Supplemental Digital Content for:

Boone PM, Liu P, Zhang F, Carvalho CMB, Towne CF, Batish SD, Lupski JR.* *Alu*-specific

microhomology-mediated deletion of the final exon of *SPAST* in three unrelated subjects with

hereditary spastic paraplegia.

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Frequency of *Alu*s at the *SPAST* locus:

163 *Alu* family members exist over 112,252 bp of sequence within and flanking *SPAST*.

These *Alu*s constitute 40,639 bp (Average of 249 bp per *Alu*)

	Alu family members	Non-*Alu* sequence/249 bp
Observed (within and flanking *SPAST*)	163	287
Expected (based on genome-wide average of 11%[1]	50	400

Pearson's chi-square test result: $\chi^2 = 287.3$; d.f. $= 1$; $p < 0.0001$

Estimation of the expected number of duplication CNVs in *SPAST*:

The number of possible contiguous, internal, exonic CNV in a gene can be calculated by $\sum_{i=1}^{n} i$, where n is the number of internal exons. Thus, in *SPAST*, which has 15 internal exons, 120 different internal exonic duplications (and 120 exonic deletions) are possible. Only 39/120 (32%) involve a multiple of three bp. Thus, 81/120 (68%) are expected to disrupt the reading frame of *SPAST* mRNA, leading to nonsense-mediated decay (NMD) and haploinsufficiency of *SPAST*. Under the lenient assumption that all possible internal exon deletions are pathogenic and no internal exon duplications that preserve the reading frame are pathogenic, an *extremely* rough estimate of 81:120 (~2:3) internal exon duplications:deletions can be made. Reported intragenic CNV (Table 1) vary considerably from this ratio.
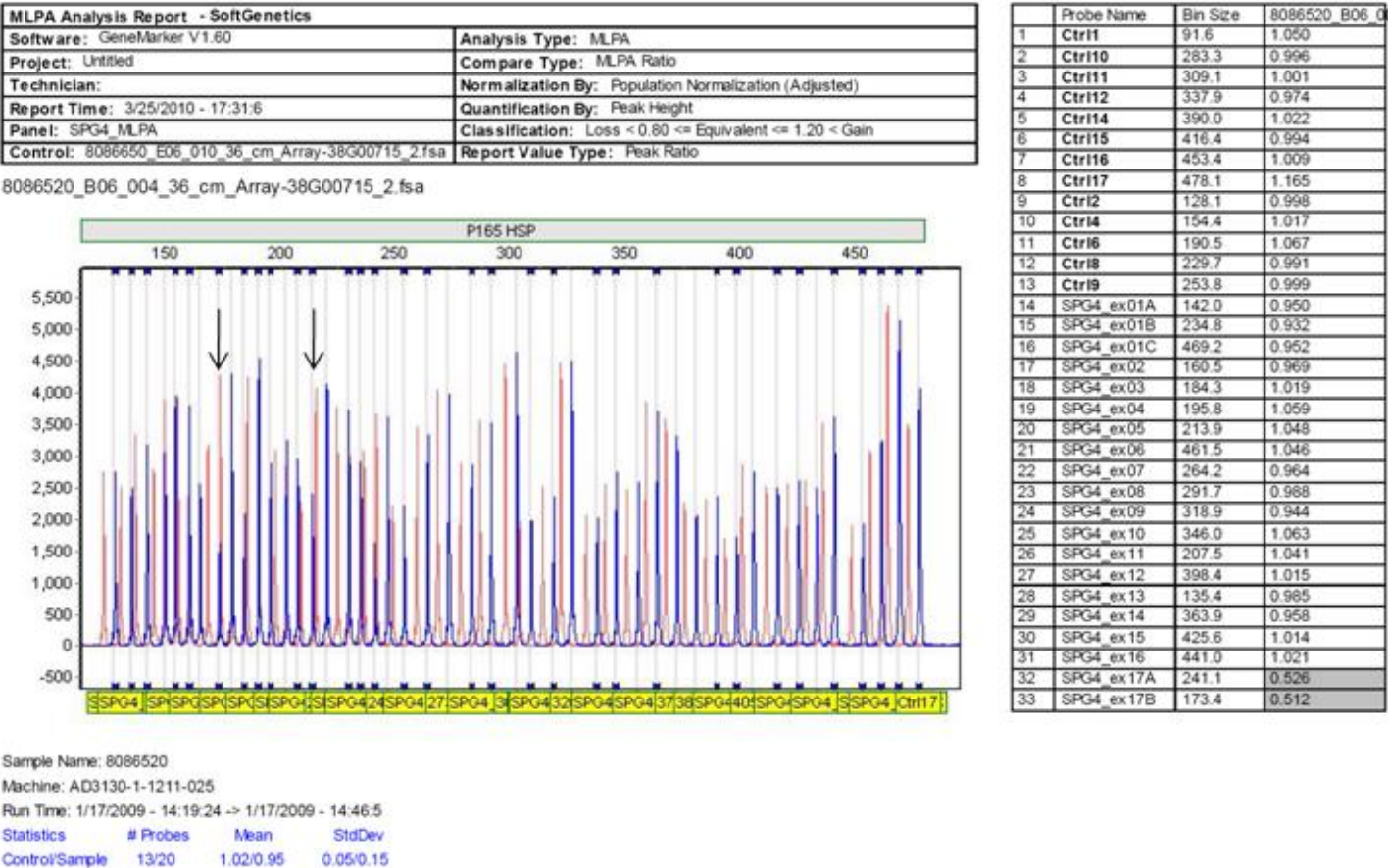
Supplemental Figures:

Patient A37

	Probe Name	Bin Size	8086520_B06_0
1	Ctrl1	91.6	1.050
2	Ctrl10	283.3	0.996
3	Ctrl11	309.1	1.001
4	Ctrl12	337.9	0.974
5	Ctrl14	390.0	1.022
6	Ctrl15	416.4	0.994
7	Ctrl16	453.4	1.009
8	Ctrl17	478.1	1.165
9	Ctrl2	128.1	0.998
10	Ctrl4	154.4	1.017
11	Ctrl6	190.5	1.067
12	Ctrl8	229.7	0.991
13	Ctrl9	253.8	0.999
14	SPG4_ex01A	142.0	0.950
15	SPG4_ex01B	234.8	0.932
16	SPG4_ex01C	469.2	0.952
17	SPG4_ex02	160.5	0.969
18	SPG4_ex03	184.3	1.019
19	SPG4_ex04	195.8	1.059
20	SPG4_ex05	213.9	1.048
21	SPG4_ex06	461.5	1.046
22	SPG4_ex07	264.2	0.964
23	SPG4_ex08	291.7	0.988
24	SPG4_ex09	318.9	0.944
25	SPG4_ex10	346.0	1.063
26	SPG4_ex11	207.5	1.041
27	SPG4_ex12	398.4	1.015
28	SPG4_ex13	135.4	0.985
29	SPG4_ex14	363.9	0.958
30	SPG4_ex15	425.6	1.014
31	SPG4_ex16	441.0	1.021
32	SPG4_ex17A	241.1	0.526
33	SPG4_ex17B	173.4	0.512

Sample Name: 8086520
Machine: AD3130-1-1211-025
Run Time: 1/17/2009 - 14:19:24 -> 1/17/2009 - 14:46:5

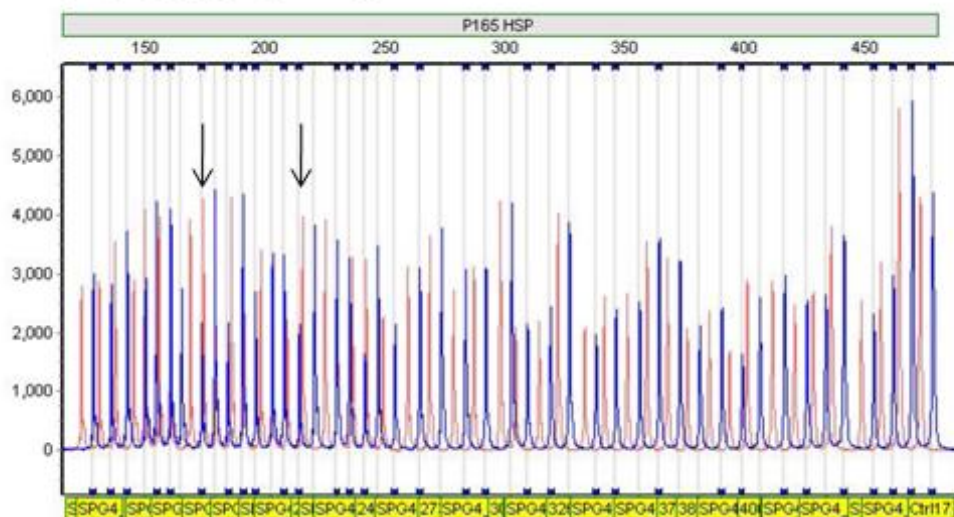| Statistics	# Probes	Mean	StdDev
Control/Sample	13/20	1.02/0.95	0.05/0.15

Figure S1. MLPA detects a genomic loss of the final exon (exon 17) of *SPAST* in patient A37. The results of automated electrophoretic analysis of MLPA amplification products are plotted as intensities (ordinate) versus product size (abscissa). Control traces are displayed in red, patient A37 traces in blue. Both probe sets mapping to *SPAST* exon 17 display diminished signal (arrows) for the patient sample, suggesting deletion of this exon.

Figure S2. MLPA detects a genomic loss of the final exon (exon 17) of *SPAST* in patient A39. The results of automated electrophoretic analysis of MLPA amplification products are plotted as intensities (ordinate) versus product size (abscissa). Control traces are displayed in red, patient A39 traces in blue. Both probe sets mapping to *SPAST* exon 17 display diminished signal (arrows) for the patient sample, suggesting deletion of this exon.

Patient A38

	Probe Name	Bin Size	08048030_D06_C
1	Ctrl1	91.6	1.191
2	Ctrl10	283.3	0.890
3	Ctrl11	309.1	0.990
4	Ctrl12	337.9	0.936
5	Ctrl14	390.0	0.978
6	Ctrl15	416.4	0.886
7	Ctrl16	453.4	0.981
8	Ctrl17	478.1	1.043
9	Ctrl2	128.1	1.006
10	Ctrl4	154.4	1.053
11	Ctrl6	190.5	1.038
12	Ctrl8	229.7	1.018
13	Ctrl9	253.8	0.958
14	SPG4_ex01A	142.0	0.949
15	SPG4_ex01B	234.8	1.033
16	SPG4_ex01C	469.2	1.012
17	SPG4_ex02	160.5	0.994
18	SPG4_ex03	184.3	1.036
19	SPG4_ex04	195.8	0.944
20	SPG4_ex05	213.9	1.054
21	SPG4_ex06	461.5	0.870
22	SPG4_ex07	264.2	0.974
23	SPG4_ex08	291.7	1.024
24	SPG4_ex09	318.9	0.961
25	SPG4_ex10	346.0	0.893
26	SPG4_ex11	207.5	1.021
27	SPG4_ex12	398.4	0.991
28	SPG4_ex13	135.4	1.097
29	SPG4_ex14	363.9	0.931
30	SPG4_ex15	425.6	1.006
31	SPG4_ex16	441.0	0.919
32	SPG4_ex17A	241.1	0.537
33	SPG4_ex17B	173.4	0.546

MLPA Analysis Report - SoftGenetics

Software: GeneMarker V1.60
Project: Untitled
Technician:
Report Time: 3/25/2010 - 17:34:52
Panel: SPG4_MLPA
Control: 08047306_H05_015_36_cm_Array-38C00223_2.fsa

Analysis Type: MLPA
Compare Type: MLPA Ratio
Normalization By: Population Normalization (Adjusted)
Quantification By: Peak Height
Classification: Loss < 0.80 <= Equivalent <= 1.20 < Gain
Report Value Type: Peak Ratio

08048030_D06_008_36_cm_Array-38C00223_2.fsa
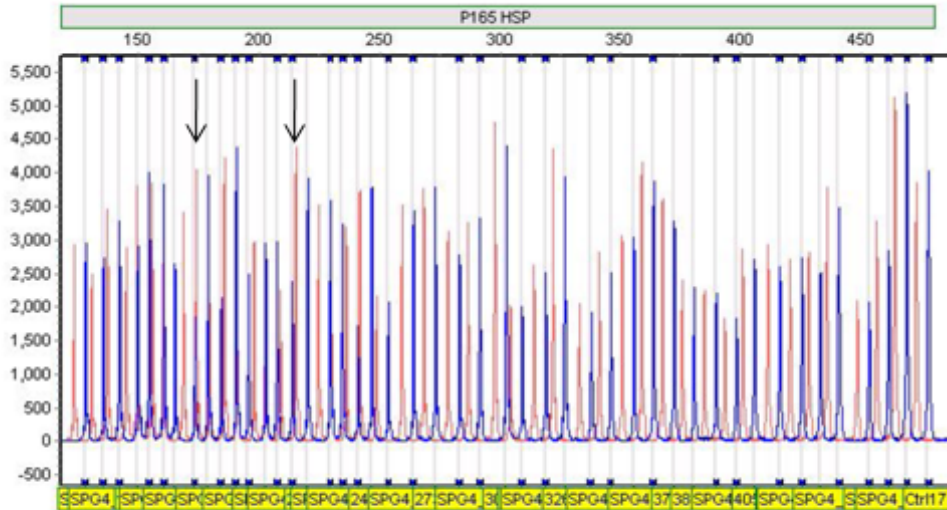
Sample Name: 08048030
Machine: 3130-1-1211-025
Run Time: 7/31/2008 - 8:52:44 -> 7/31/2008 - 9:29:43

Figure S3. MLPA detects a genomic loss of the final exon (exon 17) of *SPAST* in patient A38. The results of automated electrophoretic analysis of MLPA amplification products are plotted as intensities (ordinate) versus product size (abscissa). Control traces are displayed in red, patient A38 traces in blue. Both probe sets mapping to *SPAST* exon 17 display diminished signal (arrows) for the patient sample, suggesting deletion of this exon.

References:

1.   International Human Genome Sequencing Consortium. Initial sequencing and analysis of the human genome. *Nature* 2001;409:860-921.