JVDI: Supplementary material

Andrade DGA, et al. Description of the D4/D4 genotype in Miniature horses with dwarfism

Supplementary Table 1. Primer sequences used for D1, D2, D3* and D4 *ACAN* variants (RefSeq 100033876 and ENSECAG00000007493).

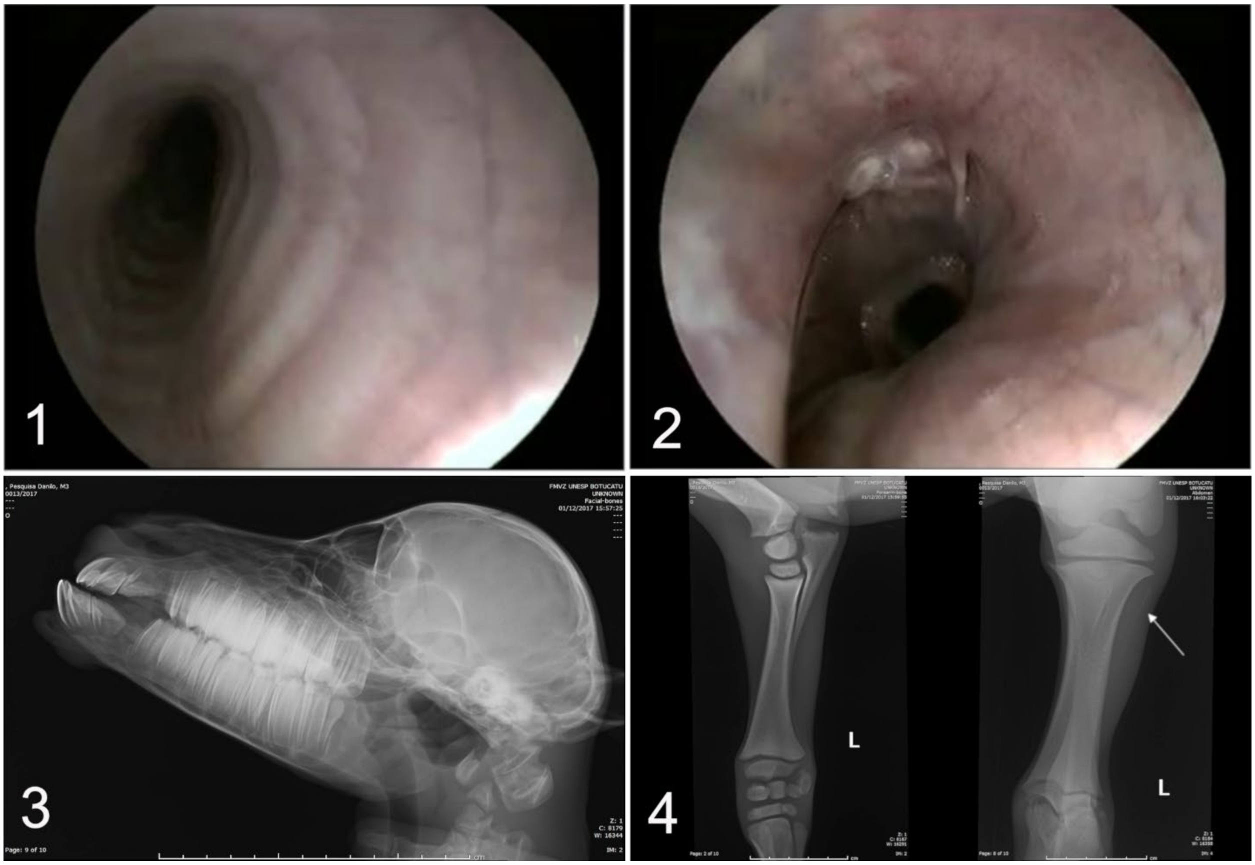
Primer	Sequence (5'–3')	Amplicon length (bp)	Coding exon
JP_ACAN_D1_Forward	ACCCTGACAACTCGCTGA	336	2
JP_ACAN_D1_Reverse	TCACCTCGCAGCGATAGAT		
JP_ACAN_D2_Forward	AGATGCCACTGCCACAAA	268	6
JP_ACAN_D2_Reverse	GTGGTCACCTGTACCACAAG		
JP_ACAN_D3*_Forward	GGAAAGAGGGAATGAACAGAGG	462	7
JP_ACAN_D3*_Reverse	AGTGACTGAATTAACCCACAGG		
JP_ACAN_D4_Forward	CGGTGAGGCCAGTTCTTT	461	14
JP_ACAN_D4_Reverse	TCAGCTCCAATCTGCTTGTC		

Supplementary Table 2. Primer sequences used for sequencing the exons of the *ACAN* gene (RefSeq 100033876 and ENSECAG00000007493).

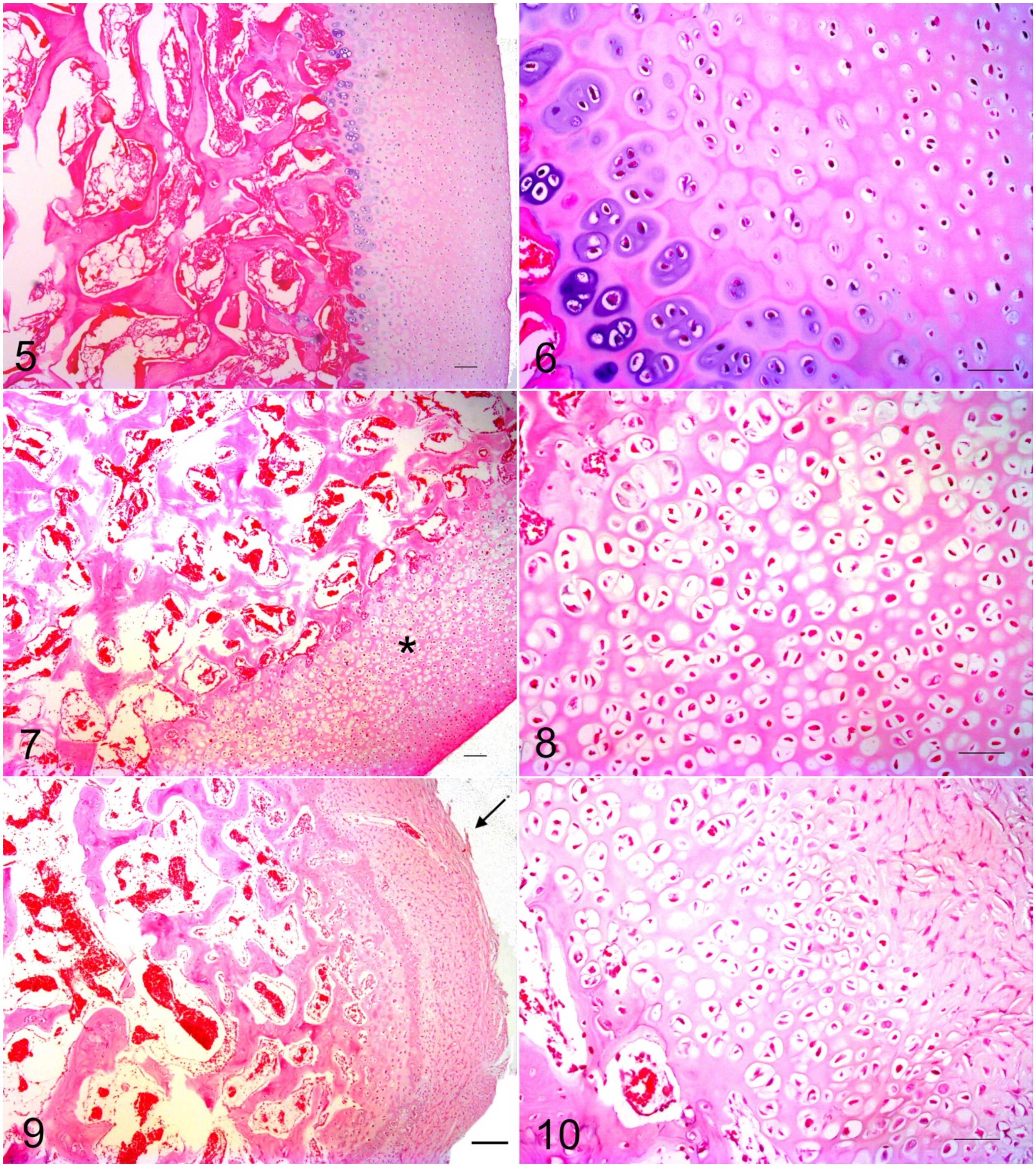
Primer	Template	Sequence (5'–3')	Amplicon length (bp)	Coding exon
DG_ACAN_Eq_F47	DNA	CACGTACAGCCTCCTTATCTTG	259	1
DG_ACAN_Eq_R47		CTTCAGCGCCTGTGGATAAA		
JP_ACAN_D1_Forward	DNA	ACCCTGACAACTCGCTGA	336	2
JP_ACAN_D1_Reverse		TCACCTCGCAGCGATAGAT		
DG_ACAN_Eq_F34	DNA	CTCCAATGACTCTGGCATCTATC	283	2
DG_ACAN_Eq_R34		CAGGGTCCAGAAAGAACGATAC		
DG_ACAN_Eq_F35	DNA	CCTCTGACTATCGCCGTAATTC	421	3
DG_ACAN_Eq_R35		AAGGATTTGGGACAGGTCATC		
DG_ACAN_Eq_F36	DNA	CCACAAGGGAGTCAGATACAAG	268	4
DG_ACAN_Eq_R36		TGTGATCCCGAGTGGTAGT		
DG_ACAN_Eq_F37	DNA	ACCCGGGCTTTGAGAATTTA	554	5
DG_ACAN_Eq_R37		CTGACATGAGGTTCAGGTATCC		
DG_ACAN_Eq_F38	DNA	GACGTCTTGTGCTATGATGACT	336	6
DG_ACAN_Eq_R38		CTTCACCCTCGGTGATGTTT		

JP_ACAN_D2_Forward	DNA	AGATGCCACTGCCACAAA	268	6
JP_ACAN_D2_Reverse		GTGGTCACCTGTACCACAAG		
DG_ACAN_Eq_F48	DNA	AGGCGGAGAACGAGACT	216	6
DG_ACAN_Eq_R48		GGCCAAGTTCCTTCCACTT		
JP_ACAN_D3*_Forward	DNA	GGAAAGAGGGAATGAACAGAGG	462	7
JP_ACAN_D3*_Reverse		AGTGACTGAATTAACCCACAGG		
DG_ACAN_Eq_F50	DNA	AGAACAGGCCCTCATTCTG	255	8
DG_ACAN_Eq_R50		GGTGAAGGTAATGCCCTCT		
DG_ACAN_Eq_F51	DNA	GTCGTTTCCGTGCAGAGT	261	9
DG_ACAN_Eq_R51		CCGGCGTAGCACTTGTC		
DG_ACAN_Eq_F39	DNA	CACGGGCCAGCTCTATG	314	9
DG_ACAN_Eq_R39		CTCCTTTCTCTGATGGATGGG		
DG_ACAN_Eq_F40	DNA	CTGGTAAGGAGGAACCTTCAC	355	10
DG_ACAN_Eq_R40		GGGAAGCACAGCTAGGG		
DG_ACAN_Eq_F41	DNA	TTTCGCTTTGGAAGGAGTAGAG	416	11
DG_ACAN_Eq_R41		GAGCCCACTGCAGAGATAAG		
DG_ACAN_Eq_F6	DNA	ACCTCAGTGGACTTCCTTCT	547	11
DG_ACAN_Eq_R6		GGCTCAACATGAATCTCTCCTC		
DG_ACAN_Eq_F7	DNA	CCCTGGAATAGAGGATCTCAGT	318	11
DG_ACAN_Eq_R7		GAGGGAGTAGTCTCCAGATGAA		
DG_ACAN_Eq_F12	DNA	CTTCTGGAGCCTTGGACTTT	802	11
DG_ACAN_Eq_R12		ACGGATCCCAAACCTTCTTC		
DG_ACAN_Eq_F13	DNA	CAGTGGGACAACATCTGGAA	803	11
DG_ACAN_Eq_R13		CTTCTCCACTGGACTCAACAA		
DG_ACAN_Eq_F29	DNA	GCCTCAGGACTTCCAGAAATTA	450	11
DG_ACAN_Eq_R29		GTAGGTGGTGGTTGACACTC		
DG_ACAN_Eq_F42	DNA	CTTGTTGAGTCCAGTGGAGAAG	734	11
DG_ACAN_Eq_R42		TGTGCTCAGAACCTTCAATCTC		
DG_ACAN_Eq_F43	DNA	CTCTTGAGGGCATGACATTGA	359	12
DG_ACAN_Eq_R43		TGCTGACTTCTGGCAAGTG		
DG_ACAN_Eq_F44	DNA	GCCTCTATGAGCTCAACTTTCT	245	13
DG_ACAN_Eq_R44		CATGCCGTCCGAATGTATCT		
JP_ACAN_D4_Forward	DNA	CGGTGAGGCCAGTTCTTT	461	14
JP_ACAN_D4_Reverse		TCAGCTCCAATCTGCTTGTC		
DG_ACAN_Eq_F45	DNA	CATCACAGGAGCCCTTTCT	223	15
DG_ACAN_Eq_R45		CGGTCTGTGCAGGTGAT		
DG_ACAN_Eq_F52	DNA	ACGATGAGGAGGGCTCA	261	16
DG_ACAN_Eq_R52		TGGAATTCCTTAGAGGACAGAAAG		
DG_ACAN_Eq_F1	cDNA	GCCGCTCCAGGTGTATG	540	1, 2, 3
DG_ACAN_Eq_R1		GGCTCTGTAATGGAACACGA		

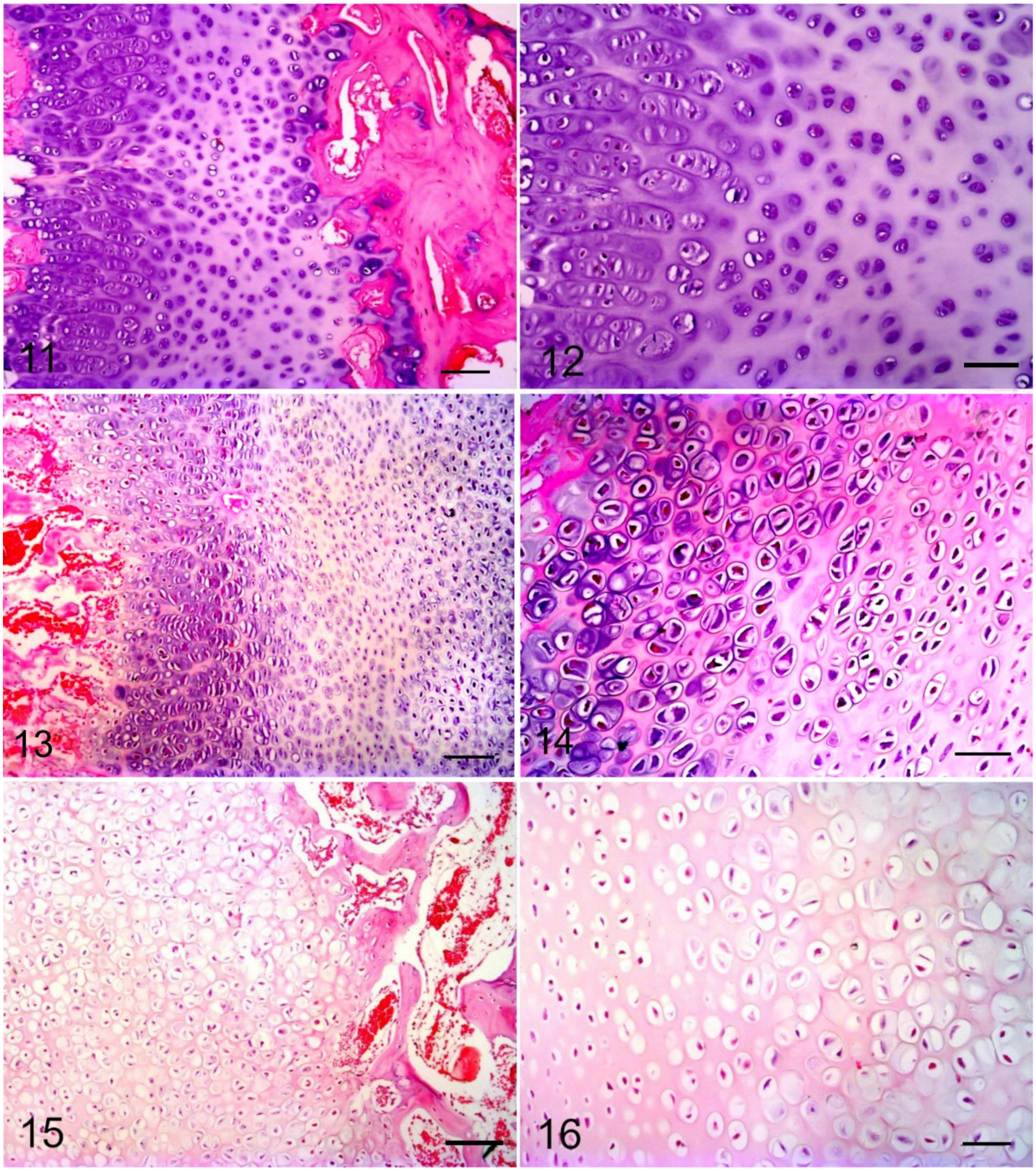
DG_ACAN_Eq_F18	cDNA	GAACCTGCGCTCCAATGA	422	2, 3, 4
DG_ACAN_Eq_R18		CCGGAGACGTTGCGTAAA		
DG_ACAN_Eq_F19	cDNA	TACGACGTGTACTGCTTTGC	436	4, 5, 6
DG_ACAN_Eq_R19		TTTGTGGCAGTGGCATCT		
DG_ACAN_Eq_F3	cDNA	AACTTCTTCGCTGTGAGTGG	815	6, 7, 8, 9
DG_ACAN_Eq_R3		CCGGCGTAGCACTTGTC		
DG_ACAN_Eq_F4	cDNA	AAACCTATGATGTCTACTGCTACG	603	8, 9, 10
DG_ACAN_Eq_R4		CTTCTGTGCTCTCCTCTGTTG		
DG_ACAN_Eq_F6	cDNA	ACCTCAGTGGACTTCCTTCT	547	11
DG_ACAN_Eq_R6		GGCTCAACATGAATCTCTCCTC		
DG_ACAN_Eq_F7	cDNA	CCCTGGAATAGAGGATCTCAGT	318	11
DG_ACAN_Eq_R7		GAGGGAGTAGTCTCCAGATGAA		
DG_ACAN_Eq_F11	cDNA	ACTGCCTCTGGAGTAGAAGA	659	11
DG_ACAN_Eq_R11		CCAGACGGAAGTCCACTAAAG		
DG_ACAN_Eq_F12	cDNA	CTTCTGGAGCCTTGGACTTT	802	11
DG_ACAN_Eq_R12		ACGGATCCCAAACCTTCTTC		
DG_ACAN_Eq_F13	cDNA	CAGTGGGACAACATCTGGAA	803	11
DG_ACAN_Eq_R13		CTTCTCCACTGGACTCAACAA		
DG_ACAN_Eq_F29	cDNA	GCCTCAGGACTTCCAGAAATTA	450	11
DG_ACAN_Eq_R29		GTAGGTGGTGGTTGACACTC		
DG_ACAN_Eq_F30	cDNA	TCTGAGTGCAGCCACCT	216	11
DG_ACAN_Eq_R30		CTGTCAGTCCTGTCTCTGGAA		
DG_ACAN_Eq_F31	cDNA	CTACCACGTTGGCACAGAG	308	11
DG_ACAN_Eq_R31		TGACGGCTGTCTCCGATA		
DG_ACAN_Eq_F32	cDNA	GCTTCCATCCCAGCTTCTC	234	11, 12
DG_ACAN_Eq_R32		CTGGTAGTCTTGGGCATTGT		
DG_ACAN_Eq_F33	cDNA	GCCACTGTTACCGCTACTTT	560	12, 13, 14, 15, 16
DG_ACAN_Eq_R33		CTTCTGTAGTCTGCGCTTGTAG		
DG_ACAN_Eq_F16	cDNA	CACCGAGGGCTTTGTCC	265	15, 16
DG_ACAN_Eq_R16		GAGGACAGAAAGCGACAAGAA		



Supplementary Figures 1–4. Endoscopic and radiographic findings in Miniature horses with dwarfism. **Figure 1.** Mild tracheal collapse. **Figure 2.** Dorsal displacement of the soft palate. **Figure 3.** Mandibular prognathism. **Figure 4.** Incomplete ulna with irregular and thick proximal physis. Enlargement of the proximal and distal epiphyses of the radius secondary to chondrodysplasia. Incomplete and filiform fibula (arrow) with incomplete bone mineralization (secondary to age) and reaching the middle third of the tibia.

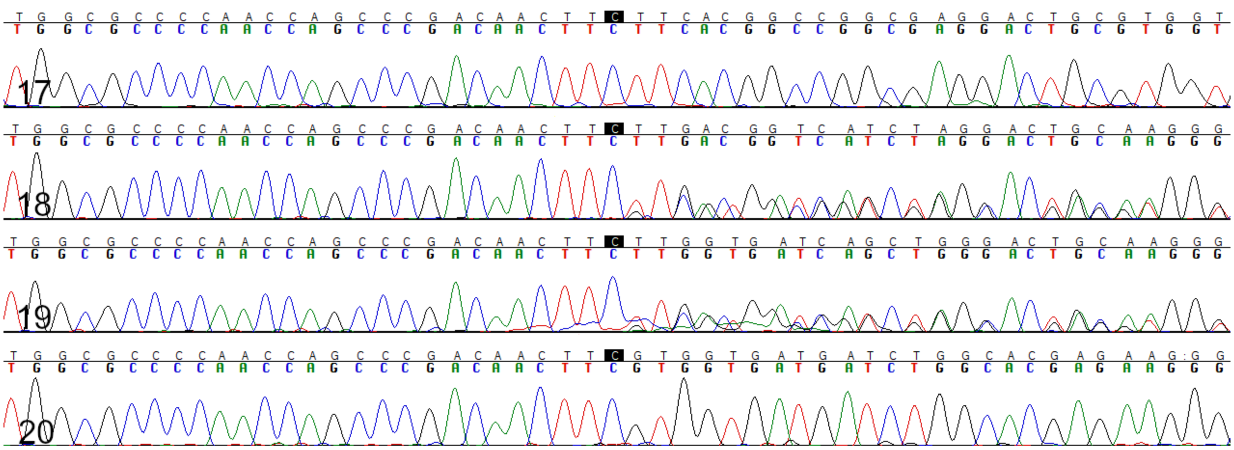


Supplementary Figures 5–10. Histologic images of the articular cartilage. H&E. **Figure 5.** One-day-old Miniature horse (control animal) without dwarfism: with normal cartilage structure. Bar = 100 µm. **Figure 6.** Higher magnification of Fig. 5. Bar = 50 µm. **Figure 7.** One-day-old Miniature horse (filly) with dwarfism: articular cartilage with mild lesion, note the disorganization of the layers of chondrocytes (*) and reduced extracellular matrix. Bar = 100 μm. **Figure 8.** Higher magnification of Fig. 7. Bar = 50 μm. **Figure 9.** One-day-old Miniature horse (colt) with dwarfism: articular cartilage with severe lesion, note the disorganization of the tissue, absence of differentiated chondrocytes, and clefts in the surface (arrow). Bar = 100 μm. **Figure 10.** Higher magnification of Fig. 9, with the absence of differentiated chondrocytes. Bar = 50 μm.



Supplementary Figures 11–16. Histologic images of the physis cartilage. H&E. **Figure 11.** One-day-old Miniature horse (control animal) without dwarfism: with normal structure. Bar = 100 µm. **Figure 12.** Higher magnification of Fig. 11. Bar = 50 µm. **Figure 13.** One-day-old Miniature horse (filly) with dwarfism: physis cartilage with mild lesion. Bar = 100 µm. **Figure**

14. Higher magnification of Fig. 13. Bar = 50 µm. **Figure 15.** One-day-old Miniature horse (colt) with dwarfism: articular cartilage with severe lesion. Bar = 100 µm. **Figure 16.** Higher magnification of Fig. 15, with severe swelling of chondrocytes and reduced extracellular matrix production. Bar = 50 µm.



Supplementary Figures 17–20. Partial chromatogram obtained from the assembly of *ACAN* gene sequences from a control foal (**Fig. 17**), stallion (**Fig. 18**), mare (**Fig. 19**), and affected foal (**Fig. 20**). A double peak from the selected cytosine ("C" in black) was observed in the heterozygote sequences (**Figs. 18, 19**), and a deletion of 21 base pairs (CTTCACGGCCGGCGAGGACTG) from the selected base was observed in the homozygote sequence (**Fig. 20**).