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A novel nonsense mutation in *KDM5C/JARID1C* gene causing intellectual disability, short stature and speech delay

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ABSTRACT

Mutations in the Jumonji AT-rich interactive domain 1C (*JARID1C/SMCX/KDM5C*) gene, located at Xp11.22, are emerging as frequent causes of X-linked intellectual disability (XLID). *KDM5C* encodes for a member of an ARID protein family that harbors conserved DNA-binding motifs and acts as a histone H3 lysine 4 demethylase, suggesting a potential role in epigenetic regulation during development, cell growth and differentiation. In this study, we describe clinical and genetic findings of a Brazilian family co-segregating a novel nonsense mutation (c.2172C > A) in exon 15 of *KDM5C* gene with the intellectual disability phenotype. The transition resulted in replacement of the normal cysteine by a premature termination codon at position 724 of the protein (p.Cys724X), leading to reduced levels of *KDM5C* transcript probably due to nonsense mediated mRNA decay. The clinical phenotype of the proband, who has two affected brothers and a mild cognitively impaired mother, consisted of short stature, speech delay, hyperactivity, violent behavior and high palate, besides severe mental retardation. Our findings extend the number of *KDM5C* mutations implicated in XLID and highlight its promise for understanding neural function and unexplained cases of XLID.

Intellectual disability (ID), formerly known as mental retardation (MR), is characterized by substantial limitations in both intellectual functioning and adaptative behavior before the age of 18 years, and has long been recognized as a common and etiologically heterogeneous disorder, affecting 1–3% of the general population [4,12]. Over 10% of ID cases with a Mendelian inheritance pattern have been assigned to the X chromosome, even though it carries only about 4% of all human protein-coding genes, leading to a marked excess of hemizygous males with intellectual disability as compared to females [15]. Up to now, more than 90 genes have been associated to X-Linked Intellectual Disability (XLID) [6]. Therefore, mutation frequencies in the majority of them are low, especially for the non syndromic forms of XLID (NS-XLID), which represent ~2/3 of all XLID cases. Consequently, many of the genetic determinants of NS-XLID remain to be elucidated. Mutations that give rise to NS-XLID are not evenly distributed along the X-chromosome and seem to be clustered in regions Xp11, Xp22 and Xq26-q28 [16]. Genes located at these regions have been shown to encode for proteins involved in signaling pathways, responsible for regulating cytoskeleton organization and synaptic efficacy, besides acting in other essential functions, like chromatin remodeling dynamics [19].

Conversely to other NS-XLID genes, mutations in Jumonji AT-rich interactive domain 1C (*JARID1C/SMCX/KDM5C*; MIM #300534), a non-pseudoautosomal brain-expressed gene located at Xp11.22, have emerged as a potential common cause for XLID. *KDM5C* contains 26 exons and encodes an ubiquitinous 1560-aa protein that catalyzes the removal of methyl groups from di- and tri-methylated lysine 4 on histone H3 lysine 4 (H3K4). It suggests a central transcriptional repressive role for KDM5C in chromatin dynamics and epigenetic regulation during cell growth, differentiation and development [5,9,21,24]. Furthermore, the strongest expression of the predominant *KDM5C* transcript was observed in skeletal muscle [8] and brain, mainly in the hippocampus [23] and a biological role for this gene in neuronal survival and dendritic development has been provided [7].

KDM5C protein is homologous to three other proteins of the human JARID1 family (JARID1A, JARID1B and JARID1C). This family shares several evolutionarily conserved domains, including the catalytic JmjC domain, JmjN domain, Arid/Bright domain, C5HC2 zinc-finger domain and two plant homeodomains (PHD), a motif

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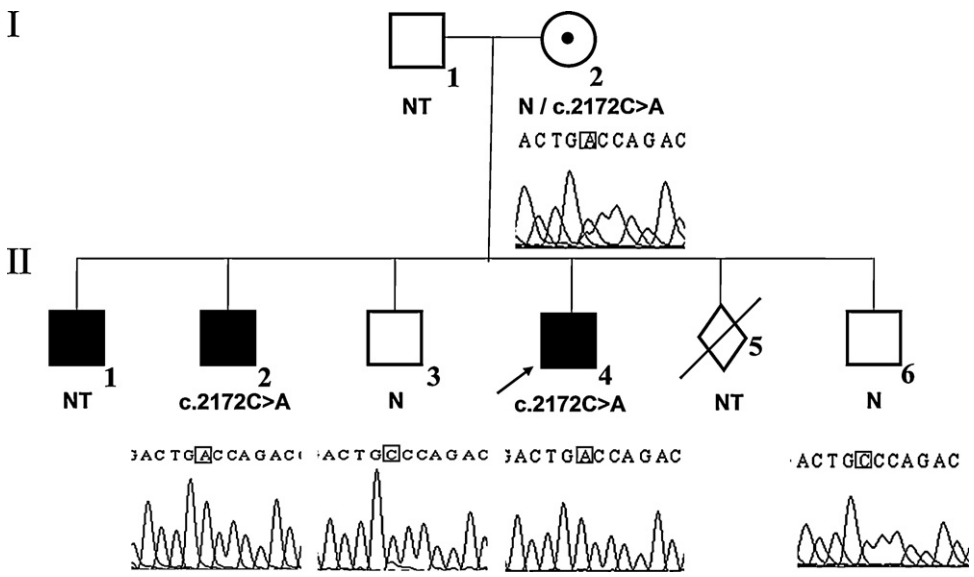

Fig. 1. Family pedigree showing the segregation of the c.2172C > A in the *KDM5C* gene. Solid squares represent boys with intellectual disability and circle with a dot represents their mother, who has mild intellectual impairment. Open squares represent unaffected males. An arrow indicates the proband (II-4). A diamond represents a pregnancy ended at the fifth month, whose malformed newborn died 24 h after being born. "N" indicates *KDM5C* gene without the mutation. NT, is for 'not available to be tested'. Chromatograms showing the mutations or wild-type sequences are shown below the tested individuals.

that binds methyl-lysine residues [5,7]. Recent evidence demonstrates that KDM5C protein can be post translationally modified by ubiquitin in vitro, suggesting that this modification is functionally relevant for maintaining the proper balance between histone demethylase and methyltransferase activities [13].

Herein, we describe clinical and genetic findings from a Brazilian family segregating a novel nonsense mutation (c.2172C > A) in *KDM5C* gene compatible with ID phenotype. The mutation generates a premature termination codon at position 724 of the protein (p.Cys724X) in the C5HC2 zinger finger domain, leading to reduction of *KDM5C* expression levels.

The Brazilian family described here consists of three affected brothers and a mild cognitively impaired mother. The propositus (TRP), a 13-year-old boy (year of birth 1996), is the fourth of five children of a young and unrelated couple. He was born by vaginal delivery after an uncomplicated pregnancy with no major intercurrence during neonatal period (birth weight = 2.54 kg; unknown length), except for the fact that he was submitted to phototherapy during the first week of life due to jaundice. At the age of 24 months, a general developmental delay was recognized (head control at 6th month of age; sitting position at 8th month of age; walking at 24th month of age and first words at 24th month of age), as well as other features such as failure to thrive, learning difficulties, aggressive behavior, hyperactivity and regular respiratory/urinary infections. Clinical investigation included normal results for inborn errors of metabolism, computed tomography (CT) scan and abdominal ultrasonography. Due to an idiopathic familial history of intellectual disability, compatible with an X-linked pattern, TRP was referred to the Human Genetics Service of the State University of Rio de Janeiro in 2005. He exhibited a normal 550 band resolution karyotype. Mutations in *FMR1*, *FMR2*, *MECP2* and *ARX* genes were subsequently ruled out, as well as, submicroscopic duplications/deletions across chromosome X (through Multiplex Ligation-dependent Probe Amplification). At the age of 12 years and 6 months, he spoke just some words, without making a sentence and exhibited proportionate short stature (height = 130 cm, below 3rd percentile), low weight (28.85 kg, below 3rd percentile), microcephaly (head circumference = 51 cm, below 3rd percentile), high palate, slight maxillary hypoplasia and small feet, besides being severely mental retarded. Clinical features of two affected siblings are similar to the proband's, including severe ID and short stature (Fig. 1). Both are institutionalized, but biological samples were available for only one of them.

As a short stature phenotype segregates with ID in the family, molecular analysis of *KDM5C* coding sequence and flanking intronic regions was performed by standard PCR according to Jensen et al. [8], followed by bidirectional direct sequencing with the BigDye Terminator kit on an ABI3100 automated sequencer (Applied Biosystems, Foster City, USA). We found a novel c.2172C > A transition in exon 15 of *KDM5C* gene, which was confirmed in a new PCR from a second DNA sample from the patient. *KDM5C* sequencing from available members of the family proved that the truncating mutation segregates completely with the ID phenotype, since the variant was present in all genotyped individuals with ID (Fig. 2; individuals II.2 and II.4) and absent in unaffected males (Fig. 2; individuals II.3 and II.6).

For *KDM5C* expression analysis, total RNA was obtained from whole blood of two affected brothers and two unaffected brothers using the RiboPure-Blood kit (Ambion, Austin, TX). The integrity of the RNA was visualized on ethidium stained agarose gels and quantified using a ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE). For quantitative RT-PCR, 50 ng total RNA was reverse transcribed into cDNA in the presence of 1.5 μg random hexamers pdN_6 (Invitrogen, Carlsbad, CA) and 0.33 mM dNTP in a total volume of 30 μl using the Roche (Mannheim, Germany) First Strand cDNA Synthesis Kit. PCR amplifications were performed in triplicates of 25 μl reactions in the presence of SYBR green (Applied Biosystems, Foster City, CA). Quantitations were performed using the absolute quantification setting on an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA) and a standard curve generated by serial dilution (factor 2) of a control cDNA. The PCR primers contain the following sequences: KDM5C_F: 5′-CATCATGGTGCAAGAAGAGC-3′; KDM5C_R: 5′- ATGTGGGAAAGGCAGACAAG-3′; GAPDH_F: 5′-CCACCCATGGCAAATTCC-3′ and GAPDH_R: 5′-TGGGATTTCCATTGATGACAAG-3′. The institutional Ethics Committee has approved all procedures and samples from TRP patient and relatives were obtained after receiving informed consent.

We identified a novel c.2172C > A transition in exon 15 of *KDM5C* gene, which resulted in substitution of the original cysteine by