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Supplemental Data

Mutations in *C5ORF42* Cause Joubert Syndrome in the French Canadian Population

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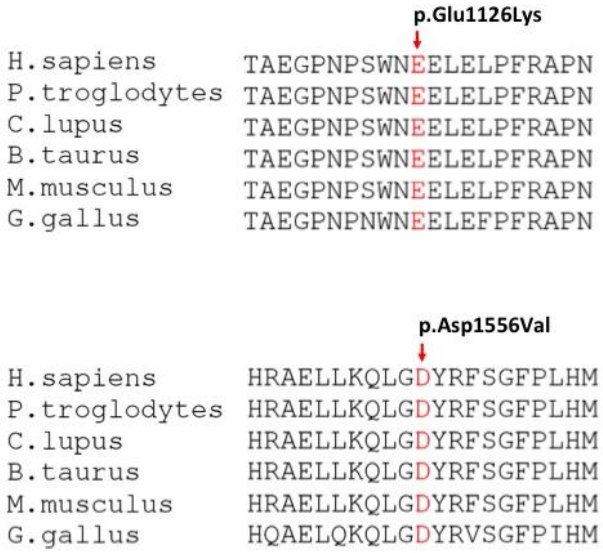

Figure S1. Amino Acid Conservation of the Residues Affected by the c.4667A>T (p.Asp1556Val) and c.3376G>A (p.Glu1126Lys) Mutations in *CC2D2A*

Amino acid alignments were generated using homologene (NCBI).

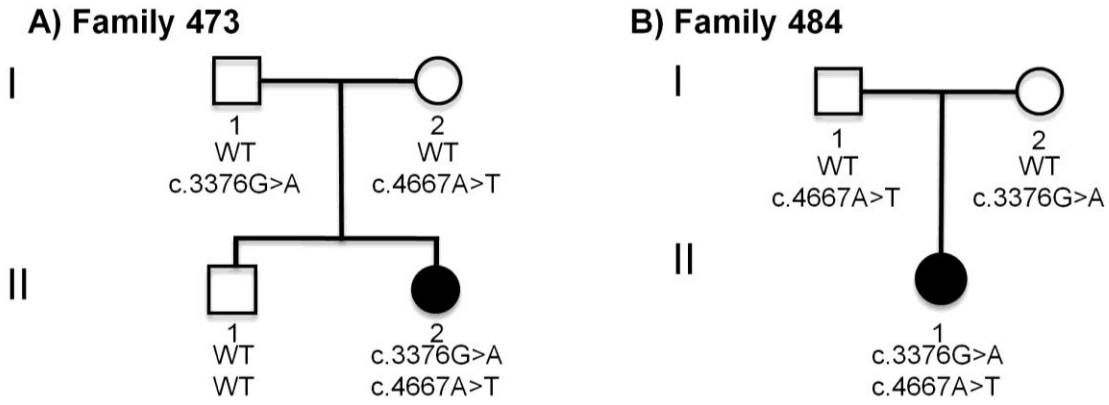

Figure S2. Segregation of c.3376G>A and c.4667A>T *CC2D2A* Mutations

We identified compound heterozygous mutations in *CC2D2A* (c.3376G>A [p.Asp1556Val] and c.4667A>T [p.Glu1126Lys], numbering according to Refseq NM_001080522.2) in two unrelated individuals with JBTS. These mutations are predicted to be damaging according to SIFT and Polyphen-2. Both individuals had a mild phenotype with oculomotor apraxia and mild developmental delay.

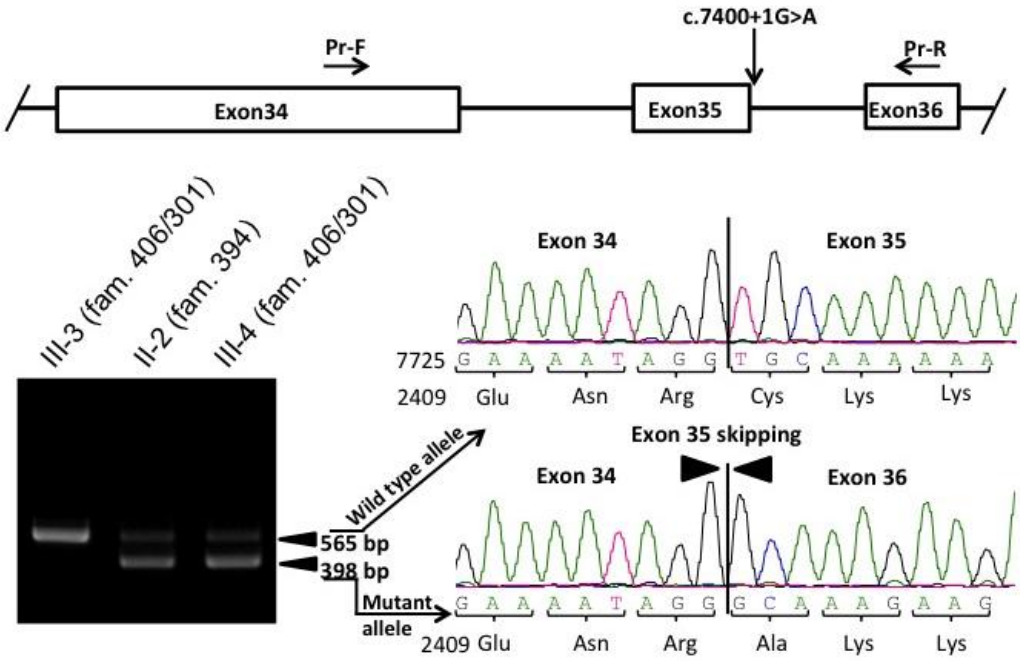

Figure S3. Mutation c.7400+1G>A Causes Skipping of Exon 35 of *C5ORF42*

Total RNA was extracted from blood samples obtained from 2 individuals carrying the heterozygous c.7400+1G>A mutation (II-2 from family 394 and III-4 from family 406/301) and from 1 individual (III-3 from family 406/301) lacking this mutation, and used for RT-PCR with primers targeting exon-34 (Forward primer[Pr-F]:5'-CCTAATCATGTGAACTTGGATCAAT) and exon-36 (Reverse primer [Pr-R]: 5' TAATTATGGAATTCTCTGGTCGAAA-3'), which flank the splicing site c.7400+1G (exon-35/intron-35 junction) of *C5ORF42* (NM_023073.3) (upper panel). Shown in the lower panel is an agarose gel electrophoresis of the RT-PCR products obtained. The expected 565-bp (Wild type allele; WT) PCR product was observed in all samples, while a smaller product (Mutant allele; MT) (~ 398 bp) was only detected from the 2 individuals with the c.7400+1G>A mutation. Sequencing of both the WT and MT fragments showed that the former corresponded to the wild type *C5ORF42* transcript, while the latter revealed a *C5ORF42* transcript lacking exon-35, resulting in a frameshift that extends for 5 amino acids followed by a premature stop codon (p.Cys2401AlafsX5).

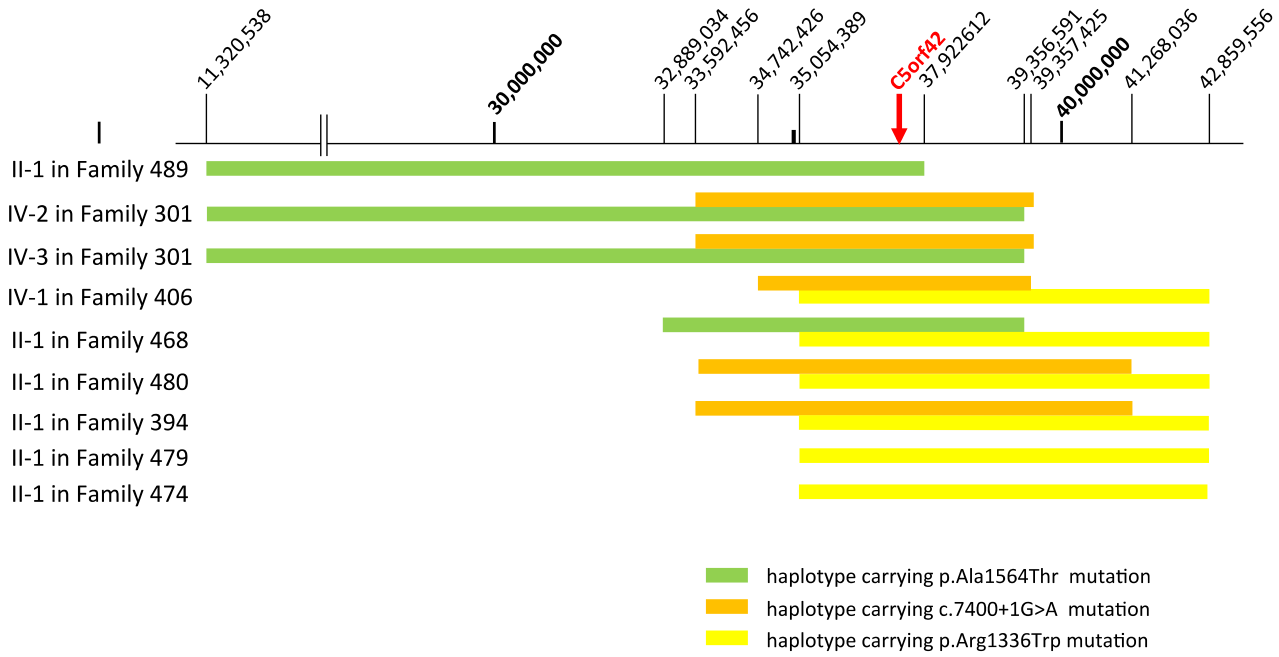

Figure S4. Schematic Diagram Showing Shared Haplotypes in Region Surrounding *C5ORF42* in Individuals with JBTS

Subject numbers are noted in the left-hand column. Chromosome 5 coordinates are noted on the horizontal axis (hg19). Position of *C5ORF42* is identified by the red arrow. The Homozygosity Haplotype (HH) method [1, 2] was used to assess whether individuals with identical *C5ORF42* mutations shared a common haplotype around *C5ORF42*. Briefly, instead of formally phasing haplotypes, the HH method uses a reduced haplotype described only by the homozygous SNPs (the heterozygous SNPs are removed) from the high density SNP genotyping data. Subjects who inherited the same mutation from a common ancestor share a chromosomal segment identical by descent around the mutation, and do not have discordant homozygous calls in that region. Analysis shows that subjects with identical mutations share a common haplotype around *C5ORF42* spanning 5.03Mb (chr5:32,889,034-37,922,612) for the c.4690G>A (p.Ala1564Thr) mutation, 4.61Mb (chr5:34,742,426-39,357,425) for the c.7400+1G>A mutation and 7.8Mb (chr5:35,054,389-42,823,549) for the c.4006C>T (p.Arg1336Trp) mutation.

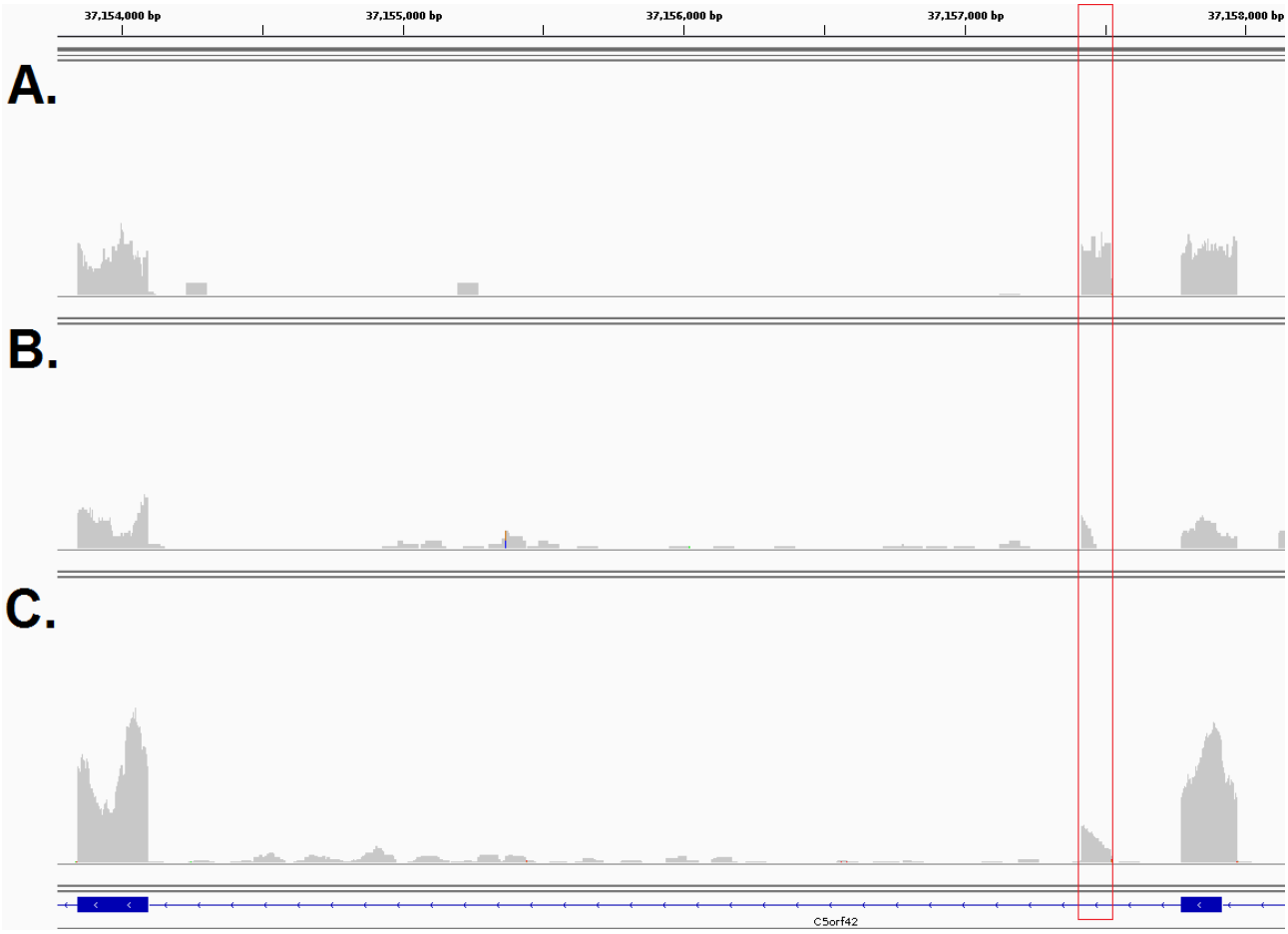

Figure S5. Coverage Histograms of RefSeq Unannotated *C5ORF42* Exon 40a (in Red Box) in Different Tissues

The vertical grey bars indicate the number of reads from (a) testis, (b) brain and (c) a mixture of adipose, adrenal, breast, colon, heart, kidney, liver, lung, lymph, ovary, prostate, skeletal muscle, thyroid, and white blood cell tissues. The proportions of reads containing the target exon versus the ones that do not are respectively (a) 21/23, (b) 13/18 and (c) 44/130. Reads were controlled for quality using FastX tools, assembled with TopHat, indexed with SAMtools and displayed with IGV. The tissue mixture (c) was obtained by merging the reads from the various tissues using SAMtools.

Table S1. Regions of Identical Homozygosity Shared by Three or More Individuals with Joubert Syndrome

Chromosome	Size (Mb)	Start Position	End Position	# of Individuals	Genes in Interval
12	1.01	38,120,168	39,128,892	5	*ALG10B, CPNE8*
8	1.15	48,439,013	49,589,706	4	*KIAA0145, CEBPD, PRKDC, MCM4, UBE2V2*
4	0.96	151,184,163	152,147,262	4	*LRBA, MAB21L2, RPS3A, SNOD73A, SH3D19*
7	0.57	119,321,200	119,887,000	4	*KCND2*
7	0.37	118,773,000	119,239,292	4	none
2	1.07	135,745,129	136,740,556	3	*YSK4, RAB3GAP1, ZRANB3, R3HDM1, MIR128-1, UBXN4, LCT, LOC100507600, MCM6, DARS*
2	0.58	186,303,487	186,895,680	3	*FSIP2*
12	0.28	39,288,892	39,566,178	3	*CPNE8*
4	0.25	150,854,889	151,103,870	3	*DCLK2*

In each subject, Plink was used to identify regions of homozygosity >30 consecutive SNPs and >1Mb in size. Thereafter, Excel was used to identify overlapping regions of identical homozygosity shared by 3 or more individuals. RefSeq genes from the intervals (hg19) are noted in the table.

Table S2. Frequency of Rare Mutations in the Candidate Genes

Gene	Rank (out of 20,870) by Mutation Frequency	Number of Rare Mutations
TTN	2	191
MUC5B	4	130
FLG	9	88
PLEC	10	82
LAMA5	12	73
FAT3	74	37
C5ORF42	2,248[a]	9

[a]*C5ORF42* is tied with 519 other genes.

We have observed that certain genes frequently have private mutations in our previous samples. To quantify this, we determined the number of "rare" mutations in each of 20870 genes annotated in Ensembl, where we defined a rare mutation as one with a 1000 genomes minor allele frequency < 0.005 and which was seen in two or fewer of our control exomes. The table shows the number of such rare mutations in our 261 control exomes for the frequently mutated genes in which multiple mutations were found in 3 JBTS families each.

References

1. Miyazawa, H., Kato, M., Awata, T., Kohda, M., Iwasa, H., Koyama, N., Tanaka, T., Huqun, Kyo, S., Okazaki, Y. et al (2007). Homozygosity haplotype allows a genomewide search for the autosomal segments shared among patients. Am J Hum Genet *80*, 1090-1102.
2. Jiang, H., Orr, A., Guernsey, D.L., Robitaille, J., Asselin, G., Samuels, M.E., Dube, M.P. (2009). Application of homozygosity haplotype analysis to genetic mapping with high-density SNP genotype data. PLoS. One. *4*, e5280.