Splice Mutations and Digital Anomalies Extend the Genotypic and Phenotypic Spectrum of Kim-Gusella Syndrome in PHF21A Patients

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Introduction

Kim-Gusella syndrome (KGS) is a rare neurodevelopmental disorder caused by heterozygous mutations in the *PHF21A* gene at 11p11.2. In 2012, *PHF21A* was identified as the causative gene for intellectual disability (ID) and craniofacial anomalies (CFA) through breakpoint mapping of balanced translocations and comparative deletion mapping. This discovery explained these two partial phenotypes observed in Potocki-Shaffer syndrome (PSS), a contiguous gene disorder resulting from the minimal 2.1 Mb interstitial deletion of 11p11.2. PSS also exhibits additional skeletal anomalies, including multiple exostoses caused by EXT2 and parietal foramina caused by ALX4. These three genes in the PSS region at 11p11.2 manifest full spectrum of PSS phenotypes.

Subsequent to the identification of *PHF21A* by positional cloning, 14 intragenic mutations were reported in KGS patients with additional clinical features, including autism, ADHD, and epilepsy. These mutations consisted of 11 frameshift, two nonsense, and one missense alterations.

In our present study, we present 14 unrelated KGS patients with novel variants in *PHF21A*, including six frameshift (resulting from four nucleotide deletions, one nucleotide duplication, and one nucleotide insertion), three nonsense, two missense, and three splice mutations. Notably, the identification of splice variants in this gene is novel and further supports the loss-of-function mechanism associated with KGS. Most KGS patients exhibited developmental delay, intellectual disability, learning disabilities, and language/speech delays. Additionally, several patients displayed digital anomalies such as clinodactyly, syndactyly, and tapering fingers, confirming previously reported phenotypes, and establishing these digital anomalies as novel features associated with KGS.

Clinical Photos

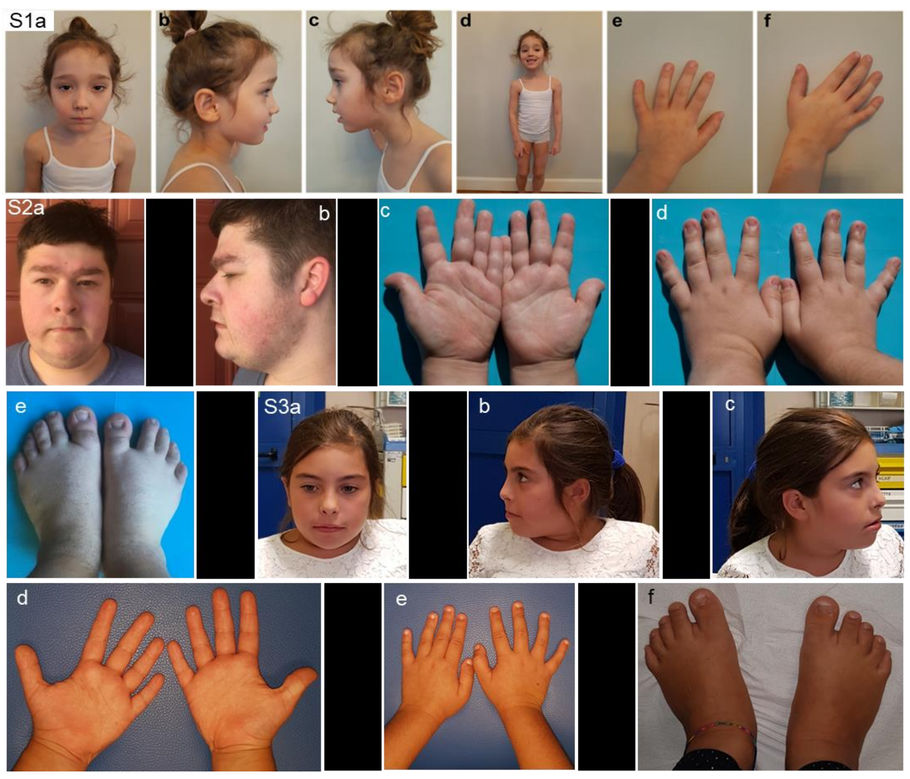

Figure 1: Facial and limb pictures of individuals with *PHF21A* variants. S1a. Facial and head appearance showing hypertelorism of eyes and short philtrum S1b-c. Lateral facial feature showing metopic ridge S1d. The posture of the whole-body. S1e-f. Right and left hands showing clinodactyly of fifth fingers. S2a. Subject 2 is a 21-year-old Hispanic male with DDM/ID (LD, speech delay) with autism, behavioral issues, anxiety, hypotonia and impaired motor skills and dysmorphic features. Subject 2 has a history of undescended testicles at birth, latent microcephaly at age 9 months, presents strabismus right eye, small short underdeveloped and highly implanted ears with narrow canals and hearing loss. Toes look short (most likely, in agreement with fingers). Hands are distinctive with peculiar appearance of fingers that are short (brachydactyly: measured F3: 15mm, palm 23.5, total 38.5), so the middle finger as percentage of total hand is 39.26 being below the 3rd centile. The vertical palmar flexion creases are short and there are Sydney lines. S3a. Subject 3 has some tendency to hypertelorism and/or telecanthus. The ala nasi looks small. S3b and c. subject 3 show in the left side, the ear is mildly posteriorly rotated and low-set. S3d. apparent asymmetric short 5th fingers, worse at left. In the left hand the index looks like the longest finger, meanwhile at rt is the middle (normal), but in S3e palms and fingers look longer except the 5th fingers (especially the right) that look short. The left index here is OK. S3f. Subject 3 shows a questionable gap between the halluces and 2nd toes, and minor apparent syndactyly between toes 2 and 3.

Conclusion

The identification of splice variants in *PHF21A* is novel and further supports the loss-of-function mechanism associated with KGS. Our findings expand the genotypic and phenotypic spectrum of KGS and enhance our understanding of the role of *PHF21A* in the pathogenesis and potentially improve diagnostic and therapeutic strategies.

Variants in PHF21A and functional domain structure

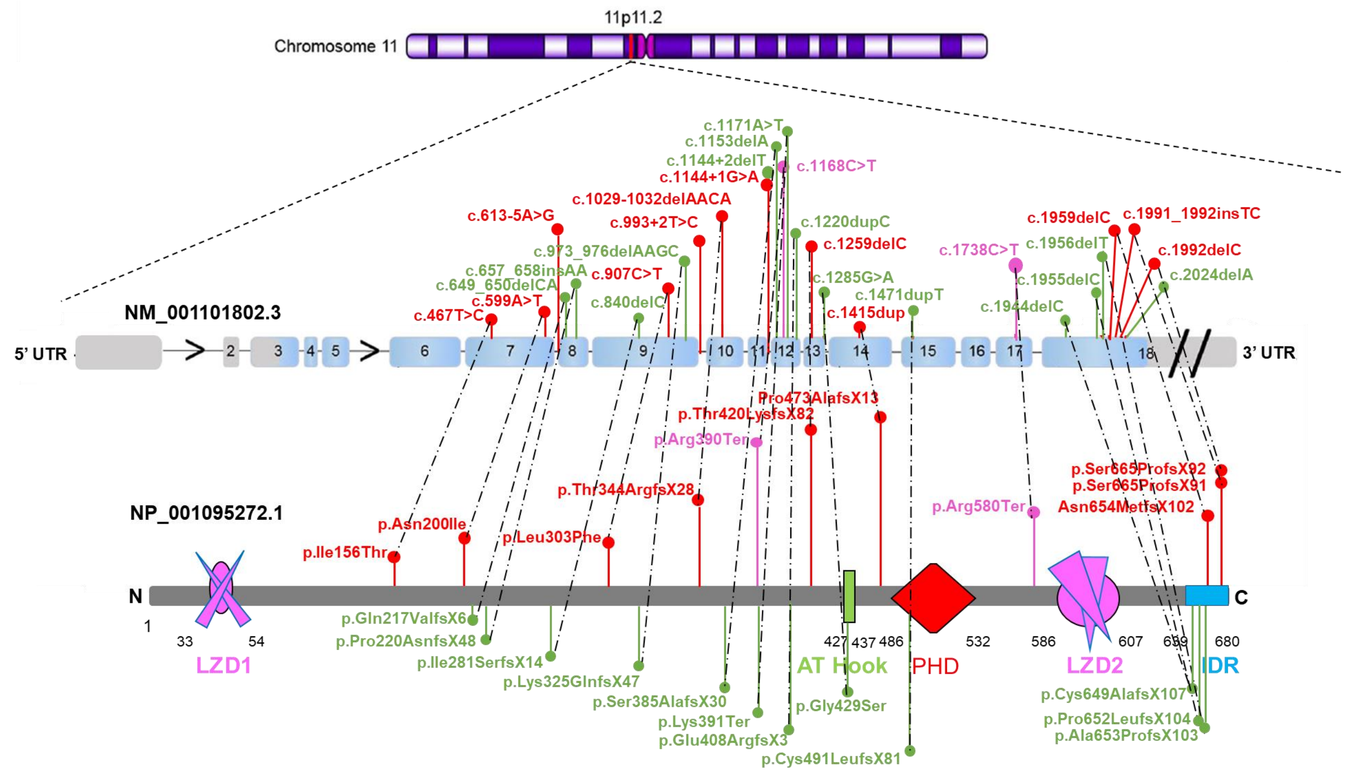

Figure 1. Mutations in *PHF21A* and domain structure of the protein. 28 mutations located in corresponding exons and introns are depicted. 18 exons are represented by light blue boxes with corresponding numbers below connected by a horizontal black line representing the introns. The arrow in the intron shows the transcription direction. The UTRs are depicted by grey boxes and the diagonal lines indicate not to scale. Note that the size of exons, mutation location, and protein domains are to scale, however, the size of the introns and UTRs are not. The identified deletions, duplication, and point mutations on the c.DNA level are depicted in red above the exons. 12 New variants found in this study are depicted in red, 14 reported variants are in green, two variants found in this study and already reported are in magenta.

Clinical features of 14 subjects with variants *in PHF21A*

Subject ID	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6	Subject 7	Subject 8	Subject 9	Subject 10	Subject 11	Subject 12	Subject 13	Subject 14
nucleotide Change (NM_001101802.3)	c.993+2T>C	c.1168C>T	c.1029-1032delAACA	c.1144+1G>A	c.1992delC	c.599A>T	c.907C>T	c.613-5A>G	c.1738C>T	c.1259delC	c.467T>C	c.1959delC	c.1415dup	c.1991_1992insTC
Exon	intron 9	exon 12	exon 10	intron 11	exon 18	exon 7	exon 9	intron 7	exon 17	exon 13	exon 7	exon 18	exon 14	exon 18
Sex	F	F	M	M	M	M	F	M	M	M	M	F	F	M
Predicted effect on protein (NP_001095272.1)	p.Thr332GlufsX45	p.Arg390Ter	p.Thr344ArgfsX28	p.Leu367SerfsX32	p.Ser665ProfsX91	p.Asn200Ile	p.Leu303Phe	abnormal splicing	p.Arg580Ter	p.Thr420LysfsX82	p.Ile156Thr	Asn654MetfsX102	Pro473AlafsX13	p.Ser665ProfsX92
Inheritance	de novo	de novo	de novo	de novo	de novo	de novo	de novo	unknown	de novo	Unknown	maternal	de novo	de novo	de novo
ACMG classification	PVS1,PS2,, PS4,PM2,PP3 Pathogenic	PVS1,PS1, PS2, PS4,PM2,PP3 Pathogenic	PVS1,PS2, PS4,PM2,PP3 Pathogenic	PVS1, PS2, PS4, PM2,PP3 Pathogenic	PVS1,PS2, PS4, PM2, Pathogenic	PS2, PS4,PM2,PP3 Pathogenic	PVS1, PS2, PS4,PM2,PP3 pathogenic	PVS1, PS4,PM2,PP3 Likely pathogenic	PVS1,PS1,PS2,PS4,PM2,PM4,PP3 Pathogenic	PVS1, PS4, PM2 Likely pathogenic	PS4, PM2, PP3 VUS	PVS1, PS2, PS4, PM2 Likely Pathogenic	PVS1, PS2, PS4, PM2 Pathogenic	PVS1, PS2, PS4, PM2 Pathogenic
CADD score	34	36	NA	34	NA	27.6	39	22.4	37	NA	23.9	NA	NA	NA
M-CAP	NA	NA	NA	NA	NA	Possibly Pathogenic	NA	NA	NA	NA	Possibly Pathogenic	NA	NA	NA
VARSOME	Likely pathogenic	Pathogenic	NA	Likely pathogenic	Pathogenic	uncertain significance	uncertain significance	Likely pathogenic	Pathogenic	Pathogenic	uncertain significance	Pathogenic	Pathogenic	Pathogenic
MAF (gnomAD)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Method of detection	trio exome	trio exome	trio exome	trio exome	trio exome	trio exome	trio exome	exome	trio exome	duo Exome with mother	exome proband only	trio exome	trio exome	trio Genome
Age	5 years and 4 months	45 years	3 years /6 years now	10 years and 6 months	8 years and 5 months	21 years	3 years and 5 months	9 months	19 years	22 years	8 years	11 years	6 years	19 years
Ancestry	NA	NA	European	NA	NA	European/hispanic	Ashkenazi Jewish\sephardic Jewish	Southeast Asian	European	NA	African Sudanese	European	European	European Italian/Hungarian
Developmental delay	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intellectual disability	+	+	+	+	+	+	+	+	+	+	+	+	_	+
Autism	_	NA	+ mild	_	+ mild	_	_	NA	_	+	_	_	+	+
ADHD	_	NA	+	+	+	_	_	_	+	_	Under evaluation	_	_	_
Epilepsy/seizures/spasms	_	NA	+	+	+	_	_	+	+	+	+	+	_	_
Cranial anomalies	+	NA	NA	_	+	NA	_	+	_	_	+	_	+	NA
Dysmorphic features	+	+	+	+ minor	+	+	+	_	+	_	NA	+	+	_
Learning disability	+	+	+		+	+	NA	NA	+	+	+	+	NA	+ Severe ID
Language/speech delay	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hand/finger/feet/toe anomalies	+	+	_	+	_	_	_	_	_	_	+	_	_	_
Behavioral problems	_	NA	+	+	+	+	_	NA	+	+	Under evaluation	_	+	+
Anxiety Disorder	+	NA	_	_	_	NA	_	_	+	+	_	+	NA	suspected
Hypotonia	+	NA	+	_	+	+	+	+	+	+	+	+	+	+
Impaired motor skills	+	NA	+	+	+	+	+	+	+	+	NA	+	+	+