ORIGINAL REPORT

Dental Abnormalities in Schimke Immuno-osseous Dysplasia

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APPENDIX

Cell Culture

Dermal fibroblasts from SIOD patients were isolated and cultured from skin biopsies of the forearm. The fibroblasts were grown in high glucose Dulbecco's Modified Eagles Medium (Invitrogen, Burlington, ON) supplemented with 10% fetal bovine serum (Invitrogen, Burlington, ON), and 1% antibiotic-antimycotic (Invitrogen, Burlington, ON).

Immunofluorescence

5×10^5 cells were grown overnight on a coverslip in a 6-well plate. Cells were fixed with 4% paraformaldehyde and then permeabilized with 0.5% Triton X-100 for 15 minutes each at room temperature. Non-specific binding sites were blocked overnight with Blocker Casein in PBS (Pierce, Rockford, IL, USA) containing 10% normal horse serum at 4°C. The cells were then incubated with rabbit anti-SMARCAL1 serum (1:200) (Kilic *et al*., 2005) and anti-prolyl 4-hydroxylase (1:50, 5B5, Abcam, Cambridge, MA, USA) diluted in blocking buffer overnight at 4°C. Alexa Fluor-conjugated secondary antibodies Alexa 488 and Alexa 555 (1:1000, Molecular Probes, Burlington, ON, Canada) were used to detect the primary antibodies. Cells were mounted in Vectashield containing 4′,6-diamidino-2-phenylindole (DAPI, Vector Laboratories, Burlington, ON, Canada). Images were acquired using a 100×/1.30 oil Plan-NEOFLUAR objective lens, a Zeiss Axiovert 200 inverted microscope, a Zeiss AxiocamMR camera, and the Zeiss Axiovision imaging system.

Immunoblot

Cell lysates were fractionated by 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene fluoride membrane. The membrane was blocked in PBS containing 0.2% I-Block (Applied Biosystems, Foster City, CA, USA) and

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0.1% Tween 20 overnight at 4°C. Anti-SMARCAL1 (1:2000) (Kilic *et al.*, 2005) and anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH, 1:2000, 6C5, Advanced ImmunoChemical Inc., Long Beach, CA, USA) were used as primary antibodies. Alkaline phosphatase-conjugated secondary antibodies (1:10,000, Bio-rad Laboratories, Mississauga, ON, Canada) were used to detect the primary antibodies. The bound antibody was detected by chemiluminescence using CDP-Star (Applied Biosystems, Streetsville, ON, Canada) according to the manufacturer's specifications.

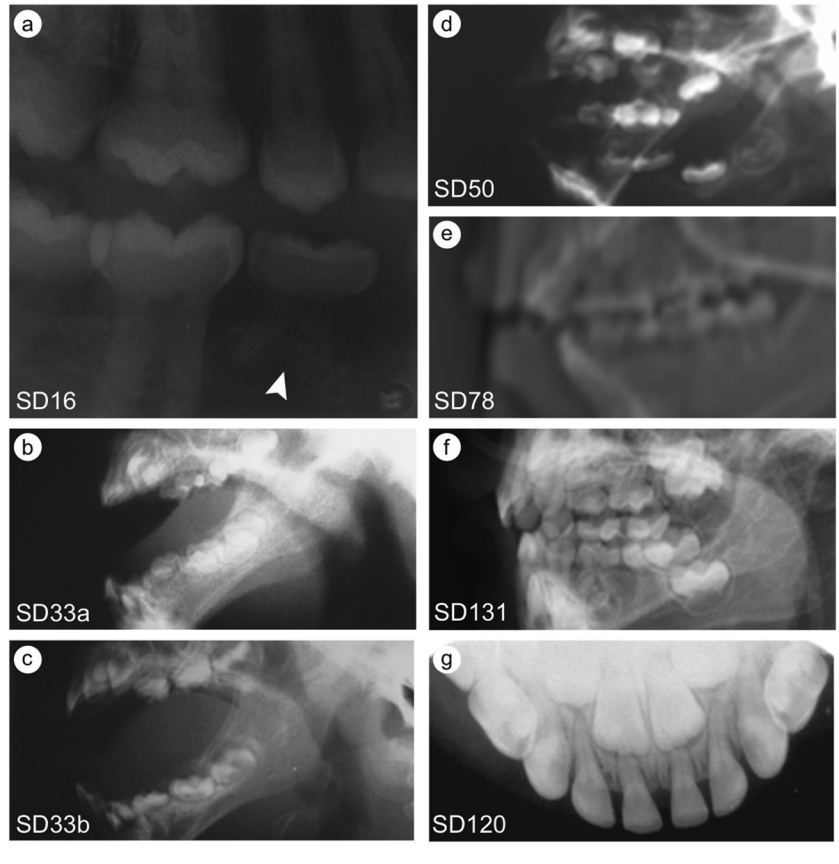
MTT Assay

3×10^3 cells were cultured in triplicate for each sample in a 96-well plate and cell viability and proliferation were assessed after 24 and 48 hours using the MTT assay (M5655, Sigma-Aldrich, Oakville, ON) as previously described (Mosmann, 1983). The relative viability and proliferation rates were calculated for the 24-hour interval, and each SIOD cell line was compared to control fibroblasts.

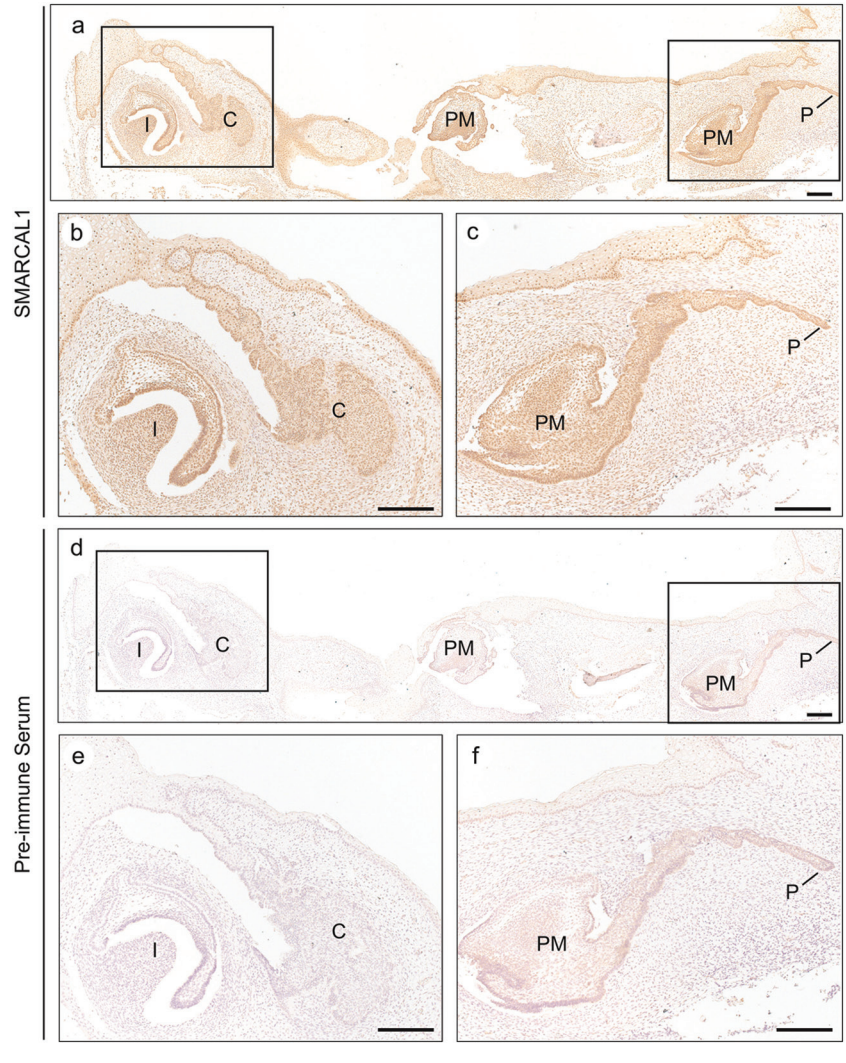
Polymerase Chain Reaction (PCR)

Following reverse transcription, 1.25 µl of cDNA (equivalent to 50 ng RNA) served as template for each reaction and was amplified with the HotStarTaq Plus Master Mix Kit (Qiagen, Toronto, ON, Canada). The following conditions used for amplification: 1 cycle of 95°C for 5 minutes, followed by 30 cycles of 94°C for 30 seconds, 55°C for 30 seconds, 72°C for 1 minute, and a final extension at 72°C for 10 minutes. PCR was performed using the primers listed in Appendix Table 1.

Appendix Figure 1.
Additional dental radiographs showing the tooth pathology of patients with identified bi-allelic *SMARCAL1* mutations. (**a**) Bitewing radiograph of SD16 showing a mild dental phenotype with normal molar roots and crowns. The white arrow indicates a retained deciduous molar. (**b-f**) Lateral skull radiographs of SD33a, SD33b, SD50, SD78, and SD131 illustrating the distinctive bulbous crowns and thin molar roots. (**g**) Occlusion radiograph of SD120.

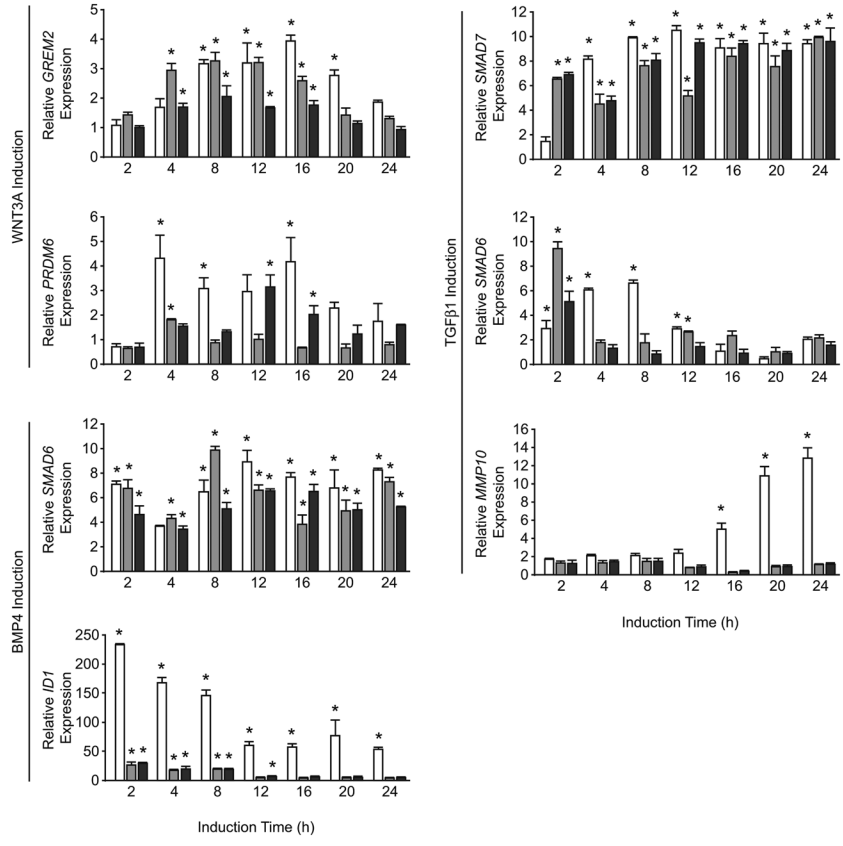


Appendix Figure 2.
Analysis of SMARCAL1 protein expression in the developing incisor, canine, and premolar. (**a-c**) Photomicrographs of SMARCAL1 immunohistochemical staining of the incisor, canine, and premolar. (**a**) Overview of the cross-section of the jawbone of a 98-day-gestation fetus. Twenty tooth buds give rise to the deciduous teeth, and each half jaw consists of 2 incisors, 1 canine, and 2 premolars at this stage of development. Four of the 5 tooth buds present in a developing half jaw can be observed in this section. (**b**) SMARCAL1 is expressed in the incisor and the canine. (**c**) SMARCAL1 is expressed in the premolar. Note that the bud of the permanent premolar also showed expression of SMARCAL1. (**d-f**) Photomicrographs of pre-immune staining of an adjacent section showed minimal non-specific staining. The boxed regions correspond to the higher-magnification images. Abbreviations: C, canine; I, incisor; P, permanent premolar; PM, premolar. Scale bars: 200 μm.



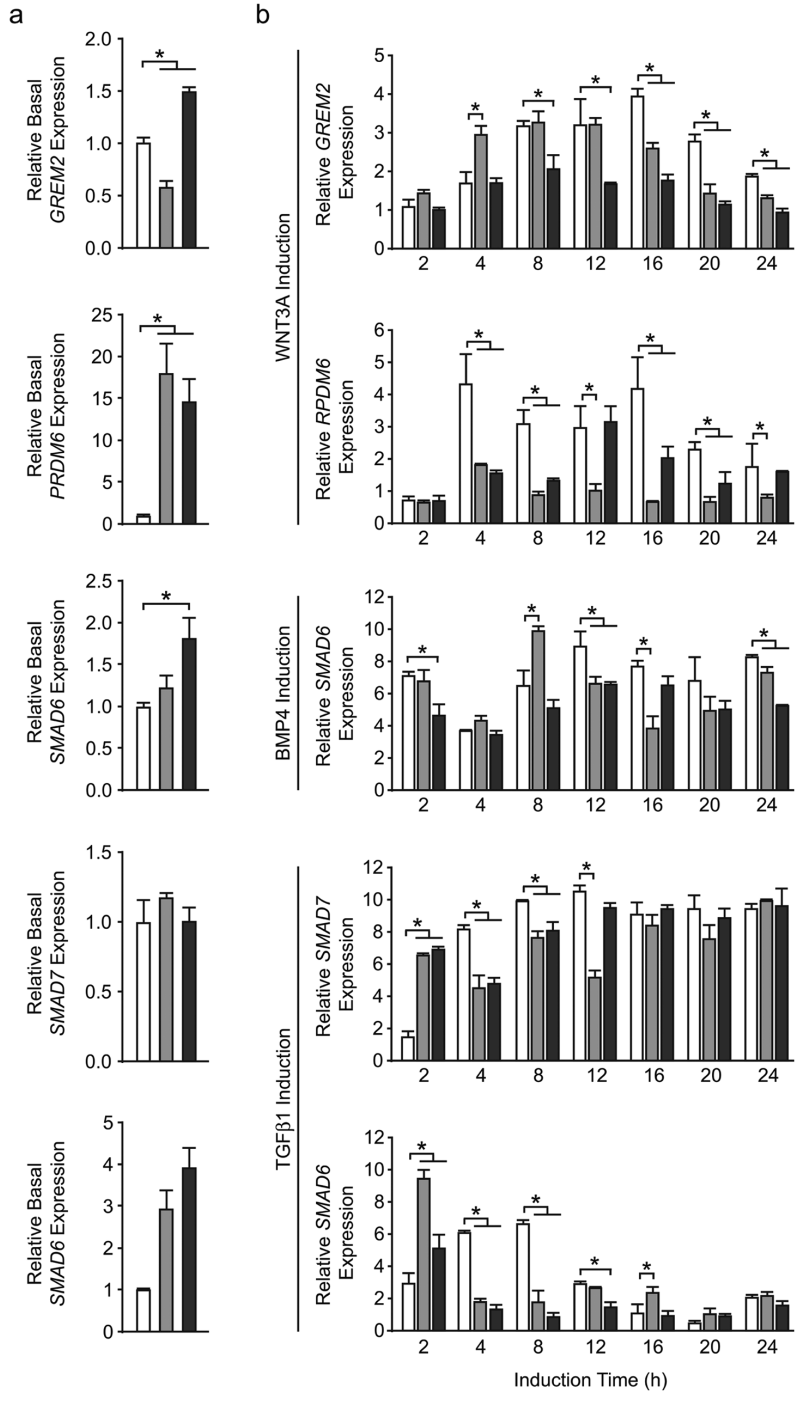
Appendix Figure 3.

Transcriptional responses of SIOD patient dermal fibroblasts stimulated with WNT3A, BMP4, or TGFβ1, comparing relative gene expression between time-points. The transcriptional responses of fibroblasts from an unaffected control (white bars) and patients SD120 (light grey bars) and SD123 (dark grey bars) were measured by qRT-PCR following induction with WNT3A, BMP4, or TGFβ1 for 0, 2, 4, 8, 12, 16, 20, or 24 hrs. Expression of housekeeping gene *GAPDH* was used as the internal control; expression of each gene was first normalized to *GAPDH* expression and then graphed relative to its expression in the relevant cell line at time = 0 hrs. Asterisks denote significant gene expression changes within a cell line between the time-point of interest and time = 0 hrs. * = $p < 0.05$.



Appendix Figure 4.

Unaltered or minimally altered transcriptional responses of SIOD patient dermal fibroblasts stimulated with WNT3A, BMP4, or TGFβ1, comparing relative gene expression between patient and unaffected control fibroblasts at each time-point. **(a)** The relative basal gene expression levels of fibroblasts from an unaffected control (white bars) and patients SD120 (light grey bars) and SD123 (dark grey bars) were measured by qRT-PCR. Expression of housekeeping gene *GAPDH* was used as the internal control; expression of each gene was first normalized to *GAPDH* expression and then graphed relative to the expression of the unaffected control. Error bars represent one standard deviation. * = $p < 0.05$. **(b)** The transcriptional responses of fibroblasts from an unaffected control (white bars) and patients SD120 (light grey bars) and SD123 (dark grey bars) were measured by qRT-PCR following induction with WNT3A, BMP4, or TGFβ1 for 0, 2, 4, 8, 12, 16, 20, or 24 hrs. Expression of housekeeping gene *GAPDH* was used as the internal control; expression of each gene was first normalized to *GAPDH* expression and then graphed relative to its expression in the relevant cell line at time = 0 hrs. * = $p < 0.05$.



Appendix Table 1.
Primer Sequences Used in This Study

Primer	Sequence
GAPDH-cDNA-F	CTTTTGCGTCGCCAGCCGAG
GAPDH-cDNA-R	GGTGACCAGGCGCCCAATACG
SMARCAL1-cDNA1-F	CCTCTACAAGGACCCAAAGCAGCAG
SMARCAL1-cDNA1-R	TCCAGGGTGTCTCCCATGTTCTGG
GREM2-F	GGCGGCGGGAGACCAAACTTA
GREM2-R	CTTCCAGAACATCCTGCAATGACGT
ID1-F	GCTATGCGGGGGTGCCTAAGG
ID1-R	GGAGGCGCTTCAGCGACACAA
MMP10-F	TCGCCCAGTTCCGCCTTTCG
MMP10-R	AGAGGCAGGGGGAGGTCCGTA
PRDM6-F	AGGTTTCCGGGCGGCACAATC
PRDM6-R	CGGCGCCTCGAACTGAAAACT
SMAD6-F	CCGGGTGAATTCTCAGACGCC
SMAD6-R	AGCCGATCTTGCTGCGCGTT
SMAD7-F	ACGCGGGAGGTGGATGGTGT
SMAD7-R	ACCCCAGCCCTTCACAAAGCTG

Appendix Table 2.
Dental Findings in SIOD Patients with Bi-allelic *SMARCAL1* Mutations

			Dental Findings			
Pedigree No.	*SMARCAL1* Mutations	Disease Severity Score[a]	Microdontia	Hypodontia	Molar Root Hypoplasia	Other
SD8	c.[1190delT];[?][b]	6	+	–	NR	–
SD16	c.[1933C>T];[1643T>A]	3	+	+	+	Retained deciduous molar
SD18a	c.[1756C>T];[1756C>T]	3	–	+	NR	–
SD18c	c.[1756C>T];[1756C>T]	2	–	+ 15, 16, 18, 28, 35, 37, 38, 42, 47, 48	–	Retained 75
SD22	c.[2459G>A];[2459G>A]	5	–	NR	NR	NR
SD23	c.[2542G>T];[2542G>T]	4	–	–	NR	NR
SD27	c.[1940A>C];[1940A>C]	3	+	+ 15, 17, 18, 24, 25, 27, 28, 34, 35, 37, 38, 44, 45, 47, 48	+	Increased caries, retained 64, 75
SD28	c.[1696A>T;1698G>C;1702delG];[1696A>T;1698G>C;1702delG]	5	–	–	–	–
SD29	c.[1934delG];[862+1G>T]	7	–	+	+	Abn superior incisors, delayed dentition
SD33a	c.[1146_1147delAA;1147+1_2delGT];[1097-2A>G]	4	–	–	+	NR
SD33b	c.[1146_1147delAA;1147+1_2delGT];[1097-2A>G]	6	–	–	+	NR
SD35	c.[1736C>T];[2321C>A]	6	–	–	NR	NR
SD38	c.[1096+1G>A];[1096+1G>A]	6	+	+ 14, 15, 18, 24, 25, 28, 35, 38, 45, 47, 48	+	Delayed dentition, retained 54, 55, 64, 65, 74, 75, 84, 85
SD44	c.[2321C>A];[1191delG]	5	–	+	+	NR
SD47	c.[2459G>A];[?][b]	4	+	NR	NR	NR
SD48	c.[1939A>C];[1939A>C]	6	+	+	NR	NR

(continued)

Appendix Table 2. (Continued)

SD49	c.[2321C>A];[1920_1921insG]	6	+	NR	NR	NR
SD50	c.[2542G>T];[2542G>T]	4	+	+	+	Abn enamel
SD51	c.[2542G>T];[2459G>A]	4	+	+	NR	–
SD57	c.[955C>T];[955C>T]	5	+	+ 14, 15, 18, 24, 25, 28, 34, 35, 36, 44, 45, 46	+	Retained 54, 55, 64, 65, 73, 74, 75, 83, 84, 85
SD60	c.[2542G>T];[2542G>T]	5	+	+ 38	+	–
SD61	c.[1146_1147delAA;1147+1_2delGT];[1146_1147delAA;1147+1_2delGT]	5	–	–	–	NR
SD65a	c.[2542G>T];[836T>C]	1	–	–	–	–
SD65b	c.[2542G>T];[836T>C]	3	–	–	–	–
SD66	c.[1933C>T];[1933C>T]	5	+	+	NR	Increased caries
SD70	c.[340_341insAGTCCAC];[836T>C]	6	+	+	NR	Abn dentin
SD74	c.[1736C>T];[?][b]	3	+	+ 14, 15, 18, 25, 28, 38, 35, 45, 48	+	NR
SD78	c.[2264T>G];[1439C>T]	4	NR	NR	+	NR
SD79	c.[2459G>A];[?][b]	4	–	+	+	NR
SD84	c.[2104T>G];[1248_1249insC]	6	+	NR	NR	NR
SD96	c.[1427G>A];[1427G>A]	4	+	NR	NR	NR
SD99	c.[1402G>C];[1402G>C]	4	+	–	NR	NR
SD106	c.[1682G>A];[1682G>A]	4	–	–	NR	NR
SD107	c.[2542G>T];[2542G>T]	4	–	+	NR	Abn enamel
SD108a	c.[1798C>T];[1798C>T]	3	–	–	NR	NR
SD108b	c.[1798C>T];[1798C>T]	1	–	–	NR	NR
SD111	c.[1129G>C];[1592T>C]	6	+	–	NR	Abn enamel

(continued)

Appendix Table 2. (Continued)

SD112a	c.[1934G>A];[2542G>T]	4	–	–	NR	NR
SD112b	c.[1934G>A];[2542G>T]	3	–	–	NR	NR
SD114	c.[1898T>C];[1898T>C]	4	+	+	+	Discoloration
SD115	c.[1437_1438insG];[1437_1438insG]	5	NR	–	–	NR
SD119	c.[2449C>T];[2542G>T]	4	–	+	+	–
SD120	c.[2291G>A];[2542G>T]	5	+	+	+	NR
SD121	c.[1382G>A];[2542G>T]	4	–	–	NR	NR
SD123	c.[49C>T];[49C>T]	4	+	–	NR	NR
SD124	c.[1920_1921insG];[1920_1921insG]	2	–	–	NR	NR
SD127	c.[1736C>T];[1736C>T]	5	+	+	+	Increased caries, abn enamel and dentin, discoloration
SD131	c.[1026C>A];[2264T>G]	7	NR	+	+	NR
SD133a	c.[1097-2A>G]; [2343_2347delGCTGT]	4	–	–	NR	–
SD138	c.[2542G>T];[2542G>T]	3	–	–	–	–

Abbreviations: +, feature present; –, feature not present; abn, abnormal; NR, not reported.
[a]To group patients according to disease severity, each patient's signs and symptoms were scored as previously described (Clewing *et al.*, 2007).
[b][?] represents alleles with non-coding *SMARCAL1* mutations as previously described (Clewing *et al.*, 2007).

Appendix Table 3.
Summary of Immunohistochemical Analysis of SMARCAL1 Expression in the Developing Human Tooth

	Expression Level*		
Developmental Stage and Cell Type	**59-day-gestation Fetus**	**98-day-gestation Fetus**	**105-day-gestation Fetus**
Bud Stage			
Oral epithelium	+ + +	NA	NA
Dental lamina	+ +	NA	NA
Mesenchymal cell	+	NA	NA
Cap Stage			
Oral epithelium	NA	+ + +	NA
Dental lamina	NA	+ +	NA
Outer dental epithelium	NA	+ + +	NA
Stellate reticulum	NA	+ +	NA
Inner dental epithelium	NA	+ + +	NA
Primary enamel knot	NA	+ + +	NA
Dental papilla	NA	+ +	NA
Bell Stage			
Oral epithelium	NA	NA	+ + +
Dental lamina	NA	NA	–
Outer dental epithelium	NA	NA	+ +
Stellate reticulum	NA	NA	+ +
Stratum intermedium	NA	NA	+ / –
Inner dental epithelium	NA	NA	+ + +
Dental papilla	NA	NA	+ / –

*Expression levels were all judged relative to the oral epithelium, which was scored as + + +. Abbreviations: –, no detectable expression; + - + + +, level of detectable expression; NA, not available.

Appendix Table 4.
Summary of Tooth Anomalies Associated with Disorders of DNA Repair or Genomic Instability

Disorder	Gene(s)	Dental Phenotype	Reference
DNA Repair			
Xeroderma pigmentosum	*XPA* *ERCC3* *ERCC2* *DDB2* *ERCC4* *ERCC5* *ERCC1* *POLH*	None reported	
Cockayne syndrome	*ERCC6* *ERCC8*	Increased dental caries	Tan *et al.,* 2005
Trichothiodystrophy	*ERCC2* *ERCC3* *GTF2H5*	None reported	
Lynch syndrome	*MSH2*	None reported	
Genomic instability			
Bloom syndrome	*RECQL3*	None reported	
Werner syndrome	*RECQL2*	None reported	
Rothmund-Thomson syndrome	*RECQL4*	Hypodontia, short roots, sensitive gingiva	Haytac *et al.*, 2002; Roinioti and Stefanopoulos, 2007
Fanconi anemia	*FANCA* *FANCB* *FANCC* *BRCA2* *FANCD2* *FANCE* *FANCF* *FANCG* *FANCI* *BRIP1* *FANCL* *FANCM* *PALB2* *RAD51C* *SLX4*	Microdontia, hypodontia, increased dental caries, gingivitis, periodontitis, transposition, supernumerary teeth	Acikgoz *et al.*, 2005; Tekcicek *et al.*, 2007
Ataxia telangiectasia	*ATM*	None reported	
Nijmegen breakage syndrome	*NBS1*	None reported	
Seckel syndrome	*ATR*	Microdontia, hypodontia, short roots, malocclusion, taurodontism, dentinal dysplasia, enamel hypoplasia	Kjaer *et al.*, 2001; Seymen *et al.*, 2002; Regen *et al.*, 2010
Dyskeratosis congenita	*DKC1* *TERC* *TERT* *TINF2* *NHP2* *NOP10*	Short roots, mild taurodontism	Atkinson *et al.*, 2008; Abdel-Karim *et al.,* 2009

Appendix Table 5.
The Target Genes of Morphogens for Which Transcriptional Responses Have Been Defined in Dermal Fibroblasts, Which Were Investigated in This Study

Morphogen	Target Gene	Reference
WNT3A	*GREM2*	Klapholz-Brown *et al.*, 2007
WNT3A	*PRDM6*	Klapholz-Brown *et al.*, 2007
BMP4	*ID1*	Fessing *et al.*, 2010
BMP4	*SMAD6*	Fessing *et al.*, 2010
TGFβ1	*SMAD6*	Afrakhte *et al.*, 1998
TGFβ1	*SMAD7*	Afrakhte *et al.*, 1998
TGFβ1	*MMP10*	Ishikawa *et al.*, 2010

Appendix Table 6.
Relative Basal Gene Expression Levels of the SIOD Patient Dermal Fibroblasts (SD120 and SD123) Used in This Study

Morphogen and Target Gene	Sample	Relative Basal Gene Expression[a]	P-value[b]
WNT3A			
GREM2	SD120	0.6	2.5×10^{-3}
GREM2	SD123	1.5	1.9×10^{-3}
WNT3A			
PRDM6	SD120	18.0	2.0×10^{-3}
PRDM6	SD123	14.6	2.0×10^{-3}
BMP4			
ID1	SD120	15.5	6.4×10^{-3}
ID1	SD123	9.7	3.8×10^{-2}
BMP4			
SMAD6	SD120	1.2	NS
SMAD6	SD123	1.8	2.7×10^{-3}
TGFβ1			
SMAD6	SD120	3.0	NS
SMAD6	SD123	3.9	NS
TGFβ1			
SMAD7	SD120	1.2	NS
SMAD7	SD123	1.0	NS
TGFβ1			
MMP10	SD120	1.8	NS
MMP10	SD123	1.4	NS

[a]Expression of each gene was first normalized to *GAPDH* expression and then graphed relative to the expression level of the unaffected control dermal fibroblast cell line.
[b]p values were calculated by the Tukey *post hoc* test following one-way ANOVA analysis and represent the statistical significance between the relative gene expression of the patient cell line of interest and the unaffected control cell line.
Abbreviation: NS, not significant.

Appendix Table 7.
Relative Gene Expression Changes in SIOD Patient Dermal Fibroblasts in Response to the Morphogens WNT3A, BMP4, or TGFβ1 over 24 hrs

Morphogen and Target Gene	Sample	Time-point (hrs after induction)	Relative Gene Expression[a]	P-value[b]
WNT3A				
GREM2	Control	2	1.10	
GREM2	SD120	2	1.45	NS
GREM2	SD123	2	1.02	NS
GREM2	Control	4	1.71	
GREM2	SD120	4	2.97	7.8×10^{-3}
GREM2	SD123	4	1.72	NS
GREM2	Control	8	3.20	
GREM2	SD120	8	3.29	NS
GREM2	SD123	8	2.08	4.5×10^{-2}
GREM2	Control	12	3.22	
GREM2	SD120	12	3.23	NS
GREM2	SD123	12	1.69	4.8×10^{-2}
GREM2	Control	16	3.97	
GREM2	SD120	16	2.62	1.1×10^{-3}
GREM2	SD123	16	1.79	2.4×10^{-4}
GREM2	Control	20	2.80	
GREM2	SD120	20	1.45	1.6×10^{-3}
GREM2	SD123	20	1.16	5.2×10^{-4}
GREM2	Control	24	1.89	
GREM2	SD120	24	1.33	5.5×10^{-3}
GREM2	SD123	24	0.95	1.2×10^{-3}
WNT3A				
PRDM6	Control	2	0.74	
PRDM6	SD120	2	0.68	NS
PRDM6	SD123	2	0.72	NS
PRDM6	Control	4	4.32	
PRDM6	SD120	4	1.84	1.3×10^{-2}
PRDM6	SD123	4	1.59	5.4×10^{-3}
PRDM6	Control	8	3.11	
PRDM6	SD120	8	0.90	3.7×10^{-4}
PRDM6	SD123	8	1.36	1.8×10^{-3}
PRDM6	Control	12	2.99	
PRDM6	SD120	12	1.05	1.5×10^{-2}

(continued)

Appendix Table 7. (Continued)

PRDM6	SD123	12	3.18	NS
PRDM6	Control	16	4.93	
PRDM6	SD120	16	0.67	2.5×10^{-3}
PRDM6	SD123	16	2.06	1.5×10^{-2}
PRDM6	Control	20	2.32	
PRDM6	SD120	20	0.70	6.6×10^{-4}
PRDM6	SD123	20	1.26	8.8×10^{-3}
PRDM6	Control	24	1.78	
PRDM6	SD120	24	0.83	3.9×10^{-2}
PRDM6	SD123	24	1.63	NS
BMP4				
ID1	Control	2	233.98	
ID1	SD120	2	27.17	1.5×10^{-5}
ID1	SD123	2	30.06	1.5×10^{-5}
ID1	Control	4	168.62	
ID1	SD120	4	17.89	1.7×10^{-5}
ID1	SD123	4	20.37	1.3×10^{-5}
ID1	Control	8	146.54	
ID1	SD120	8	20.06	1.6×10^{-5}
ID1	SD123	8	19.59	2.3×10^{-5}
ID1	Control	12	60.99	
ID1	SD120	12	5.41	3.1×10^{-6}
ID1	SD123	12	7.34	3.8×10^{-6}
ID1	Control	16	58.07	
ID1	SD120	16	4.73	1.7×10^{-5}
ID1	SD123	16	6.84	1.3×10^{-5}
ID1	Control	20	77.8	
ID1	SD120	20	5.25	7.9×10^{-4}
ID1	SD123	20	5.86	8.9×10^{-4}
ID1	Control	24	54.24	
ID1	SD120	24	4.87	3.5×10^{-5}
ID1	SD123	24	5.43	3.7×10^{-5}
BMP4				
SMAD6	Control	2	7.13	
SMAD6	SD120	2	6.80	NS
SMAD6	SD123	2	4.67	4.6×10^{-2}
SMAD6	Control	4	3.71	

(continued)

Appendix Table 7. (Continued)

SMAD6	SD120	4	4.35	NS
SMAD6	SD123	4	3.47	NS
SMAD6	Control	8	6.51	
SMAD6	SD120	8	9.91	1.5×10^{-2}
SMAD6	SD123	8	5.14	NS
SMAD6	Control	12	8.96	
SMAD6	SD120	12	6.65	1.5×10^{-2}
SMAD6	SD123	12	6.60	2.2×10^{-2}
SMAD6	Control	16	7.71	
SMAD6	SD120	16	3.87	1.3×10^{-2}
SMAD6	SD123	16	6.54	NS
SMAD6	Control	20	6.83	
SMAD6	SD120	20	4.97	NS
SMAD6	SD123	20	5.05	NS
SMAD6	Control	24	8.29	
SMAD6	SD120	24	7.34	2.9×10^{-2}
SMAD6	SD123	24	5.27	1.0×10^{-3}
TGFβ1				
SMAD6	Control	2	2.96	
SMAD6	SD120	2	9.49	6.6×10^{-4}
SMAD6	SD123	2	5.16	4.7×10^{-2}
SMAD6	Control	4	6.12	
SMAD6	SD120	4	1.83	3.9×10^{-4}
SMAD6	SD123	4	1.36	3.2×10^{-4}
SMAD6	Control	8	6.67	
SMAD6	SD120	8	1.81	1.6×10^{-4}
SMAD6	SD123	8	0.90	7.1×10^{-5}
SMAD6	Control	12	2.94	
SMAD6	SD120	12	2.69	NS
SMAD6	SD123	12	1.49	2.1×10^{-3}
SMAD6	Control	16	1.12	
SMAD6	SD120	16	2.39	4.6×10^{-2}
SMAD6	SD123	16	0.96	NS
SMAD6	Control	20	0.51	
SMAD6	SD120	20	1.07	NS
SMAD6	SD123	20	0.94	NS
SMAD6	Control	24	2.10	

(continued)

Appendix Table 7. (Continued)

SMAD6	SD120	24	2.20	NS
SMAD6	SD123	24	1.62	NS
TGFβ1				
SMAD7	Control	2	1.52	
SMAD7	SD120	2	6.58	1.3×10^{-5}
SMAD7	SD123	2	6.95	1.4×10^{-5}
SMAD7	Control	4	8.19	
SMAD7	SD120	4	4.55	2.7×10^{-3}
SMAD7	SD123	4	4.83	1.4×10^{-3}
SMAD7	Control	8	9.92	
SMAD7	SD120	8	7.67	1.4×10^{-2}
SMAD7	SD123	8	8.11	2.5×10^{-2}
SMAD7	Control	12	10.54	
SMAD7	SD120	12	5.20	1.1×10^{-3}
SMAD7	SD123	12	9.53	NS
SMAD7	Control	16	9.11	
SMAD7	SD120	16	8.42	NS
SMAD7	SD123	16	9.45	NS
SMAD7	Control	20	9.47	
SMAD7	SD120	20	7.60	NS
SMAD7	SD123	20	8.90	NS
SMAD7	Control	24	9.47	
SMAD7	SD120	24	9.95	NS
SMAD7	SD123	24	9.64	NS
TGFβ1				
MMP10	Control	2	1.75	
MMP10	SD120	2	1.34	NS
MMP10	SD123	2	1.29	NS
MMP10	Control	4	2.14	
MMP10	SD120	4	1.36	1.8×10^{-2}
MMP10	SD123	4	1.49	4.2×10^{-2}
MMP10	Control	8	2.17	
MMP10	SD120	8	1.52	4.1×10^{-2}
MMP10	SD123	8	1.54	4.6×10^{-2}
MMP10	Control	12	2.42	
MMP10	SD120	12	0.83	1.8×10^{-3}
MMP10	SD123	12	0.92	1.4×10^{-3}

(continued)

Appendix Table 7. (Continued)

MMP10	Control	16	5.05	
MMP10	SD120	16	0.80	5.6×10^{-4}
MMP10	SD123	16	0.87	4.2×10^{-4}
MMP10	Control	20	10.91	
MMP10	SD120	20	0.93	1.0×10^{-4}
MMP10	SD123	20	0.95	7.2×10^{-5}
MMP10	Control	24	12.86	
MMP10	SD120	24	1.17	5.7×10^{-5}
MMP10	SD123	24	1.21	4.0×10^{-5}

[a]Expression of each gene was first normalized to *GAPDH* expression and then graphed relative to its expression in the relevant cell line at time = 0 hrs.
[b]P-values were calculated by the Tukey *post hoc* test following one-way ANOVA analysis and represent the statistical significance between the relative gene expression of the patient cell line of interest and the unaffected control cell line at each time-point.
Abbreviation: NS, not significant.

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