

Effective Valproic Acid Treatment in Motor Function Is Caused by Possible Mechanism of Elevated Survival Motor Neuron Protein Related With Splicing Factor Gene Expression in Spinal Muscular Atrophy

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Supplementary Materials

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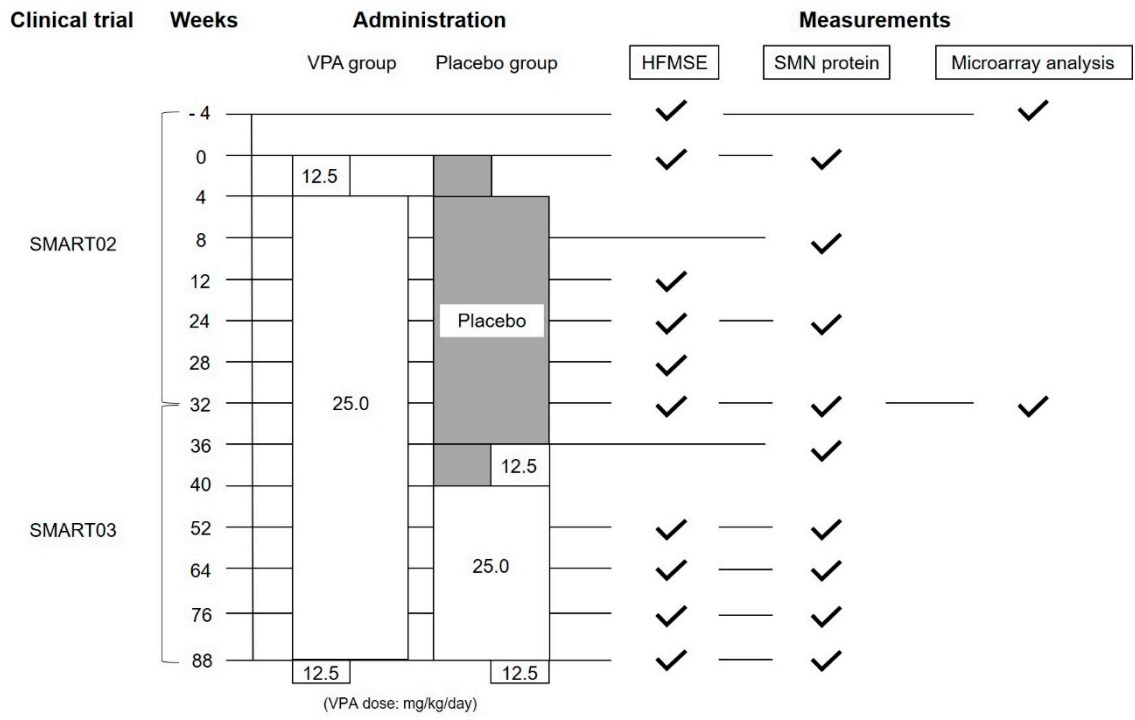
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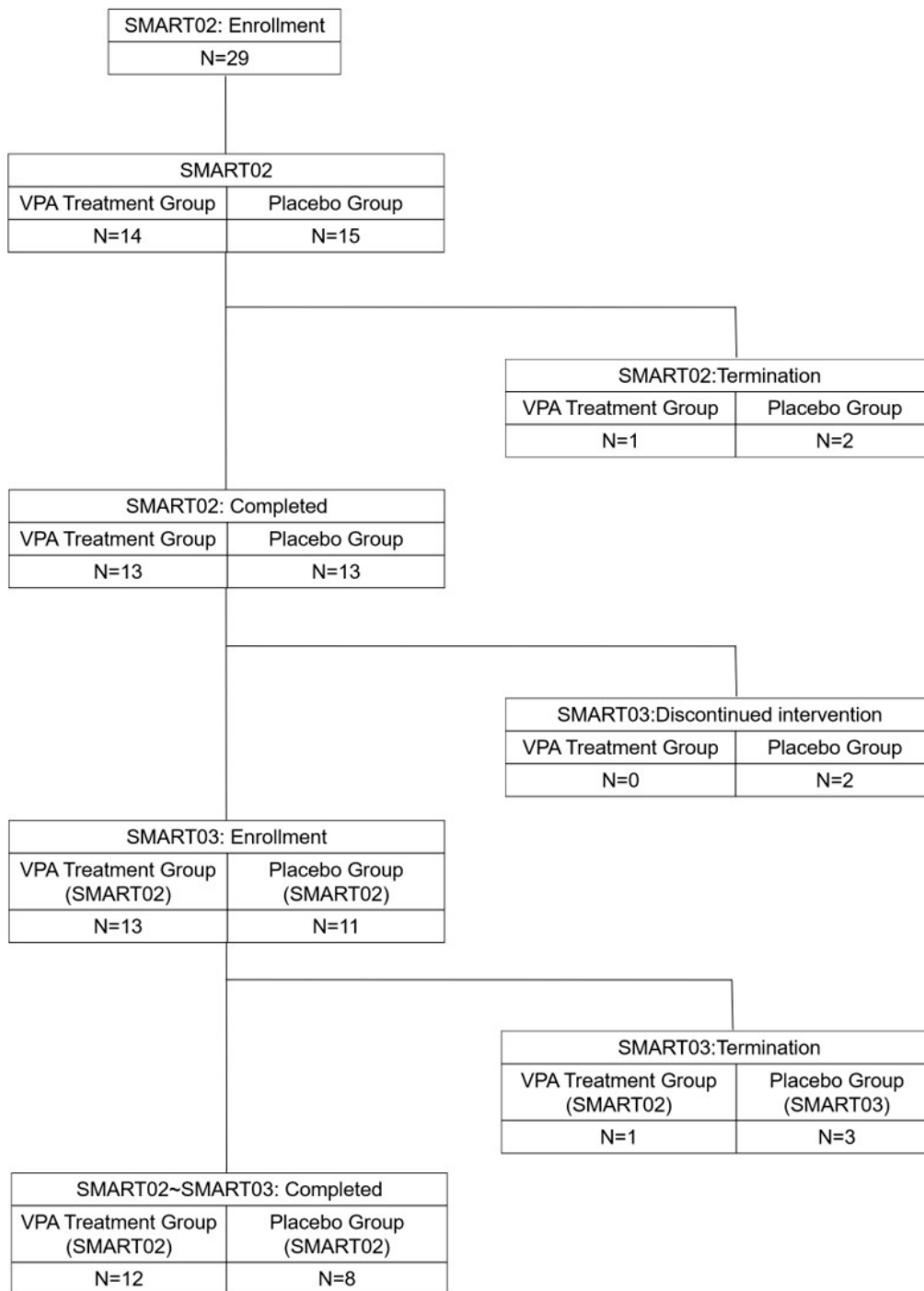
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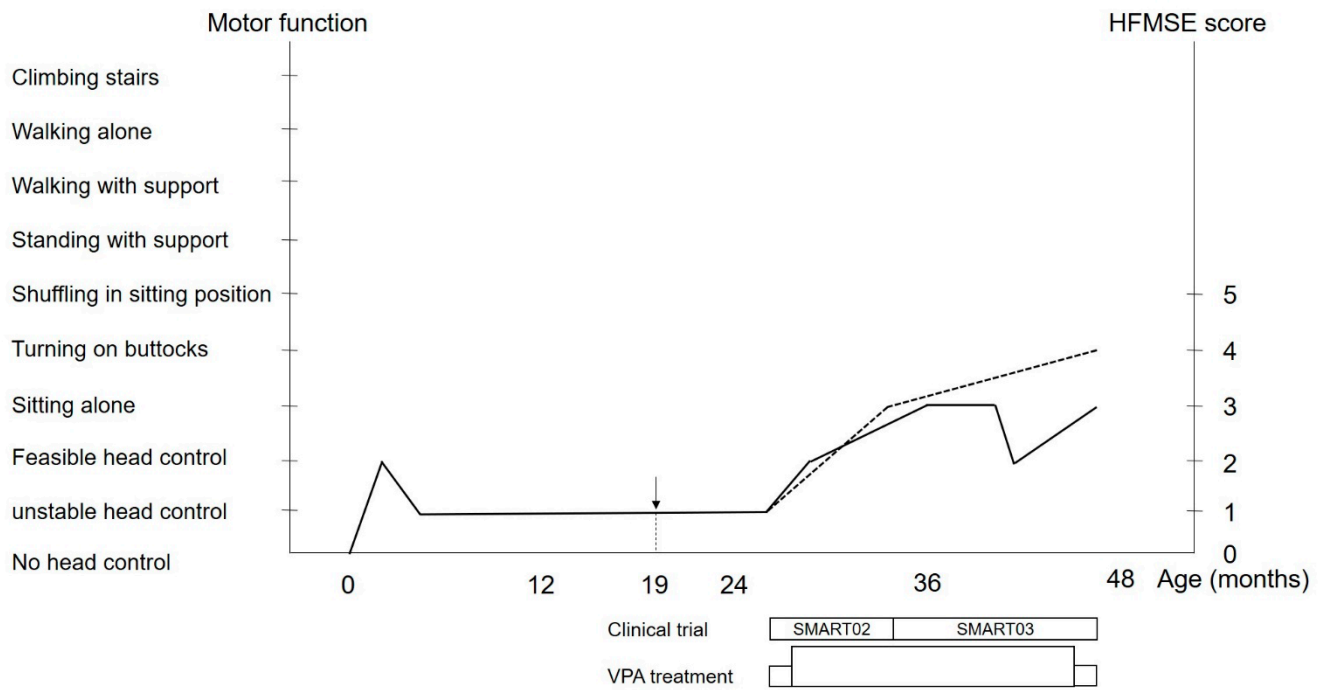


Supplementary Figure 1. Study design.

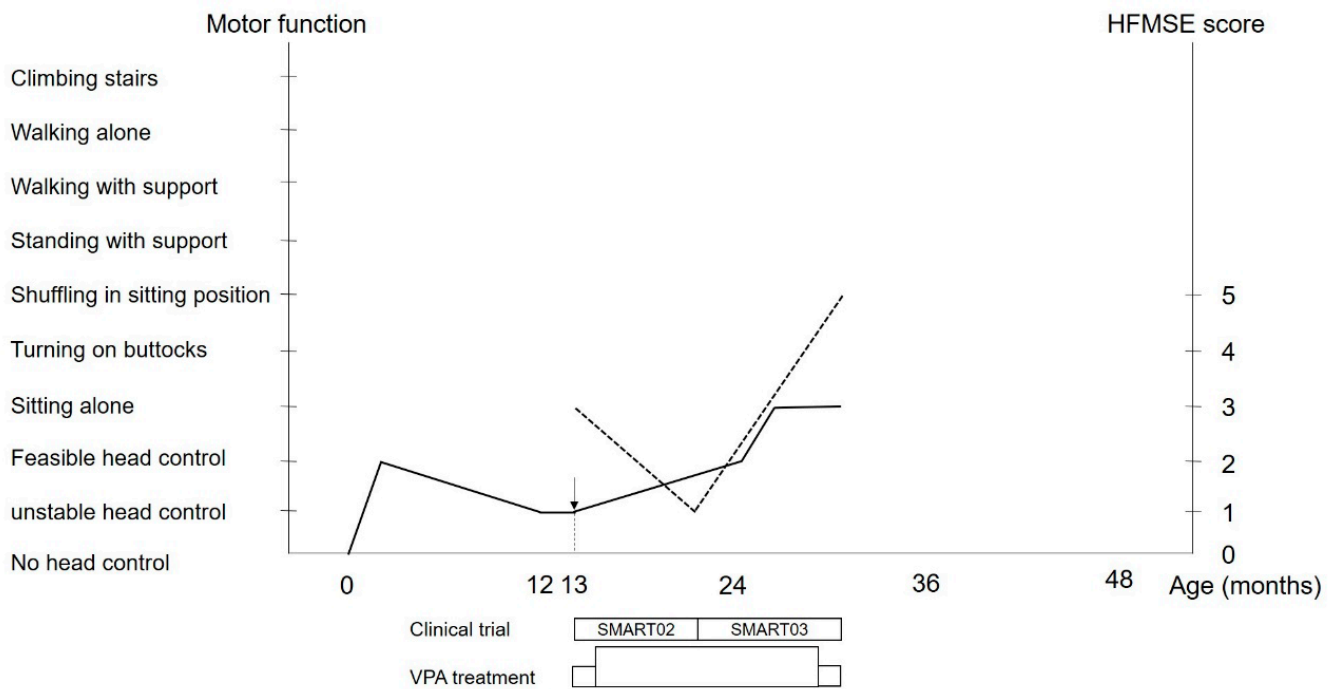


Supplementary Figure 2. Participant flow diagram.

(A)



(B)



Supplementary Figure 3. Change in motor function in response to valproic acid (VPA) treatment. The solid line indicates changes during motor milestones. Motor milestones were classified into 10 levels, and the items modified were based on those presented by Okawa et al. [Okawa Y, Eto F, Ueda S. The analysis of the course of disability in Werdnig–Hoffmann disease (in Japanese). *Rehabilitation Igaku* (Tokyo) 1986;23:115–20.]. The dashed line indicates changes in the Hammersmith Motor Function Scale Expanded (HFMSE) score. The arrow indicates the onset of spinal muscular atrophy (SMA).

(A) Group A: Case 5. At disease onset, the patient was 19 months old and participated in the clinical trial when he was 25 months of age. He was able to sit with support to maintain balance during the SMA Research and Treatment (SMART)-02 trial, although he could not sit completely before the VPA treatment. By the end of the clinical trial, he could sit upright without support. He also acquired the ability to turn from the supine to prone position. His HFMSE score improved up to 4 points from 1 point during the SMART02 trial. Among the cases classified in Group A, Case 2 was able to crawl on all four limbs during SMART02, and she was able to use a manual wheelchair on her own during SMART03.

(B) Group C: Case 12. At disease onset, the patient was 13 months of age, and that is

when he participated in the clinical trial. Although he lost antigravity motion during the SMART02 trial, he was able to sit with one hand support by the end of the entire clinical trial. The HFMSE score was 3 points before VPA treatment; however, it decreased to 2 points at the end of the SMART02 trial. His motor function improved during the SMART03 trial, as stated above, and his HFMSE score increased up to 5 points. Among the cases classified in Group C, in Case 13, motor function gradually regressed before the SMART03 trial; however, the patient was able to move his limbs after the SMART03 trial.

Supplementary Table 1. Classification of participants according to changes in HFMSE score.

| Group | Case | Sex | Age ¹ (months) | Disease Duration (months) | Type (subtype) ² | SMN2 copy number | | HFMSE score – change from baseline ³ |
|-------|------|-----|------------------------------|---------------------------------|--------------------------------|---------------------|--------|---|
| | | | | | | exon 7 | exon 8 | |
| A | 1 | F | 38 | 16 | II (IIb) | 3 | 3 | 8 |
| | 2 | F | 44 | 23 | II (IIa) | 3 | 3 | 7 |
| | 3 | M | 82 | 68 | II (IIa) | 3 | 3 | 5 |
| | 4 | M | 79 | 60 | II (IIb) | 3 | 3 | 5 |
| | 5 | M | 25 | 6 | I (Ib) | 2 | 3 | 3 |
| B | 6 | M | 54 | 33 | II (IIb) | 3 | 3 | 2 |
| | 7 | F | 46 | 36 | II (IIa) | 3 | 3 | 2 |
| | 8 | M | 59 | 47 | II (IIa) | 3 | 3 | 2 |
| | 9 | F | 44 | 29 | II (IIa) | 3 | 3 | 2 |
| | 10 | M | 41 | 22 | II (IIb) | 2 | 2 | 1 |
| C | 11 | M | 24 | 0 | II (IIb) | 3 | 3 | -1 |
| | 12 | M | 13 | 0 | I (Ib) | 2 | 3 | -2 |
| | 13 | M | 33 | 2 | II (IIb) | 3 | 3 | -3 |

¹ Age of the participant when written informed parental consent and assent was obtained.

² According to the subtype classification of Kaneko et al.²⁴, type I is further classified into

type Ia (without head control) and type Ib (head control possible), and type II is further classified into type IIa (can sit independently after 8 months of age) and type IIb (can sit independently before or at 8 months of age).³ To define the efficacy of VPA, the participants were classified into three groups, A, B, and C, by subtracting the best pre-treatment HFMSE score (week -4 or 0) from the best HFMSE score at weeks 24, 28, and 32. HFMSE, Hammersmith Motor Function Scale Expanded; VPA, valproic acid.

Supplementary Table 2. Changes in the HFMSE score in the VPA groups over 88 weeks.

| Group | Case | Week -4 | Week 0 | Week 12 | Week 24 | Week 28 | Week 32 | Week 52 | Week 64 | Week 76 | Week 88 |
|----------|------|---------|--------|---------|---------|---------|---------|---------|---------|---------|---------|
| A | 1 | 12 | 11 | 17 | 20 | 11 | 16 | 14 | 16 | 16 | - |
| | 2 | 22 | 18 | 29 | 29 | 28 | 29 | 29 | 31 | 30 | 29 |
| | 3 | 17 | 18 | 21 | 23 | 22 | 20 | 16 | 18 | 20 | 19 |
| | 4 | 19 | 24 | 26 | 28 | 28 | 29 | 20 | 19 | 29 | - |
| | 5 | 1 | 0 | 4 | 4 | 3 | 3 | 3 | 4 | 4 | - |
| B | 6 | 4 | 4 | 4 | 3 | 4 | 6 | 6 | 5 | 5 | 5 |
| | 7 | 2 | 2 | 3 | 1 | 4 | 4 | 4 | 4 | 2 | 4 |
| | 8 | 6 | 6 | 6 | 6 | 6 | 8 | 5 | 4 | 3 | 6 |
| | 9 | 2 | 5 | 6 | 5 | 6 | 7 | 5 | 7 | 8 | 10 |
| | 10 | 21 | 24 | 16 | 25 | 25 | 25 | 20 | 23 | 21 | 19 |
| C | 11 | - | 28 | 10 | 27 | 26 | - | - | - | 31 | - |
| | 12 | 3 | 2 | 0 | 1 | 1 | 1 | 4 | 5 | - | - |
| | 13 | 31 | 36 | 27 | 30 | 33 | 26 | 36 | 37 | - | - |

Supplementary Table 3. Changes in percentage of SMN-spot⁺ cells in the VPA and placebo groups over 88 weeks.

| Group | | Week 0 | Week 8 | Week 24 | Week 32 | Week 36 | Week 52 | Week 64 | Week 76 | Week 88 |
|---------------|------|--------|----------|----------|----------|----------|-----------|----------|---------|---------|
| A | N | 5 | 3 | 5 | 5 | 5 | 5 | 5 | 4 | 2 |
| | Mean | 9.08 | 17.87 | 21.86 #1 | 25.32 #2 | 19.46 #3 | 15.98 ##4 | 12.08 #5 | 24.68 | 20.05 |
| | SD | 3.39 | 5.46 | 6.26 *5 | 10.55 | 6.99 | 5.24 | 4.98 | 18.73 | 7.85 |
| B | N | 5 ***1 | 2 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| | Mean | 12.24 | 11.85 *2 | 11.76 | 21.34 | 21.42 #6 | 17.42 | 13.96 | 14.80 | 30.30 |
| | SD | 1.22 | 2.05 **3 | 3.16 | 12.63 | 6.93 | 6.64 | 4.83 | 3.45 | 23.94 |
| C | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 1 | 0 |
| | Mean | 26.47 | 26.90 | 27.57 | 20.33 | 19.43 | 29.80 | 24.47 | - | - |
| | SD | 2.84 | 10.46 | 8.69 **6 | 12.55 | 8.42 | 17.69 | 8.76 | - | - |
| VPA treatment | N | 13 | 8 **4 | 13 | 13 | 13 | 13 | 13 | 10 | 7 |
| | Mean | 14.31 | 19.75 | 19.29 #7 | 22.64 #8 | 20.21 #9 | 19.72 | 15.66 | 20.87 | 27.37 |
| | SD | 7.47 | 9.04 | 8.51 | 11.03 | 6.72 | 10.46 | 7.40 | 13.21 | 20.43 |
| Placebo | N | 13 | 8 | 13 | 13 | 10 | 10 | 9 | 7 | 5 |
| | Mean | 15.54 | 17.94 | 18.64 | 20.28 | 17.17 | 14.99 | 14.47 | 15.60 | 14.96 |
| | SD | 6.73 | 6.55 | 4.93 | 9.19 | 4.21 | 6.74 | 5.15 | 9.99 | 5.22 |

*p-values are from the two-sample t-test between groups. *p < 0.05, **p < 0.01, ***p < 0.001; ***1: p = 0.0005, *2: p = 0.017, **3: p = 0.008, **4: p = 0.002, *5: p = 0.018, **6: p = 0.005. #p-values are from the one-sample t-test compared between week 0 and each time point in VPA group. #p < 0.05, ##p < 0.01; #1: p = 0.011, #2: p = 0.023, #3: p = 0.018, ##4: p = 0.003, #5: p = 0.021, #6: p = 0.026, #7: p = 0.047, #8: p = 0.044, #9: p = 0.045. VPA, valproic acid.

Supplementary Table 4. Gene Ontology (GO) analysis of probe sets significantly

difference in expression changes between Group A and C.

| Term | Count | % | p-value |
|--|-------|------|----------|
| alternative splicing | 477 | 44.8 | 6.45E-13 |
| splice variant | 476 | 44.7 | 1.23E-12 |
| phosphoprotein | 420 | 39.5 | 2.64E-05 |
| GO:0007507~heart development | 26 | 2.4 | 6.97E-05 |
| GO:0016879~ligase activity, forming carbon-nitrogen bonds | 28 | 2.6 | 7.33E-05 |
| GO:0016881~acid-amino acid ligase activity | 24 | 2.3 | 3.35E-04 |
| GO:0019787~small conjugating protein ligase activity | 21 | 2.0 | 4.11E-04 |
| Proto-oncogene | 25 | 2.3 | 4.64E-04 |
| GO:0004842~ubiquitin-protein ligase activity | 19 | 1.8 | 6.48E-04 |
| GO:0060589~nucleoside-triphosphatase regulator activity | 38 | 3.6 | 9.46E-04 |
| GO:0007167~enzyme linked receptor protein signaling pathway | 32 | 3.0 | 9.49E-04 |
| GO:0000151~ubiquitin ligase complex | 13 | 1.2 | 0.00110 |
| GO:0030695~GTPase regulator activity | 37 | 3.5 | 0.00120 |
| hsa04360:Axon guidance | 16 | 1.5 | 0.00145 |
| chromosomal rearrangement | 27 | 2.5 | 0.00150 |
| GO:0034097~response to cytokine stimulus | 12 | 1.1 | 0.00178 |
| hsa04020:Calcium signaling pathway | 19 | 1.8 | 0.00222 |
| metal-binding | 180 | 16.9 | 0.00226 |
| GO:0004714~transmembrane receptor protein tyrosine kinase activity | 11 | 1.0 | 0.00236 |
| GO:0046332~SMAD binding | 9 | 0.8 | 0.00237 |
| GO:0005626~insoluble fraction | 59 | 5.5 | 0.00250 |
| GO:0016528~sarcoplasm | 8 | 0.8 | 0.00292 |
| region of interest:Catalytic | 9 | 0.8 | 0.00303 |
| cytoplasm | 198 | 18.6 | 0.00309 |
| GO:0001882~nucleoside binding | 109 | 10.2 | 0.00341 |
| GO:0051056~regulation of small GTPase mediated signal transduction | 24 | 2.3 | 0.00379 |

| Term | Count | % | p-value |
|---|-------|------|---------|
| GO:0001883~purine nucleoside binding | 108 | 10.2 | 0.00384 |
| GO:0005730~nucleolus | 50 | 4.7 | 0.00389 |
| GO:0017076~purine nucleotide binding | 126 | 11.8 | 0.00405 |
| GO:0030427~site of polarized growth | 9 | 0.8 | 0.00422 |
| GO:0043228~non-membrane-bounded organelle | 151 | 14.2 | 0.00430 |
| GO:0043232~intracellular non-membrane-bounded organelle | 151 | 14.2 | 0.00430 |
| GO:0010604~positive regulation of macromolecule metabolic process | 61 | 5.7 | 0.00450 |
| GO:0030554~adenyl nucleotide binding | 106 | 10.0 | 0.00462 |
| GO:0000166~nucleotide binding | 144 | 13.5 | 0.00499 |
| IPR013761:Sterile alpha motif-type | 11 | 1.0 | 0.00530 |
| GO:0005096~GTPase activator activity | 22 | 2.1 | 0.00558 |
| GO:0032469~endoplasmic reticulum calcium ion homeostasis | 4 | 0.4 | 0.00571 |
| nucleotide-binding | 107 | 10.1 | 0.00573 |
| GO:0042995~cell projection | 49 | 4.6 | 0.00612 |
| guanine-nucleotide releasing factor | 14 | 1.3 | 0.00686 |
| GO:0005624~membrane fraction | 55 | 5.2 | 0.00705 |
| GO:0042802~identical protein binding | 49 | 4.6 | 0.00715 |
| GO:0008237~metallopeptidase activity | 19 | 1.8 | 0.00730 |
| GO:0019933~cAMP-mediated signaling | 12 | 1.1 | 0.00751 |
| atp-binding | 86 | 8.1 | 0.00776 |
| GO:0032553~ribonucleotide binding | 119 | 11.2 | 0.00843 |
| GO:0032555~purine ribonucleotide binding | 119 | 11.2 | 0.00843 |
| IPR001715:Calponin-like actin-binding | 10 | 0.9 | 0.00851 |
| GO:0042175~nuclear envelope-endoplasmic reticulum network | 24 | 2.3 | 0.00892 |
| GO:0005083~small GTPase regulator activity | 25 | 2.3 | 0.00917 |
| GO:0005789~endoplasmic reticulum membrane | 23 | 2.2 | 0.00930 |
| GO:0007179~transforming growth factor beta receptor signaling pathway | 9 | 0.8 | 0.00943 |

| Term | Count | % | p-value |
|---|-------|------|---------|
| GO:0016529~sarcoplasmic reticulum | 7 | 0.7 | 0.00966 |
| GO:0005524~ATP binding | 98 | 9.2 | 0.00979 |
| autophosphorylation | 8 | 0.8 | 0.00989 |
| calcium channel | 9 | 0.8 | 0.01000 |
| GO:0000267~cell fraction | 69 | 6.5 | 0.01019 |
| kinase | 49 | 4.6 | 0.01020 |
| GO:0002237~response to molecule of bacterial origin | 11 | 1.0 | 0.01025 |
| GO:0000228~nuclear chromosome | 16 | 1.5 | 0.01036 |
| GO:0032559~adenyl ribonucleotide binding | 99 | 9.3 | 0.01049 |
| hsa04010:MAPK signaling pathway | 23 | 2.2 | 0.01067 |
| repeat:ANK 1 | 21 | 2.0 | 0.01149 |
| repeat:ANK 2 | 21 | 2.0 | 0.01201 |
| GO:0045935~positive regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process | 45 | 4.2 | 0.01250 |
| GO:0004713~protein tyrosine kinase activity | 17 | 1.6 | 0.01322 |
| GO:0044454~nuclear chromosome part | 13 | 1.2 | 0.01327 |
| GO:0046872~metal ion binding | 244 | 22.9 | 0.01331 |
| GO:0007178~transmembrane receptor protein serine/threonine kinase signaling pathway | 12 | 1.1 | 0.01344 |
| polymorphism | 605 | 56.9 | 0.01346 |
| GO:0030426~growth cone | 8 | 0.8 | 0.01352 |
| ATP | 21 | 2.0 | 0.01358 |
| GO:0042383~sarcolemma | 9 | 0.8 | 0.01377 |
| GO:0070647~protein modification by small protein conjugation or removal | 16 | 1.5 | 0.01380 |
| GO:0032496~response to lipopolysaccharide | 10 | 0.9 | 0.01404 |
| IPR008253:Marvel | 6 | 0.6 | 0.01415 |
| GO:0044432~endoplasmic reticulum part | 27 | 2.5 | 0.01480 |
| nucleotide phosphate-binding region:ATP | 64 | 6.0 | 0.01496 |
| GO:0016567~protein ubiquitination | 13 | 1.2 | 0.01546 |

| Term | Count | % | p-value |
|---|-------|------|---------|
| GO:0051254~positive regulation of RNA metabolic process | 36 | 3.4 | 0.01590 |
| ank repeat | 21 | 2.0 | 0.01605 |
| GO:0001666~response to hypoxia | 14 | 1.3 | 0.01622 |
| GO:0044459~plasma membrane part | 126 | 11.8 | 0.01652 |
| GO:0004672~protein kinase activity | 45 | 4.2 | 0.01674 |
| domain:CH | 7 | 0.7 | 0.01686 |
| repeat:ANK 3 | 18 | 1.7 | 0.01687 |
| GO:0043169~cation binding | 245 | 23.0 | 0.01712 |
| dna-binding | 113 | 10.6 | 0.01741 |
| nucleus | 240 | 22.6 | 0.01750 |
| GO:0008637~apoptotic mitochondrial changes | 6 | 0.6 | 0.01752 |
| domain:DH | 9 | 0.8 | 0.01762 |
| domain:Protein kinase | 35 | 3.3 | 0.01805 |
| GO:0043167~ion binding | 248 | 23.3 | 0.01822 |
| IPR002110:Ankyrin | 21 | 2.0 | 0.01836 |
| SM00033:CH | 10 | 0.9 | 0.01843 |
| zinc | 130 | 12.2 | 0.01863 |
| GO:0006875~cellular metal ion homeostasis | 18 | 1.7 | 0.01864 |
| IPR011616:bZIP transcription factor, bZIP-1 | 6 | 0.6 | 0.01864 |
| GO:0016605~PML body | 6 | 0.6 | 0.01884 |
| IPR001478:PDZ/DHR/GLGF | 15 | 1.4 | 0.01896 |
| GO:0048666~neuron development | 27 | 2.5 | 0.01932 |
| GO:0043087~regulation of GTPase activity | 13 | 1.2 | 0.01961 |
| compositionally biased region:Ala-rich | 15 | 1.4 | 0.02038 |
| hsa04270:Vascular smooth muscle contraction | 12 | 1.1 | 0.02042 |
| GO:0051173~positive regulation of nitrogen compound metabolic process | 45 | 4.2 | 0.02057 |
| GO:0006874~cellular calcium ion homeostasis | 17 | 1.6 | 0.02059 |
| binding site:ATP | 39 | 3.7 | 0.02071 |
| hsa04912:GnRH signaling pathway | 11 | 1.0 | 0.02082 |
| GO:0008270~zinc ion binding | 142 | 13.3 | 0.02150 |
| GO:0005262~calcium channel activity | 10 | 0.9 | 0.02158 |

| Term | Count | % | p-value |
|--|-------|------|---------|
| phosphotransferase | 18 | 1.7 | 0.02199 |
| domain:RGS | 6 | 0.6 | 0.02240 |
| GO:0050770~regulation of axonogenesis | 8 | 0.8 | 0.02245 |
| GO:0010975~regulation of neuron projection development | 9 | 0.8 | 0.02261 |
| GO:0045893~positive regulation of transcription, DNA-dependent | 35 | 3.3 | 0.02270 |
| IPR000219:Dbl homology (DH) domain | 9 | 0.8 | 0.02276 |
| coiled coil | 120 | 11.3 | 0.02297 |
| GO:0045941~positive regulation of transcription | 40 | 3.8 | 0.02356 |
| GO:0070482~response to oxygen levels | 14 | 1.3 | 0.02370 |
| 22.Cytokine-chemokine_CNS | 4 | 0.4 | 0.02381 |
| calcium transport | 10 | 0.9 | 0.02435 |
| IPR008266:Tyrosine protein kinase, active site | 11 | 1.0 | 0.02477 |
| GO:0006816~calcium ion transport | 14 | 1.3 | 0.02495 |
| GO:0034976~response to endoplasmic reticulum stress | 6 | 0.6 | 0.02540 |
| GO:0035023~regulation of Rho protein signal transduction | 11 | 1.0 | 0.02548 |
| GO:0055074~calcium ion homeostasis | 17 | 1.6 | 0.02574 |
| GO:0030534~adult behavior | 10 | 0.9 | 0.02707 |
| IPR000719:Protein kinase, core | 35 | 3.3 | 0.02733 |
| alternative promoter usage | 7 | 0.7 | 0.02741 |
| GO:0055065~metal ion homeostasis | 18 | 1.7 | 0.02743 |
| domain:Ig-like C2-type 1 | 17 | 1.6 | 0.02780 |
| GO:0005085~guanyl-nucleotide exchange factor activity | 15 | 1.4 | 0.02782 |
| compositionally biased region:Gly-rich | 20 | 1.9 | 0.02878 |
| GO:0019935~cyclic-nucleotide-mediated signaling | 13 | 1.2 | 0.02880 |
| GO:0043193~positive regulation of gene-specific transcription | 10 | 0.9 | 0.02891 |
| domain:Ig-like C2-type 2 | 17 | 1.6 | 0.02900 |
| GO:0046914~transition metal ion binding | 167 | 15.7 | 0.02905 |

| Term | Count | % | p-value |
|--|-------|-----|---------|
| ligase | 24 | 2.3 | 0.02978 |
| IPR000342:Regulator of G protein signalling | 6 | 0.6 | 0.03021 |
| IPR000299:FERM domain | 7 | 0.7 | 0.03023 |
| IPR019748:FERM central domain | 7 | 0.7 | 0.03023 |
| GO:0022604~regulation of cell morphogenesis | 13 | 1.2 | 0.03033 |
| GO:0031175~neuron projection development | 21 | 2.0 | 0.03116 |
| GO:0043596~nuclear replication fork | 4 | 0.4 | 0.03177 |
| GO:0032446~protein modification by small protein conjugation | 13 | 1.2 | 0.03192 |
| zinc finger region:PHD-type; atypical | 3 | 0.3 | 0.03239 |
| GO:0009214~cyclic nucleotide catabolic process | 3 | 0.3 | 0.03252 |
| h_Ccr5Pathway:Pertussis toxin-insensitive CCR5 Signaling in Macrophage | 4 | 0.4 | 0.03280 |
| GO:0031344~regulation of cell projection organization | 10 | 0.9 | 0.03286 |
| GO:0016323~basolateral plasma membrane | 17 | 1.6 | 0.03294 |
| GO:0007626~locomotory behavior | 22 | 2.1 | 0.03333 |
| GO:0046578~regulation of Ras protein signal transduction | 18 | 1.7 | 0.03350 |
| GO:0070161~anchoring junction | 15 | 1.4 | 0.03504 |
| hsa05414:Dilated cardiomyopathy | 10 | 0.9 | 0.03507 |
| site:Susceptible to oxidation | 4 | 0.4 | 0.03559 |
| nucleotide phosphate-binding region:cNMP | 4 | 0.4 | 0.03559 |
| GO:0010628~positive regulation of gene expression | 40 | 3.8 | 0.03568 |
| GO:0035091~phosphoinositide binding | 11 | 1.0 | 0.03575 |
| PIRSF002400:GTP-binding regulatory protein Gs alpha chain | 4 | 0.4 | 0.03586 |
| lipid moiety-binding region:S-palmitoyl cysteine | 16 | 1.5 | 0.03658 |
| IPR001245:Tyrosine protein kinase | 12 | 1.1 | 0.03673 |
| Breast cancer, susceptibility to | 3 | 0.3 | 0.03674 |
| IPR014721:Ribosomal protein S5 domain 2-type fold | 4 | 0.4 | 0.03725 |
| GO:0046700~heterocycle catabolic process | 9 | 0.8 | 0.03736 |

| Term | Count | % | p-value |
|--|-------|-----|---------|
| GO:0010769~regulation of cell morphogenesis involved in differentiation | 9 | 0.8 | 0.03736 |
| domain:MARVEL | 5 | 0.5 | 0.03792 |
| GO:0030016~myofibril | 11 | 1.0 | 0.03846 |
| GO:0005089~Rho guanyl-nucleotide exchange factor activity | 9 | 0.8 | 0.03857 |
| GO:0009187~cyclic nucleotide metabolic process | 6 | 0.6 | 0.03899 |
| tumor suppressor | 13 | 1.2 | 0.03904 |
| GO:0019842~vitamin binding | 13 | 1.2 | 0.03916 |
| IPR001331:Guanine-nucleotide dissociation stimulator, CDC24, conserved site | 8 | 0.8 | 0.03924 |
| IPR019749:Band 4.1 domain | 7 | 0.7 | 0.03944 |
| IPR019747:FERM conserved site | 7 | 0.7 | 0.03944 |
| GO:0005088~Ras guanyl-nucleotide exchange factor activity | 10 | 0.9 | 0.03981 |
| GO:0012505~endomembrane system | 49 | 4.6 | 0.04002 |
| GO:0030055~cell-substrate junction | 11 | 1.0 | 0.04052 |
| GO:0007005~mitochondrion organization | 13 | 1.2 | 0.04271 |
| SM00325:RhoGEF | 9 | 0.8 | 0.04382 |
| GO:0040036~regulation of fibroblast growth factor receptor signaling pathway | 3 | 0.3 | 0.04405 |
| GO:0046782~regulation of viral transcription | 3 | 0.3 | 0.04405 |
| IPR001019:Guanine nucleotide binding protein (G-protein), alpha subunit | 4 | 0.4 | 0.04417 |
| IPR001660:Sterile alpha motif SAM | 10 | 0.9 | 0.04554 |
| GO:0031674~I band | 7 | 0.7 | 0.04555 |
| GO:0008047~enzyme activator activity | 26 | 2.4 | 0.04557 |
| IPR013089:Kelch related | 9 | 0.8 | 0.04599 |
| ubl conjugation pathway | 35 | 3.3 | 0.04687 |
| domain:PDZ | 11 | 1.0 | 0.04708 |
| GO:0007189~activation of adenylate cyclase activity by G-protein signaling pathway | 6 | 0.6 | 0.04717 |

| Term | Count | % | p-value |
|---|-------|------|---------|
| GO:0010578~regulation of adenylate cyclase activity involved in G-protein signaling | 6 | 0.6 | 0.04717 |
| GO:0010579~positive regulation of adenylate cyclase activity by G-protein signaling pathway | 6 | 0.6 | 0.04717 |
| GO:0019838~growth factor binding | 11 | 1.0 | 0.04743 |
| domain:SAM | 9 | 0.8 | 0.04776 |
| SM00228:PDZ | 15 | 1.4 | 0.04800 |
| SM00315:RGS | 6 | 0.6 | 0.04839 |
| GO:0030182~neuron differentiation | 31 | 2.9 | 0.04845 |
| hsa05200:Pathways in cancer | 24 | 2.3 | 0.04941 |
| proteoglycan | 7 | 0.7 | 0.04948 |
| GO:0006917~induction of apoptosis | 24 | 2.3 | 0.04952 |
| GO:0045761~regulation of adenylate cyclase activity | 10 | 0.9 | 0.04958 |
| GO:0008589~regulation of smoothed signaling pathway | 4 | 0.4 | 0.04970 |
| GO:0010033~response to organic substance | 47 | 4.4 | 0.05010 |
| IPR000910:High mobility group, HMG1/HMG2 | 7 | 0.7 | 0.05026 |
| IPR004827:Basic-leucine zipper (bZIP) transcription factor | 7 | 0.7 | 0.05026 |
| IPR004843:Metallophosphoesterase | 5 | 0.5 | 0.05075 |
| GO:0043279~response to alkaloid | 7 | 0.7 | 0.05092 |
| GO:0008629~induction of apoptosis by intracellular signals | 7 | 0.7 | 0.05092 |
| GO:0012502~induction of programmed cell death | 24 | 2.3 | 0.05125 |
| GO:0000152~nuclear ubiquitin ligase complex | 4 | 0.4 | 0.05131 |
| GO:0045178~basal part of cell | 5 | 0.5 | 0.05212 |
| GO:0008624~induction of apoptosis by extracellular signals | 11 | 1.0 | 0.05256 |
| sequence variant | 621 | 58.4 | 0.05279 |
| compositionally biased region:Pro-rich | 60 | 5.6 | 0.05349 |
| GO:0044463~cell projection part | 18 | 1.7 | 0.05395 |

| Term | Count | % | p-value |
|--|-------|-----|---------|
| GO:0010557~positive regulation of macromolecule biosynthetic process | 43 | 4.0 | 0.05437 |
| IPR011510:Sterile alpha motif homology 2 | 6 | 0.6 | 0.05457 |
| h_Par1Pathway:Thrombin signaling and protease-activated receptors | 4 | 0.4 | 0.05478 |
| GO:0007190~activation of adenylate cyclase activity | 7 | 0.7 | 0.05485 |
| GO:0006873~cellular ion homeostasis | 27 | 2.5 | 0.05505 |
| GO:0006508~proteolysis | 65 | 6.1 | 0.05530 |
| GO:0015674~di-, tri-valent inorganic cation transport | 15 | 1.4 | 0.05673 |
| SM00248:ANK | 21 | 2.0 | 0.05701 |
| domain:LIM zinc-binding | 4 | 0.4 | 0.05721 |
| GO:0008088~axon cargo transport | 4 | 0.4 | 0.05751 |
| IPR017441:Protein kinase, ATP binding site | 32 | 3.0 | 0.05764 |
| zinc-finger | 100 | 9.4 | 0.05796 |
| GO:0031279~regulation of cyclase activity | 10 | 0.9 | 0.05820 |
| GO:0045637~regulation of myeloid cell differentiation | 8 | 0.8 | 0.05895 |
| GO:0045762~positive regulation of adenylate cyclase activity | 7 | 0.7 | 0.05897 |
| activator | 35 | 3.3 | 0.05919 |
| Genome-wide association of echocardiographic dimensions, brachial artery endothelial function and treadmill exercise responses in the Framingham Heart Study | 3 | 0.3 | 0.05936 |
| repeat:TPR 9 | 5 | 0.5 | 0.05956 |
| GO:0007188~G-protein signaling, coupled to cAMP nucleotide second messenger | 9 | 0.8 | 0.06082 |
| GO:0030005~cellular di-, tri-valent inorganic cation homeostasis | 18 | 1.7 | 0.06149 |
| GO:0019725~cellular homeostasis | 32 | 3.0 | 0.06181 |
| GO:0043292~contractile fiber | 11 | 1.0 | 0.06246 |

| Term | Count | % | p-value |
|---|-------|-----|---------|
| GO:0055082~cellular chemical homeostasis | 27 | 2.5 | 0.06313 |
| GO:0031281~positive regulation of cyclase activity | 7 | 0.7 | 0.06327 |
| palmitate | 17 | 1.6 | 0.06364 |
| GO:0031399~regulation of protein modification process | 22 | 2.1 | 0.06380 |
| GO:0019941~modification-dependent protein catabolic process | 38 | 3.6 | 0.06426 |
| GO:0043632~modification-dependent macromolecule catabolic process | 38 | 3.6 | 0.06426 |
| GO:0030817~regulation of cAMP biosynthetic process | 10 | 0.9 | 0.06445 |
| GO:0051339~regulation of lyase activity | 10 | 0.9 | 0.06445 |
| glycolysis | 6 | 0.6 | 0.06472 |
| GO:0001654~eye development | 12 | 1.1 | 0.06519 |
| GO:0005924~cell-substrate adherens junction | 10 | 0.9 | 0.06526 |
| domain:SH3 3 | 4 | 0.4 | 0.06551 |
| GO:0042219~cellular amino acid derivative catabolic process | 4 | 0.4 | 0.06585 |
| SM00295:B41 | 7 | 0.7 | 0.06595 |
| GO:0005912~adherens junction | 13 | 1.2 | 0.06599 |
| GO:0030018~Z disc | 6 | 0.6 | 0.06611 |
| GO:0031981~nuclear lumen | 82 | 7.7 | 0.06782 |
| GO:0051130~positive regulation of cellular component organization | 15 | 1.4 | 0.06828 |
| IPR013027:FAD-dependent pyridine nucleotide-disulphide oxidoreductase | 4 | 0.4 | 0.06838 |
| GO:0005100~Rho GTPase activator activity | 5 | 0.5 | 0.06886 |
| dna replication | 9 | 0.8 | 0.06929 |
| hirschsprung disease | 3 | 0.3 | 0.06977 |
| GO:0009125~nucleoside monophosphate catabolic process | 3 | 0.3 | 0.07071 |
| GO:0009791~post-embryonic development | 8 | 0.8 | 0.07072 |
| GO:0030814~regulation of cAMP metabolic process | 10 | 0.9 | 0.07111 |

| Term | Count | % | p-value |
|---|-------|-----|---------|
| cell adhesion | 29 | 2.7 | 0.07123 |
| hsa04120:Ubiquitin mediated proteolysis | 12 | 1.1 | 0.07127 |
| metalloprotease | 13 | 1.2 | 0.07132 |
| GO:0044427~chromosomal part | 26 | 2.4 | 0.07172 |
| GO:0051349~positive regulation of lyase activity | 7 | 0.7 | 0.07240 |
| IPR003613:U box domain | 3 | 0.3 | 0.07269 |
| IPR001327:Pyridine nucleotide-disulphide oxidoreductase, NAD-binding region | 3 | 0.3 | 0.07269 |
| GO:0005694~chromosome | 30 | 2.8 | 0.07425 |
| GO:0030516~regulation of axon extension | 4 | 0.4 | 0.07471 |
| IPR001841:Zinc finger, RING-type | 22 | 2.1 | 0.07506 |
| serine/threonine-specific protein kinase | 7 | 0.7 | 0.07516 |
| GO:0051603~proteolysis involved in cellular protein catabolic process | 39 | 3.7 | 0.07548 |
| h_p35alzheimersPathway:Deregulation of CDK5 in Alzheimers Disease | 3 | 0.3 | 0.07627 |
| IPR017907:Zinc finger, RING-type, conserved site | 21 | 2.0 | 0.07645 |
| GO:0030900~forebrain development | 13 | 1.2 | 0.07714 |
| SM00219:TyrKc | 12 | 1.1 | 0.07728 |
| GO:0030054~cell junction | 33 | 3.1 | 0.07748 |
| IPR006586:ADAM, cysteine-rich | 4 | 0.4 | 0.07754 |
| short sequence motif:PDZ-binding | 8 | 0.8 | 0.07868 |
| GO:0005887~integral to plasma membrane | 68 | 6.4 | 0.07870 |
| GO:0051336~regulation of hydrolase activity | 24 | 2.3 | 0.07907 |
| GO:0044257~cellular protein catabolic process | 39 | 3.7 | 0.07942 |
| GO:0014070~response to organic cyclic substance | 11 | 1.0 | 0.07968 |
| GO:0005543~phospholipid binding | 15 | 1.4 | 0.07974 |
| GO:0006195~purine nucleotide catabolic process | 5 | 0.5 | 0.07975 |
| GO:0008630~DNA damage response, signal transduction resulting in induction of apoptosis | 5 | 0.5 | 0.07975 |
| wnt signaling pathway | 11 | 1.0 | 0.08021 |
| GO:0030163~protein catabolic process | 40 | 3.8 | 0.08141 |
| GO:0046983~protein dimerization activity | 37 | 3.5 | 0.08163 |

| Term | Count | % | p-value |
|--|-------|-----|---------|
| h_ctcfPathway:CTCF: First Multivalent Nuclear Factor | 4 | 0.4 | 0.08218 |
| h_atmPathway:ATM Signaling Pathway | 4 | 0.4 | 0.08218 |
| SM00398:HMG | 7 | 0.7 | 0.08270 |
| SM00338:BRLZ | 7 | 0.7 | 0.08270 |
| GO:0006096~glycolysis | 6 | 0.6 | 0.08319 |
| GO:0048638~regulation of developmental growth | 6 | 0.6 | 0.08319 |
| GO:0050801~ion homeostasis | 28 | 2.6 | 0.08330 |
| GO:0030003~cellular cation homeostasis | 19 | 1.8 | 0.08390 |
| GO:0046058~cAMP metabolic process | 4 | 0.4 | 0.08407 |
| GO:0001836~release of cytochrome c from mitochondria | 4 | 0.4 | 0.08407 |
| GO:0050792~regulation of viral reproduction | 4 | 0.4 | 0.08407 |
| GO:0048524~positive regulation of viral reproduction | 3 | 0.3 | 0.08555 |
| GO:0031226~intrinsic to plasma membrane | 69 | 6.5 | 0.08580 |
| GO:0040008~regulation of growth | 24 | 2.3 | 0.08644 |
| SM00454:SAM | 10 | 0.9 | 0.08662 |
| cell projection | 16 | 1.5 | 0.08690 |
| short sequence motif:Nuclear localization signal | 23 | 2.2 | 0.08849 |
| IPR013151:Immunoglobulin | 16 | 1.5 | 0.08867 |
| domain:FERM | 6 | 0.6 | 0.08868 |
| GO:0043065~positive regulation of apoptosis | 29 | 2.7 | 0.08871 |
| GO:0055066~di-, tri-valent inorganic cation homeostasis | 18 | 1.7 | 0.08903 |
| hsa05020:Prion diseases | 5 | 0.5 | 0.08964 |
| IPR013098:Immunoglobulin I-set | 12 | 1.1 | 0.09093 |
| IPR002909:Cell surface receptor IPT/TIG | 5 | 0.5 | 0.09095 |
| PIRSF037110:transcription factor with zinc finger motifs, PRDM3/PRDM16 types | 2 | 0.2 | 0.09112 |
| PIRSF000496:trans-prenyltransferase | 2 | 0.2 | 0.09112 |
| PIRSF500696:plasmolipin | 2 | 0.2 | 0.09112 |
| SM00504:Ubox | 3 | 0.3 | 0.09112 |

| Term | Count | % | p-value |
|---|-------|------|---------|
| sarcoplasmic reticulum | 4 | 0.4 | 0.09229 |
| GO:0005886~plasma membrane | 196 | 18.4 | 0.09234 |
| h_CCR3Pathway:CCR3 signaling in Eosinophils | 4 | 0.4 | 0.09241 |
| h_gpcrPathway:Signaling Pathway from G-Protein Families | 4 | 0.4 | 0.09241 |
| GO:0004222~metalloendopeptidase activity | 10 | 0.9 | 0.09335 |
| binding site:FAD | 4 | 0.4 | 0.09343 |
| domain:N-terminal Ras-GEF | 4 | 0.4 | 0.09343 |
| GO:0007169~transmembrane receptor protein tyrosine kinase signaling pathway | 17 | 1.6 | 0.09406 |
| GO:0030017~sarcomere | 9 | 0.8 | 0.09454 |
| GO:0006984~ER-nuclear signaling pathway | 5 | 0.5 | 0.09455 |
| GO:0003713~transcription coactivator activity | 17 | 1.6 | 0.09465 |
| GO:0031328~positive regulation of cellular biosynthetic process | 43 | 4.0 | 0.09502 |
| GO:0007163~establishment or maintenance of cell polarity | 6 | 0.6 | 0.09556 |
| GO:0000785~chromatin | 15 | 1.4 | 0.09572 |
| region of interest:Amphipathic | 2 | 0.2 | 0.09694 |
| region of interest:Zinc-binding | 2 | 0.2 | 0.09694 |
| site:Required for Cu(2+) reduction | 2 | 0.2 | 0.09694 |
| short sequence motif:NPXY motif; contains endocytosis signal | 2 | 0.2 | 0.09694 |
| binding site:Dimethylallyl diphosphate | 2 | 0.2 | 0.09694 |
| region of interest:Regulatory domain | 2 | 0.2 | 0.09694 |
| GO:0031440~regulation of mRNA 3'-end processing | 2 | 0.2 | 0.09717 |
| GO:0032897~negative regulation of viral transcription | 2 | 0.2 | 0.09717 |
| GO:0046666~retinal cell programmed cell death | 2 | 0.2 | 0.09717 |
| IPR001762:Blood coagulation inhibitor, Disintegrin | 4 | 0.4 | 0.09734 |
| GO:0043068~positive regulation of programmed cell death | 29 | 2.7 | 0.09744 |

| Term | Count | % | p-value |
|---|-------|-----|---------|
| GO:0007568~aging | 10 | 0.9 | 0.09769 |
| GO:0030802~regulation of cyclic nucleotide biosynthetic process | 10 | 0.9 | 0.09769 |
| GO:0030808~regulation of nucleotide biosynthetic process | 10 | 0.9 | 0.09769 |
| GO:0005856~cytoskeleton | 77 | 7.2 | 0.09777 |
| GO:0043233~organelle lumen | 99 | 9.3 | 0.09814 |
| tyrosine-protein kinase | 10 | 0.9 | 0.09861 |
| GO:0010942~positive regulation of cell death | 29 | 2.7 | 0.09933 |
| Colorectal cancer | 3 | 0.3 | 0.09966 |

Supplementary Table 5. Splicing factor genes tested for association with expression changes and response to VPA.

| | | |
|------------------|----------------|-----------------|
| <i>HNRNPA0</i> | <i>SRSF6</i> | <i>IMP3</i> |
| <i>HNRNPA1</i> | <i>HNRNPD</i> | <i>SRSF1</i> |
| <i>SRSF10</i> | <i>HNRNPM</i> | <i>HNRNPH3</i> |
| <i>LSM5</i> | <i>LSM2</i> | <i>HNRNPUL2</i> |
| <i>LSM14A</i> | <i>HNRNPK</i> | <i>SRSF2</i> |
| <i>SRSF5</i> | <i>SRSF3</i> | <i>SRSF11</i> |
| <i>SNRNP48</i> | <i>SNRNP25</i> | <i>HNRNPC</i> |
| <i>HNRNPA3</i> | <i>LSM12</i> | <i>LSM6</i> |
| <i>HNRNPH1</i> | <i>SNRNP35</i> | <i>SF3B4</i> |
| <i>SF3B3</i> | <i>HNRNPU</i> | <i>SF3A3</i> |
| <i>SNRNP200</i> | <i>SNRNP70</i> | <i>U2AF1L4</i> |
| <i>LSM4</i> | <i>SRSF4</i> | <i>RRP8</i> |
| <i>HNRNPA2B1</i> | <i>U2AF2</i> | <i>SF3B1</i> |
| <i>HNRNPAB</i> | <i>SRSF9</i> | <i>SRSF8</i> |
| <i>SF3A1</i> | <i>HNRNPH2</i> | <i>HNRNPF</i> |
| <i>SNRNP27</i> | <i>SRSF12</i> | <i>HNRNPDL</i> |
| <i>HNRNPR</i> | <i>SF3B2</i> | <i>HNRNPUL1</i> |
| <i>LSM7</i> | <i>U2AF1</i> | <i>LSM3</i> |
| <i>SRSF7</i> | <i>RBMX</i> | <i>SYNCRIP</i> |
| <i>ELAVL1</i> | <i>SFPQ</i> | <i>PUF60</i> |
| <i>RBM10</i> | <i>KHDRBS1</i> | <i>IMP4</i> |
| <i>HNRNPL</i> | <i>SNRNP40</i> | <i>SF3A2</i> |
| <i>SF3B5</i> | <i>RALY</i> | <i>LSM10</i> |
| <i>RRP9</i> | <i>LSM1</i> | <i>LSM8</i> |
| <i>SF1</i> | <i>SNRPD3</i> | <i>SON</i> |
| <i>SNRPB2</i> | <i>TARDBP</i> | <i>TIA1</i> |
| <i>ZRANB2</i> | <i>CHERP</i> | <i>SNRPB</i> |
| <i>TRA2B</i> | <i>SMN</i> | |