HHS Public Access

Author manuscript

J Med Genet. Author manuscript; available in PMC 2024 September 27.

Published in final edited form as:

J Med Genet. 2021 October ; 58(10): 712–716. doi:10.1136/jmedgenet-2020-107137.

De novo missense variants in *LMBRD2* are associated with developmental and motor delays, brain structure abnormalities and dysmorphic features

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Competing interests AM, DLP and RT are full-time employees of Illumina, Inc. AP is an employee of CeGaT GmbH, Germany.

Patient consent for publication Parental/guardian consent obtained.

Provenance and peer review Not commissioned; externally peer reviewed.

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Abstract

Objective—To determine the potential disease association between variants in *LMBRD2* and complex multisystem neurological and developmental delay phenotypes.

Methods—Here we describe a series of de novo missense variants in *LMBRD2* in 10 unrelated individuals with overlapping features. Exome sequencing or genome sequencing was performed on all individuals, and the cohort was assembled through GeneMatcher.

Results—*LMBRD2* encodes an evolutionary ancient and widely expressed transmembrane protein with no known disease association, although two paralogues are involved in developmental and metabolic disorders. Exome or genome sequencing revealed rare de novo *LMBRD2* missense variants in 10 individuals with developmental delay, intellectual disability, thin corpus callosum, microcephaly and seizures. We identified five unique variants and two recurrent variants, c.1448G>A (p.Arg483His) in three cases and c.367T>C (p.Trp123Arg) in two cases. All variants are absent from population allele frequency databases, and most are predicted to be deleterious by multiple in silico damage-prediction algorithms.

Conclusion—These findings indicate that rare de novo variants in *LMBRD2* can lead to a previously unrecognised early-onset neurodevelopmental disorder. Further investigation of individuals harbouring *LMBRD2* variants may lead to a better understanding of the function of this ubiquitously expressed gene.

INTRODUCTION

Exome and genome sequencing have been instrumental in the identification of novel variants in genes associated with disease as well as identification of novel gene–disease relationships. The latter is of great importance in providing clinical correlations in patients in whom a genetic condition is suspected, but no variants are identified based on single

gene tests, panels, microarray or karyotyping. In this paper, we describe individuals with neurological and developmental phenotypes who carry variants in the *LMBR1 domain-containing 2* (*LMBRD2)* gene. *LMBRD2* encodes a membrane-bound protein with a poorly described function, but high interspecies conservation including the ancestral *Drosophila melanogaster* and *Caenorhabditis elegans* homologues *CG8135* and *C47G2.4*, respectively. The predicted LMBRD2 protein structure shows alternating cytoplasmic, helical and transmembrane domains (figure 1) with high levels of amino acid conservation (figure 1, online supplementary figures S1 and S2). *LMBRD2* is widely expressed across tissues in humans, with notable expression in the brain in multiple species including humans, mice, *D. melanogaster* and *Xenopus laevis* suggesting a possible role in brain function and neurodevelopment (online supplementary figures S3 and S4).

LMBRD2 is paralogous to three genes: *LMBR1 domain-containing 1* (*LMBRD1*), *limb development membrane protein 1* (*LMBR1*) and *limb region 1 homologue like* (*LMBR1L*). *LMBR1* and *LMBRD1* are associated with polydactyly[1] and methylmalonic aciduria with homocystinuria,[2] respectively, and recent reports have shown that LMBRD1 is essential for gastrulation.[3] *LMBRD2* has not yet been linked to human disease, but Paek *et al*[4] have suggested that LMBRD2 might be a potential regulator of β2 adrenoceptor signalling through involvement in G protein receptor signalling. Additionally, *LMBRD2* was identified in a study under review by Kaplanis *et al*,[5] which evaluated rare (minor allele frequency <0.01%) de novo variants (variants in coding regions including synonymous variants) found in a cohort of 31 058 individuals with developmental delay for novel gene-disease associations. Taken together, this suggests a possible role for *LMBRD2* in developmental and neurological processes.

Through GeneMatcher,[6] we identified a total of 10 patients with de novo missense variants in *LMBRD2* with a broad spectrum of neurological and developmental phenotypes. The missense z-score [7] of *LMBRD2* is 2.27, suggesting that the gene is intolerant to missense variation. The case series presented here indicates that *LMBRD2* is associated with a novel multisystem disorder that predominantly affects the nervous system.

PATIENTS AND METHODS

This cohort of affected individuals was assembled using GeneMatcher.[6]Written informedconsent was obtained with ethics committee approval for individuals 1, 3–5, 7, 9, and 10. The patients from Canada (individual 2), Switzerland (individual 6) and Italy (individual 8) were examined in a diagnostic setting.

Exome sequencing or genome sequencing was performed for all individuals to investigate unexplained clinical presentations (table 1). Prior to exome sequencing or genome sequencing, all individuals had undergone at least one other type of genetic test including chromosomal microarray, Mendeliome sequencing, karyotyping and gene panel testing, none of which resulted in identification of candidate variants. Details for each individual are provided in table 1 and in the online supplementary materials.

Exome sequencing was conducted for individuals 1–3 and 5–10; individual 4 had genome sequencing. All parents also had exome sequencing or genome sequencing to determine inheritance patterns. For exome sequencing, average coverage across the affected individuals and parents was 90×, with exome capture conducted using Agilent (Santa Clara, California, USA) SureSelect technology and sequencing using Illumina HiSeq or NovaSeq (San Diego, California, USA) platforms. Variant types evaluated included SNVs and small indels within exons and exon-intron boundaries. Individual 6 also had larger multiexon CNVs evaluated. Genome sequencing for individual 4 was conducted using the Illumina HiSeq X system[8] at an average of 30× coverage that evaluated single nucleotide variants (SNVs), small indels, copy number variants and mitochondrial DNA SNVs. The *LMBRD2* variants were confirmed using Sanger sequencing in individuals 1, 3, 5, 7, 8 and 10. Additional details on the testing methodology are provided in the online supplementary materials.

In silico tools were used to evaluate the potential impact of the variants and included Primate AI,[9] SIFT, PolyPhen, Combined Annotation Dependent Depletion (CADD),[10] Revel,[11] Deleterious Annotation of genetic variants using Neural Networks (DANN)[12] and Constrained Coding Region (CCR) score.[13] Additional details on these methods are provided in online supplementary table S1. In addition, allele frequencies for each variant were assessed by querying the Genome Aggregation Database (gnomAD).

RESULTS

We identified a total of 10 individuals harbouring de novo variants in *LMBRD2*, none of which were detected in the control population from gnomAD. Five presented with unique missense variants (c.532G>A (p.Glu178Lys), c.577T>C (p.Trp193Arg), c.820A>G (p.Lys274Glu), c.976C>G (p.Gln326Glu) and c.1436T>G (p.Met479Arg)), two shared the same variant, c.367T>C (p.Trp123Arg), and three more shared another recurrent variant, c.1448G>A (p.Arg483His) (table 1). No other candidate variants were identified in all but one individual (individual 7), where three additional variants were reported, but all with very weak evidence (online supplementary materials). We observed a range of phenotypic features across individuals, with all showing signs of developmental or neurological abnormalities (table 1; online supplementary figures S5 and S6 in online supplementary materials). For example, intellectual disability and dysmorphic facial features were each noted in six individuals and microcephaly was found in seven individuals. Likewise, five individuals presented with ocular abnormalities ranging from mild features, including hyperopia and hypertelorism, to more severe presentations of congenital cataracts and microphthalmia. Six individuals also showed brain structure abnormalities (online supplementary figure S5). Brain MRIs of individuals 1–3, who harbour the recurring p.Arg483His variant, revealed a thin corpus callosum. Similarly, individual 9 (p.Met479Arg), individual 10 (p.Trp123Arg) and individual 4 (p.Trp193Arg) showed a thin corpus callosum, with white matter volume loss and delayed myelination also noted in individual 4. All the individuals with the p.Arg483His recurrent variant also presented with motor and language delays. Additional overlapping clinical features across the cohort included seizures (n=5), developmental regression (n=2) and spasticity (n=3). We also noted features described in individual patients that are consistent with a broad neurodevelopmental disorder that included hypotonia, hypertonia and short stature.

All seven variants show features consistent with a role in disease pathology. All are absent from gnomAD and five are predicted to be damaging by all seven prediction algorithms evaluated in this study (online supplementary table S1). The remaining two variants, c.532G>A (p.Glu178Lys) and c.820A>G (p.Lys274Glu), show mixed results but both have a CADD score >20,[10] which is suggestive of pathogenicity (online supplementary table S1). Using the CCR score[13] as an assessment of genomic conservation revealed that the recurrent p.Arg483His variant and the p.Trp193Arg variant fall within the 99th and 95th most conserved percentiles (online supplementary table S1). Two variants (p.Trp123Arg and p.Trp193Arg) involve a substitution of a highly conserved tryptophan to an arginine, both of which are near the cytosolic region of transmembrane domains 3 and 5, respectively (figure 1).

DISCUSSION

Here we have described 10 cases with *LMBRD2* variants with a broad spectrum of neurodevelopmental phenotypes. Five unique missense variants and two recurrent variants, c.1448G>A (p.Arg483His) and c.367T>C (p.Trp123Arg), were identified, all de novo and highly conserved. The c.1448G>A (p.Arg483His) recurrent variant was also found in three individuals in a study currently under review by Kaplanis *et al*[5] describing a cohort of 31 058 individuals with developmental delay. Evaluation of this region suggests that a CpG dinucleotide at this position might drive the mutability of this site. The probability of loss of function intolerance (pLI) score[7] is ~0 for this gene, which suggests haploinsufficiency is unlikely to be the principal disease mechanism. However, the loss-of-function observed/expected fraction provided in the Genome Aggregation Database ranges from 0.23 to 0.55, which does not completely preclude haploinsufficiency as a disease mechanism. It is more likely that the de novo variants identified here result in a gain of function, which affects G protein-coupled receptor (GPCR) regulation including the adrenergic receptor pathway.[4] Recurring variants are often associated with a gain of function or a dominant negative effect,[14] which may explain the low pLI score. Furthermore, the missense z-score of *LMBRD2* is >2. Positive z-scores are indicative of increased selective constraint. A score of 3.09 has been suggested as a threshold for significance (p=0.001). However, z-scores can vary based on the conditions evaluated, for example, average z-scores when evaluating variants in genes associated with autism spectrum disorder and intellectual disability were 1.68 and 2.68, respectively.[15] Therefore, a score of 2.27 for *LMBRD2* is indicative of relative intolerance to missense variation.

All affected individuals had an age of onset in infancy, and while there is some variability in phenotype, 9 of the 10 cases had developmental delay, which is consistent with the observations by Kaplanis *et al*.[5] Furthermore, intellectual disability (n=6), dysmorphic facial features (n=7), microcephaly (n=7), seizures (n=5) and spasticity (n=3) are also observed among several individuals. Phenotypic variability is observed even among patients harbouring the same variant. For example, spasticity or epilepsy is found in only one of the three individuals with the p.Arg483His variant while microcephaly is not present in one individual. Taken together, these data suggest that missense variants in *LMBRD2* can cause a broad-spectrum neurodevelopmental disorder.

All the variants identified here fall in different exons (online supplementary table S1), and it is possible that they have different mechanisms of action resulting in variable phenotypes with varying degrees of severity. Of note, four of the seven variants are located in a transmembrane domain (figure 1). The seven individuals with these variants appear to have a more severe phenotype. A larger cohort and functional evaluation will be required to better delineate this phenotype/genotype correlation.

LMBRD2 protein levels were shown to be strongly upregulated by β2-adrenoreceptor (β2AR) signalling agonist and to a lesser level by angiotensin II type 1 receptor agonist.[4] β2AR was mainly studied for its role concerning cardiovascular function regulation. However, consistent with the phenotype of the described individuals, β2AR is also known to regulate several neurotransmitters such as GABA[16] and Mglur[17] and has been shown to mediate synaptic plasticity and brain development.[18] In addition, like LMBRD2, β2AR is strongly expressed in astrocytes and neurons.[19] Characterising the impact of the *LMBRD2* variants on β2AR cerebral function may provide insight into its biological role and potential therapeutic avenues.

In summary, the presence of de novo missense variants in *LMBRD2*, including two recurrent variants, in the 10 affected individuals described here suggests association with a neurological phenotype. Given the high evolutionary conservation of *LMBRD2*, highly ubiquitous expression and its potential emerging role as a regulator of GPCR signalling, further studies of the mechanism of pathogenicity in these individuals may shed light on the extent of its mechanism.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We would like to thank all the individuals who participated in this study. In addition, we acknowledge the Baylor-Hopkins Center for Mendelian Genomics (NHGRI UM1 HG006542).

Funding

This study was funded by Baylor-Hopkins Center for Mendelian Genomics (NHGRI UM1 HG006542).

REFERENCES

1. Lettice LA, Horikoshi T, Heaney SJH, van Baren MJ, van der Linde HC, Breedveld GJ, Joosse M, Akarsu N, Oostra BA, Endo N, Shibata M, Suzuki M, Takahashi E, Shinka T, Nakahori Y, Ayusawa D, Nakabayashi K, Scherer SW, Heutink P, Hill RE, Noji S. Disruption of a long-range cis-acting regulator for Shh causes preaxial polydactyly. Proc Natl Acad Sci U S A 2002;99:7548–53. [PubMed: 12032320]
2. Rutsch F, Gailus S, Miousse IR, Suormala T, Sagné C, Toliat MR, Nürnberg G, Wittkampf T, Buers I, Sharifi A, Stucki M, Becker C, Baumgartner M, Robenek H, Marquardt T, Höhne W, Gasnier B, Rosenblatt DS, Fowler B, Nürnberg P. Identification of a putative lysosomal cobalamin exporter altered in the cblF defect of vitamin B12 metabolism. Nat Genet 2009;41:234–9. [PubMed: 19136951]
3. Buers I, Pennekamp P, Nitschke Y, Lowe C, Skryabin BV, Rutsch F. Lmbrd1 expression is essential for the initiation of gastrulation. J Cell Mol Med 2016;20:1523–33. [PubMed: 27061115]

4. Paek J, Kalocsay M, Staus DP, Wingler L, Pascolutti R, Paulo JA, Gygi SP, Kruse AC. Multidimensional tracking of GPCR signaling via peroxidase-catalyzed proximity labeling. Cell 2017;169:338–49. [PubMed: 28388415]
5. Kaplanis J, Samocha KE, Wiel L, Zhang Z, Arvai KJ, Eberhardt RY, Gallone G, Lelieveld SH, Martin HC, McRae JF, Short PJ, Torene RI, de Boer E, Danecek P, Gardner EJ, Huang N, Lord J, Martincorena I, Pfundt R, Reijnders MRF, Yeung A, Yntema HG, Study DDD, Vissers L, Juusola J, Wright CF, Brunner HG, Firth HV, FitzPatrick DR, Barrett JC, Hurles ME, Gilissen C, Retterer K. Integrating healthcare and research genetic data empowers the discovery of 49 novel developmental disorders. bioRxiv 2019.
6. Sobreira N, Schiettecatte F, Valle D, Hamosh A. GeneMatcher: a matching tool for connecting Investigators with an interest in the same gene. Hum Mutat 2015;36:928–30. [PubMed: 26220891]
7. Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, O'Donnell-Luria AH, Ware JS, Hill AJ, Cummings BB, Tukiainen T, Birnbaum DP, Kosmicki JA, Duncan LE, Estrada K, Zhao F, Zou J, Pierce-Hoffman E, Berghout J, Cooper DN, Deflaux N, DePristo M, Do R, Flannick J, Fromer M, Gauthier L, Goldstein J, Gupta N, Howrigan D, Kiezun A, Kurki MI, Moonshine AL, Natarajan P, Orozco L, Peloso GM, Poplin R, Rivas MA, Ruano-Rubio V, Rose SA, Ruderfer DM, Shakir K, Stenson PD, Stevens C, Thomas BP, Tiao G, Tusie-Luna MT, Weisburd B, Won H-H, Yu D, Altshuler DM, Ardissino D, Boehnke M, Danesh J, Donnelly S, Elosua R, Florez JC, Gabriel SB, Getz G, Glatt SJ, Hultman CM, Kathiresan S, Laakso M, McCarroll S, McCarthy MI, McGovern D, McPherson R, Neale BM, Palotie A, Purcell SM, Saleheen D, Scharf JM, Sklar P, Sullivan PF, Tuomilehto J, Tsuang MT, Watkins HC, Wilson JG, Daly MJ, MacArthur DG, Consortium EA, Exome Aggregation Consortium. Analysis of protein-coding genetic variation in 60,706 humans. Nature 2016;536:285–91. [PubMed: 27535533]
8. Scocchia A, Wigby KM, Masser-Frye D, Del Campo M, Galarreta CI, Thorpe E, McEachern J, Robinson K, Gross A, Ajay SS, Rajan V, Perry DL, Belmont JW, Bentley DR, Jones MC, Taft RJ, ICSL Interpretation and Reporting Team. Clinical whole genome sequencing as a first-tier test at a resource-limited dysmorphology clinic in Mexico. NPJ Genom Med 2019;4.
9. Sundaram L, Gao H, Padigepati SR, McRae JF, Li Y, Kosmicki JA, Fritzilas N, Hakenberg J, Dutta A, Shon J, Xu J, Batzoglou S, Li X, Farh KK-H. Predicting the clinical impact of human mutation with deep neural networks. Nat Genet 2018;50:1161–70. [PubMed: 30038395]
10. Rentzsch P, Witten D, Cooper GM, Shendure J, Kircher M. Cadd: predicting the deleteriousness of variants throughout the human genome. Nucleic Acids Res 2019;47:D886–94. [PubMed: 30371827]
11. Ioannidis NM, Rothstein JH, Pejaver V, Middha S, McDonnell SK, Baheti S, Musolf A, Li Q, Holzinger E, Karyadi D, Cannon-Albright LA, Teerlink CC, Stanford JL, Isaacs WB, Xu J, Cooney KA, Lange EM, Schleutker J, Carpten JD, Powell IJ, Cussenot O, Cancel-Tassin G, Giles GG, MacInnis RJ, Maier C, Hsieh C-L, Wiklund F, Catalona WJ, Foulkes WD, Mandal D, Eeles RA, Kote-Jarai Z, Bustamante CD, Schaid DJ, Hastie T, Ostrander EA, Bailey-Wilson JE, Radivojac P, Thibodeau SN, Whittemore AS, Sieh W. REVEL: an ensemble method for predicting the pathogenicity of rare missense variants. Am J Hum Genet 2016;99:877–85. [PubMed: 27666373]
12. Quang D, Chen Y, Xie X. DANN: a deep learning approach for annotating the pathogenicity of genetic variants. Bioinformatics 2015;31:761–3. [PubMed: 25338716]
13. Havrilla JM, Pedersen BS, Layer RM, Quinlan AR. A map of constrained coding regions in the human genome. Nat Genet 2019;51:88–95. [PubMed: 30531870]
14. Lelieveld SH, Wiel L, Venselaar H, Pfundt R, Vriend G, Veltman JA, Brunner HG, Vissers LELM, Gilissen C. Spatial clustering of de novo missense mutations identifies candidate neurodevelopmental disorder-associated genes. Am J Hum Genet 2017;101:478–84. [PubMed: 28867141]
15. Samocha KE, Robinson EB, Sanders SJ, Stevens C, Sabo A, McGrath LM, Kosmicki JA, Rehnström K, Mallick S, Kirby A, Wall DP, MacArthur DG, Gabriel SB, DePristo M, Purcell SM, Palotie A, Boerwinkle E, Buxbaum JD, Cook EH, Gibbs RA, Schellenberg GD, Sutcliffe JS, Devlin B, Roeder K, Neale BM, Daly MJ. A framework for the interpretation of de novo mutation in human disease. Nat Genet 2014;46:944–50. [PubMed: 25086666]

16. Deng W-K, Wang X, Zhou H-C, Luo F. L-type Ca $^{2+}$ channels and charybdotoxin-sensitive Ca^{2+}-activated K^{+} channels are required for reduction of GABAergic activity induced by β2-adrenoceptor in the prefrontal cortex. Mol Cell Neurosci 2019;101.

17. Stoppel LJ, Auerbach BD, Senter RK, Preza AR, Lefkowitz RJ, Bear MF. β-arrestin2 couples metabotropic glutamate receptor 5 to neuronal protein synthesis and is a potential target to treat Fragile X. Cell Rep 2017;18:2807–14. [PubMed: 28329674]

18. Stowell RD, Sipe GO, Dawes RP, Batchelor HN, Lordy KA, Whitelaw BS, Stoessel MB, Bidlack JM, Brown E, Sur M, Majewska AK. Noradrenergic signaling in the wakeful state inhibits microglial surveillance and synaptic plasticity in the mouse visual cortex. Nat Neurosci 2019;22:1782–92. [PubMed: 31636451]

19. Gao V, Suzuki A, Magistretti PJ, Lengacher S, Pollonini G, Steinman MQ, Alberini CM. Astrocytic β2-adrenergic receptors mediate hippocampal long-term memory consolidation. Proc Natl Acad Sci U S A 2016;113:8526–31. [PubMed: 27402767]

20. Krogh A, Larsson B, von Heijne G, Sonnhammer EL. Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. J Mol Biol 2001;305:567–80. [PubMed: 11152613]

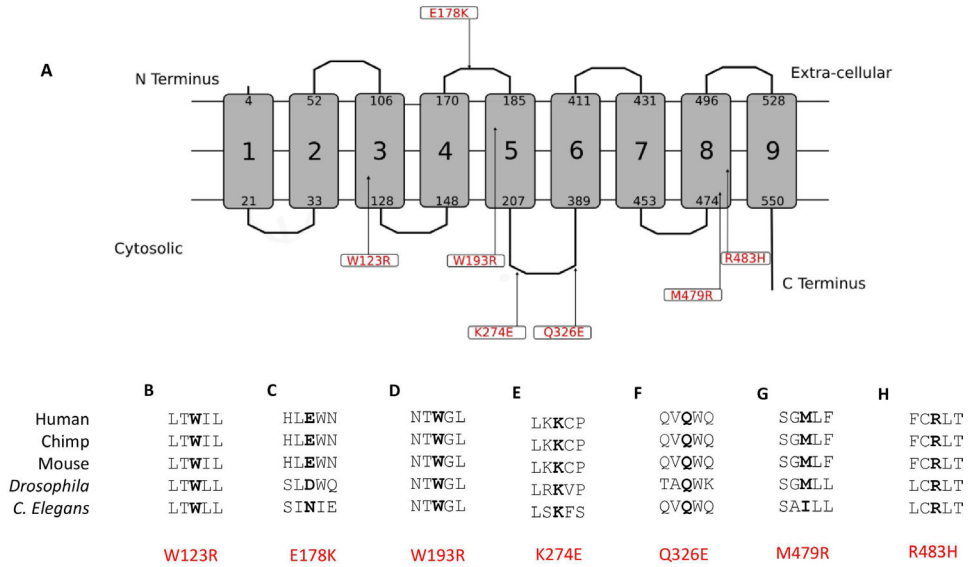

Figure 1.

(A) Schematic representation of *LMBRD2* and variants described. Membrane spanning helices were predicted using transmembrane helices hidden Markov model. [20] Conservation across species for (B) p.Trp123Arg, (C) p.Glu178Lys, (D) p.Trp193Arg, (E) p.Lys274Glu, (F) p.Gln326Glu, (G) p.Met479Arg and (H) p.Arg483His.

Table 1

Clinical features and variant information in individuals with de novo missense variants in *LMBRD2*

	Individual 1	Individual 2	Individual 3	Individual 4	Individual 5	Individual 6	Individual 7	Individual 8	Individual 9	Individual 10
Demographics										
Sex	F	F	F	M	M	M	M	M	F	F
Age of onset	Birth	Birth	4 months	4 months	2 years	Birth	Unknown	<1 year	7 months	<6 months
Age at exam	14 years	6 years	15 months	21 months	3 years	16 years	6 years	25 years	3 years	4 years
Country of origin	France	Canada	China	USA	China	Switzerland	USA	Italy	Japan	Belarus
LMBRD2 variant (chromosome 5; NM_001007527)										
Genomic position (GRCh37/hg19)	g.36115211C>T	g.36115211C>T	g.36115211C>T	g.36136581A>G	g.36141210A>G	g.36124295T>C	g.36122526G>C	g.36137380C>T	g.36116562A>C	g.36141210A>G
cDNA and amino acid change	c.1448G>A p.Arg483His	c.1448G>A p.Arg483His	c.1448G>A p.Arg483His	c.577T>C p.Trp193Arg	c.367T>C p.Trp123Arg	c.820A>G p.Lys274Glu	c.976C>G p.Gln326Glu	c.532G>A p.Glul 78Lys	c.1436T>G p.Met479Arg	c.367T>C p.Trp123Arg
Inheritance	de novo	de novo	de novo	de novo	de novo	de novo	de novo	de novo	de novo	de novo
Testing method	Exome sequencing	Exome sequencing	Exome sequencing	Genome sequencing	Exome sequencing	Exome sequencing	Exome sequencing	Exome sequencing	Exome sequencing	Exome sequencing
Phenotype										
Intellectual disability	Yes (severe)	Yes	Not specified	Not specified	Yes	No	No	Yes	Yes	Yes
Developmental delay	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes
Microcephaly	No	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes
Seizures	No	No	Yes	Yes	Yes	No	No	Yes	Yes	No
Thin corpus callosum	Yes	Yes	Yes	Yes	No	No	No	Not specified	Yes	Yes
Motor delay	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes
Language delay	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes
Dysmorphic features	Yes (mild)	Yes	No	No	No	Yes	Yes	Yes	Yes	Yes

	Individual 1	Individual 2	Individual 3	Individual 4	Individual 5	Individual 6	Individual 7	Individual 8	Individual 9	Individual 10
Ocular abnormalities	No	No	No	Yes (congenital alternating esotropia)	No	Yes (cataract, glaucoma and microphthalmia)	Yes (astigmatism, hyperopia and amblyopia)	Yes (long palpebral fissures)	No	Nystagmus

LMBRD2, LMBR1 domain-containing 2.