A novel c.2T > C mutation of the KDM5C/JARID1C gene in one large family with X-linked intellectual disability

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ABSTRACT

Mutations in the KDM5C gene (lysine (K)-specific demethylase 5C; also known as JARID1C and SMCX; MIM 314690) were recently associated with X-linked intellectual disability (XLID). To date only two case reports and five studies that screen for mutations in the KDM5C gene have been published, with 21 mutations reported. Herein we present a large family with XLID caused by a novel mutation c.2T > C in the start codon of the KDM5C gene, presumably leading to loss of gene translation. Six sibs out of seven (two sons and four sisters) and their mother carry this mutation. Two affected males presented the distinctive clinical phenotype, characterized by moderate short stature, clumsy gait, ataxia, increased muscle tone and brisk tendon reflexes. They constantly bore a happy and smiling facial expression, with a protruding tongue. We hereby offer the first thorough description of five affected females with the KDM5C gene mutation. Most frequent clinical features were short stature, facial dysmorphism and developmental problems. X-chromosome inactivation study showed completely skewed inactivation pattern of mutation-carrying chromosome in all affected female patients.

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1. Introduction

In the past 20 years great advances have been made in identifying the molecular basis of X-linked intellectual disability (XLID). Although the human X-chromosome carries only about 4% of the protein-coding genes in the human genome, X-linked gene defects are thought to be responsible for about 8–12% of the intellectual disability (ID) found in males [1]. Mutations in more than 80 genes have been associated with an XLID phenotype [1–3]. Jensen et al. [4] screened brain expressed genes from the Xp region in 210 families with XLID, and identified seven different mutations (nonsense, missense and frame-shift) in KDM5C (lysine (K)-specific demethylase 5C; also known as JARID1C or SMCX; MIM 314690) gene. This gene contains 26 exons and encodes a transcription factor that possesses several DNA binding motifs and shows histone demethylation activity specific for dimethylated and trimethylated lysine 4 of histone H3 [5–7]. KDM5C is expressed in all human tissues, including the brain, heart, skeletal muscles, liver, pancreas and lungs [4,8]. Nevertheless, brain tissue, in particular fetal brain tissue, has higher levels of KDM5C transcript than other tissues [9].

Thus far five reported studies have highlighted the role of KDM5C as a cause of XLID [4,10–13]. Two additional case reports have been described [14,15], bringing the total number of known mutations and described families to 21. We present a new large family with a novel mutation c.2T > C (g.1135T > C) in the start codon of the KDM5C gene.

2. Family report

2.1. Affected males

2.1.1. III-9 (Fig. 1), 22-year-old male A.K.

He was born at term with a low birth weight of 2700 g (−2 SD), length 49 cm (−1 SD) and head circumference 34 cm (−1 SD). Apgar scores were 3/6/8. Developmental delay was noticed during the first year of life. At the age of 4.5 years a strong suspicion of Angelman syndrome (AS) was raised due to the following clinical...
features — severe developmental delay, profound speech impairment, ataxia, jerky movements, happy disposition with frequent laughing, protruding tongue, prognathia, strabismus, hyperactive deep tendon reflexes and short stature (Fig. 2a). A generalized seizures disorder developed in this patient a few years later. Metylation-specific PCR of the SNRPN exon 1 region was carried out, excluding deletion, uniparental disomy and imprinting mutation in the 15q11-13 region. In addition, exons 7–16 of the UBE2A gene were sequenced, but no AS causing mutation was found.

When 10 years later a brother (III-15) with similar clinical features, including microcephaly, was born, our principal clinical diagnosis remained atypical AS. However, given the pedigree structure (Fig. 1), we also considered an X-linked cause for their ID. Two XLID genes — MECP2 and SLC9A6, which may cause atypical AS, were directly sequenced, but no pathogenic mutations were detected [16].

At the age of 20 years he had severe ID (by Griffiths Scales), profound speech impairment, short stature — height 155 cm (−3.5 SD), weight 51.5 kg (−2.5 SD) and head circumference 55 cm (−1.5 SD), a constantly happy and smiling face, deep-set eyes, a high nasal bridge, divergent strabismus, a high palate, dysmorphic ears, brachydactyly, mild ataxia, a clumsy gait, increased muscle tone, brisk tendon reflexes, aggressiveness and skin pigmentation abnormalities (Figs. 2b and 3a). Brain MRI showed cavum septum pellucidum.

2.2. Affected females

2.2.1. II-8, 42-year-old female S.K., the mother of affected sibs

She had short stature — height 153 cm (−3 SD), obesity (BMI of 32.5), normal head circumference of 54 cm (−1 SD), deep-set and almond-shaped eyes, divergent strabismus, a high and broad nasal bridge, a high palate, mildly dysmorphic ears, a smiling face and brachydactyly (Fig. 4a). She has finished 4 classes in primary school, which corresponds to mild ID.

2.2.2. III-12, 17-year-old female R.K.

She was the first of the monozygous triplets, and was born prematurely at 34 weeks of gestation. Her birth weight was 1320 g (−2.5 SD), length 40 cm (−2.5 SD), and Apgar scores were 6/7/8. Speech delay was noticed at the age of 2 years.

At the age of 16 years she had moderate ID (by Griffiths Scales), a height of 150.5 cm (−3 SD), weight 42.5 kg (−2 SD), head circumference 53.5 cm (−1 SD), a high and narrow forehead, deep-set and almond-shaped eyes, epicanthic folds, strabismus, a high and broad nasal bridge, a high and narrow palate, mildly dysmorphic ears, joint laxity, slim fingers and toes and clinodactyly of toes II–III (Fig. 4b).

2.2.3. III-13, 17-year-old female J.K.

She was the second of the monozygous triplets. Her birth weight was 1800 g (−1.5 SD) and length 43 cm (−1.5 SD). Her development has been in the normal range. She is studying at a regular school.

At the age of 16 years she had a height of 158 cm (−1.5 SD), weight 43 kg (−2 SD), head circumference 53 cm (−1.5 SD), a high forehead, a high and broad nasal bridge, a high and narrow palate, mildly dysmorphic ears, joint laxity, slim fingers and toes (Fig. 4c).

2.2.4. III-14, 17-year-old female K.K.

She was the third of the monozygous triplets. Her birth weight was 1600 g (−2 SD), length 40 cm (−2.5 SD), and Apgar scores were 5/7/8. She started to walk at 12 months. Speech development was delayed, and therefore speech therapy was provided. IQ was evaluated with Wechsler Intelligence Scale for Children and specific learning disabilities were diagnosed at school-age.
At the age of 16 years her height was 155 cm (−2 SD), weight 43 kg (−2 SD), and head circumference 53 cm (−1.5 SD). She is studying in a regular school, but needs some assistance. Phenotypically she has a high forehead, almond-shaped eyes, a high and wide nasal bridge, a high and narrow palate, mildly dysmorphic ears, slim fingers and toes (Fig. 4d). Brain MRI was normal.

2.2.5. III-16, 5-year-old female M.K.
She was born at term with birth weight 3200 g (−0.5 SD) and length 48 cm (−1 SD). Early development has been normal.
At the age of 3.5 years she had developmental delay (by Griffiths Scales), relatively short stature − 92.5 cm (−1.5 SD), her weight was 13.5 kg (−1.5 SD) and her head circumference was 47.5 cm (−2 SD). She had speech delay, mixed-type developmental problems, deep-set eyes, a broad nasal bridge, a happily smiling face and mildly dysmorphic ears (Fig. 4e). Her brain MRI was normal.

2.3. Healthy investigated family members
The father (II-9), one of the sisters (III-11) and her son (IV-1) had normal mental development and growth.

3. Material and methods

3.1. Mutational analysis of KDM5C gene
The KDM5C gene consists of 26 exons and is located at Xp11.2. The KDM5C gene encodes seven transcripts, four of which are protein coding.
Genomic DNA was extracted, and the complete KDM5C coding sequence and adjacent splice sites were amplified on the Rotor-Gene 5-PLEX PCR cycling in 32 independent PCR reactions from genomic DNA. Following the PCR reaction, a High Resolution Melt (HRM) analysis in the presence of the Syto® 9 dye was performed. When an aberrant HRM pattern was detected, PCR products were sequenced in both directions using the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA) according to the manufacturer’s instructions, and sequencing reactions were analyzed on an ABI3130 sequencer (Applied Biosystems).
HRM analysis and direct sequencing of exon 1 (using primers 5'-GCCTAGCTTGGACATT and 5'-ACACTTGGCGCTGGCAT) revealed a point mutation in translation start codon ATG, converting it into ACG and presumably leading to null function.
The pathogenicity of the sequenced mutation (in silico analysis) was evaluated using the following programs: (a) Sorting Intolerant From Tolerant (SIFT) http://sift.jcvi.org/www/SIFT_BLink_submit.html [17]; (b) PolyPhen: prediction of functional effect of human nsSNPs (http://genetics.bwh.harvard.edu/pph) [18]; (c) PMut (http://mmb2.pcb.ub.es:8080/PMut) [19], and NCBI Comparative Genomics Developments database (http://www.dcode.org/).

3.2. X-chromosome inactivation studies

For evaluating X-inactivation status, the androgen receptor (AR) (CAG)_n variable repeat region was used. X-chromosome inactivation (XCI) pattern was determined by comparative quantitative detection of fluorescent-labeled PCR products using intact and methylation-sensitive restriction enzyme HpaII-digested DNA as template [20]. The inactivation status of the KDM5C gene was assessed by PCR amplification of the first exon using intact and HpaII-digested DNA.

The study was approved by the Research Ethics Committee of the University of Tartu. Informed consent was obtained from the children’s parents.

4. Results

In one large family with XLID a novel change (T > C) at nucleotide 2 in exon 1 of the KDM5C gene was found, resulting in a change in the start codon Methionine to Threonine (p.Met1Thr) (Fig. 1). This mutation was found in the mother, who passed it to two of her sons and four of her daughters, and was not identified in healthy family members. According to the NCBI Comparative

Fig. 3. (a) Affected male patient III-9 at the age of 20 years; (b) affected male patient III-15 at the age of 10 years.
Genomics Developments database, the beginning of exon 1 of the KDM5C gene is a highly conserved gene region. Based on this data and results of mutation impact prediction programs, this amino acid substitution was considered to be clinically significant, as it would lead to loss of translation of KDM5C.

The XCI study with AR methylation assay showed that the X-chromosome carrying the c.2T>C mutation (maternal allele) had a completely skewed (100%) inactivation pattern in all affected female patients (4 sibs) available for analysis (mother carried the same number of CAG repeats in both alleles and was therefore uninformative in this analysis). XCI pattern was random in the healthy daughter. However, the KDM5C gene methylation assay showed that the KDM5C gene is completely unmethylated in both X-chromosomes in all investigated females regardless of their clinical status (PCR amplification of the KDM5C first exon failed completely after DNA treatment with methylation-sensitive restriction enzyme HpaII).

5. Discussion

In this paper we present a large family with XLID caused by a novel mutation c.2T>C in the KDM5C gene, affecting six sibs out of seven and their mother. In the described family two affected males presented a distinctive clinical phenotype characterized by a moderate short stature, clumsy gait, ataxia, increased muscle tone and brisk tendon reflexes (Fig. 3). Their faces bore a constantly happy and smiling appearance with a protruding tongue, especially when younger (Fig. 2). We summarized the clinical features of previously described male cases in Table 1 [4,10–15]. Profound verbal deficiency (80%), short stature (64%) and spasticity (42%); but also epilepsy (28%), microcephaly (28%), aggressive behavior (28%), dysmorphic features (24%) and strabismus (20%) have been most frequently noted in previous publications.

The variable phenotype of KDM5C mutations may be determined by the location of the mutation. The mutation in our described family (c.2T>C) is a novel mutation that replaces a translation start codon Methionine with Threonine in KDM5C, and therefore no KDM5C protein is synthesized. This may also be the cause of a more pronounced and distinctive clinical phenotype in our described family. Jensen et al. [8] have shown that nonsense mutations impair KDM5C activity and thereby also disturb the function of its target genes. However, we cannot exclude the possibility that some downstream methionine is used as an alternative translation start codon leading to the synthesis of a KDM5C protein with a different N-terminal sequence.
Herein we offer the first thorough description of five affected females with the KDM5C gene mutation. Most frequent clinical features were facial dysmorphism (100%) (Fig. 4), developmental problems (80%) and short stature (60%) (Table 1). However, monozygous triplet females (III-12, 13 and 14) were born prematurely. Therefore, we cannot fully exclude that this prematurity has had an impact on their developmental history. Few authors have described KDM5C gene mutation carrier females with ID, but no detailed clinical description has been given [10,15]. Regardless of the extremely skewed inactivation of the X-chromosome with the mutated KDM5C, our affected females still have clinical symptoms. Johnston et al. [21] have shown that in human lymphoblast cell lines 94.6% of X-chromosome genes are dosage compensated, so that similar expression is achieved in males and females. Nevertheless, a subset of six genes (including KDM5C) that escape X-inactivation, are expressed in considerably higher level in females than in males [21]. Similar difference in the expression pattern of Kdm5c was found in male and female adult mice brain [9]. Therefore, mutations abolishing KDM5C protein synthesis from one allele can still affect female phenotype in spite of otherwise skewed inactivation of the mutation-carrying chromosome.

The mother transmitted the c.2T > C mutation in KDM5C gene to four out of five offsprings (one is monozygous triplet pregnancy). This may be accidental, but it is tempting to speculate that there may be some contributing factor(s) that influenced the cell division in meiosis.

Of 354 tested families from the European XLID Consortium, pathogenic mutations in KDM5C were found in twelve, which corresponds to a mutation frequency of 3.4% for XLID [4,13]. Thus, KDM5C appears to be one of the more frequently mutated genes in patients with XLID, and its mutation frequency is comparable to that described for the creatine transporter SLC6A8 (MIM 300036) [22–24].

**Conflict of interest**

The authors declare no conflict of interest.

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