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KIAA0586 is mutated in Joubert syndrome

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Abstract

Joubert syndrome (JS) is a recessive neurodevelopmental disorder characterized by a distinctive mid-hindbrain malformation. JS is part of a group of disorders called ciliopathies, based on their overlapping phenotypes and common underlying pathophysiology linked to primary cilium dysfunction. Bi-allelic mutations in one of 28 genes, all encoding proteins localizing to the primary cilium or basal body, can cause JS. Despite this large number of genes, the genetic cause can currently be determined in about 62% of individuals with JS. To identify novel JS genes, we performed whole exome sequencing on 35 individuals with JS and found bi-allelic rare deleterious variants (RDVs) in *KIAA0586*, encoding a centrosomal protein required for ciliogenesis, in one individual. Targeted next-generation sequencing in a large JS cohort identified bi-allelic RDVs in eight additional families, for an estimated prevalence of 2.5% (9/366 JS families). All affected individuals displayed JS phenotypes toward the mild end of the spectrum.

Keywords

Joubert syndrome; KIAA0586; Talpid3; ciliopathy; sonic hedgehog

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Addendum

During the review process, Roosing et al. published mutations in *KIAA0586* as a cause of Joubert syndrome (Roosing et al., 2015).

The authors have no conflicts of interest related to this work.

Joubert syndrome (JS; MIM# 213300) is a recessive neurodevelopmental disorder characterized by a pathognomonic hindbrain malformation recognized as the “Molar Tooth sign” on axial MRI and consisting of cerebellar vermis hypoplasia, thickened and horizontally oriented superior cerebellar peduncles and a deep interpeduncular fossa (Maria et al., 1997). Clinically, individuals with JS present with abnormal eye movements, respiratory control disturbances, cognitive impairment, hypotonia and ataxia (Parisi and Glass, 2003; Brancati et al., 2010). Involvement of other organ systems in various combinations is present in over 60% of individuals with JS, including retinal dystrophy, ocular coloboma, tubulo-interstitial kidney disease, liver fibrosis or polydactyly (Bachmann-Gagescu et al., 2015). JS belongs to an expanding group of disorders collectively named ciliopathies, based on their overlapping phenotypes and common underlying pathophysiology linked to primary cilium dysfunction (Badano et al., 2006). Primary cilia are microtubule-based organelles present at the surface of most differentiated cells where they are involved in transduction of sensory, chemical or mechanical signals (Singla and Reiter, 2006). Cilia are also required for transduction of signaling pathways, in particular hedgehog signaling (Goetz and Anderson, 2010), during development and to maintain homeostasis. Similar to other ciliopathies, JS is genetically heterogeneous, resulting from recessive mutations in >28 genes (one X-linked), all of which encode proteins localizing to the primary cilium and basal body (Romani et al., 2013). Despite this large number of genes, the causal mutations can currently be identified in about 62% of individuals with JS, suggesting that additional genes may be involved (Bachmann-Gagescu et al, 2015).

To identify novel genetic causes of JS, we performed whole exome sequencing in a cohort of 35 individuals with a clinical diagnosis of JS from the University of Washington (UW) Joubert Syndrome Research Program. Inclusion criteria comprised the presence of clinical findings of JS (intellectual impairment, hypotonia, ataxia and/or oculo-motor apraxia), diagnostic brain imaging findings (MTS), and lack of mutations in 28 JS-associated genes (*NPHP1*, *AHI1*, *CEP290*, *RPGRIP1L*, *TMEM67*, *CC2D2A*, *ARL13B*, *INPP5E*, *OFD1*, *TMEM216*, *CEP41*, *TMEM237*, *TCTN2*, *KIF7*, *TCTN1*, *TMEM138*, *MKS1*, *C5ORF42*, *TMEM231*, *TCTN3*, *CSPP1*, *PDE6D*, *IFT172*, *ZNF423*, *TTC21B*, *B9D1*, *B9D2*, and *C2CD3*) by targeted sequencing (O’Roak et al., 2012, Bachmann-Gagescu et al, 2015). All participants or their legal representatives provided written informed consent for the study, which was approved by the Institutional Review Boards at the UW and Seattle Children’s Hospital. Exome sequencing was performed as previously described (Chong et al., 2015) using Roche Nimblegen SeqCap EZ Human Exome Library v2.0 capture probes (~36.5 Mb of coding exons) and paired-end 50 base pair reads on an Illumina HiSeq sequencer. In accordance with the Genome Analysis ToolKit’s (GATK) best practices, we mapped sequence reads to the human reference (hg19) using the Burrows-Wheeler Aligner (BWA v. 0.6.2), removed duplicate reads (PicardMarkDuplicates v1.70), and performed indel realignment (GATK IndelRealigner v.1.6) and base-quality recalibration (GATK TableRecalibration v1.6). We called variants using the GATK UnifiedGenotyper and flagged with VariantFiltration to mark potential false positives that did not pass the following filters: Heterozygous Allele Balance (ABHet) > 0.75, Quality by Depth (QD) > 5.0, Quality (QUAL) >= 50.0, Homopolymer Run (Hrun) < 4.0 and low depth (<6X). We used SeattleSeq for variant annotation (http://snp.gs.washington.edu/

SeattleSeqAnnotation137/) and the Combined Annotation Dependent Depletion (CADD) score to determine deleteriousness of identified missense variants (http://cadd.gs.washington.edu/; Kircher et al, 2014). Based on CADD score data for causal variants in other Joubert-associated genes, we used a CADD score cutoff of 11 to define deleterious variants (Bachmann-Gagescu et al, 2015).

We identified three samples that had one or more rare (minor allele frequency <1% in the exome variant server database (EVS; http://evs.gs.washington.edu/EVS/)), predicted-deleterious variants (RDVs) in *KIAA0586* (NM_001244189.1): UW176-3 carries two rare predicted deleterious variants, a maternally inherited canonical splice site mutation (c.1413-1G>C) and a paternally inherited frameshift mutation (c.428delG, p.R143Kfs*4); the other genes with two rare variants in this sample only harbor missense or in-frame deletions of 1–2 codons.UW325-3 and UW077-3 both carry the same frameshift mutation (c.428delG, p.R143Kfs*4). Given that *KIAA0586* is the homologue of the mouse and chicken *KIAA0586* (*TALPID3)* gene, encoding a centrosomal protein required for ciliogenesis and hedgehog signaling (Bangs et al., 2011; Yin et al., 2009; Ben et al., 2011), and that we identified truncating mutations in three samples, we considered it a good candidate for further validation.

To identify further families with */KIAA0586* mutations, we used the molecular inversion probe technique (O'Roak et al., 2012, Boyle, et al., 2014) to capture all exons of this gene in 366 families, followed by paired-end 101 base pair read sequencing on an Illumina HiSeq sequencer. Using this method, we identified affected individuals in nine additional families who carried two RDVs or a homozygous RDV in *KIAA0586* (Figure 1A and Table 1). In one family, the mother carried the same homozygous p.D1360G missense variant that was identified in the affected child; this missense variant is present in ExAC (http://exac.broadinstitute.org/) in seven control samples in the homozygous state, so we excluded this family from further analysis. The variants segregated appropriately in the other six families for whom we had parental DNA (Table 1), yielding a total of nine out of 366 families sequenced (=2.5%). In seven additional individuals, we identified single rare, heterozygous truncating *KIAA0586* variants (Table 1). All mutations have been submitted to ClinVar (http://www.ncbi.nlm.nih.gov/clinvar).

At least five isoforms are described *for KIAA0586.* The canonical isoform (transcript NM_001244189.1) encodes a protein of 1644 amino acids, with 4 coiled-coil domains (predicted by N-coil, Lupas et al., 1991) and one C-terminal proline-rich region (Figure 1A). UW174-3 carries a frameshift mutation (p.E477Gfs*7) along with a synonymous substitution near a splice site (last basepair of exon 23). This synonymous variant leads to use of a cryptic splice site in exon 23 and deletion of 56 basepairs resulting in a frameshift and premature truncation of the protein (Figure 1B). Individual UW180-3 carries a homozygous missense mutation (p. D566V) with a CADD score of 27.4, supporting its pathogenicity (Kircher et al., 2014). We detected a recurrent frameshift mutation (c.428delG, p.R143Kfs*4) in 7/9 families with bi-allelic mutations (and in four additional individuals as single heterozygous *KIAA0586* variants). In 1/7 families, this frameshift is present in the homozygous state, while in 4/7, it occurs with a second frameshift mutation, and in 2/7, it occurs with a canonical splice site mutation. R143Kfs*4 is described in ExAC

and EVS with an allele frequency of 0.39%, but is never found homozygous in these databases, supporting its pathogenicity. Moreover, the prevalence of this mutation in our JS cohort (11/732 alleles = 1.5%) is significantly increased compared to those described in ExAC or EVS ($p<0.0001$, Chi-square test).

The clinical presentation of the nine affected individuals with bi-allelic *KIAA0586* mutations is relatively homogeneous, consisting mainly of JS neurological features without other organ involvement (Table 1). Only UW180-3 has polydactyly, and individual UW174-3 has coloboma, but no reported retinal, renal or liver involvement. Individual UW174-3 also has sensorineural hearing loss and an atrial septal defect. All individuals have the Molar Tooth Sign (MTS) on brain MRI and additional brain anomalies include brainstem heterotopia in two individuals (UW174-3 and UW177-3), dysplasia of the cerebellar hemispheres in one individual and a thick and dysplastic corpus callosum in a fourth individual (Figure 2). Given the relative lack of additional clinical features and the mildly abnormal neurodevelopmental outcome of three of the four individuals for whom detailed information is available, it appears that *KIAA0586* mutations may be associated with forms of JS at the mild end of the disease spectrum.

The identification of *KIAA0586* mutations in individuals with JS fits with the extensive functional work in the literature. KIAA0586 protein localizes to centrioles (Kobayashi et al., 2014) and plays a role in centrosome migration during ciliogenesis (Yin et al., 2009; Stephen et al., 2013). Ultrastructural studies of chick KIAA0586 mutant cells in the neural tube demonstrated apically located centrosomes that were not associated with a ciliary vesicle, potentially explaining the ciliogenesis defect (Yin et al., 2009). Phenotypically, mice and chicks lacking KIAA0586 display typical ciliopathy phenotypes including neural tube patterning defects, cystic kidney disease, laterality defects and polydactyly (Davey et al., 2006; Bangs et al., 2011). Originally, the association of limb patterning and neural tube defects in the chick *KIAA0586* mutant was linked to disrupted hedgehog signaling (Davey et al., 2006), and this is consistent with the now well-documented role of primary cilia in hedgehog signaling (Goetz and Anderson, 2010). Given the pervasive polydactyly in animal models and other data demonstrating a key role for KIAA0586 in sonic hedgehog signaling, it is surprising that only one of nine individuals (11%) has polydactyly, compared to 19% in our large cohort of individuals with JS (Doherty, 2009). Likewise, the nine individuals with *KIAA0586*-related JS in this study are relatively mildly affected compared to what would be expected from a major ciliogenesis defect, therefore suggesting that either *KIAA0586* is less important for development in humans compared to animal models, or that the mutations identified in our cohort are hypomorphic. In summary, we show that bi-allelic *KIAA0586* mutations are associated with relatively mild JS in ~2.5% of families with JS.

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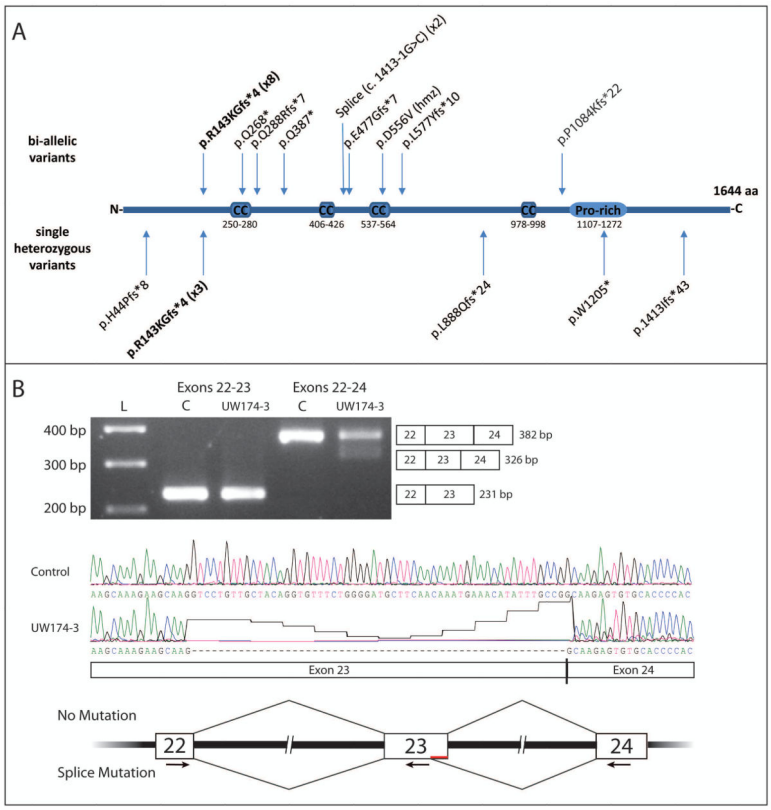

Figure 1.

(A) KIAA0586 protein ideogram with schematic position of the identified mutations. Mutations on the top row were identified in individuals with bi-allelic *KIAA0586* rare deleterious variants (RDVs), while those in the bottom row were identified only as single heterozygous *KIAA0586* RDVs. (B) Splicing studies for the synonymous-near-splice mutation detected in UW174-3: RT-PCR using the exon 22-24* primer pair (arrows in lower panel) yields a single, ~400bp product in the control (C), while in UW174-3 an additional shorter (~350bp) product is present. No difference in PCR product size is observed between the control and UW174-3 using the exon 22-23* primer pair (B, upper panel). Sequence tracings of the shorter PCR product from UW174-3 reveal a 56bp deletion of the 3′ portion of exon 23 compared to the control (B, middle panel). The longer PCR product sequence from UW174-3 was concordant with the control sequence (data not shown). The lower panel diagrams the altered splicing due to the synonymous-near-splice mutation (B, lower panel). The bar in exon 23 (red in the online version) indicates the 56bp sequence removed by aberrant splicing. *Primer sequences available upon request.

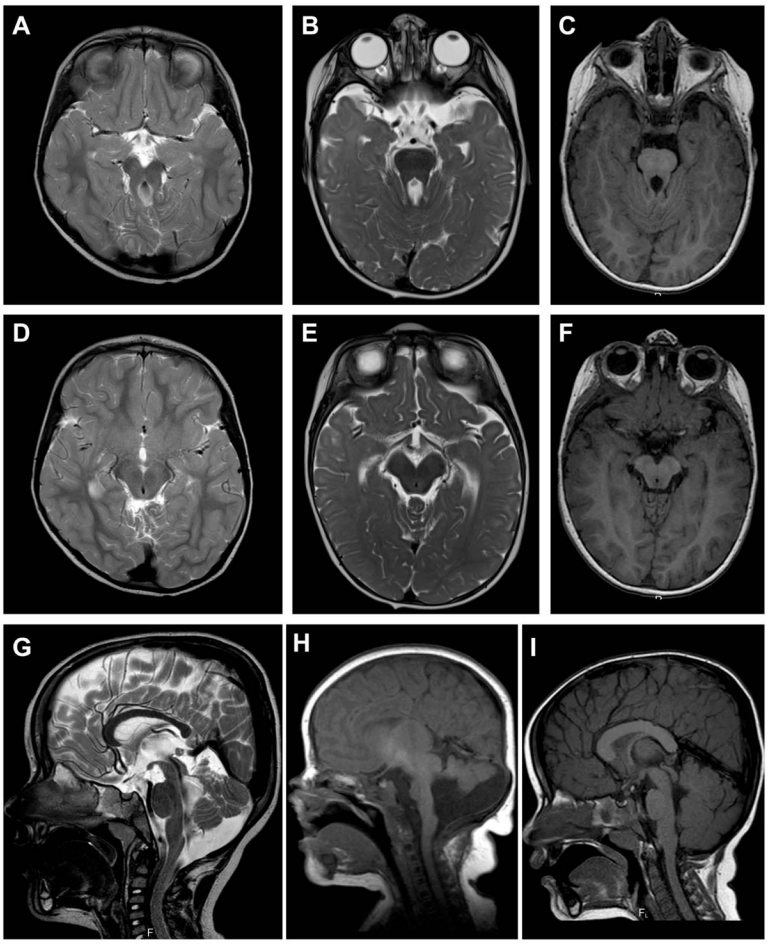

Figure 2. Brain imaging features in individuals with KIAA0586-related Joubert syndrome

(A–C) Molar tooth sign (A) and long superior cerebellar peduncles (B–C). (D–F) superior cerebellar dysplasia. (G–I) Elevated roof of the 4th ventricle (G and I) and superior cerebellar peduncle (H). Images for UW175-3 are presented in panels (A, D, G), UW177-3 in (B, E), UW179-3 in (C, F), UW174-3 in (H), and UW181-3 in (G). Panels (A, B, D, E) are axial T2-weighted images, (C, F) are axial T1-weighted images, (G) is sagittal T2-weighted, and (H, I) are sagittal T1-weighted.

Table 1

Clinical features in patients with *KIAA0586*-related Joubert syndrome

Biallelic RDVs	Mutation 1 *KIAA0586*	Mutation 2 *KIAA0586*	Age (yrs)	DD	Other brain anomalies	A/T	Abnl eye mvts	Retina	Colob	Kidney	Liver	PD	Other	Reported ethnicity	Additional RDVs in other JS genes
UW173-4	**c.428delG p.R143Kfs*4** (m)	c.1413-1G>C p.? (p)	22	mod	−	A+T	NA	−	−	−	−	−		Mixed EU	htz *IFT172* p.R1134L
UW174-3	Indel[1] p.E477Gfs*7 (m)	r.3248_3303del[2] p.P1084Kfs*22 (?)	9	sev	CM het	A+T	+	NA	+	−	−	−	SNHL ASD	Asian (Laos)	−
UW175-3	**c.428delG p.R143Kfs*4** (m)	c.1159C>T p.Q387* (?)	9	NA	Cb dysplasia	A+T	+	−	−	−	−	−		Fr CA/Native Am/ Mixed EU	−
UW176-3	**c.428delG p.R143Kfs*4** (p)	c.1413-1G>C p.? (m)	5	mild		−	+	−	−	−	−	−		Mixed EU	−
UW177-3	**c.428delG p.R143Kfs*4** (p)	**c.428delG p.R143Kfs*4** (m)	4	NA	Midbrain het	T	+	−	−	−	−	−		Middle Eastern (Lebanon)	htz *C5ORF42* p.T1598K
UW178-3	**c.428delG p.R143Kfs*4** (?)	c.863_864delAA p.Q288Rfs*7 (?)	10	NA		NA	NA	NA	NA	NA	NA	NA		Latino (Brazil)	−
UW179-3	c.1730_1734delTATCT p.L577Yfs*10 (m)	**c.428delG p.R143Kfs*4** (p)	8	mild		−	+	−	−	−	−	−		EU (Spain)	−
UW180-3	c.1697A>T p.D566V (p)	c.1697A>T p.D566V (m)	2	NA		−	NA	−		−	−	+		Middle Eastern (Turkey)	−
UW181-3	**c.428delG p.R143Kfs*4 (p)**	c.802C>T p.Q268* (m)	5	mild	dyspl CC	A+T	+	−	−	−	−	−		EU (UK)	−
Single RDVs															
UW324-3	c.4236_4239delTCTC p.L1413Ifs*43 (p)		23	NA			NA	−	+	−	NA	−	Pyloric stenosis	Mixed EU	htz *TCTN3* p.Q357R
UW325-3	**c.428delG p.R143Kfs*4** (m)		17	NA			+	−	NA	−	−	−		Mixed EU	−
UW077-3	**c.428delG p.R143Kfs*4** (m)		16	mild			+	−	−	−	−	−	sz, ADHD, PDD	AA/mixed EU	−
UW096-3	c.3614G>A p.W1205* (?)		10	NA			NA	+	NA	NA	−	+	Jeune absent R 12th rib	Trinidad, Jamaica	htz *C2CD3* p.1832G and p.G1831W[4]
UW326-3	**c.428delG p.R143Kfs*4** (?)		Unk	NA			NA	NA	NA	NA	NA	NA		Middle Eastern (Turkey)	−
UW327-3	c.130dupC p. H44Pfs*8(?)		14[3]	sev			NA	NA	NA	−	−	−	Autism, self-inj, O2	Hispanic	−
UW328-3	c.2663_2667delTGTCT p.L888Qfs*24 (m)		4	mild			+	−	+	−	−	−	GH	Mixed EU	htz *AHI1* p.Y933C

[1] c.1430_1434delAGCTAinsGAAG;

[2] chr14:g.58949430G>A, c.3303G>A;

[3] deceased,

[4] heterozygous *C2CD3* variants in *cis*

A: Apnea, AA: African American, Abnl: Abnormal, ADHD: Attention Deficit Hyperactivity Disorder, ASD Atrial Septal Defect, Cb: Cerebellar, CC: corpus callosum, CM: Cervicomedullary, Colob: coloboma, DD: Developmental Disability, EU: European, mod: moderate, Fr CA: French Canadian, htz heterozygous, inj: injury, MTS: Molar Tooth Sign, mvts movements, NA: not available, O2: supplemental oxygen, PD polydactyly, PDD: pervasive developmental disorder, R: right, sev: severe, SNHL: sensorineural hearing loss, spl: splice site, T: Tachypnea UK: United Kingdom, Unk: unknown.

(m) maternal allele, (p) paternal allele, (?): unknown parental origin

The common frameshift mutation is indicated in bold. cDNA and protein positions are indicated based on transcript ENST00000619416, NM_001244189.1, and numbering starts at the ATG codon.