Supplementary Material and Methods

Genetic screening confirms heterozygous mutations in *ACAN* as a major cause of idiopathic short stature

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Supplementary methods:

Patients

In this study, we included patients with idiopathic short stature defined by a height of 2 SD below the population specific age- and sex-related average or 2 SD below the estimated target family height. All patients have been evaluated by experts in pediatric endocrinology and medical genetics to exclude identifiable known causes of short stature including growth hormone deficiency.

Whole exome sequencing & variant evaluation

Whole exome sequencing was performed for 200 of the patients after enrichment accomplished by SureSelect targeted capturing. Sequencing was conducted on a SOLiD or HiSeq2500 device and image analysis as well as base calling was performed using the corresponding software with default parameters. We performed read alignment with BWA[1] version 0.7.8 to the human genome assembly hg19 (GRCh37). Local re-alignment around potential InDel sites was performed with the Genome Analysis Toolkit[2] version 3.1. Single-nucleotide variants and small insertions and deletions (indels) were detected using five different callers: HaplotypeCaller and UnifiedGenotyper[2], SNVer[3], freeBayes[4] and Platypus[5]. Variant annotation was performed using ANNOVAR.[6] We achieved an average SureSelect target coverage of 160x and average coverage of the *ACAN* gene of 137.4x (Supplementary Figure 1). We included only variants called with GATKHap, GATKUG[2,7] or SNVer[3] which were covered by at least 10 % of the average coverage of the patient's exome and for which at least 5 novel alleles were detected. We then confirmed the selected variants (Combined Annotation Dependent Depletion (CADD) score >10, frequency in Exome Aggregation Consortium (ExAC) database $<10^{-3}$)[8,9] and their inheritance by Sanger Sequencing. 120 patients were analyzed by multigene panel sequencing using an Illumina Nextera® Rapid Capture CustomKit for enrichment and an Illumina MiSeq system for analysis. This multigene panel included a total of 329 genes related to short stature and RAS-MAPK signaling (a complete list of genes is available on request). The obtained sequence data covered all exons and the adjacent intronic nucleotides of *ACAN* with a minimal coverage of >40x. Variants were filtered according to population frequency and predicted impact on the gene product using the Illumina's VariantStudio v2.2 and Alamut software suite (Interactive Biosoftware, Rouen, France) software tools. Rare or novel variants of possible pathogenic impact were confirmed by Sanger sequencing and their segregation in respective families was further evaluated.

Supplementary Table 1: ACMG Scoring of identified disease-related variants in *ACAN*

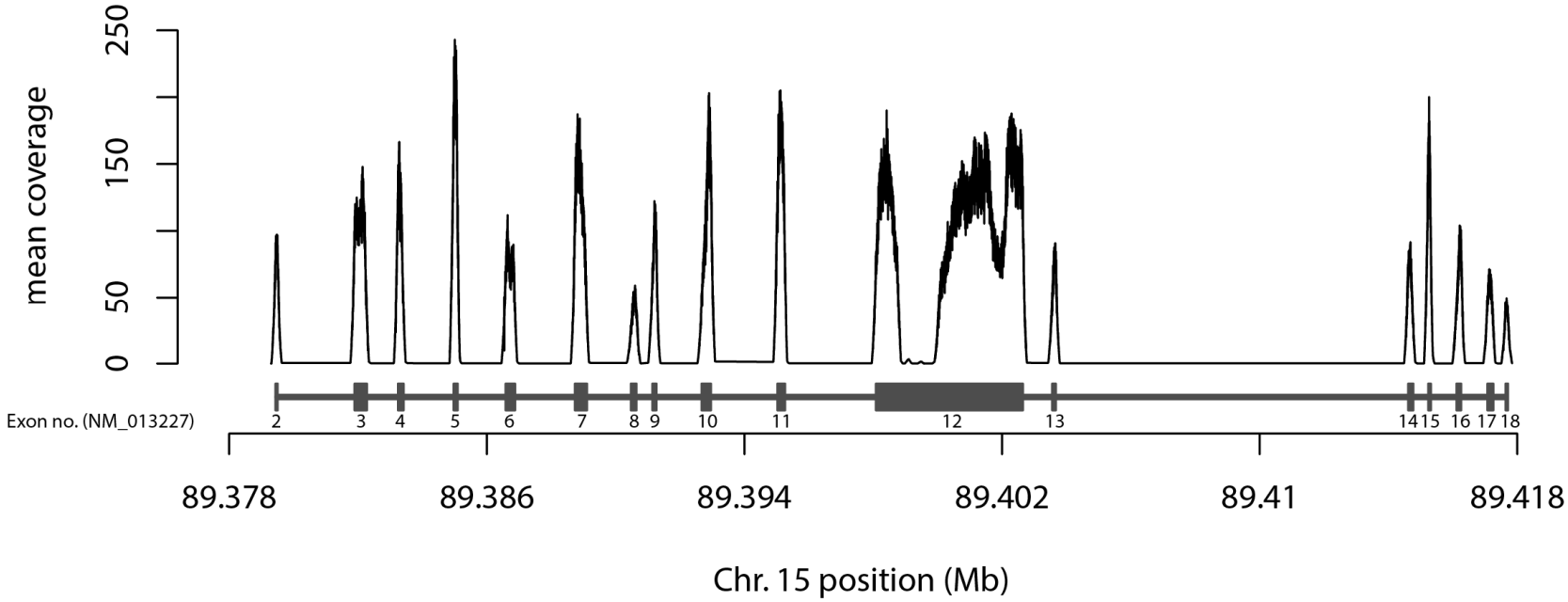
Patient	**cDNA level (NM_013227.3)**	**Protein level**	**ACMG Subcategories**	**ACMG prediction**
P1	c.151T>G	p.(Cys51Gly)	PM2, PM7, PP1, PP2, PP3, PP4	Likely pathogenic (V)
P2	c.515del	p.(Gln172Argfs*59)	PVS1, PM2, PM7, PP1, PP4	Pathogenic (Ib)
P3	c.1180C>T	p.(Arg394*)	PVS1, PM2, PM7, PP1	Pathogenic (Ib)
P4	c.1702G>A	p.(Asp568Asn)	PM7, PP1, PP2, PP3, PP4	Likely pathogenic (VI)
P5	c.1774C>T	p.(Gln592*)	PVS1, PM2, PM7, PP1, PP4	Pathogenic (Ib)
P6	c.5597C>A	p.(Ser1866*)	PVS1, PS2, PM2, PM7, PP4	Pathogenic (Ia)

Supplementary Table 2: Overview of *ACAN mutations* (see also Figure 1c)

cDNA level (NM_013227.3)*	Protein level*	ACMG Category	Affected domain	Inheritance	Reference
c.6_13del	p.(Thr3Leufs*21)	na	na	paternal	[10]
c.61G>T	p.(Glu21*)	na	G1	maternal	[11]
c.151T>G	p.(Cys51Gly)	Likely pathogenic	G1	paternal	P1
c.223T>C	p.(Trp75Arg)	na	G1	maternal	[11]
c.272delA	p.(Arg93Alafs*41)	na	G1	maternal	[11,12]
c.492C>G	p.(Tyr164*)	na	G1	maternal	[11]
c.515del	p.(Gln172Argfs*59)	Pathogenic	G1	maternal	P2
c.532A>T	p.(Asn178Tyr)	na	G1	maternal	[11]
c.661del	p.(Tyr221Metfs*10)	na	G1	maternal	[10]
c.903G>C	p.(Trp301Cys)	na	G1	paternal	[11]
c.916A>T	p.(Ser306Cys)	na	G1	maternal	[11]
c.1047_1048delinsAC	p.(Tyr349*)	na	G1	maternal	[11]
c.1120_1123del	p.(Thr374*)	na	IGD	paternal	[10]
c.1180C>T	p.(Arg394*)	Pathogenic	IGD	maternal	P3
c.1443G>T	p.(Glu415*)	na	IGD	maternal	[11]
c.1425delA	p.(Val478Serfs*14)	na	G2	paternal	[11]
c.1526C>A	p.(Ser509*)	na	G2	paternal	[11]
c.1608C>A	p.(Tyr536*)	na	G2	maternal	[13]
c.1702G>A	p.(Asp568Asn)	Likely pathogenic	G2	maternal	P4
c.1744delT	p.(Phe582Serfs*69)	na	G2	maternal	[14]
c.1774C>T	p.(Gln592*)	Pathogenic	G2	maternal	P5
c.2026+1G>A	p.?	na	G2	maternal	[11,12]
c.3758dupC	p.(Gly1254Trpfs*175)	na	GAG	unknown	[15-17]
c.4657G>T	p.(Glu1553*)	na	GAG	maternal	[11]
c.4762_4765del	p.(Gly1588Cysfs*26)	na	GAG	paternal	[13]
c.5391delG	p.(Gln1798Serfs*53)	na	GAG	maternal	[11,18]
c.5597C>A	p.(Ser1866*)	Pathogenic	GAG	de novo	P6

c.7064T>C	p.(Leu2355Pro)	na	G3	maternal	[11,12]
c.7090C>T	p.(Gln2364*)	na	G3	maternal	[13]
c.7141G>A	p.(Asp2381Asn)	na	G3	maternal & paternal	[19]
c.7153G>A	p.(Glu2385Lys)	na	G3	maternal	[11]
c.7203G>A	p.(Trp2401*)	na	G3	paternal	[11]
c.7249G>A	p.(Val2417Met)	na	G3	maternal	[11,20,21]
c.7276G>T	p.(Glu2426*)	na	G3	maternal	[11]

* All variants have been updated to the actual recommendations of the “Human Genome Variation Society”[22] using the Mutalyzer software[23].



Supplementary Figure 1: Mean coverage of the coding regions of the ACAN gene from whole exome analysis. All coding exons and the conserved splice-sites were included in the analysis (read depth at least 20x). A complex repetitive region within the coding region (exon 12) was excluded from the analysis.

Supplementary References

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