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Supplemental Data

Mesomelia-Synostoses Syndrome Results

from Deletion of *SULF1* and *SLCO5A1* Genes at 8q13

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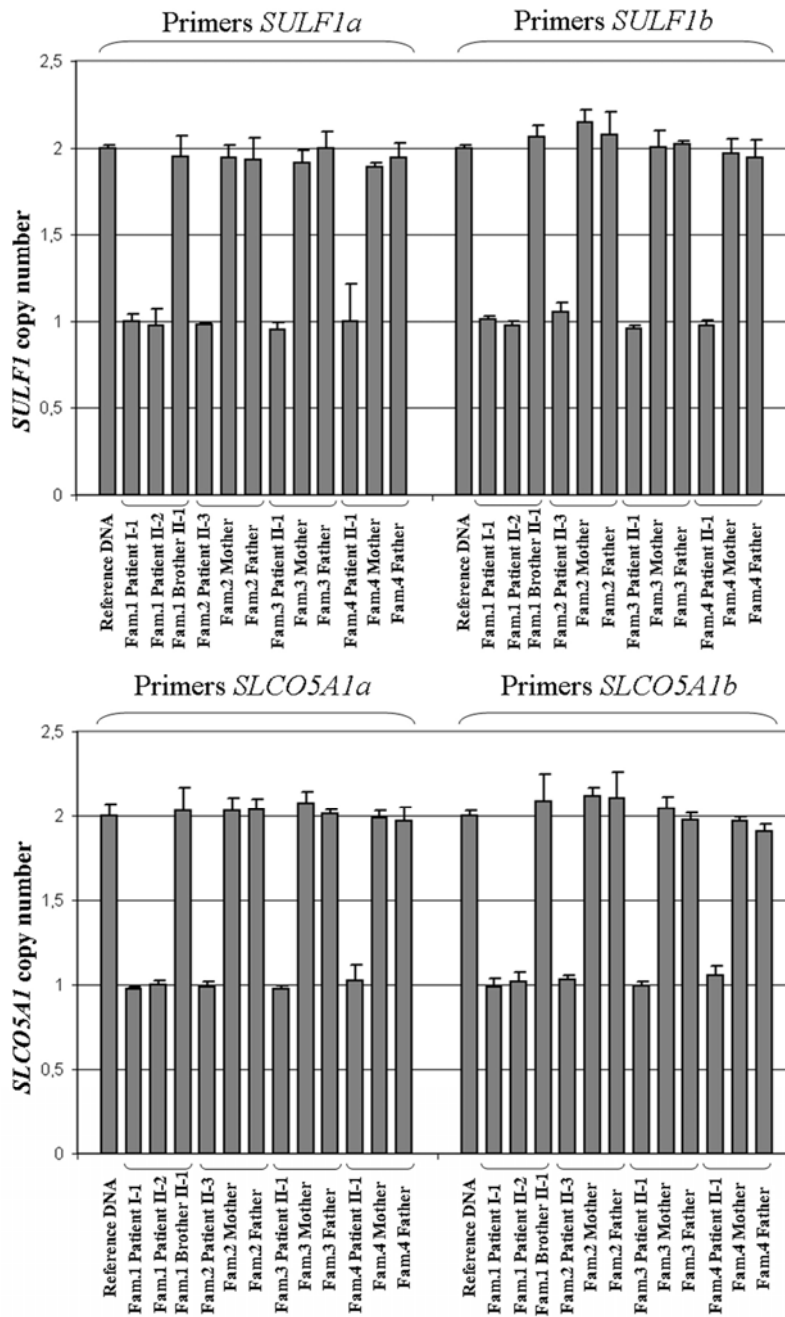


Figure S1. Real-time quantitative PCR (qPCR) results for *SULF1* and *SLCO5A1*. qPCR results showing the copy number for the *SULF1* gene and the *SLCO5A1* gene in the patients, their parents and the controls. The number of alleles present was quantified with the comparative threshold cycle (dCt) method with *ALBUMIN* as reference gene. Each reaction was performed in triplicate. The sequences of the primers used are given in Supplementary Table 3. Fam.: family.

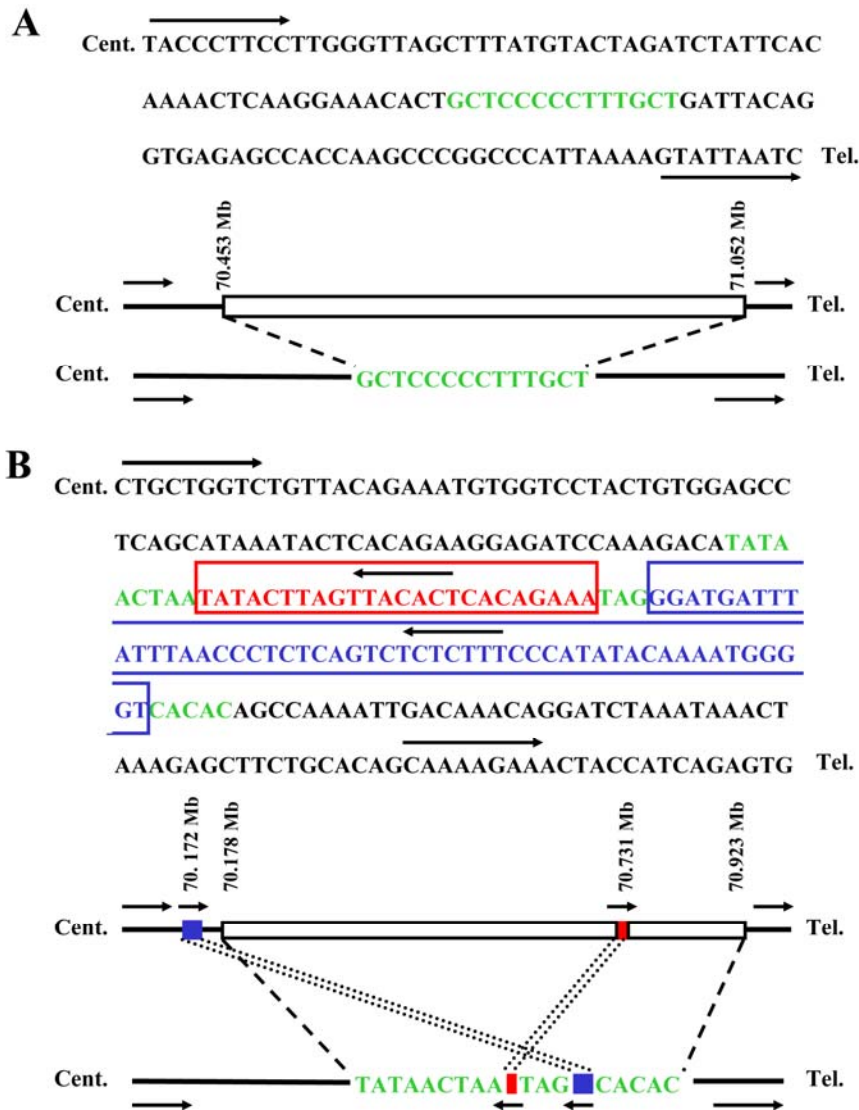


Figure S2. Results of breakpoint sequencing.

Panel A. Sequence data for the deletion junction in patient II-1 in family 4. The 607 Kb deletion may have been mediated by non-homologous end joining (NHEJ) with the additional bases GCTCCCCCTTTGCT bridging the breakpoints (depicted in green). Panel B. Sequence data for the junction of the 744 Kb deletion in patient II-2 in family 1. Sequence analysis of breakpoint junctions revealed sequence complexity. A 553 Kb deletion is followed by a 24 bp segment with no copy-number change (depicted in red), a 52 bp segment with no copy-number change (depicted in blue) and a 191 Kb deletion. The additional bases of unknown origin are depicted in green. Black boxes indicate the deleted regions. Arrowheads show direction of DNA relative to the positive strand. Figure is not drawn to scale and approximate positions are given in megabases (Mb) (hg18). Cent.: centromeric position; tel.: telomeric position.

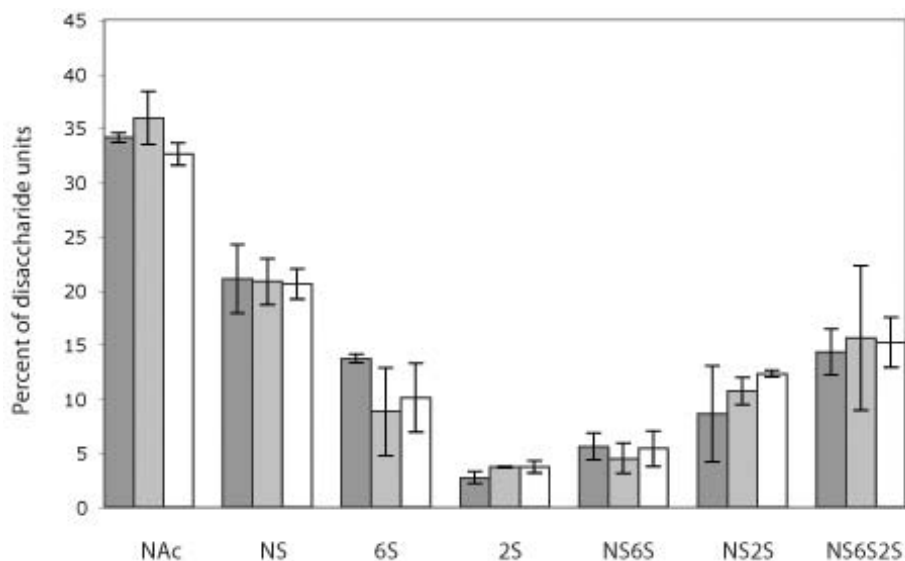


Figure S3. Disaccharide composition of heparan sulfate recovered from the urine of patient II-2 of Family 1.

Heparan sulfate purified from 10 ml samples of urine from two healthy controls and patient II-2 of family 1 were subjected to disaccharide compositional analyses by reversed phase ion pairing-HPLC after digestion with heparinase I, II and III (Ledin J, Staatz W, Li JP, Götte M, Selleck S, Kjellén L, Spillmann D.(2004) J Biol Chem. 279, 42732-42741). Two samples from control 1 (dark grey staples) and three samples from control 2 (light grey staples) and patient II-2, family 1 (white staples) were analyzed. Average values +/- standard deviation are shown.

Non-sulfated disaccharide: **NAc** (HexA-GlcNAc);

Monosulfated disaccharides: **NS** (HexA-GlcNS), **6S** (HexA-GlcNAc(6OS)),

2S (HexA(2OS)-GlcNAc);

Disulfated disaccharides: **NS6S** (HexA-GlcNS(6OS), **NS2S** (HexA(2OS)-GlcNS);

Trisulfated disaccharide: **NS6S2S** (HexA(2OS)-GlcNS(6OS));

where HexA is hexuronic acid, GlcNAc is *N*-acetyl-glucosamine and GlcNS is *N*-sulfated glucosamine.

Table S1. Types of Agilent Human Genome CGH oligonucleotide array used in the present study.

	Pangenomic 44K	Pangenomic 400K
Fam.1 ; Patient I-1		X
Fam.1 ; Individual I-2	X	
Fam.1 ; Individual II-1	X	
Fam.1 ; Patient II-2		X
Fam.2; Individual I-1	X	
Fam.2; Individual I-2	X	
Fam.2 ; Patient II-3		X
Fam.3; Individual I-1	X	
Fam.3; Individual I-2	X	
Fam.3 ; Patient II-1		X
Fam.4; Individual I-1	X	
Fam.4; Individual I-2	X	
Fam.4 ; Patient II-1		X
Patient K1 with Kantaputra syndrome	X	
Patient K2 with Kantaputra syndrome	X	

Table S2. 400K array CGH results for the 8q13 deletions.

		Fam.1 Patient I-1	Fam.1 Patient II-2	Fam.2 Patient II-3	Fam.3 Patient II-1	Fam.4 Patient II-1
Last normal copy-number probe (centromeric side)	Agilent probe reference	A_16_P18342627	A_16_P18342627	A_18_P16520270	A_16_P18342932	A_16_P38436215
	Physical position of the probe (start-end)(hg18)	70176105-70176164	70176105-70176164	70337713-70337772	70309160-70309219	70442152-70442210
First deleted probe (centromeric side)	Agilent probe reference	A_16_P38435619	A_16_P38435619	A_16_P38436025	A_18_P16521930	A_16_P18343273
	Physical position of the probe (start-end)(hg18)	70179295-70179354	70179295-70179354	70353405-70353464	70326013-70326072	70453476-70453535
Last deleted probe (telomeric side)	Agilent probe reference	A_16_P18344511	A_16_P18344511	A_16_P18344545	A_16_P18344511	A_14_P126822
	Physical position of the probe (start-end)(hg18)	70917620-70917679	70917620-70917679	70935409-70935468	70917620-70917679	71052720-71052779
First normal copy-number probe (telomeric side)	Agilent probe reference	A_16_P01937896	A_16_P01937896	A_16_P18344553	A_16_P01937896	A_16_P18344798
	Physical position of the probe (start-end)(hg18)	70926798-70926857	70926798-70926857	70944715-70944774	70926798-70926857	71083900-71083959
Minimum size of the deletion (in Kb)		738 Kb	738 Kb	582 Kb	591 Kb	599 Kb
Maximum size of the deletion (in Kb)		750 Kb	750 Kb	607 Kb	617 Kb	641 kb

Table S3. Real-time quantitative PCR (qPCR) assays.

Primer sequences and PCR conditions for *SULF1*, *SLCO5A1* and the reference gene *ALB*

oligo	forward primer	reverse primer	physical position (Hg19)	amplicon size (bp)	annealing temperature
ALB	GCTGTCATCTCTTGTGGGCTGT	AAACTCATGGGAGCTGCTGGTT	chr4:74284555-74284695	141	59
SULF1intr1	CCCCAAGAAATGGTCACTA	CAGGCAAGACTGCCCTAGAC	chr8:70245160-70245287	128	59
SULF1ex6	CCACCTTCATCAATGCCTTT	GGGAAGAGCAGTTCTCGTTG	chr8:70650811-70650930	120	59
SLCO5A1intr5	CCATGCCAACCATAAAAAAG	GGGAAGGGAGAGAATTTTGC	chr8:70649951-70650064	114	59
SLCO5A1intr2	GGGTCCTATGGGTTGACCTT	CCCTGTTGGGTTTTTGTGAT	chr8:70684188-70684351	164	59

Table S4. Real-time quantitative RT-PCR assays.

Primer sequences and PCR conditions for *SULF1* and *SLCO5A1*. Two different reference genes, *GAPDH* and *HPRT1*, have been used for the study.

oligo	forward primer	reverse primer	amplicon size (bp)	annealing temperature
SULF1-ex22-24	GAGCTCAGAAGCTGTCAAGGA	AGACGGGGCTGATTAACCTT	138	60
SLCO5A1-ex7-8	TGAGCCAGTCTGTGGATCAG	ATCACTTGGCGACTTTGGAC	132	60
GAPDH	GTGAAGGTCGGAGTCAACG	TGAGGTCAATGAAGGGGTC	112	60
HPRT1	GCCAGACTTTGTGGATTTG	CTCTCATCTTAGGCTTTGTATTTG	114	60

Table S5. *SULF1* and *SLCO5A1* sequencing performed on two patients with mesomelic dysplasia, Kantaputra type.

No deleterious mutations were identified in both patients. Variants were identified in patient K1 (Siwicka et al.) (one variant in intron 10 of *SULF1*: rs1155965) and in patient K2 (Shears et al.) (two variants located in the 3'UTR of *SULF1*: rs1441204 in exon 2 and rs13264163 in exon 3 and one variant in exon 2 of *SLCO5A1*: rs3750266 in exon 2).

	oligo	forward primer	reverse primer	physical position (Hg19)	amplicon size (bp)	annealing temperature
SLCO5A1	SLC2aF/SLC2aR	AGGTGGGCAAGACTGACTG	GACCACGTAGAGGCACCTTC	chr8:70744541-70745015	478	60
	SLC2a2F/SLC2a2R	AGCAAAACCTTCTCGGTGTC	AGCCACGTAGACCCAGTGAT	chr8:70744115-70744606	492	60
	SLC2a3F / SLC 2a3R	CCCCTACCAGATCCAAGAGT	ACAGCAAGGCTAAAACGTAAAA	chr8:70743858-70744250	393	60
	SLC3F / SLC 3R	AAGGGCAAGCTTCAAAACC	GACGTAGCTTTCTTTGGGATG	chr8:70673814-70674287	451	60
	SLC4F / SLC 4R	TTTGCTCAACTTCTACTGCTT	ACAGTGGGTAGACAGCATGG	chr8:70667569-70668019	451	60
	SLC5F / SLC 5R	GGATGGTTTCTAATCGTCATCA	TCCAGGATTTCTCTCTCTA	chr8:70650123-70650598	476	60
	SLC6aF / SLC 6aR	TCTGGATGTGAGTCCGTTGT	AGATGCACAAAACCCACAGG	chr8:70617196-70617659	464	60
	SLC7F / SLC7R	GGCTTTGATTCATGGGAAG	TTGGAGCCTCTGAAAAGAAA	chr8:70594307-70594699	393	60
	SLC8F / SLC 8R	TTAGCTTTGAGGGGCACA	TTACAACCTTTCTTCCCTTCC	chr8:70591422-70591901	480	60
	SLC9F / SLC9R	CAAAGCCGACTTGATTTGAC	CAAACAACAGAACCTGTCAGC	chr8:70588661-70588986	326	60
SLC10F / SLC10R	CCTCCCCTCAACTGGTTTAA	CTTTTTGCAGGCCAGTCTCT	chr8:70585215-70585672	458	60	
SULF1	SULF1-6F / SULF1-6R	ATTTGTAGCCCTTCTCTGC	TAGGGTGTGGTTAATGTGC	chr8:70476068-70476504	437	58
	SULF1-7F / SULF1-7R	ACAATCGAAATAGGCATTCA	CATGACACTTCTTCTGAGTCT	chr8:70488143-70488513	371	58
	SULF1-8F / SULF1-8R	CCGTCTCTTTTCAAGTCATAA	TAGAAAGCCAATCTTGTGGA	chr8:70498500-70498843	344	58
	SULF1-9F / SULF1-9R	TGAATTGCTTCTGAGGAAAG	TGAAGAAAGCCTTGTGGATA	chr8:70501117-70501494	378	58
	SULF1-10F / SULF1-10R	CACAACCTCGAGATATTTCC	CTGGGCCAAAACACTACT	chr8:70512766-70513030	265	58
	SULF1-11F / SULF1-11R	GTGGCAACACTATTGAGCAT	TCGAAATTAACCAGCCTACA	chr8:70513819-70514199	381	58
	SULF1-12F / SULF1-12R	AGAAAAAGCAGCCCTAAGTG	TTCACAAGCAGCTGAGAAAT	chr8:70515357-70515705	349	58
	SULF1-13F / SULF1-13R	AAATAAGCTGTAGCACAGATCC	AAATACTCAGCAGGAAAGC	chr8:70515761-70515960	200	58
	SULF1-14F / SULF1-14R	GGAGTCATGTGACAGGGTAA	GAGGTGTCTCAGTGAGCTTG	chr8:70516941-70517312	372	58
	SULF1-15F / SULF1-15R	AATAACACCTGGAGCAGAATC	GCAACCCTTTCGTCTCTA	chr8:70533220-70533564	345	58
	SULF1-16F / SULF1-16R	TCACCTTCTCCCTTGTAAGC	CCAAGTGCAAAATGGTGATA	chr8:70536114-70536515	402	58
	SULF1-17F / SULF1-17R	CAATATTTCCATTGATGCTG	GTATGTCCCACCAGACACAG	chr8:70539290-70539624	335	58
	SULF1-18F / SULF1-18R	ACAGAACAGAGAAAACAAGGTG	GCTAATGCCTCTGGCTTGT	chr8:70539918-70540167	250	58
	SULF1-19F / SULF1-19R	CAATGCAAGGCTATGAAGTC	GCACTGCAGGAAACACAGG	chr8:70540361-70540569	209	58
	SULF1-20-2F / SULF1-20-2R	TCACAGCAACAAGCCTATTT	TTATGAGTCGCTGTCTCTGC	chr8:70541666-70541973	321	58
	SULF1-21+22F / SULF1-21+22R	CCTGCCTAAGGTTTTCACT	AGGCAGAAAAGCTCAGTAACA	chr8:70550680-70551162	483	58
	SULF1-23F / SULF1-23R	TGCATTTGAACCTAATAGGAA	CTCTGTGCAACACGGTAAGA	chr8:70552877-70553176	300	58
	SULF1-24_1F / SULF1-124_1R	GAGGGGAAAATGCTTTTGAG	CAACCTTATGCCTTGGGTCT	chr8:70570615-70571080	466	58