Novel variants identified in CKAP2L in two siblings with Filippi syndrome

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Abstract

Pathogenic variants in CKAP2L have previously been reported in Filippi syndrome (FS), a rare autosomal recessive, craniodigital syndrome characterized by microcephaly, syndactyly, short stature, intellectual disability, and dysmorphic facial features. To date, fewer than 10 patients with pathogenic variants in CKAP2L associated with FS have been reported. All of the previously reported probands have presumed loss-of-function variants (frameshift, canonical splice site, starting methionine), and all but one have been homozygous for a pathogenic variant. Here we describe two brothers who presented with microcephaly, micrognathia, syndactyly, dysmorphic features, and intellectual disability. Whole-exome sequencing of the family identified a missense variant, c.2066G > A;p.(Arg689His), in trans with a frameshift variant, c.1169_1173del;p.(Ile390LysfsTer4), in CKAP2L. To our knowledge, these are the first patients with FS to be reported with a missense variant in CKAP2L and only the second family to be reported with two variants in trans.

[Supplemental material is available for this article.]

CASE PRESENTATION

The male proband was first seen at 14-yr-old presenting with microcephaly, dysmorphic facial features including a broad forehead, micrognathia, and thin lips, abnormal hair growth patterns including broad eyebrows and long eyelashes, developmental delay, serrated incisors, 2–3 toe syndactyly, and a seizure disorder. Seizures began at age 3. Electroencephalograms (EEGs) showed an abundance of epileptiform activity, sometimes occupying 90% of the record. He was prescribed Lamictal and has not had a seizure in the past 6 yr. His most recent head circumference measurement at age 17 was 50.2 cm, a –3.8 standard deviation, and height was in the third percentile. In addition, he had small testes secondary to congenital cryptorchidism, which was repaired via orchiopexy at age 1. He was born at 41 wk after an uncomplicated pregnancy weighing 3260 g. Case 2 is the 5-yr-old brother of the proband, who is similarly affected with intellectual disability, dysmorphic facial features, microcephaly, small testes, syndactyly of his second and third toes, and congenital cryptorchidism. Given his brother’s seizure disorder, an EEG analysis was performed that found abnormal EEG activity, putting him at a higher seizure risk. However, seizures have never been documented, and he is not on an anticonvulsant. No further EEGs have been performed. He was born weighing 3266 g at 40 wk after an uncomplicated pregnancy. He has undergone a right inguinal orchiopexy, right inguinal hemia repair, and frenotomy. His most recent head circumference measurement at age 8 yr 4 mo was 48.1 cm, a –3.5 standard deviation, and height was in the sixth percentile. Both siblings’ cases of toe syndactyly were surgically repaired. The constellation of features in both patients are consistent with the...
common features shared among patients with Filippi syndrome (Table 1; see Supplemental Fig. 1). A third male sibling and both parents were asymptomatic and did not share any features with the two affected siblings. No consanguinity was reported in the family.

### Table 1. Features exhibited by the proband and his brother

| Filippi syndrome clinical featuresa | Proband | Sibling | Comments |
|-----------------------------------|---------|---------|----------|
| **Growth**                        |         |         |          |
| Low weight                        | Y       | Y       |          |
| Intrauterine growth retardation   | N       | N       |          |
| Postnatal growth retardation      | Y       | Y       |          |
| **Head and neck**                 | Microcephaly | Y | Y |          |
| Broad forehead                    | Y       | Y       |          |
| Hairy forehead                    | N       | N       |          |
| Short philtrum                    | N       | N       |          |
| **Eyes**                          | Microcephaly | Y | Y |          |
| Poor vision                       | N       | N       |          |
| Retrobulbar venous varix          | N       | N       |          |
| Proptosis                         | N       | N       |          |
| Optic atrophy                     | N       | N       |          |
| **Nose**                          | Prominent columella | N | N |          |
| Broad nasal bridge                | N       | N       |          |
| Hypoplastic alae nasi             | N       | N       |          |
| **Mouth**                         | Straight mouth | N | N |          |
| Thin lips                         | Y       | Y       |          |
| Small teeth                       | N       | N       |          |
| Abnormally shaped teeth           | Y       | Y       |          |
| Hypodontia (rare)                 | N       | N       |          |
| Serrated incisors (rare)          | Y       | Y       |          |
| **Cardiovascular**                | Ventricular septal defect | N | N | Echocardiography performed to verify absence following negative auscultation. |
| **Genitourinary**                 | Cryptorchidism | Y | Y |          |
| Ambiguous genitalia               |         |         |          |
| **Skeletal**                      | Bilateral cutaneous syndactyly of second, third, and fourth fingers | N | N |          |
| **Hands**                         | Fifth-finger clinodactyly | Y | Y |          |
| **Feet**                          | Bilateral cutaneous syndactyly of second and third toes | Y | Y | Second and third—surgically repaired in both. |
| Hypoplasia of fifth toes          |         |         |          |
| **Hair**                          | Abnormal hair growth pattern | Y | Y |          |
| Sparse hair (rare)                | N       | N       |          |
| Hypertrichosis (rare)             | N       | N       |          |
| **Neurologic**                    | Developmental delay | Y | Y | Sibling had abnormal EEG and is therefore high seizure risk, but has not had seizures. |
| Seizures                          | Y       | N       |          |
| Dystonic movements                | N       | N       |          |
| Dystonic tongue protrusion        | N       | N       |          |
| Cerebellar atrophy                | Unknown | Unknown |          |
| Arachnoidal cyst                  | Unknown | Unknown |          |
| Diffuse enlargement of subarachnoid spaces and lateral ventricles (rare) | Unknown | Unknown |          |
| Megacisterna magna (rare)         | Unknown | Unknown |          |
| MRI abnormalities                 | Unknown | Unknown |          |

*aThe list of clinical features is based on the Online Mendelian Inheritance in Man (OMIM) clinical synopsis (# 272440).
TECHNICAL ANALYSIS

An Affymetrix CytoScan HD chromosomal microarray with a resolution of 30 kb for deletions and 60 kb for duplications was performed on the proband’s younger brother by the Mayo Medical Laboratory. Data was analyzed and reported using human genome build 37.1 (hg19) and did not reveal significant copy number changes. Whole-exome sequencing was performed by the Sanford Medical Genetics Laboratory on genomic DNA isolated from both affected siblings, the mother, and the father. The unaffected sibling was not tested. Exome capture was performed using the SureSelect Clinical Research Exome kit V1 (Agilent Technologies) and sequencing was performed on an Illumina HiSeq2500 both according to manufacturers’ protocols. The sequence reads were converted to FASTQ format. Alignment, variant calling, and analysis were performing using Codified software utilizing the Burrows–Wheeler aligner (BWA) to align to the reference human genome (hg38) and Platypus to call variants. Data was interpreted and classified according to American College of Medical Genetics and Genomics (ACMG) guidelines (Richards et al. 2015). Both affected siblings were found to be compound heterozygous for a paternally inherited frameshift variant, c.1169_1173del;p.(Ile390LysfsTer4), and a maternally inherited missense variant, c.2066G > A;p.(Arg689His), in the CKAP2L gene (Table 2). Both variants and trans phase in the siblings were confirmed by targeted Sanger sequencing analysis of all family members.

VARIANT INTERPRETATION

CKAP2L contains nine exons that encode a 745-amino acid protein. The carboxy-terminal 319 amino acids encode for a cytoskeleton-associated protein-2 domain that is homologous to CKAP2, a microtubule-associated protein involved in mitotic progression (Seki and Fang 2007). To date, eight other frameshift variants in CKAP2L have been reported in the literature in affected individuals (Hussain et al. 2014; Capecchi et al. 2018; Sabir et al. 2019; Karakaya et al. 2021). All are suspected of leading to premature termination (PT) and nonsense-mediated decay (NMD) and are found in the amino-terminal half of the protein that does not encode for the CKAP2 domain, with a majority found in exon 4. The c.1169_1173del;p.(Ile390LysfsTer4) variant is consistent with these previously reported variants and is also predicted to result in NMD. It is therefore also classified as a pathogenic variant.

In contrast, the c.2066G > A;p.(Arg689His) variant is located in the last exon of the gene. It is present in the gnomAD (Genome Aggregation Database) population database only in the European (non-Finnish) population (8/113,738 alleles). The arginine at position 689 is highly conserved in vertebrate species from humans to zebrafish. Prediction algorithms

| Table 2. Genomic findings |
|---------------------------|
| Gene | Genomic location | HGVS cDNA | HGVS protein | Zygosity | Parent of origin | Variant interpretation |
| CKAP2L | GRCh37: Chr 2:113513775-113513782 | NM_152515.5 | p.Ile390LysfsTer4 | Heterozygous | Paternal | Pathogenic |
| CKAP2L | GRCh37: Chr 2:113496572 | c.1169_1173del | p.Ile390LysfsTer4 | Heterozygous | Paternal | Pathogenic |
| CKAP2L | GRCh37: Chr 2:113496572 | c.2066G > A | p.Arg689His | Heterozygous | Maternal | Variant of uncertain significance |
are discordant as to the pathogenicity of this variant (SIFT: Deleterious; PolyPhen-2: Probably Damaging; Align-GVGD: Class C0), and there are currently no studies about the effect of this variant on protein function. Therefore, the c.2066G > A;p.(Arg689His) variant is classified as a variant of uncertain significance (VUS).

DISCUSSION

Filippi syndrome (FS) was first described in 1985 in three Sardinian siblings with syndactyly, microcephaly, shared facial abnormalities, and growth and developmental delays (Filippi 1985). Since the original description, fewer than 40 cases have been reported. Additional features such as cleft palate, hypertrichosis, coarse hair, seizures, cerebellar atrophy, precocious puberty, and multiple dental and skeletal abnormalities have been reported in patients, suggesting a variable clinical presentation (Orrico and Hayek 1997; Walpole et al. 1999; Schorderet et al. 2002; Battaglia et al. 2008; Sandhu et al. 2013; Sabir et al. 2019). However, it is important to note that many of these patients either did not have molecular testing or are consanguineous and therefore these additional features may be caused by variants in other genes resulting in a blended phenotype. The molecular identification of variants in \( CKAP2L \) provides a definitive diagnosis.

At the cellular level, loss of \( CKAP2L \) protein causes a defect in spindle organization and subsequent disruption of chromosomal segregation (Hussain et al. 2014). However, studies on the mouse homolog gene \( radmis \) also suggest a gain-of-function mechanism. Yumoto et al. (2013) showed that overexpression of \( CKAP2L \) in vitro also results in defects in spindle formation and mitotic arrest through hyperstabilization of microtubules. The role of \( CKAP2L \) in neural progenitor cell division and a potential role in regulating apoptosis purportedly lead to the phenotypic microcephaly and syndactyly of FS, respectively (Hussain et al. 2014).

In summary, we present here two siblings with novel \( CKAP2L \) variants associated with FS. This is only the second family reported with two different variants in \textit{trans}. Although the frameshift variant is consistent with previously reported pathogenic variants, to our knowledge this is the first missense variant in \( CKAP2L \) reported to be associated with FS. It is possible that the c.2066G > A;p.(Arg689His) variant leads to a more stable protein resulting in the gain-of-function phenotype observed in the mouse studies. However, it is also possible that there is a yet undetected causative variant in \textit{cis} with the missense variant. Although one of the siblings had a negative single-nucleotide polymorphism (SNP) microarray result, the possibility of a genomic deletion too small to be detected by array yet also too large to be detected by exome sequencing cannot be ruled out. Additionally, the presence of a deep intronic variant leading to abnormal mRNA splicing in \textit{cis} with the c.2066G > A;p.(Arg689His) also cannot be ruled out. Additional studies are warranted to determine what affect the c.2066G > A;p.(Arg689His) VUS has on cell spindle organization and chromosomal segregation.

ADDITIONAL INFORMATION

Data Deposition and Access
The c.1169_1173del;p.(Ile390LysfsTer4) and c.2066G > A;p.(Arg689His) variants were submitted to ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) and can be found under accession numbers VCV001292042.1 and VCV001040806.1, respectively.
Ethics Statement
This study was approved by the Sanford Research institutional review board, and all participants provided written informed consent for genetic testing. The family provided written consent for publication of identifiable images.

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Author Contributions
R.J.P. and M.L.L. wrote the manuscript. L.D.-K. and J.W. reviewed the manuscript. M.L.L. analyzed and interpreted the sequencing data. L.D.-K. oversaw patient care.

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REFERENCES

Battaglia A, Filippi T, Pusceddu S, Williams CA. 2008. Filippi syndrome: further clinical characterization. Am J Med Genet A 146A: 1848–1852. doi:10.1002/ajmg.a.32400

Capecchi G, Baldassarri M, Ferranti S, Guidoni E, Cioni M, Nürnberg P, Mencarelli MA, Renieri A, Grosso S. 2018. CKAP2L mutation confirms the diagnosis of Filippi syndrome. Clin Genet 93: 1109–1110. doi:10.1111/cge.13188

Filippi G. 1985. Unusual facial appearance, microcephaly, growth and mental retardation, and syndactyly. A new syndrome? Am J Med Genet 22: 821–824. doi:10.1002/ajmg.1320220416

Hussain MS, Battaglia A, Szczepanski S, Kaygusuz E, Toliat MR, Sakakibara S, Altmüller J, Thiele H, Nürnberg G, Moosa S, et al. 2014. Mutations in CKAP2L, the human homolog of the mouse Radmis gene, cause Filippi syndrome. Am J Hum Genet 95: 622–632. doi:10.1016/j.ajhg.2014.10.008

Karakaya T, Bilgic AE, Eris D, Baser B, Mermer S, Yildiz O. 2021. Identification of a novel pathogenic variant in CKAP2L and literature review in a child with Filippi syndrome and congenital talipes equinovarus. Am J Med Genet A 185: 2198–2203. doi:10.1002/ajmg.a.62223

Orrico A, Hayek G. 1997. An additional case of craniodigital syndrome: variable expression of the Filippi syndrome? Clin Genet 52: 177–179. doi:10.1111/j.1399-0004.1997.tb02540.x

Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, et al. 2015. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 17: 405–424. doi:10.1038/gim.2015.30

Sabir A, Walker JK, Hart R. 2019. Expanding the phenotype of Filippi syndrome: a patient with early onset puberty. Clin Dysmorphol 28: 224–226. doi:10.1097/MDC.0000000000000267

Sandhu M, Malik P, Saha R. 2013. Multiple dental and skeletal abnormalities in an individual with Filippi syndrome. Case Rep Dent 2013: 845405. doi:10.1155/2013/845405

Schorderet DF, Addor MC, Maeder P, Roulet E, Junier L. 2002. Two brothers with atypical syndactylies, cerebellar atrophy and severe mental retardation. Genet Couns 13: 441–447.

Seki A, Fang G. 2007. CKAP2 is a spindle-associated protein degraded by APC/C-Cdh1 during mitotic exit. J Biol Chem 282: 15103–15113. doi:10.1074/jbc.M701688200

Walpole IR, Parry T, Goldblatt J. 1999. Expanding the phenotype of Filippi syndrome: a report of three cases. Clin Dysmorphol 8: 235–240. doi:10.1097/00019605-199910000-00001

Yumoto T, Nakadate K, Nakamura Y, Sugitani Y, Sugitani-Yoshida R, Ueda S, Sakakibara S. 2013. Radmis, a novel mitotic spindle protein that functions in cell division of neural progenitors. PLoS ONE 8: e79895. doi:10.1371/journal.pone.0079895

Competing Interest Statement
The authors have declared no competing interest.

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