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Supplemental Data

Biallelic Mutations in Citron Kinase Link Mitotic Cytokinesis to Human Primary Microcephaly

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Supplemental Data

Supplemental Data include 3 figures and 2 tables.

Figure S1

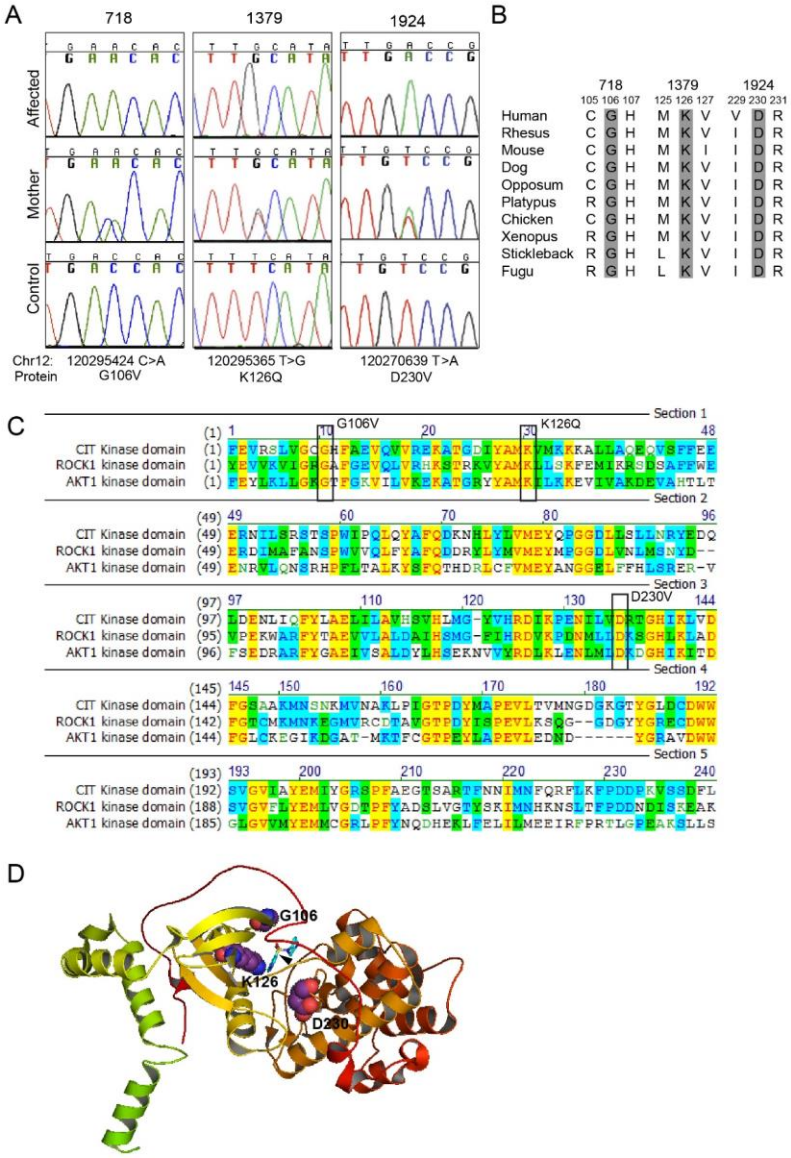

Figure S1. Mutations in the kinase domain of CIT-K cause primary microcephaly
(A) Sequencing chromatograms illustrating *CIT* mutations in the affected children. (B) Evolutionary conservation of the residues with patient mutations across the animal kingdom. (C) Kinase domain conservation among CIT-K and other AGC kinases. The residues with patient mutations are marked with black boxes. The sequence alignment was performed using Vector NTI (Invitrogen). (D) The crystal structure of the kinase domain of ROCK1, with residues with patient mutations labeled. Hydroxyfasudil, a competitive inhibitor of ATP, is marked with arrowhead showing the ATP binding pocket. The kinase domain and residues of ROCK1 were viewed by PyMol (https://www.pymol.org).

Figure S2

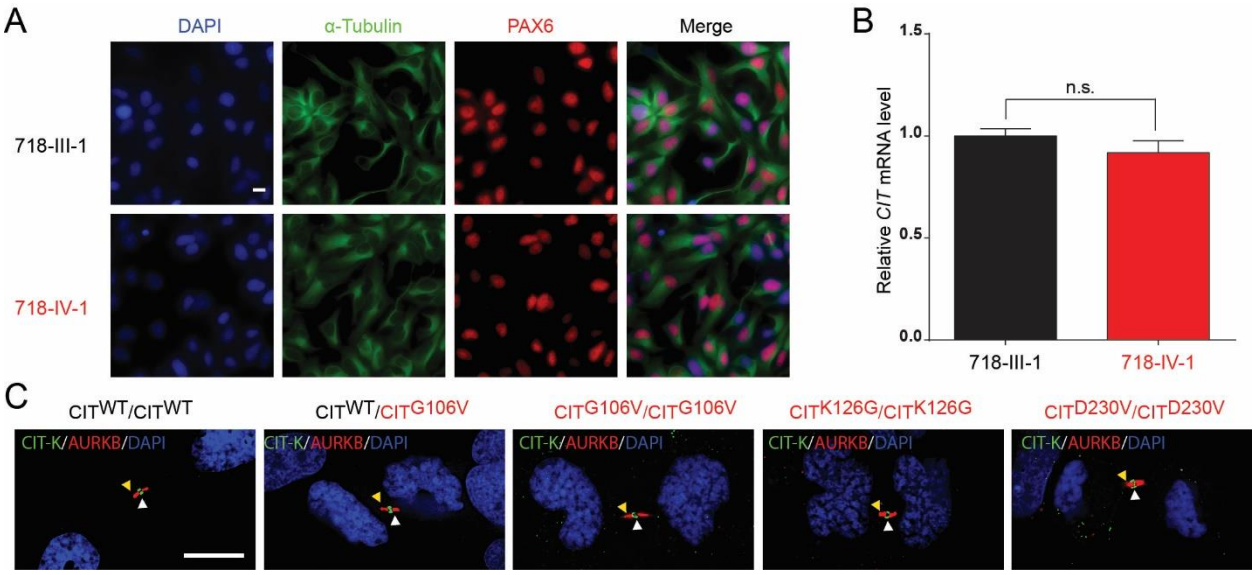

Figure S2. Expression level and localization of CIT-N in patient-derived NPCs
(A) Representative immunocytochemistry images of PAX6+ NPCs differentiated from iPSCs. Scale bar = 10μm. (B) Relative mRNA level of *CIT* in affected and control NPCs. (n=3 cultures for each group). Student's t test. Bar graph: mean ± s.e.m (C) Representative immunocytochemistry images for CIT-K (white arrowhead) and midbody marker Aurora kinase B (AURKB) (yellow arrowhead) during cytokinesis in NPCs with absent, heterozygous or homozygous *CIT* mutations. The localization of CIT-K and AURKB was indistinguishable among all conditions. Scale bar = 10μm.

Figure S3

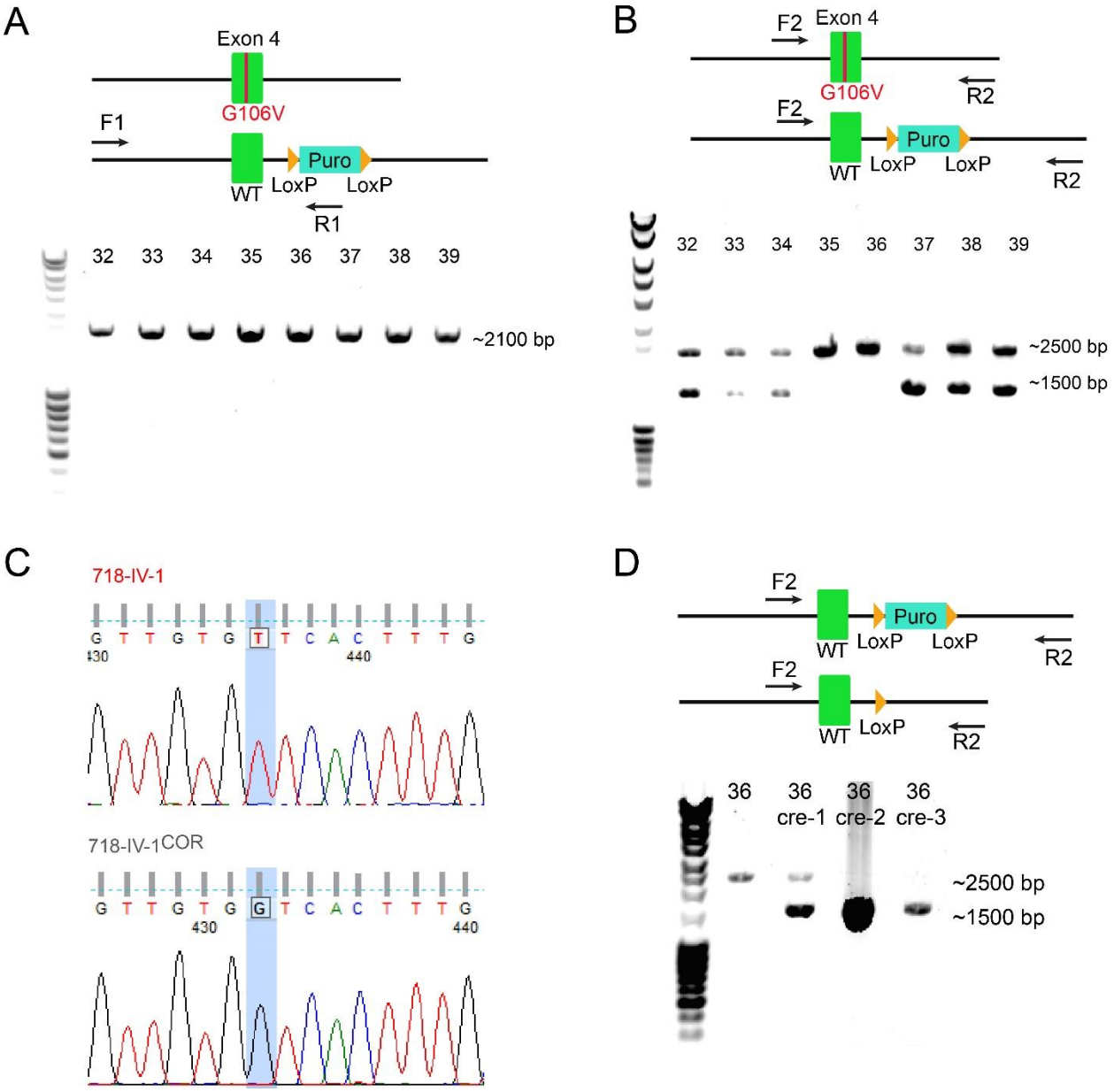

Figure S3. Identification of correctly-targeted iPSC clones in genome editing
(A) Forward primer F1 outside the 5' recombination arm, reverse primer R1 inside the puromycin cassette. This PCR allowed identification of iPSC clones with homologous targeting vector recombination. (B) Forward (F2) and reverse (R2) primers outside of Exon4 and the puromycin cassette. A ~2500 bp band represented the recombined allele, and a ~1500bp band represented the unmodified allele. Clone #35 and #36 were homozygous for homologous recombination, and were chosen for downstream studies. (C) Sanger sequencing confirmed c.317G>T mutation corrected in clone #36. (D) PCR identification of clones with LoxP-Puro-LoxP cassette removal. A ~2500 bp band represented the corrected allele, and a ~1500bp band represented removal of the LoxP-Puro-LoxP cassette. Clone #36-2 and #36-3, with LoxP-Puro-LoxP cassette removal on both alleles, were used for downstream studies.

Table S1 Genetic variants identified in each family from exome sequencing

Family 718

chrom	pos	dbSNP	ref	mut	gene	functionGVS	cDNA Position	AA_Change	PolyPhen Val	SIFTval	CADD PHRED	accession
3	98620028		G	A	DCBLD2	missense	143	PRO/LEU	0.118	0.5	20.6	NM_080927.3
3	112729916	rs115971253	C	T	C3orf17	missense	889	VAL/ILE	0.165	0.08	12.29	NM_015412.3
3	113377150		C	A	KIAA2018	missense	3380	VAL/LEU	0.05	0.05	23	NM_001009899.2
3	122274792		T	C	PARP9	missense	331	ARG/GLY	0.467	0.01	24	NM_031458.2
3	124141662		T	C	KALRN	missense	2540	ILE/THR	0.596	0.02	26.4	NM_003947.4
10	23399192		A	G	MSRB2	missense	241	ASN/ASP	0.051	0.65	11.51	NM_012228.3
10	25313059	rs116200992	G	T	THNSL1	missense	907	ALA/SER	0.169	0.49	11.04	NM_024838.4
10	27389200		C	A	ANKRD26	missense	56	ARG/LEU	0.003	0.72	10.13	NM_014915.2
10	30317861		C	G	KIAA1462	missense	1220	GLY/ARG	0.971	0.01	23.8	NM_020848.2
12	120295424		C	A	CIT	missense	317	GLY/VAL	1	0	32	NM_007174.2
12	122748141	rs114821540	T	G	VPS33A	missense	274	ILE/LEU	0.058	0.89	15.87	NM_022916.4
16	89347612	rs75362060	C	T	ANKRD11	missense	5340	ALA/THR	0.002	0.95	0.024	NM_013275.4

Family 1379

chrom	pos	dbSNP	ref	mut	gene	functionGVS	cDNA Position	AA_Change	PolyPhen Val	SIFTval	PHRED	accession
1	230846446		A	G	AGT	missense	151	CYS/ARG	0.987	0.01	23.7	NM_000029.3
1	234565060		G	A	TARBP1	missense	2882	SER/PHE	0.736	0.04	28.3	NM_005646.3
2	179203724		C	T	OSBPL6	missense	734	ALA/VAL	0.999	0.13	28.2	NM_145739.2
2	179457681	rs116592778	A	G	TTN	missense	32546	VAL/ALA	NA	NA	14.63	NM_133437.3
2	186664898		A	G	FSIP2	missense	11132		0.023	0.02	18.14	
2	231380146	rs61733089	G	A	SP100	missense	2431	ALA/THR	NA	NA	4.163	NM_003113.3
3	73024182		T	C	GXYLT2	missense	1204	PHE/LEU	0.411	1	16.73	NM_001080393.1
4	9336408		A	G	LOC72837	missense	25	ARG/GLY	0	1	0.002	NM_001242328.1
5	75596565		T	C	SV2C	missense	1648	TYR/HIS	0.827	0.5	24.2	NM_014979.1
5	79025441		C	T	CMYA5	missense	853	ARG/CYS	0.046	0.02	13.24	NM_153610.3
5	89930940		G	A	GPR98	missense	1849	VAL/MET	0.038	0.03	23.1	NM_032119.3
5	135513083		A	AT	SMAD5	frameshift	0		0.069	0.12	12.56	
10	103900766	rs17855877	C	G	PPRC1	missense	2501	PRO/ARG	0.787	0.04	23.2	NM_015062.3
10	124091985		A	G	BTBD16	missense	1121	ASP/GLY	0.997	0.02	33	NM_144587.2
10	125521435	rs112981683	T	C	CPXM2	missense	1730	LYS/ARG	1	0.06	23	NM_198148.2
11	78369281		C	T	ODZ4	missense	8132	ARG/GLN	0.013	0.28	23.1	NM_001098816.2
11	111228529	rs35664667	T	C	POU2AF1	missense	181	THR/ALA	0.012	0.46	6.234	NM_006235.2
12	120295365		T	G	CIT	missense	376	LYS/GLN	1	0	26.7	NM_007174.2
12	121853964		G	A	RNF34	ding-synonym	12		NA	NA	16.6	NM_194271.1
14	105417500		C	T	AHNAK2	missense	4288	GLU/LYS	0.086	0.2	3.783	NM_138420.2
20	3804572		G	T	C20orf29	missense	231	MET/ILE	0.003	0.39	10.15	NM_018347.2
20	3843058		C	A	MAVS	missense	623	ALA/GLU	0.522	0.01	23.5	NM_020746.4
21	33694796		C	T	URB1	missense	5332	ASP/ASN	0.127	0.15	22	NM_014825.2
21	37444851		G	C	LOC1001	missense	505		0.05	0.22	20.4	NM_001757.2
21	43287457		TC	T	PRDM15	frameshift	0		NA	NA	12.37	
21	43531659		C	T	UMODL1	missense	2327	PRO/LEU	0.851	0.08	19.24	NM_173568.3

Family 1924

chrom	pos	dbSNP	ref	mut	gene	functionGVS	cDNA Position	AA_Change	PolyPhen Val	SIFT val	PHRED	Accession
2	179440696	rs55853138	C	T	TTN	missense	43568	Arg14448Gln	1	0.006	23.6	NM_133432.3
2	190338924		G	A	WDR75	missense	2122	Glu688Lys	0.998	0.04	35	NM_032168.1
2	190338928		G	A	WDR75	missense	2126	Ser689Asn	0.966	0.313	21.8	NM_032168.1
3	51749962	rs151188856	G	A	GRM2	missense	2412	Asp725Asn	1	0.017	33	NM_000839.3
3	49849583		C	T	UBA7	missense	922	Gly251Arg	0.999	0	25.4	NM_003335.2
12	123444667		C	T	ABCB9	missense	426	Arg39His	0.968	0.138	29.9	NM_019625.3
12	120270639		T	A	CIT	missense	745	Asp230Val	0.98	0	35	NM_001206999.1
12	120884493	rs149168717	C	T	G00000257	missense	158	His39Tyr	0.085	0.454	22.8	NM_176818.2
12	120662133		G	A	PXN	missense	918	Arg19Trp	0.999	0.001	25.1	XM_005253915.1
13	95673848		A	G	ABCC4	missense	4078	Ile1320Thr	0.954	0.002	23.2	NM_005845.3
18	580778		A	G	CETN1	missense	410	Lys124Glu	0.017	0.108	14.51	NM_004066.1
19	41198211	rs144343899	T	C	ADCK4	missense	1431	Tyr455Cys	0.998	0	22	XM_005259272.1
19	41205970		AC	C	ADCK4	splice_donor					27.1	
19	40964447	rs144111604	C	T	BLVRB	missense	195	Glu29Lys	0.013	0.56	25.4	XM_005259157.1
19	47883001	rs200643374	A	G	DHX34	missense	3090	Asn914Ser	0.028	0.145	35	NM_014681.5
19	44536034		C	A	ZNF222	stop_gained	422	Cys109X			11.1	NM_001129996.1
X	79979316		A	C	BRWD3	missense	1844	His527Gln	0.743	0.251	22.9	NM_153252.4
X	131203569		G	A	G00000134	missense	908	Asp221Asn	0.07	0.08	26.5	XM_005262424.1

Abbreviations. Chrom, chromosome; pos, position; ref, reference; mut, mutation.

Table S2 Additional variants identified in 1379-B1-1 from exome sequencing

We identified additional compound heterozygous variants and dominant variants in subject 1379-B1-1.

Compound heterozygous variants

CHROM	POS	effect	gene_name	dbSNP	HGVS_c	HGVS_p	transcript_id	impact
2	201488623	missense_variant	AOX1	rs369839297	c.2041G>A	p.Asp681Asn	ENST00000374700	MODERATE
2	201501730	missense_variant	AOX1	rs144419430	c.2443G>A	p.Gly815Arg	ENST00000374700	MODERATE
6	157488190	missense_variant	ARID1B	rs34786733	c.2857G>A	p.Gly953Ser	ENST00000350026	MODERATE
6	157522223	missense_variant	ARID1B	rs34870395	c.4456A>T	p.Met1486Leu	ENST00000350026	MODERATE
2	29287937	splice_region_variant	C2orf71		c.3669-5_3669-4insCA		ENST00000331664	LOW
2	29287938	splice_region_variant	C2orf71		c.3669-6_3669-5insA		ENST00000331664	LOW
1	17256337	splice_region_variant	CROCC		c.352-4G>A		ENST00000375541	LOW
1	17256531	splice_region_variant	CROCC	rs371339969	c.537+5G>A		ENST00000375541	LOW
1	156499967	missense_variant	IQGAP3	rs149623112	c.4334G>A	p.Arg1445His	ENST00000361170	MODERATE
1	156520100	missense_variant	IQGAP3	rs59573847	c.1778G>T	p.Arg593Leu	ENST00000361170	MODERATE
16	4714702	splice_region_variant	MGRN1	rs199769383	c.562-8C>G		ENST00000262370	LOW
16	4733217	splice_region_variant	MGRN1	rs200737185	c.1483-8T>C		ENST00000262370	LOW
3	195512549	inframe_deletion	MUC4		c.5854_5901delCCTCTT	p.Pro1952_Thr1967del	ENST00000463781	MODERATE
3	195518118	frameshift_variant	MUC4		c.326_332delATGTGAT	p.Asn109fs	ENST00000463781	HIGH
5	141324975	missense_variant	PCDH12	rs3833449	c.3526G>A	p.Gly1176Ser	ENST00000231484	MODERATE
5	141324976	missense_variant	PCDH12	rs13188049	c.3525A>C	p.Arg1175Ser	ENST00000231484	MODERATE
10	3187831	missense_variant	PITRM1	rs34837384	c.2120G>A	p.Arg707Gln	ENST00000451104	MODERATE
10	3214939	missense_variant	PITRM1	rs199766052	c.26G>T	p.Gly9Val	ENST00000380989	MODERATE
9	8331582	frameshift_variant&s	PTPRD	rs200157286	c.4303_4304insT	p.Ser1435fs	ENST00000397611	HIGH
9	8331584	stop_gained&splice_r	PTPRD		c.4302C>A	p.Cys1434*	ENST00000397611	HIGH
19	50037548	missense_variant	RCN3		c.341T>A	p.Ile114Lys	ENST00000270645	MODERATE
19	50040307	missense_variant	RCN3	rs77227069	c.463G>A	p.Val155Met	ENST00000270645	MODERATE
15	42977810	missense_variant	STARD9	rs140924205	c.4034T>G	p.Ile1345Ser	ENST00000290607	MODERATE
15	42978141	missense_variant	STARD9	rs376229251	c.4365A>C	p.Glu1455Asp	ENST00000290607	MODERATE
15	42977810	missense_variant	STARD9	rs140924205	c.4034T>G	p.Ile1345Ser	ENST00000290607	MODERATE
15	42982583	missense_variant	STARD9	rs115491632	c.8807G>T	p.Gly2936Val	ENST00000290607	MODERATE
15	42978141	missense_variant	STARD9	rs376229251	c.4365A>C	p.Glu1455Asp	ENST00000290607	MODERATE
15	42982583	missense_variant	STARD9	rs115491632	c.8807G>T	p.Gly2936Val	ENST00000290607	MODERATE
6	152623062	missense_variant	SYNE1	rs150376715	c.17270C>G	p.Thr5757Arg	ENST00000423061	MODERATE
6	152647218	missense_variant	SYNE1	rs35493783	c.15100G>A	p.Asp5034Asn	ENST00000423061	MODERATE

Abbreviations. Chrom, chromosome; pos, position; ref, reference; mut, mutation; HGVS, Human Genome Variation Society; HGVS_c, cDNA location; HGVS_p, protein location.

Dominant variants

CHROM	POS	effect	gene_nan	dbSNP	HGVS_c	HGVS_p	transcript_id	impact
1	1254762	stop_gained	CPSF3L		c.256A>T	p.Lys86*	ENST00000545578	HIGH
1	120277963	missense_variant	PHGDH		c.689G>A	p.Arg230His	ENST00000369409	MODERATE
1	156499967	missense_variant	IQGAP3	rs149623112	c.4334G>A	p.Arg1445His	ENST00000361170	MODERATE
1	156520100	missense_variant	IQGAP3	rs59573847	c.1778G>T	p.Arg593Leu	ENST00000361170	MODERATE
2	74274744	missense_variant	TET3	rs148646838	c.1295T>C	p.Val432Ala	ENST00000409262	MODERATE
2	110922668	missense_variant	NPHP1	rs113450177	c.503C>T	p.Ala168Val	ENST00000355301	MODERATE
2	152483510	splice_region_variant	NEB	rs141088433	c.10347+6C>T		ENST00000397345	LOW
2	171240217	splice_region_variant	MYO3B	rs146172688	c.1186-3C>T		ENST00000408978	LOW
2	201488623	missense_variant	AOX1	rs369839297	c.2041G>A	p.Asp681Asn	ENST00000374700	MODERATE
3	45049073	splice_region_variant	EXOSC7	rs145650051	c.771+6G>A		ENST00000265564	LOW
3	47452676	missense_variant	PTPN23	rs138329311	c.3388G>A	p.Gly1130Ser	ENST00000265562	MODERATE
3	52430897	missense_variant	DNAH1		c.11624C>T	p.Thr3875Met	ENST00000420323	MODERATE
3	186947662	missense_variant	MASP1		c.1327C>T	p.Arg443Trp	ENST00000337774	MODERATE
5	98115646	stop_gained	RGMB	rs201449079	c.622G>T	p.Gly208*	ENST00000308234	HIGH
5	141324975	missense_variant	PCDH12	rs3833449	c.3526G>A	p.Gly1176Ser	ENST00000231484	MODERATE
5	141324976	missense_variant	PCDH12	rs13188049	c.3525A>C	p.Arg1175Ser	ENST00000231484	MODERATE
6	56342136	splice_region_variant	DST	rs34892827	c.13806+7G>A		ENST00000244364	LOW
6	152623062	missense_variant	SYNE1	rs150376715	c.17270C>G	p.Thr5757Arg	ENST00000423061	MODERATE
6	152623062	missense_variant	SYNE1	rs150376715	c.17483C>G	p.Thr5828Arg	ENST00000367255	MODERATE
6	152647218	missense_variant	SYNE1	rs35493783	c.15100G>A	p.Asp5034Asn	ENST00000423061	MODERATE
6	157488190	missense_variant	ARID1B	rs34786733	c.2896G>A	p.Gly966Ser	ENST00000346085	MODERATE
6	157522223	missense_variant	ARID1B	rs34870395	c.4456A>T	p.Met1486Leu	ENST00000350026	MODERATE
7	12443318	missense_variant	VWDE		c.25G>C	p.Val9Leu	ENST00000275358	MODERATE
7	134719612	missense_variant	AGBL3		c.1270C>T	p.Arg424Cys	ENST00000436302	MODERATE
7	143096736	splice_region_variant	EPHA1	rs149923216	c.835+8C>T		ENST00000275815	LOW
8	33361259	splice_region_variant	TTI2	rs199720231	c.1115+7C>G		ENST00000360742	LOW
8	52384860	missense_variant	PXDNL		c.699C>A	p.Ser233Arg	ENST00000356297	MODERATE
8	120814146	missense_variant	TAF2		c.680A>G	p.His227Arg	ENST00000378164	MODERATE
8	141034062	missense_variant	TRAPPC9		c.2671A>C	p.Thr891Pro	ENST00000438773	MODERATE
8	144995672	missense_variant	PLEC	rs200683827	c.8221G>A	p.Ala2741Thr	ENST00000398774	MODERATE
9	13158047	missense_variant	MPDZ		c.3422C>T	p.Thr1141Ile	ENST00000541718	MODERATE
9	98677946	splice_region_variant	ERCC6L2	rs367808564	c.822-4A>G		ENST00000288985	LOW
9	135940049	missense_variant	CEL		c.249C>A	p.Phe83Leu	ENST00000372080	MODERATE
10	3187831	missense_variant	PITRM1	rs34837384	c.2120G>A	p.Arg707Gln	ENST00000451104	MODERATE
10	3214939	missense_variant	PITRM1	rs199766052	c.26G>T	p.Gly9Val	ENST00000380989	MODERATE
10	32856748	frameshift_variant	CCDC7		c.1351_1354delAC	p.Thr451fs	ENST00000277657	HIGH
10	37433935	missense_variant	ANKRD30A		c.1238C>T	p.Pro413Leu	ENST00000361713	MODERATE
10	60562867	missense_variant	BICC1	rs77500675	c.2046A>T	p.Glu682Asp	ENST00000373886	MODERATE
11	20907067	missense_variant	NELL1	rs115437355	c.584A>G	p.Gln195Arg	ENST00000357134	MODERATE
11	34129870	inframe_insertion	NAT10	rs139367378	c.100_102dupAAA	p.Lys34dup	ENST00000257829	MODERATE
11	46766098	missense_variant	CKAP5		c.5554G>A	p.Val1852Met	ENST00000312055	MODERATE
11	62656158	missense_variant	SLC3A2	rs201220940	c.1583C>T	p.Ala528Val	ENST00000338663	MODERATE
11	65487790	inframe_deletion	RNASEH2(	rs141875736	c.268_270delAAG	p.Lys90del	ENST00000308418	MODERATE
12	57864531	missense_variant	GLI1	rs138680284	c.1624G>T	p.Ala542Ser	ENST00000543426	MODERATE
12	103246735	splice_region_variant	PAH	rs62508624	c.707-7A>T		ENST00000553106	LOW
12	109702166	missense_variant	ACACB	rs149917930	c.6917G>A	p.Arg2306Gln	ENST00000338432	MODERATE
13	25479878	missense_variant	CENPJ	rs79951875	c.2298T>A	p.Asp766Glu	ENST00000381884	MODERATE
13	44454253	splice_region_variant	LACC1	rs73465585	c.-37C>A		ENST00000325686	LOW
15	42977810	missense_variant	STARD9	rs140924205	c.4034T>G	p.Ile1345Ser	ENST00000290607	MODERATE
15	42978141	missense_variant	STARD9	rs376229251	c.4365A>C	p.Glu1455Asp	ENST00000290607	MODERATE
15	42982583	missense_variant	STARD9	rs115491632	c.8807G>T	p.Gly2936Val	ENST00000290607	MODERATE
15	75644499	missense_variant	NEIL1		c.482T>C	p.Ile161Thr	ENST00000355059	MODERATE
15	84506964	missense_variant	ADAMTSL	rs112527144	c.724A>G	p.Lys242Glu	ENST00000286744	MODERATE
16	27715275	missense_variant	KIAA0556	rs143325975	c.1345G>A	p.Gly449Ser	ENST00000261588	MODERATE
16	56875738	missense_variant	NUP93	rs147381896	c.1973G>A	p.Arg658His	ENST00000564887	MODERATE
16	67298319	missense_variant	SLC9A5		c.1907T>G	p.Val636Gly	ENST00000299798	MODERATE
16	71571175	missense_variant	CHST4	rs141141955	c.595C>T	p.His199Tyr	ENST00000338482	MODERATE
17	649703	missense_variant	GEMIN4		c.1580A>G	p.Asn527Ser	ENST00000319004	MODERATE
17	7671259	missense_variant	DNAH2	rs146539788	c.3717C>A	p.Asp1239Glu	ENST00000389173	MODERATE
17	16068429	missense_variant	NCOR1		c.155C>T	p.Ser52Leu	ENST00000395848	MODERATE
17	62024398	splice_region_variant	SCN4A	rs142270113	c.3441+7G>A		ENST00000435607	LOW
19	8161433	missense_variant	FBN3	rs115948457	c.5434A>G	p.Ile1812Val	ENST00000270509	MODERATE
19	9868536	frameshift_variant	ZNF846		c.1213_1216delAA	p.Asn405fs	ENST00000397902	HIGH
19	18256548	splice_region_variant	MAST3	rs73925428	c.2953-5C>T		ENST00000262811	LOW
19	50037548	missense_variant	RCN3		c.341T>A	p.Ile114Lys	ENST00000270645	MODERATE
19	50040307	missense_variant	RCN3	rs77227069	c.463G>A	p.Val155Met	ENST00000270645	MODERATE