Supplementary information

De novo mutations of *SETBP1* cause Schinzel-Giedion syndrome

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Supplementary Table 1: Overview of exome-sequencing performance

Patient	Total reads	Uniquely mapped reads	Reads overlapping with targets	Unique reads	Mean coverage	Number of called bases
Patient 1	89,949,891	58,189,014	46,075,589	29,610,185	47.32	196,123,687
Patient 2	86,104,150	55,453,009	45,014,396	27,565,627	47.43	178,688,964
Patient 3	88,542,531	52,783,914	40,266,883	28,586,694	40.43	195,062,516
Patient 4	61,480,688	52,684,110	38,528,450	27,384,374	36.71	213,431,228

Supplementary Table 2: Overview of all variants identified by exome sequencing in 4 patients with Schinzel-Giedion syndrome

	Patient 1	Patient 2	Patient 3	Patient 4	Mean	No. of genes with variants in all 4 samples
Total variants called	22,916	22,602	22,152	19,528	**21,800**	**4,735**
Exonic + SpliceSites(SS) (+/- 2nts intronic) variants	12,196	12,255	11,796	10,498	**11,686**	**3,331**
Non-synonymous (NS) + SS variants	5,556	5,618	5,427	4,802	**5,351**	**1,634**
Unknown SNP variants (dbSNP130)	405	401	390	387	**396**	**35**
Unknown variants (in-house database)	299	289	275	288	**288**	**12**
Genes showing variants in at least 2 different genomic positions						**2**

Supplementary Table 3: Further clinical details of all 13 patients with Schinzel-Giedion syndrome

Individuals ID	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Gender	F	M	F	M	M	F	F	F	M	F	M	M	M
Current Age	-	-	1 yr 10m	-	-	1 yr	-	-	-	2 yrs	-	3 yr 1 m	-
Age at death	4 yr 6 m	2 yr 3 m	-	7 m	2 yr 6 m	-	6 m	9 yr 3 m	u	-	3 m	-	7 wks
Craniofacial findings													
Head circumference	<P3	u	<P3	<P3	u	P10	<P3	<P3	p25	P3	P10	<P3	P90
Large anterior fontanelle/sutures	-	+	u	+	+	+	-	+	+	+	+	+	+
Prominent forehead	+	+	+	+	+	+	+	+	+	-	+	+	+
Bitemporal narrowing	+	+	+	+	+	+	u	+	+	-	-	+	+
Mid-face retraction	+	+	+	+	+	+	+	+	+	+	+	+	+
Prominent eyes/shallow orbits	+	+	+	+	+	+	+	+	+	+	+	+	+
Hypertelorism	+	+	+	+	+	+	+	+	+	+	-	+	+
Deep groove under eyes	+	+	+	+	+	+	u	+	+	+	+	+	+
Abnormal helices and pinnae	+	+	+	+	+	+	+	+		+	+	+	+
Low-set ears	+	+	+	+	+	+	+	+	+	+	+	+	-
Short, upturned nose	+	+	+	+	+	+	+	+	+	+	+	+	+
Macroglossia	+	u	-	-	-	-	+	+	+	-	+	-	u
Macrostomia	-	u	-		+	-	u	+	+	-	u	-	+
Capillary malformation	-	u	-	-	-	+	u	+	-	-	-	-	-
Short neck	+	+	+	+	+	+	+	+	+	+		+	+
Hypertrichosis	-	u	-	-	-	+	+	+	+	-	+	-	-
Other	upslanting palpebral fissures	hyperconvex nails	cutis marmorata			upslanting palpebral fissures; cutis marmorata		hypoplastic nipples; hyperconvex nails	upslanting palpebral fissures	hypoplastic toenails	hypoplastic nipples		cleft palate
Congenital anomalies													
Hydronephrosis/vesicoureteral reflux	+	+	+	+	+	+	+	+	+	+	+	+	+
Genital abnormalities	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiac defects	-	+	+	-	-	+	+	+	+	-	+	-	-
Choanal stenosis	+	-	+	-	-	+	u	-	-	-	-	+	-
Post-axial polydactyly	-	+	-	-	-	-	-	-	-	-	-	-	-
other		tracheo-malacia	hip dysplasia		Laryngomalacia nodular growth in forearm of u origin			club feet	hydrocele			pharyngeal incoordination; renal calculi	tracheo-malacia; subglottic cysts; hydrocele
Neurodevelopmental anomalies													
Developmental delay	+	+	+	+	+	+	+	+	+	+	u*	+	u*
Seizures	+	+	+	+	+	+	+	+	+	+	-	+	+
Vision impairment	+	+	+	u	u	+	+	+	+	u	+	-	u
Hearing impairment	+	+	+	u	u	+	-	+	+	u	+	+	u

Brain MRI/CT													
Ventriculomegaly	+	u	u	-	-	-	u	+	+	-	-	+	+
Underdeveloped corpus callosum/septum	+	+	u	+	+	+	u	+	+	+	+	-	+
Cortical atrophy	-	+	u	-	+	u	u	+	-	u	-	+	+
Choroid plexus cysts	+	u	u	+	+	u	u	-	-	u	+	-	-
Other	polymicrogyria				cortical dysplasia		dysmorphic ventricles	Polymicrogyria					
Ophthalmological													
Abnormal fundoscopy	u	u	-	+	u	u	u	-	-	u	+	-	u
Alacrima with corneal hypoesthesia	+	+	-	na	u	u	+	+	+	+	+	+	u
Progressive failure to thrive	+	+	-	+	+	+	u	+	+	+		+	u
Radiological findings													
Skull													
Sclerotic base or mastoid	u	+	+	+	+	+	+	+	+	+	+	-	+
Wide occipital synchondrosis	u	u	-	+	+	+	+	-	u	+	+	+	u
Steep base	u	u	+	+	+	u	u	-	u	+	u	+	+
Wormian bones	u	-	-	-	+	u	u	-	u	-	u	-	u
Extremities													
Cortices: dense, thickened, undertubulated	u	u	-	-	-	+		u	u	+	u	-	u
Hypoplastic distal phalanges	u	u	-	+	+	+	+	u	u	+	u	+	+
Broad metaphyses	u	u	-	-	-	+		u	+	-	u	+	u
Short first metacarpal	u	u	-	+	-	+	+	+	u	+	u	-	+
Chest													
Broad ribs	+	+	+	-	+	+	+	-	+	+	u	+	-
Abnormal (long/irregular) clavicles	-	u	-	-	-	+	u	+	u	+	u	-	u
Hypoplastic first rib(s)	-	u	-	-	+	u	u	u	u	-	u	-	+
Pelvis													
Hypoplastic/underossified pubic bones	+	u	-	u	+	+	u	+	u	+	u	+	u
Other	left kidney lesion of unknown origin		bowed forearm bones; radio ulnar synostosis			wide sutures	wide sutures; hydrocephaly	congenital hip dislocation	-	accessory ribs	cystic mass in spine		mild shortening humeri and femora
Tumors						Recurrent sacrococcygeal PNET					PNET		
u=unknown, PNET = primitive neuroectodermal tumor, * died shortly after birth													
Table adapted from Lehman et al.[1]													

Supplementary Figure 1: Comparison of fold coverage of the targeted exomes for all 4 samples.

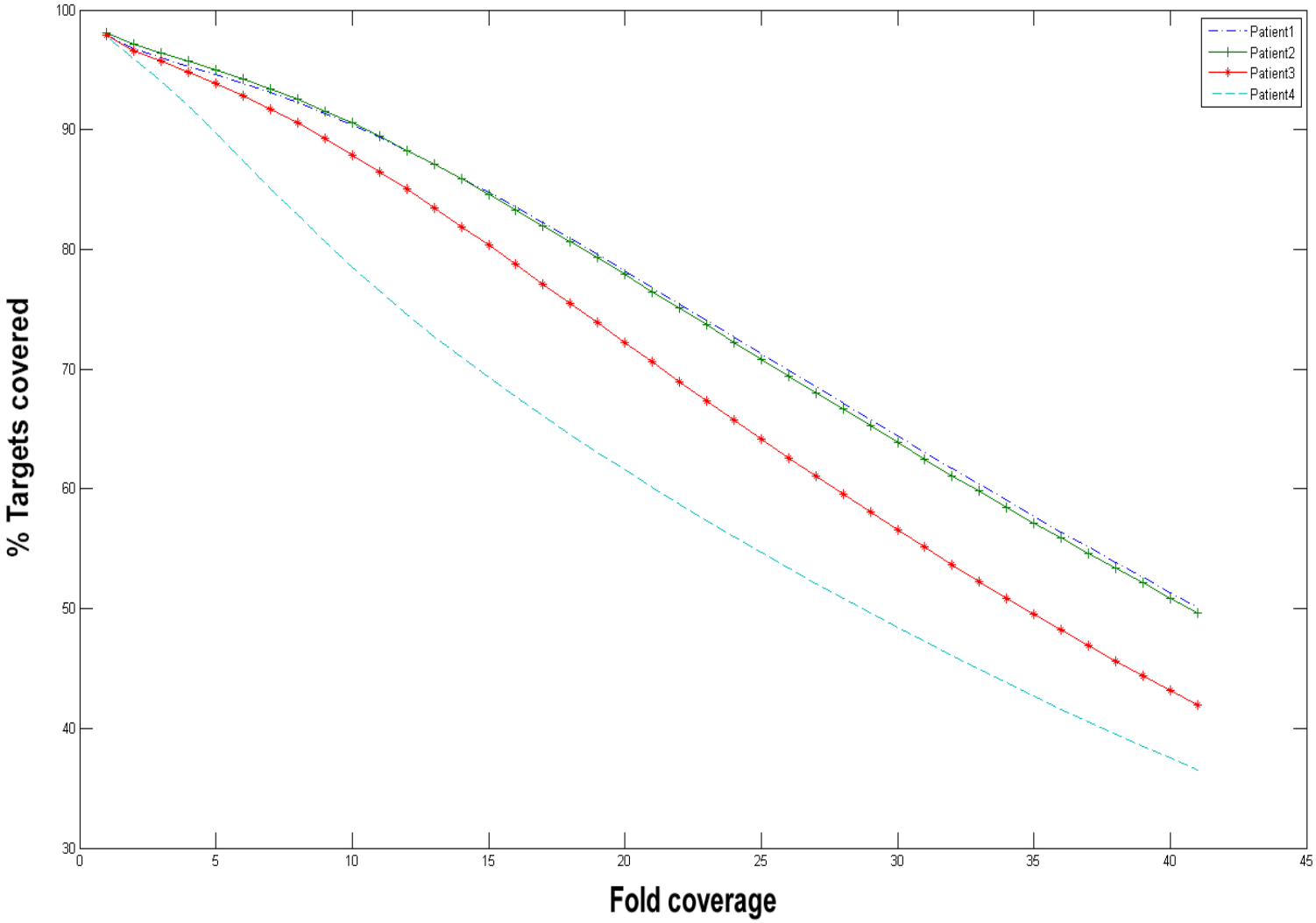

Figure legend:

Comparison of fold coverage (x-axis) and percentage of covered targets (y-axis) for all 4 samples.

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Supplementary Figure 2: Comparison of fold coverage of the *SETBP1* gene for all 4 samples.

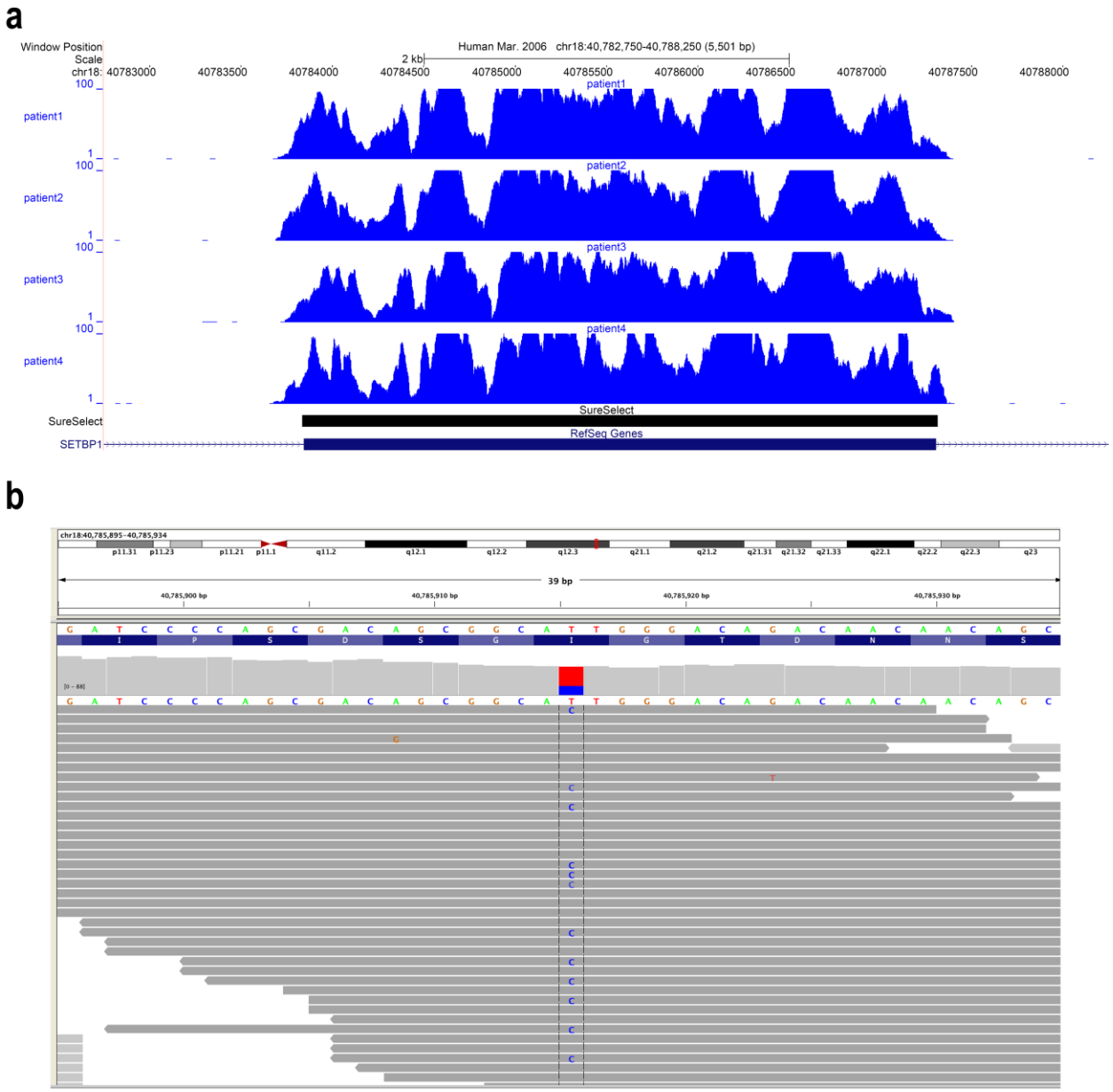

Figure legend:

a) Coverage histogram of read depth per basepair of exon 4 of *SETBP1*, displayed in hg18 version of the UCSC genome browser.

b) SOLiD sequencing reads displayed for patient 1. Reads overlapping the mutation in exon 4 of *SETBP1* (bp position g.40785915, hg18, NCBI36) show the heterozygous T>C change.

Supplementary Figure 3: Confirmation of *de* novo SETBP1 mutations by Sanger sequencing

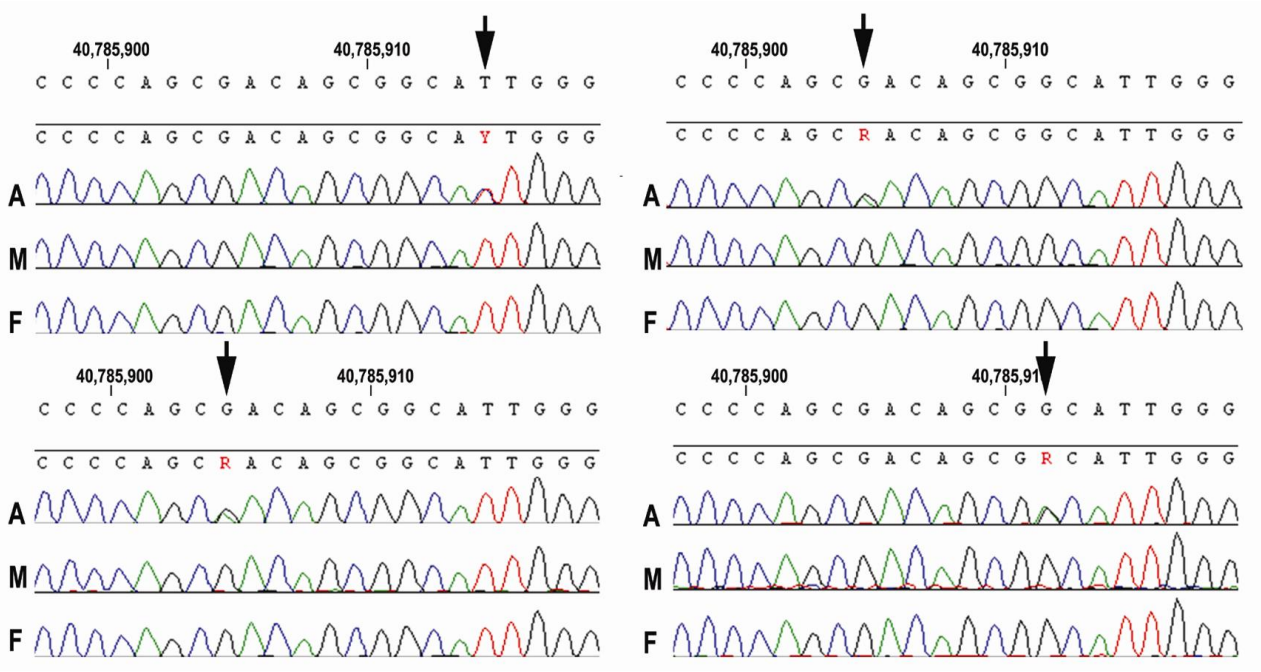

Figure legend:

Confirmation and *de novo* proof by Sanger sequencing, shown for Patient 1-4 (A = affected), in comparison with their respective parents (M = mother, F = father).

Supplementary Note:

Clinical samples

All 13 Schinzel-Giedion syndrome patients fulfilled the diagnostic criteria suggested by Lehman et al.[1] (Table 1 and supplementary Table 3) and are from various parts of the world (Europe (n=7), New-Zealand (n=3), Australia (n=2) and the USA (n=1)). The DNA samples studied from 13 subjects and 94 normal control DNAs were isolated from peripheral blood leucocytes following standard techniques. This study was approved by the Medical Ethics Committee of the Radboud University Nijmegen Medical Center. Written informed consent to participate in the study was obtained for all patients as well as informed consent to publish clinical photos for cases 1, 3 and 4.

Exome Sequencing

DNA was obtained from peripheral blood of all patients. This study was approved by the Medical Ethics Committee of the Radboud University Nijmegen Medical Centre. For exome enrichment 3µg genomic DNA was required. We used a AB SOLiD optimized SureSelect human exome kit (Agilent, Santa Clara, CA, USA), which contained exonic sequences of ~18.000 genes, covering a total of 37Mb genomic sequences, following the manufacturer's instructions. The enriched exome libraries were subsequently used for e-PCRs following manufacturer's instructions (Life Technologies, Carlsbad, CA, USA), based on a library concentration of 0.5pM. For each sample one quad of a SOLiD sequencing slide (Life Technologies, Carlsbad, CA, USA) was used.

Color space reads were mapped to the hg18 reference genome with the SOLiD bioscope software which utilizes an iterative mapping approach. Single nucleotide variants were subsequently called by the DiBayes algorithm[2] using the conservative default call stringency. Small insertions and deletions were detected using the SOLiD Small InDel Tool. Called SNP variants and indels were combined and annotated using a custom analysis pipeline.

For prioritization we excluded known SNPs from dbSNP v1.30 and variants from our in-house database which consists of data from in-house targeted resequencing projects (6,595 variants), the 1000 genomes project (http://www.1000genomes.org) as well as published data from various studies[3-5] (2,535,563 variants).

Sanger Sequencing validation

Validation and *de novo* testing for the mutation hotspot was performed by classical Sanger sequencing using following primers. Additional 94 DNAs from normal controls were analyzed for the same amplicon.

No.	Name	Sequence
1	SETBP1_exon4_F	CTTCACCAGCAGCTATGCAC
2	SETBP1_exon4_R	CGGTGGGAGATTCTGAACAC

To exclude additional changes in all individuals with Schinzel-Giedion syndrome the following primers have been used to generate amplicons for all coding exons:

No.	Name	Sequence
3	SETBP1_Exon1_Fw	GGTAAGGAAAGGGGTGGG
4	SETBP1_Exon1_Rev	CAAGAAAGAGACTCAAATCCATTG
5	SETBP1_Exon2.1_Fw	GGGCCTCTGGAACTTAGATTG
6	SETBP1_Exon2.1_Rev	CCGCCCTGAGCCTAGTTC
7	SETBP1_Exon2.2_Fw	TTTGCTCTCCACTCCAGGAC
8	SETBP1_Exon2.2_Rev	GACCCTAAGAAGTTGAGGATAAAATG
9	SETBP1_Exon3_Fw	TGGTAAGTCCATTGCTGGTC
10	SETBP1_Exon3_Rev	ATCCAAGGTTCGGGTTTCTG
11	SETBP1_Exon4.1_Fw	CATGCTCATCTTTGTTTCTCTCTC
12	SETBP1_Exon4.1_Rev	ATGCTTTCTGGGCATTCTTG
13	SETBP1_Exon4.2_Fw	CCCCAGAACCACCTACGG
14	SETBP1_Exon4.2_Rev	TTTGGATGCTGGATTTCTGG
15	SETBP1_Exon4.3_Fw	CCCAGGAGGTGTGTCTAAGC
16	SETBP1_Exon4.3_Rev	CTTTTGCCTTCAGAGCAACG
17	SETBP1_Exon4.4_Fw	AGAAAGTTGGAAAGCTCGGC
18	SETBP1_Exon4.4_Rev	GGACAGCGTGATTTCCTTTAG
19	SETBP1_Exon4.5_Fw	ACACAGTGGAACCTGGAAGC
20	SETBP1_Exon4.5_Rev	AGGCCTACCACGCTTCTTC

21	SETBP1_Exon4.6_Fw	ACCACGAGAATCCATATCCC
22	SETBP1_Exon4.6_Rev	CTTTGTCTGCGCTACTCAGC
23	SETBP1_Exon4.7_Fw	CACAAGCATAAGCACAAGGAAG
24	SETBP1_Exon4.7_Rev	ATGTGTCTGAGGTGCAAAGC
25	SETBP1_Exon5_Fw	TGTTGTCTATCTTCCTGTTCCC
26	SETBP1_Exon5_Rev	TCAACAGGCCATTCTCAGTG
27	SETBP1_Exon6.1_Fw	TTGAAGGCACCTTGCATC
28	SETBP1_Exon6.1_Rev	TTCACCTCCTCTTCCTGGG
29	SETBP1_Exon6.2_Fw	GGTCATCCACATGGCCC
30	SETBP1_Exon6.2_Rev	AATTTGCAGCTTTTCTCCCC
31	SETBP1_Exon6.1_Fw2	AATAATGCATCTTGGGCACG
32	SETBP1_Exon6.1_Rev2	TGTGTTTCCTCTTTCCGCC
33	SETBP1_Exon6.2_Fw2	CCACCCAGTTCGATGAGG
34	SETBP1_Exon6.2_Rev2	TCTGGAGAGAAGGGCGTG
35	SETBP1_Exon6.3_Fw	GCACGGAGACATACCCATAGC
36	SETBP1_Exon6.3_Rev	CTTCTGGAGAGAAGGGCGTG
37	SETBP1_Exon6.4_Fw	AAAAGGAATAATGCATCTTGGG
38	SETBP1_Exon6.4_Rev	AGAGAAGGGCGTGGGTGTC

Parentage testing

To confirm parentage in all 10 trios, 16 STR markers have been tested with the AmpFlSTR® Identifiler® PCR Amplification Kit (Applied Biosystems Inc., Foster City, CA, USA). Parentage was proven for all trios.

Mutations interpretation

The missense mutations have been tested for mutational effect by the amino acid substitution (AAS) prediction methods SIFT and PolyPhen prediction programs the prediction output is stated in Table 1.

Amino acid conservation

Accession number of the *SETBP1* protein sequences used for amino acid sequence comparison (Figure 1B) are as follows: Homo sapiens (NM_015559.2); Pan troglodytes (ENSPTRT00000018330); Pongo pygmaeus (ENSPPYT00000010655); Macaca mulatta (ENSMMUT00000007041); Rattus norvegicus (ENSRNOT00000021717); Mus musculus (ENSMUST00000025430); Canis familiaris (ENSCAFT00000028030); Felis catus (ENSFCAT00000007348); Bos taurus (ENSBTAT00000024078); Gallus gallus (ENSGALT00000002566); Xenopus tropicalis (ENSXETT00000017900).

Web resources

For variant visualization we used the IGV browser developed at:

http://www.broadinstitute.org/igv

For in-house variant database:

http://www.1000genomes.org

For genome browser annotations:

http://genome.ucsc.edu

For variant effect of identified mutations:

http://sift.jcvi.org/

http://genetics.bwh.harvard.edu/pph/

Supplementary Reference List

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