Identification of the *SPG15* Gene, Encoding Spastizin, as a Frequent Cause of Complicated Autosomal-Recessive Spastic Paraplegia, Including Kjellin Syndrome

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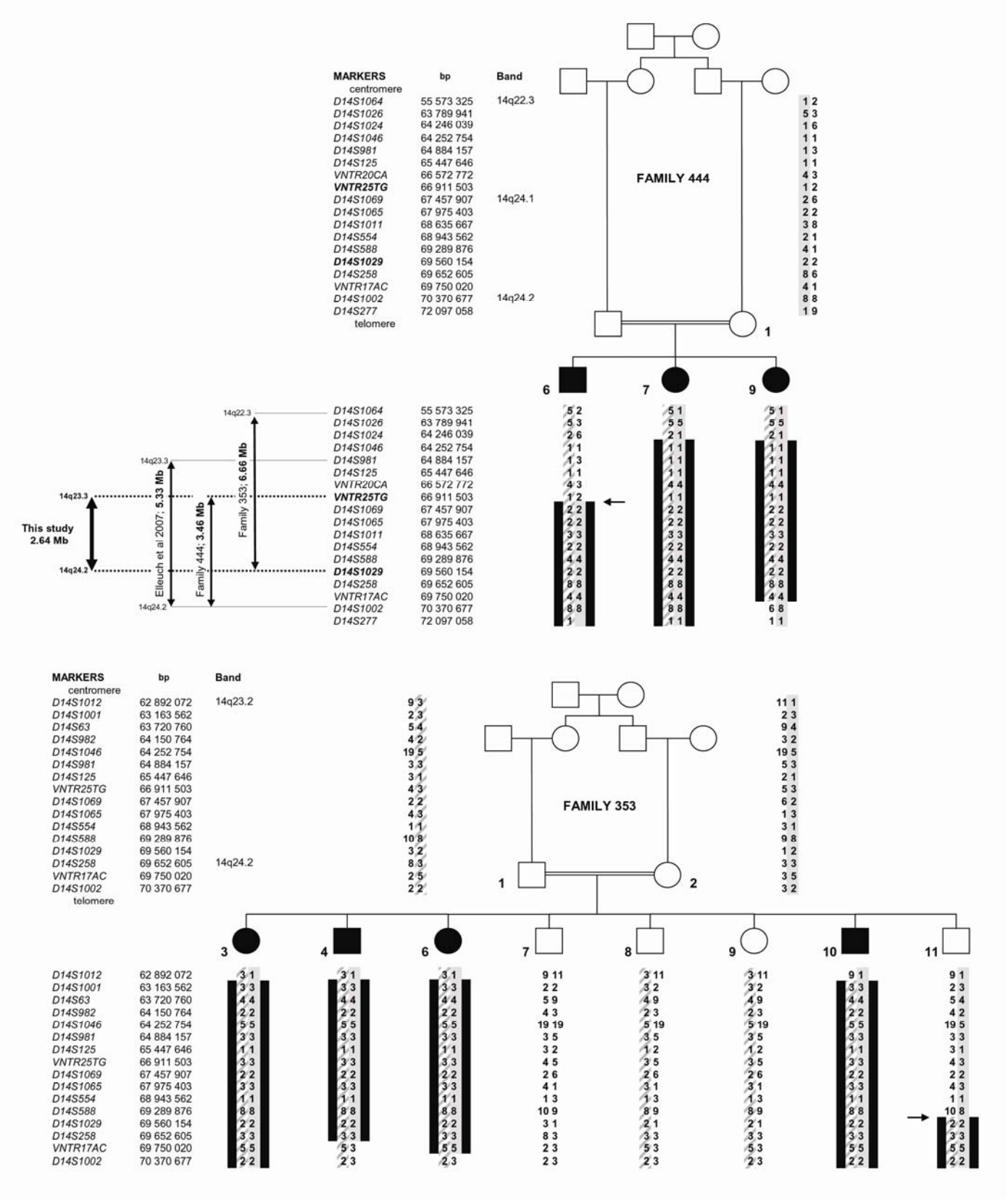
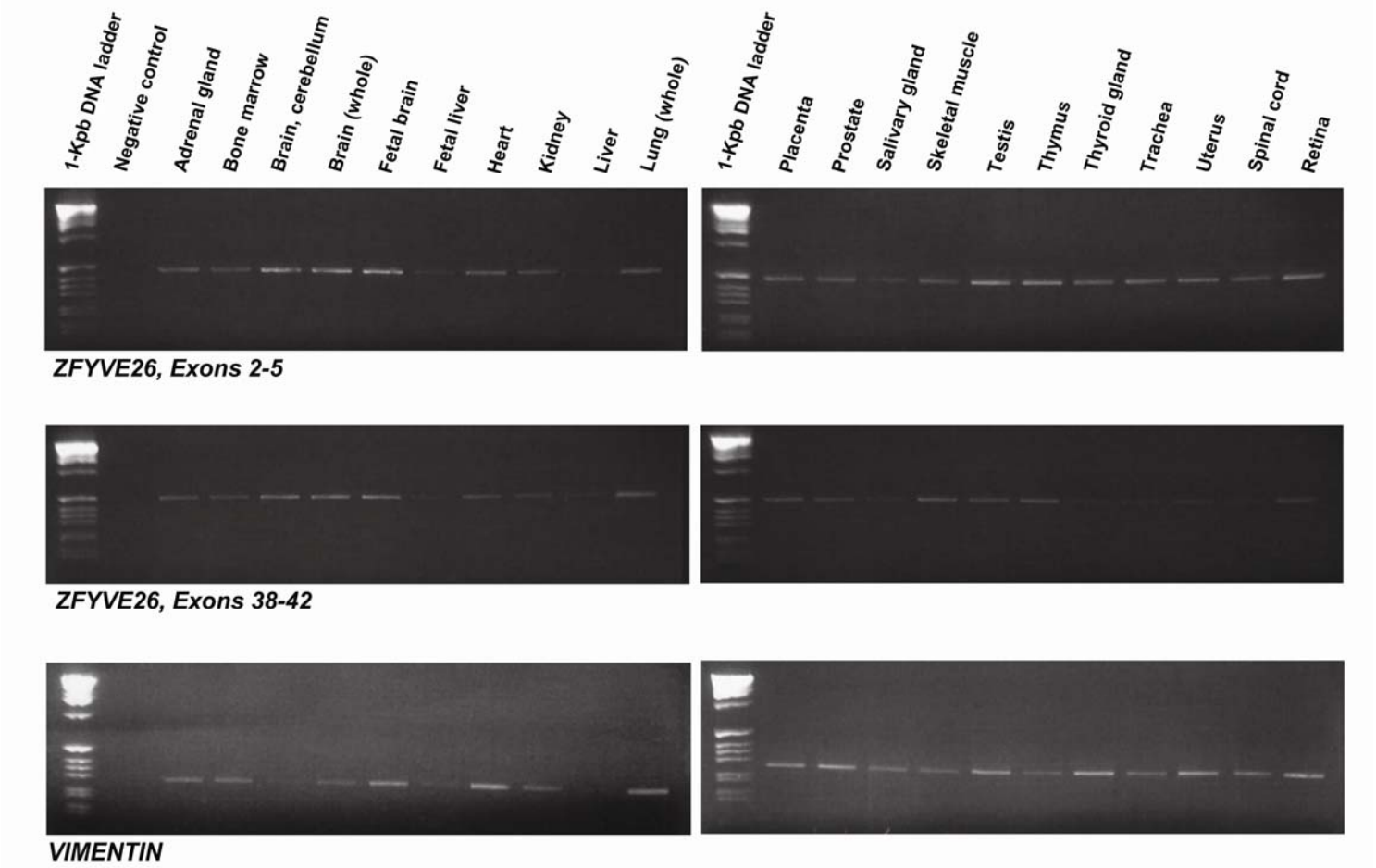
Figure S1. Refinement of the *SPG15* Locus and Pedigree Structure of Families 444 and 353 with Haplotype Reconstruction for Informative Markers on Chromosome 14q23.3-q24.2



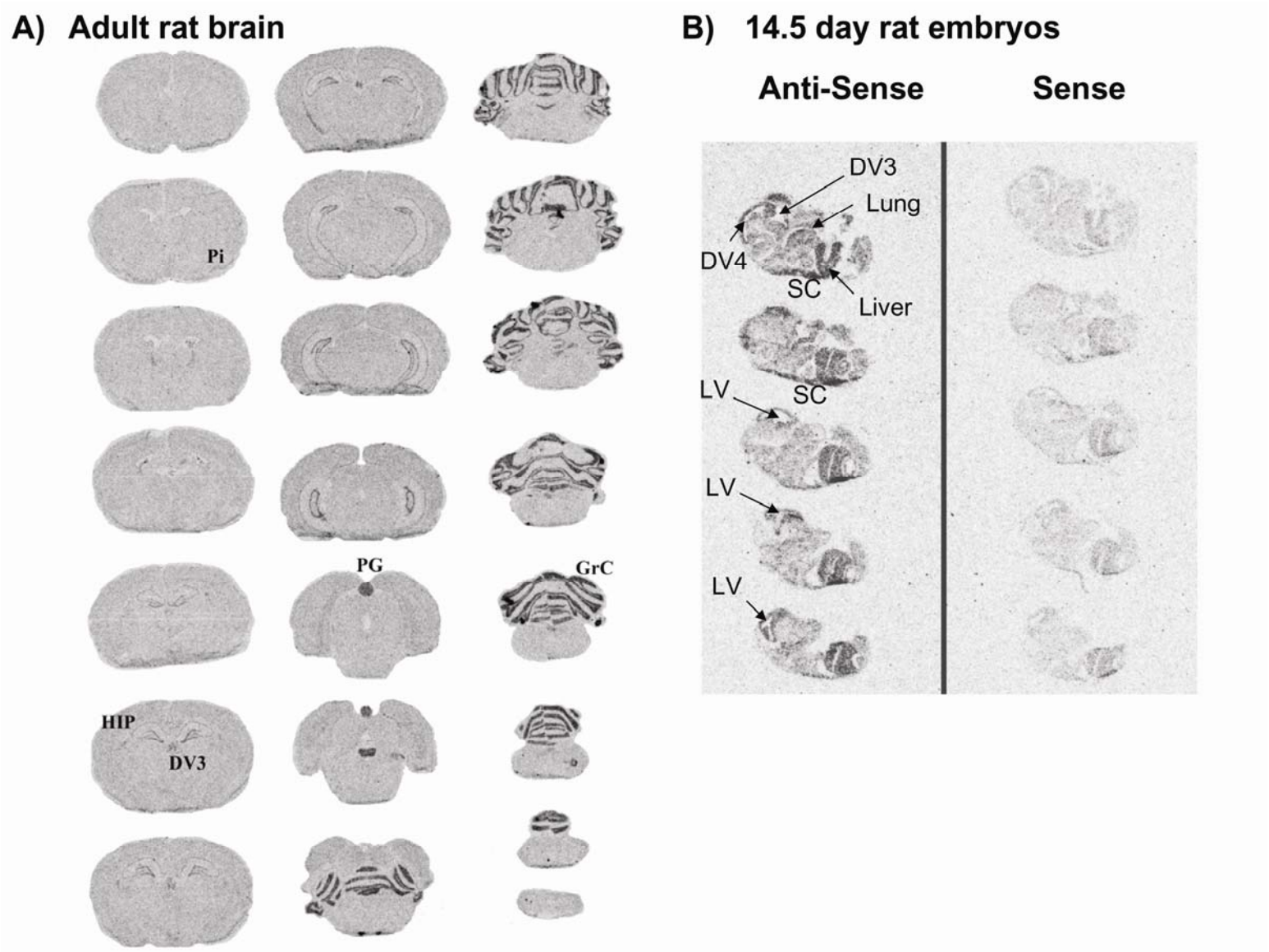
Black circles (women) and squares (men) indicate affected members. The code numbers of all sampled individuals are given below the symbols. VNTR denotes variable number of tandem repeat chosen from the Human Genome Working Draft at UCSC. Chromosomal positions of microsatellite markers are indicated in base pairs (bp) according to the human genome draft sequence (UCSC and Ensembl databases). The homozygous haplotype in which the mutated gene is most likely located in affected patients is flanked by black boxes. Arrows indicate the position of key recombination events that were used to restrict the candidate interval. The *SPG15* interval was refined to 2.64 Megabases (Mb) between loci *VNTR25TG* (primers in the supplementary table) and *D14S1029* because of obligatory recombinations observed between loci *VNTR25TG* and *D14S1069* (patient 444-6) and loci *D14S588* and *D14S1029* (Individual 353-11, who is still unaffected at age 18).

Figure S2. *ZFYVE26* Expression



Semiquantitative analysis of *ZFYVE26* expression with RT-PCR in adult human tissues, in comparison with vimentin (NM_003380). Two probes, which covered exons 2 to 5 and exons 38 to 42, gave similar results, showing widespread expression of the gene in all tissues but predominantly in adrenal gland, bone marrow, brain, fetal brain, lung, placenta, prostate, skeletal muscle, testis, thymus, and retina.

Figure S3. In Situ Hybridization in Adult P68 Rat Brain and in E14.5 Embryos



Sections were probed with a pool of three antisense *ZFYVE26* probes. The same results were obtained with the pool of three probes or with each probe independently.

LV: lateral ventricles; HIP: hippocampus; PG: pineal gland; Pi: piriform cortex; GrC: granular cell layer of the cerebellum; DV3: third ventricle; DV4: fourth ventricle, and SC: spinal cord.

NOTE: Labelling in 14.5 day embryos concerns mainly the liver, lungs, and nervous system—particularly the spinal cord and the cortical, hippocampal, cerebellar and thalamic neuroepithelia, as well as the inferior and superior colliculi and the tegmental and basal telencephalic areas. In the adult brain, labeling is stronger in the edges of the ventricles, the hippocampus, the granular layer of the cerebellum, and the pineal gland.

Table S1. Forward and Reverse Primer Sequences

Sequences of forward and reverse primers used for amplification of (A) four new polymorphic markers developed from the UCSC sequence draft at http://genome.ucsc.edu/ and of (B) exons of the *ZFYVE26* gene (Genbank accession number NM_ 015346).

(A) Polymorphic Markers

VNTRs / Chromosomal Position	Forward Sequence (5′–3′)	Reverse Sequence (5′–3′)
VNTR20CA (66.57Mb)	tctaatcaaagcgctaggc	tgttgactttgtacccctgc
VNTR25TG (66.91Mb)	gcagcagcaaagcaaagatag	cctgtaatctcaaacattcc
VNTR17CA (66.66Mb)	caaggacctaatgaattcct	ggaattttcattctctgggc
VNTR17AC (69.75Mb)	gtgtgtagctgtcagtcaga	ttgaagacagctccccttatc

VNTR denotes “Variable Number of Tandem Repeat.”

(B) *ZFYVE26*

Exon	Forward Sequence (5′–3′)	Reverse Sequence (5′–3′)
1	cagccaggtagctgatttcc	aattcagcaggaacctcccta
2	ataggaatccgcgtgaagag	gcagccaggcttacattcag
3	caccgcacttggctaatttt	ggcacaagactcatggtggt
4	tgcttcatcttagagaaatagcagaa	atgggcaacatcttggagac
5	ctgaaaaagaggaaagcatgaa	ttacgaaagagcatcgcacc
6	tgaagctcccaagggaagta	cgatgtaaaatgactgcaactg
7–8	tacaggcatgagccactacg	ggtcaacattgccaactcaa
9	ggccctttctaggacctttc	agacctcctcaccaccctct
10	aggaagtgcagggaactgaa	ccctgggtgaaataaaacca
11a	taaatgagctaaagttgcgagaa	cctgaggaaggcccctatt
11b	gaagtcagaacggggttcc	ggtgacgatatgccctgagt
12	tcagaacactggggtatgctc	gcatggaaaatttctgaaagg
13	acccaggtgaactctgttgc	gctaaaatctggccatctgc
14	gtttgcccttcatttgagga	cttgatgtggacccctgagt
15	tgaggctttggtggttttct	tggacgtatcaggtttgctg
16	gaaaaagccctccctcatct	ccatctgcctcctccaataa
17–18	ccaaaatggcacagcatgta	gagacatgccctggctaact
19	ctggctgggaatcacttgtc	gccagagatgaataagagagga
20	gagagcaggagttggctgtc	agtgcagagtcacccactga
21a	caattaggaacttttatttacatttgc	actcccgggctacctgct
21b	ctctgccttggcctttctta	gggcttctctctagagttaccg
22	tcttccttctgaaagtctcatgg	atgcaaagcaaaacccagac
23	tcctggataggttcactctgc	ccgcctcggccagaatgtg
24	tgaacagtaagcctgcttcaa	agctgagattgcatgggatt
25	gagaaagggttagtccaaaatga	ggcaaaagagccattgaaaa
26	cccctcatctggtgaaggta	tcctccaagaccaagatctctc
27	gtttgtttttcgaggcgttt	ttctgaaggatagaataaggcaaga
28	tcaggaggcacacaatgttc	atggctgtttgagggtgtct
29	gcccatcagctgacagatatt	tggcatttcagtgtgaatgtt
30	cgcataggaaggaagacaca	ggctgatacaaatgccaagaa
31	aagcaaacaaaaggaaccaagg	ccaagatgttcattattttctgc
32	gctttcttgtagaatctggttcc	ggaagaacacttgagatctgg

33	gaatcgtttgaacccaggag	gtcatgtccccgattctacc
34	ggcagatagtgggaatgagg	cttgatgctgagccaggact
35	cacaacgtgcaggtttgttac	gttgtgcagagtcccctgtt
36	aggagagaagtgaagcagtcg	gctctagggtcagccaaaca
37	ccagtcagtgcacttcagga	caggattcaaggaatggacaa
38	tggcacataggtgctcaataa	gaggcagccatcaaacaaac
39	tggtgatcaggtccattttg	tcttgaatttgacccagttctgt
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41	caggcagacattttcattctga	gtccatgttcacctgctcct
42	gcatatgtccagaatattgaaaga	tgcgtgaaaggtcctatcct