) and polyunsaturated (LPC 18:2, 20:4 and 20:3: percent increases of 254%, $P = 0.002$; 117%, $P = 0.007$; and 238%, $P = 0.002$, respectively) fatty acyl chains, but not in the levels of the most abundant plasma LPCs, which contain saturated acyl chains (C16:0 and C18:0: percent increases of 20%, $P = 0.105$ and 23%, $P = 0.166$, respectively). Plasma concentrations of LPCs containing DHA were below quantifiable detection in all subjects, which is consistent with the lack of marine fish[7] in the diet of the families investigated, who are resident in a geographically land-locked region of Pakistan. We note that LPCs with saturated fatty acyl chains are lower-affinity ligands for MFSD2A than LPCs with unsaturated acyl chains[1], and affinity for MFSD2A was inversely correlated with physiological levels (**Fig. 3b**). Therefore, these findings confirm that

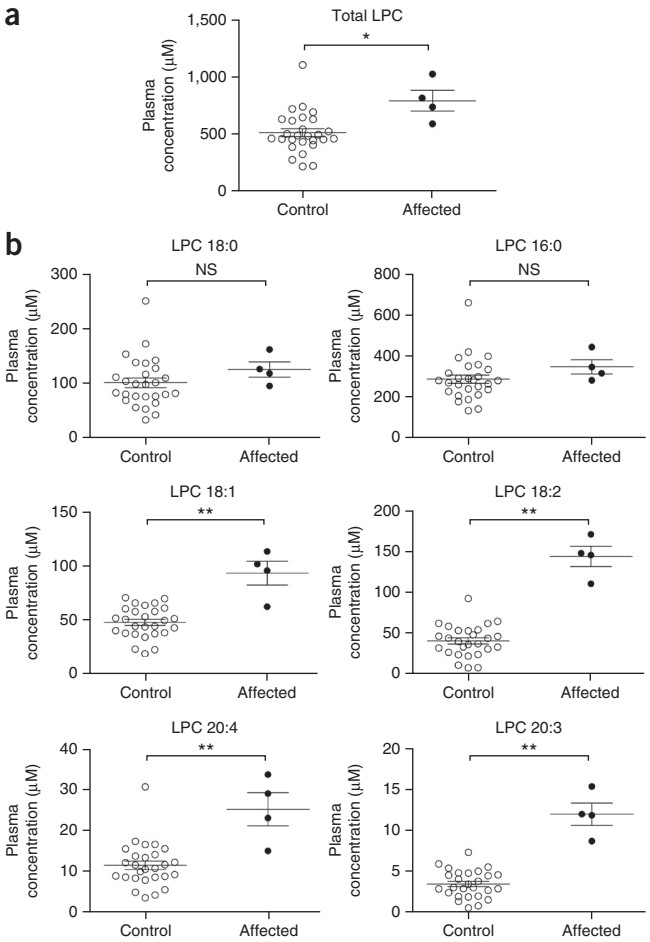


**Figure 3** Total plasma LPC concentration and concentrations of individual LPC species by lipidomic mass spectrometry. (**a**,**b**) Total LPC concentration (**a**) and concentrations of common individual LPC species (**b**) in plasma from age-matched controls, including 34 unrelated children from the same geographical location, and 1 parent (II:3) and 1 unaffected sibling (III:5) from the pedigree. Analysis was performed once with two technical replicates. Values are represented as means ± s.e.m. $^{*}P < 0.05$, $^{**}P < 0.01$; NS, not significant.

partial inactivation of MFSD2A (by p.Ser339Leu alteration) does not result in significant changes in plasma LPC concentrations for lower-affinity ligands but does affect the plasma levels of LPCs that are higher-affinity ligands for MFSD2A. In contrast, complete loss of MFSD2A activity[4] results in an increase in the concentrations of all detectable LPC species. These findings indicate that LPCs with mono- and polyunsaturated fatty acyl chains are the major plasma LPC species required for human brain growth.

There are notable phenotypic parallels between individuals homozygous for the variant encoding p.Ser339Leu alteration and mice null for *Mfsd2a*, as both groups display severe microcephaly, motor dysfunction and progressive worsening of neurological phenotypes with age, and we anticipate that individuals homozygous for the p.Ser339Leu variant would also display a severe deficiency for DHA in the brain, similarly to *Mfsd2a*-knockout mice. The DHA deficiency in *Mfsd2a*-knockout mice can be explained by the finding that LPC is the primary carrier of DHA to the brain, which does not synthesize DHA. The presentation of microcephaly in both *Mfsd2a*-knockout mice and humans homozygous for the p.Ser339Leu variant suggests the possibility that LPC uptake by the brain provides a preformed phospholipid source for membrane biogenesis. This is possible because LPCs, which are found at low levels in cellular membranes, are converted biochemically into membrane phosphatidylcholine through the action of lysophosphatidylcholine acyltransferase enzymes[8]. Moreover, given that the syndrome exhibited by individuals homozygous for the p.Ser339Leu alteration appears progressive, it seems likely that LPC transport is required in the adult brain to maintain brain function, perhaps by providing LPCs for membrane repair and turnover. In conclusion, our data establish that LPCs are critical for human brain growth and function and defines LPCs as potential therapeutic agents for further exploration in disorders of brain growth and neurodegenerative disease.

## METHODS

Methods and any associated references are available in the online version of the paper.

*Note: Any Supplementary Information and Source Data files are available in the online version of the paper.*


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### AUTHOR CONTRIBUTIONS

A.H.C. and D.L.S. conceived, designed and supervised the project. V.A., D.Q.Y.Q., B.A.C., A.C.-G., L.N.N., M.R.W. and M.N.W. performed experiments. A.H. aided in family recruitment. A.H., A.Q.A., T.T.W., M.A.P., P.R. and E.L.B. aided in assessment of clinical data. A.S.-N. was involved in the genetic studies. A.H.C., D.L.S. and E.L.B. wrote the manuscript.

### COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.




1. Nguyen, L.N. *et al.* Mfsd2a is a transporter for the essential omega-3 fatty acid docosahexaenoic acid. *Nature* **509**, 503–506 (2014).
2. Ethayathulla, A.S. *et al.* Structure-based mechanism for $Na^+$/melibiose symport by MelB. *Nat. Commun.* **5**, 3009 (2014).
3. Berger, J.H., Charron, M.J. & Silver, D.L. Major facilitator superfamily domain–containing protein 2a (MFSD2A) has roles in body growth, motor function, and lipid metabolism. *PLoS ONE* **7**, e50629 (2012).
4. Guemez-Gamboa, A. *et al.* Inactivating mutations in *MFSD2A*, required for omega-3 fatty acid transport in brain, cause a lethal microcephaly syndrome. *Nat. Genet.* doi:10.1038/ng.3311 (25 May 2015).
5. Baisted, D.J., Robinson, B.S. & Vance, D.E. Albumin stimulates the release of lysophosphatidylcholine from cultured rat hepatocytes. *Biochem. J.* **253**, 693–701 (1988).
6. Croset, M., Brossard, N., Polette, A. & Lagarde, M. Characterization of plasma unsaturated lysophosphatidylcholines in human and rat. *Biochem. J.* **345**, 61–67 (2000).
7. Innis, S.M. Dietary (*n*-3) fatty acids and brain development. *J. Nutr.* **137**, 855–859 (2007).
8. Shindou, H., Hishikawa, D., Harayama, T., Eto, M. & Shimizu, T. Generation of membrane diversity by lysophospholipid acyltransferases. *J. Biochem.* **154**, 21–28 (2013).

## ONLINE METHODS

**Study conduct.** All tissue samples were taken with informed consent in accordance with all ethical standards and protocols and governed by the Institute of Biomedical and Genetic Engineering (Pakistan).

**Genetic studies.** SNP genotyping was carried out using Illumina Human CytoSNP-12v2.1 arrays. Multipoint linkage analysis was performed with SimWalk2 (ref. 9) under a model of autosomal recessive inheritance with full penetrance, using a disease allele frequency estimated at 0.0001. Unique primers for sequencing and microsatellite analysis (Sigma-Aldrich) were designed using online Primer3web software[10] with sequences from the UCSC Genome Browser. Bidirectional dideoxy DNA sequencing was performed on an ABI3130 XLA capillary sequencer (Applied Biosystems), with analysis carried out using Finch TV 1.4.0 (Geospiza) and Gene Tool 1.0.0.1 (Bio Tools). Whole-exome sequencing was performed by the Otogenetics Corporation using the SureSelect Human All Exon V4 (Agilent Technologies) exome enrichment kit on an Illumina HiSeq 2000. Exome sequencing produced 31,783,299 mapped reads, corresponding to 93% of the targeted sequences being covered sufficiently for variant calling (>10× coverage; mean depth of 45×).

**PNGase F treatment.** For treatment with PNGase F, 1 µl of 10× Glycoprotein Denaturing Buffer (New England BioLabs), 2 µl of 10× G7RB, 2 µl of 10% NP-40 and 1,000 U of PNGase F (New England BioLabs) were added to 7 µl of HEK293 whole-cell lysate (30 µg protein). The reaction was incubated for 2 h at 37 °C. Treated and untreated lysates were then evaluated by immunoblotting using our polyclonal antibody against MFSD2A[3]. Immunoblots were quantified, with data representing means ± s.e.m. Statistical analysis was performed using a Student's *t* test. HEK293 cells were from the American Type Culture Collection and were negative for mycoplasma.

**Transport assays.** Transport assays using HEK293 cells was performed as previously described[1]. Briefly, plasmids encoding wild-type and Ser339Leu MFSD2A were transfected into HEK293 cells. Uptake assays were performed after 24 h of transfection with a range of LPC-[$^{14}$C]DHA, LPC-[$^{14}$C]oleate and LPC-[$^{3}$H]palmitate concentrations. Experiments were repeated twice in triplicate in 12-well plates. Uptake activity is expressed in dpm per well. Radiolabeled LPC-[$^{14}$C]DHA, LPC-[$^{14}$C]oleate and LPC-[$^{3}$H]palmitate were purchased from ARC. Non-radiolabeled LPC-DHA was synthesized as described previously[3]. LPC-oleate and LPC-palmitate were obtained from Avanti Polar Lipids.

**Immunoblotting and immunofluorescence microscopy.** Immunoblotting and immunofluorescence microscopy for MFSD2A were carried out as previously described using a rabbit polyclonal antibody against MFSD2A[1,11].

**Lipidomic analysis of plasma samples.** For human plasma samples, a single plasma sample each for the father, mother and affected child and duplicate plasma samples for the father, mother and affected child from the family were used for LPC analysis. Plasma samples from 34 age-matched healthy children (male and female) between 0.1 and 18 years old (mean = 4.3 years, s.d. = ±4.4 years) and the mother and 1 non-carrier sibling in the pedigree were used as controls. Lysophospholipids were extracted using a methanol-based protocol described previously[12]. Briefly, plasma samples (2 µl) were resuspended in 198 µl of methanol containing 453 pmol/ml LPC 20:0 as an internal standard (Avanti Polar Lipids) and were then vortexed for 30 s and sonicated for 30 min on ice. Samples were centrifuged at 20,000*g* for 10 min at 4 °C to remove debris. The supernatants (2 µl) were injected into a liquid chromatography–tandem mass spectrometry (LC-MS/MS) instrument.

**Mass spectrometry analysis.** Samples were randomized for injection into the LC-MS/MS instrument. Each sample was analyzed in technical duplicates. Injections with blank were carried out every six samples to avoid carry-over. The stability of signal throughout the analysis was monitored by regular injection of a quality control sample (chromatographic analysis was undertaken on a 1290 Liquid Chromatography System, Agilent Technologies) using a Kinetex HILIC stationary phase (column length, 150 mm; column internal diameter, 2.1 mm; particle size, 2.6 µm; pore size, 100Å; Phenomenex). Gradient elutions were performed with solvents A (95% acetonitrile/5% 10 mM ammonium formate/0.1% formic acid) and B (50% acetonitrile/50% 10 mM ammonium formate/0.1% formic acid), with a gradient range from 0.1 to 75% solvent B in 6 min, 75 to 90% solvent B in 1 min, and 90 to 0.1% solvent B in 0.1 min followed by 0.1% solvent B for 3 min (total run time of 10.1 min). Under these conditions, LPC species elute in ~4.9 min with a flow rate of 0.5 ml/min. LPC species were quantified using Multiple Reaction Monitoring (MRM) on a 6460 triple-quadruple mass spectrometer (Agilent Technologies) with gas temperature of 300 °C, gas flow of 5 l/min, sheath gas flow of 11 l/min and capillary voltage of 3,500 V. MRM transitions were from precursor ions to the choline head fragment (mass/charge ratio ($m/z$) = 184) with a collision energy of 29 V. We monitored 36 transitions simultaneously with a dwell time of 20 ms. Quantification data were extracted using MassHunter Quantitative Analysis (QQQ) software, and data were manually curated to ensure correct peak integration. Areas under the curve (AUCs) for the extracted ion chromatogram peaks for each MRM transition and lipid species were normalized to an internal standard. The total LPC concentration and concentrations for individual LPC species from human and mouse samples are expressed in µM. A Mann-Whitney non-parametric, non-paired, two-tailed *t* test was used to determine statistical significance.

**Mutagenesis of *MFSD2A*.** Human *MFSD2A* in pSport6 (Open Biosystems) was amplified using primers hMFSD2ABamHI and hMFSD2AXbaI and cloned into pcDNA3.1 in the BamHI and XbaI sites. Mutagenesis of the Ser339 codon to encode leucine in human *MFSD2A* was carried out by PCR using the primers indicated in **Supplementary Table 2**. The mutated cDNA was subsequently cloned into pcDNA3.1 and sequenced to verify the mutation.


9. Sobel, E., Sengul, H. & Weeks, D.E. Multipoint estimation of identity-by-descent probabilities at arbitrary positions among marker loci on general pedigrees. *Hum. Hered.* **52**, 121–131 (2001).
10. Rozen, S. & Skaletsky, H. Primer3 on the WWW for general users and for biologist programmers. *Methods Mol. Biol.* **132**, 365–386 (2000).
11. Ben-Zvi, A. *et al.* Mfsd2a is critical for the formation and function of the blood-brain barrier. *Nature* **509**, 507–511 (2014).
12. Zhao, Z. & Xu, Y. An extremely simple method for extraction of lysophospholipids and phospholipids from blood samples. *J. Lipid Res.* **51**, 652–659 (2010).





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