Moderate to severe short stature and joint involvement in individuals with ACAN deletions

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Moderate to Severe Short Stature and Joint Involvement in Individuals With *ACAN* Deletions

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ABSTRACT

Objective: In recent years, numerous heterozygous ACAN variants have been identified in individuals with short stature. The phenotypic spectrum includes mild to moderate short stature, advanced to delayed bone age, mild dysmorphic features, brachydactyly and/or other mild skeletal defects and joint pathology in early adulthood which often requires surgery. However, only one multiexonic *ACAN* deletion has been reported to date, in a family with short stature, advanced bone age in childhood and early osteoarthritis and spine deformity in the father. Here, we describe 15 individuals from 6 families with *ACAN* deletions.

Design, Patients and Measurements: All probands were referred for short stature to paediatric endocrinology and genetic clinics in different European hospitals. After molecular studies detected the deletion, segregation studies were performed when available. Clinical and radiological features were evaluated in all cases.

Results: Three complete and three intragenic deletions were detected. Patients present with moderate to severe short stature, mildly disproportionate growth and mild dysmorphic features. The majority suffer from joint involvement (osteochondritis dissecans and/or early onset osteoarthritis) which has required early surgical intervention.

Conclusions: Although individuals with heterozygous *ACAN* deletions exhibit phenotypic variability, it is noticeable that they present with a quite homogeneous and age-dependent phenotype with a high proportion of severe joint problems.

1 | Introduction

Aggrecan is a large chondroitin sulphated proteoglycan, the most abundant non-collagenous protein in cartilage, which is essential for its structure and function and plays a pivotal role in skeletal development. It is encoded by *ACAN*, located on chromosome 15q26.1. Biallelic *ACAN* pathogenic variants cause a severe skeletal dysplasia, spondyloepimetaphyseal dysplasia Aggrecan type (MIM 612813) [1, 2], while heterozygous variants are associated with spondyloepiphyseal dysplasia Kimberley type (MIM 608361) [3], as well as short stature with/without advanced bone age and sometimes osteochondritis dissecans (MIM 165800) [4–7].

We and others have detected a significant number of heterozygous *ACAN* variants in children small for gestational age (SGA) and/or short stature with mild skeletal defects [8–10]. In some cohorts, heterozygous *ACAN* variants have been observed to be the second most common monogenic cause of idiopathic short stature, after defects in *SHOX* or its enhancers [11].

During recent years, the phenotypic spectrum has broadened, with individuals having mild to moderate short stature, advanced to delayed bone age and mild dysmorphic features, brachydactyly, other mild skeletal defects and joint pathology in early adulthood, often requiring surgery. Osteochondritis dissecans may occur during puberty and individuals may present with early-onset osteoarthritis and/or disc degeneration [12, 13].

Different variant types have been described but no genotype:-phenotype correlation has been observed. To date, only one multiexonic *ACAN* deletion has been reported, in a family with short stature, advanced bone age in the children and early osteoarthritis and spine deformities in their father [14]. Here, we report the clinical, radiological and genetic features of 15 individuals from 6 families all of whom have a deletion in *ACAN*.

2 | Materials and Methods

All probands were referred for short stature to paediatric endocrinology and genetic clinics in different European hospitals (Spain, Netherlands and Portugal). All participants provided informed consent according to local protocols. Blood samples were extracted from the proband and family members, when available. The deletions were detected in Probands 1, 3, 5 and 6 using a custom-designed large skeletal dysplasia Next generation sequencing (NGS) panel using KAPA HyperChoice and KAPA HyperCapture (Roche) and sequenced on a Novoseq. 6000 or HiSeq. 2500 (Illumina). A customized *ACAN* MLPA (multiplex ligation probe amplification) assay was used to confirm these deletions and for family testing. This included probes for Exons 1, 2, 5, 7, 11, 12 (two probes), 15, 16 and 17. SNP arrays were employed to delimit the deletion extensions in Probands 1, 3 and 5. The deletions in Probands 2 and 4 were detected by a SNP-array and whole exome sequencing, respectively.

3 | Results

A summary of the clinical, radiological and molecular findings of the 15 individuals, 6 probands and 9 affected family members is shown in Figure 1 and Table 1 whilst the principal skeletal

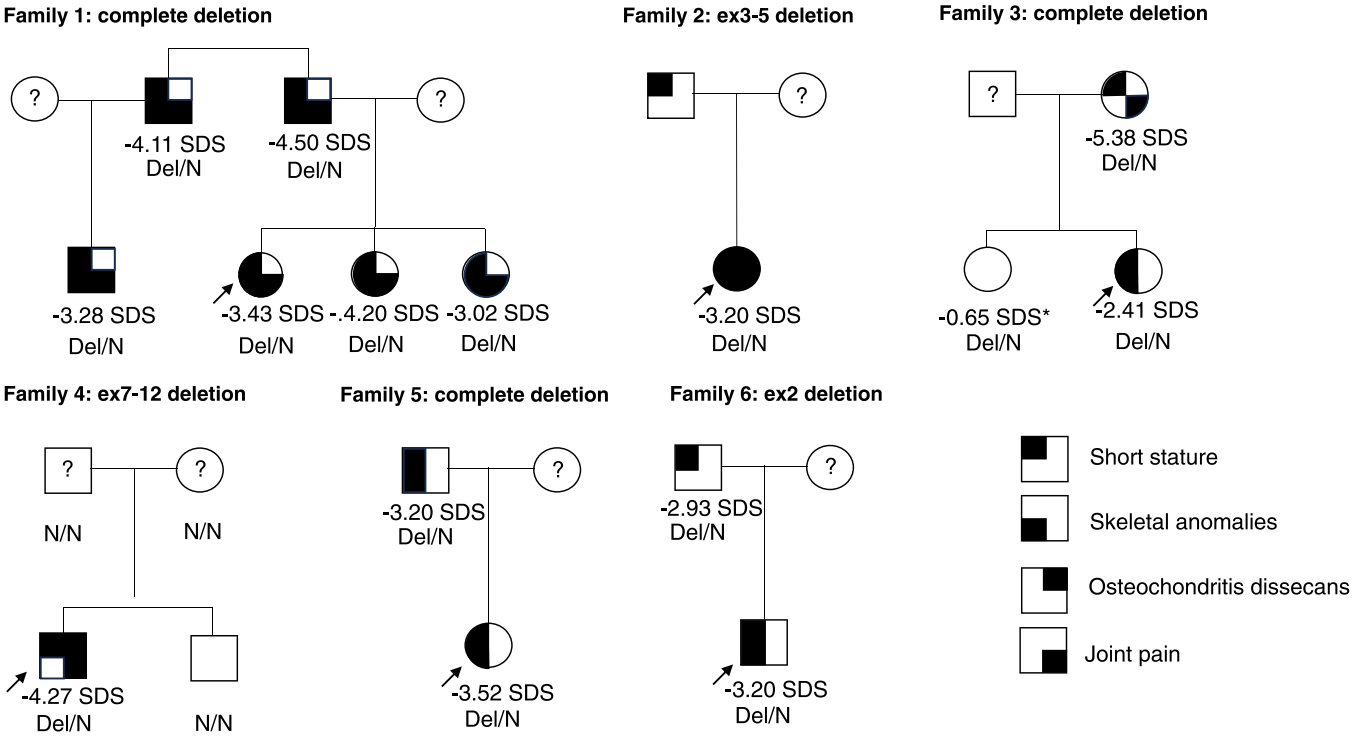

FIGURE 1 | Pedigrees of the six families showing their clinical and radiological characteristics. Below each individual is their height SDS and whether the ACAN deletion genotype. *The elder sister of Proband 3 (Family 3) currently has normal proportionate stature; nonetheless, she had advanced menarche with poor pubertal spurt, so her final height is predicted to be short.

TABLE 1 | Clinical and radiological features of the individuals with *ACAN* deletions from the six families.

	Htz *ACAN* deletion coordinates (hg19) (size)	Genes or exons included in deletion	Sex (M/F) Age (years)	Height (SDS)	Arm span/ Height	**Upper/Lower segment* or Sitting height/ Height	Facial dysmorphic features	Skeletal features	Osteochondritis dissecans	Joint involvement	Other
Familiy 1 (Portugal) **Proband 1**	15:89,330,574–89,449,621 × 1 (~119 kb)	***ACAN,*** *HAPLN3, MFGE8*	F 25	−3.43	1.010	**1.07*	Macrocephaly	Scoliosis Short trunk Brachydactyly	No	Progressive joint pain	
Sister 1			F 22	−4.20	0.964	**1.00*			No	Progressive joint pain	
Sister 2			F 17	−3.02	0.960	**1.00*			No	Progressive joint pain	
Father			M 59	−4.50	0.979	**1.13*			No	Progressive joint pain Bilateral hip arthroplasty (47 and 57 y)	
Paternal uncle			M 62	−4.11	0.948	**1.19*			No	Progressive joint pain Bilateral hip arthroplasty (57 and 58 y)	
Paternal cousin			M 29	−3.28	0.974	**0.990*			No	Progressive joint pain	
Family 2 (Netherlands) **Proband 2**	15:89,277,326–89,490,661 × 1 (~213 kb)	***ACAN*** **ex 3-5**	M 15.7	−3.20	1.030	NR	Broad forehead, pointed chin	Brachydactyly, Broad first metacarpal	Bilateral osteochondritis dissecans	Persistent knee pain	Early puberty, Poor pubertal spurt
Family 3 (Spain) **Proband 3**	15 89,106,233–89,474,659 × 1 (~368 kb)	***ACAN,*** *HAPLN3, MFGE8*	F 6.5	−2.41	1.020	0.539	Prominent forehead, micrognathia	Genu valgo, cubitus valgus	No	No	Muscular hypertrophy
Mother			F 41	−5.38	1.045	0.564	No	NA	No	Knee arthroplasty 40 y	Poor pubertal spurt, Menarche 12 y
Sister			F 11	−0.65	1.010	0.541	No	No	No	No	Muscular hypertrophy, Poor pubertal spurt, Menarche 10 y
Family 4 (Spain) Proband 4	(min 9.9 kb)	***ACAN*** **ex 7–12**	M 14	−4.27	1.044	0.511	Frontal bossing, depressed nasal bridge	Short trunk	Bilateral osteochondritis dissecans	Frequent knee blocking and pain	Poor pubertal spurt

(Continues)

TABLE 1 | (Continued)

	Htz *ACAN* deletion coordinates (hg19) (size)	Genes or exons included in deletion	Sex (M/F) Age (years)	Height (SDS)	Arm span/ Height	***Upper/Lower segment* or Sitting height/ Height**	Facial dysmorphic features	Skeletal features	Osteochondritis dissecans	Joint involvement	Other
Family 5 (Spain) **Proband 5** Father	15:85,090,367–89,413,037 × 1 (~43,322 kb)	*ZSCAN2,* ***WDR73,*** *NMB, C11A, Sec 11A, ZNF592, C28A1,* ***SLC28A1,*** *PDE8A, GOLGA6L3, AKAP13, KHL25,* ***AGBL1*,*** *NTRK3, MRPS11, DET1, AEN, ISG20,* ***ACAN***	F 7	−3.52	1.031	0.527	No	Advanced bone age, broad first metacarpal	No	No	
			M 43	−3.20	1.076	NA	Macrocephaly	Short trunk	No	No	Poor pubertal spurt
Family 6 (Spain) **Proband 6** Father	NA	***ACAN*** **ex 2**	M 4	−3.20	0.982	0.566	Pectus excavatum	Advanced bone age, rib widening, hyperlordosis	No	No	GH treatment from 4–13 y
			M 45	−2.93	NA	0.540	No	No	No	No	

Note: Deletion details are also shown. Genes in bold are related to an autosomal recessive disorder except that marked with an asterisk, which is associated with an autosomal dominant disorder. Loss of function variants in *AGBL1* cause autosomal dominant Fuchs corneal dystrophy (MIM 615523) with onset in the 3–5th decades of life. ACAN deletion is underlined in the table.
Abbreviations: *F*, female; M, male; NA, not available; ND, not determined.

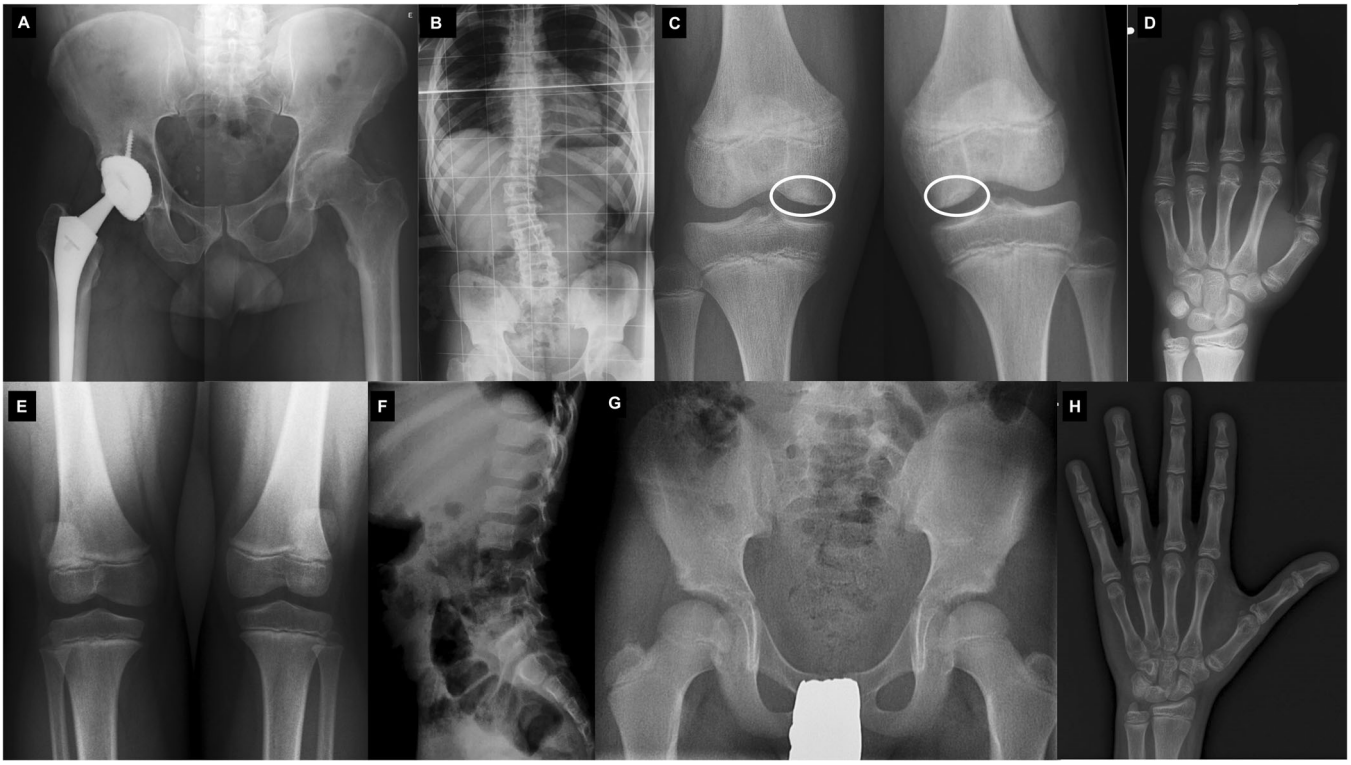

FIGURE 2 | (A) Hip radiograph from *Proband's 1 father* at age 57 years old. Total arthroplasty is present at the right hip. The left hip also shows signs of degenerative articular disease, mild deformity of femoral head with osteophytes, joint space narrowing and subchondral sclerosis. (B) Mild capital femoral changes and lumbar scoliosis are present in *Proband's 1 cousin* at the age of 18. (C) Knee radiographs from *Proband 2*. Bilateral osteochondral fragments are seen in both medial femoral condyles, corresponding to osteochondritis dissecans (ovals). The fragments are not displaced and there is no sign of instability. (D) Hand and wrist radiograph from *Proband 2* at 11.5 years old. Bone age corresponds to a 12.5-year-old male. Mildly broad and short first metacarpal and slightly short and diverted distal phalanges (2nd and 3rd) are shown. (E) Knee radiographs from *Proband 3*. No osteochondral knee defects are noted. Radiographs from *Proband 4*. (F) Lateral lumbar spine radiograph at 7 years. No spinal features are seen. (G) Normal hips at 8 years. (H) Hand and wrist at 14 years; no epiphyseal defects or brachydactyly. Bone age corresponds with chronological age.

features are presented in Figure 2. Growth charts from paediatric patients of the cohort are also available (Figure S1).

Family 1 comprises of six adults with short stature ranging from −3.02 to −4.50 SDS, short trunk, brachydactyly and macrocephaly. Major reported health problems include progressive joint pain in all family members, scoliosis and early-onset osteoarthritis, requiring hip replacement in the two elderly affected individuals. No data about bone maturation, menarche and pubertal spurt were available.

Proband 2, from Family 2, is a 15-year-old boy with proportionate short stature (−3.20 SDS), mild dysmorphic features, brachydactyly and bilateral osteochondritis dissecans. He suffers from persistent knee pain and limited physical activity. During childhood, he was mildly disproportionate and entered puberty at 9.5 years old. He was treated with GnRH analogues until the age of 12 years, but a poor pubertal spurt was noted. No other family members were studied but individuals from four generations are suspected to have had the *ACAN* variant as they had short stature (between −3 and −4.5 SDS), osteochondritis dissecans from an early age and early-onset osteoarthritis.

Family 3 consists of a 6-year-old girl with proportionate short stature (−2.41 SDS), mild skeletal defects and dysmorphic features. Her mother has severe micromelic short stature (−5.3 SDS) and underwent knee arthroplasty at age 40. Her sister currently has normal proportionate stature; nonetheless, she had advanced menarche with poor pubertal spurt, so her final height is predicted to be short. All three have the deletion. Other unstudied adult family members are presumed to be affected as they all have severe short stature (heights between 135 and 140 cm) and have suffered from early hip or knee osteoarthritis with some cases requiring prosthesis and discopathy.

Family 4 is composed of a 15-year-old Spanish boy with proportionate short stature (−4.27 SDS), mild dysmorphic facial features and osteochondritis dissecans (Figures 2 and 3). No skeletal defects were detected in the skeletal survey. Poor pubertal spurt was also reported. Both parents and his brother were tested for the familial *ACAN* deletion but all tested negative, suggesting that the deletion was de novo.

The proband from Family 5, from Spain too, is a 7-year-old girl with proportionate short stature (−3.52 SDS), advanced bone age and brachydactyly. Her father is also short (−3.2 SDS) with a short trunk and macrocephaly.

Family 6 comprises of a 4-year-old boy with mildly disproportionate short stature (−3.2 SDS), advanced bone age and

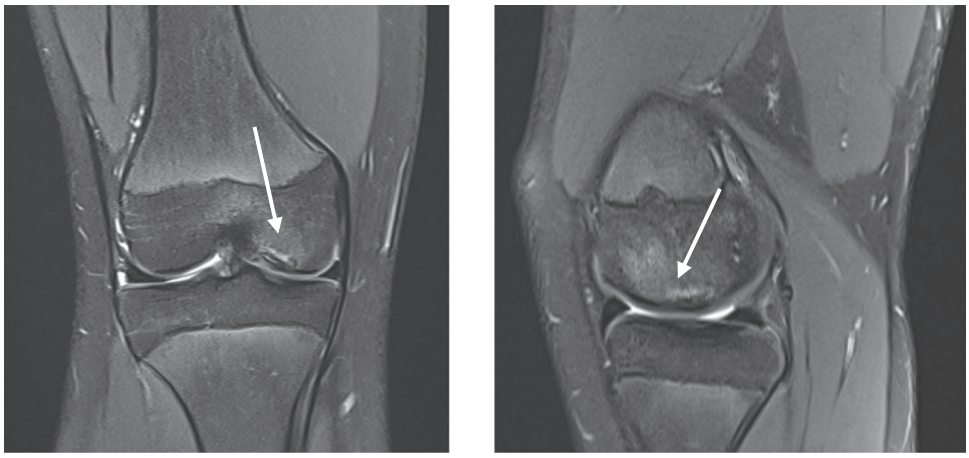

FIGURE 3 | Coronal and sagital images of right knee of *Proband 4*. An 8 mm fragment with moderate periferic hyperintensity is seen in the internal femoral condylar surface (arrow), compatible with osteochondritis dissecans. No fragment shift is present. Mild local bone oedema is present.

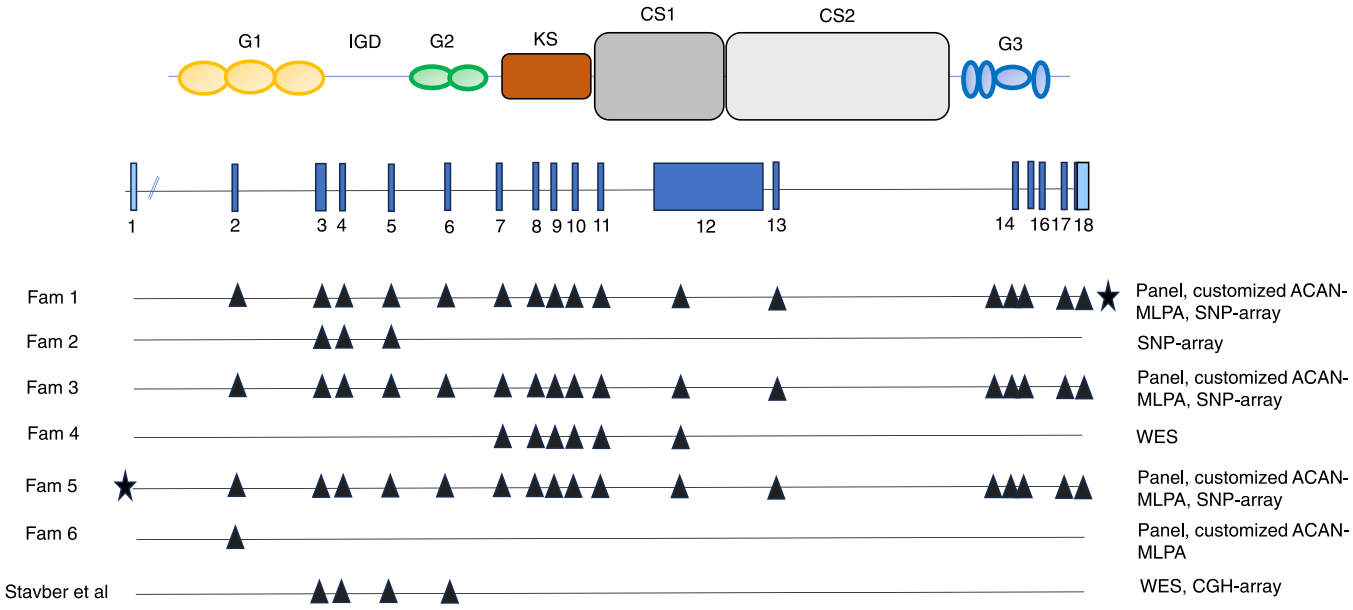

FIGURE 4 | Schematic diagram of aggrecan protein, gene and deletions observed in the six families reported here and that reported by Stavber et al. [14]. In the upper panel, the functional domains of aggrecan are indicated. G1–G3, globular domains; IGD, intergobular domain; KS, keratin sulphate interaction domain; CS1 and CS2, chondroitin sulphate interaction domain. G1 interacts with hyaluronan and link proteins formatting stable ternary complexes in the extracellular matrix. G2 is involved in the processing of products. G3 regulates attachment of glycosaminoglycan chains and aggrecan secretion. The genomic structure is depicted in the middle panel. Coding exons are in dark blue and non-coding exons in light blue. The exons are aligned with the functional domains of the protein although the localization is not fully drawn to scale. In the lower panel the deletions identified in the six families and that previously reported [14] are shown by black triangles. The asterisks indicate that adjacent genes are included in the deletion, downstream of aggrecan in Family 1 and upstream of aggrecan in Family 5 (see Table 1 for further details). The detection and delimitation methods are indicated to the right of the diagram. WES, whole exome sequencing, MLPA, multiplex ligation probe amplification; SNP-array, single nucleotide polymorphism array; CGH-array, comparative genomic hybridization array.

minor skeletal defects. His father is also short (−2.93 SDS). None of them have so far reported joint problems.

3.1 | Genetic Studies

Heterozygous *ACAN* deletions were detected by different techniques in the six families (Figure 4). In Families 1, 3 and 5, the deletion spans not only the entire *ACAN* but also extends to adjacent gene loci, all currently not implicated in any disorder except *AGBL1*, present in Family 5. Loss of function variants in this gene cause autosomal dominant Fuchs corneal dystrophy (MIM 615523). Intrafamilial variability in severity is present but the penetrance is 100%. However, environmental factors play a part on the age of onset, ranging from the 3rd to the 5th decades of life [15].

Families 2, 4 and 6 have intragenic *ACAN* deletions: extending from Exons 3–5, Exons 7–12 and Exon 2, respectively.

4 | Discussion

We report the largest study to date of 15 individuals from 6 unrelated families with heterozygous *ACAN* deletions, 3 complete deletions and 3 intragenic deletions. The pathogenic mechanism of the three families with complete deletions (Families 1, 3 and 5) is haploinsufficiency. The single exon deletion detected in Family 6 deletes Exon 2 which contains the initiation codon thus we would predict that no ACAN to be translated and thus, haplosufficiency would likely be the pathogenic mechanism too. In Families 2 and 4 (Exons 3–5 and 7–12), the 5′ and 3′ ends of the deleted exons are in frame thus aberrant proteins lacking important functional domains may be produced. The Exons 3–5 deletion would lack the majority of the G1 domain which anchors aggrecan to hyaluronan in the extracellular matrix. Exon 6 encodes the final part of the G1 domain. Thus, we predict that this deletion may result in a misfolded protein and undergo endoplasmic reticulum stress. Family 4 deletion could result in an aberrant protein lacking the domains, KS, CS1, CS2, where all the glycosaminoglycan attachment sites are found. Without these glycan chains, the protein would not act as a proteoglycan and thus act as if aggrecan haploinsufficiency occurs. The previously reported deletion by Stavber, the deletion of Exons 3–6, is predicted to lack the entire G1 domain, thus impeding the interactions with hyaluronan and link proteins, thus the function of the protein would be significantly impeded.

Thus, the pathogenic mechanism in 4/6 ACAN deletions described in this study would be haploinsufficency whilst the remaining two and that previously reported, could result in a mutant aggrecan protein translation. Taking these predictions into account, we compared the clinical and radiological aspects for these two mechanistic actions.

We focus on the different phenotypic presentations during childhood and in adulthood.

Joint pathology was present in 4/6 families with individuals from Families 1 and 3, requiring prosthesis from the 4th decade onwards. No adults or children from Families 5 and 6 have reported joint complaints to date. Joint problems manifest as weight-bearing joint pain or osteochondritis dissecans in the first years of life with probable evolution to early osteoarthritis. Osteochondritis dissecans is characterized by the separation of articular cartilage and subchondral bone fragments from the articular surface. This fragmented tissue may then become avascular and exist as a 'loose body' within the joint space. Osteochondritis dissecans is not an easily recognizable entity, and many clinicians are not accustomed to seeing it, thus it may be underdiagnosed [16]. In this cohort, osteochondritis dissecans was formally diagnosed during puberty in Families 2 and 4, with Proband 4 suffering from disabling knee pain and frequent blocking. However, X-rays of both knees failed to show any remarkable injury and magnetic resonance imaging was required to diagnose the injury. Interestingly, these two families had deletions which are likely to generate non-functional mutant proteins whereas haploinsufficiency is thought to be the pathogenic mechanism in the other families where osteochondritis dissecans has not been observed. The identification of further cases will determine if this holds true.

Besides these concrete features, other non-specific clinical and radiological aspects have been observed in this cohort. As reported previously by Gerver et al. [17], an increased arm span/height ratio above 1 was observed in 6/11 of affected individuals. A total of 5/11 patients present with mild shortening of lower limbs (upper segment/lower segment > 1 or sitting height/height > 0.555), which coincides with other previous studies [7]. Short trunk was observed in various members from Families 1 and 4, probably due to the scoliosis present in the members from Family 1 but unexplained for Proband 4. We therefore conclude that body disproportion is not a consistent feature in patients with heterozygous *ACAN* deletions.

Dysmorphic features are variable too. Frontal bossing was present in 3/5 affected children and macrocephaly was reported in the adults from Family 1, thus it could be considered as a continuum.

Radiological anomalies appear to be uncommon during the early years. Brachydactyly is present in some individuals but without significant phalangeal or metacarpal involvement. Advanced bone maturation was reported in Probands 5 and 6. As mentioned previously, scoliosis not related to platyspondyly was present in Family 1 members. Paediatric patients did not present with epiphyseal alterations; however, adults affected with osteoarthritis do.

Thus, the six complete or exonic *ACAN* deletions reported in this current study and that reported by Stavber et al. [14], cause a moderate and age-dependent phenotype, characterized by at least moderate to severe short stature and high risk of joint involvement, in the form of osteochondritis dissecans and/or early osteoarthritis. The size of the gene deletion does not seem to affect the phenotype except for the observation that osteochondritis dissecans occurred in the two children in whom mutant aggrecan proteins are predicted to be translated rather than haploinsufficiency. Otherwise complete deletions and intragenic ones show superimposable features (Families 5 and 6). However, this report still only describes a limited number of individuals. Therefore, detailed description of larger pedigrees and the evolution of paediatric patients included in this cohort/will help us to improve our knowledge of the phenotypic variability and natural history of this disorder.

No specific therapy is currently available for this condition. In view of these findings, the main two aspects to consider are: (1) Promoting good joint health and preventing osteoarthritis is a priority in affected individuals. Osteochondritis dissecans should be investigated in teenagers with persistent knee pain. Obesity should be avoided and if present should be acted on. Additionally, guided sport practice should be promoted, avoiding cargo exercises. However, at present, joint replacement is the only treatment that allows functional and pain improvement when advanced osteoarthritis is present. (2) Short stature: Proband 6 has received growth hormone (GH) treatment from the

age of 4 to present (13 years old) with a favourable response (2 SDS gain) and lack of adverse effects. His final height is still unknown. Some previous reports describe short-term use of recombinant GH, GnRH analogues and aromatase inhibitors in patients with heterozygous *ACAN* variants, resulting in safe but modest improvement of height after 1–3 years of treatment [18–21]. On the contrary, one recent report describes GH treatment as a potential trigger of multiple osteochondritis dissecans lesions in an individual with an *ACAN* nonsense variant [22]. Although this is the only described to date, clinicians should be aware of this. Nonetheless, final height studies are necessary to make a stronger recommendation. At present, GH therapy is an option to consider given the poor height prognosis in patients with *ACAN* variants.

In summary, heterozygous *ACAN* deletions appear to cause more severe short stature and articular involvement than nonsense, frameshift or missense variants but are well integrated in the known 'aggrecanopathies' spectrum. Copy number variants (CNV) evaluation in patients with a suspected aggrecan phenotype should be taken into consideration.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data are available on request from the authors.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.