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Supplemental Data

Association between Rare Variants in *AP4E1*, a Component of Intracellular Trafficking, and Persistent Stuttering

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Figure S1

Sequence conservation of Valine V517 and Glutamic acid E801 across different species.

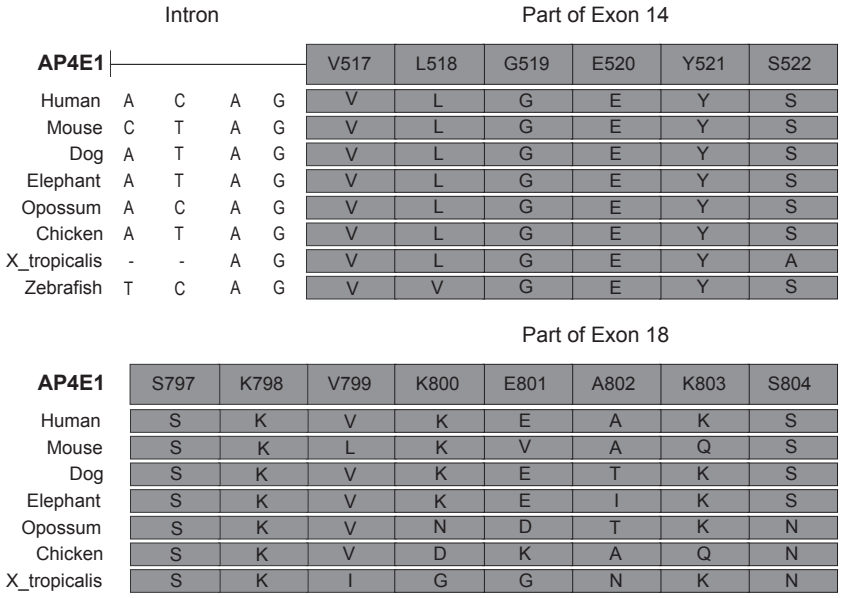

Figure S1

Sequence conservation of Valine (V517) and Glutamic acid (E801) across different species.

Figure S2

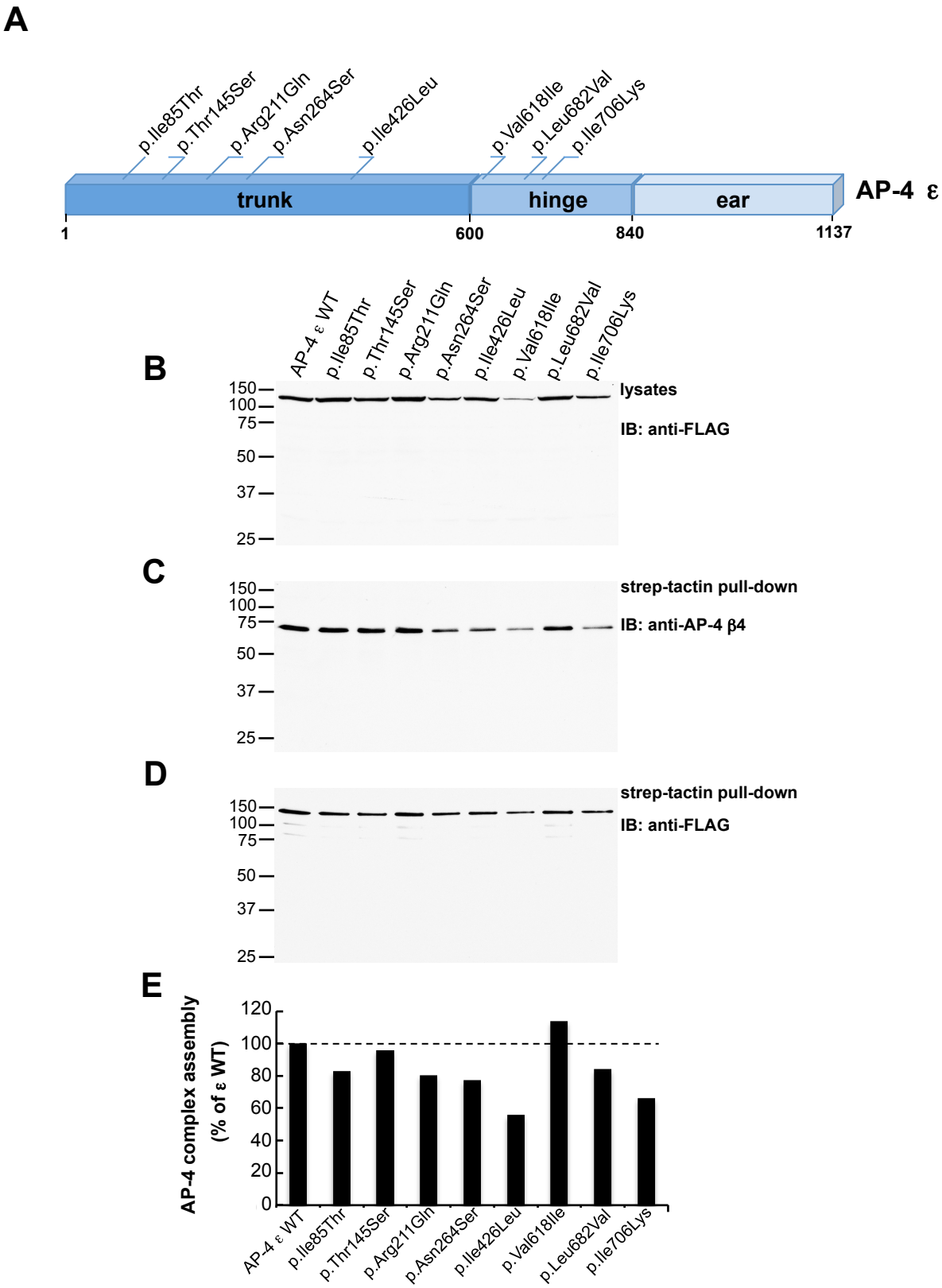
A
p.Ile85Thr
p.Thr145Ser
p.Arg211Gln
p.Asn264Ser
p.Ile426Leu
p.Val618Ile
p.Leu682Val
p.Ile706Lys
trunk
hinge
ear
AP-4 ε
1
600
840
1137
B
AP-4 ε WT
p.Ile85Thr
p.Thr145Ser
p.Arg211Gln
p.Asn264Ser
p.Ile426Leu
p.Val618Ile
p.Leu682Val
p.Ile706Lys
150
100
75
50
37
25
lysates
IB: anti-FLAG
C
strep-tactin pull-down
IB: anti-AP-4 β4
D
strep-tactin pull-down
IB: anti-FLAG
E
AP-4 complex assembly
(% of ε WT)
120
100
80
60
40
20
0

FIGURE S2

Assembly of AP-4 complexes by rare non-synonymous AP-4 ε subunit variants identified in controls.

(A) Schematic representation of the AP-4 ε subunit including trunk, hinge and ear domains and highlighting the non-synonymous ε subunit variants identified in controls. The numbering in the scheme corresponds to the human AP-4 ε isoform comprising 1137 residues (accession number NP_031373.2); assignment of domains is as in Boehm and Bonifacino 2001. (B) Expression of WT and variant human AP-4 ε constructs tagged at their N-termini with two Strep and one FLAG epitopes (TSF-tagged ε constructs) in HEK293T cells. Cell lysates of transfected cells were subjected to SDS-PAGE followed by immnoblotting with anti-FLAG antibody. (C) Assembly of WT and variant TSF-tagged ε constructs into AP-4 complexes. Lysates of transfected HEK293T cells were pulled-down with StrepTactin Sepharose beads, eluted with d-desthiobiotin and subjected to SDS-PAGE and immunoblotting with anti-AP-4 β4 antiserum (Dell'Angelica et al., 1999). D) Immunoblot membranes with samples of the StrepTactin pull-down assays shown in panel C were stripped and subjected to additional immunoblotting with anti-FLAG antibody. (E) Assembly of AP-4 complexes by WT and variant constructs. Immunoblots shown in panels B and C were subjected to densitometric analysis using the Image J software. The assembly of AP-4 complexes by WT and variant ε constructs (pulled-down complexes detected by anti-β4; blot in panel C) was calculated relative to the total expression of the cognate ε constructs in transfected HEK293T cells (blot in panel B). Results were expressed as percentage of assembly measured for WT ε (% of WT ε).

Figure S3
Sub-pedigree structure of Cameroonian stuttering family B.

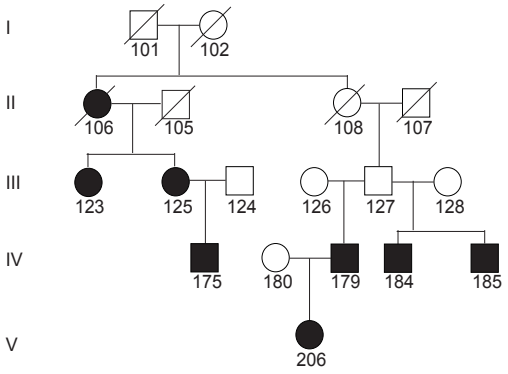

Figure S3
Sub-pedigree structure of Cameroonian stuttering family B.

Individuals that were used for whole exome sequencing are III-125, IV-175 and IV-184.

Table S1A: Summary of rare *AP4B1* (NM_001253852.1) variants in unrelated stuttering cases vs population matched controls

cDNA change	Amino acid change	SNP ID	Unrelated stuttering cases (609)			Population matched controls (558)	Population databases	
			STCR (93)	PKST (132)	NA (384)		1000G	ESP6400
c.1-11C>T	N/A	Unknown	-	PKST3	-	-	-	-
c.99C>G	p.Ile33Met	Unknown	-	-	NA351	-	-	-
c.319C>T	p.Arg107Trp	rs74361335	CAMST02	-	-	-	(1)	(1)
c.767C>T	p.Thr256Ile	rs143286419	-	PKST10	NA312	(3)	(2)	(14)
c.755T>C	p.Val252Ala	rs141417436	-	PKSTR47	NA147	-	(2)	(25)
c.901C>T	p.Arg301Cys	Unknown	-	PKSTR64	-	-	-	-
c.898G>T	p.Val300Leu	rs111785152	-	-	NA138	(1)	(2)	(16)
c.647C>A	p.Aal216Asp	Unknown	-	PKST82	0	-	-	-
c.1160_1161del2	p.Thr387Arg*fs**30	Unknown	-	-	NA167	-	-	(1)
c.1318A>C	p.Ile440Leu	Unknown	-	-	NA208	-	-	-
c.1460G>A	p.Arg487Gln	Unknown	-	-	NA217	-	-	(1)
c.1727C>T	p.Ser576Phe	Unknown	-	-	NA70	-	-	-
c.1747C>T	p.Arg582Cys	Unknown	-	-	NA163	-	-	-
c.1843T>G	p.Ser615Ala	rs148748734	-	-	NA304	-	(1)	(4)
c.2200A>G	p.Ile734Val	rs79050956	-	-	-	-	(1)	-
c.305G>A	p.Arg102Gln	rs377192417	-	-	-	(1)	-	(1)
c.967T>A	p.Ser322Thr	rs149335605	-	-	-	(1)	(2)	(6)
c.1048G>C	p.Glu350Gln	Unknown	-	-	-	(1)	-	-
c.695A>G	p.Glu232Gly	Unknown	-	-	-	(1)	-	-
c.938G>A	p.Ser313Asn	Unknown	-	-	-	(1)	-	-
c.1180C>A	p.Gln394Lys	Unknown	-	-	-	(1)	-	-
c.1244G>A	p.Cys415Tyr	rs200590674	-	-	-	(2)	-	(4)
c.1510_1510+1del2	p.Glu504Arg*fs**20	Unknown	-	-	-	(1)	-	-
c.1497G>T	p.Leu499Phe	rs147573619	-	-	-	(1)	-	(2)
c.1679T>G	p.Phe560Cys	Unknown	-	-	-	(1)	-	-

1000G = Genomes Data base (www.1000genomes.org), ESP6400 = Exome Sequencing Project Data base (evs.gs.washington.edu/EVS/), CAMST02 = Cameroonian family, STCR = Cameroonian unrelated stuttering cases, PKST = Pakistani stuttering family probands, PKSTR = Pakistani unrelated stuttering cases, NA = Unrelated North American stuttering cases, - = Variants with zero allele frequency, () = Total number of individuals, N/A = Not applicable
All annotations are based on human genome assembly hg19

Table S1B: Summary of rare *AP4M1* (NM_004722.3) variants in unrelated stuttering cases vs population matched controls

cDNA change	Amino acid change	SNP ID	Unrelated stuttering cases (609)			Population matched controls (558)	Population databases	
			STCR (93)	PKST (132)	NA (384)		1000G	ESP6400
c.77G>A	p.Gly26Asp	Unknown	CAMST01	-	-	(7)	-	-
c.220G>A	p.Val74Ile	Unknown	-	PKSTR20	-	-	-	(1)
c.361T>C	p.Tyr121His	Unknown	-	PKST27	-	-	-	-
c.742A>G	p.Ile248Val	Unknown	167,176,177	-	-	(1)	-	(2)
c.740G>C	p.Gly247Ala	rs145887263	-	-	NA236, 242	-	-	(11)
c.889C>T	p.Arg297Trp	rs199982051	-	PKSTR18	-	-	-	-
c.953G>A	p.Arg318Gln	Unknown	-	PKST75	-	-	-	-
c.1024A>G	p.S342G (near splice)	Unknown	STCR119	-	-	-	-	(1)
c.1079C>A	p.Ala360Asp	rs145979929	STCR120, CAMST01	-	-	-	(2)	(9)
c.1100G>A	p.Arg367Gln	rs139861201	-	PKST31	-	(1)	-	(5)
c.380C>T	p.Thr127Met	rs147261925	-	-	-	(1)	-	(4)
c.544-6T>G	N/A (near splice)	Unknown	-	-	-	(1)	-	(10)
c.803G>A	p.Arg268Gln	Unknown	-	-	-	(1)	-	-
c.908A>G	p.Gln303Arg	Unknown	-	-	-	(1)	-	-
c.930G>A	p.Arg310Arg (near splice)	rs141754568	-	-	-	(3)	-	(4)
c.1357A>G	p.Ile453Val	Unknown	-	-	-	(1)	-	-

1000G = Genomes Data base (www.1000genomes.org), ESP6400 = Exome Sequencing Project Data base (evs.gs.washington.edu/EVS/), CAMST01 = Cameroonian family, STCR = Cameroonian unrelated stuttering cases, PKST = Pakistani stuttering family probands, PKSTR = Pakistani unrelated stuttering cases, NA = Unrelated North American stuttering cases, - = Variants with zero allele frequency, () = Total number of individuals, N/A = Not applicable
All annotations are based on human genome assembly hg19

Table S1C: Summary of rare *AP4S1* (NM_007077.4) variants in unrelated stuttering cases vs population matched controls

cDNA change	Amino acid change	SNP ID	Unrelated stuttering cases (609)			Population matched controls (558)	Population databases	
			STCR (93)	PKST (132)	NA (384)		1000G	ESP6400
c.1-6A>G	N/A (near splice)	Unknown	-	-	NA362	-	-	(3)
c.98C>G	p.Thr33Arg	Unknown	-	-	NA242	-	-	-
c.228C>T	p.Asn76Asn (near splice)	rs144109125	-	-	NA52	(1)	(1)	(4)
c.295-3C>A	N/A (near splice)	rs185246578	-	-	NA24	-	(2)	-
c.343C>G	p.Gln115Glu	Unknown	-	PKSTR25	-	-	-	-
c.348G>A	p.Met116Ile	rs74807133	-	-	NA277	-	(8)	(2)
c.367-11_367-10insT	N/A (near splice)	Unknown	-	-	NA292,329	(1)	-	(27)
c.373A>G	p.Ile125Val (near splice)	rs200969079	-	-	NA284	-	(1)	(3)
c.430delG	p.Gly144Asp*fs**23	Unknown	-	-	NA394	(4)	-	-
c.154T>C	p.Tyr52His	rs150801272	-	-	-	(4)	-	(10)
c.179G>A	p.Arg60Gln	Unknown	-	-	-	(1)	-	(1)
c.181C>G	p.Gln61Glu	Unknown	-	-	-	(1)	-	-
c.367-7T>G	N/A (near splice)	Unknown	-	-	-	(1)	-	-
c.418A>G	p.Lys140Glu	Unknown	-	-	-	(1)	-	-
c.407G>A	p.Cys136Tyr	Unknown	-	-	-	(1)	-	-

1000G = Genomes Data base (www.1000genomes.org), ESP6400 = Exome Sequencing Project Data base (evs.gs.washington.edu/EVS/), STCR = Cameroonian unrelated stuttering cases, PKST = Pakistani stuttering family probands, PKSTR = Pakistani unrelated stuttering cases, NA = Unrelated North American stuttering cases, - = Variants with zero allele frequency, () = Total number of individuals, N/A = Not applicable

All annotations are based on human genome assembly hg19

SUPPLEMENTAL REFERENCES

Boehm, M., and Bonifacino, J.S. (2001). Adaptins: the final recount. Mol. Biol. Cell *12*, 2907-2920.

Dell'Angelica, E.C., Mullins, C., and Bonifacino, J.S. (1999) AP-4, a novel protein complex related to clathrin adaptors. J. Biol. Chem. *274*, 7278-85.

Image J, version 1.48v, Wayne Rasband, NIH, http://imagej.nih.gov)