**Supplementary Material**

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| **Amino acid change** | **Nucleotide change** | **Domain** | **Reference** |
| c.97G>A | p.G33S | TPR1 | De Michele et al.# |
| c.170C>T | p.P57L | TPR2 | Lieto et al. |
| c.199G>A | p.A67T | TPR2 | Lieto et al. |
| c.673C>T | p.R225\* | U-box | Lieto et al. |
| c.682C>T | p.P228S | U-box | De Michele et al. |
| c.689\_692delACCT | p.Y230Cfs\*9 | U-box | Lieto et al.  |
| c.721C>T | p.R241W | U-box | Lieto et al. |
| **c.731\_732delGC** | **p.C244Yfs\*24** | **U-box** | **Current study#** |
| c.791\_792delTG | p.V264Gfs\*4 | U-box | Lieto et al. |
| c.818\_819dupGC | p.P274Afs\*3 | U-box | Lieto et al. |
| c.823\_824delCT | p.L275Dfs\*16 | U-box | Genis et al.#, Lieto et al., Palvadeau et al.  |

**Table e-1. Overview of the 11 heterozygous *STUB1* gene mutations reported up till now in SCA48**The majority of the mutations is located in the U-box domain.
#extensive segregation analysis including >2 patients in ≥2 branches of the pedigree.

**References**1. De Michele G, Lieto M, Galatolo D, et al. Spinocerebellar ataxia 48 presenting with ataxia associated with cognitive, psychiatric, and extrapyramidal features: A report of two Italian families. Parkinsonism Relat Disord 2019;65:91-96.
2. Genis D, Ortega-Cubero S, San Nicolas H, et al. Heterozygous STUB1 mutation causes familial ataxia with cognitive affective syndrome (SCA48). Neurology 2018;91:e1988-e1998.
3. Lieto M, Riso V, Galatolo D, et al. The complex phenotype of spinocerebellar ataxia type 48 in eight unrelated Italian families. Eur J Neurol 2019.
4. Palvadeau R, Kaya-Gulec ZE, Simsir G, et al. Cerebellar cognitive-affective syndrome preceding ataxia associated with complex extrapyramidal features in a Turkish SCA48 family. Neurogenetics 2020;21:51-58.

**Sanger sequencing validation**

To confirm the identified novel mutation, and to check for segregation within the family, a fragment containing the *STUB1* mutation (NM\_005861:exon6:c.731\_732delGC:p.Cys244Tyrfs\*24) was PCR amplified for all the available samples from the family, and sequenced in an ABI3730 sequencer (Applied Biosystems). The primers used are shown below. The sequencing results of the *STUB1* mutation of two family members (one affected, one unaffected) are presented in Figure 1. Chromatograms of the other family members are available upon request.

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|  | **Sequence (5’-3’)** |
| STUB1-Ex6-Forward | TTCAGCCTCTGACCGTGTGC |
| STUB1-Ex6-Reverse | CACGCTGCAGTGACAAGAAGG |

Primers used for PCR amplification and sequencing of the *STUB1* gene mutation.





**Figure e-1:** Chromatogram showing the results of the Sanger DNA sequencing of the affected carrier III-13 (upper panel) and the unaffected non-carrier III-15 (lower panel).The red bar indicates the location of the mutation.