Genetic Variants in *SPOUT1* are associated with epileptic encephalopathy, microcephaly and severe developmental delay

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Background:

SPOUT1, a member of the SPOUT family of methyltransferases, contains a catalytic domain crucial for RNA methylation. This study investigates the role of bi-allelic missense variants in SPOUT1 and their association with refractory seizures and severe global developmental delay in affected infants.

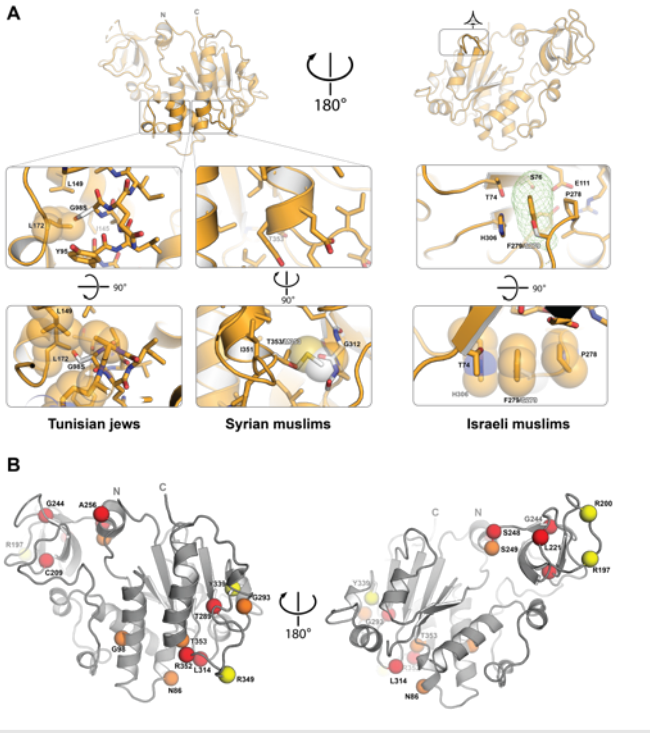
Results:

WGS identified three homozygous missense variants in SPOUT1 in three families: c.292G>A (p.Gly98Ser), c.1058C>T (p.Thr353Met), and c.836T>C (p.Phe279Ser). Structural modeling predicted that these variants destabilize the SPOUT1 protein, potentially leading to its degradation. Immunoblotting revealed significantly reduced SPOUT1 protein levels in fibroblasts carrying the G98S variant compared to controls. Immunofluorescence showed abnormal nuclear speckle organization in patient fibroblasts, likely contributing to the observed neurological symptoms.

Genetic and Clinical manifestation of SPOUT1 related disease

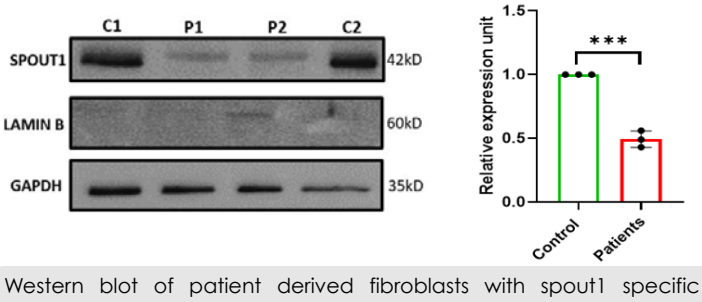
(A) Pedigree of the Tunisian Jewish family **(B)** Sanger sequencing of the c.292G>A; p.Gly98Ser variant **(C)** Clinical presentation of IV-5 patient

Structural analysis of the SPOUT1 variants

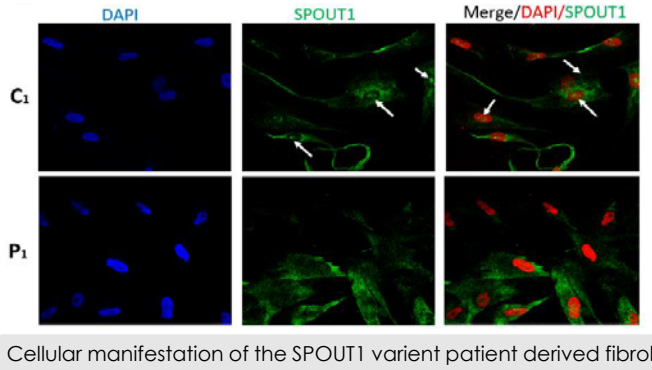


(A) A ribbon representation of the crystal structure of SPOUT1 protein (PDB ID: 4RG1), and the mutation's sites. In the magnification boxes is a stich representation of the relevant residues. Spheres represents VDW radius. **(B)** The distribution of the known mutations (including the ones from the literature) on the SPOUT1 structure.

SPOUT1 variants show low levels of spout1 protein



Western blot of patient derived fibroblasts with spout1 specific antibody **(A)** and spout1 protein level quantification **(B)**



Cellular manifestation of the SPOUT1 varient patient derived fibroblast. Abnormality nuclear speckles organization in SPOUT1 deficiency patients. White arroes indicate the cellular speckles

Conclusions:

This study links SPOUT1 deficiency to neurodevelopmental disorders with refractory seizures and severe developmental delay. It underscores SPOUT1's role in nuclear speckle organization and RNA processing, offering insights into epilepsy's molecular mechanisms. Adding SPOUT1 to the global developmental delay and seizure gene panel could aid in diagnosing affected infants.