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**CLINICAL
ENDOCRINOLOGY**

Heterozygous aggrecan mutations cause short stature with brachydactyly: Description of 16 probands and a review of the literature.

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2 1 ***Heterozygous aggrecan mutations cause short stature with brachydactyly:***
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4 2 ***Description of 16 probands and a review of the literature.***
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9 4 **Short title:** ACAN Clinical spectrum
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1
2 50 **SUMMARY**
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5 51 **Objective:** Mutations in the aggrecan gene (ACAN) have been identified in two autosomal
6 52 dominant skeletal dysplasias, Spondyloepiphyseal dysplasia, Kimberley type (SEDK) and
7 53 osteochondritis dissecans, as well as in a severe recessive dysplasia,
8 54 Spondyloepimetaphyseal dysplasia, aggrecan type. Next generation sequencing (NGS) has
9 55 aided the identification of heterozygous ACAN mutations in individuals with short stature,
10 56 minor skeletal defects and mild facial dysmorphisms, some of whom have advanced bone
11 57 age (BA), poor pubertal spurt and early growth cessation as well as precocious osteoarthritis.

12 58 **Design & methods:** Clinical and genetic characterization of 16 probands with heterozygous
13 59 ACAN variants, 14 with short stature and mild skeletal defects (group 1) and two with SEDK
14 60 (group 2). Subsequently, we reviewed the literature to determine the frequency of the
15 61 different clinical characteristics in ACAN positive individuals.

16 62 **Results:** A total of 16 ACAN variants were located throughout the gene, six pathogenic
17 63 mutations and 10 variants of unknown significance (VUS). Interestingly, brachydactyly was
18 64 observed in all probands. Probands from group 1, with a pathogenic mutation tended to be
19 65 shorter and 60% had an advanced BA compared to 0% in those with a VUS. A higher
20 66 incidence of coxa valga was observed in individuals with a VUS (37% v 0%). Nevertheless,
21 67 other features were present at similar frequencies.

22 68 **Conclusions:** ACAN should be considered as a candidate gene in patients with short stature
23 69 and minor skeletal defects, particularly those with brachydactyly, and in patients with
24 70 spondyloepiphyseal dysplasia. It is also important to note that advanced BA and
25 71 osteoarticular complications are not obligatory conditions for aggrecanopathies/aggrecan-
26 72 associated dysplasias.

27 73

28 74 **Key words:** skeletal dysplasia, short stature, aggrecan, ACAN, brachydactyly.

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2 75 **INTRODUCTION**
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5 76 Longitudinal bone growth occurs at the growth plate as a result of chondrogenesis. It
6 77 is regulated by a complex network of signals from endocrine and paracrine systems as well
7 78 as interactions between cellular growth factors and extracellular matrix. Mutations in many of
8 79 these pathways result in growth delay and/or skeletal defects.
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11 80 Short stature is one of the most common reasons for referral to a pediatric
12 81 endocrinologist. Next generation sequencing (NGS) has permitted the identification of
13 82 genetic defects in subgroups of short stature individuals, including heterozygous mutations in
14 83 the aggrecan gene (*ACAN*).
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17 84 Aggrecan is a major structural component of the cartilage growth plate. Until recently,
18 85 *ACAN* mutations had been observed in a few families with spondyloepiphyseal dysplasia,
19 86 Kimberley type (SEDK, MIM 608361)¹, spondyloepimetaphyseal dysplasia, aggrecan type
20 87 (SEMD, MIM 612813)², and osteochondritis dissecans (MIM 165800).³ More recently,
21 88 through the implementation of NGS, heterozygous *ACAN* mutations have been reported in
22 89 individuals with a milder skeletal dysplasia, presenting with short stature and advanced bone
23 90 age (BA).⁴⁻¹⁰ This led to the creation of an International Aggrecan Consortium for the clinical
24 91 and genetic evaluation of 103 *ACAN* heterozygotes from 20 families.⁶ Height appeared to be
25 92 less affected during childhood (median -2 SDS), and most had advanced BA compared to
26 93 chronological age (CA).
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29 94 Aggrecan consists of an N-terminal domain, two globular domains (G1 and G2), two
30 95 inter-globular domains (CS and KS attachment regions), a selectin-like domain (G3) and a C-
31 96 terminal domain.¹¹ Mutations are located throughout the protein and no genotype-phenotype
32 97 correlations have been observed.^{6,10} The pathogenic mechanisms for the accelerated bone
33 98 maturation, cartilage degradation and the clinical heterogeneity remain elusive.
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36 99 We present a retrospective study of the clinical and genetic findings of 16 probands
37 100 with heterozygous *ACAN* variants, detected during routine genetic studies using a skeletal
38 101 dysplasia NGS panel. We also review all cases reported in the literature to determine the
39 102 frequency of the different clinical characteristics related to aggrecanopathies.
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104 **PATIENTS AND METHODS**

105 All participants provided informed consent for the performed studies and ethical
106 approval was obtained from the Hospital La Paz ethical committee.

107 The 16 probands were referred for molecular study from Spanish and Portuguese
108 endocrinology and genetic clinics. **Ten formed part of a cohort of 100 children with short**
109 **stature and mild skeletal defects in either the proband or one of their parents, in whom SHOX**
110 **defects had been previously excluded using MLPA (P018G1, MRC Holland) and DNA**
111 **sequencing. Endocrine disorders including GH-IGF1 related conditions were also excluded**
112 **by biochemical analysis. The remaining six probands were referred for routine skeletal**
113 **dysplasia genetic diagnosis (n>1000 patients). SHOX mutations were similarly excluded in**
114 **four of these, not performed in probands 15-16. BA and skeletal surveys were performed.**

115 Blood samples were extracted from the proband and family members, when available.

116 All probands were analysed using a custom designed Skeletal dysplasia Next
117 generation sequencing (NGS) panel, SKELETALSEQ.V3-6 (n=315-368 genes) and
118 sequenced on a MiSeq/NextSeq sequencer (Illumina, San Diego, CA, USA). Bioinformatic
119 analyses were performed as previously described.¹¹ Conservation, pathogenicity prediction
120 analysis and population frequencies of the identified ACAN variants was carried out using
121 CADD V1.3 (<http://cadd.gs.washington.edu/>), GerpRS
122 (<http://mendel.stanford.edu/SidowLab/downloads/gerp/>) and Alamut V2.10 (Interactive
123 Biosoftware, France) and gnomAD database (<http://gnomad.broadinstitute.org>). Variants
124 were subsequently validated by Sanger sequencing as was family testing. Kinship was
125 confirmed using microsatellite marker analysis (Devyser Complete QF-PCR, Stockholm,
126 Sweden).

127 **After the identification of an ACAN variant**, each referring clinician was asked to
128 complete a specific aggrecanopathy clinical questionnaire including personal and familial
129 records, anthropometric measures, facial dysmorphisms, age of puberty onset and pubertal
130 spurt and other associated medical conditions, similar to that previously published.⁶

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2 131 Advanced BA was defined as a BA greater than one year compared to the CA, whilst
3 132 delayed BA was defined as BA less than one year relative to the CA. Brachydactyly was
4 133 defined as short metacarpals and/or fingers. The clinical data, BA and skeletal surveys were
5 134 then revised by three experts (LS-M, AO, MP). Subsequently, the 16 probands were divided
6 135 into two clinical groups: those presenting with short stature and mild skeletal defects (group
7 136 1, probands 1-14) and two with SEDK (group 2, probands 15-16).
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11 138 **RESULTS**
12
13 139 *Molecular genetics*
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15 140 A total of 16 heterozygous ACAN variants were identified (Figure 1, Table 1). **No**
16 141 other pathogenic mutation or variant of unknown significance (VUS) associated with short
17 142 stature and skeletal defects, including brachydactyly, was detected in the probands. Six
18 143 variants were classified as pathogenic mutations (4 nonsense, 1 frameshift and 1 splicing)
19 144 whilst the remaining ten were classified as VUS (Table 1) using the American Society of
20 145 College of Genetics and Genomics (ACMG) recommendations for classifying variants.¹²
21
22 146 Family testing was performed in all 16 probands. Mutations were inherited in all but
23 147 one case (proband 9) which appears to have arisen as a *de novo* event or due to germinal
24 148 mosaicism. The variants were identified in a total of 20 family members, 19 adults and one
25 149 child, all with short stature and/or mild skeletal defects or SEDK (fathers of probands 15 and
26 150 16). Unfortunately, further cosegregation studies from multiple generations were not
27 151 possible.
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153 *Clinical group 1 (patients 1-14) with short stature and mild skeletal defects*
154 154 Thirteen of the 14 probands were children (age range 1.5-18 years, median 10.2
155 155 years). Probands 1 and 13 were previously included in the International Aggregation
156 156 consortium study.⁶ Clinical characteristics are shown in Table 2. Anthropometric
157 157 measurements were assessed in the 13 children of group 1. The median height SDS was
158 158 below average (-2.9), sitting height-to-height ratio was in the normal range (0.54) and BMI

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3 159 was -0.31 SDS. Advanced BA was observed in three probands whilst equal or delayed BA
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5 160 with respect to CA was determined in ten. Three probands had reached their final height, two
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7 161 of whom had had a poor pubertal spurt. Examples of growth patterns are shown in Supp. Fig.
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9 162 1. Radiological features and hand photos are shown in Supp. Fig. 2 and 3, respectively.
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11 163 Brachydactyly (short fingers and/or short metacarpals) was observed in all probands in group
12
13 164 1. Seven probands (53%) showed a similar phenotype with frontal bossing, depressed nasal
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15 165 bridge and/or mid-facial dysplasia (Table 2). Three of the patients (probands 2, 9, 12) have
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17 166 recently initiated growth hormone therapy, but no response data is currently available. To
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19 167 determine if the clinical characteristics were similar or different in individuals with a clearly
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21 168 pathogenic mutation compared to those with a VUS, we performed a comparison of the
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23 169 clinical and radiological characteristics of these probands from group 1 (Table 3).
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27 171 **Group 2 (probands 15-16) – SEDK**
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29 172 Heterozygous ACAN mutations were identified in the two probands with moderate
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31 173 skeletal anomalies including platyspondyly, whom were subsequently diagnosed as having
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33 174 SEDK (Table 1). Proband 15 was found to have a mutation in the canonical splice acceptor
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35 175 site of intron 1 (c.-7-2A>C) which is predicted to result in the removal of exon 2, where the
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37 176 initiation codon is located. We demonstrated, using a minigene assay, that this mutation
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39 177 indeed ablated the intron 1 splice acceptor site, thus, confirming the pathogenicity of this
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41 178 variant (Supp. Fig. 4). The second case, proband 16, has a missense variant (VUS) in ACAN
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43 179 (p.Thr533Ile). Clinical and radiological characteristics of both probands are shown in Table 4
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45 180 and Supp. Fig.2, respectively.
46
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48 181
49 182 **DISCUSSION**
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51 183 A total of 16 heterozygous variants were detected throughout ACAN, 14 in individuals
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53 184 with short stature, mild skeletal defects and/or facial dysmorphisms (group 1) and two with
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55 185 SEDK (group 2). No other mutation/variant was identified in the skeletal dysplasia patient in
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57 186 the 16 probands which could explain their phenotype. As functional characterization is not
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3 187 currently feasible for confirming the pathogenicity of the ACAN VUS variants, we compared
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5 188 the clinical features in probands from group 1 with a pathogenic mutation (nonsense,
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7 frameshift, splicing, n=5) and those with VUS (missense variants, n=8) (Table 3). Individuals
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9 190 with a pathogenic mutation were shorter (median -3.33 v -2.7 SDS) and 60% had an
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11 191 advanced BA compared to 0% in those with a VUS. Advanced BA:CA was only observed in
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13 192 3/5 individuals with a pathogenic mutation, thus, two individuals had a BA equal or delayed
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15 with respect to the CA. To date, a total of 58 probands and 106 family members (total =164
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17 193 heterozygous ACAN positive individuals with short stature and mild skeletal defects) have
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19 194 been reported in this study and in the literature (Table 5).⁴⁻¹⁰ Although advanced BA is a
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21 195 good indicator for the presence of mutations in ACAN, it cannot be the principal selection
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23 196 criteria. The other major difference was that a third of the individuals with a VUS had coxa
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25 197 valga whereas no individual with a pathogenic mutation presented with this clinical feature.
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27 199 Nevertheless, other features such as skeletal defects, facial dysmorphisms and precocious
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29 200 arthropathy or discopathy were present at similar frequencies in both variant classification
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31 201 groups. An interesting observation and in contrast to previous data, brachydactyly was
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33 202 observed in all probands.

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35 203 After analysing the individuals according to the variant classification, we performed a
36
37 204 study of the clinical features of the probands and affected family members from group 1
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39 205 (n=32; 14 children and 18 adults). No sex or ethnic differences were observed. Only 23% of
40
41 206 the children had advanced BA, once again significantly lower than that previously described
42
43 207 (Table 5). The degree of short stature was also very variable. Another previously
44
45 208 undescribed feature was the presence of mild hip abnormalities in five probands (38%).
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47 209 Parents of two of these probands suffer with osteoarthritis. Osteoarthritis and disc disease
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49 210 were uncommon in our cohort with only three parents having these medical complications
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51 211 (23%) (Table 5).

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53 212 Clinical heterogeneity occurred in some families, as previously reported in a few
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55 213 cases.^{6,10} Proband 3 (p.Ala248Thr- **VUS**) presents with normal stature although in the lower
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57 range (-1.8 SDS), BA equal to CA but has brachydactyly and minor skeletal defects. He has
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3 215 not yet reached adult height and early growth cessation occurs in this growth disorder. His
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5 216 mother, with the same variant, presents with short stature (-3 SDS), precocious osteoarthritis
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7 217 and discopathy. Thus, the differences in height and clinical presentation are likely to be
8
9 218 associated with age. In a similar way proband 12 (p.Glu2426Lys- **VUS**) presents with short
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11 219 stature (-2.5 SDS), BA equal to CA, mild dysmorphic features and skeletal defects. Her
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13 220 father has the same variant but has normal stature although within the lower limit (-1.79
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15 221 SDS) and only brachydactyly. This clinical heterogeneity is similarly observed in other
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17 222 skeletal dysplasias such as those associated with heterozygous *SHOX* or *NPR2* mutations.¹³

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19 223 Two heterozygous *ACAN* variants (1 pathogenic, 1 VUS) were identified in two
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21 224 individuals with SEDK. To date, only one SEDK case with an *ACAN* mutation in the CS1
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23 225 domain, has been reported in the literature.¹ Prior to the implementation of NGS patients with
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25 226 this form of spondyloepiphyseal dysplasia were generally tested for mutations in *COL2A1*
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27 227 and if negative, remained molecularly undiagnosed. Thus, further cases may be identified in
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29 228 the future.

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31 229 Interestingly, proband 6 presented with Madelung deformity and proband 15 and his
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33 230 father, considered to have SEDK, have curved radii and limited elbow extension. Madelung
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35 231 deformity is typically observed in individuals with Léri-Weill dyschondrosteosis (MIM 127300),
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37 232 isolated or due to post-traumatic conditions.¹⁴ *SHOX* defects have been excluded in all
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39 233 probands. This observation is not that surprising since *SHOX* binds to *SOX5* and *SOX6*
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41 234 which along with *SOX9* (SOX trio) activate an aggrecan enhancer, thus, participating in
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43 235 common regulatory pathways in chondrogenesis.¹⁵

44
45 236 The description of our cohort supports the undertaking of a detailed clinical
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47 237 examination and skeletal survey in short stature individuals with suspicion of a mild skeletal
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49 238 dysplasia. Our observation of an association with brachydactyly may help clinicians to
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51 239 request genetic analysis of *ACAN*.

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53 240 For now, the diagnosis of this dysplasia is paramount for patients and their families.
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55 241 Individuals with *ACAN* mutations are at risk of short stature, early growth cessation and poor
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57 242 pubertal spurt, and other health related problems such as obesity and orthopaedic problems

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2 243 should be prevented or their effects reduced. Careful monitoring of patients with ACAN
3 244 mutations may help us to identify important genotype-phenotype correlations and to
4 245 understand their long-term clinical outcomes. Additional familial studies, analysis of larger
5 246 cohorts, generation of animal models and functional analysis will be also required in order to
6 247 determine the incidence of ACAN mutations and the pathogenic mechanism(s)¹⁶.
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16 248
17 249 **References**
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21
22
23

24 250 1. Gleghorn L, Ramesar R, Beighton P, Wallis G. A mutation in the variable repeat region
25 251 of the aggrecan gene (AGC1) causes a form of spondyloepiphyseal dysplasia
26 252 associated with severe, premature osteoarthritis. *Am J Hum Genet*. 2005; 77(3):484-
27 253 490.
28
29
30 254 2. Tompson SW, Merriman B, Funari VA, et al. A recessive skeletal dysplasia, SEMD
31 255 aggrecan type, results from a missense mutation affecting the C-type lectin domain of
32 256 aggrecan. *Am J Hum Genet*. 2009; 84(1): 72-79.
33
34 257 3. Stattin EL, Wiklund F, Lindblom K, et al. A missense mutation in the aggrecan C-type
35 258 lectin domain disrupts extracellular matrix interactions and causes dominant familial
36 259 osteochondritis dissecans. *Am J Hum Genet*. 2010; 86(2):126-137.
37
38 260 4. Nilsson O, Guo MH, Dunbar N, et al. Short Stature, accelerated bone maturation, and
39 261 early growth cessation due to heterozygous aggrecan mutations. *J Clin Endocrinol*
40 262 *Metab*. 2014; 99(8): E1510-E1518.
41
42 263 5. Quintos JB, Guo MH, Dauber A. Idiopathic short stature due to novel heterozygous
43 264 mutation of the aggrecan gene. *J Ped Endocrinol Metab*. 2015; 28(7-8):927-932.
44
45 265 6. Gkouogianni A, Andrew M, Tyzinski L et al. Clinical characterization of patients with
46 266 autosomal dominant short stature due to aggrecan mutations. *J Clin Endocrinol Metab*.
47
48 267 2016; 102(2): 460-469.
49
50 268 7. Van der Steen M, Pfundt R, Maas SJWH, Bakker-van Waarde WM, Roelof J, Odink RJ,
51 269 Hokken-Koelega ACS. ACAN gene mutations in short children born SGA and response
52 270 to Growth Hormone treatment. *J Clin Endocrinol Metab*. 2017;102(5): 1458-1467.

1
2
3 271 8. Dateki S, Nakatomi A, Watanabe S et al. Identification of a novel heterozygous mutation
4
5 of the aggrecan gene in a family with idiopathic short stature and multiple intervertebral
6
7 disc herniation. *J Hum Genet.* 2017; 62(7):717-721.
8
9 274 9. Hu X, Gui B, Su J et al. Novel pathogenic ACAN variants in non-syndromic short stature
10
11 patients. *Clin Chim Acta.* 2017;469: 126-129.
12
13 276 10. Hauer NN, Sticht H, Boppudi S et al. Genetic screening confirms heterozygous
14
15 mutations in ACAN as a major cause of idiopathic short stature. *Sci Rep.* 2017;7(1):
16
17 278 12225.
18
19 279 11. Barraza-García J, Rivera-Pedroza CI, Hisado-Oliva A et al. Broadening the phenotypic
20
21 spectrum of POP1-skeletal dysplasias: identification of POP1 mutations in a mild and
22
23 severe skeletal dysplasia. *Clin Genet.* 2017; 92(1): 91-98.
24
25 282 12. Richards S, Aziz N, Bale S et al. ACMG Laboratory Quality Assurance Committee.
26
27 Standards and guidelines for the interpretation of sequence variants: a joint consensus
28
29 recommendation of the American College of Medical Genetics and Genomics and the
30
31 Association for Molecular Pathology. *Genet Med.* 2015; 17(5):405-424.
32
33 286 13. Hisado-Oliva A, Garre-Vázquez AL, Santaolalla-Caballero F et al. Heterozygous *NPR2*
34
35 mutations cause disproportionate short stature, similar to Léri-Weil dyschondrosteosis. *J*
36
37 288 *Clin Endocrinol Metab.* 2015;100(8):E1133-1142.
38
39 289 14. Knutson EJ, Goldfarb CA, Madelung deformity. *Hand (N.Y.)*.2014; 9(3): 289-291.
40
41 290 15. Aza-Carmona M, Shears DJ, Yuste-Checa P et al. SHOX interacts with the
42
43 chondrogenic transcription factors SOX5 and SOX6 to activate the aggrecan enhancer.
44
45 292 *Hum Mol Genet.* 2011; 20(8): 1547-1559.
46
47 293 16. Gibson BG, Briggs MD. The aggrecanopathies: an evolving phenotypic spectrum of
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49 human genetic skeletal diseases. *Orphanet J Rare Dis.* 2016; 11(1):86.
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5 296 **Table 1: Details of the 16 heterozygous ACAN variants present in this cohort in individuals with short stature and mild skeletal**
6 **defects (probands 1-14) and SEDK (probands 15, 16). Gene and protein domain localization indicated. Conservation, in silico**
7 **pathogenicity predictions and number of affected family members shown for each mutation. ACMG classification also indicated.**
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Proband	Variant	Exon	Aggrecan domain	GerpRS	Amino acid conservation	CADD V1.3	SIFT	Polyphen	MutationTaster	gnomAD (MAF %)	Number of affected family members	ACMG classification
1	c.61G>T (p.Glu21*)	2	G1A	5.3	-	35	-	-		-	2	Pathogenic
2	c.371G>A (p.Arg124His)	3	G1A	5.36	High	24.9	Del	Possdam	Dis Caus	Fin:0.0046, Lat: 0.0029, NFE: 0.0017	1	VUS
3	c.742G>A (p.Ala248Thr)	5	G1B	5.36	High	24.9	Del	Probdam	Dis Caus	Eur: 0.0063 Lat: 0.0058 SA: 0.0032 Fin: 0.0011 EA: 0.0053	1	VUS
4	c.903G>C (p.Trp301Cys)	6	G1B'	5.56	High	24.7	Del	Probdam	Dis Caus	-	2	VUS
5	c.1608C>A (p.Tyr536*)	9	G2B	4.41	-	38	-	-	-	-	1	Pathogenic
6	c.1930G>A (p.Gly644Ser)	10	G2B'	5.11	High	25.2	Del	Probdam	Dis Caus	EA: 0.074 Afr: 0.0041 SA: 0.0032 NFE: 0.0008	1	VUS
7	c.1948G>A (p.Val650Met)	10	G2B'	5.11	High	25.4	Del	Probdam	Dis Caus	NFE: 0.027 Lat: 0.026 SA: 0.0065 Afr: 0.0041	1	VUS
8	c.2218A>T (p.Thr740Ser)	12	KS	5.77	High	22.1	Del	Probdam	Dis Caus	NFE: 0.008	1	VUS
9	c.2369C>G	12	KS	5.77	-	22.1	-	-	-	-	0	Pathogenic

	(p.Ser790*)											
10	c.6142C>G (p.Pro2048Ala)	12	CS2	5.26	High	18. 5	Del	Possdam	Poly	NFE: 0.022 SA: 0.022 Lat: 0.0029	1	VUS
11	c.7269delG (p.Glu2424fs*5)	15	G3	-	-	-	-	-	-	-	2	Pathogenic
12	c.7276G>A (p.Glu2426Lys)	16	G3	5.69	High	35	Del	Probdam	Dis Caus	EA: 0.084 SA: 0.035 Afr: 0.021 NFE: 0.0031 Lat: 0.0029	1	VUS
13	c.7276G>T (p.Gly2426*)	16	G3	5.69	High	56	-	-	-	-	2	Pathogenic
14	c.7342G>A (p.Gly2448Arg) ^π	17	G3	5.31	High	23.5	Del	Probdam	Dis Caus	NFE: 0.026 Afr: 0.016 Lat: 0.014 SA: 0.0032	2	VUS
15	c-7-2A>C*	Intron 1	-	-	-	-	-	-	-	-	1	Pathogenic
16	c.1598C>T p.(Thr533Ile)	9	G2B	4.49	High	23.7	Del	Probdam	Dis Caus	-	1	VUS

The coordinates are according to ACAN transcript NM_013227.3. Aggrecan domains: G1 (A, B, B') and G2 (B, B') globular domains, chondroitin (CS) and keratin (KS) sulphate attachment regions, selectin-like domain (G3). Del: Deleterious, Tol: Tolerated, Prob dam: Probably Damaging, Poss dam: Possibly Damaging, Dis Caus: Disease causing, Poly: Polymorphism. CADD V1.3 values >14 were classified as deleterious. Patients 1 and 13 were included in the International Aggrecan Consortium (Gkourogianniet al, 2017). The gnomAD MAF: T: Total; NFE: Non-Finnish European; Lat: Latin; Afr: African; EA: East Asian; Fin: Finish; SA: South Asian. Highly conserved amino acid (AlamutV2.10).^πSplicing tools in Alamut V2.10 predicted that it may affect splicing but a minigene assay did not confirm these

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5 306 predictions.* Splicing tools predict the ablation of intron 1 canonical splice acceptor site, which was subsequently confirmed using a minigene
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7 307 assay.
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For Peer Review

308 **Table 2: Clinical characteristics of probands from group 1 with short stature and mild skeletal defects. The total number presenting**
 309 **each clinical feature is based on paediatric cases (n=13), thus, excluding adult patient 14.**

Proband	Geographic origin	Mutation cDNA (protein)	Age (years)	Gender (F/M)	SGA (Y/N)	Height (SDS)	Anthropometric data		Facial dysmorphisms						Skeletal findings				Otherskeletal findings	Precocious arthropathy or dysopathy in family member	Affected family members (n)	Affected family members height (SDS)					
							Target height (SDS)	St/H	BA v CA	Macrocephaly	Frontal bossing	Mid-facial hypoplasia	Depressed nasal bridge	Broad nose and philtrum	Thin lips	High arched palate	Hypertelorism	Epicantus	Triangular face	Pubertyspurt (Menarche)	Brachydactyly	Hyperlordosis	Coxa valga				
1	Sp	c.61G>T (p.Glu21*)	4.5	F	N	-3.5	-3.0	0.56	+1.5	+	+	+	-	-	-	-	-	-	-	+	+	-	-	-	2	-3 -1.5	
2	Sp	c.371G>A (p.Arg124His)	8.0	F	N	-3.7	-2.4	0.54	-2	-	-	-	-	+	+	+	-	-	-	-	+	+	+	-	-	1	-2.7
3	Ec	c.742G>A (p.Ala248Thr)	14.5	M	N	-1.8	-2.6	0.52	=	-	-	-	-	-	-	-	-	-	-	NA	+	-	+	Mildly flattened capital femoral epiphyses, slender femora	Osteoarthritis and discopathy in mother	1	-3
4	Sp	c.903G>C (p.Trp301Cys)	7.0	F	N	-3.5	-3.6	NA	=	-	+	+	+	-	-	-	-	-	-	-	+	-	+	Slender femora Osteochondral knee mild defects	Familiar osteochondritis dissecans in affected father and uncle	2	-4.4 -3.8
5	Sp	c.1608C>A (p.Tyr536*)	4.5	F	N	-3.5	-3.0	0.6	+1	-	-	-	-	-	-	-	-	-	-	-	+	-	-	Osteochondral knee mild defects	Osteoarthritis in father	1	-4.5
6	Ch	c.1930G>A (p.Gly644Ser)	16.0	F	N	-2.1	-2.0	0.57	=	-	-	-	-	-	-	-	-	-	-	NA	+	-	-	Madelung deformity, short femoral necks, mild epiphyseal knee defects	-	1	-3.7
7	Sp	c.1948G>A (p.Val650Met)	12.0	M	N	-2.6	-1.4	0.52	-3	-	-	-	+	-	+	-	-	+	-	-	+	-	-	-	-	1	-2.6
8	Sp	c.2218A>T (p.Thr740Ser)	3.0	M	N	-3.2	NA	0.57	-1	-	+	+	-	-	-	-	+	-	-	-	+	-	-	-	-	1	-3.7

9	Sp	c.2369C>G (p.Ser790*)	14.5	M	Y	-2.2	-0.6	0.52	+2	-	+	-	-	-	+	-	-	-	NA	+	+	-	-	-	-		
10	Sp	c.6142C>G (p.Pro2048Ala)	12.5	F	Y	-2.2	-2.1	NA	+1	-	-	-	-	-	-	-	-	-	NA	+	-	-	-	-	1	-2.3	
11	Sp	c.7269delG (p.Glu2424fs*5)	1.5	M	N	-3	-3.2	NA	+2	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	2	-5.8 -3.8	
12	Sp	c.7276G>A (p.Glu2426Lys)	8.5	F	Y	-2.5	-1.8	NA	=	-	+	-	+	-	-	-	-	-	-	+	-	-	Cone-shaped epiphysis	-	1	-1.79	
13	Sp	c.7276G>T (p.Gly2426*)	18.0	M	Y	-4.3	-3.4	0.54	=	+	-	-	-	-	-	-	-	-	Poor	+	-	-	Short femoral neck	-	2	-5.0 -3.7	
14	Sp	c.7342G>A (p.Gly2448Arg)	46	F	NA	-3.7	-4.3	0.52	NA	-	+	+	-	-	-	-	-	-	Poor (11y)	+	-	-	-	-	2	-4.3 -3.5	
		TOTAL (paediatric cohort, n=13)	Median 10.2	7F 6M	4	-2.9	-2.5	0.54	3 Adv 10 Eq/Del	2	5	3	4	2	2	2	1	1	1	-	13	3	3	6	3 families	18	Median -3.77

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312 Geographic origin: Sp: Spain, Ec: Ecuador, Ch: China. Gender: M: Male; F: Female. SGA: N: No; Y: Yes. SH/H: Sitting height/height. **BA vs CA:**
313 **BA: Bone age; CA: Chronological age; +: BA>CA. -: BA=CA (equal), -: BA<CA. BA>1y CA: Advanced (Adv). BA +/- 1y CA: Equal, BA<1y:**
314 **Delayed, Eq/Del: Equal or Delayed. NA: Not available. Facial dysmorphisms and skeletal findings: +: Present; -: Absent**

315 **Table 3. Comparison of clinical features observed in group 1 child probands (n=13) with pathogenic mutations (n=5) and**
 316 **VUS (n=8) as classified by ACMG.**

Variant type (n=number of probands)	Inheritance/ De novo	SGA	Median height SDS	Target height SDS	SH/H	BA v CA (Adv or Equal/delayed) (%)	Facial dysmorphisms (%)	Brachydactyly (%)	Hyperlordosis (%)	Coxa valga (%)	Otherskeletal findings (%)	Precocious arthropathy or discopathy in family member(%)	Affected family members height SDS
Pathogenic mutation (nonsense/ frameshift) (n=5)	4 AD 1 de novo	2	-3.33	-2.64	0.55	3 Adv (60%) 2 Equal/Delayed (40%)	4 80%	5 100%	2 40%	0 0%	2 40%	1 20%	-5.8/-1.5
VUS (missense) (n=8)	8 AD	2	-2.7	-2.27	0.544	8 Equal/Delayed (100%)	5 62%	8 100%	1 12%	3 37%	4 50%	2 25%	-4.4/-1.78

332 AD: Autosomal dominant, SGA: Small for gestational age, SH/H: Sitting height/height, BA vs. CA: Bone age v Chronological age,

333 Adv: Advanced BA (>1 year), Equal/Delayed: BA equal to CA or delayed (<1 year).

334 **Table 4. Clinical and genetic features of probands 15 and 16, both with SEDK.**

Proband	Geographic origin	Mutation	Gender (M/F)	Age (years)	SGA (Y/N)	Height SDS	Target height SDS	SH/H	BA v CA	Facial dysmorphisms	Morphologic findings	Skeletal findings	Affected family members
15	Pt	c.-7-2A>C (intron 1)	M	7.5	Y	-4.20	-3.6	0.53	-2	No	Stocky appearance, short neck, limited elbow extension	Brachydactyly, curved radius, mild platyspondyly	Father's height -3.66 SDS
16	Sp	c.1598C>T (exon 8) p.(Thr533Ile) (G2 domain)	M	10	N	-0.76	-0.54	NA	=	No	Obesity, short trunk, waddling gait	Bilateral irregular femoral epiphyses, mild thoracic platyspondyly	Father's height -0.38 SDS, limp.

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336 Geographic origin: Pt: Portugal, Sp: Spain. BA vs CA: BA: Bone age; CA: Chronological age; +: BA>CA (Advanced), =: BA=CA

337 (Equal), -: BA<CA (delay); NA: Not available.

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5 338 **Table 5. Overview table of main molecular and clinical characteristics of individuals with heterozygous ACAN variants/mutations and**
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7 339 **short stature (excluding SEDK and Familial osteochondritis dissecans) reported in this current study and previously in the literature.**

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10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47	Reference (ref number)	Number of patients (Children/adults) from X families	SGA	Range of height (SDS) Children Adults	Advanced BA in children	Frontal bossing	Flat nasal bridge	Mid facial hypoplasia	Brachydactyly	Short thumbs, and/or short first metacarpal	Broad great toes	Hyperlordosis	Hip anomalies	Mild osteochondral knee defects	Earlygrowthcessation (adults)	Early-onset arthritis /OD (families)	Intervertebral disc disease (families)
Nilsson et al, 2014 (ref 4)	14 (5/9)* 3 families	2/4	-4/-1.2 -3.8/-2.3	3/5	NR	NR	6/9	6/9	3/9	NR	NR	NR	NR	NR	5/5	1/3	0/3
Quintos et al, 2015 (ref 5)	3 (1/2)** 1 family	0/1	-2.7 -4.7/-2.6	1/1	NR	NR	1/3	NR	NR	NR	NR	NR	NR	NR	2/2	0/1	0/1
Manouk van der Steen et al, 2016 (ref 7)	10 (4/6) 3 families	3/3	-3.7/-2.4 -5.4/-3.7	3/4	NR	NR	9/10	NR	3/10	6/10	3/10	NR	NR	NR	3/3	0/3	
Gkourogianni et al, 2017 (International Aggrecan Consortium) (ref 6)	102 ♀ (32/70) 20 families	NR	-4.2/-0.6 -5.9/-0.9	19/23 probands	2/32 probands	8/20	8/20	5/20	3/20	NR	NR	NR	NR	NR	23/70	13/20	8/20
Dateki et al, 2017 (ref 8)	4 (2/2) 1 family	0/2	-2.7/-2.5 -3.1/-3	2/2	NR	NR	3/4	NR	NR	NR	1/4	NR	NR	NR	0/1	1/1	
Hu et al, 2017 (ref 9)	9(3/6) 3 families	1/1	-4.3/-2.9 -5.4/-2.9	0/3	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	0/3	0/3	

	Hauer et al, 2017 (ref 10)	11(6/5) 6 families	1/6	-3.9/-2 -3.8/-1.8	2/5	3/6	NR	NR	3/6	2/6	2/6	NR	1/6	NR	NR	1/6	NR
	Current study	32 (14/18)*** 14 families	4/13	-4.3/- 1.86 -5.4/- 1.79	3/13	5/13	4/13	3/13	12/13	11/13	NR	3/13	5/13	3/13	6/6	3/14	1/14
	SUMMARY[§]	164 (59/105) 45 families	11/40	-4.7/-0.6 -5.9/-0.9	28/45	9/49	4/13	22/39	19/28	19/38	8/16	7/27	5/13	3/13	27/74	20/45	10/39

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342 Ns: nonsense mutation, Fs: frameshift mutation, Spl: splice donor site variant, Mis: missense variant, NR: not reported.* Three families are
 343 included in the International Aggrecan Consortium (5 children, 9 adults)⁶; **The family reported in this study is included in the International
 344 Aggrecan Consortium (1 child, two adults)⁶; *** Two families (two children, two adults) are included in the International Aggrecan Consortium⁶.
 345 Results shown here are related to the paediatric cohort. ¥ Gkourogianni et al. 2017, reported a large cohort of probands and relatives
 346 (International Aggrecan Consortium). Clinical and molecular characteristics are related to the 20 families, not to individuals as with the other
 347 studies. In addition, data was compiled from both adults and children. We have attempted to separate this data when possible. [§] Combined data
 348 from this current study and previous studies. The numbers documented for type of mutation, early onset arthritis/OD and intervertebral disc
 349 disease data are given as the number of families whilst the other characteristics are totals for the number of individuals (probands or adults).
 350 Once again, most of the data from the International Aggrecan Consortium (¥ ref 6) cannot be summarized as the data is presented for families
 351 rather than individuals. Patients included in three earlier studies (*, **, ***) have not been duplicated in this data.

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2 352 **Figure Legends**
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45 353 **Figure 1: Structure of ACAN and the locations of the variants identified in the 16**
6 354 **probands.** The G1 region encoded by exons 3-6, the IGD region by exon 7, the G2 region
7 355 by exons 8-10, the GAG (KS-CS1-CS2) attachment region encoded by exons 11-12 while
8 356 the G3 region encoded by exons 13-19. Missense variants written in black whilst premature
9 357 truncating (nonsense, frameshift, splicing) mutations written in red. Mutations corresponding
10 358 to SEDK written in blue. Families with osteoarthritis and/or discopathy underlined.
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For Peer Review

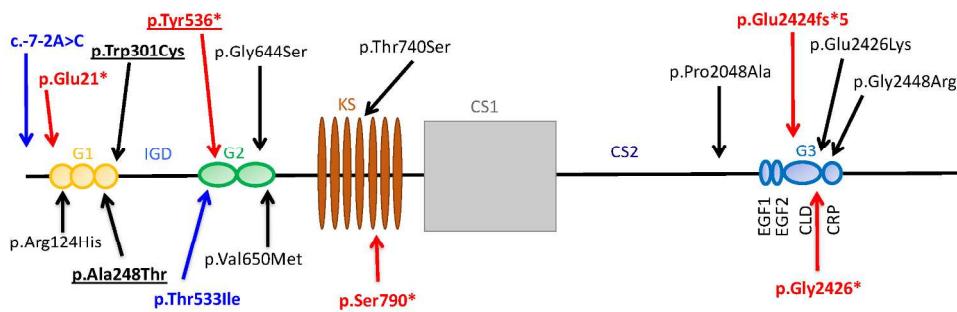
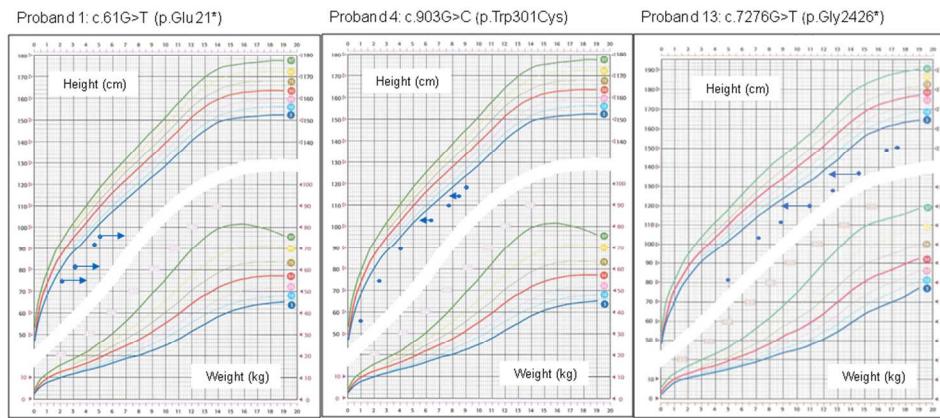


Figure 1: Structure of ACAN and the locations of the variants identified in the 16 probands. The G1 region is encoded by exons 3-6, the IGD region by exon 7, the G2 region by exons 8-10, the GAG (KS-CS1-CS2) attachment region encoded by exons 11-12 while the G3 region is encoded by exons 13-19. Missense mutations are written in black. Truncating (nonsense and frameshift) mutations are written in red. Mutations corresponding to Kimberley type dysplasia are written in blue. Families with osteoarthritis and/or discopathy are underlined.

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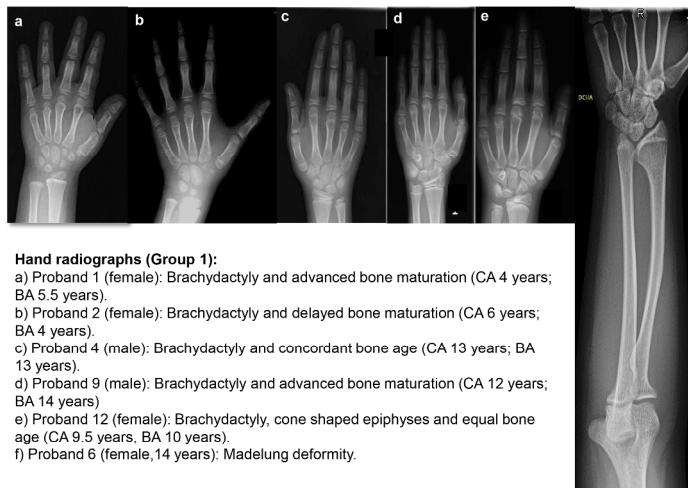
Supp Fig 1: Growth charts of 3 from 13 probands from group 1. The closed circles indicate height at the chronological ages whilst the arrows indicate the bone age for those chronological ages. Proband 1 has advanced bone age, proband 4 has equal bone age and proband 13 has delayed bone age.



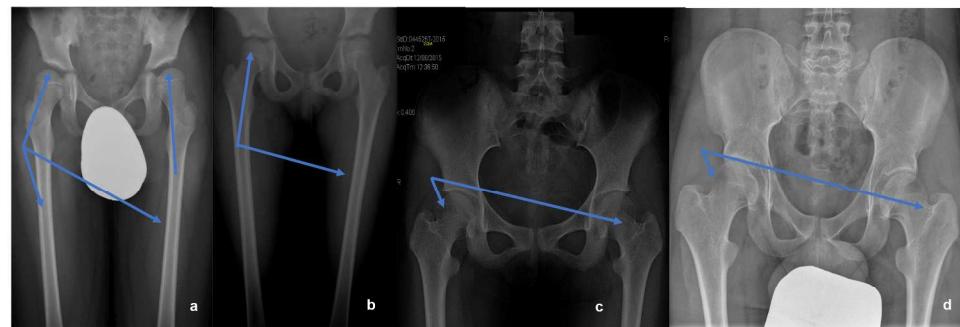
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Supp Fig 2: Main skeletal findings in probands from both group 1 and 2.



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**Hip radiographs:**

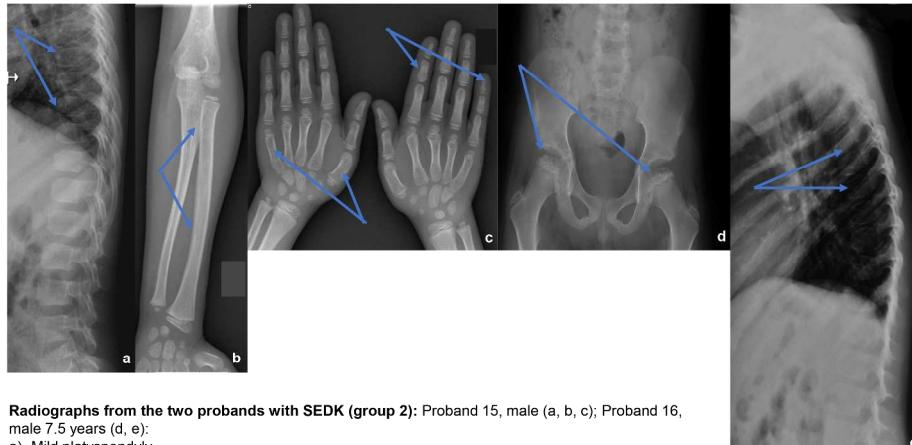
- a) Proband 3 (male, 14 years): Coxa valga, mildly flattened capital femoral epiphyses, slender femora.
- b) Proband 4 (female, 7 years): Coxa valga, slender femora.
- c) Proband 6 (female, 14 years): Short femoral necks.
- d) Proband 13 (male, 18 years): Short femoral necks.

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**Knee radiographs:**

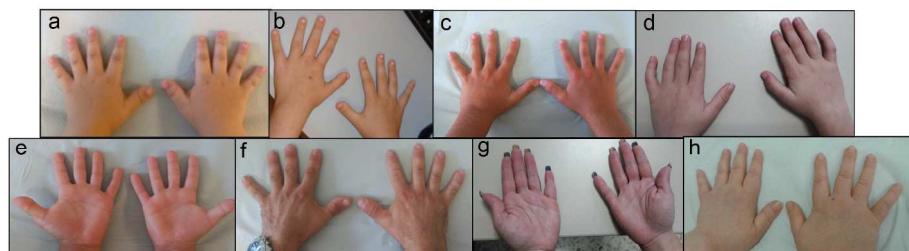
- a) Proband 4 (female 7 years): Osteochondral knee mild defects.
- b) and c) Proband 5 (female, 4.5 years): Osteochondral knee mild defects.
- d) Proband 6 (female, 14 years): Mild epiphyseal knee defects.

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20 Radiographs from the two probands with SEDK (group 2): Proband 15, male (a, b, c); Proband 16,
21 male 7.5 years (d, e):
22 a) Mild platyspondyly.
23 b) Slightly deformed/bended radius and ulna.
24 c) Brachydactyly with shortened metacarpal bones.
25 d) Irregularity in capital femoral epiphysis.
26 e) Moderate platyspondyly and kyphosis.

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Supp Fig 3: Hand photographs of various probands and parents from group 1, all showing brachydactyly.

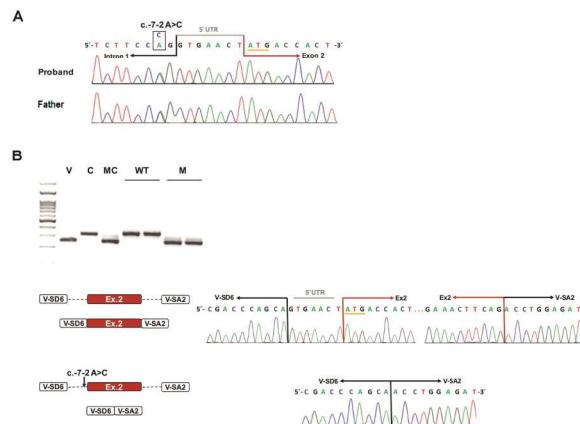
Upper panel: Dorsal images of hands from paediatric cohort of patients with heterozygous ACAN mutations.

a) Patient 1, b) Patient 4, c) Patient 7, d) Patient 8.

Lower panel: Ventral and dorsal images of hands from parents with heterozygous ACAN mutations:

e and f) Proband's 7 father, g) Proband's 8 mother, h) Proband's 13 mother.

285x190mm (300 x 300 DPI)



Supp. Figure 5. Genetic and functional analysis for the ACAN c.-7-2A>C variant. A) Sequence chromatogram of the c.-7-2 A>C variant detected in exon 2 of ACAN in the proband and his father. B) In vitro assay for the splicing mutation c.-7-2A>C using a minigene assay. On top panel, gel electrophoresis of splicing products generated from the amplification of cDNA isolated from transfected HEK293 cells (the same results were observed with COS7, data not shown) using two vector primers (PSD6 and PSA2) for empty pSPL3vector (band V), wild-type control (band C), mutant splicing control (band MC), ACAN wildtype c.-7-2A (band WT) and variant, c.-7-2C, in the non-coding portion of exon 2 (band M). On the bottom panel, schematic representation and chromatograms of the sequences obtained for the splicing PCR products for ACAN wild type (c.-7-2A) and variant (c.-7-2C). Firstly, sequencing of band D corresponds to the wild-type exon 2 of ACAN. Secondly, sequencing of band E corresponds to an aberrant transcript which demonstrated that the exon 2 splice acceptor site was ablated, thus, only the pSPL3vector sequence is observed (V-SD6/V-SA2).

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