BRIEF REPORT

Human Mutation

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A Novel Mutation in *RPL10* (Ribosomal Protein L10) Causes X-Linked Intellectual Disability, Cerebellar Hypoplasia, and Spondylo-Epiphyseal Dysplasia

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ABSTRACT: ***RPL10*** **encodes ribosomal protein L10 (uL16), a highly conserved multifunctional component of the large ribosomal subunit, involved in ribosome biogenesis and function. Using X-exome resequencing, we identified a novel missense mutation (c.191C>T; p.(A64V)) in the N-terminal domain of the protein, in a family with two affected cousins presenting with X-linked intellectual disability, cerebellar hypoplasia, and spondylo-epiphyseal dysplasia (SED). We assessed the impact of the mutation on the translational capacity of the cell using yeast as model system. The mutation generates a functional ribosomal protein, able to complement the translational defects of a conditional lethal mutation of yeast rpl10. However, unlike previously reported mutations, this novel *RPL10* missense mutation results in an increase in the actively translating ribosome population. Our results expand the mutational and clinical spectrum of RPL10 identifying a new genetic cause of SED and highlight the emerging role of ribosomal proteins in the pathogenesis of neurodevelopmental disorders.**

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KEY WORDS: RPL10; uL16; XLID; cerebellar hypoplasia; spondylo-epiphyseal dysplasia

RPL10 (MIM #312173) is located on chromosome Xq28 and encodes a highly conserved multifunctional component of the large 60S ribosomal subunit (uL16 according to the new nomenclature [Supp. Reference Ban et al., 2014]). A series of studies investigating mutant forms of RPL10 have shown that from yeast to human, it is a functionally conserved ribosomal protein, which performs a multitude of ribosome associated functions; among them are large subunit maturation, subunit export, subunit joining [West et al., 2005; Sulima et al., 2014], and differential mRNA expression [Chiocchetti et al., 2014]. Two missense mutations, p.(L206M) and p.(H213Q), in the C-terminal domain of RPL10 have been previously identified in three families with autistic features and moderate to severe ID or normal cognitive development [Klauck et al., 2006; Chiocchetti et al., 2011]. Recently, two novel RPL10 mutations in the N-terminal domain, p.(K78E) and p.(G161S), were reported in two families with X-linked intellectual disability (XLID), microcephaly, growth retardation, seizures, and minor facial anomalies [Brooks et al., 2014; Thevenon et al., 2015]. We report a family with two affected males, maternal first cousins with syndromic XLID partially overlapping, but distinct from the previously described phenotypes.

The index patient (III:1, Supp. Fig. S1A) was born at term with a weight of 2,820 g after a pregnancy complicated by placental insufficiency and poor fetal growth. Neonatal hypotonia, moderate delay in psychomotor development, and growth retardation were present; he walked at 4 years of age with an ataxic gait. Ophthalmological examination revealed retinitis pigmentosa; audiometric examination and brainstem auditory-evoked responses (ABR) were in the normal range. At the age of 5 years, he was surgically operated for cryptorchidism and inguinal hernia. Body X-ray performed at the age of 14 years showed spondylo-epiphyseal dysplasia (SED), scoliosis, and osteoporosis (Fig. 1E–G). Patient's medical history revealed previous proximal and distal tubulopathy and central hypothyroidism. Growth retardation and minor cranio-facial anomalies (dolicocephaly, long and flat philtrum, microretrognathia, protruding ears with absent anthelix) were present. An EEG performed during wakefulness showed a predominant theta rhythm at 4–6 c/s on the central posterior regions. Brain MRI showed cerebellar hypoplasia, predominantly in the vermis (Fig. 1A and C).

Additional Supporting Information may be found in the online version of this article.

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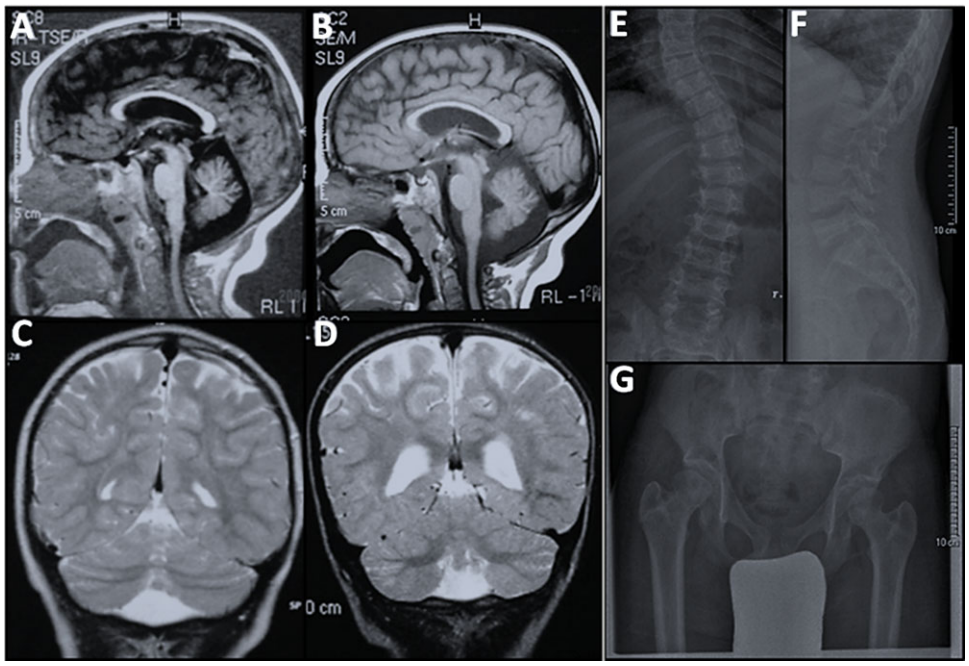

Figure 1. Neuroimaging and radiographic studies of the patients with *RPL10* mutation. **A** and **B:** Brain MRI scan of affected individuals III:1 and III:2: mid-sagittal T1-weighted image shows an overall size reduction of the cerebellum predominant in the vermis and enlarged cerebral ventricles. **C** and **D:** Brainstem and cerebral cortex are normal. Coronal T2-weighted images showing vermis hypoplasia and ventricular dilatation, more pronounced in patient III:2. **E** and **F:** Radiograph of index patient III:1 thorax and lumbar vertebrae showing platispondily and scoliosis. **G:** The pelvis radiograph shows epiphyseal dysplasia of the femoral head and neck.

Patient 2 (III:2, Supp. Fig. S1A) was born at term with a weight of 3,379 g after a regular pregnancy. He could sit at 14–15 months and started to walk at 4 years of age with an ataxic gait; speech delay was observed. He was pharmacologically treated for cryptogenic focal epilepsy. Brain MRI showed cerebellar hypoplasia (Fig. 1B and D). The boy presented with SED, dorso-lumbar scoliosis, and similar cranio-facial features as his maternal cousin; however, no endocrinological, renal, or ocular abnormalities (except for hypermetropia) were present. Both patients showed a total score of 4 and 7, respectively, in the Autism Diagnostic Observation Schedule that is considered "out of the autistic spectrum." The detailed description of the behavioral phenotype and neuropsychological assessments are shown in the Supporting Information.

To identify the disease-causing mutation in this family, we performed X-chromosome exome resequencing using DNA of the index patient as previously described by Hu et al. (2015). After filtering against public databases, we identified only one variant (*RPL10* chrX:153628144; c.191C>T; p.(A64V)) cosegregating with the disease (Supp. Fig. S1B). The variant is annotated according to *RPL10* cDNA GenBank accession no. NM_006013.3 and has been submitted to a public database (http://www.lovd.nl/RPL10).

This missense mutation alters a highly conserved alanine in the α1 N-terminal domain of the protein (Supp. Fig. S1C). The change is predicted to be damaging by in silico prediction tools SIFT (score 0.01) and MutationTaster (score 0.99), whereas Polyphen predicts it to be benign (score 0.089), probably based on the absence of major alterations in charge properties or side chain length (Supp. Materials and Methods: Bioinformatic analysis). X-inactivation analysis in the mother and maternal aunt of patient III:1 revealed completely skewed X-inactivation in both carrier females (data not shown).

The C-terminal mutations in RPL10 (p.[L206M] and p.[H213Q]), previously identified in patients with autism spectrum disorder, have been functionally analyzed in vitro in patient-derived lymphoblastoid cell lines and yeast [Chiocchetti et al., 2014]. We previously showed that a conditional, temperature-sensitive mutation in yeast rpl10[G161D] [Oender et al., 2003] is rescued by ectopic expression of human RPL10[L206M] and RPL10[H213Q], respectively [Klauck et al., 2006].

We therefore investigated whether ectopic expression of human RPL10[A64V] identified in this study was also able to functionally complement yeast rpl10[G161D]. Indeed, we observed growth of the temperature-sensitive strain rpl10[G161D] harboring RPL10[A64V] at the conditional, restrictive temperature (Fig. 2A). We conclude that the mutations rpl10[G161D] and RPL10[A64V] are able to functionally complement each other during translation as restoration of growth at the restrictive temperature is observed, albeit not at the wild-type (WT) level (Fig. 2A). This suggests that RPL10[A64V] encodes a functional ribosomal protein.

Next, by ribosomal profiles analysis, we assessed the role of RPL10[A64V] in the formation of actively translating ribosomes (Fig. 2B). Cellular extracts enriched for translating ribosomes were layered onto a sucrose gradient. Optical density tracing (A_{254}) of the gradients generates a typical profile, which when viewed from top to bottom, shows the free 40S and 60S subunits, the monosomal 80S peak, and the polysomes. The individual polysome peaks report mRNA populations harboring two, three, four, or more translating ribosomes, respectively. The 80S peak represents ribosomes that have initiated translation and the polysome peaks collectively represent the actively translating ribosome population. In a typical WT profile, the free subunit peaks are small, the 80S peak is larger than any of the polysomal peaks, and the individual polysomal peaks decrease with increasing ribosome load (Fig. 2B, a). Histogram analysis documents the area under individual ribosomal profile peaks and allows for a semiquantitative comparison (Fig. 2B, d). This can be used to monitor a possible shift in peak intensity resulting from a mutant effector of translation. Upon ectopic expression of human

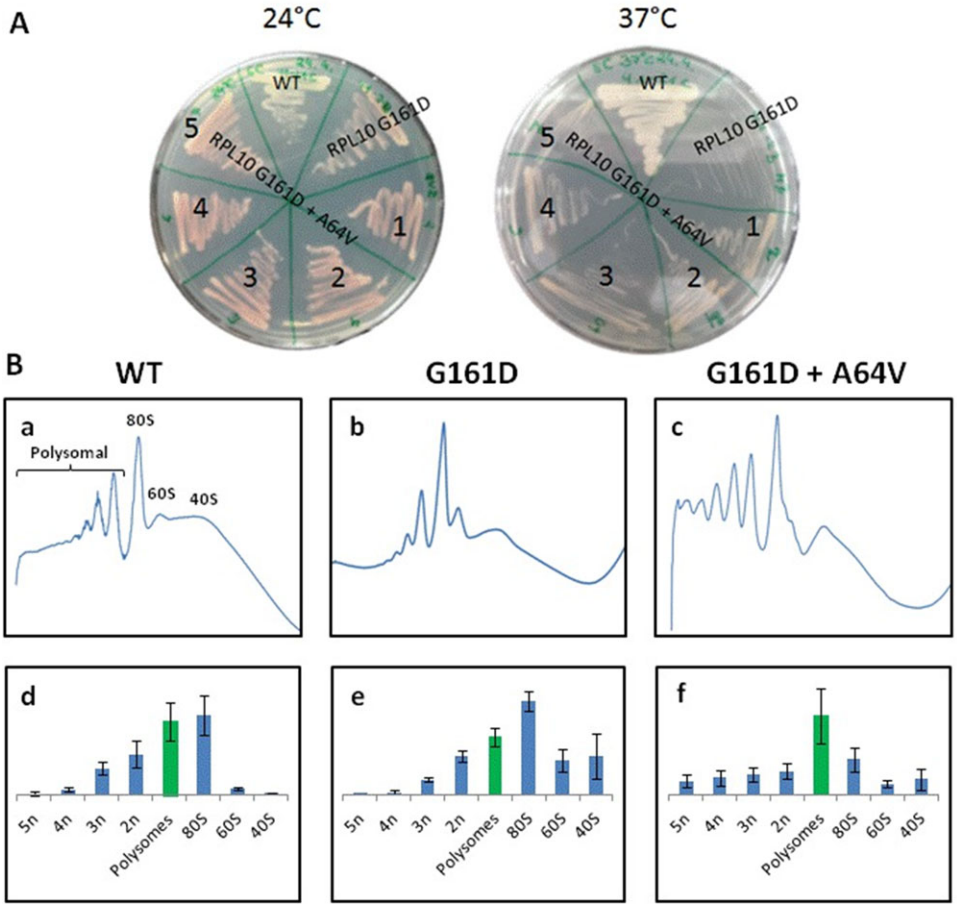

Figure 2. Temperature-sensitive rpl10[G161D] is rescued by ectopic expression of human RPL10[A64V]. Ribosomal profile analysis demonstrates competence of RPL10[A64V] to support a translationally active ribosome population. **A:** Growth of wild-type (WT), rpl10[G161D] mutant cells, and five colonies (1–5) of rpl10[G161D] mutant cells transformed with RPL10[A64V], was documented after 3 days of incubation at the indicated temperatures. Colonies 1–5 all indicate similar complementation of growth at the restrictive temperature. **B:** Ribosomal profile analyses of WT (WT BY1na), rpl10[G161D] (LK 41 2B) mutant cells, and rpl10[G161D] mutant cells transformed with RPL10[A64V] (LK 41 2B + A64V) are shown in panels a, b, and c, respectively. The corresponding histograms are shown in panels d, e, and f, respectively. Sucrose gradients from bottom to top separate polysomes, 80S single ribosomes (monosomes), and free ribosomal subunits (60S, 40S) monitored by optical density (A254) tracing. Representative recordings from individual profile analyses performed at least three times are shown including standard deviation bars.

RPL10[A64V] in yeast rpl10[G161D], a ribosomal profile is observed with small subunit peaks and a prominent polysome fraction with concomitant reduction of the mutant large 80S peak seen in rpl10[G161D) mutant cells, which there indicates a translationally reduced state (Fig. 2B, c). Of note, the large polysome population (Fig. 2B, f) indicates a significant increase in translationally active ribosomes, a phenotype that is quite different from the translationally repressed rpl10[G161D) mutant phenotype (Fig. 2B, e).

RPL10 in ribosomes from yeast to human is located on the intersubunit side of the large subunit, in a cleft between the central protuberance and the ribosomal stalk. Ribosomal topology of rpl10, here shown for yeast, is unique among ribosomal proteins (Supp. Fig. S2A). First, in the assembled ribosome, most of the rpl10 protein is covered by the small subunit and only the extreme end of the rpl10 C-terminus extends out of the translation competent ribosome, providing docking space for regulatory proteins (our unpublished observations). Second, rpl10 is one of few ribosomal proteins that is in close proximity to several major regulatory sites of the ribosome, the sarcin-ricin loop, and the GTPase center (GAC) [Spahn et al., 2001] that serve as regulators of binding sites for initiation and elongation factors of translation, respectively [Sonenberg and Hinnebusch, 2009]. Third, within the ribosome, rpl10 is positioned in such a way that every incoming tRNA has to pass by it. Fourth, rpl10, with a large, unstructured loop, extends deep down into the peptidyltransferase center (PTC), the site of peptide bond formation. Taken together, this unique topological characteristic makes human RPL10 a promising candidate for translational control of gene expression, both at a qualitative and a quantitative level [Oender et al., 2003; Chiocchetti et al., 2014].

RPL10[A64V] clusters into the mutational hot spot region between amino acids 59–94 of the protein, with identical sequence between yeast and human and known to support high frequencies of mutations that are compatible with viability [Petrov et al., 2008]. In addition, on the tertiary level of protein structure, A64 and G161 are juxtaposed to each other, and both reside within a K^+ binding site of rpl10 [Nishimura et al., 2008]. Most interestingly, human mutation RPL10 p.(G161S) precisely at the position of the yeast rpl10[G161D] was reported by Thevenon et al. (2015). Together, this suggests that RPL10[A64] and RPL10[G161] reside within a structural domain of RPL10 that can be modified without compromising viability. Indeed, Sulima et al. [2014] suggested that this domain is a structural platform that moves as a single unit to coordinate signaling

between the PTC and the elongation factor binding site. As amino acid A64 and amino acid G161 are juxtaposed at the PTC proximal side of this platform, disrupting their interaction is predicted to decrease its structural integrity, perhaps altering its range of motion/rotation, thus changing the flow of information between these two critical functional centers of the ribosome. We have shown that the recruitment of mRNAs into translating ribosomes is functional when the RPL10 [G161D] mutation is present within this structural platform. However, there are subsets of mRNA recruited, which in comparison to the WT are quantitatively and qualitatively altered [Chiocchetti et al., 2014]. It remains to be established whether such differential recruitments of mRNAs observed in the presence of a mutated ribosomal protein translate in pleiotropic phenotypes in human disease, in particular for those observed in patients carrying the RPL10 p.(A64V) mutation. The concept that changing a methyl group in a ribosomal protein—as it is the case for the exchange of alanine to valine identified in the family—would have an effect on the translational readout of selected, but not all mRNAs, is supported by our finding that changing one methyl group on a single rRNA nucleotide in the PTC alters mRNA recruitment and translational readout in oxidative stress response [Schosserer et al., 2015].

RPL10, also known as QM/Jif1, is a negative regulator of c-jun transcription factor and has been shown to interact with presenilin-1 in human cortical neurons [Imafuku et al., 1999]. Overexpression of RPL10 in neuroblastoma cell lines enhances neurite outgrowth induced by glial cell line-derived neurotrophic factor [Park and Jeong, 2006]. Interestingly, the RPL10 nuclear localization signal is located at amino acids 1-64 at the N-terminus of RPL10 interacting with karyopherins, nuclear import receptors for ribosomal proteins [Gadal et al., 2001]. Further studies are needed to determine whether the p.(A64V) mutation identified here could have an impact on the subcellular distribution of RPL10 and its possible consequences on translational activity in neuronal cells.

In addition to XLID and cerebellar hypoplasia, our patients show SED. To date, only one X-linked gene, *TRAPPC2* (MIM #300202), previously called *SEDLIN* (MIM #313400), has been associated with this skeletal disorder, characterized by mild-to-moderate short-trunked short stature and joint degeneration mainly involving the epiphyses and vertebral bodies [Gedeon et al., 1999]. TRAPPC2 is part of the conserved transport protein particle complex (TRAPP) that functions in secretory and endocytic pathways [Sacher et al., 2008]. We analyzed the entire coding region of *TRAPPC2* and its exon–intron boundaries by Sanger sequencing prior to X-exome resequencing and thereby excluded it as the candidate gene for SED in our family.

Interestingly, RPL10 is highly expressed in epiphyseal cartilage during endochondral bone development [Green at al., 2000] and defects of ribosomal biogenesis are emerging as important causes in the pathogenesis of skeletal disorders and development.

Our study expands the set of pathological mutations in *RPL10*, showing distinctive genotype–phenotype and functional associations, identifies a new form of X-linked SED, and highlights the emerging role of ribosomal proteins in neurodevelopmental disorders.

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