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Supplemental Data

Short Stature, Onychodysplasia, Facial Dysmorphism, and Hypotrichosis Syndrome Is Caused by a *POC1A* Mutation

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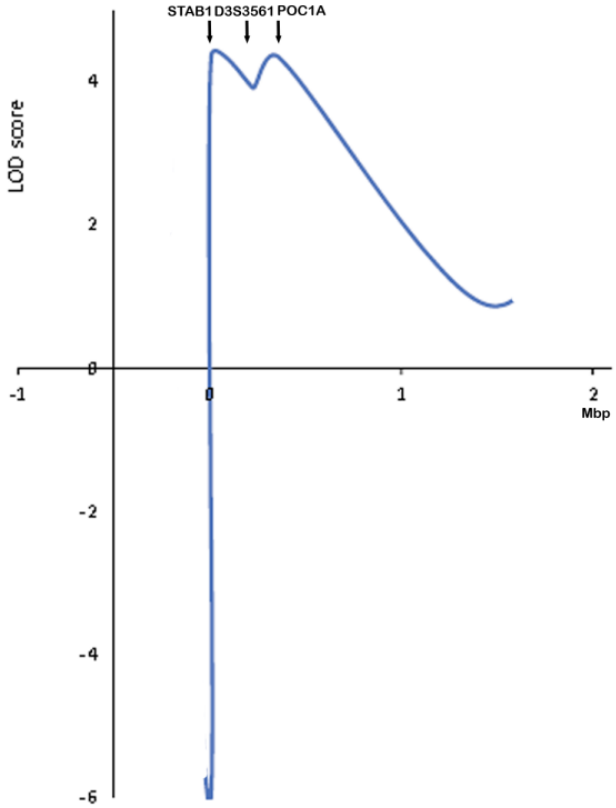

Figure S1. Linkage Analysis

LOD scores of the three highest ranked markers assessed are plotted against chromosomal locations.

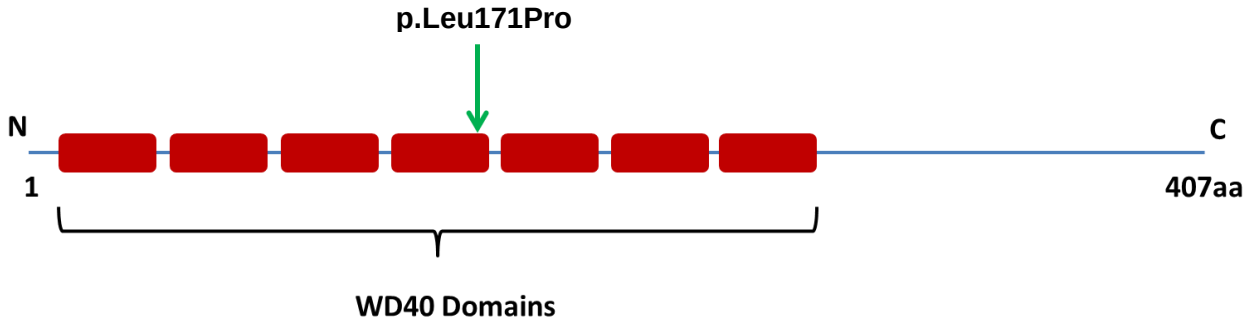

Figure S2. Scheme of POC1A Structure

The mutation position is marked by a green arrow.

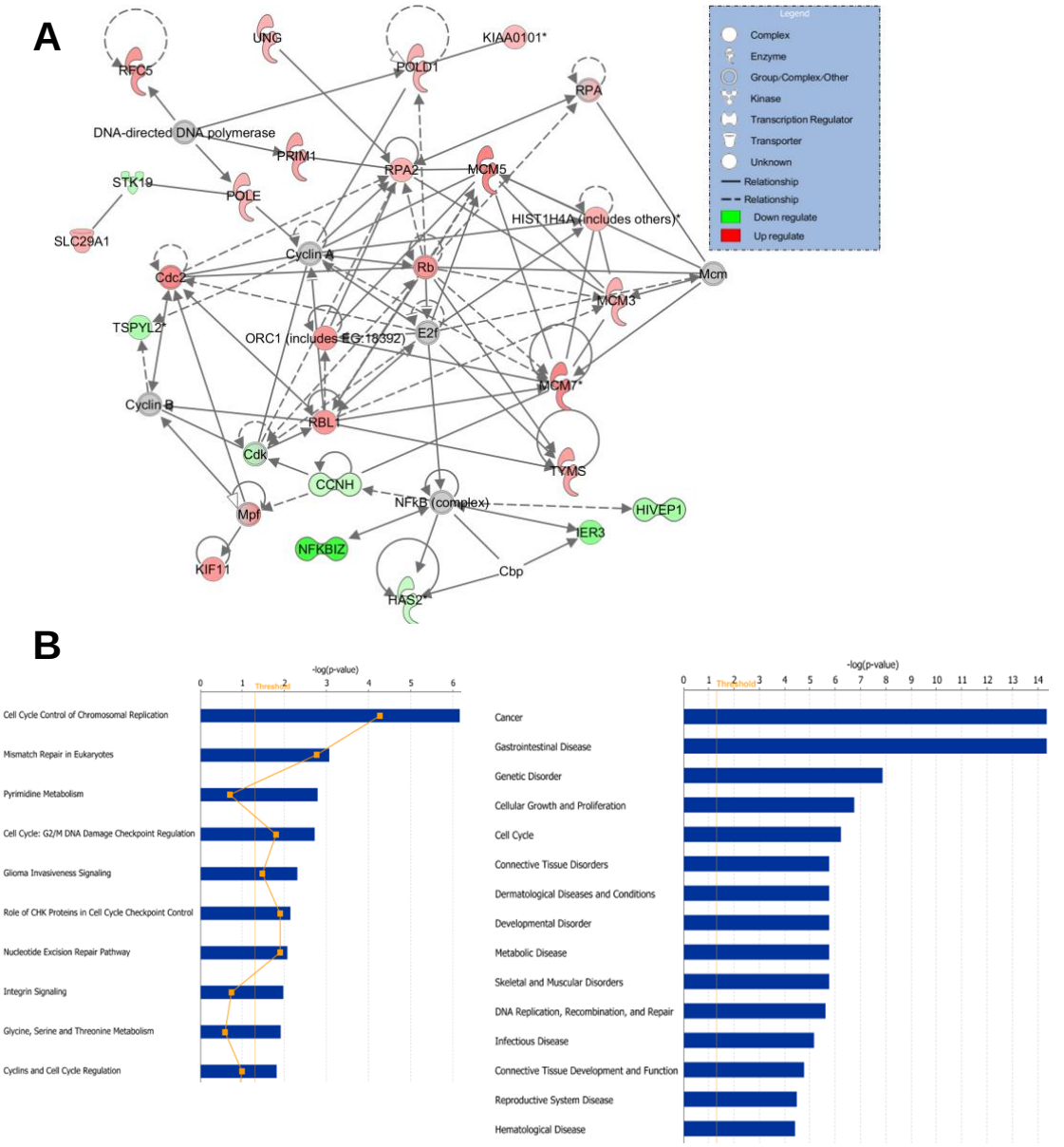

Figure S3. Pathway Analysis

To gain insight into the pathomechanisms underlying the deleterious effect of the SOFT syndrome-causing mutation, we compared transcription profiles of patient and control fibroblasts using the HumanHT-12 v3 Expression BeadChip Kit (Illumina). In brief, total RNA (200 ng) was reverse transcribed and cRNA prepared using TargetAmp-Nano Labeling Kit (Epicentre Biotechnologies**,** Madison, WI) according to the manufacturer's protocol. Biotinylated cRNA (1.5 µg) was hybridized to HumanHT-12 v3 Expression BeadChip (encompassing more than 47,000 transcript targets), washed, and scanned on a BeadArray 500GX Reader using Illumina BeadScan image data acquisition software (version 2.3.0.13). Quality control and quantile normalization of the microarray data was done by BeadStudio 3.0 software (Illumina). The scanning data were exported to JMP genomic Software (SAS, Cary, NC), log transformed normalized, and transcripts with low expression (log value < 6.5) or with low variation across all samples (variation < 0.05) were removed from the analysis (more than 6900 transcripts passed the above criteria). Genes differentially expressed in patient's fibroblasts vs. normal control cells were ascertained using one way ANOVA analysis. A data set of 227 genes that were found to be significantly differentially expressed between patient and controls cells, each consisting of gene name, fold change in expression levels, and a *P* value for each of the genes, were imported into Ingenuity Pathway Analysis (IPA) software 5.0 (Ingenuity Systems, Redwood City, CA, USA). IPA revealed strong enrichment of biological networks and functions that are associated with cell cycle as well as connective tissue and dermatology disorders (p values < 0.0005, supplementary Figure 2S and Table 3S).

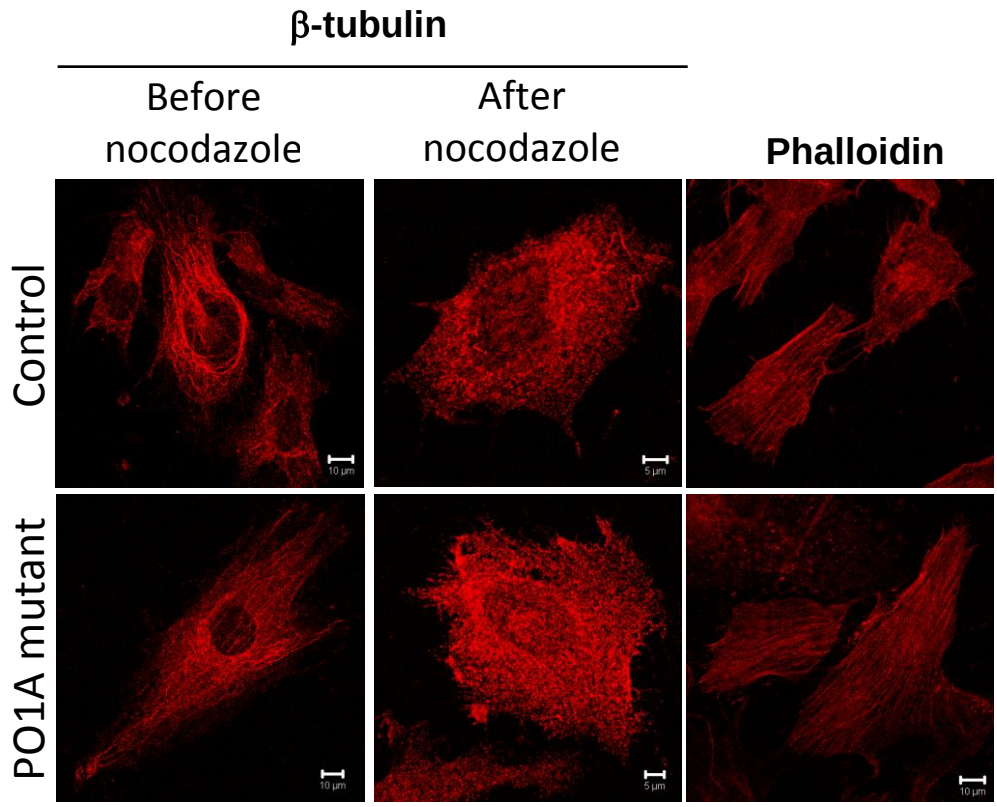

Figure S4. β-Tubulin and Phalloidin Staining

Normal and POC1A mutant fibroblasts were fixed and stained for γ-tubulin (microtubule cytoskeleton) before and after nocodazole washouts, and for F-actin (actin filaments) with phalloidin. No differences were noticed between patient and control cells (Bars, 5 µm and 10 µm).

Table S1. Candidate Genes Sequenced in the Critical Interval (between Markers D3S1573 and rs2279323)

Gene	Location
RBM15B	3p21.2
TEX264	3p21.31
GPR62	3p21.1
RPL29	3p21.3-p21.2
DUSP7	3p21
PPM1M	3p21.2
PHF7	3p21.1
PBRM1	3p21
GNL3	3p21.1
SNORD19	3p21.1
SNORD19B	3p21.1
SNORD69	3p21.1
GLT8D1	3p21.1
ITIH1	3p21.1
ITIH3	3p21.1
ITIH4	3p21.1
MUSTN1	3p21.1
PRKCD	3p21.31
TKT	3p14.3

Table S2. Exome-Sequencing Details

	Actual Number	Percent
Total sequence (bp)	6,560,365,680	100
Aligned paired reads	61,319,857	85
Median read depth	X49	
1× coverage[a]	193,479	99.2
4× coverage[a]	190,651	97.2
8× coverage[a]	186,523	95.7
20× coverage[a]	168,232	86.3
30× coverage[a]	146,406	75.1

[a] Coverage of exons in the genome (number of exons as defined by Nimblegen kit V2.0)

Table S3. Predicted Pathogenicity of the p.Leu171Pro Substitution in POC1A

Prediction Software	**Prediction category**	**Score**
PolyPhen-2	Score	0.999
	Sensitivity	0.14
	Specificity	0.99
	Prediction	**Probably damaging substitution**[a]
SIFT	Score	0.00
	Prediction	**Probably damaging substitution**[b]
Align GVGD	GV	4.85
	GD	95.38
	Prediction	**Probably damaging substitution – Class C65**[c]

[a] Probably damaging (predicted to affect protein function with a high degree of confidence).

[b] Score range from 0 to 1. The amino acid substitution is predicted to be damaging if the score is less than 0.05 and tolerated if the score is above 0.05.

[c] Substitution classification in Align GVGD: GD>=65+Tan(10)x(GV^2.5) => Class C65 <=> most likely.

Table S4. Fold change of the Expression Level of Top 100 genes that Were Differentially Expressed between Cells Obtained from Patients vs. Controls

Gene	MIM	Fold Change	Description
NTSR1	162651	23.52	neurotensin receptor 1 (high affinity)
NPTX1	602367	12.84	neuronal pentraxin I
ST8SIA5	607162	11.79	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 5
GRP	137260	10.09	gastrin-releasing peptide
RPS4Y1	470000	9.93	ribosomal protein S4, Y-linked 1
AQP1	107776	7.70	aquaporin 1 (Colton blood group)
LOC404266		6.89	hypothetical LOC404266
C13orf15	610077	6.84	chromosome 13 open reading frame 15
NDP	300658	6.33	Norrie disease (pseudoglioma)
KCTD12	610521	6.18	potassium channel tetramerisation domain containing 12
EIF1AY	400014	5.63	eukaryotic translation initiation factor 1A, Y-linked
GSTM1	138350	5.53	glutathione S-transferase mu 1
ANGPTL4	605910	4.80	angiopoietin-like 4
HOXB5	142960	4.60	homeobox B5
ST8SIA2	602546	4.52	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 2
THBS4	600715	4.37	thrombospondin 4
GPR68	601404	4.26	G protein-coupled receptor 68
OLFM1	605366	4.22	olfactomedin 1
MMP1	120353	4.22	matrix metallopeptidase 1 (interstitial collagenase)
PPAPDC3		4.13	phosphatidic acid phosphatase type 2 domain containing 3
BTBD11		4.02	BTB (POZ) domain containing 11
FOXQ1	612788	4.02	forkhead box Q1
TBX1	602054	3.99	T-box 1
CADPS	604667	3.88	Ca++-dependent secretion activator
KIAA1644		3.86	KIAA1644
PTGS1	176805	3.82	prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase)
STC1	601185	3.69	stanniocalcin 1
CNIH3		3.62	cornichon homolog 3 (Drosophila)
CLEC2B	603242	3.60	C-type lectin domain family 2, member B
TNFAIP8L3		3.55	tumor necrosis factor, alpha-induced protein 8-like 3
HOXB8	142963	3.49	homeobox B8
SQSTM1	601530	3.39	sequestosome 1
FILIP1L	612993	3.40	filamin A interacting protein 1-like
RHOJ	607653	3.40	ras homolog gene family, member J
OXTR	167055	3.41	oxytocin receptor
HERPUD1	608070	3.43	homocysteine-inducible, endoplasmic reticulum stress-inducible, ubiquitin-like domain member 1
DENND5B		3.46	DENN/MADD domain containing 5B
TSC22D3	300506	3.48	TSC22 domain family, member 3
ID3	600277	3.55	inhibitor of DNA binding 3, dominant negative helix-loop-helix protein
DACT1	607861	3.66	dapper, antagonist of beta-catenin, homolog 1 (Xenopus laevis)
A2M	103950	3.71	alpha-2-macroglobulin

CHAC1		3.72	ChaC, cation transport regulator homolog 1 (E. coli)
PSG7	176396	3.75	pregnancy specific beta-1-glycoprotein 7 (gene/pseudogene)
PDE1C	602987	3.77	phosphodiesterase 1C, calmodulin-dependent 70kDa
SLC7A5	600182	3.77	solute carrier family 7 (amino acid transporter light chain, L system), member 5
PRICKLE1	608500	3.80	prickle homolog 1 (Drosophila)
PRG1	605157	3.82	p53-responsive gene 1
EDIL3	606018	3.85	EGF-like repeats and discoidin I-like domains 3
ASS1	603470	3.86	argininosuccinate synthase 1
COL4A2	120090	3.87	collagen, type IV, alpha 2
MX1	147150	3.91	myxovirus (influenza virus) resistance 1, interferon-inducible protein p78 (mouse)
EFHD1	611617	3.93	EF-hand domain family, member D1
TGM2	190196	3.95	transglutaminase 2 (C polypeptide, protein-glutamine-gamma-glutamyltransferase)
TCF21	603306	3.95	transcription factor 21
TGFB2	190220	4.00	transforming growth factor, beta 2
IFI44L	613975	4.01	interferon-induced protein 44-like
DDIT3	126337	4.03	DNA-damage-inducible transcript 3
ATF3	603148	4.04	activating transcription factor 3
NFKBIZ	608004	4.07	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, zeta
COL11A1	120280	4.09	collagen, type XI, alpha 1
ADAMTS1	605174	4.11	ADAM metallopeptidase with thrombospondin type 1 motif, 1
HOXD11	142986	4.12	homeobox D11
MECOM	165215	4.17	MDS1 and EVI1 complex locus
RARB	180220	4.25	retinoic acid receptor, beta
ADAMTS5	605007	4.30	ADAM metallopeptidase with thrombospondin type 1 motif, 5
IFIT1	147690	4.34	interferon-induced protein with tetratricopeptide repeats 1
TRIB3	607898	4.47	tribbles homolog 3 (Drosophila)
IGFBP7	602867	4.53	insulin-like growth factor binding protein 7
EPSTI1	607441	4.56	epithelial stromal interaction 1 (breast)
MT1F	156352	4.70	metallothionein 1F
RAB27B	603869	4.73	RAB27B, member RAS oncogene family
PTGS2	600262	4.83	prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)
KCNH1	603305	4.90	potassium voltage-gated channel, subfamily H (eag-related), member 1
ADM2	608682	4.97	adrenomedullin 2
IFITM1	604456	5.01	interferon induced transmembrane protein 1
RNF150		5.14	ring finger protein 150
CLDN1	603718	5.15	claudin 1
GDF15	605312	5.22	growth differentiation factor 15
INHBE	612031	5.30	inhibin, beta E
CPA4	607635	5.33	carboxypeptidase A4
TUFT1	600087	5.35	tuftelin 1
CXCL1	155730	5.38	chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)
HAPLN1	115435	5.60	hyaluronan and proteoglycan link protein 1

PAPPA	176385	5.66	pregnancy-associated plasma protein A, pappalysin 1
IL8	146930	6.23	interleukin 8
PSG4	176393	6.34	pregnancy specific beta-1-glycoprotein 4
SULF1	610012	6.53	sulfatase 1
LRRC17		6.75	leucine rich repeat containing 17
COL4A5	303630	6.76	collagen, type IV, alpha 5
AFF3	601464	6.95	AF4/FMR2 family, member 3
USMG5		7.07	up-regulated during skeletal muscle growth 5 homolog (mouse)
CH25H	604551	7.22	cholesterol 25-hydroxylase
PTX3	602492	7.55	pentraxin 3
F2RL2	601919	7.61	coagulation factor II (thrombin) receptor-like 2
KRT18	148070	7.99	keratin 18
CXCL6	138965	8.16	chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2)
DDIT4	607729	8.24	DNA-damage-inducible transcript 4
COL4A1	120130	9.14	collagen, type IV, alpha 1
EFEMP1	601548	36.39	EGF containing fibulin-like extracellular matrix protein 1
FBN2	612570	52.78	fibrillin 2

Upregulated and downregulated genes are highlighted with red and green colors, respectively.
Data have been deposited through MIAMExpress.