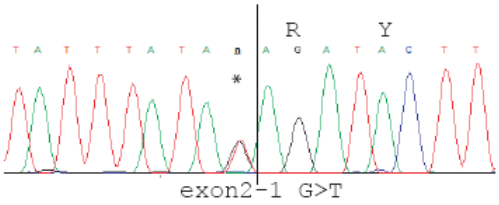
**Supplemental Data**

# Deleterious Variants of *Figure 4*, a Phosphoinositide Phosphatase, in Patients with ALS

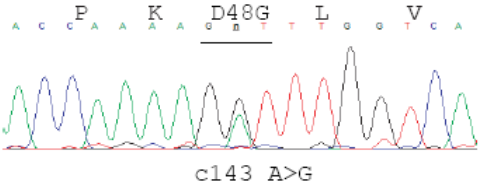
**Clement Y. Chow, John E. Landers, Sarah K. Bergren, Peter C. Sapp, Adrienne E. Grant, Julie M. Jones, Lesley Everett, Guy M. Lenk, Diane M. McKenna-Yasek, Lois S. Weisman, Denise Figlewicz, Robert H. Brown, and Miriam H. Meisler**

A) FALS G07



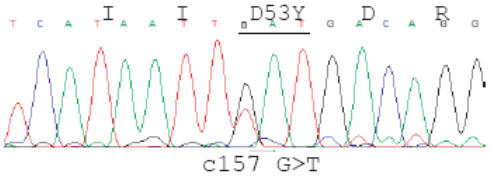

```
                                                    exon 2
wt: acattccttttttatttatagAGATA

FALS G07: acattccttttttatttatatAGATA
```

B) SALS B12 exon 2



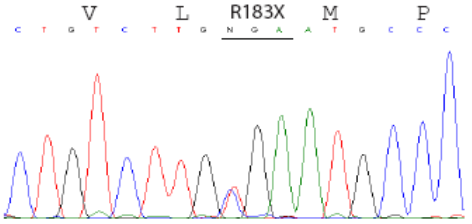

```
SALS B12    ..........G.........
Human       VLKIDRTEPK-DLVIIDDRHVY
Chimp       ....................
Mouse       ............V.......
Opossum     ..................K...
Platypus    .........R........K...
Chicken     ..................K...
Stickleback ..................K...
Yeast       I.E..L.V.RGE.TVLE.NVFF
```

C) FALS G03 exon 2



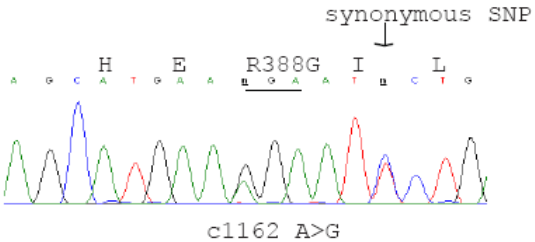

```
FALS G03    ..........Y.........
Human       TEPK-DLVIIDDRHVYTQQEV
Chimp       ....................
Mouse       ........V...........
Opossum     ............K.......
Platypus    ...R.......K.......L
Chicken     ............K.......
Stickleback ............K...N....
Yeast       .V.RGE.TVLE.NVFF.RN.I
```

D) SALS E12 exon 6



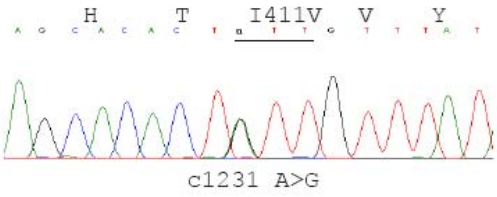


E) SALS H11 exon 11



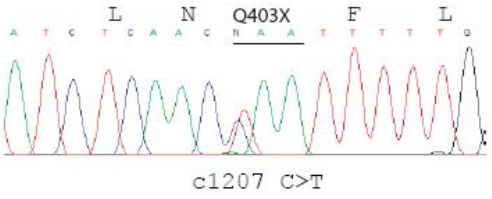

```
SALS H11    ..........G.........
Human       VKEREKRKHERILSEELVAAV
Chimp       ....................
Mouse       ....................
Opossum     ....................
Platypus    ....................
Chicken     ....................
Stickleback ..K.................YP..
Yeast       I.TK..TPR.TK.LW.FEQCI
```

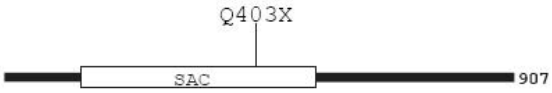
F) SALS E12 exon 11



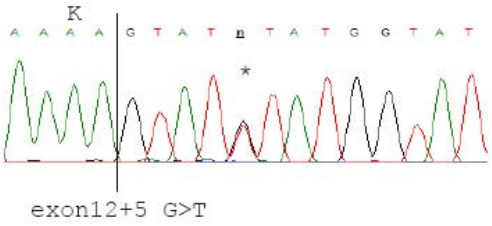

```
SALS E12      .........V.........
Human         LNQFLPPEHTIVYIPWDMAKY
Chimp         ....................
Mouse         ....................
Opossum       .........S.........
Platypus      .........A.I.......
Chicken       .........A.I.......
Stickleback   ........NC.E.LA....R.
Yeast         ..E...TLKKLD.TS...SRA
```

G) SALS 8533 exon 11

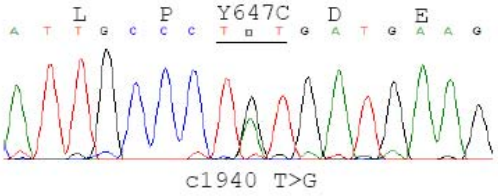



H) NINDS N0 09489

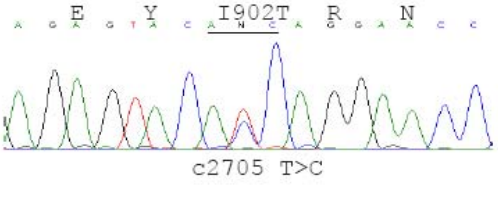



I) NINDS N0 11318




```
NINDS N0 09489  ..........C..........
Human           PEVIKHLPLPYDE-VICAVN-
Chimp           ..................A..
Mouse           ...V..............A..
Opossum         .G..N..........T.VE..
Platypus        .G..D...M......SSPE..
Chicken         TG.L......F....T.AE..
Stickleback     AG.LSY..V.....VS.EDT.
Yeast           DYNI.SVKELIN.EL.ATG.D
```

J) FALS A04 exon 23




```
FALS A04      ..........T.....
Human         KEDSSMYREYIRNRYL
Chimp         ................
Mouse         ...TA...........
Opossum       ...I...........M
Platypus      ...VLI.........M
Chicken       .D.FL..........
Stickleback   R..ML.....VK....
Yeast         RD--LCFSKD.QLDFQ
```

**Figure S1. Sequence Chromatograms and Evolutionary Conservation of the Patient Mutations of FIG4.** These mutations are discussed in detail in the text. All of the six missense mutations alter evolutionarily conserved residues and result in nonconservative amino acid substitutions. Additional information about these mutations is presented in text Table 1 and summarized in text Figure 1B.

**Table S1. Clinical Features of Patients with Sporadic and Familial Motor Neuron Disease Carrying Nonsynonymous Variants of *FIG4***

| *Patient* | *Mutation* | *Sex* | *Age of Onset (yrs)* | *Site of Onset* | *El Escorial Diagnosis* | *Duration* | *Sensory Loss* | *Conduction Velocity* | *Miscellaneous* |
|---|---|---|---|---|---|---|---|---|---|
| SALS H11 | p.R388G | M | 42 | LE | PLS | >29 | No | normal | Very prominent corticospinal tract findings<br>Minimal lower motor neuron findings<br>CSF protein 114<br>Muscle biopsy - rare atrophic fibers<br>EMG, somatosensory and visual ER's normal |
| SALS E12 | p.[R183X (+) I411V] | M | 62 | Bulbar | Probable ALS | 8.9 | No | normal | Very prominent corticospinal tract findings<br>Pseudobulbar affect<br>Moderate lower motor neuron findings<br>EMG - mild denervation, 3 extremities |
| SALS B12 | p.D48G | F | 29 | LE | Possible PLS | n.a. | No | normal | Very prominent corticospinal tract findings<br>Subtle changes in memory, attention<br>EMG - minimal denervation, 2 extremities |
| SALS 8533 | p.Q403X | F | 60 | Bulbar | Possible ALS | 25 | No | normal | Very prominent corticospinal tract findings<br>Initial EMG normal |
| SALS ND 11318 | p.Y647C | F | 65 | Bulbar | Definite ALS | >2 | No | normal | UMN and LMN signs; EMG - denervation, acute/chronic, bulbar& 4 extremities |
| SALS ND 09489 | +5G>T exon 12 | F | 57 | UE | Definite ALS | >2 | No | normal | UMN and LMN signs; EMG- denervation, acute/ chronic, bulbar, 4 extremities, thoracic |
| FALS GO3 | p.D53Y | F | 56 | Bulbar | Definite ALS | 2.6 | No | normal | Moderate corticospinal findings<br>Early EMG normal<br>Autopsy - lower motor neuron loss but corticospinal tract, Betz cells normal |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Subtle personality changes for 2 year reclusive, irritable |
| FALS G07 | -1G>T exon 2 | M | 77 | UE | Possible ALS | 1.3 | Minimal | normal | Minimal corticospinal findings<br>Reduced vibratory sense in great toes<br>EMG - denervation, 4 extremities |
| FALS A04 | p.I902T | M | 55 | Bulbar | Definite ALS | 1.7 | No | normal | Combined corticospinal and LMN findings<br>EMG - diffuse denervation |
| | | ***Average*** | 55.9 | | ***Average*** | >9.1 | | | |
| | | ***SD*** | 13.7 | | ***SD*** | 11.3 | | | |

All patients and controls were of European ethnicity. SALS, sporadic ALS; FALS, familial ALS. ND samples are from the NINDS collection (Coriell). Disease duration is measured from disease onset to death.

LE, lower extremity; UE, upper extremity; ER, evoked responses.

**Table S2. Polymorphic SNPs in the *FIG4* Gene**

| Location | nucleotide (amino acid) | MAF ALS (n) | MAF Control (n) | MAF dbSNP Caucasian |
|---|---|---|---|---|
| exon 1 | c.27C>T (p.I9I) | 0.005 (276) | 0.011 (181) | n.d. |
| intron 1 | c.67-7T>C | 0.06 (272) | 0.04 (87) | n.d. |
| intron 2 | c.165+100A>T | 0.34 (272) | 0.37 (87) | n.d. |
| intron 5 | c.497+30A>T | 0.009 (272) | 0.005 (184) | n.d. |
| intron 5 | c.498-138A>G | 0.002 (261) | 0.005 (368) | n.d. |
| intron 6 | c.647-18C>A | 0.32 (92) | n.d. | 0.32 rs2273752 |
| intron 8 | c.877-49_45delTCATT | 0.36 (273) | 0.38 (182) | n.d. rs57291908 |
| exon 10 | c.1090A>T (p.M364L) | 0.03 (276) | 0.04 (162) | 0.03 rs2295837 |
| intron 10 | c.1137+73_75delTAA | 0.33 (276) | 0.38 (90) | n.d. |
| intron 17 | c.1948+3A>G | 0.35 (272) | 0.25 (92) | 0.37 rs10499054 |
| intron 17 | c.1948+46C>A | 0.14 (272) | 0.14 (92) | 0.12 rs9320315 |

| | | | | |
|---|---|---|---|---|
| intron 17 | c.1948+91T>G | 0.006 (264) | 0.005 (92) | n.d. |
| exon 18 | c.1961T>C (p.V654A) | 0.10 (92) | 0.14 (173) | 0.16 rs9885672 |
| intron 19 | c.2180+63G>T | 0.35 (276) | 0.30 (381) | n.d. |
| exon 23 | c.2559G>A (p.S853S) | 0.25 (270) | 0.28 (268) | 0.38 rs9398218 |
| exon 23 | c.2724+29G>A | 0.02 (270) | 0.03 (268) | 0.04 rs106599 |

The listed SNPs were identified in multiple individuals. In addition, SALS-F08 (early onset, short duration) was heterozygous for a synonymous C>T substitution in residue N494 that disrupts a strongly predicted SF2/ASF binding site in exon 14 (12). FALS A02 and SALS H02 (onset in 60s, 2-3 year duration) were heterozygous for a C>G substitution in the polypyrimidine tract upstream of exon 19. Mutations at this position of the polypyrimidine tract may be pathogenic (10). The previously described CMT4J mutant allele I41T was observed in two control individuals, consistent with the predicted allele frequency of 0.001 (4).