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Supplemental Data

In-Frame Mutations in Exon 1 of *SKI*

Cause Dominant Shprintzen-Goldberg Syndrome

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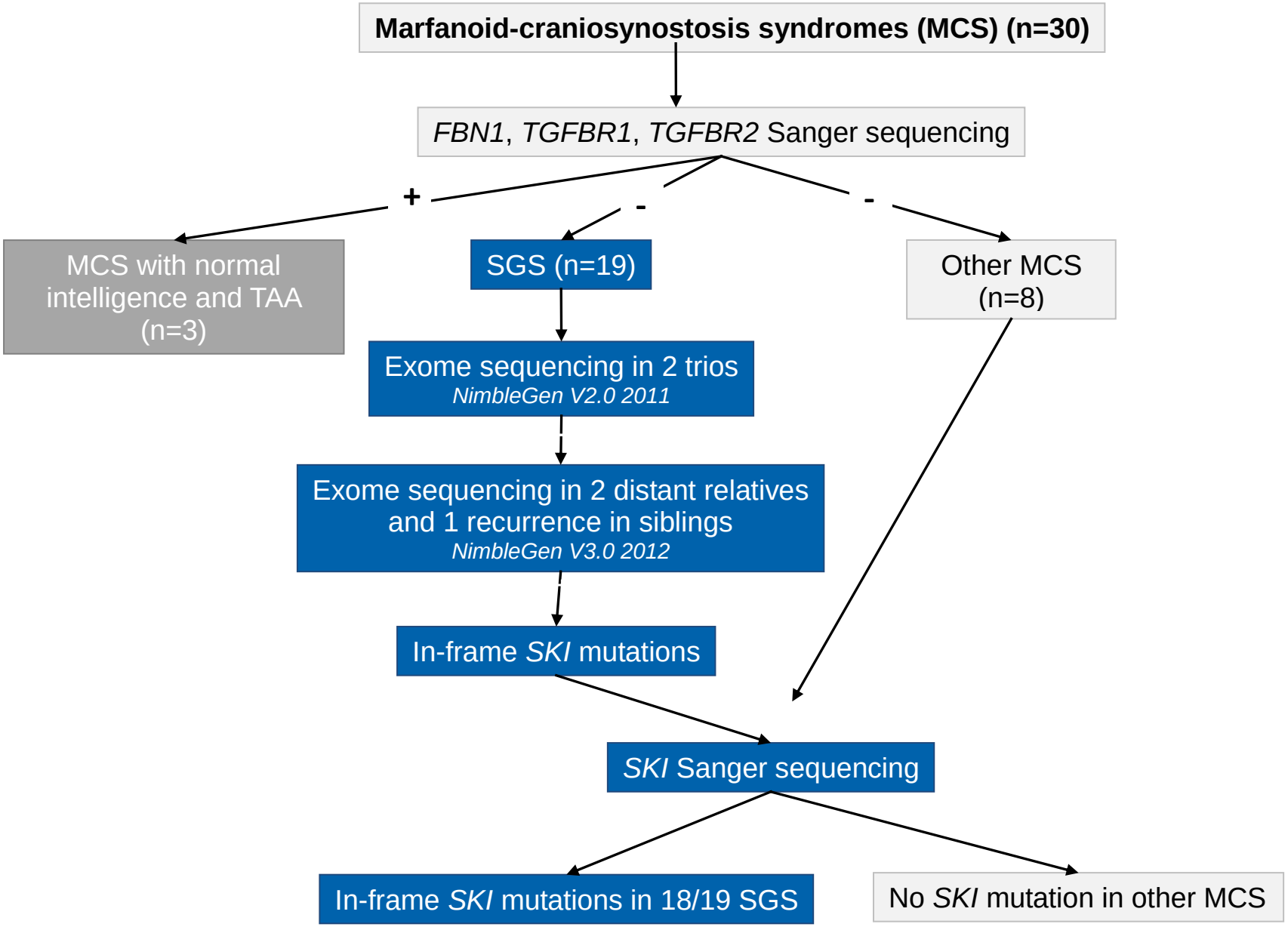

Figure S1. Design of the Study

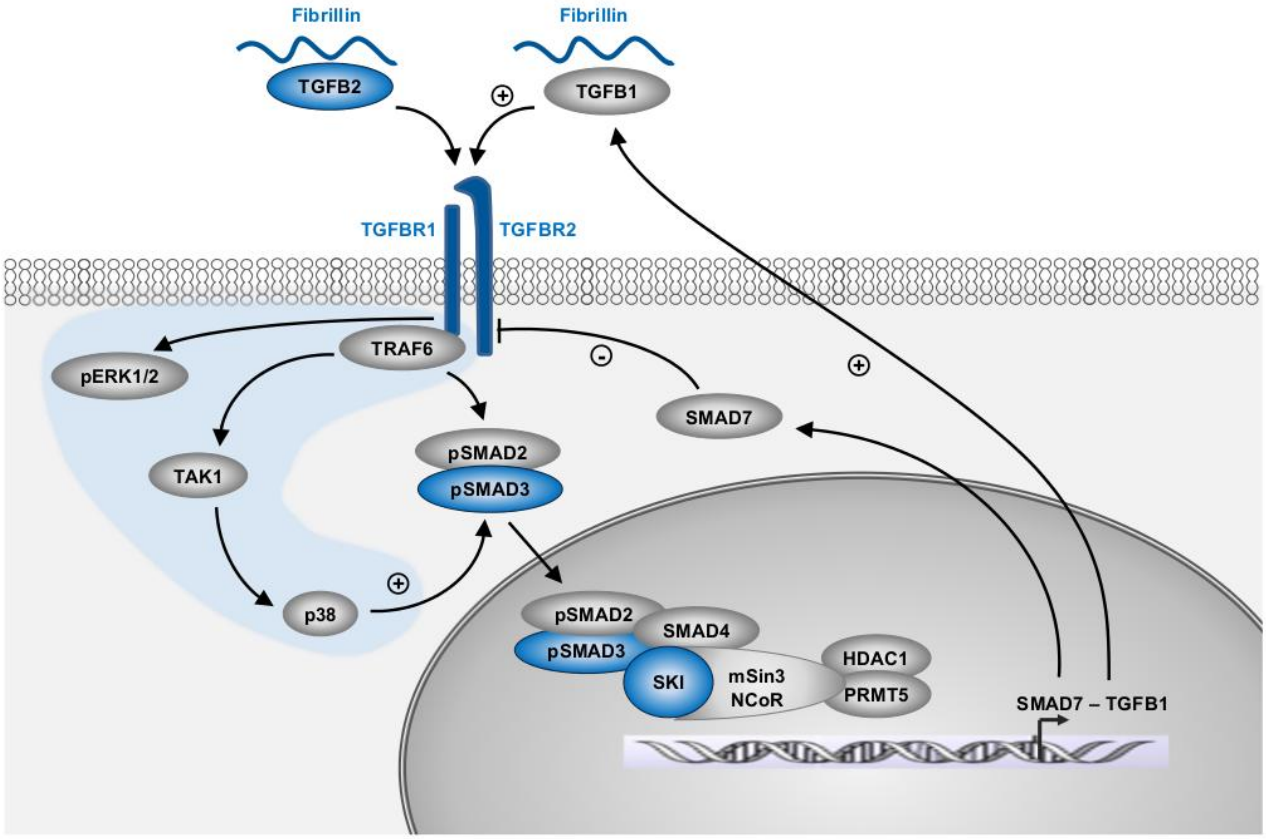

Figure S2. SKI within the TGF-β Pathway (adapted from Holm et al., 2011[19])

This diagram highlights the genes implicated in marfanoid syndromes (in blue).

Table S1. Detailed Clinical Features of Non-SGS Marfanoid-Craniosynostosis Patients

Family	F14	F15	F16	F17	F18	F19	F20	F21	F22	F23	F24	
Affected Individual	20	21	22	23	24	25	26	27	28	29	30	Total
Sex	M	M	F	F	F	M	M	F	M	M	F	6M/5F
Age (years)	15	6	8	22	22	13	23	20	19	1	21	
Craniosynostosis	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	+	**11/11**
Arachnodactyly	+	+	+	+	+	+	+	+	+	+	+	11/11
Pectus deformity	+	-	+	+	-	+	-	+	-	-	+	6/11
Scoliosis	+	+	+	+	+	+	-	-	-	-	+	7/11
Joint contractures	-	-	-	+	-	+	-	-	+	-	-	3/11
Camptodactyly	-	-	-	-	+	+	-	-	-	-	+	3/11
Foot malposition	+	+	+	+	+	+	+	-	NA	-	+	8/10
Scapho/dolichocephaly	+	+	+	+	-	+	+	NA	-	+	+	8/10
Hypertelorism	-	+	+	-	-	-	-	+	+	-	+	5/11
Proptosis	-	-	-	+	+	-	-	+	+	+	+	6/11
Downslanting palpebral fissures	-	+	+	-	-	+	-	NA	NA	-	-	3/9
Micro/retrognathia	-	-	-	-	-	-	-	NA	-	+	+	2/10
Intellectual disability	-	-	-	-	-	+	+	NA	+	+	-	4/10
Hernias	+	+	+	+	+	-	-	NA	-	+	-	6/10
Loss of subcutaneous fat	-	-	-	-	-	-	-	NA	-	-	-	0/10
Valvular anomalies	+	+	+	+	-	-	+	NA	-	NA	+	6/9
Aortic root dilatation	+[a]	+[a]	+[a]	+	-	+	-	NA	-	NA	+	6/9
Myopia	-	+	-	-	NA	NA	-	NA	-	NA	-	1/7
SKI mutation	-	-	-	-	-	-	-	-	-	-	-	0/11
Other mutations	*FBN1* p.Cys1254Tyr	*TGFBR1* p.Glu245Gly	*TGFBR2* p.Arg528His	-	-	-	-	-	-	-	-	3/11
Inheritance	*de novo*	*de novo*	*de novo*	Sporadic	Sporadic	Sporadic	Sporadic	Sporadic	Sporadic	Sporadic	Sporadic	

F : Female ; M : Male ; NA : Not available.

[a] Aortic dilatation required surgery in childhood

Table S2. Next-Generation-Sequencing Statistics

	Project 1 (2011)						Project 2 (2012)				
Family	F1	F1	F1	F2	F2	F2	F4	F4	F3	F3	F3
Individual	II-1	I-1	I-2	II-1	I-1	I-2	IV-2	III-1	II-4	I-1	I-2
NB_READS	152 949 574	188 373 140	153 270 902	164 636 014	172 788 840	188 524 030	140 793 700	123 309 166	124 054 811	110 402 922	120 007 422
PERCENT_MAPPED_READS	88,34%	88,62%	91,31%	90,08%	90,54%	88,29%	98,38	98,26	97,87	97,77	97,97
PERCENT_PAIRING	98,54%	98,95%	98,83%	98,82%	98,77%	98,93%	99,63	99,62	99,49	99,43	99,49
PERCENT_DUPLICATE_READS	7,11%	7,58%	8,44%	6,20%	6,99%	6,04%	12,76	10,94	12,77	14,13	13,36
Capture kit	Nimblegen SeqCap EZ Exome v2.0						Nimblegen exome V3				
PERCENT_NT_30X	88%	90%	88%	90%	90%	91%	88,32	87,77	86,22	84,87	86,16
PERCENT_REGIONS_0x	1%	1%	1%	1%	1%	1%	0,69	0,72	0,81	0,60	0,82
PERCENT_REGIONS_FULL_COVER	98%	98%	98%	97%%	98%	98%	97,30	97,23	96,94	97,04	96,91
MEAN_COVER	177,65	220,17	173,83	195,78	206,05	229,30	140,79	130,14	125,12	110,29	122,25
MEDIAN_COVER	142,00	177,00	139,00	158,00	179,00	187,00	111,00	103,00	99,00	87,00	97,00
Exonic heterozygous call	12 851	12 765	12 619	12 732	12 811	12 872	14 694	14 585	13 973	14 356	14 528
De novo Trio analysis	84			51			na		71		
Intrafamilial call segregation study	na			na			12 067		na		
Absent from local Exome database	50			30			314		31		
EVS6500 <1/1000 variant filter	32			18			46		24		
TGF-beta pathway filter	0			0			1		0		

na : not appropriate.

Table S3. Strategy for Application of a Biological Filter (Cellular TGF-β Pathway)

HGNC Gene Symbol	OMIM number	OMIM reference
E2F4	600659	E2F TRANSCRIPTION FACTOR 4
E2F5	600967	E2F TRANSCRIPTION FACTOR 5
FKBP1A	186945	FK506-BINDING PROTEIN 1A
FOXH1	603621	FORKHEAD BOX H1
FOXO3	602681	FORKHEAD BOX O3A
HDAC1	601241	HISTONE DEACETYLASE 1
MEN1	131100	MULTIPLE ENDOCRINE NEOPLASIA, TYPE I
MTMR4	603559	MYOTUBULARIN-RELATED PROTEIN 4
NEDD4L	606384	UBIQUITIN PROTEIN LIGASE NEDD4-LIKE
NR1I2	603065	NUCLEAR RECEPTOR SUBFAMILY 1, GROUP I, MEMBER 2
PARD6A	607484	PARTITIONING-DEFECTIVE PROTEIN 6, C. ELEGANS, HOMOLOG OF, ALPHA
PARP1	173870	POLY (ADP-RIBOSE) POLYMERASE 1
PMEPA1	606564	TRANSMEMBRANE PROSTATE ANDROGEN-INDUCED RNA
PPA1	179030	PYROPHOSPHATASE, INORGANIC, 1
PPM1A	606108	PROTEIN PHOSPHATASE, MAGNESIUM-DEPENDENT, 1A
PPP1R15A	611048	PROTEIN PHOSPHATASE 1, REGULATORY SUBUNIT 15A
RBL1	116957	RETINOBLASTOMA-LIKE 1
RHOA	165390	RAS HOMOLOG GENE FAMILY, MEMBER A
RNF11	612598	RING FINGER PROTEIN 11
RNF111	605840	RING FINGER PROTEIN 111
SKI	164780	V-SKI AVIAN SARCOMA VIRAL ONCOGENE HOMOLOG
SKIL	165340	SKI-LIKE
SMAD2	601366	MOTHERS AGAINST DECAPENTAPLEGIC, DROSOPHILA, HOMOLOG OF, 2
SMAD3	603109	MOTHERS AGAINST DECAPENTAPLEGIC, DROSOPHILA, HOMOLOG OF, 3
SMAD4	600993	MOTHERS AGAINST DECAPENTAPLEGIC, DROSOPHILA, HOMOLOG OF, 4
SMAD7	602932	MOTHERS AGAINST DECAPENTAPLEGIC, DROSOPHILA, HOMOLOG OF, 7
SMURF1	605568	SMAD-SPECIFIC E3 UBIQUITIN PROTEIN LIGASE 1
SMURF2	605532	SMAD-SPECIFIC E3 UBIQUITIN PROTEIN LIGASE 2
SP1	189906	TRANSCRIPTION FACTOR Sp1
STRAP	605986	SERINE/THREONINE KINASE RECEPTOR-ASSOCIATED PROTEIN
STUB1	607207	STIP1 HOMOLOGOUS AND U BOX-CONTAINING PROTEIN 1
TFDP1	189902	TRANSCRIPTION FACTOR DP1
TFDP2	602160	TRANSCRIPTION FACTOR DP2
TGFB1	190180	TRANSFORMING GROWTH FACTOR, BETA-1
TGFBR1	190181	TRANSFORMING GROWTH FACTOR-BETA RECEPTOR, TYPE I
TGFBR2	190182	TRANSFORMING GROWTH FACTOR-BETA RECEPTOR, TYPE II
TGIF1	602630	TRANSFORMING GROWTH FACTOR-BETA-INDUCED FACTOR
TRIM33	605769	TRIPARTITE MOTIF-CONTAINING PROTEIN 33
UCHL5	610667	UBIQUITIN CARBOXYL-TERMINAL HYDROLASE L5
USP15	604731	UBIQUITIN-SPECIFIC PROTEASE 15
WWTR1	607392	WW DOMAIN-CONTAINING TRANSCRIPTION REGULATOR 1
ZFYVE9	603755	MADH-INTERACTING PROTEIN

HGNC : HUGO Gene Nomenclature Committee ; OMIM : Online Mendelian Inheritance in Man.

Table S4. Prediction of Identified *SKI* Variants

Family	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Affected individual	**II-1**	**II-1**	**II-1, III-1, III-2, III-4, IV2**	**II-2, II-3, II-4**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
Genomic location (bp), Hg19, Chromosome 1	2160305	2160299	2160485-2160497	2160306	2160309	2160299	2160488-2160497	2160308	2160300	2160305	2160299	2160297
Codon change	GGC/TGC	CTG/GTG	TCCGACCGCTCC/-	GGC/GTC	CCG/CAG	CTG/GTG	GACCGCTCC/-	CCG/TCG	CTG/CCG	GGC/AGC	CTG/GTG	TCG/TTG
cDNA position	c.100G>T	c.94C>G	c.280_291del TCCGACCGCTCC	c.101G>T	c.104C>A	c.94C>G	c.283_291del GACCGCTCC	c.103C>T	c.95T>C	c.100G>A	c.94C>G	c.92C>T
Protein position	p.Gly34Cys	p.Leu32Val	p.Ser94_Ser97del	p.Gly34Val	p.Pro35Gln	p.Leu32Val	p.Asp95_Ser97del	p.Pro35Ser	p.Leu32Pro	p.Gly34Ser	p.Leu32Val	p.Ser31Leu
Ensembl VEP	Missense	Missense	12-bp in-frame deletion	Missense	Missense	Missense	9-bp in-frame deletion	Missense	Missense	Missense	Missense	Missense
Polyphen2	Probably damaging	Possibly damaging	NA	Probably damaging	Probably damaging	Possibly damaging	NA	Probably damaging	Benign[a]	Probably damaging	Possibly damaging	Possibly damaging
SIFT[b]	Damaging	Damaging	NA	Damaging	Damaging	Damaging	NA	Damaging	Damaging	Damaging	Damaging	Damaging
EVS	-	-	-	-	-	-	-	-	-	-	-	-
dbSNP	-	-	-	-	-	-	-	-	-	-	-	-
In house exomes	-	-	-	-	-	-	-	-	-	-	-	-

NA : Not Available ; [a] Specificity : 0.44 ; Sensitivity : 0.98 ; [b] Median Information Content : 2.58. *SKI* genomic accession number: NC_000001.10; SKI transcript accession number: NM_003036.3.

Table S5. Sequences of Primers Used to Sequence All Coding *SKI* Exons

Amplicon	Forward Primer Sequence	Reverse Primer Sequence	PCR Conditions
E1-1	TCCAGCGGCGGGACCCCTT	GATGGTCTCGCCTTCCAGTA	PCR Touchdown 60-50°C 10+30 cycles Standard Taq GC-Rich protocol (Roche)
E1-ATG	ATGCCCATGACTTTGAGGAT	GATGGTCTCGCCTTCCAGTA	PCR Touchdown 65-55°C 10+30 cycles Standard Taq Phusion GC-Rich protocol (Thermo)
E1-2	CAGGAGGCCTACAAGAAGGA	GGCTGCTGTAGAGCTCGG	PCR fixed Tm 56°C 40 cycles Standard Taq GC-Rich protocol (Roche)
E1-3	CTCATCACCAAGACGGACG	TCGGAGACCAGAGCCTGTAG	PCR Touchdown 60-50°C 10+30 cycles Standard Taq Phusion GC-Rich protocol (Thermo) DMSO 3%
E2	AGTGCATGGGGCTCTGACT	CAAGGAGAAGGGCCCAGTA	PCR Touchdown 65-55°C 10+30 cycles Taq Gold (Applied)
E3	GGGACATGAAGTGGCTTGTT	ACCCAGCCTGCAGAAACAT	PCR Touchdown 65-55°C 10+30 cycles Taq Gold (Applied)
E4	GAGCACACCTAGAGCGTTCC	AGGGAGGAGGCACAGAAAG	PCR Touchdown 65-55°C 10+30 cycles Taq Gold (Applied)
E5	CGTCTCCCTGGTGTGGAG	GTTCACCTGGTGCAGGCT	PCR Touchdown 65-55°C 10+30 cycles Taq Gold (Applied)
E6	ATGGTGAGGGGTGTGCTG	CTGCTCCAAGGCCTTTCC	PCR Touchdown 65-55°C 10+30 cycles Taq Gold (Applied)
E7	TGTCCTAGCAGGTGGAGGAG	TCTGAATTTCAAGTCTCCTTACTGG	PCR Touchdown 60-50°C 10+30 cycles Standard Taq GC-Rich protocol (Roche)

E: Exon