De Novo SOX6 Variants Cause a Neurodevelopmental Syndrome Associated with ADHD, Craniosynostosis, and Osteochondromas

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INTRODUCTION

The SOX gene family consists of twenty transcription factors that play a pivotal role in cell fate and differentiation during the development of many organ systems. These genes contain a highly conserved high mobility group (HMG) domain that has been shown to be critical for DNA binding and bending, nuclear trafficking, and protein-protein interactions. Mutations within this transcription factor family have been associated with rare congenital disorders, known as SOXopathies. These mutations are commonly de novo, heterozygous and inactivating, and exhibit gene haploinsufficiency. Of these twenty transcription factors, SOX6 is known to be involved in chondrocyte differentiation and development of the central nervous system. Although there have been reports of *SOX6* variants causing adult pathological conditions in genome wide association studies, there has yet to be a well-established association between *SOX6* variants and a developmental syndrome.

OBJECTIVES

The objective of this study was to use clinical and genetic data to examine *SOX6* variants found in 19 individuals demonstrating developmental delay and to test the transcriptional activity of the 4 missense variants *in vitro* to determine if SOX6 haploinsufficiency leads to a neurodevelopmental SOXopathy.

METHODS

Nineteen individuals were identified as carriers of *SOX6* variants, confirmed by molecular karyotyping, whole-exome sequencing, or whole-genome sequencing. Clinical pathogenicity was predicted and assessed *in silico* and *in vitro.*

Expression plasmids for SOX6 missense variants were generated by PCR mutagenesis. The four missense variants generated were: p.Trp161Cys, p.Met605Thr, p.Trp639Arg, and p.Ser746Leu, with p.Met605Thr and p.Trp639Arg located within the HMG domain. For reporter assays, HEK293 cells were transfected in triplicate cultures with 3.5 µL ViaFect Transfection Reagent and a total of 1000ng of DNA. SOX6 intracellular localization was tested by transfecting either HEK293 or COS-1 cells and cytoplasmic and nuclear extracts were prepared for Western Blot analysis. Whole cell extracts transfected with respective WT-SOX6 or variant plasmid were also prepared for electrophoretic mobility shift assay (EMSA) to test DNA binding ability.

RESULTS

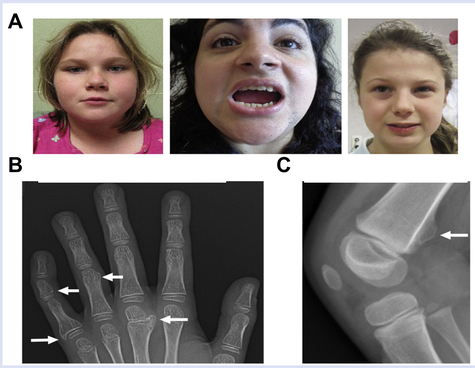

Figure 1. Clinical findings in subjects with *SOX6* variants

Patient ages ranged between 12 months and 27 years. Most individuals had intellectual disability (18/19) and abnormal behavior (14/19) such as ADHD (10/19) or autism spectrum disorders (4/19). Dysmorphic features and skeletal anomalies were noted in many individuals, including mild facial dysmorphism (**Figure 1A**). Moreover, multiple diffuse osteochondromas were present in three patients (arrowed in **Figure 1B and C**).

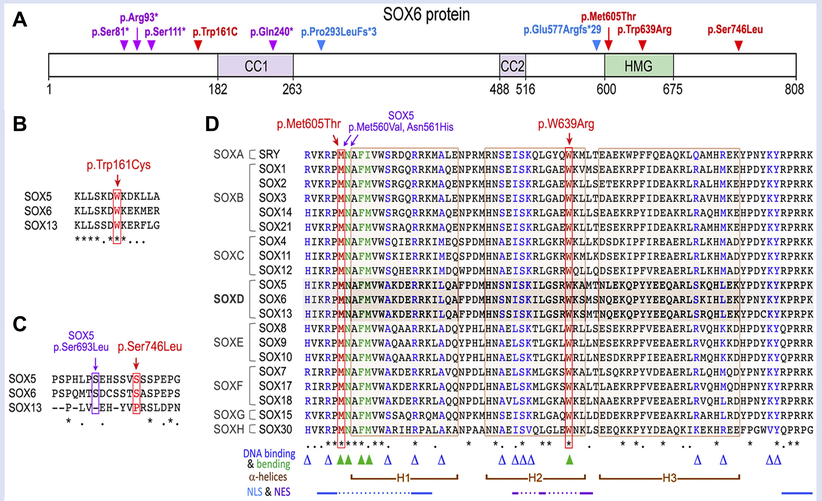

Figure 2. Single nucleotide variants (SNVs) identified in patients and their evolutionary conservation

All SNVs were distinct and corresponded to 4 nonsense, 2 frameshift, and 4 missense mutations. The nonsense and frameshift variants caused SOX6 truncation encoding a non-functional protein. The missense variants were in the HMG domain and non-functional regions (**Figure 2A**). However, they were all were conserved among the SOXD group (i.e., SOX5, SOX6 and SOX13) and the other SOX genes. Additionally, two of the affected residues are involved in DNA binding and bending (**Figure 2B-D**).

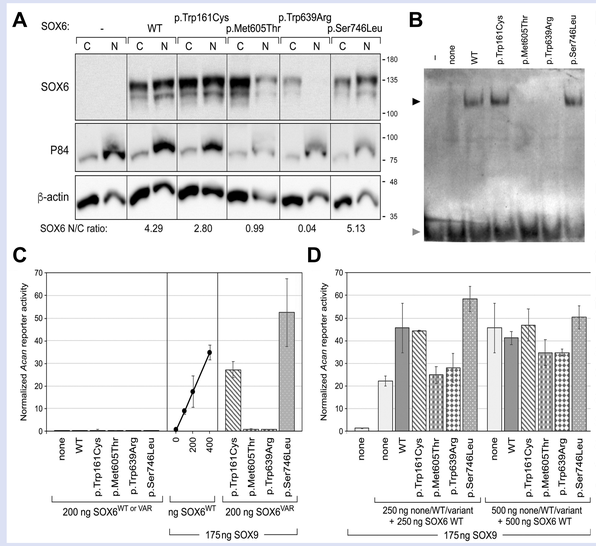

Figure 3. Functional tests of SOX6 missense variants

All variants were efficiently expressed after transfection in HEK cells. p.Met605Thr and p.Trp639Arg proteins were not translocated or retained into the nucleus as efficiently as WT SOX6 (**Figure 3A**). In addition, they failed to bind the DNA probe in the EMSA and to transactivate the reporter in luciferase assays (**Figure3B and C**).

On the contrary, pTrp161Cys and p.Ser746Leu variants showed normal protein activity. Finally, none of the variants interfered with WT SOX6 (**Figure 3D**).

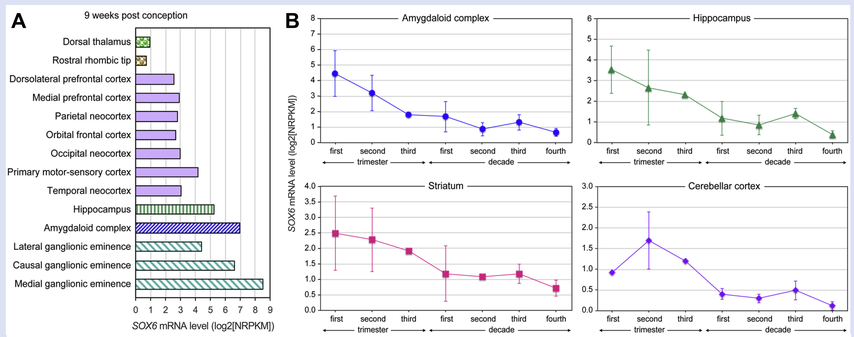

Figure 4. Analysis of *SOX6* expression in the human brain

Whole transcriptome profiling data deposited in the Brain-Span Atlas of the Developing Human Brain provided information on where and when *SOX6* is expressed in the brain of human fetuses, children, and adults. At 9 weeks after conception, fetuses were found to express *SOX6* in many prospective brain structures. *SOX6* RNA expression was highest in the ganglionic eminence, the amygdaloid complex, and the hippocampus, all of which have central roles in brain development (**Figure 4A**). The expression of *SOX6* declined in all brain structures in the final stages of gestation and in the neonatal period (**Figure 4B**).

CONCLUSION

- The findings from this study concur that SOX6 haploinsufficiency leads to a specific form of neurodevelopmental SOXopathy characterized by mild to severe intellectual disability and inconstantly associated with skeletal anomalies, such as mild facial dysmorphism, craniosynostosis, and osteochondromas.
- No clear genotype-phenotype correlations were found.
- This new syndrome has been designated in Online Mendelian Inheritance in Man (OMIM) database as #618971 Tolchin-Le Caignec syndrome (TOLCAS).

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