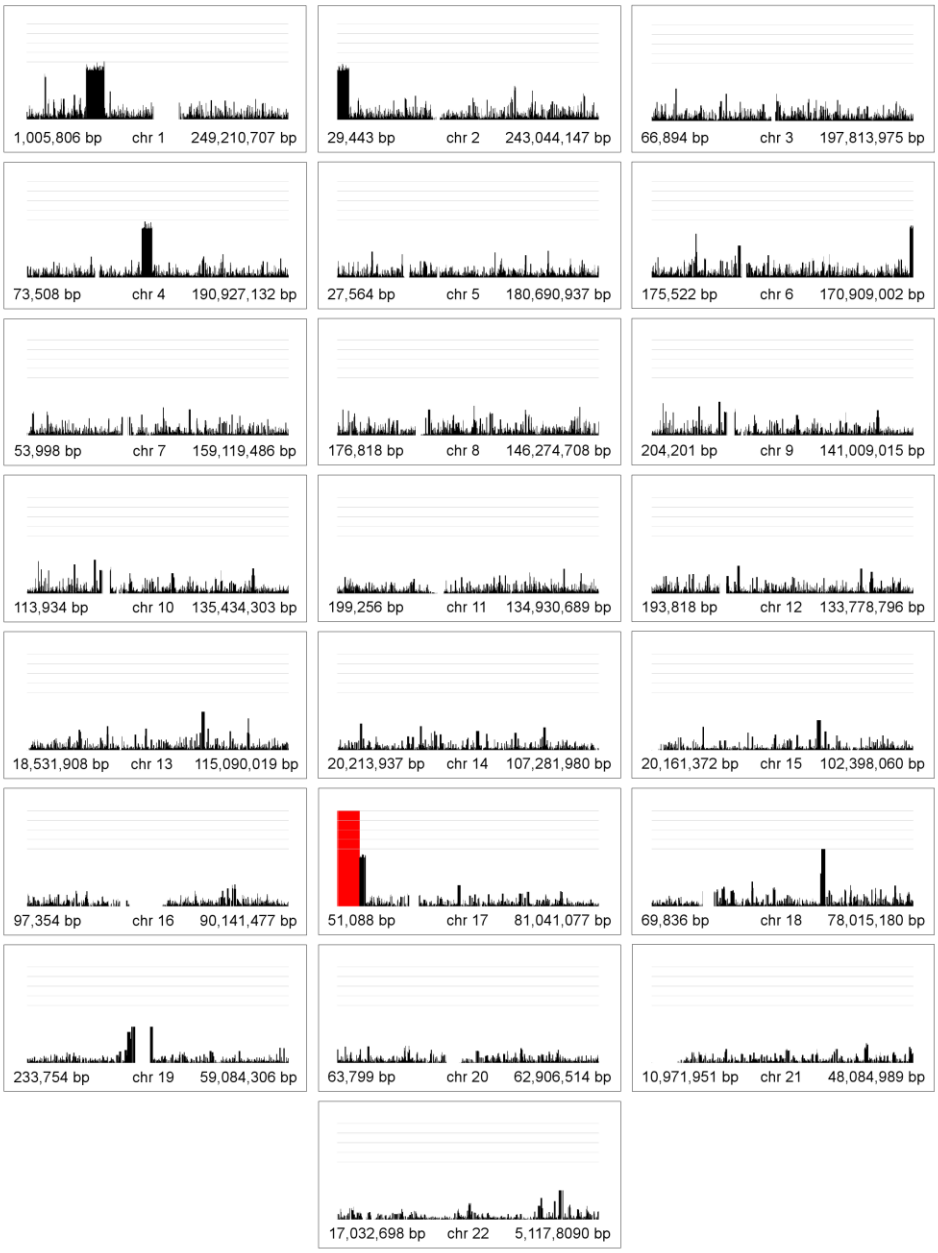
Homozygosity mapping and targeted genomic sequencing reveal the gene responsible for cerebellar hypoplasia and quadrupedal locomotion in a consanguineous kindred

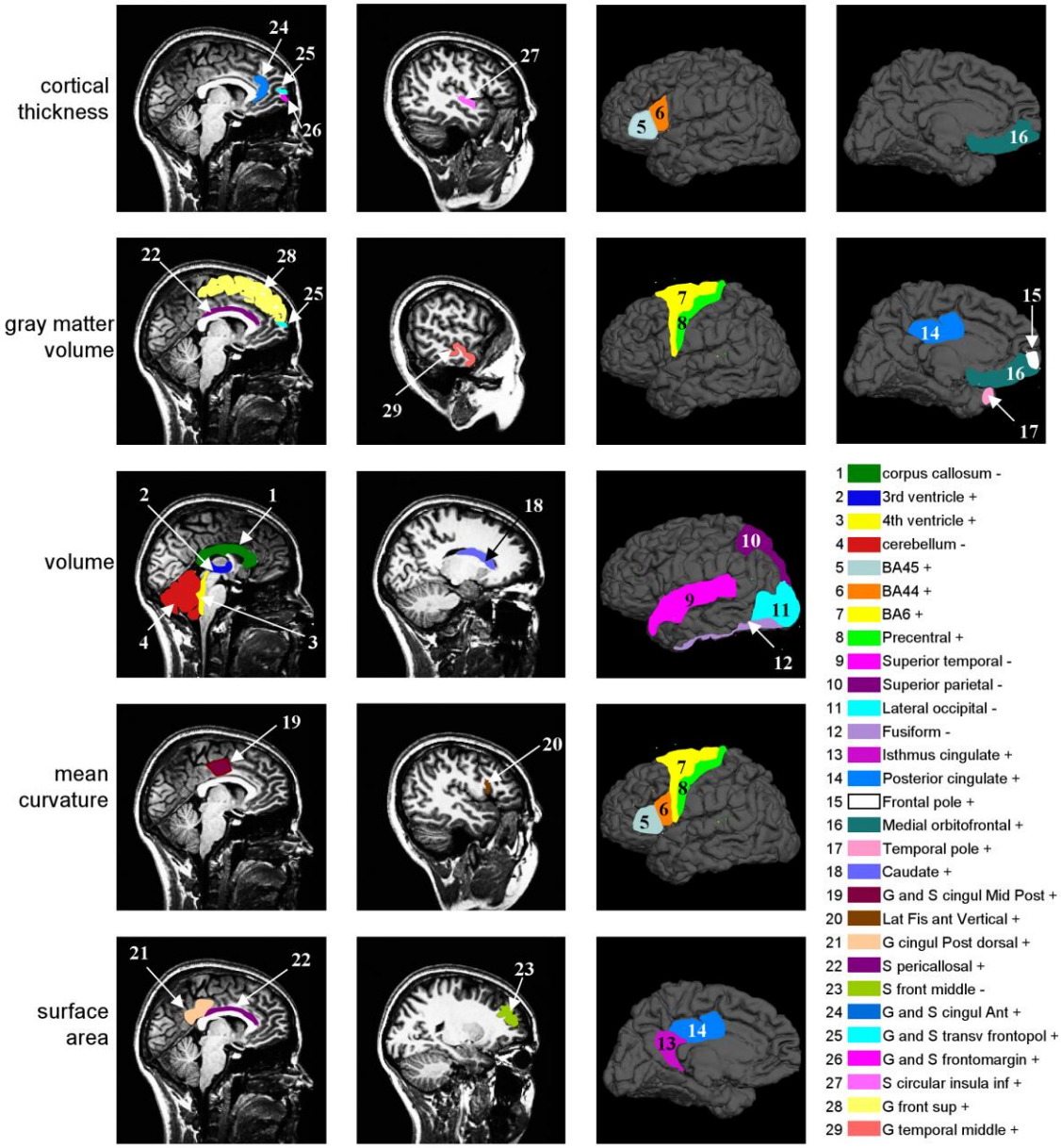
Suleyman Gulsuner, Ayse Begum Tekinay, Katja Doerschner, Huseyin Boyaci, Kaya Bilguvar, Hilal Unal, Aslihan Ors, O. Emre Onat, Ergin Atalar, A. Nazli Basak, Haluk Topaloglu, Tulay Kansu, Meliha Tan, Uner Tan, Murat Gunel, Tayfun Ozcelik

Supplemental Material

Supplemental Figures 1-5

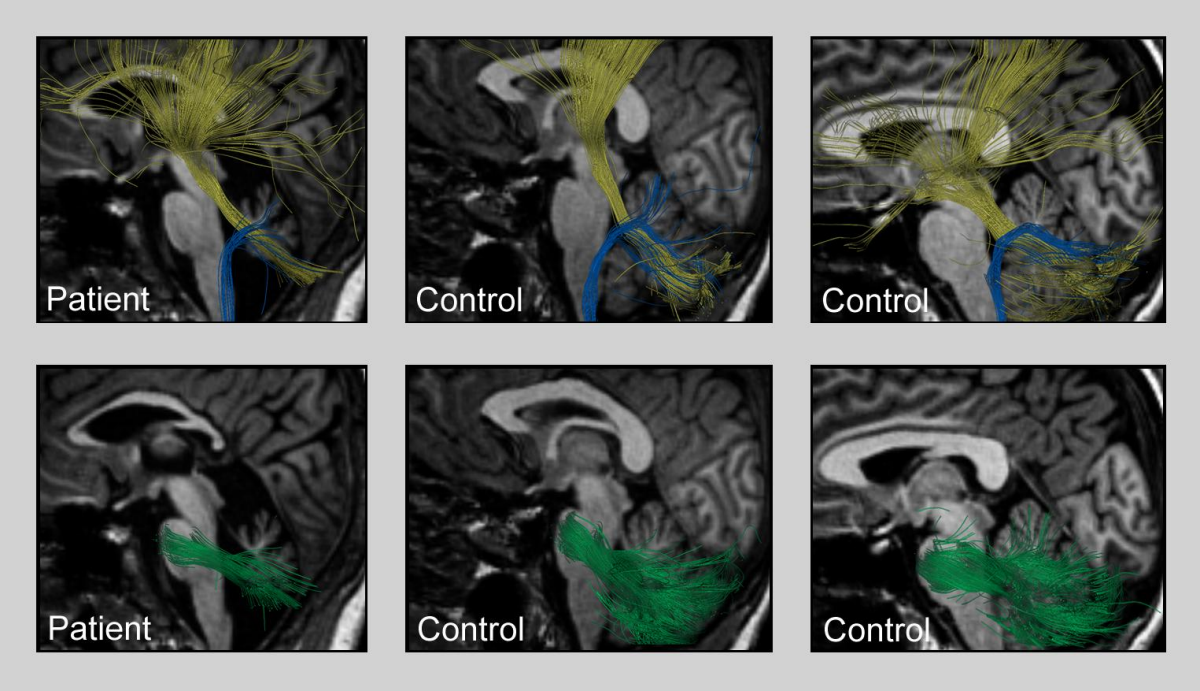


Supplemental Figure 1. Homozygosity mapping analysis of two affected individuals. Illumina 300 Duo v2 BeadChip SNP genotype data of 05-984 and 05-987 was used to verify previously reported linkage interval. A single homozygous locus was detected between 114,669-6,917,703 base pairs on chromosome 17. Y-axis of the graphs indicates genome-wide homozygosity scores (max=500) calculated by HomozygosityMapper software. Red bars refer to the most promising genomic regions.

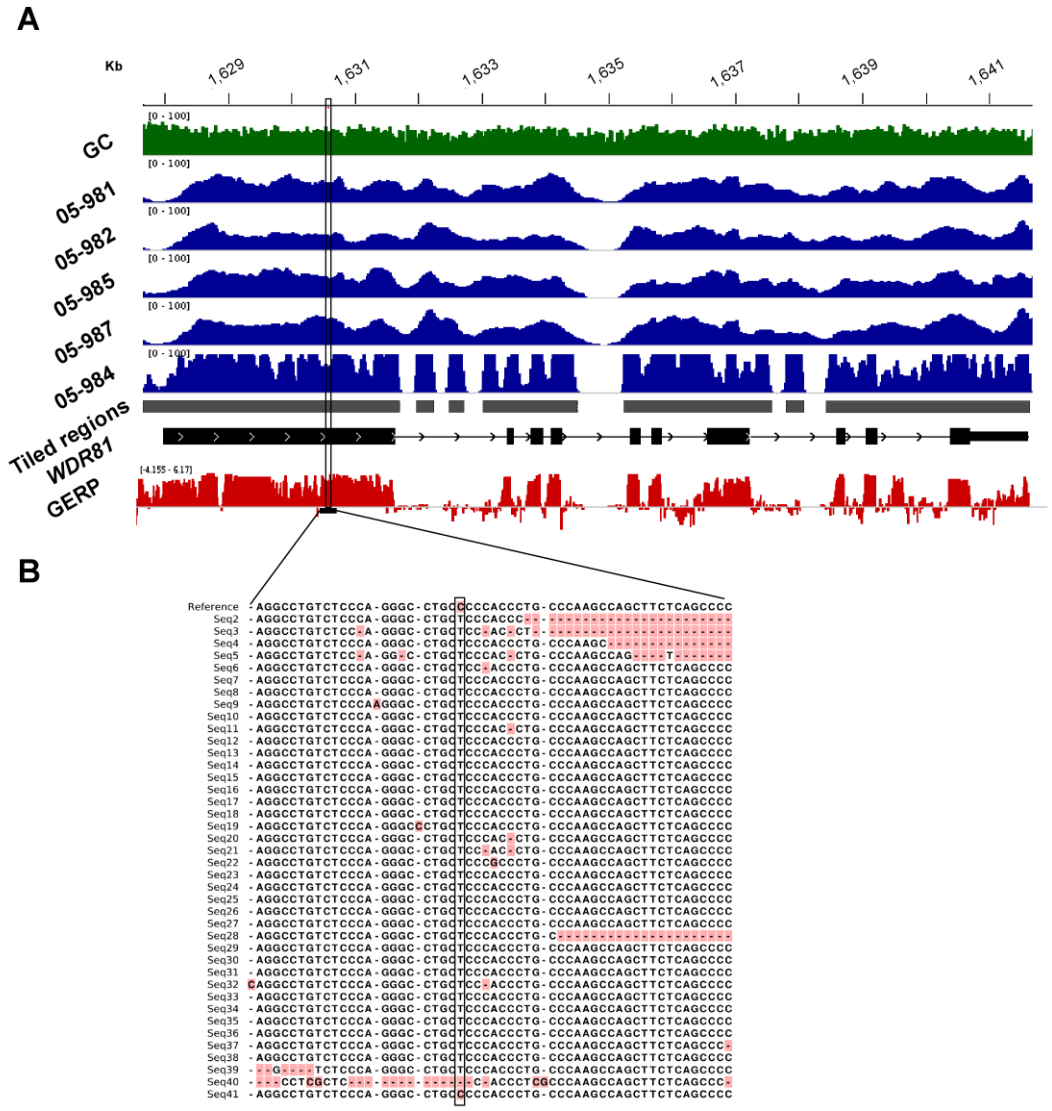


Supplemental Figure 2. A detailed account of the morphometric analyses. The regions that show significant differences between patients and controls are displayed on a reference sagittal view or lateral and medial view of the 3D-reconstructed cortex. The structures labeled as + and - indicate the areas where the curvature/thickness/area or volume is increased and decreased respectively in patients. An increase in cortical thickness primarily in the motor speech areas pars opercularis and pars triangularis, as well as in other structures such as medialorbitofrontal region, fronto-marginal gyrus (of Wernicke) and sulcus, transverse frontopolar gyri and sulci, anterior part of the cingulate gyrus and sulcus, and inferior segment of the circular sulcus of the insula were observed in patients when compared to healthy controls. Increased gray matter volumes were found in patients' motor areas (precentral gyrus, BA6), posterior

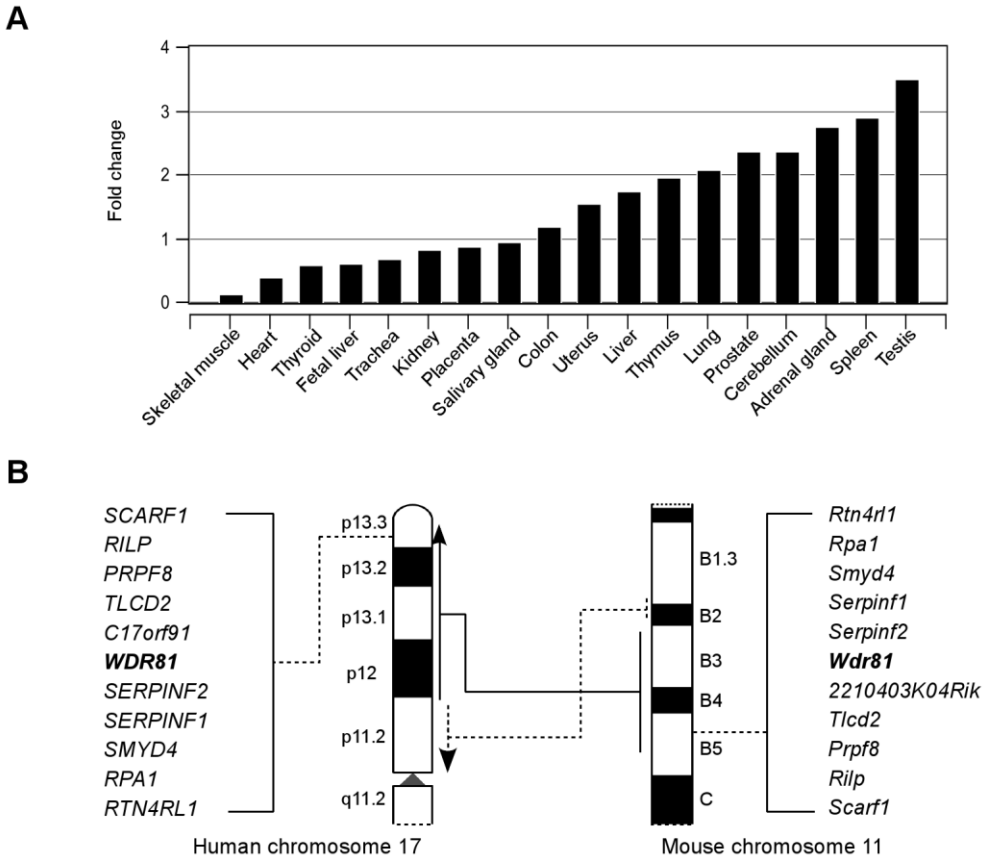
cingulate, pericallosal sulcus and medial orbitofrontal region as well as in superior frontal, frontal pole, transverse frontopolar gyri and sulci, temporal pole and middle temporal gyrus. The volume of the cerebellum, corpus callosum (posterior region, specifically), fusiform, lateral occipital, superior parietal and superior temporal regions were significantly smaller. In contrast, the cerebro spinal fluid volume in the 3rd and 4th ventricles and the caudate nucleus were increased. A more generalized calculation revealed that total cortical white matter volume and subcortical gray matter volume were smaller in the patients whereas total cortical gray matter volume was larger (Supplemental Table 1). Mean curvature analysis revealed higher cortical folding in patients' motor speech areas BA44 and BA45, precentral gyrus (primary motor area) and BA6 (supplementary motor area). Moreover, patients' middle-posterior part of the cingulate gyrus and sulcus, and vertical ramus of the anterior segment of the lateral sulcus showed significantly higher mean curvature. Surface area values showed enlargements in isthmus and posterior cingulate as well as posterior-dorsal part of the cingulate gyrus and pericallosal sulcus, whereas the middle frontal sulcus was diminished in surface area.



Supplemental Figure 3. Diffusion tensor imaging (DTI) and fiber tractography. Fibers of the inferior middle and superior cerebellar peduncles of 1 female patient and 2 controls are shown. Peduncles of the patient are atrophic compared to healthy controls; this is most obvious in the middle cerebellar peduncle.



Supplemental Figure 4. NGS coverage and alignment information of *WDR81* mutation. (A) Visualization of coverage histogram by Integrative Genomics Viewer (IGV). GC content in a five-base window, 454 (05-981, 05-982, 05-985 and 05-987) and Illumina (05-984) read depth histogram per base pair (coverage between 0 and 100-fold is displayed; >100-fold coverage is not shown), NimbleGen probe coverage (tiled regions) and Genomic Evolutionary Rate Profiling (GERP) histogram based on an alignment of 35 mammals (allHg19RS_BW track from UCSC genome browser, http://genome.ucsc.edu) across *WDR81* are diplayed. (B) Alignment of reads overlapping with the mutation is displayed for patient 05-985. 39/40 of the reads show the homozygous mutation. Vertical boxes indicate mutation site.



Supplemental Figure 5. Expression pattern of *WDR81*. (A) Initial screen in different human tissues. (B) Physical location of *WDR81/Wdr81* in human and mouse chromosomes. Approximately 15 Mb of mouse chromosome 11 is syntenic to human chromosome 17p, which also includes CAMRQ 2 linkage interval. Closely linked orthologous genes flanking *WDR81/Wdr81*, 5 on each side, is shown. Based on the similarity of sequences and conserved synteny, *Wdr81* is the mouse ortholog of human *WDR81*.

Supplemental Tables 1-13

Supplemental Table 1. Brain regions with significant differences between the patients and controls.

	CAMRQ2	Control	p	T(8)	CAMRQ2	Control	p	T(8)
				Cortical Thickness (mm)				
BA44	2.72±0.02	2.43±0.10	<0.005	3.92	2.59±0.07	2.35±0.10	<0.02	3.32
BA45	2.52±0.03	2.24±0.16	<0.05	2.41	2.49±0.04	2.15±0.11	<0.004	4.07
Medial orbito frontal	2.66±0.04	2.27±0.16	<0.02	3.35	2.66±0.04	2.27±0.16	<0.02	3.35
Pars opercularis	2.77±0.11	2.45±0.10	<0.006	3.78	2.54±0.09	2.39±0.08	<0.05	2.35
Pars triangularis	2.54±0.04	2.21±0.17	<0.04	2.62	2.49±0.02	2.20±0.09	<0.003	4.43
G and S frontomargin	2.31±0.16	2.00±0.14	<0.03	2.79	2.38±0.17	1.91±0.09	<0.0006	5.54
G and S transv frontopol	2.53±0.22	2.27±0.10	<0.04	2.57	2.59±0.03	2.23±0.10	<0.002	4.66
G and S cingul ant	2.83±0.10	2.38±0.23	<0.03	2.65	2.65±0.36	2.13±0.09	<0.004	4.05
G front inf opercular	2.94±0.02	2.71±0.13	<0.04	2.49	2.80±0.08	2.56±0.08	<0.007	3.68
G front inf triangul	2.91±0.04	2.44±0.17	<0.006	3.71	2.80±0.01	2.45±0.12	<0.005	3.97
S circular insula inf	3.14±0.15	2.76±0.21	<0.05	2.38	3.16±0.29	2.72±0.13	<0.02	3.35
				Mean Curvature (1/mm)				
BA6	0.14±0.00	0.14±0.00	<0.03	2.83	0.15±0.01	0.13±0.00	<0.0006	5.55
BA44	0.15±0.01	0.14±0.01	<0.05	2.34	0.15±0.01	0.13±0.01	<0.02	3.11
BA45	0.17±0.01	0.15±0.01	<0.04	2.46	0.17±0.00	0.15±0.01	<0.05	2.42
Pars opercularis	0.16±0.00	0.14±0.01	*<0.03*	*2.67*	0.16±0.01	0.14±0.01	<0.03	2.83
Precentral	0.13±0.00	0.13±0.00	*<0.02*	*2.95*	0.14±0.01	0.13±0.01	<0.02	3.00
G and S cingul mid post	0.16±0.00	0.15±0.00	<0.002	4.71	0.16±0.00	0.14±0.00	<0.03	4.44
G precentral	0.15±0.00	0.13±0.01	<0.04	2.54	0.16±0.01	0.14±0.01	<0.009	3.48
Lat fis ant vertical	0.14±0.02	0.12±0.01	<0.04	2.53	0.14±0.00	0.11±0.01	<0.04	2.51
				Surface Area (mm2) normalized to total surface area				
Isthmus cingulate	1.49±0.14	1.26±0.09	*<0.03*	*2.76*	1.46±0.11	1.23±0.12	*<0.05*	*2.38*
Posterior cingulate	1.79±0.06	1.52±0.13	*<0.03*	*2.84*	1.82±0.09	1.43±0.12	*<0.003*	*4.34*
G cingul post dorsal	0.58±0.05	0.48±0.05	<0.04	2.53	0.54±0.02	0.46±0.04	<0.05	2.31
S front middle	1.16±0.04	1.61±0.19	<0.02	-3.14	1.17±0.17	1.54±0.09	<0.004	-4.18
S pericallosal	1.38±0.04	1.09±0.10	<0.005	3.92	1.49±0.10	1.05±0.10	<0.0008	5.27
				Volume (mm3) normalized to supratentorial volume				
Cerebellum WM	1.31±0.04	3.08±0.29	<0.00002	-9.09	1.25±0.01	2.91±0.26	<0.00003	-8.47
Cerebellum cortex	4.61±1.06	9.94±1.06	<0.0002	-6.75	4.54±0.30	9.39±1.02	<0.0003	-6.34
Caudate	0.92±0.10	0.75±0.07	<0.02	3.01	0.92±0.07	0.70±0.06	<0.003	4.32
3rd ventricle	0.20±0.02	0.08±0.01	<0.00002	9.53	0.18±0.03	0.10±0.02	<0.003	4.30
4th ventricle	0.28±0.01	0.16±0.04	<0.004	4.15	0.29±0.05	0.13±0.05	<0.004	4.07
CSF	0.14±0.01	0.08±0.01	<0.006	3.75	0.11±0.02	0.08±0.01	<0.04	2.54
Corpus callosum posterior	0.04±0.02	0.09±0.01	<0.003	-4.41	0.05±0.03	0.09±0.01	<0.009	-3.48
Corpus callosum	0.19±0.05	0.31±0.04	<0.007	-3.68	0.24±0.08	0.32±0.03	<0.03	-2.75
Cortical wm volume	38.67±1.02	45.96±2.11	<0.002	-4.58	41.38±0.93	48.48±1.64	<0.0005	-5.68
Cortical gm volume	49.24±1.12	44.53±1.87	<0.02	3.31	46.59±1.55	42.15±1.43	<0.006	3.83
Subcortical gm volume	12.35±0.33	17.63±1.24	<0.0005	-5.71	12.51±1.05	16.93±1.17	<0.002	-4.79
Fusiform	0.99±0.05	1.28±0.13	<0.03	-2.83	1.11±0.01	1.40±0.15	<0.03	-2.71
Lateral occipital	1.46±0.04	1.67±0.09	<0.02	-3.05	1.44±0.07	1.75±0.12	<0.02	-3.29
Superior parietal	1.80±0.02	2.29±0.27	<0.04	-2.47	1.92±0.06	2.38±0.20	<0.02	-2.99
Superior temporal	1.13±0.02	1.35±0.11	<0.04	-2.63	1.18±0.10	1.42±0.09	<0.02	-3.19
				Gray Matter Volume (mm3) – normalized to supratentorial volume				
BA6	4.60±0.11	3.64±0.55	<0.05	2.35	4.05±0.23	3.45±0.22	<0.008	3.51
Medial orbito frontal	1.23±0.05	0.95±0.10	*<0.005*	*3.98*	1.03±0.05	0.84±0.05	*<0.003*	*4.42*
Posterior cingulate	0.91±0.07	0.73±0.09	*<0.04*	*2.61*	0.86±0.04	0.64±0.05	*<0.0004*	*5.86*
Precentral	3.11±0.09	2.51±0.25	*<0.02*	*3.17*	3.00±0.16	2.48±0.16	*<0.004*	*4.03*
Superior frontal	4.96±0.06	4.05±0.39	*<0.02*	*3.15*	4.70±0.24	3.91±0.20	*<0.002*	*4.79*
Frontal pole	0.21±0.04	0.16±0.02	*<0.02*	*3.27*	0.19±0.01	0.12±0.02	*<0.008*	*3.57*
Temporal pole	0.60±0.01	0.43±0.08	*<0.03*	*2.72*	0.55±0.09	0.39±0.06	*<0.02*	*3.03*
G and S transv frontopol	0.46±0.02	0.35±0.04	<0.02	3.23	0.43±0.02	0.31±0.04	<0.003	4.25
G front sup	4.04±0.24	3.10±0.36	<0.01	3.40	3.56±0.13	2.93±0.17	<0.002	4.72
G precentral	1.52±0.06	1.17±0.14	<0.02	3.35	1.42±0.09	1.16±0.10	<0.01	3.42
G temporal middle	2.07±0.33	1.64±0.17	<0.03	2.65	1.85±0.16	1.50±0.17	<0.03	2.67
Pole temporal	1.46±0.19	1.11±0.15	<0.03	2.76	1.26±0.02	0.97±0.14	<0.03	2.75
S pericallosal	0.40±0.02	0.32±0.03	<0.01	3.38	0.41±0.00	0.31±0.05	<0.02	2.92

Supplemental Table 2. Summary statistics of Roche 454 NGS data.

	05-981 (carrier)	05-982 (carrier)	05-985 (affected)	05-987 (affected)
Mapped bases (Mb)	403.5	362.7	410.2	399.2
% of total bases	99.4	99.1	99.3	99.4
Mapped reads (x10^6)	1.20	1.78	1.21	1.13
% of total reads	98.3	97.6	98.3	98.3
Target base coverage (%)	96.9	96.6	96.7	96.7
Fold enrichment	1,963	1,275	1,622	2,247
Mean coverage	48.4x	40.5x	47.4x	48.9x
All variants	26,751	27,471	24,115	24,690
dbSNP + CEU[a]	11,674	11,703	8,991	9,106
Novel variants	15,077	15,768	15,124	15,584
High confidence variants	11,942	12,801	9,805	9,592
dbSNP + CEU[a]	10,443	10,426	8,150	8,235
Novel heterozygous	678	1,020	421	332
Novel homozygous	821	1,355	1,234	1,025

[a]: CEU population 1000Genomes variants.

Supplemental Table 3. Coverage of the target region.

Depth	05-981	05-982	05-985	05-987	05-984
0	0.16	0.17	0.17	0.22	0.19
≥2	99.73	99.67	99.72	99.64	99.74
≥4	99.47	99.35	99.48	99.39	99.59
≥10	98.31	97.65	98.28	98.02	99.07
≥15	96.72	95.14	96.58	96.26	98.44
≥20	94.29	91.23	94.05	93.79	97.67
≥30	86.81	78.36	86.07	85.71	95.51

Supplemental Table 4. Gene symbols, genomic coordinates, gap sizes, tiling status, and GC percentage of coding regions with limited coverage.

Position (hg19)	Size	GC Content	Accession code	Gene	Status[a]
chr17: 263352-263385	34	73.50%	NM_001013672	*C17orf97*	Low coverage
chr17: 882713-882715	3	100%	NM_022463	*NXN*	Low coverage
chr17: 1174341-1174383	43	86%	NM_001164405	*BHLHA9*	Low coverage
chr17: 2966802-2966821	20	45%	NM_014566	*OR1D5*	Low coverage
chr17: 2966822-2966893	72	52.80%	NM_014566	*OR1D5*	Not covered
chr17: 2966894-2966901	8	50%	NM_014566	*OR1D5*	Low coverage
chr17: 4402342-4402343	2	50%	NM_001124758	*SPNS2*	Not covered
chr17: 4402344-4402351	8	50%	NM_001124758	*SPNS2*	Low coverage
chr17: 6459704-6459726	23	73.90%	NM_001165966	*PITPNM3*	Low coverage
chr17: 6459710-6459724	15	86.70%	NM_001165966	*PITPNM3*	Not covered

[a]: Not covered: <2X sequence depth. Low coverage: 2-3X sequence depth.

Supplemental Table 5: Heterozygous SNPs in the homozygous block[a].

		Illumina 300Duo V2		454 FLX NGS	
dbSNP id	**Position (hg19)**	**Genotype**	**Score**	**Genotype**	**Read / %**
rs7217872	chr17: 88,988	CT	0.63	CT	25 / 68
rs4617924	chr17: 114,669	CA	0.86	CA	84 / 50
rs719913	**chr17: 1,727,298**	**GG**	**0.88**	**AG**	**35 / 69**
rs231674	**chr17: 3,190,533**	**CC**	**0.86**	**TC**	**27 /67**
rs7338	chr17: 6,917,703	CT	0.85	CT	27 / 48
rs10521149	chr17: 6,932,140	AC	0.8	AC	46 / 46
rs312456	chr17: 6,938,335	AG	0.81	AG	27 / 63
rs13342692	chr17: 6,946,287	TC	0.82	TC	32 / 34
rs364569	chr17: 6,979,179	AG	0.81	AG	66 / 64
rs444207	chr17: 6,982,444	AG	0.86	AG	47 / 40
rs8078782	chr17: 6,996,653	CA	0.91	CA	26 / 54
rs3760352	chr17: 7,019,878	AG	0.87	AG	52 / 46
rs2062737	chr17: 7,031,638	AG	0.45	AG	46 / 46
rs12150124	chr17: 7,036,251	TC	0.93	TC	33 / 42
rs314245	chr17: 7,040,878	CT	0.92	CT	51 / 47
rs1237044	chr17: 7,042,246	TC	0.86	TC	23 / 57
rs3826408	chr17: 7,101,292	CT	0.9	CT	60 / 52
rs390200	chr17: 7,109,995	AG	0.71	AG	29 / 38
rs2074222	chr17: 7,129,974	AG	0.86	AG	34 / 38
rs222851	chr17: 7,139,238	GA	0.83	GA	17 / 59
rs222852	chr17: 7,140,606	AG	0.82	AG	61 / 43
rs1215	chr17: 7,163,350	AG	0.83	AG	25 / 64
rs4796407	chr17: 7,245,371	AG	0.91	AG	55 / 51
rs11657406	chr17: 7,249,870	GT	0.75	GT	41 / 37
rs2292068	chr17: 7,253,659	TC	0.89	TC	14 / 57

[a]: 23/1004 SNPs were called as heterozygous by both genotyping and sequencing platforms in 05-987. 2/1004 SNPs were called heterozygous only with the sequencing platform. Alignment analysis showed that these SNPs (rs719913, rs231674) are variant detection errors.

Supplemental Table 6. Previously reported nonsynonymous SNPs compatible with the Mendelian transmission of the disease allele[a].

SNP	Gene	Pos[b]	Ref	Var	05-981	05-982	05-985	05-987	Annotation	Homozygous	SIFT [c]	Polyphen2 [d]
rs9905106	*MYO1C*	chr17: 1,373,518	T	C	23 (0.43)	25 (0.44)	35 (1.00)	43 (1.00)	Q826R	+	T. (0.5)	B. (0.18)
rs2070862	*SERPINF2*	chr17: 1,648,294	C	T	47 (0.45)	39 (0.59)	40 (1.00)	37 (1.00)	A2V	+	D. LC (0)	B. (0.90)
rs216195	*SMG6*	chr17: 2,203,167	T	G	60 (0.38)	39 (0.54)	53 (1.00)	58 (1.00)	K294Q	+	D. LC (0)	B. (1.27)
rs41333251	*OR1E2*	chr17: 3,337,057	A	G	37 (0.54)	35 (0.43)	38 (1.00)	47 (1.00)	C27R	+	D. (0.01)	Pos. D. (1.83)
rs55916885	*TRPV1*	chr17: 3,495,391	T	C	32 (0.56)	33 (0.61)	37 (1.00)	36 (1.00)	Q85R	-	T. (0.76)	B. (1.36)
rs150857	*SHPK*	chr17: 3,526,637	C	T	79 (0.39)	42 (0.38)	64 (0.91)	70 (0.99)	E215K	+	D. (0.05)	B. (1.28)
rs2976230	*ITGAE*	chr17: 3,631,241	A	G	65 (0.38)	48 (0.54)	52 (1.00)	71 (1.00)	V1019A	+	T. (0.09)	B. (1.31)
rs7214723	*CAMKK1*	chr17: 3,775,848	T	C	45 (0.40)	26 (0.54)	38 (1.00)	38 (0.95)	E375G	+	T. (0.26)	B. (0.65)
rs34457931	*SPNS3*	chr17: 4,352,636	G	A	23 (0.39)	25 (0.44)	33 (1.00)	18 (1.00)	G293R	+	T. (0.09)	B. (0.45)
rs3809849	*MYBBP1A*	chr17: 4,458,598	G	C	40 (0.47)	32 (0.62)	31 (1.00)	35 (1.00)	Q8E	+	D. LC (0)	B. (1.04)
rs7216284	*GGT6*	chr17: 4,463,699	G	A	29 (0.52)	22 (0.55)	36 (0.94)	31 (1.00)	R40W	+	T. (0.1)	B. (1.13)
rs1052748	*PLD2*	chr17: 4,720,469	C	T	34 (0.41)	38 (0.47)	36 (1.00)	33 (1.00)	T577I	+	T. (0.24)	B. (0.12)
rs12761	*RPAIN*	chr17: 5,326,145	C	G	86 (0.45)	67 (0.43)	77 (1.00)	73 (1.00)	N103K	+	T. (0.97)	B. (0.188)
rs2304977	*KIAA0753*	chr17: 6,513,329	G	A	39 (0.33)	20 (0.75)	37 (0.97)	33 (0.97)	P566L	+	NS	Pro. D. (2.5)
rs61146770	*KIAA0753*	chr17: 6,524,298	T	A	42 (0.62)	34 (0.50)	43 (1.00)	45 (0.98)	E375D	+	NS	B. (1.25)
rs16956264	*FBXO39*	chr17: 6,683,684	A	G	80 (0.46)	75 (0.56)	77 (1.00)	72 (1.00)	Y166C	+	T. (0.1)	Pro. D. (2.19)
rs7213731	*FBXO39*	chr17: 6,690,164	A	G	78 (0.53)	43 (0.42)	57 (0.98)	80 (1.00)	I363M	+	T. (0.22)	B. (0.22)

[a]: Homozygous inheritance of all SNPs listed above was found in dbSNP except rs55916885, [b]: Position(hg19), [c]:SIFT score, [d]:PSIC score difference. Abbreviations T: Tolerated; D: Damaging; B: Benign; Pos. D: Possibly damaging; Pro. D: Probably damaging; NS: Not scored. Sequence depth and variant frequency for each variant is shown in the same sample column.

Supplemental Table 7. List of novel variants detected in coding regions.

Gene	Pos (hg19)	Mutation	Ref	Var	Annotation	05-981	05-982	05-985	05-987
Candidate variants									
MYO1C [a]	chr17: 1,371,325	Silent	C	T	p.E932E	42 (0.55)	24 (0.54)	26 (1.00)	39 (1.00)
WDR81	chr17: 1,630,820	Missense	C	T	p.P856L	41 (0.51)	33 (0.52)	40 (0.97)	53 (1.00)
MYBBP1A	chr17: 4,448,967	Missense	G	A	p.R671W	29 (0.52)	21 (0.48)	32 (0.97)	29 (1.00)
ZNF594	chr17: 5,085,637	Missense	G	A	p.L639F	39 (0.54)	50 (0.56)	38 (0.97)	37 (1.00)
Excluded by homozygous parents									
C17orf97	chr17: 263,287	Missense	A	G	p.D218G	15 (1.00)	13 (1.00)	9 (1.00)	14 (1.00)
C17orf97	chr17: 263,316	Silent	T	C	p.D228H	13 (0.77)	6 (1.00)	4 (0.75)	8 (0.87)
AC090282.6	chr17: 3,132,364	miRNA	-	C	-	39 (0.95)	37 (1.00)	34 (0.97)	39 (0.97)
TRPV1	chr17: 3,477,172	Frameshift	-	G	p.A620fs	28 (1.00)	17 (1.00)	21 (1.00)	39 (1.00)
ASGR2 [b]	chr17: 7,012,080	Frameshift	-	T	p.H65fs	29 (1.00)	35 (0.54)	36 (1.00)	41 (0.54)
Retained intron [c]									
PELP1	chr17: 4,578,955	Missense	G	A	T402I	46 (0.61)	37 (0.51)	45 (1.00)	60 (1.00)
False positive variants									
RPH3AL	chr17: 169,221	Frameshift	T	-	p.D114fs	46 (0.17)	29 (0.28)	31 (0.23)	51 (0.18)
SLC43A2	chr17: 1,479,053	Frameshift	C	-	p.V519fs	41 (0.15)	33 (0.21)	35 (0.23)	38 (0.16)
PRPF8	chr17: 1,563,808	Frameshift	G	-	p.T1568fs	83 (0.13)	65 (0.20)	66 (0.17)	85 (0.07)
OR1E2	chr17: 3,336,820	Frameshift	A	-	p.Y106fs	75 (0.09)	57 (0.19)	52 (0.12)	74 (0.16)
ITGAE	chr17: 3,664,330	Frameshift	-	C	p.E192fs	40 (0.15)	29 (0.10)	35 (0.11)	43 (0.07)
USP6	chr17: 5,036,205	Missense	A	C	p.K66Q	50 (0.20)	42 (0.17)	42 (0.19)	67 (0.31)
USP6	chr17: 5,036,210	Missense	T	G	I68M	50 (0.20)	42 (0.17)	43 (0.23)	68 (0.35)
DHX33	chr17: 5,353,585	Frameshift	C	-	p.E383fs	52 (0.15)	39 (0.18)	58 (0.12)	46 (0.09)
NLRP1	chr17: 5,418,095	Frameshift	T	-	p.G1467fs	75 (0.19)	54 (0.22)	50 (0.20)	77 (0.16)
KIAA0753	chr17: 6,513,435	Frameshift	T	-	p.K531fs	47 (0.17)	32 (0.22)	37 (0.16)	41 (0.15)

[a]: Nucleotide change does not alter splicing pattern. [b]: Variant is observed outside of the narrowed homozygous region and one parent was detected as homozygous. Sequence depth and variant frequency for each variant is shown in the same sample column. [c]: UCSC Genome Browser and ENSEMBL analysis suggested that, the T402I mutation is actually located in intron 10-11 of *PELP1* gene. This intron was retained in the alternative isoform of the gene (ENST00000301396, Q8IZL8-2) and annotated as an exon in the ENSEMBL database.

Supplemental Table 8. Novel untranslated variants co-inherited with the disease phenotype in Family B [a].

Gene	Pos (hg19)	Mutation	Ref	Var	05-981	05-982	05-985	05-987
ELP2P	chr17: 656,577	3'UTR	C	G	11 (0.36)	14 (0.29)	13 (0.62)	9 (1.00)
HIC1	chr17: 1,962,684	3'UTR	A	-	17 (0.53)	9 (0.67)	16 (1.00)	23 (0.96)
USP6	chr17: 5,076,345	3'UTR	G	C	23 (0.35)	21 (0.52)	26 (1.00)	22 (1.00)
ZNF594	chr17: 5,083,351	3'UTR	A	T	18 (0.61)	12 (0.33)	12 (1.00)	12 (1.00)
ZNF594	chr17: 5,083,804	3'UTR	G	C	24 (0.25)	14 (0.57)	19 (1.00)	21 (1.00)

[a]: Sequence depth and variant frequency for each variant is shown in the same sample column.

Supplemental Table 9. Population screening of missense variants.

	Control	Allele Frequencies		Expected Genotype Frequencies			Observed Genotype Frequencies		
Variant	**Choromosomes**	**A1**	**A2**	**AA**	**AB**	**BB**	**AA**	**AB**	**BB**
WDR81	0/1,098	1	0	1	0	0	1	0	0
ZNF594	4/1,098	0.99636	0.00364	0.99273	0.00726	0.00001	0.99271	0.00728	0
MYBBP1A	15/1,228	0.98779	0.01221	0.97572	0.02413	0.00015	0.97883	0.01792	0.00326

Supplemental Table 10. Conservation and prediction scores of missense variants.

Gene	Pos (hg19)	Ref	Var	Effect	SIFT[a]	PolyPhen[b]	GERP[c]
WDR81	chr17: 1,630,820	C	T	P856L	D. (0)	Pro. D. (2.724)	5.68
MYBBP1A	chr17: 4,448,967	G	A	R671W	D. (0.01)	Pro. D. (2.315)	5.22
ZNF594	chr17: 5,085,637	G	A	L639F	D. LC (0.04)	B. (0.301)	-0.665

[a]:SIFT score, [b]:PSIC score difference, [c]: GERP scores for mammalian alignments (allHg19RS_BW), [d].

Supplemental Table 11. Genes linked to neuronal phenotypes with expression profiles correlated with that of *WDR81.*

Symbol	Name	Disease	OMIM	Correlation
LTM2B	integral membrane protein 2B	Cerebral Amyloid Angiopathy, Itm2b-Related, 1	176500	0.981
PRNP	prion protein	Gerstmann-Straussler Disease; GSD	137440	0.993
LOX	lysyl oxidase	Menkes Disease	309400	0.995
DCX	Doublecortin	Lissencephaly 1; LIS1	607432	0.982
L1CAM	L1 cell adhesion molecule	MASA Syndrome	303350	0.984

Supplemental Table 12. Primers for genotyping of nonsynonymous G1 variants.

Locus	Forward	Reverse	Size	Method
MYBBP1A_V15	AGCCGCACCAAGACCATC	CAGCCCACATTCCACTCC	290	Sanger
ZNF594_361	GGTGTGACCTCTGGCTGAA	TCATTTGGCGCACAGCTT	426	Sanger
WDR81_V1	TGCCTACTCCACAGGGACA	CTGCCACAGAGCAAACACC	429	Sanger
MYBBP1A_V15	AGCCGCACCAAGACCATC	CAGCCCACATTCCACTCC	290	HpaII RD
ZNF594_361	GGTGTGACCTCTGGCTGAA	TCATTTGGCGCACAGCTT	426	BglII RD
WDR81_WT	TGTCTCCCAGGGCCTGCC	GGACATGAGTGAGAGCACGA	250	AS-PCR
WDR81_MUT	TGTCTCCCAGGGCCTGCT	GGCAACAGGCTCAAACAGAT	301	AS-PCR

RD: Restriction digestion

Supplemental Table 13. Primers for quantitative PCR analysis of WDR81.

Locus	Forward	Reverse	Len
WDR81-1_6-RT	GCAAGCTGGACCAACTGTTT	GGGAAGTAGGGTGGGAAGG	115
GAPDH	AGGTGAAGGTCGGAGTCAAC	GGGTCATTGATGGCAACA	100