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Supplemental Data

Mutations in *ANAPC1*, Encoding a Scaffold Subunit of the Anaphase-Promoting Complex, Cause Rothmund-Thomson Syndrome Type 1

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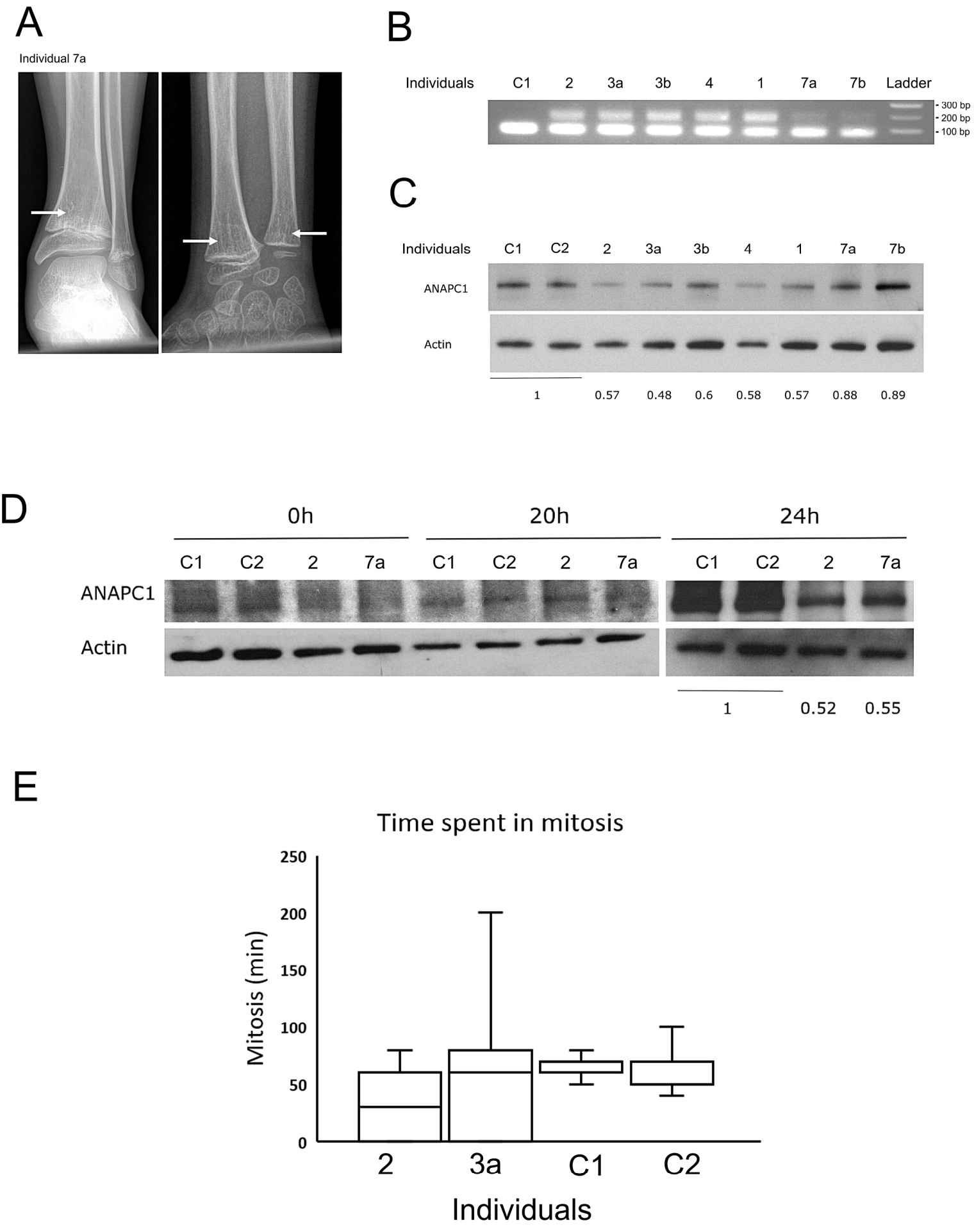
A
Individual 7a
B
Individuals
C1
2
3a
3b
4
1
7a
7b
Ladder
300 bp
200 bp
100 bp
C
Individuals
C1
C2
2
3a
3b
4
1
7a
7b
ANAPC1
Actin
1
0.57
0.48
0.6
0.58
0.57
0.88
0.89
D
0h
20h
24h
C1
C2
2
7a
C1
C2
2
7a
C1
C2
2
7a
ANAPC1
Actin
1
0.52
0.55
E
Time spent in mitosis
Mitosis (min)
250
200
150
100
50
0
2
3a
C1
C2
Individuals

Figure S1

Figure S1. Radiographs and *ANAPC1* expression studies.

A) Radiographs of individual 7a at 9 years of age show abnormal sclerotic marks near the metaphyses of the ankle (left) and wrist (right).

B) *ANAPC1* expression in RTS Type 1 available fibroblasts by qPCR. For all five homozygous individuals (2, 3a, 3b, 4, and 1) there was presence of a clear upper band corresponding to the increased size of the fragment because of the retained pseudo-exon. The upper band was also seen in both individuals with compound heterozygous mutations (7a, 7b) but with less intensity than in the other affected individuals. C1 = wildtype control

C) Western blot of ANAPC1 for the same affected individuals. Relative expression was calculated by normalizing to Actin and comparing with the ANAPC1 levels in control cells (C1, C2) using Quantity One software, version 4.6.6 (Biorad). In all five individuals homozygous for the *ANAPC1* intronic splicing variant, ANAPC1 was decreased by half while individuals with compound heterozygous mutations showed a moderate reduction.

D) ANAPC1 expression in synchronized fibroblasts. Fibroblasts from two healthy controls (C1, C2), individual 2 (homozygous for the intronic splicing mutation) and 7a (compound heterozygous mutations) were plated 1 day before serum starvation for 24h. Then the cells were supplemented again with 20% FBS and harvested after 0h, 20h, and 24h. Western blot was performed at the indicated time points. ANAPC1 expression in the two individuals was calculated as described in C. At 0h and 20h, ANAPC1 levels were very low, and no difference was detected between controls and individuals. After 24h, ANAPC1 was abundantly expressed, and a difference is marked at the 24h time point.

E) Same experiment as Figure 3a, but here showing the time spent in mitosis. There was no significant difference between any group.

Table S1. Additional clinical details.

		Individuals homozygous for the intron 22 mutation					Individuals compound heterozygous for the intron 22 mutation and another mutation					Individuals without *ANAPC1* mutations		
ID in this manuscript		1	2	3a	3b	4	5	6a	6b	7a	7b	8a	8b	9
Eyes	Bilateral juvenile cataracts	+	+	+	+	+	+	+	+	+	+	No	No	No
	Other eye findings	corneal ulcer and retinal detachment					astigmatism	microphthalmia, strabismus	strabismus		lens detachment, photodysphoria, filamentary keratitis			
Ectoderm	Poikiloderma	+	+	+	+	+	+	+	+	+	+	+	+	+
	Hyperkeratosis		+	+						+	+			
	Blistering						+	+		+	+			
	Café-au-lait spots	+								+	+			
	Other skin findings	Eczema					photosensitivity				warts		Squamous cell carcinoma of lip	erythema
	Sparse hair or alopecia		+			+	+	+	+	+	+	alopecia	alopecia	
	Absent eyebrows		+	+	+	+	+			+	+	+	+	
	Absent eyelashes			+	+	+				+	+	+	+	
	Teeth					abnormal teeth		cone shaped teeth	cone shaped teeth	small pointy teeth with caries	small misshaped teeth with caries			
	Nails							dystrophic nails	dystrophic nails	thin nails	thin nails			

Other systems	Endocrine and fertility		POF	3 children	POF		hypogonadism	hypothyroidism	hypothyroidism					
	Genito-urinary system						undescended testes, micropenis	undescended testes	undescended testes	undescended testes	undescended testes			
	Short stature		-	-	-	+	+	+	+	+	+	+	+	
	GH therapy						+	+	+	+	+			
	Skeletal	Osteoporosis, left foot fracture						delayed bone age, short metacarpals and phalanges, dysplastic aspect of the phalanges, large metaphyses of long bones, genu varum	delayed bone age, short metacarpals and phalanges	arm fracture (never healed properly), punctate sclerotic foci at multiple metaphyses	right arm fixed flexion deformity			
	Developmental delay or ID						mild developmental delay	Intellectual disability	ADHD					
	Recurrent otitis media						+			+				+
	Other						GI disturbance, adenoidal hypertrophy			history of hearing loss but improved after PET placement				history of GI disturbance, history of recurrent pulmonary issues as a toddler (bronchitis, asthma, pneumonia, sinusitis), bradycardia, benign

														peripheral flow murmur