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Supplemental Data

Cantú Syndrome Is Caused by Mutations in *ABCC9*

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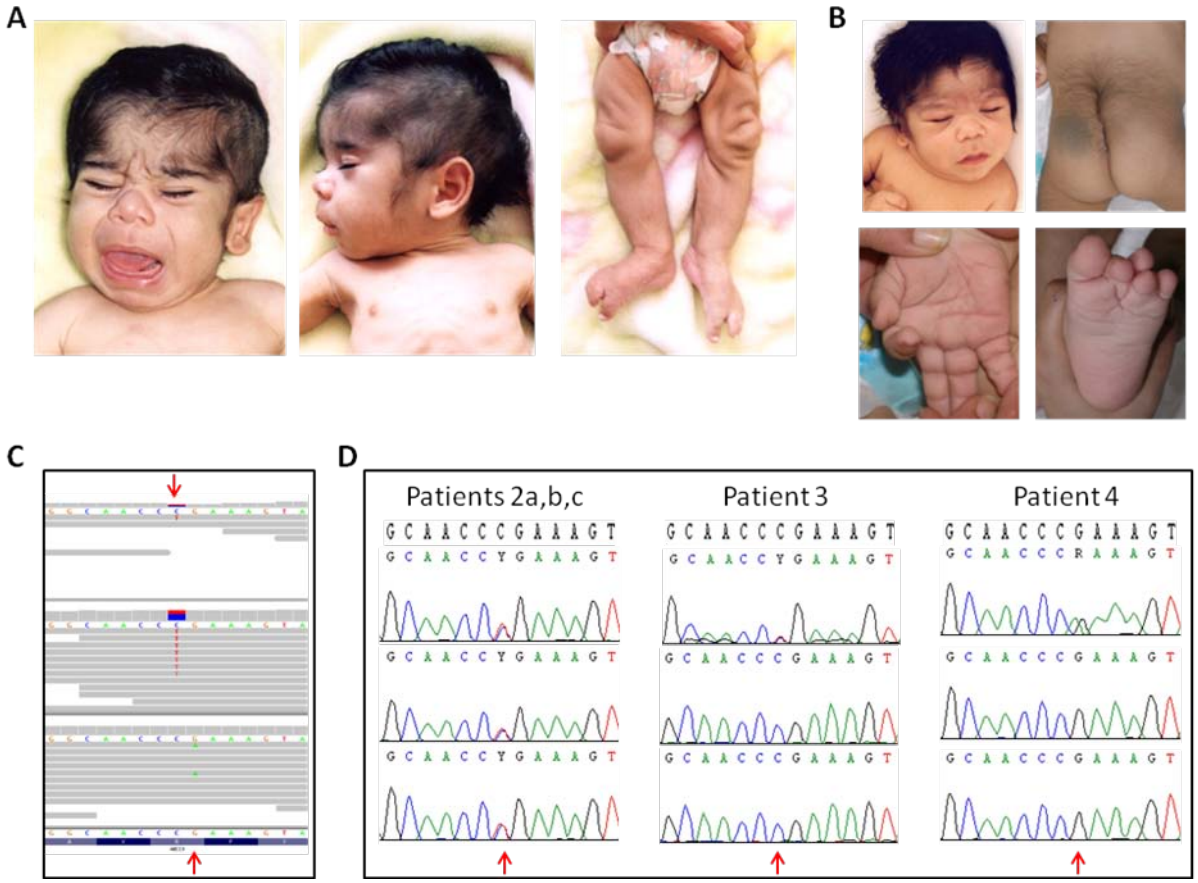

Figure S1. *ABCC9* Mutations Cause Cantú Syndrome

(A) Photographs of individual 1. Note the facial features including the typical hair pattern, short nose with broad flat nasal bridge, prominent mouth and long philtrum. In addition, the individual had wrinkled and loose skin.

(B) Photographs of individual 5. Note the facial features including the typical hair pattern, short nose with broad flat nasal bridge and long philtrum. In addition, hirsutism, deep hand/feet creases.

(C) *ABCC9* mutations identified by exome sequencing of individuals 2a, 3 and 4; the upper panel shows the next generation sequencing reads of individual 2a, followed by the reads of individual 3 (middle) and individual 4 (bottom); mutation nucleotides are marked with red arrows.

(D) Sanger validation in the individuals and family members; Sanger traces of the individuals 2a,b and c (left), individual 3 with respective parents (middle) and individual 4 with respective parents (bottom). Point mutations are marked with a red arrow. Sanger sequencing showed de novo occurrence of the *ABCC9* mutations for individual 3 and 4.

Table S1. Sequencing Statistics

	Individual 2	Individual 3	Individual 4	Individual 5	Individual 5 mat	Individual 5 pat
Agilent SureSelect kit	v1 (38Mb)	v1 (38Mb)	v1 (38Mb)	v2 (50Mb)	v2 (50Mb)	v2 (50Mb)
Reference	Hg18	Hg18	Hg18	Hg19	Hg19	Hg19
Sequence (Gigabase)	3.42	3.62	3.16	5.04	5.03	5.47
% Sequence within or near (50bp) targets	84.18%	82.57%	81.20%	87.41%	87.62%	85.68%
Median target coverage	48.7x	51.1x	44.1x	63.6x	63.8x	64.7x
Number of variants	50,677	52,782	49,608	38,575	38,268	39,920
Exonic and splice site	17,895	16,876	16,212	15,933	15,816	15,752
Non-synonymous	8,844	8,180	7,774	7,973	7,870	7,836
Novel dbSNP	2,027	1,210	1,359	830	813	668
Novel Inhouse database	724	442	503	403	389	274
High QC (>=5 variant reads, >=20%)	299	200	200	168	170	100

Statistics of exome sequencing for six Cantú syndrome individuals. Samples of individuals 2,3 and 4 were enriched with a SureSelect v1 exome (38Mb) and sequence reads were mapped on hg18 using Bioscope v1.3. Variants were called using medium stringency settings and annotated with dbSNP v130. Samples of individuals 5, 5 mat, and 5 pat were enriched with a SureSelect v2 exome (50Mb) and sequence reads were mapped on hg19 using Lifescope v2.1. Variants were called using high stringency settings calling and annotated with dbSNP v134.

Table S2. De Novo Analysis

	# Variants
Total variants	38,575
Coding	15,933
Non-genic	7,973
Non-exonic	830
dbSNP v134	403
In house database	168
Possibly *de novo*	15
Possibly *de novo* and candidate gene in the exome of at least one other individual	4
Possibly *de novo* and candidate gene in the exome of at least two other individuals	1 (*ABCC9*)

Prioritization of *de novo* variants, resulted in 15 *de novo* candidate variants, of which 4 variants overlapped genes for which variants were also identified in the initial 3 individuals that were exome sequenced. Only a single gene harboured additional mutations in two of the three individuals.

Table S3. Summary of Identified Point Mutations in *ABCC9*

		Individual 1	Individuals 2a, 2b, 2c	Individual 3	Individual 4	Individual 5	Individual 6	Individual 7	Individuals 8a, 8b
cDNA position		c.3460C>T	c.3461G>A	c.3461G>A	c.3460C>T	c.3460C>T	c.3128G>A	c.3461G>A	c.1433C>T
Genomic DNA position [hg19], chromosome 12		g.21995261G>A	g.21995260C>T	g.21995260C>T	g.21995261G>A	g.21995261G>A	g.21997818C>T	g.21995260C>T	g.22,061,033G>A
Protein position		p.(Arg1154Trp)	p.(Arg1154Gln)	p.(Arg1154Gln)	p.(Arg1154Trp)	p.(Arg1154Trp)	p.(Cys1043Tyr)	p.(Arg1154Gln)	p.(Ala478Val)
De novo?		*de novo*	Mutation is co-segregating with disease	*de novo*	*de novo*	*de novo*	*de novo*	*de novo*	Mutation is co-segregating with disease
In silico prediction	AlignGVGD	C0 (GV 241.31-GD: 94.79)	C0 (GV 241.31-GD: 0.00)	C0 (GV 241.31-GD: 0.00)	C0 (GV 241.31-GD: 94.79)	C0 (GV 241.31-GD: 94.79)	C15 (GV 111.67 - GD: 117.28)	C0 (GV 241.31-GD: 0.00)	C65 (GV 0.00-GD: 65.28)
	PolyPhen2	probably damaging (score: 1.00)	possibly damaging (score: 0.806)	possibly damaging (score: 0.806)	probably damaging (score: 1.00)	probably damaging (score: 1.00)	possibly damaging (score: 0.708)	possibly damaging (score: 0.806)	benign (0.368)
	SIFT	deleterious (score: 0.00)	deleterious (score: 0.00)	deleterious (score: 0.00)	deleterious (score: 0.00)	deleterious (score: 0.00)	deleterious (score: 0.01)	deleterious (score: 0.00)	deleterious (0.00)
	MutPred	0.752 (P = 0.04)	0.737 (P = 0.06)	0.737 (P = 0.06)	0.752 (P = 0.04)	0.752 (P = 0.04)	0.580 (P = 0.24)	0.737 (P = 0.06)	0.550 (P = 0.06)
Nucleotide conservation (PhyloP, 46 vertebrates)		2.12	5.89	5.89	2.12	2.12	4.28	5.89	6.03

RefSeq accession number NM_020297.2 was used in naming mutations

Mutations in individuals 1, 2a, 3 and 4 were initially identified by exome sequencing, and validated by Sanger sequencing. Mutations in individuals 2b, 2c, 5, 6, 7, 8a and 8b were identified by Sanger sequencing.