The American Journal of Human Genetics, Volume *104*

Supplemental Data

Bi-allelic Variants in *TONSL* Cause SPONASTRIME Dysplasia and a Spectrum of Skeletal Dysplasia Phenotypes

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Figure S1. Additional photographs. (A) Subject 2 at 6 years of age showing rhizomelia, mesomelia and genu valgum. (B) Subject 4 at 22 years of age. Additional radiographs and photographs have been published previously[8]. (C) Subject 6 at 12 years of age. (D) Twisted lateral incisors observed in subject 4 (bilateral). (E) Twisted lateral incisor observed in subject 7-2 (unilateral).

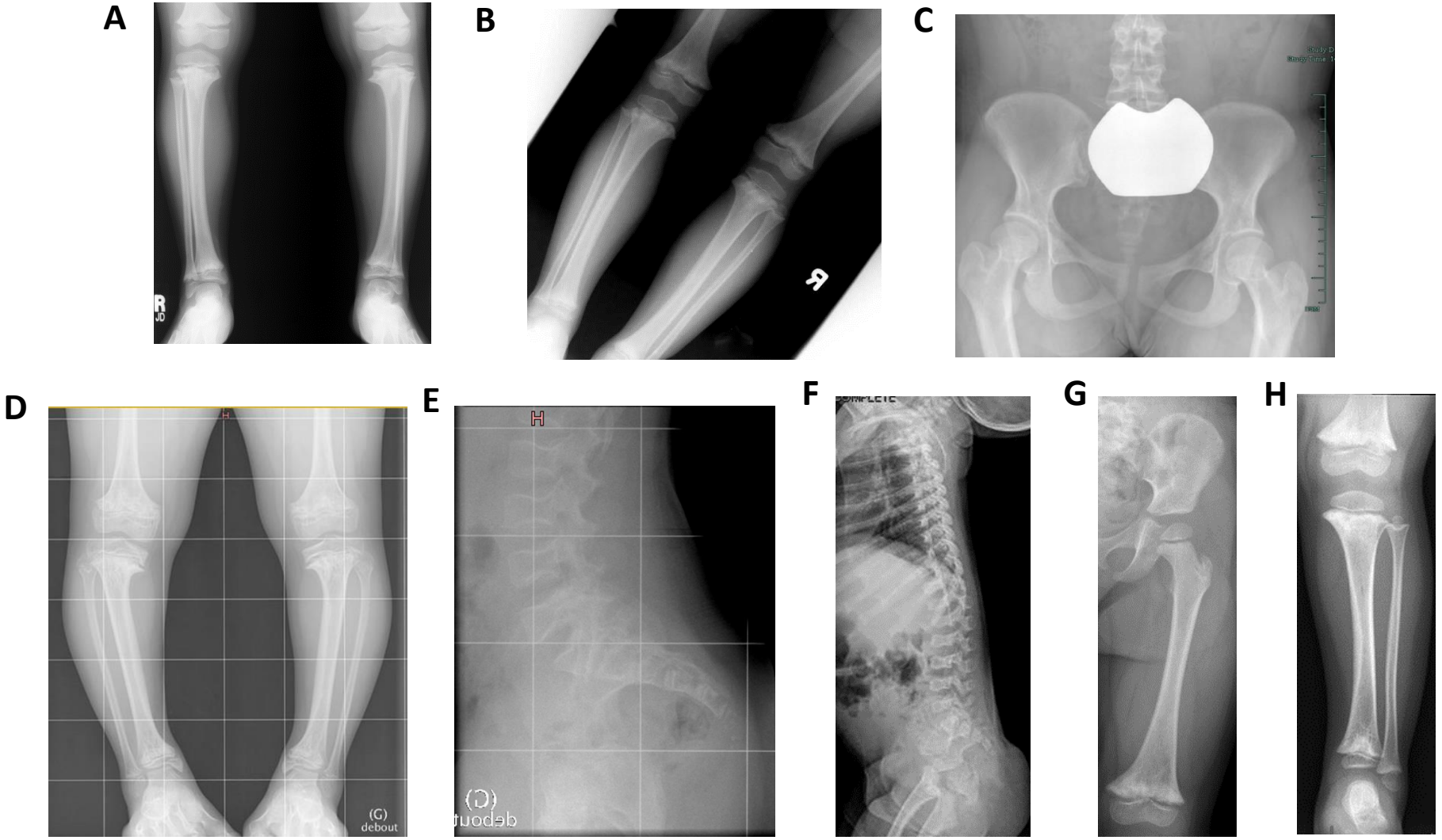


Figure S2. Additional radiographs. (A) Subject 1, 6 years 8 months. Metaphyseal widening and irregularities are apparent in tibia. (B) Subject 1, childhood, age unknown. Metaphyseal striations and irregularities are apparent in tibia and femur. (C) Subject 1, 13 years, 3 month. Femoral necks appear short and wide. (D) Subject 5, 11 years. Metaphyseal striations and irregularities are noted in tibia and femur with varus bowing. (E) Subject 5, 11 years. Platyspondyly and biconcave vertebrae are noted. (F) Subject 8, 1 year 5 months. Platyspondyly noted. Several of the vertebral bodies also have a biconcave shape. Note the distinct junction between anterior and posterior portions of the lumbar vertebral bodies. (G) Subject 8, 4 years 5 months. Widening, sclerosis, and irregularity and mild striations of the distal femoral metaphysis are noted. (H) Subject 8, 4 years 5 months. Widening, sclerosis, and irregularity and mild striations of the proximal tibial metaphysis are noted.

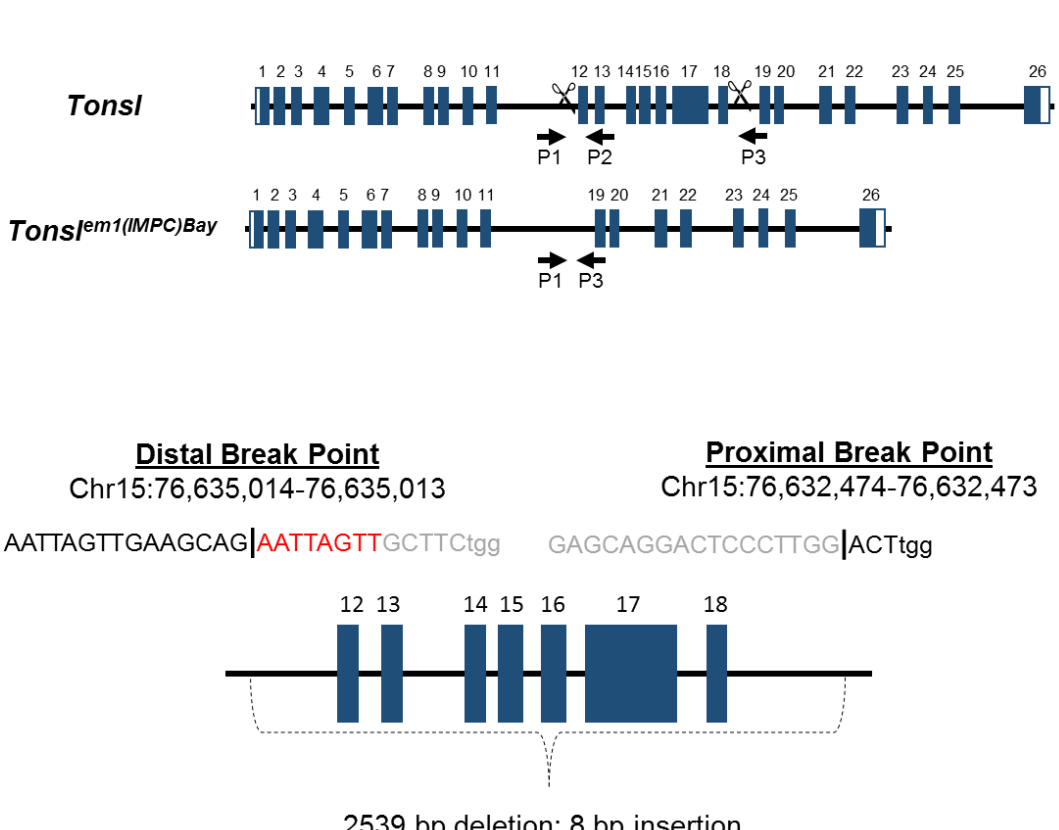
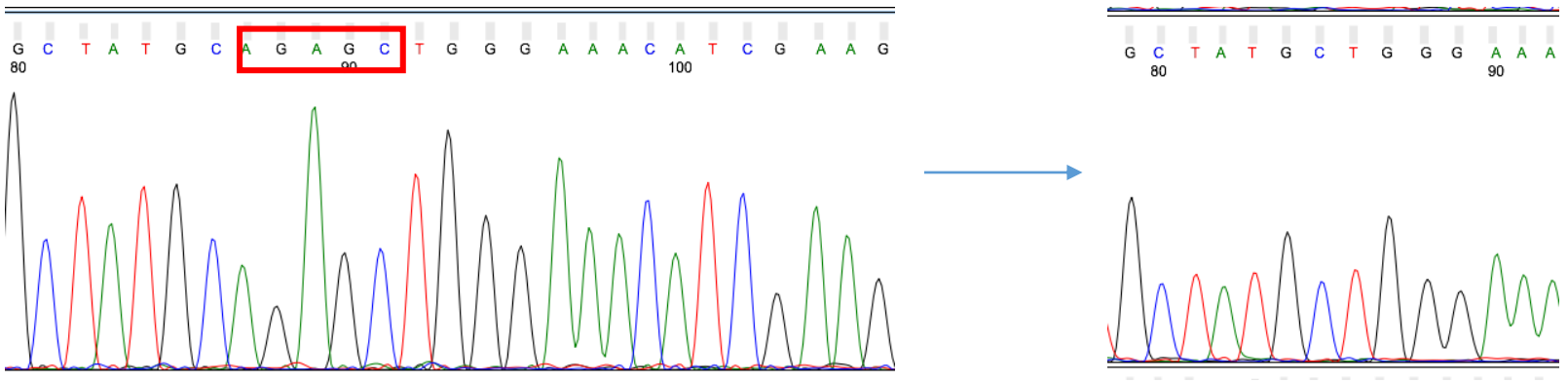


Figure S3. *Tonsl deletion analysis.* (A) Schematic representation of the *Tonsl* locus. The exon/intron structure of the wild-type and null (*Tonsl*$^{em1(IMPC)Bay}$) allele are shown. Exons 12-18 are deleted in the null allele. Blue boxes = coding sequence; White boxes = UTRs. Scissors represent sgRNA target sites. Arrows indicate primers P1, P2, and P3 used for genotyping and sequencing (P1 and P2 amplify the wild-type allele; P1 and P3 amplify the null allele). (B) Distal (Chr15:76,635,014-76,635,013; GRCm38/mm10) and proximal (Chr15:76,632,474-76,632,473; GRCm38/mm10) breakpoints of the deletion in *Tonsl*$^{em1(IMPC)Bay}$. *Tonsl* is oriented in the antisense direction in the mouse genome. Sequence text in black and gray are the sgRNA target and protospacer adjacent motif (PAM) sequences. Red sequence text is an 8 bp insertion that occurred during nonhomologous end joining (NHEJ). (C) Chromatogram showing DNA sequence of the nonhomologous end joining (NHEJ) repair junction.

tonsl^{b1347} c.234-8; p.E80G21Ter



tonsl^{b1348} c.235-47; p. E80S4Ter

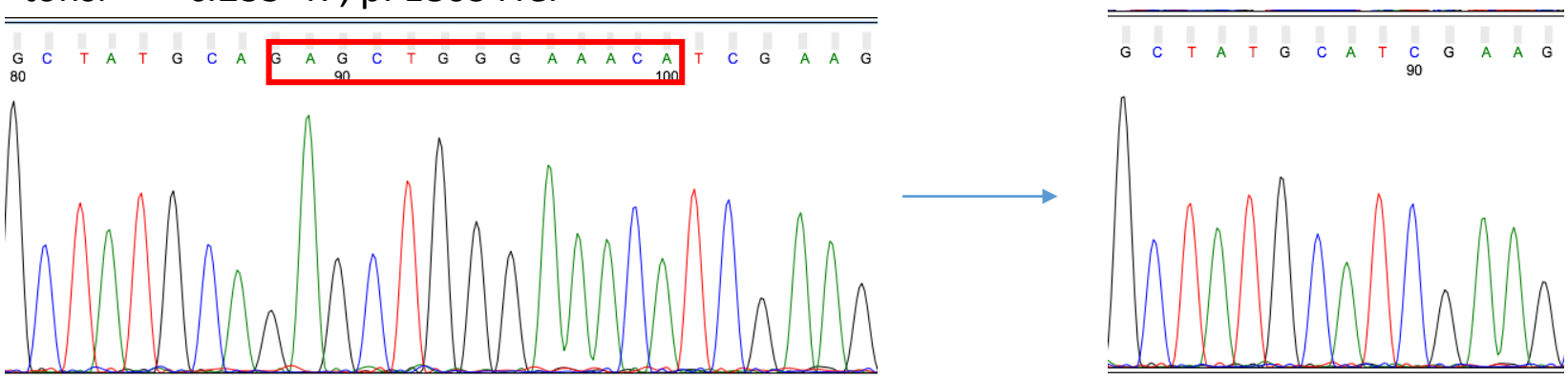


Figure S4. Zebrafish CRISPR mutants. Frameshift alleles of *tonsl* affect the two alternate reading frames of exon 3. Bases deleted in each allele are boxed in red; homozygous mutant sequence is presented on the left for each allele.

Table S1. Primers used for Sanger sequencing of human *TONSL*

Exons	5' primer	3' primer	Amplicon size (nt)
hTONSLex1-2	GGCCGACCGTACTTCCC	CTCCTGCCAGTGCTGCTC	636
hTONSLex3	CAAGGCGAAAGCCAAGG	AACCTACTCCTGCCCCAGTC	586
hTONSLex4	AGCAAGAACAGGGTCTCTGG	GCTCCAGAAGACGGGATTG	373
hTONSLex5	GGCCCAAAGCTGGAAAC	ACTTCCTCCAGGAACAAGGG	249
hTONSLex6-7	CCGTGTGGCATCAGCAG	GGCTCACCCCTGCACAC	492
hTONSLex8-9	TCACAGCTTGCAGGTGGTAG	GTCCTGAGGCAGAGACATGG	524
hTONSLex10-11	CCGTTGGACGCAGACAG	CACAGCACACCCCTCTCC	578
hTONSLex12-13	CTGCTAACCTTCACCTCCC	CACAAACGCACAGCTCCTC	380
hTONSLex14-15	TAGGCTGCAGAGCTCACG	AGTTGAGCAGGGGCACAG	483
hTONSLex16	ACTCGAAAGGTGAGCCTGG	CGGGGACTCTCAGCGTAG	263
hTONSLex17_1	GATGCTCCATCACAGGTGG	AAGGGCTTTGCTGTGGC	508
hTONSLex17_2	ACAGGGAAGCAGCCACAG	AGTGGGCTCCACCCTACAC	509
hTONSLex18	AGGCAGGTGTAGGGTGGAG	ACCCTGACATGCAAACACG	217
hTONSLex19-20	GCATTACCCCGGCTGTG	TGGTGGAGCCTGTGTGC	481
hTONSLex21	GATTCAGAGGGCAGAAAGGG	GGACCTGCAGAATGGGAAC	423
hTONSLex22	GACTGCCAAGCCAAGCC	GTCCTGGAAACCCTCAATGC	312
hTONSLex23	AGAACTTGGGGTGGGTACAG	GAGCTCCTCCCAGCAACC	313
hTONSLex24	TGCTGGGAAGCAGGCAG	CCTTCTCCCATAGGGTCCAG	212
hTONSLex25	GCAGCTTTCCTAGTGTTGGG	CACCTGGGTCTCAGGCAG	274
hTONSLex26	TCCTGGCATCTGTACCTTCC	AAGCCCGGTCTTACCCC	1008

Table S2. Additional Subject Characteristics for Subjects with Clinical Diagnosis of SPONASTRIME Dysplasia

Subject ID	1	2	3-1	3-2	4	5	13	14	15
Sex	F	F	M	M	F	F	M	F	M
Age at last follow-up	7 y 9 m	7 y 11 m	4 y 9 m	9 m	22 y	23 y	17 y 10 m	4 y	11 y
Birth Parameters									
Gestational Age	Not available	Full term	40 weeks	40 weeks	40 weeks	38 weeks	Full term	39 weeks	39 weeks
Length	Not available	-3.71	-1.16	-3.41	-2.83	-1.89	Not available	-0.23	-2.94
Weight	Not available	-2.70	-4.71	-1.78	-2.18	-1.01	Not available	-2.03	-2.11
FOC	Not available	Not available	Not available	-0.38	-2.32	-0.41	Not available	-2.26	Not available
Extra-Skeletal Features									
Dental	Unknown	Short dental roots; Malocclusion with early loss of teeth[a]	Normal	None	Shallow dental roots	Malocclusion ; Early loss of teeth; Gingival recession	Normal examination	Short dental roots	Short dental roots; Dental crowding
Facial Features	Midface hypoplasia	Midface hypoplasia; Prominent forehead	Midface hypoplasia; Frontal bossing	Midface hypoplasia; Prominent forehead	Midface hypoplasia; Frontal bossing; Short philtrum	Midface hypoplasia; Prominent forehead	Midface hypoplasia	Frontal bossing; Midface hypoplasia	Coarse, mid face hypoplasia; Flat, depressed nasal bridge; Short upturned nose and thick full lips; Frontal bossing; Mild dolichocephaly with maxillary hypoplasia
Eye	Unknown	Bilateral cataracts (age ~10 years)[a]	None	None	Bilateral cataracts (extracted at age 8 years, 15 years)	Bilateral cataracts (age ~12 years)	Normal	None	Normal
Nose	Unknown	Depressed nasal bridge; Anteverted nares	Depressed nasal bridge	Depressed nasal bridge	Depressed nasal bridge; Anteverted nares	Depressed nasal bridge; Short nose; Thick alae nasi	Short, depressed nasal bridge; Anteverted nares	Concave nasal ridge; Depressed nasal bridge	Short, upturned
Immunologic Abnormalities	None	None	Not assessed; Parental report of recurrent infections	Low neutrophil count (2 months of age)	Transient hypogamma globulinemia, Recurrent infections; Poor pneumococcal antibody response	Hypogamma globulinemi; Recurrent infections; Poor pneumococc al antibody response	Normal	None	Normal

Development	Normal	Normal	Expressive language delay	Normal	Normal	Normal	Normal	Normal	Abnormal findings
Other	Similarly affected sibling with same variants	Decreased carrying angle of elbows	Glottic and subglottic stenosis requiring tracheostomy (eventually removed)	Left cryptorchidism; subglottic stenosis requiring tracheostomy at 2 months	Possible subglottic stenosis (asymptomatic); Mild osteopenia	None	Mild joint laxity in fingers; Mild limitation of full extension at elbows.	None	At age 30 y, weight 40.8 kg (-4.3SD) and height 130.8 cm (-6.2SD). Family history includes two maternal third cousins (12 y/o female, 10 y/o male) with clinical diagnosis of SPONASTRIME dysplasia. Consanguinity was denied.

Table S3. Subjects with clinical diagnosis of SPONASTRIME dysplasia but without Biallelic Variants in *TONSL*

Subject ID	9[1]	10[2]	11[3]	12
Sex	M	M	F	M
Age at last follow-up	5 y 9 m	15 y 7 m	14 y	15 y 1 m
Height (Z-score)	-5.6	-5.36	-6.4	-1.61
Weight (Z-score)	-0.19	-4.09	-3.8	-0.61
FOC (Z-score)	Not available	0.10	-2.6	0.42
Disproportionate Short Stature	Unknown	Yes	Yes	No
Birth Parameters				
Gestational Age	Unavailable	Full Term	39 weeks	Not Available
Length	Unavailable	48.26 cm	NA	Not Available
Weight	Unavailable	2807 g	-1.3	Not Available
FOC	Unavailable	Not Available	Not Available	Not Available
Clinical Features				
Dental	Short dental roots	Mandibular overbite and wide spaced teeth	Extensive dental caries	Misaligned
Facial Features	Midface hypoplasia; Bifrontal bossing, Prominent mandible	Prominent forehead; Midface hypoplasia; Mild prognanthism; Mildly coarsened facial appearance	Frontal bossing; Midface hypoplasia	Mild malar hypoplasia; Low-set ears
Eye	Bilateral subcapsular cataracts at age 11 years	Normal dilated examination; Bilateral epicanthal folds	Strabismus	Epicanthi
Nose	Depressed nasal bridge	Depressed nasal bridge; Anteverted nares	Concave nasal ridge	Depressed and large nasal bridge
Immunologic	Hypogammaglobulinemia; Poor antibody response to tetanus and Haemophilus vaccinations	None known	Clinically normal	Clinically normal
Orthopedic	Mild genu valgum; Limitation in extension of elbows; Mild joint laxity	Bilateral genu valgum; Brachydactyly; Progressive kyphoscoliosis requiring T2-L4 posterior spinal fusion at age 11 years	No other issues	Scoliosis surgery
Development	Normal	Normal	Severe intellectual disability	Normal
Radiographic Features				
Metaphyses	Irregularities with striations	Mild metaphyseal irregularities; Metaphyseal striations evident at age 10 years.	Striations	Striations with sclerosis
Epiphyses	Epiphyseal abnormalities	Normal	Normal	Normal
Spine	Platyspondyly	Platyspondyly; Ovoid and biconcave vertebral shape,	Thoracic kyphosis; Increased lumbar lordosis indentation of	Biconvex vertebrae with reduced posterior vertical height, left

	Reduced height of vertebral bodies; Anterior portion of the vertebral body is taller than posterior portion with central anterior protuberance	hypoplastic dens without instability	vertebral endplates, thickened endplates, reduced height	thoracic scoliosis, surgically fused T4-L1 at age 12.
Other Skeletal Findings	Coxa vara	Short and wide femoral necks; J-shaped sella; Proximal pointing of the 3rd, 4th and 5th metacarpals; Mild osteopenia	Carpal ossification delay; Brachymetacarpia	Sclerotic zones in phalanges, metacarpal and metatarsal bone
Other		Mildly limited bilateral elbow extension; Hypernasal voice; Multiple otitis media requiring PE tubes; Juvenile polyp		

[1] Heterozygous variant in *TONSL* noted (NM_013432.4:c.2800C>T, p.(Arg934Trp), rs75557541) but no second variant detected. Patient was previously published[1,2].

[2] Heterozygous variant in *TONSL* noted (NM_013432.4:c.25+2dupT) but no second variant detected.

[3] Patient was previously published[3].

Table S4. Additional Subject Characteristics for Subjects without Clinical Diagnosis of SPONASTRIME Dysplasia

Subject ID	6	7-1	7-2	8
Diagnosis	Spondylometaphyseal Dysplasia	Spondylometaphyseal Dysplasia	Spondylometaphyseal Dysplasia	Spondylometaphyseal Dysplasia
Sex	F	F	M	F
Age at last follow-up	12 y	10 y 9 m	9 y 9 m	5 y 11 m
Birth Parameters				
Gestational Age	34 weeks	39 5/7 weeks	37 4/7 weeks	35 2/7 weeks
Length	-2.64	0.36	1.33	-2.54
Weight	-1.54	-0.34	-0.49	-1.91
FOC	-1.10	0.33	-0.47	-1.49
Extra-Skeletal Features				
Dental	Delayed tooth eruption, small teeth	Delayed tooth eruption; Tooth discoloration; High palate	Delayed tooth eruption; Tooth discoloration; Upper lateral incisor is malpositioned; High palate	Normal
Facial Features	High forehead, frontal bossing; Hypoplasia of supra-orbital ridges	Short philtrum	Poorly defined philtrum	Short philtrum
Eye	Acoria; Deep set eyes; Primary aphakia; Microphthalmia; Short palpebral fissures; Bilateral epicanthal folds; Glaucoma (bilateral) likely secondary to aphakia	Wears glasses	No known abnormalities	Epicanthal folds with mild synophrys
Nose	Broad and depressed nasal bridge with crease over the bridge; Anteverted nares	Bulbous tip	Broad and depressed and nasal bridge	Depressed nasal bridge
Immunologic Abnormalities	None	Hypogammaglobulinemia; Vaccine responses untested due to immunoglobulin replacement; Congenital neutropenia	Hypogammaglobulinemia; Vaccine responses untested due to immunogloblulin replacement;Congenital neutropenia; Frequent infections	Transient hypogammaglobulinemia of infancy; Low memory B cell percentages Congenital neutropenia with normocellular bone marrow with myeloid hypoplasia and left shift; Failure to respond to the polysaccharide-restricted serotypes in the 23-valent pneumococcal vaccine; Recurrent infections
Development	Normal; Vision impairment impacts her motor performance	Normal	Normal	Mild global delay, now resolved

Other	Osteopenia; Low set ears; High-arched palate	Brittle toe nails	Growth hormone deficiency via glucagon stimulation test; Absence seizures; Dystrophic toenails	Asymmetric renal length; Multiple otitis media requiring PE tubes

Supplemental References:

1. Gripp, K.W. *et al.* Expanding the phenotype of SPONASTRIME dysplasia to include short dental roots, hypogammaglobulinemia, and cataracts. *Am J Med Genet A* **146A**, 468-73 (2008).
2. Langer, L.O., Jr., Beals, R.K., LaFranchi, S., Scott, C.I., Jr. & Sockalosky, J.J. Sponastrime dysplasia: five new cases and review of nine previously published cases. *Am J Med Genet* **63**, 20-7 (1996).
3. Camera, G., Camera, A., Di Rocco, M. & Gatti, R. Sponastrime dysplasia: report on two siblings with metal retardation. *Pediatr Radiol* **23**, 611-4 (1993).

Table S5. Exome Coverage Statistics and Number of Rare or Novel Variants Identified and Analyzed in the Exomes

Individual ID	1	2	3	4	5	6	7-1	7-2	8	9	10	11	12	13	14	15
Exome coverage	143 X	117 X	75X	Sanger only	99X	NA	159 x	137 x	118 x	97X	45X	83X	114 X	141 X	44X	Sanger only
% coding regions covered at 10x	97 %	95 %	95 %		88 % at 15X	NA	98 %	98 %	96 %	91 %	98 % at 8X	94 %	98 %	97 %	89 %	
% coding regions covered at 20x	96 %	92 %	90 %		82 % at 30X	NA	97 %	97 %	95 %	87 %	89 %	90 %	97 %	97 %	73 %	
Rare or novel variants (SNVs and indels)	782	997	433		342	641	697	677	438	716	271	861	525	484	274	
Rare or novel variants in known skeletal dysplasia genes	9	14	3		NA	NA	4	4	9	11	NA	8	9	8	NA	
Rare or novel variants in genes explaining the phenotype	0	0	0		0	0	0	0	0	0	0	0	0	0	0	