SUPPLEMENTARY MATERIAL

Table S1. MLPA probe set P179 (*GLI3*, *HOXD13* and *ROR2* probes)

Length (nt)	*GLI3* exon	Ligation site NM_000168.2		Sequence at Ligation site
391	exon 1	40	41	GAGCTGAAGT-AATGAGAAGA
136	exon 1	135	136	CACTCGAACA-GATGTGAGCG
409	exon 2	201	202	TGGACAGACT-TATCACAGAG
160	exon 2	263	264	GGCTCAGCAA-AGTCAGTGAG
427	exon 3	422	421	TGAGGAGGGT-CTGAAAAGAA
190	exon 3	463	464	CCTGTACCAA-TTGATGCCAG
196	exon 4	575	576	TGCCCTTCAT-TAGGATCTCC
211	exon 5	762	763	CCCAGCAGAA-TACTATCATC
229	exon 6	1018	1019	ACGATTCTCA-ATAATTCCCG
247	exon 7	1213	1214	GCCCCAACTT-TTCCAACACA
265	exon 8	1363	1364	AGGTCCAAGA-TCAAACCCGA
283	exon 9	1438	1439	ACCCTTGTCA-AGGAGGAAGG
301	exon 10	1617	1618	GGACTGCTCA-AGAGAGCAGA
319	exon 11	1818	1819	GGCTTTCTCA-AATGCCTCTG
337	exon 12	2035	2036	AGCCATTCAC-AGTCCAGGTC
355	exon 13	2190	2191	TCAGTCTTCA-TGCAGCAGCC
373	exon 14	3355	3356	CAGTATTTAA-ATTCCCAGAA

Length (nt)	*HOXD13* exon	Ligation site NM_000523.2		Sequence at Ligation site
148	exon 1	664	665	GTATCCTTCT-ACCAGGGCTA
172	exon 2	978	979	GTATGCCATT-AACAAATTCA

Length (nt)	*ROR2* exon	Ligation site NM_004560.2		Sequence at Ligation site
142	exon 1	74	75	GGTCCTCGAA-GTGGACCCGT
436	exon 1	295	296	CCGGACTTCA-GGTAGGATCT
166	exon 2	330	331	ACGACCCTTT-AGGACCCCTT
220	exon 4	684	685	GCCCAAATCA-TAACTTTCAG
256	exon 5	772	773	CCGGACCATT-TATGTGGACT
292	exon 6	872	873	CAGTGCTCAC-AGTTCGCCAT
328	exon 7	1169	1170	GGCATGGATT-ACAGAGGAAC
364	exon 8	1415	1416	GGGATTCTGT-ACATCTTGGT
400	exon 9	1854	1855	TGAGCATGAT-CTTCAGCTAC

Table S2. MLPA probe set P180 (*SALL1*, *SALL4* and *TBX5* probes)

Length (nt)	*SALL1* exon	Ligation site NM_002968		Sequence at Ligation site
228	exon 1	33	34	GCCTCAACAT-TTCCAATCCG
220	exon 2	805	806	ACATCTTCTA-GTCCTTCTCA
290	exon 3	3872	3873	CAGAGCCCAA-TGCTCCCCTG

Length (nt)	*SALL4* exon	Ligation site NM_020436		Sequence at Ligation site
142	exon 1	47	48	CGGCTCTCCC-GGCGCCCCGA
190	exon 2	2129	2130	GATGATGTCA-TCGAAAGCAT
256	exon 3	2806	2807	AGGCAACTTA-AAGGTGGGTT
326	exon 4	2923	2924	CTCAGAAATC-TTTCCCAAGG

Length (nt)	*TBX5* exon	Ligation site NM_000192		Sequence at Ligation site
166	exon 1a	509	510	CGTTGGAAGA-AGACCTGGCC
318	exon 1b	341 in NM_181486	342	ATTCTGGGTA-AGCAGTAAAC
184	exon 2	726	727	CTGACGCAAA-AGACCTGCCC
196	exon 3	837	838	TCAAAGTGTT-TCTCCATGAA
211	exon 4	958	959	TAATCCCAAA-ACGAAGTACA
346	exon 5	1138	1139	CTTCCAGAAA-CTCAAGCTCA
247	exon 6	1213	1214	CCAGCCTAGA-TTACACATCG
148	exon 7	1340	1341	ATCACGCAAT-TAAAGATTGA
265	exon 7	1421	1422	AGAATGCAAA-GGTAGGAAAG
160	exon 8	1465	1466	GAGGCAAAAA-GTGGCCTCCA
283	exon 8	1640	1641	ATTGTACCAA-GAGGAAAGGT
172	exon 9	1714	1715	GAAGATTCCT-TCTACCGCTC
303	exon 9	2869	2870	TGCTTTGGTT-TTGTCCTGCC

Table S3. Non-synonymous variants not considered to be pathogenic

Gene	Number of Patients	Nucleotide Change	Amino Acid Change	dbSNP	SIFT score[1]	Notes
EN1	1	770A>T	N257I	ss120037533	0.18	Patient had symbrachydactyly
GLI3	1	299C>T	P100L	ss120037498	1.00	
	1	314G>A	R114K	ss120037499	-	Patient from family linked to other genomic region
	1	1393G>C	G465R	rs35488756	-	
	1	2119C>T	P707S	ss120037500	-	Patient has *ZRS* triplication. Present in unaffected father
	2	2424A>G	I808M	rs62622373	0.23	One patient has a mutation in *ZRS* of *SHH*
	6	2993C>T	P998L	rs929387	-	
	14	3083G>T and 3084C>T	S1038I	ss120037501	-	Two patients have mutations in *HOXD13*
	5	4007G>A	G1336E	rs35280470	-	
	2	4609C>T	R1537C	rs35364414	-	
HAND2	2	283C>G	P95A	ss120037538	-	Present in unaffected father
	1	594G>C	K198N	ss120037539	-	Present in unaffected mother
SALL1[3]	1	44A>C	D15A	ss120037520	0.05	
	1	90G>C	K30N	ss120037521	0.336	
	1	292A>G	M98V	rs28643388	-	
	1	1322C>A	T441N	ss120037522	-	Present in unaffected mother
	2	1878G>C	E626D	ss120037523	0.08	Patients had contrasting phenotypes
	1	1991C>T	P664L	ss120037524	-	Present in

						unaffected mother
	2	2827A>G	S943G	ss120037525	0.03	Patients had different phenotypes. Murine Sall1 has Gly at equivalent position
	1	3823G>A	V1275I	rs4614723	-	
	1	3872A>G	N1291S	ss120037526		Present in 4/141 normal controls
SALL4	9	2392A>C	I798L	rs6091375	-	
	1	3103G>A	G1035S	ss120037543	0.81	
SPRY4	1	64G>A	A32T	ss120037549	-	Present in unaffected mother
	1	530A>G	K177R	ss120037550	0.72	
TBX5	2	331G>T	D111Y	ss120037552	0.00	Patients had contrasting phenotypes. Not present in affected father
	1	1114T>A	S372T	ss120037553	0.48	

1. SIFT (Sorting Intolerant From Tolerant; http://blocks.fhcrc.org/sift/SIFT.html) attempts to predict whether an amino acid substitution affects protein function. Positions with normalised probabilities <0.05 are predicted to be deleterious, those ≥0.05 are predicted to be tolerated.

2. Previously described as a mutation causing GCPS (Wild A *et al*. Hum Mol Genet 1997; 6:1979-84)

3. In addition, we found all 7 allele lengths between 7 and 13 serines for the polyserine tract in exon 2 of *SALL1* (S150 to S158).

Tables S4-S6 Synonymous and non-coding variants not considered to be pathogenic

The tables note the number of patients carrying the variant, and whether any of these patients have a separate confirmed pathogenic mutation described elsewhere. Also noted is the presence or absence of the variant in dbSNP version 126 (www.ncbi.nih.gov/SNP). In cases where the variant was not present in any patient with a convincing pathogenic mutation, and is not a known SNP, then 60 bp of wild type and variant sequence, as well as the native splice site closest to the variant, were entered into the neural network splice site prediction program (http://www.fruitfly.org/seq_tools/splice) in order to assess whether the variant might cause aberrant splicing and therefore be pathogenic. Scores range from 0 to 1, with a higher score being more likely to produce a splice site.

Table S4. Presumed non-pathogenic variants in *GLI3*

Variant	No of patients affected (number with other identified pathogenic mutation)	dbSNP number	Neural network splice site prediction score		
			Wild type	Variant	Nearest native splice site (change with variant)
1-56G>A	1	ss120037502	<0.05	<0.05	0.96 (0)
124+61C>A	3	rs2286291	-	-	-
124+76G>A	1	ss120037503	<0.05	<0.05	0.99 (0)
231A>G	1 (1)	ss120037504	-	-	-
368-19G>A	3 (1)	ss120037505	<0.05	<0.05	0.96 (+0.01)
368-7T>G	1	ss120037506	<0.05	<0.05	0.96 (-0.14)
473+22A>G	1	ss120037507	<0.05	<0.05	0.97 (0)
473+37C>T	1 (1)	ss120037508	-	-	-
474-4C>T	1	ss120037509	<0.05	<0.05	0.96 (0)
827-75G>A	1	ss120037510	0.94	0.95	0.69 (0)
840C>G	1	ss120037511	0.51	0.23	0.69 (+0.05)
900C>T	6	rs35961850	-	-	-
1029-7C>T	1	ss120037512	<0.05	<0.05	0.74 (+0.10)
1242+8G>A	1	ss120037513	<0.05	<0.05	0.91 (+0.02)
1242+113C>G	1	ss120037514	<0.05	<0.05	0.91 (0)
1243-40A>C	1	ss120037515	<0.05	<0.05	<0.05
1356+11G>C	8 (1)	rs846273	-	-	-
1356+64C>A	5 (1)	ss120037516	-	-	-
1498-44G>A	8 (1)	rs17707162	-	-	-
1509C>T	8 (1)	rs34020684	-	-	-
2373G>A	1	rs61754622	<0.05	<0.05	0.99 (0)
2826G>C	7	rs34245321	-	-	-
2835G>C	4 (1)	rs61758978	-	-	-
3774C>G	3	rs35448119	-	-	-
4020C>T	7 (1)	rs35139358	-	-	-
4071C>T	9 (1)	rs34089404	-	-	-
4560G>T	1	ss120037517	<0.05	<0.05	0.98 (0)
4740+33G>T	9 (1)	ss120037518	-	-	-
4740+139T>A	9 (1)	ss120037519	-	-	-

Table S5. Presumed non-pathogenic variants in *SALL1* and *SALL4*

Gene	Variant	No of patients affected (number with other identified pathogenic mutation)	dbSNP number	Neural network splice site prediction score		
				Wild type	Mutant	Nearest native splice site (change with variant)
SALL1	390G>A	3	ss120037527	<0.05	<0.05	0.26 (0)
	567C>G	1	ss120037528	<0.05	<0.05	0.26 (0)
	609C>G	1	ss120037529	<0.05	<0.05	0.26 (0)
	1200G>A	1	ss120037530	<0.05	0.14	0.26 (0)
	2310C>T	2	ss120037531	0.30	0.44	0.26 (0)
	2343G>C	2	rs60270998	<0.05	<0.05	0.71 (0)
	2544A>G	5 (2)	rs45459896	-	-	-
	2574C>T	7 (1)	rs56627294	-	-	-
	3456C>T	4 heterozygous, 1 homozygous	rs11645288	-	-	-
	3534+40C>G	1	rs7184489	-	-	-
	3534+76C>T	2	ss120037532	<0.05	<0.05	0.71 (0)
SALL4	1-41delG	1	ss120037543	<0.05	<0.05	0.60(0)
	408T>C	1	ss120037545	<0.05	<0.05	0.98(0)
	540T>C	1	rs6013281	-	-	-
	645C>G	13 (1)	rs61737139	-	-	-
	1056G>A	69 (2)	rs13038893	-	-	-
	1113C>G	1	ss120037546	<0.05	<0.05	0.98(0)
	1557C>T	1	ss120037547	0.72	0.57	0.98 (0)
	1860A>G	69 (3)	rs6021437	-	-	-
	2037C>T	10 (1)	rs13043248	-	-	-
	2493A>G	1	ss120037548	<0.05	<0.05	0.96(0)
	2640G>C	36	rs17802735	-	-	-

Table S6. Presumed non-pathogenic variants in *EN1*, *HAND2*, *ZRS* of *SHH*, *SPRY4*, *TBX5* and *WNT7A*

Gene	Variant	No of patients affected (number with other identified pathogenic mutation)	dbSNP number	Neural network splice site prediction score		
				Wild type	Mutant	Nearest native splice site (change with variant)
EN1	489G>A	16 (2)	ss120037534	-	-	-
	621G>C	2	ss120037535	<0.05	<0.05	0.99(0)
	863-28delC	3	ss120037537	<0.05	<0.05	0.94(0)
	1029C>T	2	ss120037536	<0.05	0.06	0.94(0)
HAND2	565T>C	8	ss120037540	<0.05	<0.05	0.98(0)
	621G>A	1	ss120037541	<0.05	<0.05	0.98(0)
	654+51G>T	5	ss120037542	<0.05	<0.05	0.98(0)
ZRS of *SHH*	-56T>C	2	ss120037563	-		
	-46A>C	2	ss120037564	-		
	3C>G[1]	84 (2)	ss120037565	-		
	507C>G[1]	2	ss120037566	-		
	+68A>G	1	ss120037567	-		
SPRY4	537C>T	1	ss120037551	<0.05	0.06	0.75(0)
TBX5	309C>A	4	rs28730763	-	-	-
	364-82G>A	1	ss120037554	<0.05	<0.05	0.98(0)
	664+36G>T	66	rs2236017	-	-	-
	757-26G>T	1	ss120037555	<0.05	<0.05	0.99(0)
WNT7A	60C>T	3	ss120037556	<0.05	<0.05	0.99(0)
	72-10C>T	1	ss120037557	<0.05	<0.05	0.82 (+0.04)
	75C>T	1	rs35103037	-	-	-
	81C>T	1	ss120037558	0.73	0.87	0.82
	213C>T	2	ss120037559	0.14	0.17	0.82
	298+37C>A	95 (3)	rs3749319	-	-	-
	298+40C>T	1	ss120037560	<0.05	<0.05	0.97(0)
	315G>A	48	rs12639607	-	-	-
	458T>C	50 (1)	rs3762719	-	-	-
	571-15C>T	1	ss120037561	<0.05	<0.05	0.97(0)
	681G>A	1	ss120037562	<0.05	<0.05	0.97(0)

1. Previously reported as a SNP (Lettice, LA *et al*. Hum Mol Genet 2003;12:1725-35)