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Identification of a homozygous nonsense mutation in *KIAA0556* in a consanguineous family displaying Joubert syndrome

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Abstract

Joubert Syndrome (JS) is an inherited ciliopathy associated with mutations in genes essential in primary cilium function. Whole exome sequencing in a multiplex consanguineous family from India revealed a *KIAA0556* homozygous single base pair deletion mutation (c.4420del; p.Met1474Cysfs*11). Affected siblings present a mild and classical form of Joubert syndrome allowing for further delineation of the JS associated genotypic spectrum.

Keywords

Joubert syndrome; ciliopathy; *KIAA0556*; autosomal recessive

Joubert syndrome (JS) is a neurodevelopmental disorder characterized by a distinctive midbrain-hindbrain malformation, named the ‘molar tooth sign’ on brain MRI (magnetic resonance imaging), and clinically by developmental delay, oculomotor apraxia and hypotonia. Currently, 26 genes are known to cause JS when mutated in a bi-allelic or X-linked fashion (Akizu, et al., 2014; Beck, et al., 2014; Romani, et al., 2014; Romani, et al., 2013; Roosing, et al., 2015). All known mutated JS genes encode proteins localized to the primary cilium, and generally result in defective ciliation in patient cells or in animal models (Akizu, et al., 2014; Singla, et al., 2010; Valente, et al., 2013). Here, we describe a whole

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Compliance with Ethical Standards

The authors declare that they have no conflict of interest. This study has been approved by the appropriate institutional committee and has been performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

exome sequencing study in a family with JS, leading to the identification of a frameshift mutation in *KIAA0556*, thereby further expanding the genetic spectrum of JS.

In Family 1015, two affected siblings were born to first-degree cousin Indian parents (Fig. 1a). Individual II-2, an 11-year old male, had normal delivery and birth centiles. Delays in walking initiated a neurological assessment; mild ataxia and language delay were noted at age 2. Currently, he is able to read and write with some difficulty.

Neurological assessment showed mild hypotonia, oculomotor apraxia, nystagmus and bilateral ptosis, consistent with the stable clinical course in JS. An electroretinogram revealed cone dystrophy, but gross visual function was not impaired. Kidney and liver ultrasound were normal. Brain MRI demonstrated the characteristic ‘molar tooth sign’ (Fig. 1b). Individual II-4 showed nystagmus and oculomotor apraxia by 2 years, with mild hypotonia and bilateral ptosis. Ultrasonography showed normal kidney and liver parenchyma. Dysplastic left optic disc was observed in ophthalmological examinations with slightly low amplitude of cone responses, but again visual function was intact. Brain MRI documented the ‘molar tooth sign’ along with a thin corpus callosum (Fig. 1b).

Presence of hypotonia, ataxia, oculomotor apraxia, nystagmus, developmental delay along with the characteristic ‘molar tooth’ sign on brain imaging was consistent with the Joubert syndrome clinical diagnosis in the siblings. The siblings did not present with any other systemic or dysmorphic finding than aforementioned and were therefore grouped as having classical Joubert syndrome.

Whole exome sequencing, performed in both affected siblings, identified six homozygous rare potentially deleterious variants that passed standard filtering (MacArthur et al. 2014) of which only one segregated according to a recessive mode of inheritance and was recently classified as a ciliary gene based on a whole-genome siRNA study.(Roosing, et al., 2015) A single base pair deletion c.4420del; p.Met1474Cysfs*11 in *KIAA0556* (chr16:g. 27786375del [hg19]), residing in the single overlapping homozygous region of ~2.6Mb, predicted a frameshift and premature stop codon, and nonsense mediated mRNA decay. This variant was not reported in the Exome Variant Server, the Exome Aggregation Consortium (ExAC) or the 1000 Genomes databases. In the remaining 145 cases with Joubert syndrome that whole exome sequencing was performed for no other pathogenic mutations were identified.

To validate the functional impact of the gene *in vivo*, we developed a zebrafish model by knocking down the single *kiaa0556* orthologue using a morpholino oligonucleotide. Morphant *kiaa0556* zebrafish showed curly tails, smaller head size and perithoracic and abdominal edema (Fig. 1c), similar to other ciliopathy morphants. (Lee, et al., 2012) This phenotype could be rescued when *kiaa0556* morpholino was co-injected with human full-length KIAA0556 mRNA (Fig. 1d)

The *KIAA0556* predicted protein of 1618 residues is broadly expressed (Fig. 1e) with no conserved domains or paralogues, and two small splice isoforms. Several Domains of Unknown Function (DUF) are reported in NCBI, including DUF4641 and DUF4457 but these are only found in *KIAA0556* orthologues (Fig. 1f). A single coiled-coil domain is

predicted, frequently identified in JS-encoded proteins. Recently, KIAA0556 was recognized as a microtubule associated protein located at the ciliary base. (Sanders, et al., 2015) In their study a single Joubert syndrome family was identified with a homozygous nonsense mutation. Our study further establishes KIAA0556 as a ciliary component and identifies a novel *KIAA0556* mutation in a second family diagnosed as classical Joubert syndrome.

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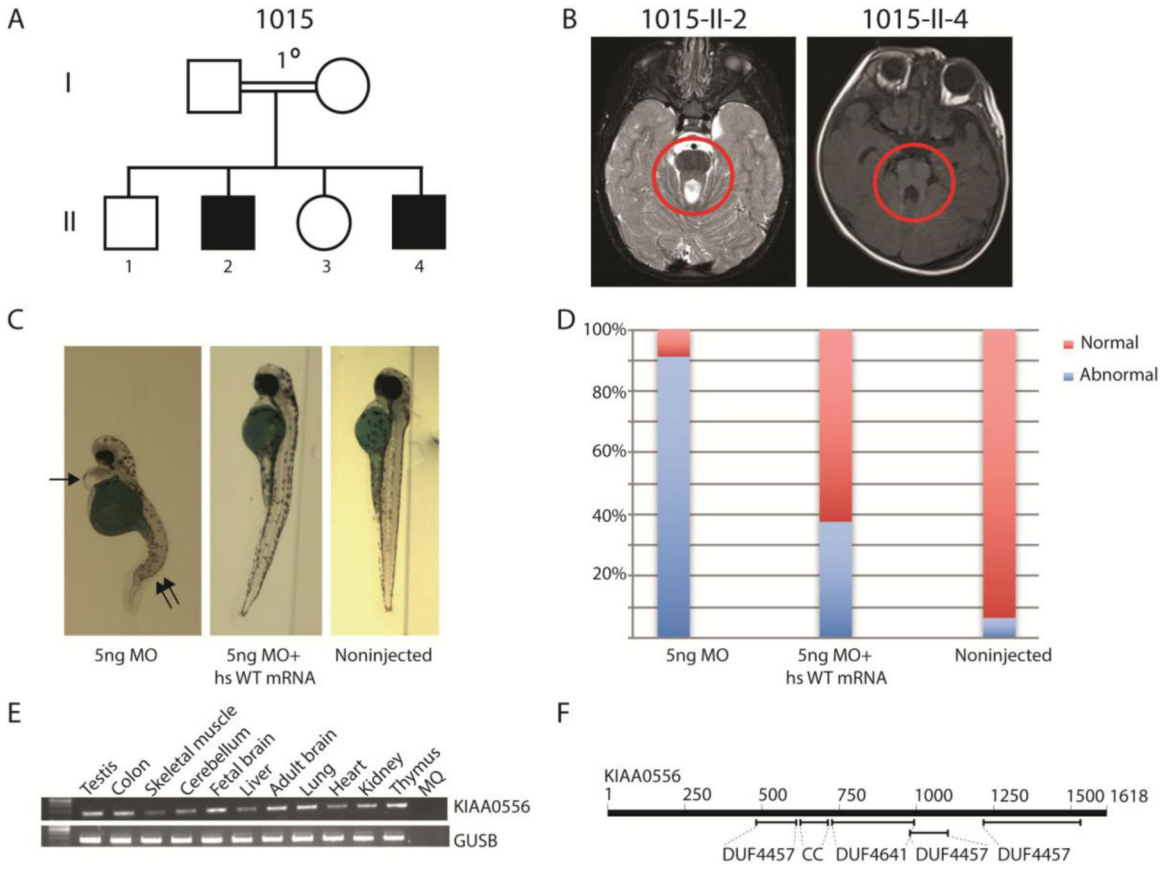

Fig. 1.

a Pedigree of the family showing two affected individuals born from a first-degree consanguineous marriage. **b** Brain MRI-cuts of the affected individuals showing the classical molar tooth sign (red circles). **c** Comparison of zebrafish injected with *kiaa0556* (5 ng) morpholino (MO), *kiaa0556* (5 ng) MO co-injected with human (hs) KIAA0556 wildtype (WT) mRNA and non-injected zebrafish 50 hours' post-fertilization. Perithoracic and abdominal edema (arrow) and curled tail (double arrow) were seen in >90% of all morphants (n>80). **d** Graphical results of phenotypes observed in *kiaa0556* (5 ng) morpholino (MO) co-injected with human KIAA0556 wildtype mRNA (200ng) and non-injected zebrafish. Analysis was performed with >80 fish per condition. **e** Expression levels of KIAA0556 in various human tissues compared to housekeeping gene GUSB. **f** Predicted protein with NCBI predicted Domains of Unknown Function (DUF) and coiled-coil domain (CC).