Supplement

Increased bone turnover, osteoporosis, progressive tibial bowing, fractures, and scoliosis in a patient with a final-exon *SATB2* frameshift mutation

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Supplemental Figures

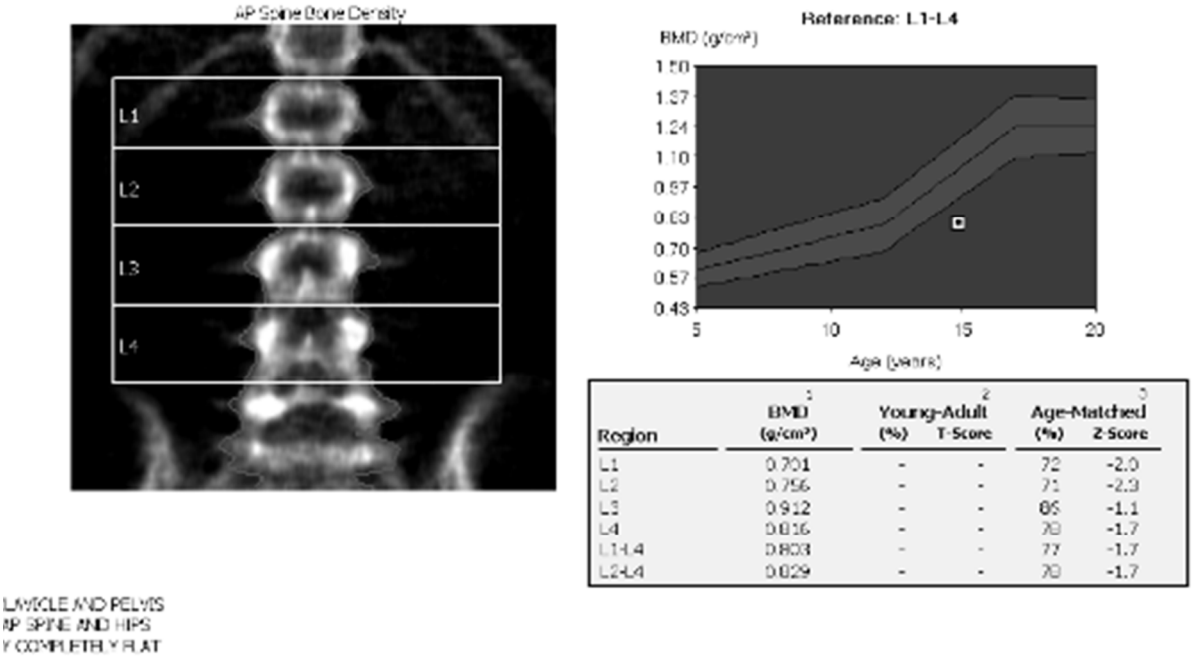

Figure S1. DXA scan at age 14 demonstrating decreased bone mineral density. Bone mineral densitometry of the lumbar spine was performed on a GE Lunar Prodigy densitometer using a standard technique. Total body and hip films were not performed as the patient was not able to completely lie flat or motionless for the examination. Lumbar spine density (L1-L4) was 0.803g/cm^3, resulting in a Z score of -1.7.

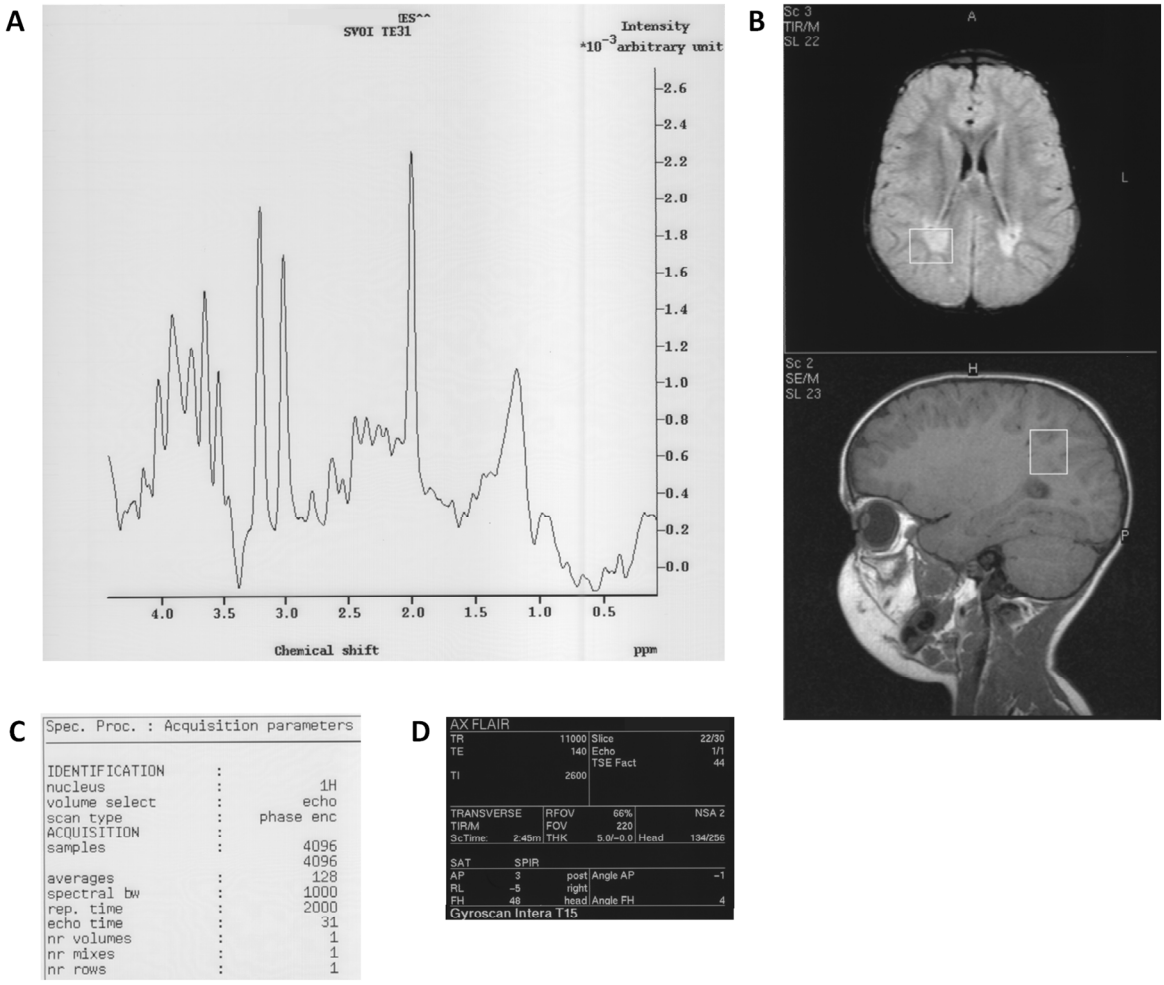

Figure S2. Magnetic resonance spectroscopy (MRS) at age 3 years. Single voxel short TE MR spectroscopy was performed in the periventricular white matter around the right atrium, demonstrating a broad lipid peak centered around 1.35 ppm. This is larger than would normally be expected and may reflect an abnormality of myelination in this region.

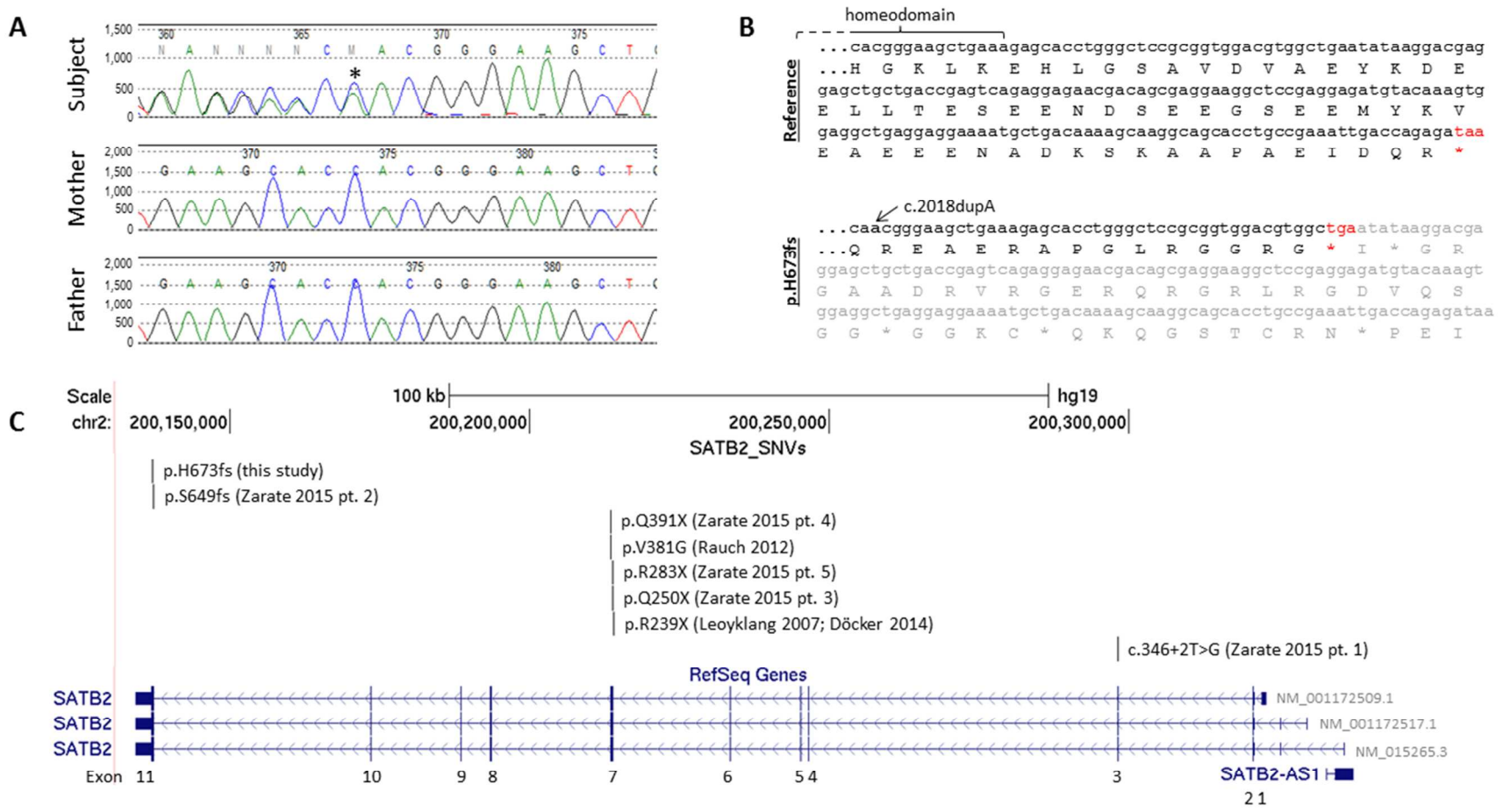

Figure S3. The *SATB2* c.2018dupA (p.H673fs) mutation is apparently *de novo* and is predicted to disrupt the SATB2 homeodomain and lead to premature truncation. a. A heterozygous *SATB2* c.2018dupA mutation (*) identified by exome sequencing is confirmed and found to be apparently *de novo* by bi-directional Sanger sequencing (displayed is 5' to 3' sequence using the

forward genomic primer). **b.** Nucleotide and protein sequence of *SATB2* (top, reference sequence; bottom, patient sequence) starting with codon 673. c.2018dupA is predicted to alter the final five amino acids of the SATB2 homeodomain, followed by truncation after 10 additional aberrant amino acids or escape from nonsense-mediated decay. **c.** *SATB2* gene structure with all known deleterious point mutations plotted. Note that the variant reported by Rauch et al. [2012] is a missense variant (predicted to be "probably damaging" by PolyPhen2) reported in a female with severe intellectual disability, mild facial dysmorphism, and a bifid uvula without cleft palate [Rauch et al., 2012].

Table S1. Laboratory results.

Test	Value (normal range if abnormal value)	Age
Bone studies		
Alkaline phosphatase	**1063 IU/L (H)** (92-468) Bone fraction 89%	13y
" "	**873 IU/L (H)** (107-340)	14y
" "	**813 IU/L (H)**	16y[b]
" "	**965 IU/L (H)**	16y[c]
Calcium, serum	10.3 mg/dL	13y
" "	9.7 mg/dL	14y
" "	9.8 mg/dL	16y[b]
" "	10.2 mg/dL	16y[c]
Calcium, random urine	4.2 mg/dL (no range available)	14y
Calcitonin	< 2 pg/mL	13y
Calcitonin	< 2 pg/mL	16y[c]
C-telopeptide	1514 pg/mL (435-2924)	16y[b]
C-telopeptide	**3165 pg/ml (H)** (435-2924)	16y[c]
N-telopeptide, urine	31,610 nmol BCE (no range available)	14y
N-telopeptide/creatinine ratio, urine	**2885 nmol BCE/mM Cr (H)**[a] (male tanner stage: I = 55-508; II = 21-423; III = 27-462; IV = <609; V = <240)	14y
Osteocalcin	**77 ng/mL (H)**	13y
" "	**105.1 ng/mL (H)**[a] (3.2-39.6)	14y
" "	**81 ng/ml (H)**	16y[b]
" "	**66 ng/mL**	16y[c]
PTH, intact	19 pg/mL	13y
" "	46 pg/mL	14y
" "	37 pg/mL	16y[b]
" "	37 pg/mL	16y[c]
Phosphorus	**4.9 mg/dL (H)** (2.5-4.5)	13y
" "	**5.0 mg/dL (H)** (2.5-4.5)	16y[b]
" "	**5.4 mg/dL (H)** (2.5-4.5)	16y[c]
Procollagen type I intact N-terminal propeptide (PINP)	**1470 mcg/ml** (normal 22-87 mcg/ml for males >18yo; no pediatric reference values exist)	16y[c]
Vitamin D, 25-OH	Total, 39 ng/mL D3, 39 ng/mL D2, <4 ng/mL	13y
" "	Total, 35.2 ng/mL	14y
" "	Total, 30 ng/mL	16y[c]
Vitamin D, 1-, 25- $(OH)_2$	Total, 67 pg/mL D3, 67 pg/mL D2, <8 pg/mL	13y
" "	Total, **186.0 pg/mL (H)**[a] (10.0-75.0)	14y

[a] Verified by repeat testing.

[b] 3-4 months post first dose of denosumab.

[c] Prior to second dose.

BCE, Bone Collagen Equivalent

Table S1, cont'd.

Genetic studies		
Karyotype	46, XY	2y
Telomere FISH	No rearrangements seen	2y
Angelman/Prader-Willi syndrome methylation studies	Normal pattern	2y
Chromosomal microarray analysis (Baylor CMA-HR+SNP, V9.1.1)	No reportable nuclear genome CNVs; no mitochondrial deletions; no increased blocks of absence of heterozygosity (AOH)	14y
Exome sequencing (Methodology: https://www.bcm.edu/research/medical-genetics-labs/)	Deleterious mutations in genes related to clinical phenotype: D*e novo* c.2018dupA (p.H673fs) mutation in *SATB2* VUSs in disease genes related to the clinical phenotype: Paternally inherited c.4598C>T (p.A1533V) mutation in *NRXN2* Paternally inherited c.107C>T (p.T36M) mutation in *SPECC1L*	14y
Metabolic studies		
Coenzyme Q10, total	0.87 µg/mL	14y
Folate (folic acid), serum	>19.9 ng/mL	14y
Lactate	1.3 mmol/L	2y
Pyruvate	0.3 mmol/L	2y
Vitamin B_{12}	399 pg/mL	14y
Vitamin K_1	0.52 ng/mL	14y
Neurological studies		
Anti-folate antibodies, serum	**6.55 pmol IgG/ml (H)**[d]	13y
Anti-Neuronal Cell Ab	**301 Units (H)** (0-54)	4y 5m
" "	**76 Units (H)** (0-54)	4y 9m
" "	**326 Units (H)** (0-54)	4y 11m
" "	**298 Units (H)** (0-54)	6y 1m
Myelin basic protein antibody, serum ELISA	IgG: 70 Units IgM: 78 Units **(H)** (0-50)	4y 9m

[d] On account of this laboratory study consistent with (but not diagnostic of) cerebral folate deficiency, the patient takes oral leucovorin (folinic acid). Other medicines include mometasone furoate nasal spray for allergies and low-dose baclofen for spasticity.

Table S1, cont'd.

Immunological Studies		
CBC	WBC: **4.08 10^3/µL (L)** (5.0-19.5) RBC: 4.59 10^6/µL (3.9-5.3) HGB: **13.9 g/dL (H)** (11.5-13.5) HCT: 38.8 % (34.0-40.0) MCV: 84.6 fL (75.0-85.0) MCH: **30.2 pg (H)** (25.0-29.0) MCHC: 35.7 g/dL (33.0-37.0) RDW: 14.4 % Platelets: 263 10^3/µL (150-450) MPV 6.7 FL TYPE AUTO SEG% 45 % 23-61 LYMPH% 46 % 28-65 MONO% 5 % 0-5 EOS% 3 % 0-3 BASO% 1 % 0-1 ANC 1.83 10^3/UL ! LYMPH# 1.75 ! MONO# 0.21 ! EOS# 0.11	4y
Lymphocyte subset panel (5-total lymphocyte enumeration)	ABS CD4: 818/µL %CD19: 22% %CD4: 43% CD4:CD8 ratio: 1.65 ABS CD8: 496/µL %CD8: 26% ABS CD3: 1450/µL %CD3: 73% ABS CD19: 461/µL ABS NK-Cells: **58/µL (L)** (60-590) %CD16+, CD56+, CD3-: **3% (L)** (4-26)	4y

Table S1, cont'd.

Other studies		
Apolipoprotein E mutation testing	APO e2/e3 genotype	6y
Ova and parasites, stool	No ova or parasites seen	4y
C Difficile tox a/b, stool	negative	4y
Trichrome stain, stool	No ova or parasites seen	4y
Occult blood stool	Negative	4y
Copper, plasma	1.22 µg/mL	14y
Copper, RBC	0.55 µg/mL	14y
Creatinine, urine	123.8 mg/dL	14y
IBD Panel (Prometheus laboratories) *The qualitative IBD FIRST STEP result is derived from evaluation of results from four modified proprietary ELISA assays: a) Anti-Omp C IgA ELISA, b) ASCA IgA ELISA, c) ASCA IgG ELISA, and d) Neutrophil-Specific Nuclear Autoantibodies ELISA (previously pANCA).	First step markers not detected	4y
measles and mumps	Measles and mumps immune (IgG)	13y
Intestinal isoenzyme	4% (2-12% for 10-13 year olds)	13y
Antigliadin IgG	**45 (H)**[e]	4y
Antigliadin IgA	8 (nl)[e]	4y
Tissue transglutaminase	IgG 17 (nl) IgM 45 (nl)	4y
Antigliadin IgG (native) Note that this test is for "detection of IgG antibodies to native gliadin in human serum to aid in the diagnosis of non-celiac gluten sensitivity in conjunction with other clinical and lab findings. Not recommended for Celiac disease screening"	39 H (0-19 neg, 20-30 weak positive, >30 moderate to strong positive)	14y
Renin activity, plasma	**5.31 ng/mL/hr (H)** (0.50 - 3.30)	14y

[e] The patient's low weight prompted a celiac disease workup at age 4 years; while an antigliadin IgG test was positive (45 Units), antigliadin IgA and tissue transglutaminase levels were normal and a duodenal biopsy showed only mild, non-specific chronic inflammation of the lamina propria, excluding the diagnosis of celiac disease.