CLINICAL/SCIENTIFIC NOTES OPEN ACCESS

A novel *PUS7* mutation causes intellectual disability with autistic and aggressive behaviors

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Neurol Genet 2019;5:e356. doi:10.1212/NXG.0000000000000356

Recently, homozygous *PUS7* mutations causing premature stop and truncation of the gene product were identified in 3 independent consanguineous families presenting with intellectual disability (ID), speech delay, short stature, microcephaly, and aggressive behavior.[1] *PUS7* encodes for a pseudouridine synthase 7 that catalyzes the isomerization of RNA uridine to RNA pseudouridine (Psi), which is the most abundant modified nucleotide found in all cellular RNAs and which may function as an RNA chaperone. The encoded protein contains a pseudouridine synthase domain of the TruD family that modifies uracil-13 in tRNA. Two homozygous mutations c.89_90del (p.Thr30Lysfs20*) and c.1348C>T (p.Arg450*) resulted in nonsense-mediated mRNA decay, meaning that mRNA transcripts containing the premature stop codons were eliminated through surveillance mechanisms, while the third mutation, consisting of a homozygous deletion encompassing the penultimate exon 15, escaped the nonsense-mediated mRNA decay to encode a mutant protein missing the C terminus including the TruD catalytic domain. All identified *PUS7* variants resulted in aberrant pseudouridylation of at least 10 cytosolic tRNAs at position 13.[1]

Clinical and scientific findings

We report a novel *PUS7* homozygous mutation resulting in p.Gly128Arg amino-acid translation in a consanguineous Afghani family presenting with similar but milder clinical features without microcephaly and short stature (table e-1, links.lww.com/NXG/A180), further confirming the pathogenic role of *PUS7* in ID syndromes with autistic features, speech delays, and aggressive behaviors.

The entire nuclear family of 2 healthy parents and 2 affected siblings (figure) was subjected to homozygosity mapping (HM) using high-throughput single nucleotide polymorphism genotyping (HumanOmniExpress Exome arrays v1.3; Illumina Inc., San Diego, CA) as previously described.[2,3] The generated single nucleotide polymorphism data were used to determine regions of homozygosity present exclusively in the affected siblings but not their healthy parents. A total of 10 different homozygous tracks were identified (table e-2, links.lww.com/NXG/A180). We then proceeded to perform whole genome sequencing (WGS) analyses in both affected siblings. WGS was carried out at the New York Genome Center and the data were analyzed as previously described.[2,4] Based on parental consanguinity and recessive inheritance (figure), novel and rare genomic variations, including nonsynonymous, frame-shift, splice site, small insertions and deletions, as well as gain/loss of stop codons, present in homozygosity or compound heterozygosity were considered as potential candidates. All genetic variations present in the regions of homozygosity were prioritized. Ten out of 303 genomic variations found to be present in

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Go to Neurology.org/NG for full disclosures. Funding information is provided at the end of the article.

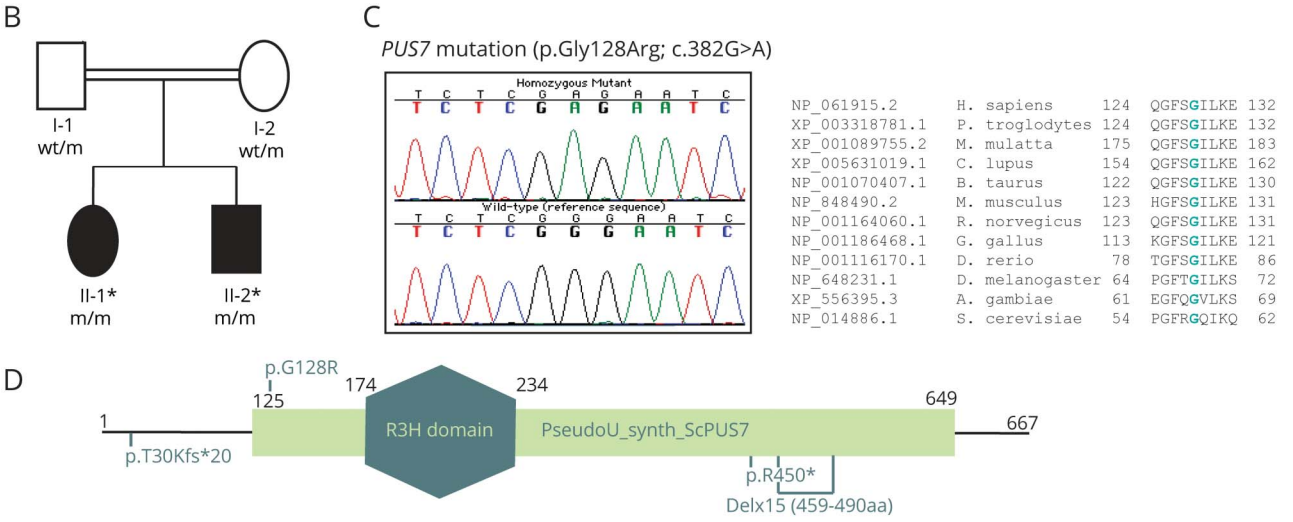
The Article Processing Charge was funded by NINDS.

Ethical approval: The local ethics committee at the Semnan University of Medical Sciences approved this study, and informed consent according to the Declaration of Helsinki was obtained from all participants.

Figure Identification of a *PUS7* homozygous mutation in a family with intellectual disability (ID), autistic features, and aggressive behaviors

A

Chr	Position	Gene (exon)	Nucleotide change	Protein change	Reference transcript	GNOMAD	CADD
7	94,037,520	*COL1A2* (exon 14)	c.665G>A	p.Arg222Lys	NM_000089	N.A	25.7
7	101,188,732	*COL26A1* (exon 7)	c.817G>C	p.Gly273Arg	NM_001278563	N.A	15.73
7	105,148,578	*PUS7* (exon 1)	c.382G>A	p.Gly128Arg	NM_001318163	N.A	23.3



(A) Genomic variants identified in the genomes of 2 siblings with ID, autistic features, and aggressive behaviors. Disease-causing mutation is highlighted in bold. CADD = combined annotation dependent depletion (cadd.gs.washington.edu/); GNOMAD = genome aggregation database (gnomad.broadinstitute.org/); and NA = not applicable. Recessive mutations in *COL1A2* cause Ehlers-Danlos syndrome. (B) Pedigree structure of the examined ID family. Wt/m indicates heterozygous carrier for the *PUS7* p.Gly128Arg mutation while m/m indicates homozygous carrier. Affected siblings are represented with a black square (male) and a black circle (female). *Indicates participants that underwent whole genome sequencing analyses. (C) *Sanger* chromatogram sequences of the *PUS7* exon 1 containing the c.382G>A mutation are shown on the left, while G128 amino-acid conservation among other species is shown on the right. (D) PUS7 protein structure. R3H domain that is predicted to bind single-stranded DNA; PseudoU_synth_ScPUS7 is a pseudouridine synthase domain of the TruD family (PMID:12756329). The *PUS7* mutation identified in this study is represented at the top while previously reported *PUS7* mutations are represented at the bottom.

both affected siblings were homozygous; however, only 3 were located within the previously determined homozygous tracks (figure, table e-2). These 3 novel genetic variations were located within the *COL1A2*, *COL26A1*, and *PUS7* genes, and none of them were present in public databases, including the Iranome browser (iranome.com/), the Greater Middle-East variome (igm.ucsd.edu/gme/), and the Genome Aggregation database (gnomAD; gnomad.broadinstitute.org/), and disease databases such as ClinVar (ncbi.nlm.nih.gov/clinvar/) and the Human Gene Mutation Database (hgmd.org).

Mutations in *COL1A2* encoding collagen of skin, tendon, and bone are associated with diseases of the connective tissues distinct from the manifestations of our family and not observed in the patients.[5] No human disease has been associated with mutations in *COL26A1*, which encodes a protein with collagen-like characteristics expressed in mouse mesenchyme of the head, skeletal muscles, and kidney (Mendelian Inheritance in Man [MIM] #608927). Given the association of *PUS1* (MIM #600462) and *PUS3* (MIM #616283) genes with ID syndromes with speech and motor impairments,[6,7] we favored the nucleotide variant in the *PUS7* gene as a causative. The recent identification of 3 different ID families with pathogenic *PUS7* mutations[1] strengthened the likelihood of c.382G>A (p.Gly128Arg) as the disease-causing mutation. The pathogenicity of this novel *PUS7* mutation is further supported by its segregation with disease status (figure A–C), its location within a region of homozygosity identified through the performed HM analyses (table e-2, links.lww.com/NXG/A180), and the alteration of an evolutionarily conserved glycine down to yeast (figure, C). The *PUS7* p.Gly128Arg mutation lies within the Pseudouridine synthase TruD domain (figure, D) such that this amino acid alteration may disrupt pseudouridylation, similar to the recently described truncation mutations.

We present genetic and clinical evidence of another family identified with ID, speech delay, motor impairments, and aggressive behavior due to pathogenic *PUS7* mutations. The absence of highly extreme phenotypes such as short stature or microcephaly in this family might reflect genotype–phenotype correlation, since this family presented with a *PUS7* missense mutation that may be hypomorphic, while previously reported families carried nonsense or frameshift mutations that may cause loss of function. This is the first report of a *PUS7* missense mutation that confirms *PUS7* as

a pathogenic gene for ID syndromes with speech impairments and aggressive behaviors.

Acknowledgment

The authors thank the patients and their relatives for their cooperation in this study.

Study funding

This work was supported by the Semnan University of Medical Sciences and the NINDS of the NIH (R01NS079388; C. Paisán-Ruiz).

Disclosure

Disclosures available: Neurology.org/NG.

Publication history

Received by *Neurology: Genetics* March 3, 2019. Accepted in final form June 24, 2019.

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