RESEARCH ARTICLE OPEN ACCESS

Multiplex Consanguineous Family Highlights *CLASP1* as a Candidate Gene for Lissencephaly

Rawan Alsafh, MD, Amal Alhashem, MD, Aly Elsyed, MD, Zafer Yüksel, MD, Kalthoum Graiess-Tlili, MD, Khalid Hundallah, MD, Farah Thabet, MD, and Brahim Tabarki, MD

Correspondence
Dr. Tabarki
btabarki@hotmail.com

Neurol Genet 2024;10:e200172. doi:10.1212/NXG.0000000000200172

Abstract

Background and Objectives

Noncentrosomal microtubules are essential cytoskeletal filaments that are important for neurite formation, axonal transport, and neuronal migration. They require stabilization by microtubule minus-end–targeting proteins including the CLASP family of molecules. To date, no human monogenic disorder has been associated with the *CLASP1* gene. In this study, we aimed to delineate the clinical and neuroradiologic phenotype associated with biallelic CLASP1 variants.

Methods

We analyzed clinical characteristics, MRI data, and genotypes of a cohort of 3 patients with homozygous variants in CLASP1.

Results

Homozygous *CLASP1* variant is associated with primary microcephaly, severe neurodevelopmental delay, and early-onset refractory epilepsy. The neuroradiologic phenotype comprises a highly recognizable combination of classic lissencephaly, with the posterior gradient more severe than the anterior gradient, a thin/hypoplastic splenium of the corpus callosum, mild enlargement of the lateral ventricles primarily posteriorly with a squared pattern, and pontine hypoplasia.

Discussion

This study underscores the role of CLASP1 in brain development and suggests that the identified variant disrupts CLASP1 interaction with the microtubule cytoskeleton, contributing to lissencephaly pathogenesis.

Background

Lissencephaly is a severe brain developmental disorder characterized by reduced brain folding due to underlying cortical layering defects. These aberrations arise during embryonic development owing to defective neuronal migration. Progress in molecular genetics has aided the identification of at least 31 lissencephaly-associated genes, with an overall diagnostic yield of over 80%.[1] Many of these genes encode microtubule structural proteins (tubulin) or microtubule-associated proteins, which play major roles in regulating cytoskeleton dynamics during neuronal migration.[2] Cytoplasmic linker-associated protein 1 (CLASP1) belongs to a group of proteins known as CLASPs, which are nonmotor microtubule-associated proteins that interact with Cap-Gly domain-containing linker proteins (CLIPs), members of the microtubule plus-end tracking protein family.[3] Considerable evidence now implicates CLASP1 in growth cone orientation, axon guidance, and the regulation of neuronal migration

From the Division of Pediatric Neurology (R.A., K.H., B.T.); Division of Genetics (A.A.), Department of Pediatrics, Prince Sultan Military Medical City, Riyadh, Kingdom of Saudi Arabia; Department of Pediatrics (A.E.), King Fahad Military Medical Complex, Dhahran, Saudi Arabia; Bioscientia Human Genetics (Z.Y.), Bioscientia Institut für Medizinische Diagnostik GmbH, Ingelheim, Germany; Department of Radiology (K.G.-T.), Sahloul University Hospital, Sousse; and Department of Pediatrics (F.T.), Fattouma Bourguiba University Hospital, Monastir, Tunisia.

Go to Neurology.org/NG for full disclosures. Funding information is provided at the end of the article.

The Article Processing Charge was funded by the authors.

Glossary

CLASP1 = cytoplasmic linker-associated protein 1; **WES** = whole-exome sequencing.

in mice.[4] Nevertheless, no human monogenic disorder has been associated with the *CLASP1* gene. In this study, we propose a possible phenotypic association between biallelic CLASP1 variants and lissencephaly in humans.

Methods

Whole-exome sequencing (WES) analysis was performed in 3 siblings diagnosed with lissencephaly, along with their parents. We performed this analysis with informed consent from the family, focusing on genes already associated with the clinical manifestations of the patients, including but not limited to lissencephaly and other protein-coding genes not yet associated with a phenotype. The genomic DNAs were fragmented, and the exons of known genes in the human genome and the corresponding exon-intron boundaries were enriched, amplified, and sequenced simultaneously using Illumina technology (San Diego, CA). The targeted regions were sequenced to obtain at least 20x coverage depth for approximately 99% of the regions of interest. We performed short-read whole-genome sequencing and segregation analyses in this family to rule out other possible genetic etiologies.

Standard Protocol Approvals, Registrations, and Patient Consents

Written informed consent was obtained from the guardian of the participants. This study was approved by Research Ethics Committee at Prince Sultan Military Medical City/Riyadh (IRB: 943/2023).

Data Availability

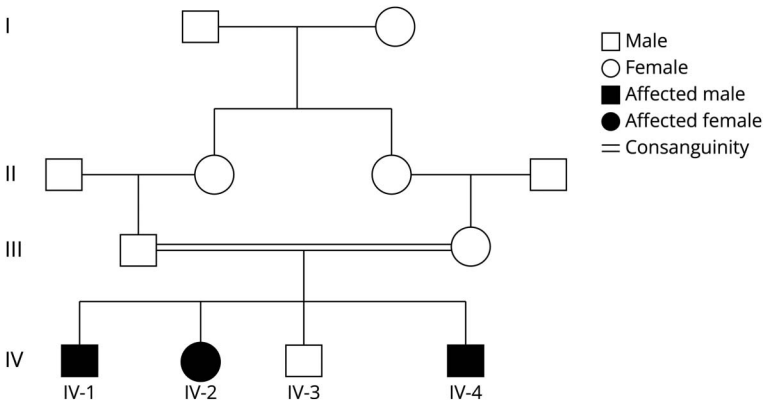
The data supporting the findings of this study are available within the article.

Results

We evaluated 3 affected siblings from a large consanguineous Saudi family (Figure 1). The Table 1 summarizes the main clinical and MRI features. WES revealed a novel homozygous variant, c.4442G>A p.(Arg1481His), in the CLASP1 gene for all 3 affected siblings (IV-1, IV-2, IV-4) and heterozygous in their healthy, consanguineous parents (III-1, III-2). Sanger sequencing revealed that the unaffected male sibling (IV-3) is a heterozygous carrier of the variant. The variant is absent in controls in the Genome Aggregation Database (gnomAD v2.1.1) (PM2). Three affected siblings (III1, III2, III4) exhibited the variant in a homozygous state, whereas the unaffected sibling (III3) and parents (II1 and II2) were heterozygous carriers, suggesting a possible autosomal recessive inheritance. In accordance, with the disease-specific ACMG/AMP guidelines for autosomal recessive segregation evidence,[5] the criteria were adjusted (PP1_Moderate). A computational analysis of the CLASP1 variant indicated that the 3 predictive tools (PolyPhen-2, Sorts Intolerant From Tolerant, and MutationTaster) used to assess the potential pathogenicity of the variant exhibited a damaging/pathogenic effect (PP3). In light of the aforementioned criteria, the CLASP1 change can be classified as a variant of uncertain significance. It is worth noting that the CLASP1 variant was the only novel homozygous coding/splicing variant shared by all 3 affected siblings identified through WES and WGS, with no variants in other lissencephaly-related genes detected in any of the sequencing analyses.

At birth, all had low weight and microcephaly, with head circumference ranging from 27 to 32 cm (<third percentile). The patients presented with clinical features of microcephaly (ranging from −3SD to −4SD), early-onset spasticity,

Figure 1 Pedigree



Pedigree of the study family showing the degree of consanguinity between the parents.

Table Clinical and Genetic Characteristic of *CLASP1*-Associated Neurophenotypes

Patient/Sex	Patient IV1	Patient IV2	Patient IV3
Neonatal parameters			
Birth weight (kg)	2	1.6	2.3
Height (cm)	45	44	46
Head circumference (cm)	30	27	32
Age at the last assessment	17 y	5 y	11 mo
Microcephaly	−4SD	−4SD	−3SD
Spasticity	+	+	+
Developmental delay	Profound	Profound	Profound
Epilepsy			
Age at onset (mo)	4	4	6
Seizure type	GTC	GTC	Tonic
EEG	Multifocal epileptiform discharges	Multifocal epileptiform discharges	Multifocal epileptiform discharges
Response to ASM	Refractory, on 3 ASM	Refractory, on 3 ASM	Fairly controlled, on 1ASM
Brain MRI	• Lissencephaly with a posterior-more-severe-than-anterior gradient • Reduced white matter volume • Splenium of corpus callosum hypoplastic • Pontine hypoplasia	• Lissencephaly with a posterior-more-severe-than-anterior gradient • Reduced white matter volume • Splenium of corpus callosum hypoplastic • Pontine hypoplasia	• Lissencephaly with a posterior-more-severe-than-anterior gradient • Reduced white matter volume • Splenium of corpus callosum hypoplastic • Pontine hypoplasia
***CLASP1* variant**	c.4442G>A	c.4442G>A	c.4442G>A
Zygosity	Homozygous	Homozygous	Homozygous
Protein	Arg1481His	Arg1481His	Arg1481His

Abbreviations: ASM = antiseizure medication; GTC = generalized tonic-clonic seizures; IUGR = intrauterine growth retardation.

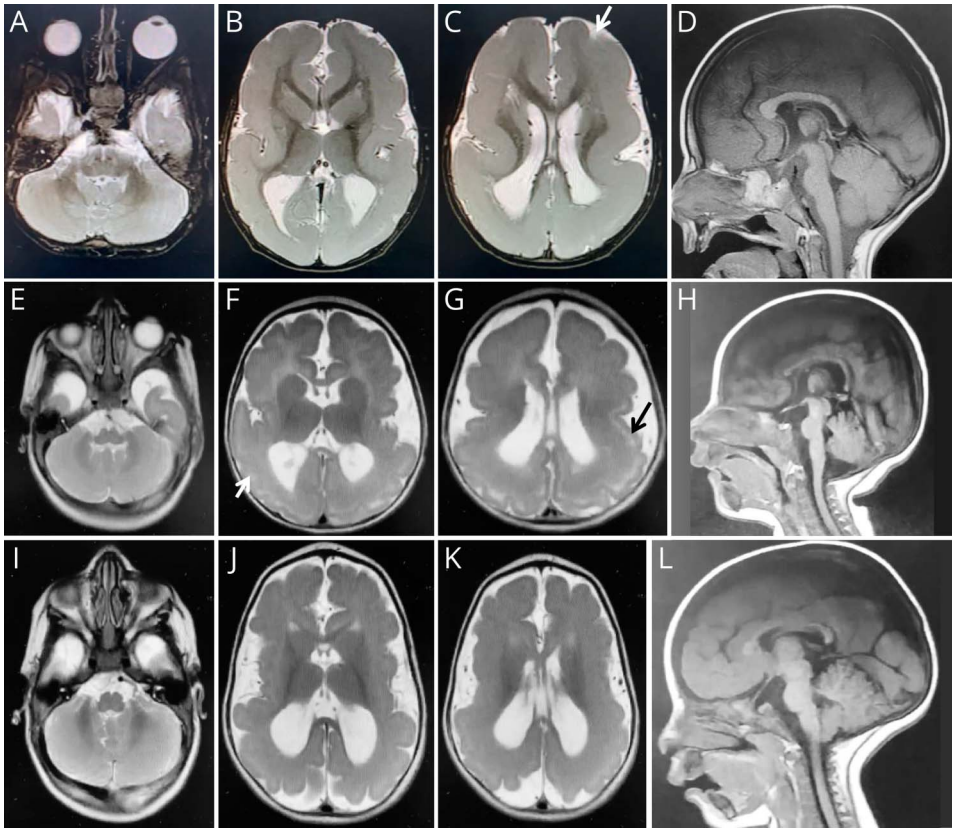
intractable epilepsy, and profound developmental delay. All 3 patients had epilepsy, with seizures starting as early as 4 months of age. Two patients had generalized tonic-clonic seizures while the other patient had tonic seizure. Two patients had medically intractable epilepsy, and the condition of one patient was fairly controlled (but he was relatively younger than the others and with limited follow-up). Neuroimaging revealed multiple brain abnormalities (Figure 2). MRI in all 3 patients showed lissencephaly, reduced white matter volume, hypoplastic corpus callosum, and a pontine hypoplasia.

Discussion

We describe 3 affected siblings from a large consanguineous family with a novel homozygous variant in the CLASP1 gene. All affected individuals presented early in life with severe to profound developmental delays, primary microcephaly, seizures refractory to antiepileptic treatment, and lissencephaly. Lissencephaly represents a spectrum of malformations in cortical development, including agyria, pachygyria, and subcortical band heterotopia.[1] CLASP1 belongs to a group of proteins known as CLASPs. These proteins are highly expressed in the CNS and are nonmotor microtubule-associated proteins that interact with CLIPs, a member of the microtubule plus-end tracking protein family that promotes the stabilization of dynamic microtubules in migrating cells.[3,6] Loss of the CLASP1 C-terminus weakness MT plus-end binding. The clinical and neuroradiologic abnormalities observed in CLASP1, such as lissencephaly with a posterior-more-severe-than-anterior gradient, thin/hypoplastic corpus callosum, and pontine hypoplasia, are analogous to the findings seen in *CAMSAP1*-related neuronal migration disorder, a phenotype consistent with a severe tubulinopathy.[7] The close alignment of neuroradiologic features between the *CAMSAP1*-related neuronal migration disorder and *CLASP1*-related lissencephaly suggests a shared pathomolecular mechanism that underlies these diseases. This observation underscores the significance of the minus end of the microtubule in neuronal migration disorders.

In conclusion, we propose that the biallelic p.(Arg1481His) variant disrupts the interaction of CLASP1 with the microtubule cytoskeleton, as a candidate gene for lissencephaly.

Figure 2 Neuroimaging in 3 Individuals With the *CLASP1*-Related Lissencephaly



Row 1 (A–D) is patient IV:1 aged 4 years. T2-weighted axial images (A–C) show posterior-more-severe-than-anterior gradient with areas exhibiting nearly absent gyration or agyria with extremely shallow frontal sulci (white arrow) and a slightly wide and shallow Sylvian fissure. Reduced white matter volume, particularly poorly developed posteriorly, and mild enlargement of the lateral ventricles, mainly posteriorly with a squared pattern. T1-weighted midline sagittal image (D) shows a thin/hypoplastic splenium of the corpus callosum and pontine hypoplasia. Row 2 (E–H) is patient IV:2 aged 2 months, and row 3 (I–L) is patient IV:4 aged 9 months. T2-weighted images (E–G and I–K) show diffuse thick cortex with reduced gyration/pachygyria, slightly more severe posteriorly, along with almost age-appropriate myelination of the reduced white matter. In figures F–G, there is thin T2-hyperintense band within the thick cortex (black arrow) representing a sparse cell layer zone between the thick arrested neuronal layer and the thin superficial molecular layer. Mild enlargement of the lateral ventricles, mainly posteriorly with a squared pattern. T1-weighted midline sagittal images (H, L) show the diffuse hypoplastic corpus callosum and pons.

Study Funding

The authors report no targeted funding.

Disclosure

The authors report no relevant disclosures. Go to Neurology.org/NG for full disclosures.

Publication History

Received by *Neurology: Genetics* May 13, 2024. Accepted in final form June 10, 2024. Submitted and externally peer reviewed. The handling editor was Deputy Editor Stefan M. Pulst, MD, Dr med, FAAN.

Appendix Authors

Name	Location	Contribution
Rawan Alsafh, MD	Division of Pediatric Neurology, Department of Pediatrics, Prince Sultan Military Medical City, Riyadh, Kingdom of Saudi Arabia	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data
Amal Alhashem, MD	Division of Genetics, Department of Pediatrics, Prince Sultan Military Medical City, Riyadh, Kingdom of Saudi Arabia	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data
Aly Elsyed, MD	Department of Pediatrics, King Fahad Military Medical Complex, Dhahran, Saudi Arabia	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data
Zafer Yüksel, MD	Bioscientia Human Genetics, Bioscientia Institut für Medizinische Diagnostik GmbH, Ingelheim, Germany	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data
Kalthoum Graiess-Tlili, MD	Department of Radiology, Sahloul University Hospital, Sousse, Tunisia	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data
Khalid Hundallah, MD	Division of Pediatric Neurology, Department of Pediatrics, Prince Sultan Military Medical City, Riyadh, Kingdom of Saudi Arabia	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data

Appendix *(continued)*

Name	Location	Contribution
Farah Thabet, MD	Department of Pediatrics, Fattouma Bourguiba University Hospital, Monastir, Tunisia	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data
Brahim Tabarki, MD	Division of Pediatric Neurology, Department of Pediatrics, Prince Sultan Military Medical City, Riyadh, Kingdom of Saudi Arabia	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data

References

1. Koenig M, Dobyns WB, Di Donato N. Lissencephaly: update on diagnostics and clinical management. *Eur J Paediatr Neurol.* 2021;35:147-152. doi:10.1016/j.ejpn.2021.09.013
2. Romero DM, Bahi-Buisson N, Francis F. Genetics and mechanisms leading to human cortical malformations. *Semin Cell Dev Biol.* 2018;76:33-75. doi:10.1016/j.semcdb.2017.09.031
3. Mimori-Kiyosue Y, Grigoriev I, Lansbergen G, et al. CLASP1 and CLASP2 bind to EB1 and regulate microtubule plus-end dynamics at the cell cortex. *J Cell Biol.* 2005;168(1):141-153. doi:10.1083/jcb.200405094
4. Sayas CL, Basu S, van der Reijden M, et al. Distinct functions for mammalian CLASP1 and -2 during neurite and axon elongation. *Front Cell Neurosci.* 2019;13:5. doi:10.3389/fncel.2019.00005
5. Oza AM, DiStefano MT, Hemphill SE, et al. Expert specification of the ACMG/AMP variant interpretation guidelines for genetic hearing loss. *Hum Mutat.* 2018;39(11):1593-1613. doi:10.1002/humu.23630
6. Cushion TD, Leca I, Keays DA. MAPping tubulin mutations. *Front Cell Dev Biol.* 2023;11:1136699. doi:10.3389/fcell.2023.1136699
7. Khalaf-Nazzal R, Fasham J, Inskeep KA, et al. Bi-allelic CAMSAP1 variants cause a clinically recognizable neuronal migration disorder. *Am J Hum Genet.* 2022;109:2068-2079. doi:10.1016/j.ajhg.2022.09.012