# Homozygous *XYLT2* variants as a cause of spondyloocular syndrome

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## ABSTRACT

Spondyloocular syndrome (SOS) is a rare autosomal recessive skeletal disorder. Two recent studies have shown that it is the result of biallelic sequence variants in the *XYLT2* gene with pleiotropic effects in multiple organs including retina, heart muscle, inner ear, cartilage, and bone. The *XYLT2* gene encodes xylosyltransferase 2, which catalyzes the transfer of xylose (monosaccharide) to the core protein of proteoglycans (PG) leading to initiating the process of proteoglycan assembly.

SOS was originally characterized in two families A and B of Iraqi and Turkish origin, respectively. Using DNA from affected members of the same two families we performed whole exome sequencing, which revealed two novel homozygous missense variants (c.1159C>T, p.Arg387Trp) and (c.2548G>C, p.Asp850His). Our findings extend the body of evidence that SOS is caused by homozygous variants in the *XYLT2* gene. In addition, this report has extended the phenotypic description of SOS by adding follow-up data from five affected individuals in one of the two families, presented here.

**Keywords:** spondyloocular syndrome, SOS, *XYLT2*, WES, skeletal dysplasia, missense variants

## INTRODUCTION

Proteoglycans (PGs) are a class of extracellular matrix and surface-associated proteins involved in many physiological processes such as signal transduction, cellular homeostasis, membrane integrity, co-repressor activity, morphogen gradient formation, lipid catabolism and scaffolding (1). PGs consist of a core protein, which is linked to glycosaminoglycan (GAG) disaccharide chains. Structurally, PGs are very diverse having different lengths and composition of disaccharides, with various types of modifications of phosphorylation, sulfation, and different combinations of core proteins (2). Assembly of GAGs on the core protein results in different groups of sulfated PGs such as chondroitin sulfate (CSPGs), heparan sulfate (HSPGs) and modified form of CSPGs the dermatan sulfate (DSPGs) (3). Synthesis of a common tetrasaccharide linker chain is the initial step in heterogeneous CSPGs, HSPGs and DSPGs formation.

Xylosyltransferases (XylTs) catalyzes the transfer of xylose to the core protein serine. Two enzymes XYLT1 (MIM 608124) and XYLT2 (MIM 608125) with xylosyltransferase activity have been described to catalyze this reaction in humans (4). Additional sugar residues are added by specific enzymes including B4GALT7 (GalT-I), B3GALT6 (GalT-II) and B3GAT3 (GlcAT-1). A defect in any of these steps causes severe autosomal recessive disorders (MIM 604327, 615291, 606374) (5).

Spondyloocular syndrome (SOS; MIM 605822) is caused by biallelic sequence variants in the *XYLT2* gene (6). Here, we have reported the clinical follow-up of two families originally used to describe SOS. In addition, using WES we have identified two homozygous missense variants in the *XYLT2* in the same two families.

## MATERIALS AND METHODS

### Study Approval

The present study was performed according to the declaration of Helsinki protocols and approved by Institutional Review Board (Technical University, Munich, Germany). Written informed consent for publication of images was obtained from all members.

### Research Subjects

A consanguineous family A, of Iraqi origin, was clinically described previously by Schmidt *et al*. (7) and Rudolph *et al*. (8). Similarly, family B, of Turkish origin, was described previously by Alanay *et al*. (9).

### Genetic Analysis

In family A, genome-wide linkage analysis was performed using the Affymetrix 10K Mapping Array. The statistical analysis was carried out with Allegro (10). Whole exome sequencing was performed in both the families (A and B) using HiSeq 2500 systems (Illumina, San Diego, CA, USA) (11). All the filtered variants were inserted into an in-house database. Subsequently, we queried the database to identify only rare homozygous variants, thus two homozygous variants were identified in the families, which were further validated by Sanger sequencing.

## RESULTS

### Clinical description

Family A, of Iraqi origin, has been extensively described previously in 2001 and 2003 (7, 8). Characteristic features such as facial hypotonia, facial dysmorphism, short trunk, reduced lumbal

lordosis, immobile spine with kyphosis, platyspondyly and mild osteoporosis was present in all six affected family members. In addition, they showed features of poor eyesight due to dense cataract, retinal degeneration/detachment, and consecutive phtisis bulbi.

All six affected members underwent a follow-up examination 13 years after the family was reported (7, 8), however molecular analysis was performed in five of these individuals. Their current age ranges between 27 and 37 years. They had normal weight (59-96 kg), height (155-185 cm) and showed no sign of cognitive impairment (Fig. 1a). They were presented with a barrel-shaped chest, long fingers, and broadened fingertips. Over the years, the ocular condition has progressed to complete blindness characterized by ocular phthisis and untreated retinal detachment. Fractures had been observed in the affected individuals and two of them are suffering from continuous vertebral pain. Marked kyphosis was apparent in four individual (II-3, II-5, II-6, II-7), while the fifth member (II-4) showed no such phenotype. Cardiac abnormalities were recorded in two individuals. The individual (II-6) had a heart murmur, while individual II-5 revealed a systolic heart murmur. A sonography of the kidney showed no abnormalities (Table S1).

As described previously by Alanay *et al.* (9), a single affected member in family B, had SOS related phenotypic spectrum including bilateral cataract, generalized platyspondyly and osteoporosis, facial hypotonia, thin upper lip, ventricular septal defect and speaking difficulties. In addition, the affected member showed unique features including short stature, hyperextensible joints, hyperelastic skin and thoracic kyphosis. Follow-up examination for family B was not available.

**Linkage Analysis**

In family A, genome-wide linkage analysis was performed using Affymetrix 10K Mapping Array. A multipoint LOD score of above 3 was obtained with SNP markers mapped on chromosome 17q21.2-q22 (SNP_A-1510482 - SNP_A-1516550) (Fig. 1c). The maximum LOD score of 3.61 was obtained with a marker SNP_A-1518025 (Fig. 1b). Fine mapping using eleven microsatellite markers narrowed down the region to 6cM between D17S931 and D17S788, with a maximum 3.27 LOD score obtained with marker D17S1868 (Fig. 1d).

**WES and Sanger Sequencing**

Using DNA of affected members of both the families (A and B), WES was performed at the Institute of Human Genetics, Helmholtz Zentrum Munich, Germany (11). After following a step-by-step filtering process for screening homozygous variants, two potential homozygous missense variants were identified in the *XYLT2* gene (NM_022167.3). In family A, the missense variant (c.1159C>T, p.Arg387Trp) was located in exon 6, while in family B (c.2548G>C, p.Asp850His) it was located in exon 11 (Table S2). Both the variants affect highly conserved amino acid positions (Fig. 2a-c) and were predicted pathogenic by *in silico* online tools such as PolyPhen-2, CADD, and SIFT. These variants were absent in different databases (ExAC, genomeAD, 1000 Genome) and 7000 in-house exomes. WES was followed by Sanger sequencing all available members of the families (Fig. 2d, e).

## DISCUSSION

Proteoglycans play a key role in multiple cellular processes and represent a group of glycosylated macromolecules, mostly expressed on the cell surface and in the extracellular matrix. Since xylosyltransferase 2 catalyzes the initiation of GAG assembly to the PG core protein serine (12, 13), it is crucial to PG function. Any pathogenic mutation in core protein or in

the GAGs chain modifying enzymes affects proliferation, ossification, and maturation of chondrocytes (14, 15). Therefore, it is certain that an addition of xylose to the serine residue at the core protein of PGs is the crucial first step in GAGs initiation, which is catalyzed by xylosyltransferase (16). Two xylosyltransferases (XYLT1, XYLT2) are paralogs having a high homology in the catalytic domains (13). Although, both XYLT1 and XYLT2 show no functional differences in vitro tissue-specific expression differences have been reported in soft tissues in humans (13, 16) and mice (17), where deficiency in mice is associated with polycystic kidney disease (17).

The clinical entity spondyloocular syndrome (SOS) was first described in two families of Iraqi and Turkish origin (7, 9). These two families were characterized at a genetic level in the present study. Recently, two groups have described SOS in seven other families of different ethnic backgrounds (6, 18, 19). Further, they have reported disease-causing mutations in the *XYLT2* gene. Clinical features reported in affected individuals by Munns *et al*. (6) such as ureter dilatation and hearing impairment were not observed in affected individuals of the two families presented here. However, features including learning difficulties, fractures, bilateral cataract, osteopenia, platyspondyly, and retinal detachment reported by Munns *et al*. (6) and Taylan *et al*. (18) were observed in patients of our two families. A cardiovascular defect observed in our two families was not reported by Taylan *et al*. (18). Details of clinical features, observed have been summarized in Table 1.

Using human genome scan and whole exome sequencing (WES), we have found two novel disease-causing mutations (p.Arg387Trp, p.Asp850His) in the *XYLT2* gene in the two families, presented here. To date, only **seven** homozygous variants including a nonsense, **three missense**,

a frameshift duplication and **two frameshift deletions** in the *XYLT2* gene have been reported (6, 18, **19**).

The *XYLT2* gene comprises of 11 exons encodes an 865 amino acids protein and located on chromosome 17q21.3-q22. The XYLT2 is composed of an N- terminus domain, a xylosyltransferase terminal domain (catalytic domain), a core2/I-branching enzyme domain and a C-terminus domain (Fig. 2b). The two missense mutations p.Arg387Trp and p.Asp850His, identified in our families here, are located in the Core2/I-binding domain and C-terminus domain, respectively. Previously reported two frameshift truncated variants (6) are located in the N-terminus trans-membrane domain. Three other variants including two missense and a nonsense (18) are located in the catalytic and C-terminus domain, respectively. Diversity in the clinical features observed in our patients and those reported previously suggests a possible residual XYLT2 activity that might depend on the nature and/or location of the identified variants. In addition, involvement of different modifiers in defining the severity of disorder cannot be ruled out. The identified two mutations (p.Arg387Trp, p.Asp850His), are highly conserved (Fig. 2c) and might possibly affect secondary structure leading to non-functional XYLT2 protein.

In conclusion, we have reported two novel variants in the *XYLT2* gene in the two families originally used to define the SOS syndrome. This increases the mutation spectrum, signifying and supporting a greater role of *XYLT2* in the pathogenesis of SOS. Furthermore, our finding will also help in determining proper genotype-phenotype correlation in patients suffering from *XYLT2* related pathogenesis.

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## Figures legends:

*Fig. 1*: (a) Recent photograph of family A. From left to right five affected members (II-5, II-7, II-6, II-4, II-3) and a normal father (I-1). (b) Multipoint linkage analysis with subsequent fine mapping and haplotype analysis revealed LOD score of 3.61. (c) Haplotypes using SNP markers established linkage to chromosome 17q12-q22. The disease-associated haplotype is marked in pink. (d) Haplotypes generated after fine mapping was performed with microsatellite markers showing disease-associated haplotype in dark blue.

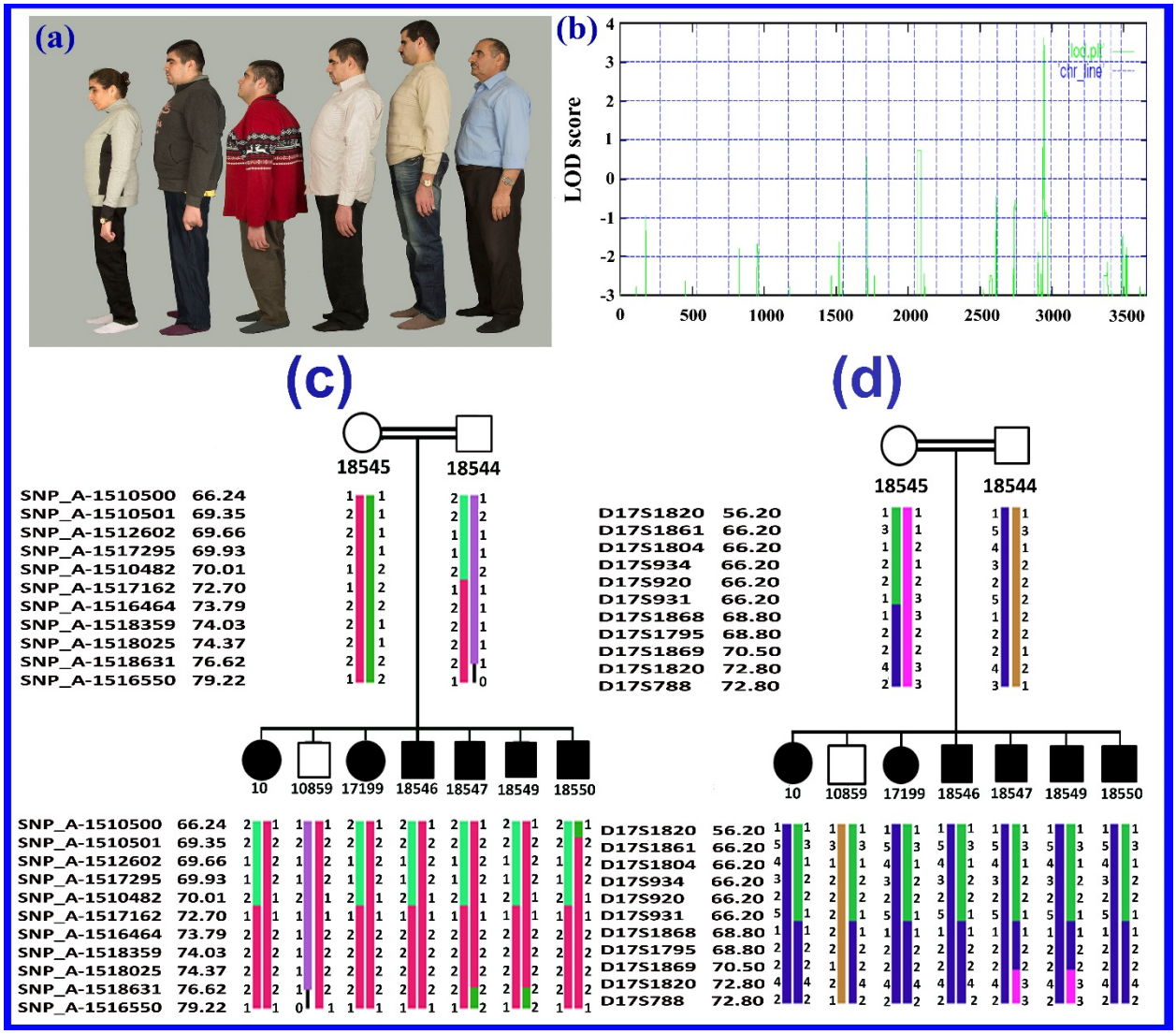

*Fig. 2*: (a) Cartoon diagram showing *XYLT2* gene. Red arrows showing two novel variants identified in the present study. (b) Protein cartoon diagram of various domains of XYLT2 protein. Red arrows showing two novel mutations identified in the present study. (c) Partial XYLT2 amino acid sequence revealed Arginine (R) 387 and aspartate (D) 850, conservation across different species. (d, e) Pedigrees and Sanger sequencing electropherograms of family A and family B. Unaffected carriers are indicated by clear symbols with a dot and arrows indicate the position of the variants.

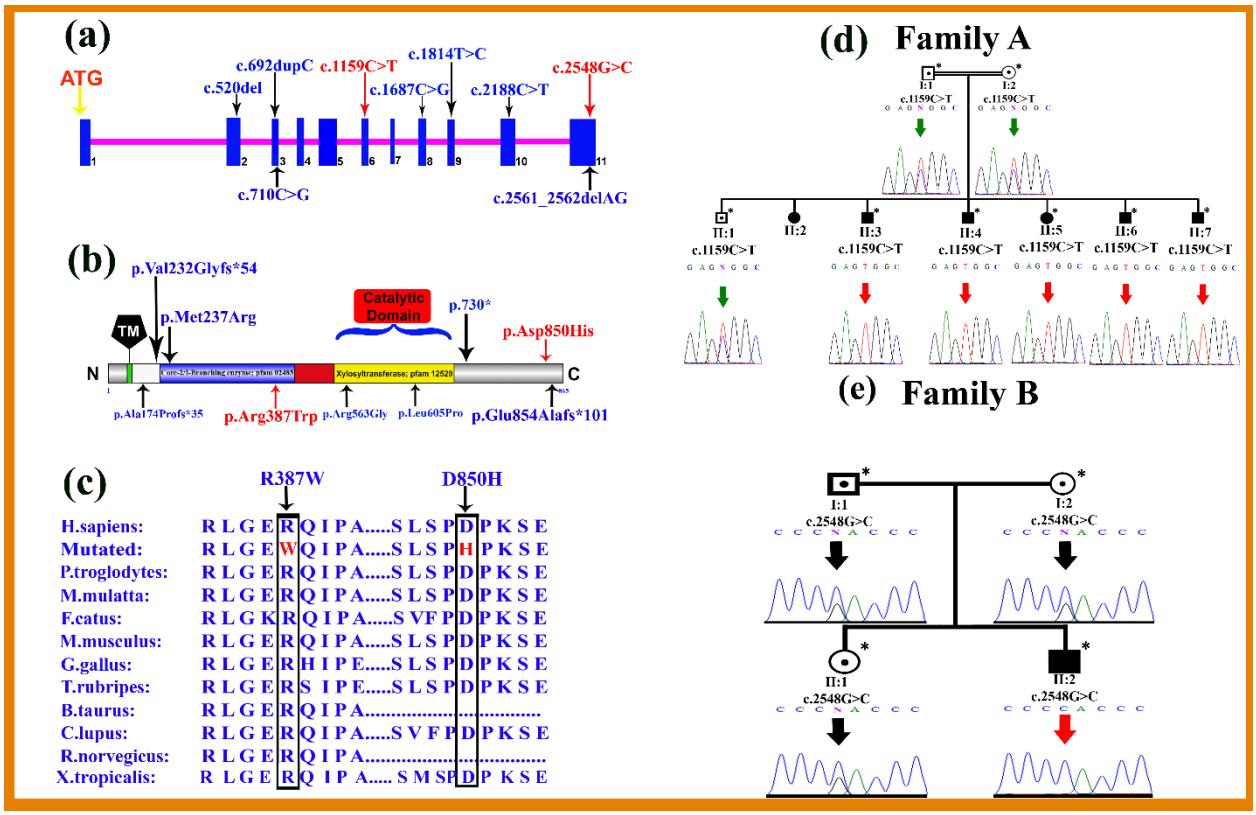

**Table 1.** SOS Phenotypic comparison of affected individuals from different reported studies.

| | **Present study** | **Present study** | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Schmidt *et al*., 2001 and Rodulph *et al*., 2003 (7,8) | Alanay *et al*., 2006 (9) | Munns *et al*., 2015 (6) | | | Taylan *et al*., 2016 (18) | | | |
| | **SOS (Family A)** | **SOS (Family B)** | **Individual 1** | **Individual 2** | **Individual 3** | **Patient1** | **Patient2** | **Patient3** | **Patient4** |
| **Ethnic origin** | Iraq | Turkish | European Australian | | | Turkish | Canada | | Iraq |
| **Consanguinity** | + | - | +/- | +/- | + | + | + | + | + |
| **Mutation** | c.1159C>T; p.Arg387Trp | c.2548G>C (p.Asp850His) | c.692dupC; p.Val232Glyfs*54 | | c.520delA; p.Ala174Profs*35 | c.2188C>T; p.Arg730* | c.1687C>G; p.Arg563Gly | | c.1814T>C; p.Leu605Pro |
| **Normal height** | + | - | + | + | + | + | + | + | + |
| **Platyspondyly** | + | + | + | + | + | + | + | + | + |
| **Long bone fractures** | + | Not reported | + | + | + | + | + | + | + |
| **Fragile bones** | + | - | + | + | + | + | + | + | + |
| **Flat feet** | + | Not reported | + | + | + | Not reported | Not reported | Not reported | Not reported |
| **Cataract** | + | + | + | + | + | + | + | + | + |
| **Retinal detachment** | +/- | - | + | - | + | - | - | - | - |
| **Heart defect** | +/- | - | + | + | - | + | - | - | - |
| **Hearing loss** | - | - | + | + | + | - | - | + | - |
| **Ureter dilatation** | - | - | + | + | Not reported | - | - | - | - |
| **Reduced spine mobility** | + | + | + | + | + | + | + | + | + |
| **Facial dysmorphism** | + | + | + | + | + | + | + | + | + |
| **Intellectual disability** | - | Mild | - | - | - | - | - | - | - |
| **Learning difficulties** | Not reported | + | + | + | - | + | + | + | + |
| **Dental anomaly** | - | - | - | - | - | - | + | - | - |