

# IDENTIFICATION OF ADAMTS6 AS A NOVEL CANDIDATE GENE FOR IDIOPATHIC SHORT STATURE WITH ADVANCED BONE MATURATION

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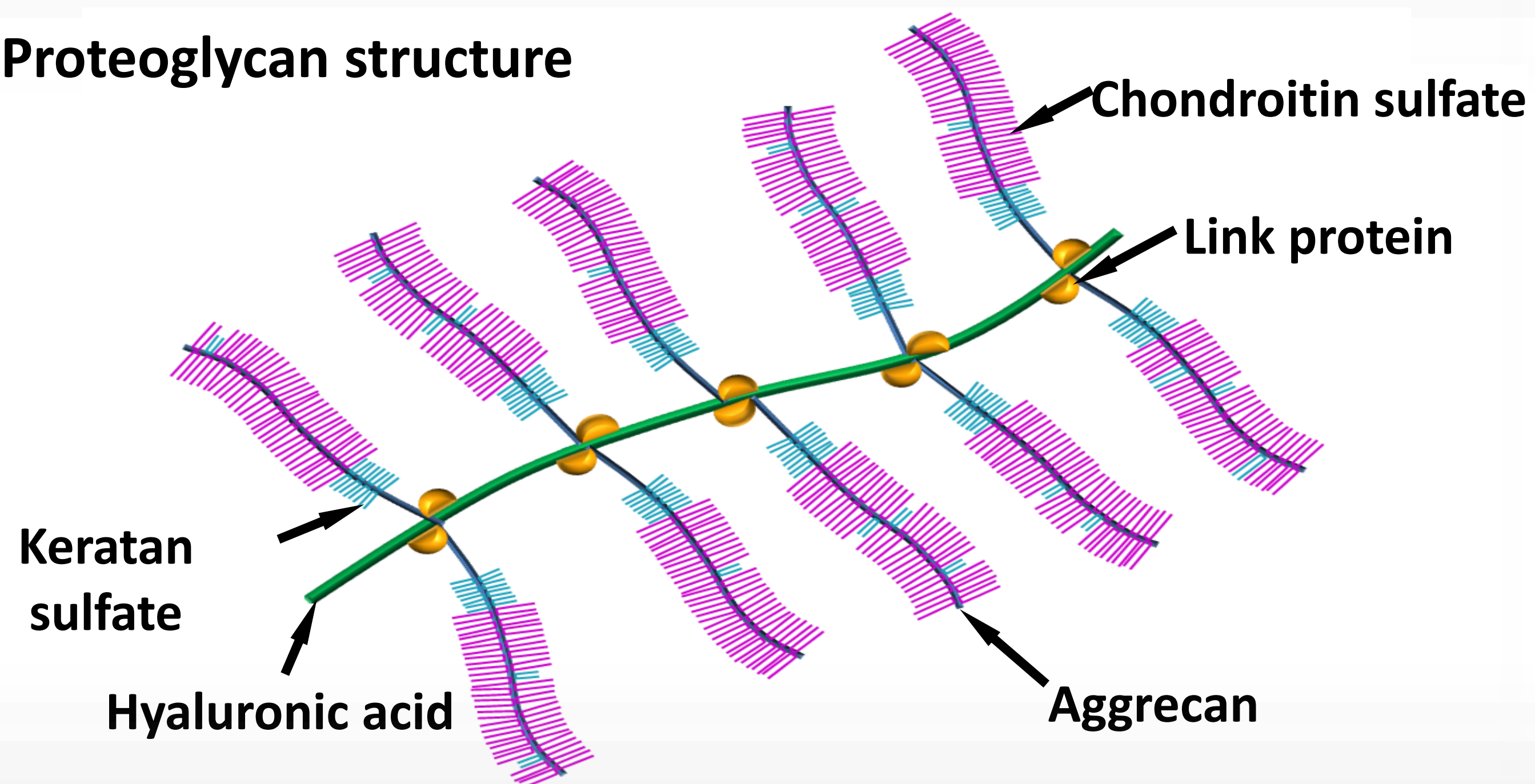
P1-P174



## Introduction

Aggrecan (ACAN) is the major proteoglycan in the articular cartilage, critical for the structure and function of growth plate cartilage. Recently, heterozygous ACAN gene mutations have been reported in families with idiopathic short stature, advanced bone maturation and premature growth cessation.

## Proteoglycan structure



## Exome Sequencing

A library of genomic DNA extracted from the patient's whole blood was prepared with the SureSelect XT2 Human All Exon V4 kit (Agilent Technologies) and was sequenced using 100 bp paired-end reads on the Illumina HiSeq 4000. Mean depth of target regions was 94,6X. Variants were called with the GATK Unified Genotyper and annotated with SnpEff. Variants were analyzed using a tiered filtering strategy based on inheritance patterns, functionality, *in silico* prediction tools, amino acid conservation scores, mutation references phenotype and population frequencies, as appropriate.

Whole exome sequencing (WES) revealed no deleterious variants in the ACAN gene. WES generated more than 83,000 variants, filtering strategy constricted the list to 61 variants.

We identified a potential candidate gene: **ADAMTS6** (Gene ID: 11174) with 2 heterozygous variants in the same allele:

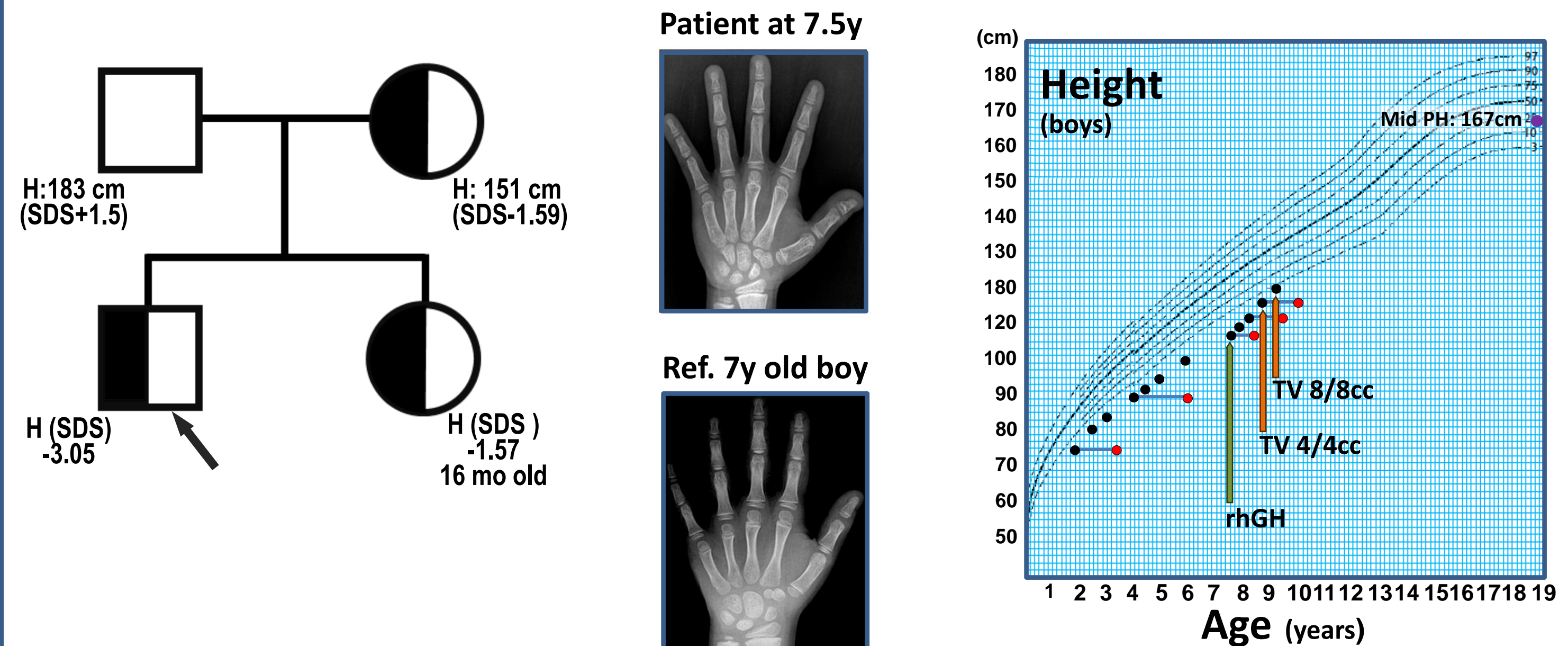
c.[2424T>G;2425C>T], p.[Asn808Lys;Leu809Phe].

These changes were predicted to be damaging by all *in silico* prediction algorithms, and were not found in any database. Segregation was confirmed by Sanger sequencing, revealing that his mother and sister were also heterozygous for these variants.

## Clinical Summary

This is a 11-year-old (y) boy admitted to Garrahan Pediatric Hospital at 1.8 y of chronological age (CA), due to poor growth rate. He had been born full-term (AGA: weight 3250 g, length 49 cm), after an unremarkable gestation and normal delivery. At initial physical examination, he presented a peculiar face with open ears, short neck and mammary hypertelorism and prepubertal external genitalia (two scrotal testes, 1 cc volume each). Neurologic maturation was normal. Initial bone age (BA) was estimated at 3.3 y ( $\Delta$  BA-CA: 1.5 y). Routine and hormonal laboratory evaluations were normal.

He was initially considered as a rare case of idiopathic short stature with advanced BA. No spontaneous catch up growth was observed during the first 4 years of follow up (CA: 5.8 y, height SDS: -3.19)



CA (y)	Height (H) (cm)	H (SDS)	BA (y)	$\Delta$ BA-CA (y)	GV (cm/y)	Weight (g)	W(SDS)
1,8	76	-2.75	3,3	1,5		9.30	-2.42
2,3	80	-2.56			8.00	10.4	-1.78
2,9	85.1	-2.35			8.70	11.0	-2.31
4,1	90.5	-3.19	6	1,9	4.62	12.4	-2.08
4,9	95.7	-2.98	7	2,1	6.50	13.6	-2.01
5,8	99.5	-3.19			4.14	16.0	-1.95
7,5	107.9	-3.03	8,5	1	4.80	19.0	-1.85
8,2	113	-2.62	9,5	0,8	7,28	20.4	-1.85
9,2	119.5	-2.20			6,50	23.9	-1.47
10,5	126.4	-1.79	11,5	1	5,30	28.4	-0.98
10,8	127.4	-1.77			5,20	28.5	-1.09

rhGH

puberty

At 7.5y of CA and a BA slightly advanced (8.5y), even though a normal serum GH levels response to pharmacological arginine/clonidine stimulatory test was found, rhGH treatment (0.33 mg/Kg/day) was initiated. Moreover, at 10.5 y of CA, clinical evaluation showed increase of testicular volume (TV) (4/4 cc) and Tanner Stage (TS): G1, PH 1. At 10.8y, early and accelerated pubertal progression was observed (TV: 8/8 cc, TS: G3, PH: 3) Hormonal studies confirm onset of puberty (serum basal LH: 0.58 ( $\geq$ 0.35), FSH: 1.57mIU/ml and Testosterone: 0.25ng/ml levels). His affected sister showed at 16 months of life stunted growth with mild advanced BA ( $\Delta$  BA-CA: 0.5y)

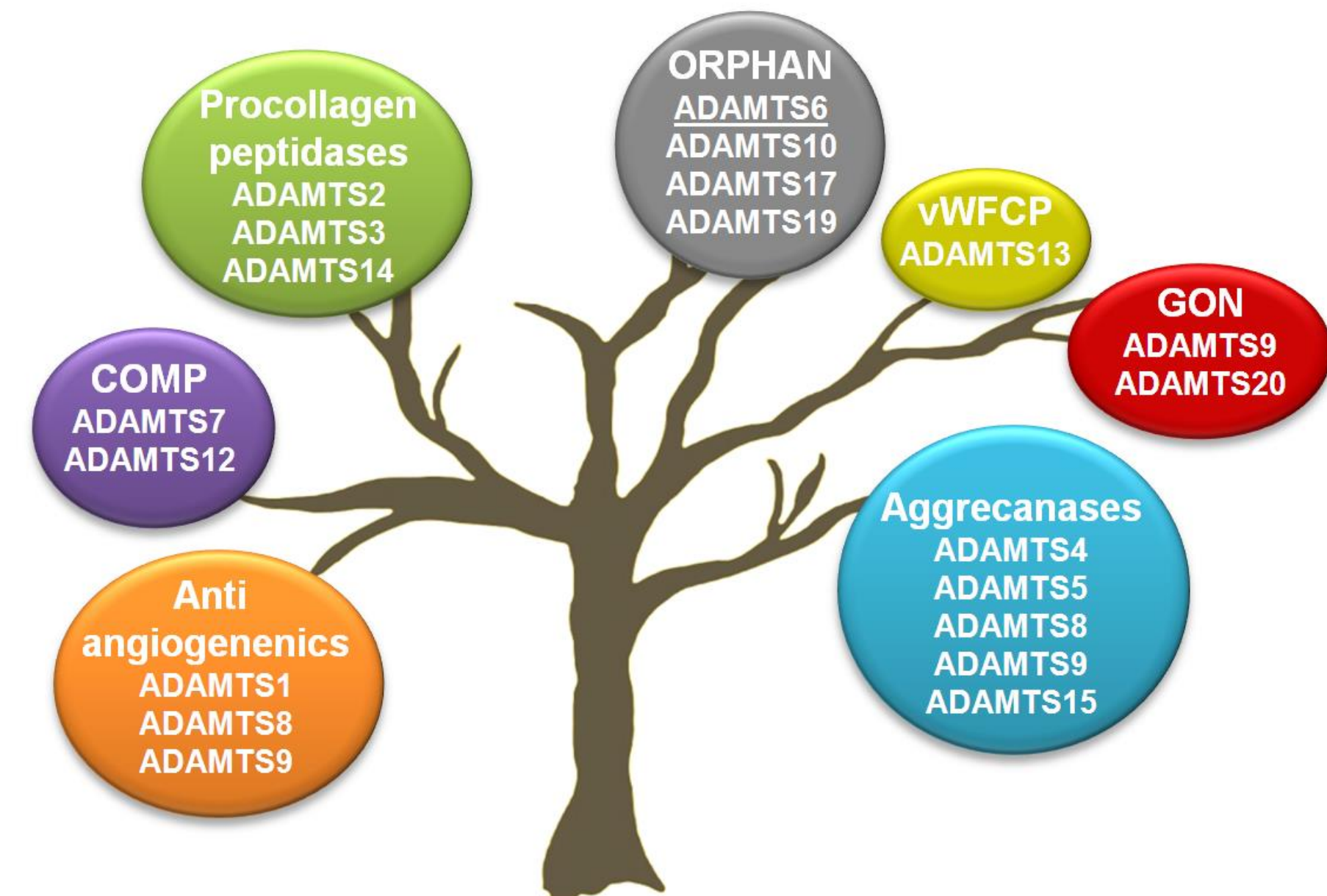
## Clinical comments

Regarding to growth response, we have considered that the rhGH treatment was beneficial for the patient, since the height gained was 19.5 cm ( $\Delta$ HSDS:+1.26). According to present knowledge, there is no explanation to justify why the patient presented early onset of puberty and accelerated pubertal development. We speculate that ADAMTS6 mutation might be involved in the regulation of the early onset of puberty and accelerated pubertal tempo. Currently, in order to improve the prognosis of adult height combined rhGH and LHRH analog treatment has been started.

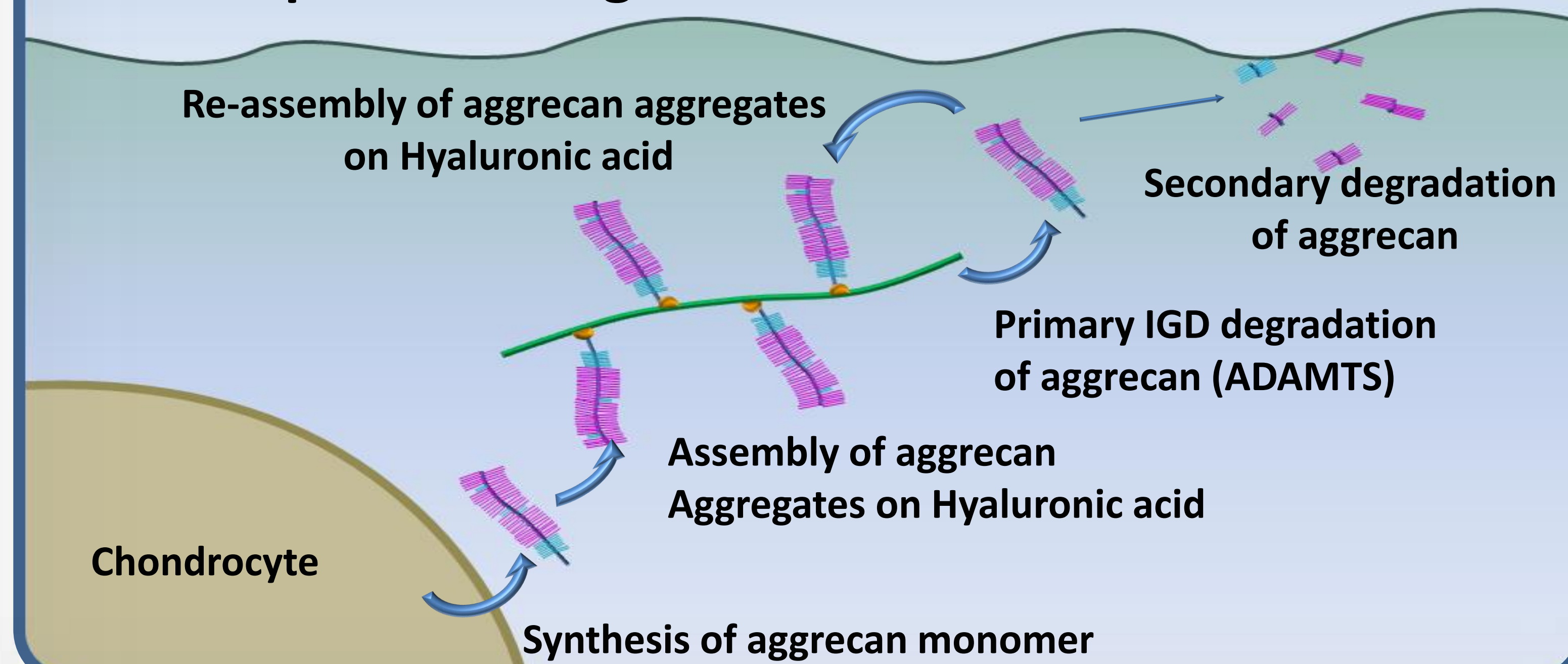
## ADAMTS family

The ADAMTS6 protein is a member of the ADAMTS (A Disintegrin and Metalloproteinase with Thrombospondin motifs) family. It has been demonstrated that ADAMTS1, 4, 5, 8, 9 and 15 cleave the major cartilage proteoglycan Aggrecan, and have thus been termed 'aggrecanases' while ADAMTS6 is a orphan ADAMTS, with no known function or substrate to date.

- ADAMTS6 is widely expressed in several tissues, including cartilage and CNS.
- ADAMTS6 is named like "interesting height candidate gene" on GWA study.
- A translocation has been reported t(5;6)(q11;q25.3) in which the breakpoints disrupted the ADAMTS6, in an 8 ys old boy with short stature and clinodactyly.
- ADAMTS6-knockout mouse shows developmental alterations, short snout, abnormal vertebrae and hindlimb morphology and unfused sternum among others.
- Recently it was reported that ADAMTS6 is necessary for expression of gap junction protein connexin 43 (Cx43) in mouse myocardium; and Cx43 promotes cartilage differentiation.



## Growth plate cartilage turnover



## Discussion

Even though the patient has not reached adult height (AH), it could be suggested that the growth response to rhGH treatment might have improved the prognostic of AH. It might be speculated that early puberty observed in our case could be related to ADAMTS6 mutation. Since aggrecan protein is involved in the regulation of developmental neural plasticity, we propose that the interaction between this metalloprotease and aggrecan protein might be involved in the mechanism of early onset of puberty and accelerated pubertal tempo. Finally we propose ADAMTS6 as a candidate gene for idiopathic short stature and recommend further investigation to confirm this hypothesis.