KIAA1109 gene mutation in surviving patients with Alkuraya-Kučinskas syndrome: a review of literature

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Abstract

Background: Alkuraya-Kučinskas syndrome is an autosomal recessive disorder characterized by brain abnormalities associated with cerebral parenchymal underdevelopment, arthrogryposis, club foot and global developmental delay. KIAA1109, a functionally uncharacterized gene is identified as the molecular cause for Alkuraya-Kučinskas syndrome. Most of the reported mutations in KIAA1109 gene result in premature termination of pregnancies or neonatal deaths while a few mutations have been reported in surviving patients with global developmental delay and intellectual disability. To our knowledge, only three surviving patients from two families have been reported with missense variants in KIAA1109. In this study, we describe four surviving patients from two related families (a multiplex family) with global developmental delay and mild to severe intellectual disability with no other systemic manifestations. There were no miscarriages or neonatal deaths reported in these families.

Methods: X-chromosome exome panel sequencing was carried out in one patient and whole exome sequencing was carried out on the remaining three affected individuals and the unaffected father of the index family. Data analysis was carried out followed by variant filtering and segregation analysis. Sanger sequencing was carried out to validate the segregation of mutation in all four affected siblings and unaffected parents from both families.

Results: A novel homozygous missense mutation in a conserved region of KIAA1109 protein was identified. Sanger sequencing confirmed the segregation of mutation in both families in an autosomal recessive fashion.

Conclusion: Our study is the second study reporting a KIAA1109 variant in surviving patients with Alkuraya-Kučinskas syndrome. Our study expands the spectrum of phenotypic features and mutations associated with Alkuraya-Kučinskas syndrome.

Keywords: Neonatal death, Premature termination of pregnancy, Prenatal diagnosis, KIAA clones, Mental retardation, Miscarriages, Developmental delay, Club foot, Arthrogryposis

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Background
Alkuraya-Kucinskas syndrome (MIM: 617822) is an autosomal recessive disorder characterized by severe brain malformations, arthrogryposis and club foot. The brain abnormalities include cerebral parenchymal underdevelopment, lissencephaly, ventriculomegaly, reduced white matter volume, corpus callosum agenesis, dysplasia of cerebellum, hypoplasia of the pons and brainstem dysgenesis. Besides, abnormalities in multiple organ systems such as cardiac, renal and ophthalmologic were reported [1]. Loss of function mutations in KIAA1109 have been reported as the cause for premature termination of pregnancies and perinatal deaths while some missense mutations were reported in surviving patients with global developmental delay and learning disabilities. The clinical features of the surviving patients with KIAA1109 mutations are distinct as compared to the features observed in fetuses/new-borns carrying KIAA1109 mutations that causes miscarriages and perinatal deaths. Surviving patients with KIAA1109 mutations exhibited global developmental delay, mild to moderate learning disability, no development of speech, inability to stand or walk without support, muscle hypotonia, atrophy, stereotypic movements, dysmorphic features and early-onset epilepsy [1]. Behaviorally, the surviving patients exhibited poor concentration, self injuring behavior such as head-banging to express anger or frustration. Thus far, three surviving patients from two families have been reported with compound heterozygous missense variants [1].

In addition to Alkuraya-Kucinskas syndrome, KIAA1109 has been reported as a significantly associated molecule in several autoimmune disorders including moderate to severe asthma [2], ulcerative colitis and celiac disease [3–5], rheumatoid arthritis [6], psoriatic arthritis [7], type 1 diabetes [8], anterior uveitis [9] and allergic sensitization [10]. It has also been identified to be associated with prostate cancer [11] and endometrial cancer [12].

In order to understand and characterize previously unidentified genes of larger size (> 50 KDa) in brain, Ohara et al and Kikuno et al cloned and sequenced 2031 cDNA clones from human brain library that includes 27 clones from fetal brain [13, 14]. The sequences of these genes are made publicly available in a repository known as Human Unidentified Gene-Encoded (HUGE) (http://www.kazusa.or.jp/huge/). As part of this project, KIAA1109 gene was first sequenced from an adult human brain library that encodes a large protein product of 5055 amino acids. The GRCh38 annotation of KIAA1109 gene in RefSeq lists one transcript (NM_015312.3) with experimental evidence and displays several other predicted transcripts including several non-protein coding transcripts of unknown significance [14]. KIAA1109 is abundantly expressed in human ovary followed by the amygdala, cerebellum, subthalamic nucleus, thalamus and spinal cord with moderate to low expression in the hippocampus, caudate nucleus, corpus callosum, substantia nigra, fetal liver and fetal brain [14]. Low expression in fetal brain was detected at protein level as shown in the Human Proteome Map [15] (https://www.humanproteomemap.org/protein.php?hpm_id=84162). The GTEx portal shows ubiquitous expression of KIAA1109 gene in 53 tissues that includes 13 different brain tissues (https://www.gtexportal.org/home/gene/KIAA1109). KIAA1109 gene was discovered two decades ago, however, it remains uncharacterized. However, the published studies on this gene support its possible role in embryonic development [16] and the regulation of phagocytosis [17]. Tweek, the Drosophila ortholog of KIAA1109 is involved in regulating synaptic vesicle recycling [18], thus suggesting its role in brain related functions.

In this study, we report four individuals from two related Indian families affected with intellectual disability and global developmental delay. Exome sequencing revealed a novel potentially pathogenic mutation in KIAA1109 gene that well segregated in multiple members tested in this family. While several studies reported mutations in KIAA1109 in fetal demises and neonatal deaths, this is the second study reporting a novel KIAA1109 mutation in surviving patients with intellectual disability.

Methods
In this study, we aimed at identifying the genetic cause of intellectual disability in four patients from two related families. Blood samples were collected from the parents and affected individuals. To identify the potentially pathogenic variants, we first carried out G-banded karyotyping in two patients, chromosome microarray analysis in two patients and exome sequencing in affected and unaffected members in the families. Sanger sequencing was performed to confirm the segregation of the potentially pathogenic variant identified in the family.

Blood sample collection and karyotyping
Blood samples were collected from three affected male siblings from the index family and an affected male maternal cousin and the parents from both families. DNA isolation was carried out using QIAamp DNA minikit (Qiagen) as per the instructions given in the manufacturer’s protocol. G-banded karyotyping was first carried out on two affected individuals (F1.III-2 and F2.III-2) representing one patient in each family, as described previously [19].

Chromosome microarray analysis
In order to identify larger genomic alterations, chromosomal microarray (CMA) analysis was performed on two
patients (F1.IV-1 and F2.IV-2) representing one patient in each family, using a CytoScanTM 750 K array (Affymetrix, CA, USA). This microarray contains a comprehensive list of oligonucleotide probes (750 K) including a unique set of non-polymorphic probes (550 K) and a collection of bi-allelic single nucleotide polymorphism (SNP) probes (200 K) that enables genome wide screening of the variants. Analysis was carried out according to manufacturer’s protocol. Approximately, 250 ng of genomic DNA isolated from each of two affected individuals was digested with a restriction enzyme NspI. The digested fragments were then ligated with adapter sequences. These fragments were then PCR amplified using a single pair of primers that recognize the adapter sequences. Titanium Taq amplified PCR products of size 120 bp to 2000 bp were purified using AMP pure beads and fragmented to a range of product size of 25 bp to 125 bp. Subsequently, the fragmented PCR products were end labelled with biotin and the amplified and size selected products were then hybridized on CytoScan 750 K gene chip, and then scanned. Data analysis was performed using Chromosome Analysis Suite v1.2 (ChAS) (Affymetrix Inc., USA) based on human reference genome (GRCh37/hg19) using default parameters. The quality filtered raw data is subjected to CNV analysis. To identify the genes in CNVs and to evaluate the pathogenicity of the identified CNVs, we queried the following databases: i) Database of Genomic Variants (DGV) (http://dgv.tcag.ca/dgv/app/home), ii) DECIPHER (https://decipher.sanger.ac.uk/), iii) UCSC Genome browser (http://genome.ucsc.edu/) and iv) PubMed (http://www.ncbi.nlm.nih.gov/pubmed). In addition to the CNV analysis, loss of heterozygosity (LOH) analysis was also carried out using ChAS software.

**Exome sequencing**

In order to identify single nucleotide variants and small insertions and deletions, we carried out exome sequencing on all affected indivuduals and an unaffected father (II-2). X-chromosome exome panel (X-panel) sequencing was carried out on the index patient and whole exome sequencing was carried out on the remaining three patients and unaffected father of index family. X-panel and whole exome sequencing and data analysis were carried out as described previously [19]. Briefly, paired end sequencing (2 × 150) reads were generated with a target depth of coverage of 100x. Quality filtered raw reads were aligned to the human reference genome (hg19) and post alignment quality recalibrated reads were subjected to joint variant calling across all five datasets. Variants were annotated using ANNOVAR [20] and rare variants with minor allele frequency < 0.01 were retained after comparing the variants with variants in 1000 genomes project [21], Exome Aggregation Consortium (ExAC) [22] and gnomAD (https://www.biorxiv.org/content/10.1101/531210v2). Subsequently, potential pathogenicity of the identified variants were evaluated using the publicly available tools such as SIFT [23], Polyphen-2 [24], MutationTaster-2 [25] and CADD [26]. Further, autosomal recessive variants, compound heterozygous variants and X-linked recessive variants were identified from filtered variants data. Compound heterozygous variants were identified using the following filtering steps: i) heterozygous variants in unaffected father and the same heterozygous variant in the patients, ii) a different heterozygous variant in the same gene found in patients but not present in the father which could have either been inherited from the mother or occurred as de novo mutations and iii) the potentially pathogenic nature of variants were interpreted manually.

**Sanger sequencing**

Sanger sequencing was performed on the potentially pathogenic KIAA1109 gene variant using the following procedure: Sanger confirmation was done on the index family (three affected siblings and their parents) and their cousin’s family (one affected individual and his parents). Primer Quest Tool (http://eu.idtdna.com/Primerquest/Home/Index) was used to design the primers (5′-TGCCAAGCGAGCTATGTGAAG-3′ and 5′-ATGTAAAGTCCTTGAACACACCA-3′) to amplify a 726 bp region of KIAA1109. OneTaq 2X Master Mix was used as per the manufacturer’s protocol. The DNA molecules were denatured at 95 °C for 30 s, followed by 35 cycles of denaturation at 95 °C for 30 s. The primers annealing temperature was set to 60 °C for 30 s and extended for 45 s at 68 °C. The final extension was at 68° for 5 min. The PCR products were cleaned and Sanger sequencing was carried out. The sequences obtained from sequencing were aligned to the reference genome to look for the mutations using BLAST tool. The chromatograms were analyzed manually to visualize the KIAA1109 mutation to study its segregation in the patients and obligate carrier parents from both families.

**Results**

**Patients**

The family described here is a multiplex family from the state of Karnataka in India. The multiplex family comprises four adult males affected with intellectual disability and global developmental delay. Two male siblings (F1.II-2 and F2.II-3) in this multiplex family had consanguineous marriages with the daughters (F1.III-1 and F2.III-4) of one of their sisters (II-4). The index family (F1) comprises three affected male siblings (F1.IV-1, F1.IV-2 and F1.IV-3). The second family (F2) comprises one affected male (F2.IV-2) and two unaffected females (Fig. 1a). The degree of intellectual disability varies
among the affected siblings. The parents and the females in both families are phenotypically normal. The clinical photographs describing the phenotypic features of patients F1.IV-2 and F2.IV-2 representing each family is depicted in Fig. 1b.

All four boys were born by uncomplicated vaginal delivery following uneventful gestation. Patients F2.IV-2 and F1.IV-3 displayed delays in gross motor milestones with the former learning to walk independently only by 3 years, and the latter by about 18 months. These two patients also had delayed language milestones, learning to say two words by only about 5 and 7 years, respectively.

The four boys were brought to medical attention at the same time after the parents noticed F1.IV-1 underperform at school. They were brought to our clinic about 10 years ago when their ages were 28 (F1.IV-1), 21 (F1.IV-2), 24 (F1.IV-3), and 13 (F1.IV-3). They were diagnosed with intellectual disability (ID) of varying severity, with F2.IV-2 recording the lowest IQ of 29 as a case of severe ID. While F1.IV-3 was diagnosed with mild to moderate ID, it was also noted that he showed signs of social anxiety. F1-IV-1 was noted to show some dysmorphic features including a high arched palate with low set ears. None of the patients suffers from any other systemic illness.

Patients F1.IV-2 and F2.IV-2 were re-examined recently, however, the other two siblings declined to be assessed. F2.IV-2 at 24 years of age continues to have severe ID, with his speech restricted to phrases and short sentences. While he can read the clock and sign his name, he is unable to count beyond ten. His parents reported that he could care for himself, but being shy and asocial, he did not help with any outdoor errands. He
also had a history of being impulsive and irritable with occasional headbanging. Though bashful during the examination he smiled when making eye contact. He had a lanky appearance with an elongated face, his arm span matching his height. His oral cavity showed a high-arched palate and protruding teeth on large gums. He had cubitus valgus and a palmar simian crease (right hand). Neurological examination revealed decreased muscle mass but was otherwise unremarkable. The rest of the systemic examination did not reveal anything significant.

At 31 years of age, F1.IV-2 is also intellectually disabled, though to a milder degree and he was more cooperative and amenable to being examined. He spoke

| Table 1 Clinical phenotypic features of all four patients |
|-----------------|-----------------|-----------------|-----------------|
|                 | F1.IV-1 | F1.IV-2 | F1.IV-3 | F2.IV-2 |
| Age             | 38      | 31      | 23      | 24      |
| Miscarriages/   | Nil     | Nil     | Nil     | Nil     |
| neonatal deaths |         |         |         |         |
| Labour          | FTND    | FTND    | FTND    | FTND    |
| Delayed         | Yes     | Yes - mild | Yes - mild | Gross delay |
| Milestones –    |         |         |         |         |
| general         |         |         |         |         |
| Walking         | 1.5 years | 1.5 years | 1.5 years | 3 years |
| independent     |         |         |         |         |
| Speaking two    | 1.5 years | 1.5 years | 6–7 years | 4–5 years |
| meaningful      |         |         |         |         |
| words           |         |         |         |         |
| Other salient   | NS      | NS      | NS      | Brain imaging done ~ 5 y back, reportedly abnormal, records not available |
| historical points |         |         |         |         |
| ID severity     | Mild    | Mild    | Moderate with expressive speech delay | severe |
| IQ              | ND      | 57      | 43      | 29      |
| Behavior        | Shy, uncooperative | Cooperative, self-care fair | Social anxiety | Impulsive, irritable, short tempered, stubborn, occasional head-banging, shy/asocial |
| Height/cm       | 163     | 161     | Could not be measured | 164 |
| Head Circumference/cm | Could not be measured | 82       | Could not be measured | 72     |
| Chest Circumference/cm | Could not be measured | 82       | Could not be measured | 72     |
| Mid-arm         | Could not be measured | 24       | Could not be measured | 18     |
| Circumference/cm |         |         |         |         |
| Vitals          | Could not be measured | BP 124/80 mmHg | Could not be measured | BP 100/60 mmHg |
| Major           | Nil     | Nil     | Nil     | Nil     |
| Congenital      |         |         |         |         |
| Anomalies       |         |         |         |         |
| Minor           | High arched palate, low set ears, prominent eyebrows, curly hair, mild beaking of the nose | High arched palate | Could not be examined | Malformed dentition, high arched palate, pointed ear on rt. side, long face, simian crease rt. hand, long thumb, small sub-mental region, cubitus valgus, high-arched foot |
| Congenital      |         |         |         |         |
| Anomalies       |         |         |         |         |
| External        | Normal  | Could not be examined | Could not be examined | normal |
| genitalia       |         |         |         |         |
| Neurological    | No apparent abnormalities | No apparent abnormalities | No apparent abnormalities | Muscle bulk less, rest of the systemic examination. No apparent abnormalities |
| Examination,    |         |         |         |         |
| salient findings |         |         |         |         |

Patients F1.IV-2 and F2.IV-2 were examined on 20 Dec 2019. The physical examination findings of the other two patients are from the archives. None of the patients has a history of seizures or other systemic illnesses. NS Nothing significant, ND Not done
| Reference     | This study, F1.IV-1 | This study, F1.IV-2 | This study, F1.IV-3 | This study, F2.IV-2 | Gueneau, et al., 2018 [1] | Gueneau, et al., 2018 [1] | Gueneau, et al., 2018 [1] |
|---------------|---------------------|---------------------|---------------------|---------------------|--------------------------|--------------------------|--------------------------|
| **Sex, Age**  | Male, 38 yo         | Male, 31 yo         | Male, 23 yo         | Male, 24 yo         | Male, 13 yo              | Female, 7 yo              | Female, 11 yo              |
| **Mutation**  | g. 123140678A > G; g. 123140678A > G; c.2431A > G (hom) | g. 123140678A > G; g. 123140678A > G; c.2431A > G (hom) | g. 123140678A > G; g. 123140678A > G; c.2431A > G (hom) | g. 123140678A > G; c.2431A > G (hom) | Chr4:123160823; Chr4:123170727; c.5599G > A (het) | Chr4:123160823; Chr4:123170727; c.5599G > A (het) | Chr4:123164200; Chr4:123171679; c.5873G > A (het) |
| **Protein Change** | p.Thr811Ala | p.Thr811Ala | p.Thr811Ala | p.Thr811Ala | p.Tyr1329Cys and p.Val1867Met | p.Tyr1329Cys and p.Val1867Met | p.Met1573Ile and p.Arg1958Cln |
| **ID**        | Mild               | Mild               | Moderate           | Severe             | Severe                   | Severe                   | Moderate                   |
| **Neuropsychiatric** | Delayed motor milestones, shy and uncooperative | Delayed motor milestones, delayed motor milestones, expressive speech delay, delayed motor milestones, social anxiety | Delayed motor milestones, expressive speech delay, delayed motor milestones, social anxiety | Delayed motor milestones, expressive speech delay, delayed motor milestones, social anxiety | Global developmental delay, no language, cannot stand or walk without support, early onset epilepsy | Global developmental delay, no language, cannot sit or stand without support, stereotypic movements, early onset epilepsy | Global developmental delay, mild to moderate learning disability, poor concentration, immature behavior with minor self-harm (head-banging) when angry/frustrated |
| **Imaging**   | Not done           | Not done           | Not done           | Not available       | Post-natal brain MRI: small posterior fossa arachnoid cyst, discrete vermian atrophy, slight increase in the fluid-filled retro- and infracer-ebellar space and mild enlargement of subarachnoid spaces of frontal regions | Post-natal brain MRI: discrete parenchymal rarefaction involving the frontal lobes | Prenatal imaging (US and MRI): major microcephaly (HC −5 SD) with reduced white matter volume and mild ventriculomegaly |
| **Head and neck** | High arched palate, low set ears, prominent eyebrows, mild beaking of the nose | High arched palate | – | Malformed dentition, high arched palate, pointed ear on right side, long face, simian crease right hand, long thumb, small submental region | Plagiocephaly, refractive errors of the eyes, delayed dentition | Plagiocephaly, refractive errors of the eyes, strabismus | Hypertelorism, slightly upslanting palpebral fissures, ocular motor apraxia, refractive errors of the eye, strabismus, dental crowding, high palate |
| **Skeletal System** | – | – | – | Cubitus valgus, high-arched foot, reduced muscle bulk | Mild contractures of large joints, syndactyly of 2nd and 3rd toes, limb paresis at birth, talipes valgus, muscle hypotonia and atrophy | Mild contractures of large joints, paretic position of hands and feet in infancy, talipes valgus, muscle hypotonia and atrophy | Asymmetry of the thorax, mild bilateral talipes, syndactyly of 2nd and 3rd toes, 5th toe clinodactyly, hallucus valgus |
| **GI**        | –                   | –                   | –                   | –                   | –                        | Chronic constipation       | Gastroesophageal reflux |
| **Heart**     | –                   | –                   | –                   | –                   | –                        | Tetralogy of Fallot        | Tetralogy of Fallot with pulmonary atresia |
| **Urogenital**| –                   | –                   | –                   | –                   | Scrotal hypoplasia        | –                        | –                        |
clearly and coherently in short sentences. He was gain-
fully employed in the family business of weaving. Phys-
ical examination revealed a high arched palate, but the
rest of the examination was unremarkable.

Table 1 describes the clinical findings from all four pa-
tients. A detailed comparison of phenotypic features of
previously reported surviving patients and the pheno-
types observed in these patients along with the identi-
fied/reported mutations are provided in Table 2. Unlike
previously reported patients with KIAA1109 mutations,
there is no unexplained intrauterine fetal demise in these
families. These patients did not have any overlapping
features of patients reported previously with perinatal
deaths.

Karyotyping and chromosome microarray analysis
In order to look for any chromosomal abnormalities, G-
banded karyotyping was carried out on two affected sib-
lings F1.IV-2 and F2.IV-2 representing one patient in
each family. The chromosomes were normal with 46,
XY complement. Since the patients exhibited non-
syndromic phenotypes including developmental delay
and intellectual disability, the diagnostic yield of G-
banded karyotyping is ~3% and chromosome microarray
analysis is often recommended as a first-tier diagnostics
test [27]. CytoScan 750 K Array was used since it has a
comprehensive list of probes with higher sensitivity and
specificity to detect copy number variations in two af-
ected individuals representing one in each family. Kar-
yotypes were checked first which revealed normal chromo-
osomes with 46, XY complement, consistent with G-
banded karyotyping. CNV analysis was carried out
using ChAS software and identified with CNVs and
LOH regions. CNVs devoid of gene content or reported
in general population were removed and the clinical
relevance of the identified CNVs were evaluated
manually. The CNVs identified in both the patients were
compared and we found that there is no potentially
pathogenic CNVs addressing the phenotypes observed in
the patients. Similarly, LOH analysis also did not yield
any significant clinically relevant variants.

Exome sequencing and identification of a novel mutation
in KIAA1109
Based on the inference from the pedigree, because only
males are affected, we initially carried out X-panel
sequencing of the index patient (F1.IV-1) expecting X-
linked recessive inheritance. Data analysis revealed no
significant X-linked variants that fit the phenotypes ob-
served in the patients. Whole exome sequencing was
then carried out on the remaining patients (F1.IV-2,
F1.IV-3 and F2.IV-2) and unaffected father (F1.II-2) of
the index family (F1). Joint variant calling identified a
total of 1,613,810 unfiltered variants. Exonic, and splice
site variants were retained and common variants with
minor allele frequency < 0.01 were removed. A list of
qualifying rare variants are provided in Supplementary
Table 1. After applying autosomal recessive mode of inher-
tance pattern, a novel variant g.123140678A > G was
identified that was found to be a homozygous in all three
affected individuals (F1.IV-2, F1.IV-3 and F2.IV-2) and heterozygous in the unaffected father (F1.II-2). This vari-
ant is located at 4q27, a locus of autoimmune disorders
[28]. This mutation was found in exon 19 of KIAA1109
gene (c.2431A > G) resulting in a missense variant alter-
ing threonine to alanine at position 811, p.Thr811Ala in
KIAA1109 protein (RefSeq: NM_015312.3 (transcript)
and NP_056127.2 (protein)) (Table 2). KIAA1109 en-
codes for a large protein that has no known functional
domains or motifs. Although KIAA1109 gene is discov-
ered two decades ago, it remains functionally uncharac-
terized. This variant is not reported in dbSNP, 1000
genomes project, ExAC and gnomAD databases.
Polyphen-2 predicted the effect of p.Thr811Ala mutation
as probably damaging (Score: 0.994), SIFT predicted the
effect of the mutation as damaging (score: 0.05). Mutati-
onTaster predicted the effect of the mutation as disease-causing (score: 1).

In order to validate the KIAA1109 variant
g.123140678A > G and to study the segregation of muta-
tion in the family, we carried out Sanger sequencing on
the three affected individuals in the index family (F1.IV-
1, F1.IV-2, F1.IV-3), their parents (F1.II-3, F1.III-1), af-
fected cousin (F2.IV-2) and his parents (F2.II-3, F2.III-4).
The chromatograms of Sanger results confirmed the
homozygous mutation in all affected patients and het-
erozygous mutation in all unaffected parents confirming
the segregation of mutation in the family (Fig. 2a &
Fig. 2b). Conservation analysis of a region spanning
p.Thr811Ala across species shows the conservation of
the mutated residue and the residues around the site of
the mutation (Fig. 3a).
Discussion

KIAA1109 is a large and evolutionarily conserved protein with no known domains or motifs. A number of mutations in KIAA1109 have been reported in miscarriages and/or neonatal deaths and only one study has reported surviving patients with KIAA1109 mutations (Fig. 3b).

**KIAA1109 mutations causing miscarriages and neonatal deaths**

The association of KIAA1109 with neurogenic disorders was first reported from a single family of a large cohort study of 143 consanguineous families comprising of two female patients (deceased) with Dandy-Walker malformation, hydrocephalus, flexed deformity, club feet, micrognathia, and pleural effusion [29]. Another study identified a large homozygous deletion spanning exons 28 to 55 in four neonatal deaths with patients exhibiting severe arthrogryposis and axillary pterygium [30]. The reason for death and age of death were not indicated. A subsequent study screening 44 families with terminating pregnancies identified two unrelated families with KIAA1109 mutations: i) a truncating mutation was...
reported in a patient with hydrocephalus, hypoplastic cerebellum, skin edema and bilateral talipes with a history of two preceding intrauterine fetal deaths at 6 months of pregnancy with similar presentations (severe hydrocephalus, spina bifida, and polyhydramnios) and ii) a splice site mutation was identified in a patient with hydrocephalus and arthrogryposis [16]. Subsequently, the same group published 13 patients from 10 families with KIAA1109 mutations in fetuses with miscarriages and neonatal deaths. In addition to these, this study has reported three surviving patients with global developmental delay [1]. In a Russian family with a history of two miscarriages, two intronic compound heterozygous variants were identified both altering splicing leading to premature termination of the translation with a deletion of 46 amino acids of KIAA1109 protein [31]. These studies indicate that KIAA1109 plays a crucial role in fetal development. Although fetal malformations can be detected in utero using advanced imaging techniques, molecular diagnosis provides additional clues for a more accurate diagnosis.

**KIAA1109 mutations in surviving patients with intellectual disability and developmental delay**

Interestingly, only one previous study has reported missense mutations in KIAA1109 in surviving patients in a Lithuanian family with a male and a female children of ages 13 and 7 years, respectively exhibiting severe developmental delay and no speech (Table 2) [1]. Another British family with an 11 years old child exhibiting global developmental delay and mild to moderate learning disabilities has been reported (Table 2) [1]. We report here the third family with KIAA1109 mutation in surviving patients with global developmental delay and intellectual disability (Table 2). Unlike the other surviving cases reported, none of the patients in this study had major congenital anomalies or musculoskeletal involvement such as congenital heart disease, arthrogryposis, ocular manifestations or dysmorphic features. Thus, compared to surviving cases reported earlier, these patients had much milder phenotypes with varying degrees of ID as the
predominant feature that expands the genotype and phenotype spectrum of Alkuraya-Kučinskis Syndrome.

Conclusions
Our description of the four surviving patients significantly expands the phenotypic features of Alkuraya-Kučinskis Syndrome. Our study is the second study to provide evidence for a role of KIAA1109 in intellectual disability in surviving patients. Identification of such mutations differentiating the mutations responsible for fetal demise and intellectual disability will greatly enhance the accuracy of prenatal diagnosis to aid informed decision making by prospective parents. A high degree of precision is essential for accurate genetic counseling and the pursuit of preventive options such as preimplantation genetic diagnosis and prenatal diagnosis. Molecular experiments to understand and differentiate the regions of KIAA1109 protein in manifesting these groups of disorders are warranted.

Supplementary information
Supplementary information accompanies this paper at https://doi.org/10.1186/s12881-020-01074-2.

Additional file 1: Supplementary Table S1. List of qualitative rare variants identified from four patients and unaffected father of index family.

Abbreviations
MIM: Mendelian inheritance in man; CDNA: Complementary deoxyribonucleic acid; HUGE: Human unidentified gene-encoded; GRCh38: Genome reference consortium human build 38; BWA: Burrows-Wheeler aligner; PCR: Polymerase chain reaction; GVCF: Genomic variant call format; GATK: Genome analysis toolkit; ExAC: Exome aggregation consortium; gnomAD: Genome aggregation database; CADD: Combined annotation dependent depletion; CMA: Chromosome microarray; SNP: Single nucleotide polymorphism; ChAS: Chromosome analysis suite; CNV: Copy number variation; DGV: Database of genomic variants; LOH: Loss of heterozygosity;_ucsc: University of california santa cruz; BLAST: Basic local alignment search tool; ID: Intellectual disability

Acknowledgements
The authors thank the families for participating in this study and for providing samples and written informed consent. We declare that the preprint of abstract of this manuscript is made available in Authorea website.

Authors’ contributions
BM and SCG designed the study, revised and edited the manuscript. BM analyzed the data, interpreted the results and wrote the manuscript. KK and BM helped SCG for the clinical assessment of the patients, wrote clinical details of the patients were obtained from the parents from both families as all four patients lacked mental capacity to consent. Due to this, we obtained written informed consent to participate in this study from the parents of both families.

Consent for publication
Written informed consents for publication of identifying images and the clinical details of the patients were obtained from the parents from both families as all four patients lacked mental capacity to consent.

Competing interests
The authors declare no competing interests.

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Received: 2 March 2020 Accepted: 19 June 2020
Published online: 26 June 2020

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