**The American Journal of Human Genetics**

**Supplemental Data**

# A Missense Mutation in *KCTD17* Causes Autosomal Dominant Myoclonus-Dystonia

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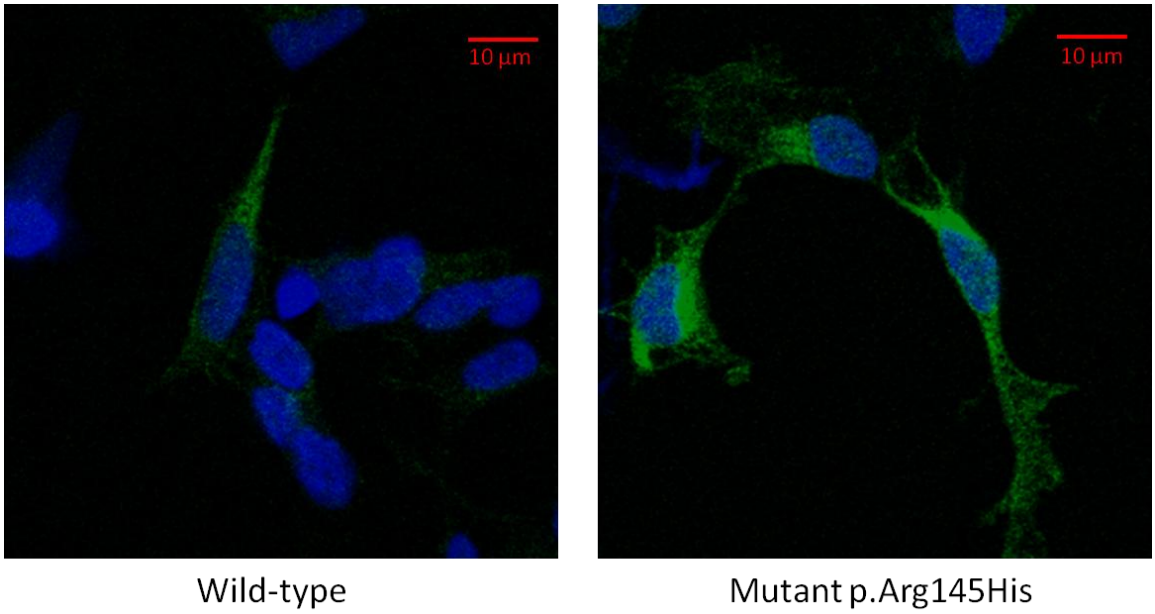


**Figure S1. Immunocytochemistry in stably transfected SH-SY5 cells showing no difference between wild-type and mutant KCTD17 subcellular localization.**

The mutation c.434 G>A p.(Arg145His) was inserted by recombinant PCR. Both N- and C-terminal HA tagged wild-type and mutant cDNAs were inserted with a 1-step recombinant PCR into pcDNA3.1 constructs for expression in mammalian cells. Stable SH-SY5 cells were generated by electroporating 5 µg linearized tagged WT and mutant plasmids into ~1 million cells and G418 (InvivoGen) selection at 250 mg/l over at least 4 weeks and at least 6 passages. A control cell line expressing the empty vector was obtained in parallel. After fixation with either 4% PFA in PBS or ice-cold 50% methanol/50% acetone, cells were blocked in PBS+2% BSA%, 3% normal goat serum, 1% NP-40, 0.5% sodiumdesoxycholate for 30 minutes and primary antibodies added in block diluted 1:1 with PBS at 4°C overnight. The Roche 3F10 monoclonal antibodies were used for detection of the HA tag, at 1:1000 dilution. After washing, secondary detection used Alexa-dye labelled, highly cross-absorbed anti-rat (Invitrogen, UK) in 0.5x block, with 1 mg/l DAPI. Microscopy was performed on a Zeiss confocal microscope. HA-tagged KCTD17 is shown in green.

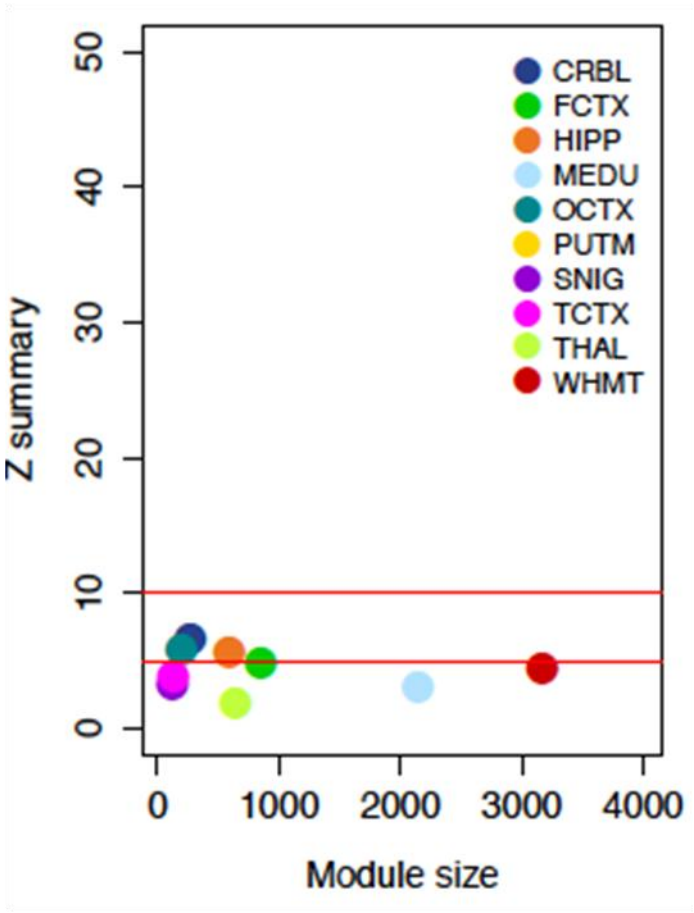


**Figure S2. Putamen *KCTD17*-containing module preservation across other brain regions.**

Module preservation statistics were calculated (z score) to assess how well modules from one tissue are reproducible (or preserved) in another brain region.[1] Previously proposed thresholds were considered (z score of <2 indicates no evidence of module preservation, z score between 2 and <10 indicates weak to moderate evidence, and z score of ≥10 indicates strong evidence). The module is poorly conserved across other brain regions, indicating its specificity to the putamen.

**Table S1 - Summary of novel variants detected by whole-exome sequencing and shared by individuals V-3 and IV-14.**

| Chr | Position (hg19) | Gene (Transcript) | Variant | GERP score[a] | CADD C-score[b] | SIFT | Provean | PolyPhen-2 HumVar | Mutation Taster | Gene previously associated with disease? | Linkage analysis (LOD score) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 152276386 | *FLG* (NM_002016.1) | c.10976 C>T p.(Ser3659Phe) | 2.48 | 11.15 | T (0.06) | N (-0.8) | D (0.78) | P (0.99) | Yes, skin diseases (e.g. ichthyosis vulgaris, and/or eczema)[2] | < -2 |
| 1 | 228401208 | *OBSCN* (NM_052843.3) | c.1055 T>G; p.(Phe352Cys) | 5.58 | 21 | D (0) | D (-3.8) | D (0.94) | D (0.99) | Hypertrophic cardiomyopathy[3] | < -2 |
| 8 | 133622476 | *LRRC6* (NM_012472.4) | c.1076 A>C; p.(Lys359Thr) | 3.5 | 15.74 | D (0.01) | D (-3.1) | B (0.39) | D (0.99) | Recessive primary ciliary dyskinesia[4] | < -3 |
| 22 | 37453460 | *KCTD17* (NM_001282684.1) | c.434 G>A; p.(Arg145His) | 4.46 | 28.8 | D (0) | D (-4.8) | D (0.53) | D (0.99) | No | 2.4 |

B=benign; D=deleterious/damaging/disease-causing; N=neutral; P=polymorphism; T=tolerated

[a]Positive scores represent a substitution deficit and indicate that a site may be under evolutionary constraint. Negative scores indicate that a site is probably evolving neutrally. Positive scores scale with the level of constraint, such that the greater the score, the greater the level of evolutionary constraint inferred to be acting on that site.

[b]C-scores greater or equal 10 indicates that the variant is predicted to be the among the 10% most deleterious substitutions that you can do to the human genome; a score of greater or equal 20 indicates the 1% most deleterious.

**Table S2. *KCTD17* primers used for Sanger sequencing**

| | |
|---|---|
| Exon 1 FOR | AGGCGCGGACTACAGCTC |
| Exon 1 REV | CCACGGCAATGGGTACATC |
| Exon 2 FOR | TCTCCCTCCACTCTCCTTC |
| Exon 2 REV | TCCTGGTTGTCCAAATGG |
| Exon 3 FOR | GGAGGGAACAAGAGGAGAATG |
| Exon 3 REV | TCCCAACCTCCTCTGCTTC |
| Exon 4 FOR | TCTTCTTTGGGTATGTTGCG |
| Exon 4 REV | TGGTCAGAGGCTAGGAGGTC |
| Exon 5 FOR | GAGGTCTGTCGTATCCTGCC |
| Exon 5 REV | AGAGGTGGAGGGATGGTG |
| Exon 6 FOR | CTTTCACCTTGCCTGAGACC |
| Exon 6 REV | AGGCAAGTGGCTGAGCTAAC |
| Exon 7 FOR | CAGGGTTAGCTCAGCCACTT |
| Exon 7 REV | AGGCAGGGTGCAGATGAGAT |
| Exon 8 FOR | TCTGTGCCCACTAACCCTG |
| Exon 8 REV | TCAAGAGATGAGCACCCTCC |
| Exon 9 FOR | CACCCGTCAATCTCCTCTC |
| Exon 9 REV | AGGCAGGAGTAAGTCACAGC |

**Table S3. Disease haplotype of the families with the *KCTD17* c.434 G>A p.(Arg145His)**

| Marker | Chromosomal position | Genotype UK family | Genotype German family |
|---|---|---|---|
| rs5756370 | 37242476 | A | A |
| rs6000449 | 37251377 | A | A |
| rs4821542 | 37252918 | G | G |
| rs909483 | 37260474 | A | A |
| rs2413429 | 37289869 | G | A |
| rs4821558 | 37308785 | G | A |
| rs11705394 | 37329676 | A | A |
| rs9622506 | 37338286 | A | G |
| rs8137446 | 37347959 | G | G |
| rs9622521 | 37350881 | G | G |
| rs4821576 | 37357169 | G | G |
| rs8142593 | 37363121 | A | A |
| rs877166 | 37369148 | C | C |
| rs5756437 | 37375668 | G | G |
| rs1157557 | 37381674 | G | G |
| rs5756477 | 37407527 | G | A |
| rs5756492 | 37424991 | G | G |
| Microsatellite 19xAG | 37446300 | 17[a] | 18/14[a] |
| ***KCTD17* c.434G>A** | **37453460** | **A** | **A** |
| rs2160906 | 37493178 | G | G |
| rs228924 | 37507250 | A | G |
| rs11914132 | 37509087 | G | G |
| rs228942 | 37524619 | C | C |
| rs3218258 | 37544245 | A | G |
| rs229483 | 37553619 | G | A |
| rs12167757 | 37567490 | G | G |
| rs229518 | 37577872 | A | A |
| rs11913300 | 37580627 | A | A |
| rs5756540 | 37582205 | G | G |
| rs5756546 | 37589805 | G | G |
| rs64547 | 37592504 | A | A |
| rs9610680 | 37621951 | A | G |
| rs8137698 | 37624236 | G | A |
| rs739042 | 37625419 | G | G |
| rs2285110 | 37628145 | G | G |
| rs9607431 | 37629938 | C | A |
| rs5995404 | 37632938 | C | C |

SNP markers on chromosome 22 located ~0.5 Mb up- and down-stream the *KCTD17* C.434 G>A mutation were analysed and compared. In the British family, the haplotype of the identified *KCTD17* mutation was determined using MERLIN.[5] The German case was genotyped using the same array, HumanCytoSNP-12 DNA Analysis BeadChip Kit (Illumina, San Diego). In the German case SNP phasing was possible only for homozygous alleles. The *KCTD17* c.434 G>A mutation is marked in red. All alleles where the haplotype of the UK family differs from that of the German family are highlighted in yellow. The physical position of the markers refers to the human genome assembly hg19.

[a]These values indicate the number of AG repeats

**Supplemental references**


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