**Appendix e-1**

**Exclusion criteria**

Children with transient varicella related cerebellitis, classic opsoclonus-myoclonus ataxia, clinically defined leukodystrophies (n=5, who were recruited to another research project),and acquired forms of ataxia, such as ataxia following brain trauma, brain tumors, congenital infection and ischemic anoxic lesions were excluded from this study.

**Exome sequencing technical details**

Exome sequencing was done with the Agilent V5 whole exome, the VCRome v2.1, Agilent Clinical Research kit, NimbleGen MedExome or the SureSelect Human All Exon V6 capture kit on an Illumina HiSeq sequencing platform.

**Patients either prescreened or screened during the study for pathologically expanded trinucleotide repeats**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **ID** | **Group** | **Gene** | **Spinocerebellar ataxia (SCA)** | **Friedreich ataxia** |
| P2 | Confirmed diagnosis | *COQ8A* | SCA1, 2, 7 | yes |
| P5 | Confirmed diagnosis | *ITPR1* | affected mother tested for SCA1, 2, 3, 7, 8, 12, 17 | no |
| P13 | Confirmed diagnosis | *CLN5* | SCA2, 7 | no |
| P14 | Confirmed diagnosis | *NKX2-1* | no | yes |
| P15 | Confirmed diagnosis | *STUB1* | SCA1, 7, 8 | no |
| P19 | Confirmed diagnosis | *ATP1A3* | no | yes |
| P28 | No diagnosis |   | no | yes |
| P35 | No diagnosis |  | no | yes |
| P40 | No diagnosis |  | no | yes |
| P44 | No diagnosis |  | SCA1, 2, 6, 7, 8, 17 | no |
| P46 | No diagnosis |  | SCA6 | no |
| P49 | No diagnosis |  | SCA7 | no |

**Primers used in Sanger sequencing**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Chr** | **pos** | **Gene** | **Forward** | **Reverse** |
| 1 | 43394882 | *SLC2A1* | ttccctttagaccccaagcc | aacctagcaactcaccagca |
| 1 | 227169808 | *COQ8A* | tatcaggtggggcttgcc | atcttgcccactgagctttg |
| 1 | 227174171 | *COQ8A* | gtggagggctctggtgtc | acgggaatcaggttgtggat |
| 2 | 166245891 | *SCN2A* | atgtacatcgcggtcatcct | tagcgtctgtaagccctctg |
| 2 | 191116992 | *HIBCH* | ggtttgtgtgatctacggca | aagtctgtggctctcaacca |
| 2 | 191159358 | *HIBCH* | cacacagtgtactcatctagca | tgattcagggagagagacaaca |
| 2 | 219525942 | *BCS1L* | ccccacaaaaggagggtatt | gaatccatttcccccgatac |
| 2 | 219526485 | *BCS1L* | ggcacagctccacctaattg | cttttcattcctcttaatctctgg |
| 3 | 4687363 | *ITPR1* | cttgggggcagcagtcta | ttgtaggatagagagagagagagccta |
| 3 | 4709128 | *ITPR1* | catcaggaaacattgctgctt | tcaaaaagcctctccagacc |
| 3 | 4856866 | *ITPR1* | atatccctcctggctgtgtc | tcccagcagaataaaggcca |
| 6 | 131919485 | *MED23* | acttctctatgcagggctcc | tagggaagtgacagcaatgc |
| 8 | 145138112 | *GPAA1* | ggactccgggtttaggtctc | agacacgaagggagcctctg |
| 8 | 145139371 | *GPAA1* | accacgtcctccattagcac | aggaagaaggactggtgcag |
| 10 | 131640542 | *EBF3* | cccagccagatattgcacac | agaacagaacgctacggaca |
| 10 | 131676043 | *EBF3* | agtgggttgagatggtaggc | tgcaccaattccagtttgct |
| 10 | 131676045 | *EBF3* | agtgggttgagatggtaggc | tgcaccaattccagtttgct |
| 11 | 6636680 | *TPP1* | tcccattgttccttttcgtc | tcagaaagtgcagccacatc |
| 12 | 111057639 | *TCTN1* | gcatctctgtaggcaaagca | tggacggtatggcttcagaa |
| 12 | 111085142 | *TCTN1* | cagtttcaggtgatgtcggc | agaaccatcgtcagtgtttct |
| 12 | 52080889 | *SCN8A* | tcctcacttccttcctgctg | tgcttgccttctcccacata |
| 13 | 77575055 | *CLN5* | ttcaaaccacatttgccaac | ctgggtccaaaggtcctaca |
| 14 | 36987093 | *NKX2-1* | atcctaatgctctgacccgg | ttgtagcggtggttctggaa |
| 16 | 732184 | *STUB1* | cacaggacaagtacatggcg | gtccaacagcagaacttggg |
| 16 | 732442 | *STUB1* | cacaggacaagtacatggcg | gtccaacagcagaacttggg |
| 17 | 57775212 | *PTRH2* | aaacagggtgcaggcataag | atttttgcatgggccaataa |
| 18 | 6950834 | *LAMA1* | gaaacagttcccggcagac | tccctgccttcaagacttgt |
| 18 | 6999503 | *LAMA1* | tgggtggattgtcatgtgga | tcaccacttctttcccgaca |
| 19 | 13346507 | *CACNA1A* | ttttcctgttggttggcttc | gcactgtcttcctcccttgt |
| 19 | 42474691 | *ATP1A3* | gcaggagaatggcgtgaac | agatagctcactggttggtcc |
| 19 | 42474692 | *ATP1A3* | gcaggagaatggcgtgaac | agatagctcactggttggtcc |
| X | 41495865 | *CASK* | ccctcatttcagattgcttacca | ccggcccctaattccaattc |

**P12/TCTN1 Western blot**

P12 has compound heterozygous variants in *TCTN1* and parental samples were unavailable to confirm that the variants segregated. This study used western blot with rabbit anti-TCTN1 (Proteintech Group Cat#15004-1-AP, RRID:AB\_10644442) to show complete lack of TCTN1 in P12 fibroblasts. *Antibody reference: Chih B, Liu P, Chinn Y, Chalouni C, Komuves LG, Hass PE, et al. A ciliopathy complex at the transition zone protects the cilia as a privileged membrane domain. Nature Cell Biology 2011; 14: 61.*

**Supplementary Figure 1. TCTN1 western blot of P12 fibroblasts**

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**{Chih, 2011 #1714}P3/3-Hydroxyisobutyryl-CoA hydrolase (HIBCH) activity**

HIBCH-enzyme activity in cultured skin fibroblasts was measured in a clinical laboratory and was found to be 2.1 nmol/(min.mg protein) (reference value 5.3-10.5.), reduced but not fully deficient.

**Supplementary Figure 2. {Chih, 2011 #1714}P8/*EBF3* Sanger sequencing chromatogram**

Sanger sequencing of the *EBF3* c.1183 C>T variant in P8 and the clinically unaffected parents of P8. In the mother the peak height of the variant allele is less than what is observed in a conventional heterozygote and raised suspicion of somatic mosaicism in the mother.



**Proband phenotype**

**P27/*MED23***

P27 was found to have a *de novo* variant in *MED23*. She was investigated because of tremor, and was found to have hypotonia and upper limb predominating ataxia at the age of 1.5 years. At the age of six years old, her ataxia and tremor of the upper limbs has remained stable and she has a diagnosis of mixed disorder of scholastic skills. Brain MRI at the age of 2, 3 and 4 years of age was normal. Metabolic screening and other laboratory tests were within normal range. Molecular karyotype and testing for Fragile-X syndrome were normal.