Supplementary information

Novel missense *ACAN* gene variants linked to familial osteochondritis dissecans cluster in the C-terminal globular domain of aggrecan

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Supplemental table 1

Overview of skeletal effects of *ACAN* variants (December 2021)

cDNA level [1]	Protein level [1]	Affected domain	Phenotype [2]	Inheritance	Ref
Translocation t(10;15)	-	Intron 1	SS	AD	[1]
c.-7-2A>C*	-	Intron 1	SEDK	AD	[2]
c.6_13delCACTTTAC [4]	p.Thr3Leufs*21	Signal pept	SS	AD	[3]
c.6_17delinsTCT	p.Thr3_Trp6delinsLeu	Signal pept	SS	AD	[4]
c.61G>T	p.Glu21*	G1 A	SS, AdvBA, facial dysmorphism, mild skeletal effects	AD	[2, 5]
c.71_1051del	p.His25_Thr350del	G1 AB	SS, AdvBA, OA, Spine deformities	AD	[6]
c.116dupT	p.Arg40Glufs*51	G1 A	SS, facial dysmorphism, mild skeletal effects	AD	[7]
c.151T>G [5]	**p.Cys51Gly** [5]	G1 A	SS	AD	[8]
c.217delA	p.Ile73Serfs*12	G1 A	SS	Not analyzed	[9]
c.223T>C	**p.Trp75Arg**	G1 A	SS, AdvBA, OA, IDD	AD	[5]
c.272delA	p.Arg93Alafs*41	G1 A	SS, AdvBA, IDD, mild midface hypoplasia, short thumbs	AD	[5, 10]
c.301C>T	p.Gln101*	G1 A	SS, DelBA, OA, IDD	AD	[6]
c.350C>T	**p.Thr117Ile**	G1 A	SS	AD	[11]
c.371G>A	**p.Arg124His**	G1 A	SS, DelBA, mild skeletal defects	AD	[2, 12]
c.410_418delinsTGGA	p.His137Leufs*31	G1 A	SS, AdvBA	AD	[6]
c.436delC	pLeu146Trpfs*5	G1 A	SS	AD	[4]
c.492C>G	p.Tyr164*	G1 B	SS, AdvBA, OA, IDD	AD	[5]
c.512C>T	**p.Ala171Val**	G1 B	SS, AdvBA	AD	[13]
c.515delA	p.Gln172Argfs*59	G1 B	SS	AD	[8]
c.532A>T	**p.Asn178Tyr**	G1 B	SS, AdvBA	AD	[5, 14]
c.560dupA	p.Leu188Alafs*13	G1 B	SS, DelBA	AD	[11]
c.631_632insA	p.Tyr211* [3]	G1 B	SS, AdvBA	Not analyzed	[11]
c.661delT	p.Tyr221Metfs*10	G1 B	SS	AD	[3]
c.742G>A	**p.Ala248Thr**	G1 B	SS, mild skeletal defects	AD	[2]
c.903G>C	**p.Trp301Cys**	G1 B'	FOCD, SS, facial dysmorphism, mild skeletal effects, OA, IDD	AD	[2, 5, 12]

c.916A>T	**p.Ser306Cys**	G1 B’	SS, AdvBA, IDD	AD	[5]
c.973G>A	**p.Val325Met**	G1 B’	SS, craniofacial dysmorphism, kyphosis, trident hands	Not analyzed (compound heterozygous for c.2902T>C)	[15]
c.1046A>G	**p.Tyr349Cys**	G1 B’	SS only	Not analyzed	[16]
c.1047_1048delinsAC	p.Tyr349*	G1 B’	SS	AD	[5]
c.1117_1120delCAGA	p.Thr374*	IGD	SS	AD	[3]
c.1180C>T	p.Arg394*	IGD	FOCD, SS	Compound heterozygous for NPR2 mutation	[8]
c.1243G>T [3]	p.Glu415*	IGD	SS, OA, IDD	AD	[5]
c.1411C>T	p.Gln471*	IGD	SS	AD	[11, 17]
c.1425delA	p.Val478Serfs*14	G2 B	SS, OA	AD	[5]
c.1467C>G	p.Tyr489*	G2 B	SS, AdvBA	de novo	[11]
c.1505G>C	**p.Arg502Pro**	G2 B	SS only	Not analyzed	[16]
c.1526C>A	p.Ser509*	G2 B	SS, OA	AD	[5]
c.1531-1532delGA	p.Glu511Alafs*10	G2 B	SS	Not analyzed	[18]
c.1551C>G	p.Tyr517*	G2 B	SS	AD	[9]
c.1598C>T	**p.Thr533Ile**	G2 B	SEDK	AD	[2]
c.1608C>A	p.Tyr536*	G2 B	SS, AdvBA, mild osteochondral defects, OA, mild midface hypoplasia, broad great toes	AD	[2, 12, 19]
c.1702G>A	**p.Asp568Asn**	G2 B	OCD, SS	AD	[8]
c.1733-1G>A		G2 B	SS	AD	[17]
c.1744delT	p.Phe582Serfs*69	G2 B’	SS, IVD	AD	[20]
c.1762C>T	p.Gln588*	G2 B’	SS	AD	[17]
c.1774C>T	p.Gln592*	G2 B’	SS	AD	[8]
c.1817C>A	**p.Ala606Asp**	G2 B’	SS	AD	[17]
c.1861A>T	p.Lys621*	G2 B’	SS, AdvBA	Not analyzed	[11]
c.1880_1883dupTGGC	p.Asp629Glyfs*31	G2 B’	SS, AdvBA	AD	[11]
c.1930G>A	**p.Gly644Ser**	G2 B’	SS, mild skeletal defects	AD	[2, 12]
c.1948G>A	**p.Val650Met**	G2 B’	SS, DelBA, mild skeletal defects	AD	[2, 12]
c.1979C>T	**p.Thr660Met**	G2 B’	SS only	Not analyzed	[16]
c.2026+1G>A		G2 B’	SS, mild midface hypoplasia, brachydactyly	AD	[5, 10]

c.2099G>A	p.Trp700*	KS	SS, AdvBA, Spine deformities	AD	[6]
c.2164C>G	**p.Pro722Ala**	KS	SS	AD	[21]
c.2173delG	p.Glu725Argfs*6	KS	SS, AdvBA	AD	[11]
c.2218A>T	**p.Thr740Ser**	KS	SS, facial dysmorphism, mild skeletal effects	AD	[2, 12]
c.2266G>C	**p.Gly756Arg**	KS	SS	AD	[17]
c.2367delC	p.Ser790Glnfs*20	KS	SS, vertebral column,	AD	[7]
c.2369C>G	p.Ser790*	KS	SS, facial dysmorphism, mild skeletal effects	De novo	[2, 12]
c.2441C>G	p.Ser814*	KS	SS, early onset OA, vertebral column	AD	[22]
c.2535_2536insTTCA	p.Pro846Phefs*9	KS	SS only	Not analyzed	[16]
c.2863_2864delAG	p.Ser955Trpfs*473	GAG, CS-1	SS	AD	[4]
c.2902T>C	p.Ser968Pro	GAG, CS-1	SS, craniofacial dysmorphism, kyphosis, trident hands	Not analyzed (compound heterozygous for c.973G>A)	[15]
c.3758dupC	p.Gly1254Trpfs*175	GAG, CS-1	SEDK	AD	[23]
c.4138G>T	**p.Val1380Phe**	GAG, CS-1	SEMD (mild)	Not analyzed (compound heterozygour for c.5061T>A)	[24]
c.4259A>G	**p.Glu1420Gly**	GAG, CS-1	SS only	Not analyzed	[16]
c.4390delG	p.Val1464*	GAG, CS-1	fOCD, SS, craniofacial dysmorphism, brachydactyly	AD	[25]
c.4634delT	p.Leu1545Profs*11	GAG, CS-1	SS, IDD	AD	[26]
c.4657G>T	p.Glu1553*	GAG, CS-1	SS, OA, IDD	AD	[5]
c.4762_4765delGGGT	p.Gly1588Cysfs*26	GAG, CS-1	SS, AdvBA, mild midface hypoplasia, broad great toes	AD	[19]
c.4852C>T	p.Gln1618*	GAG, CS-2	SS, DelBA, OA	AD	[27]
c.5061T>A	**p.Ser1687Arg**	GAG, CS-2	SEMD (mild)	Not analyzed (compound heterozygous for c.4138G>T	[24]
c.5391delG	p.Gln1798Serfs*53 [3]	GAG, CS-2	SS, AdvBA,	AD	[5, 28]

			mild midface hypoplasia		
c.5443delC	p.Leu1815Trpfs*36	GAG, CS-2	SS, DelBA	Not analyzed	[11]
c.5579delC	p.Gly1861Glufs*41	GAG, CS-2	SS, AdvBA	Not analyzed	[11]
c.5597C>A	p.Ser1866*	GAG, CS-2	SS	de novo	[8]
c.5658delG	p.Phe1887Leufs*15	GAG, CS-2	OCD, SS	AD	[29]
c.6142C>G	**p.Pro2048Ala**	GAG, CS-2	SS, mild skeletal defects	AD	[2, 12]
c.6404delC	p.Ala2135Aspfs	GAG, CS-2	Mild SS, AdvBA	de novo	[27]
c.6530T>C	**p.Val2177Ala**	GAG, CS-2	SS only, polymorphism	Not analyzed	[16]
c.6861delC	p.Cys2288Valfs*28	G3, EGF1	SS, DelBA	Not analyzed	[11]
c.7121dup (c.7007dup)	p.Asp2374Glyfs*9 (p.Asp2337Glyfs*9)	G3, CLD	SS, AdvBA	AD	[30]
c.7084T>C (c.6970T>C)	**p.Trp2362Arg** (p.Trp2324Arg)	G3, CLD	OCD, SS, facial dysmorphism, mild skeletal effects	AD	[31]
c.7085G>A (c.6971G>A	p.Trp2362* (p.Trp2324*)	G3, CLD	SS	Not analyzed	[18]
c.7155delG (c.7041delG)	p.Cys2386Valfs*8 (p.Cys2348Valfs*8)	G3, CLD	OCD, SS, DelBA, OA	AD	[6]
c.7156T>A (c.7042T>A)	**p.Cys2386Ser** (p.Cys2348Ser)	G3, CLD	FOCD, SS, OA	AD	This study
c.7178T>C (c.7064T>C)	**p.Leu2393Pro** (p.Leu2355Pro)	G3, CLD	OCD, SS, AdvBA, OA, short thumbs	AD	[5, 10]
c.7183A>T (c.7069A>T)	**p.Ser2395Cys** (p.Ser2357Cys)	G3, CLD	SS, DelBA	AD	[6]
c.7196C>T	**p.Pro2399Leu**	G3, CLD	SS	AD	[11]
c.7204C>T (c.7090C>T)	p.Gln2402* (p.Gln2364*)	G3, CLD	OCD, SS, AdvBA, OA, mild midface hypoplasia, joint problems, broad great toes	AD	[19]
c.7205A>C (c.7091A>C) [3]	**p.Gln2402Pro** (p.Gln2364Pro)	G3, CLD	SS, mild skeletal defects	AD	[32]
c.7207-7209delGAG (c.7093_7095delGAG)	p.Glu2403del (p.Glu2365del)	G3, CLD	SS only	Not analyzed	[16]
c.7250T>C (c.7136T>C)	**p.Leu2417Pro** (p.Leu2379Pro)	G3, CLD	FOCD, SS, OA	AD	This study
c.7255G>A (c.7141G>A)	**p.Asp2419Asn** (p.Asp2381Asn)	G3, CLD	SEMD	AR	[33]
c.7267G>A (c.7153G>A)	**p.Glu2423Lys** (p.Glu2385Lys)	G3, CLD	SS, OA, IDD	AD	[5]
c.7317G>A (c.7203G>A)	p.Trp2439* (p.Trp2401*)	G3, CLD	SS, OA, IDD	AD	[5]
c.7336dupA (c.7222dupA)	p.Asn2446Lysfs*22 (p.Asn2407Lysfs*22)	G3, CLD	SS, AdvBA	Not analyzed	[34]
c.7358A>T (c.7244A>T)	**p.Asp2453Val** (p.Asp2415Val)	G3, CLD	FOCD, SS, OA	AD	This study

c.7361G>A (c.2416G>A)	**p.Cys2454Tyr** (p.Cys2416Tyr)	G3, CLD	SS	AD	[35]
c. 7363G>A (c.7249G>A)	**p.Val2455Met** (p.Val2417Met)	G3, CLD	FOCD, SS, OA, IDD	AD	[5, 36]
c.7381delG (c.7267delG) [3]	p.Glu2461Argfs*148 (p.Glu2423Argfs*148)	G3, CLD	SS, AdvBA, facial dysmorphism, mild skeletal effects	AD	[2]
c.7390G>A (c.7276G>A)	**p.Glu2464Lys** (p.Glu2426Lys)	G3, CLD	SS, facial dysmorphism, mild skeletal effects	AD	[2, 12]
c.7390G>T (c.7276G>T)	p.Glu2464* (p.Glu2426*)	G3, CLD	SS, facial dysmorphism, mild skeletal effects	AD	[2, 5]
c.7456G>A (c.7342G>A)	**p.Gly2486Arg** (p.Gly2448Arg)	G3, Sushi	SS, facial dysmorphism, mild skeletal effects	AD	[2]
c.7583G>A (c.7469G>A)	**p.Cys2528Tyr** (p.Cys2490Tyr)	G3, Sushi	SS	AD	[17]

[1] Sequence numbering is based on the full length ACAN variant 3 (NM_001369268.1). This sequence is identical to ACAN variant 2 (NM_013227.3) until nucleotide 6946, when the alternatively spliced EGF2 repeat is inserted in variant 3. For entries after this point, numbering based on NM_013227.3 is given in parenthesis.

[2] SS, short stature
SS only, no craniofacial, skeletal or joint symptoms and parents of normal height.
AdvBA, advanced bone age vs chronological age
DelBA, delayed bone age vs chronological age
OA, osteoarthritis
OCD, osteochondritis dissecans (reported only for proband)
fOCD, familiar OCD linked to the ACAN variant
IDD, intervertebral disc disease

[3] Labeled differently in original publication

[4] Gray background indicates non-globular parts of the aggrecan core protein

[5] Bold typeface marks missense variants

Referenses for Supplemental Table 1

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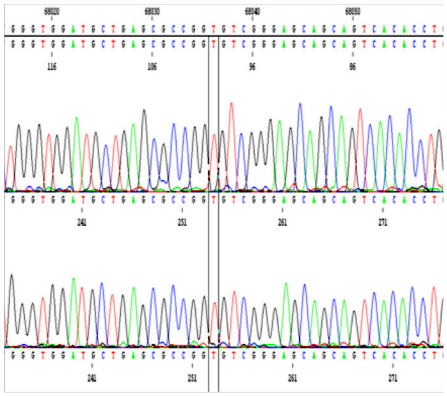
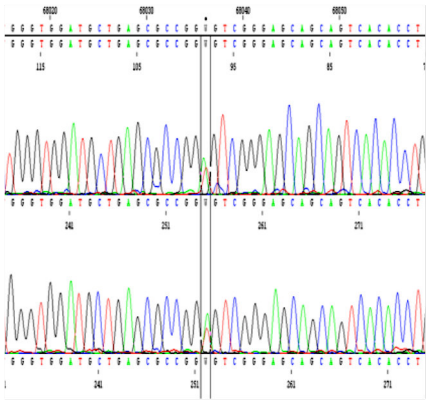
a) **Family 1**

II:1



II:2



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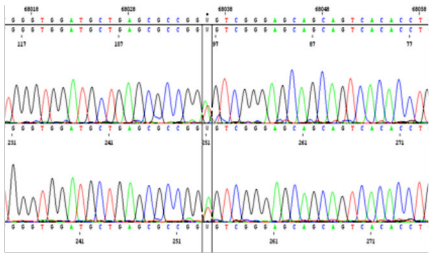

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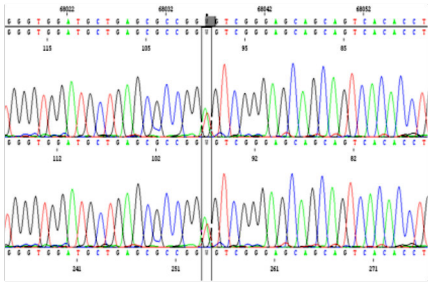
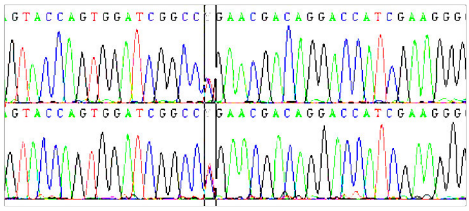
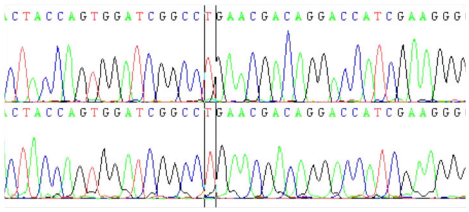


b) **Family 2**

III:2



II:1



Supplemental figure 1. Genomic DNA sequencing

a) Sequence traces from Family 1 proband (III:1), sister (III:2), mother (II:2) and father (II:1).

b) Sequence traces from Family 2 proband (III:2) and father (II:1). The sibling (III:1) and mother (II:2) of the proband showed the same C>T variation as III:2.

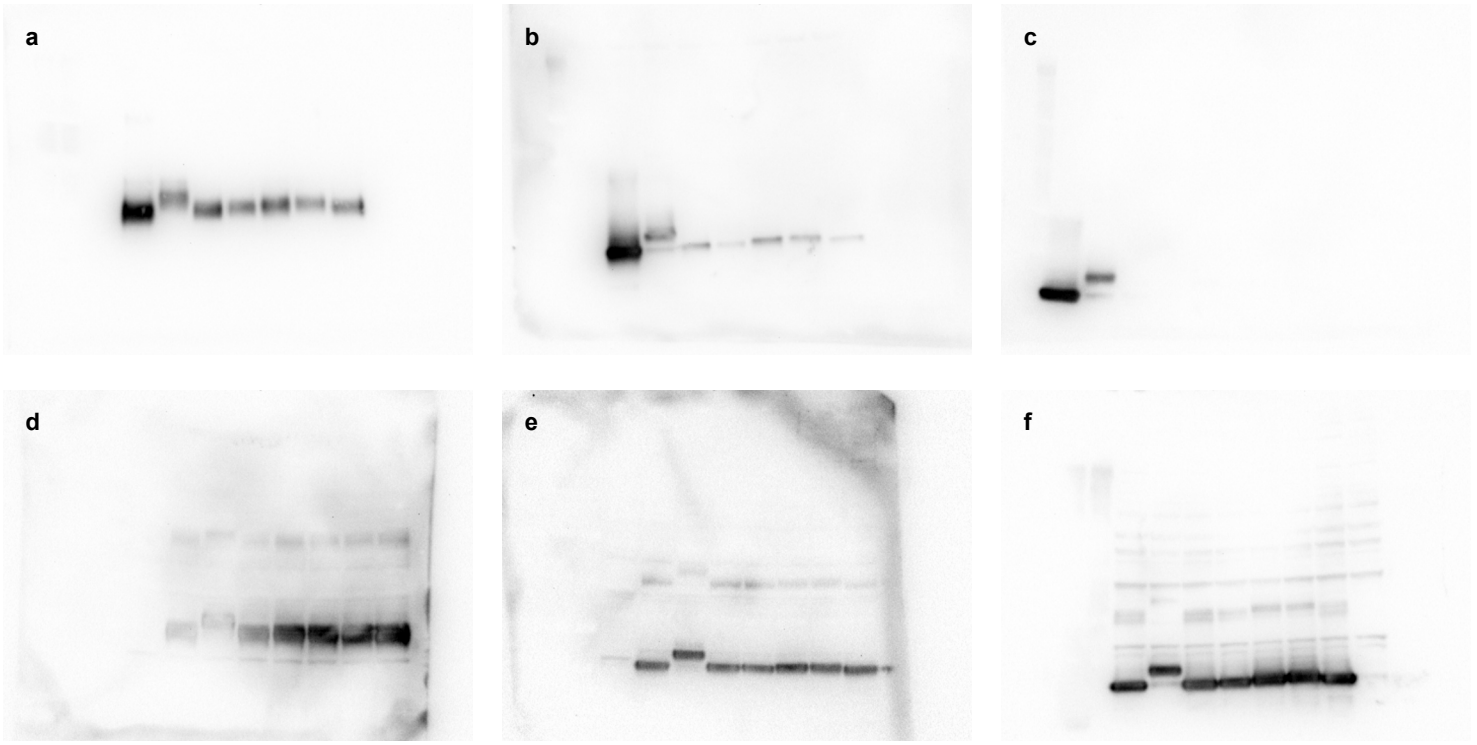


Figure S2. Un-cropped western blots.

Original digital images of western blots, shown cropped in Figure 5a-f.