Supplemental Figure S1

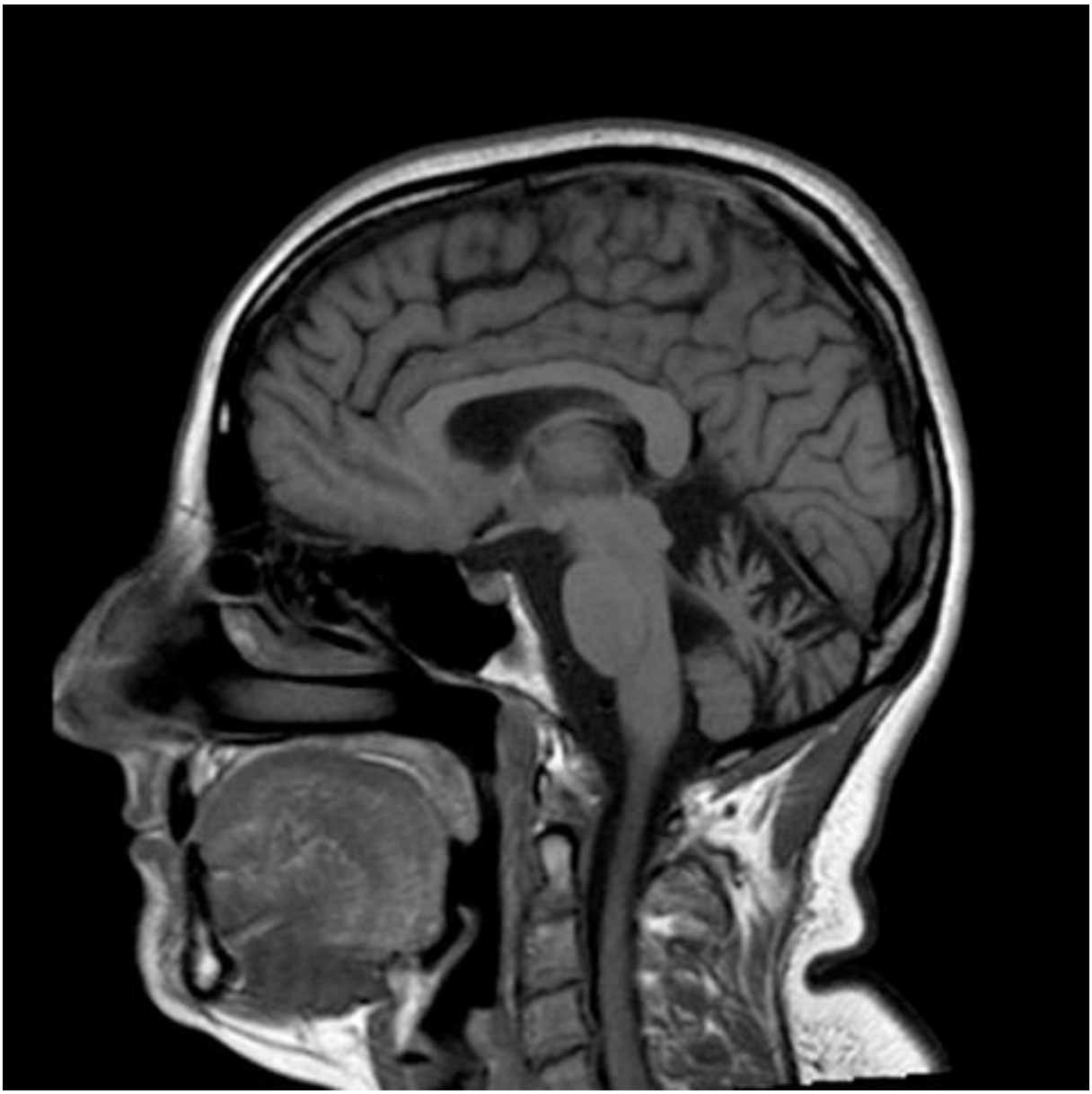

Figure S1. MRI findings in individual AAD-SAL-233-25 (IV-16 in Figure 2)

Cerebral MRI in individual AAD-SAL-233-25 (IV-16 in Figure 2) at age 49. Sagittal view of a T1-weighted sequence showing superior cerebellar vermian atrophy, normal pons and cerebellar amygdala.

Supplemental Figure S2

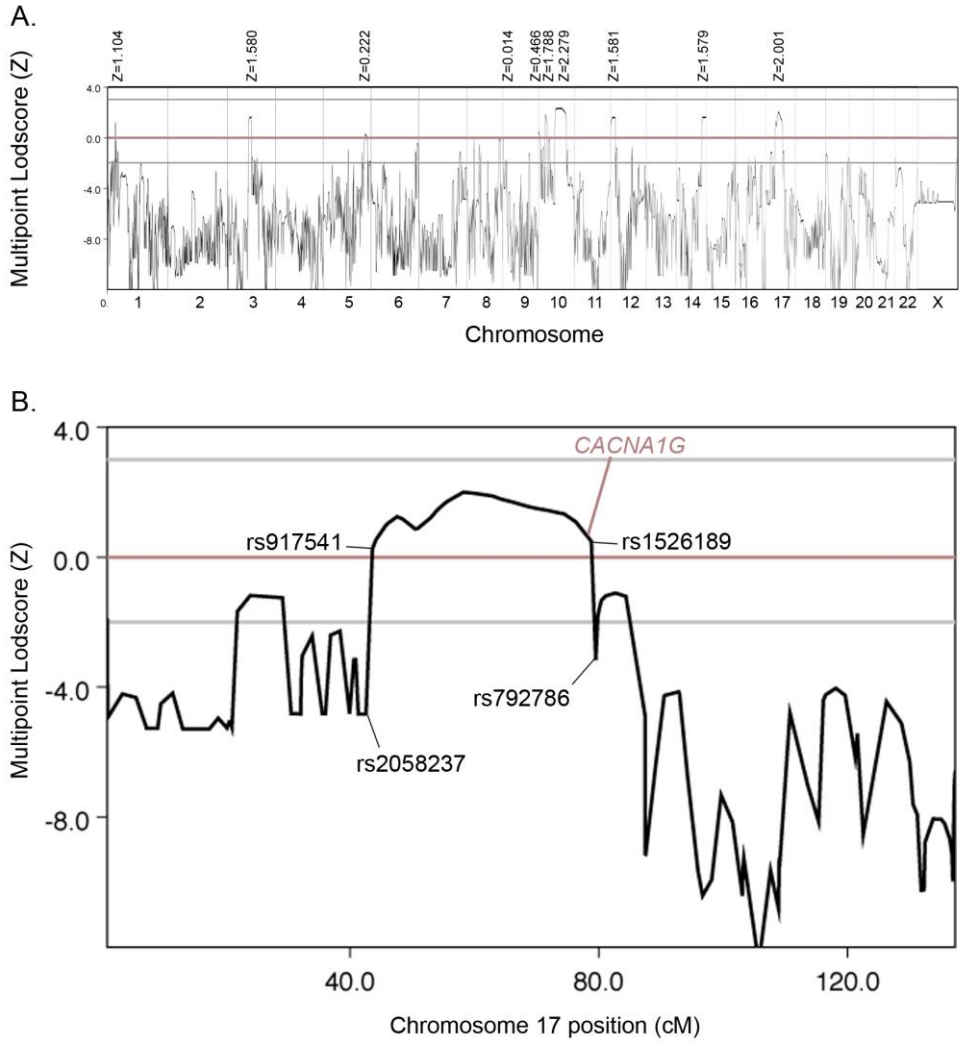

Figure S2. Whole genome linkage analysis in family AAD-SAL-233.

(A) Multipoint linkage analysis on all the autosomes in AAD-SAL-233 family. Whole genome linkage analysis was performed using Illumina LINKAGE_12 microarrays (6090 SNP markers). Genotypes were determined using Beadstudio (Illumina) and analyzed with MERLIN 1.0,[1] assuming an autosomal dominant transmission under a 0.80 penetrance model with equal allele frequencies, similar recombination fractions between males and females, and a disease frequency of 0.00001. Genotypes were available for 12 individuals, including affected subjects 9, 14, 20, 23, 25, 45, 46 (III-3, III-9, IV-4, IV-7, IV-16, V-5 and V-6 in Figure 2) and their relatives, 11, 15, 21, 24, 29 (III-5, III-10, IV-5, IV-8 and IV-11 in Figure 2). Whole genome linkage analysis could not lead to the identification of a unique well-defined associated locus due to pedigree structure and size limitations. Instead, various putatively linked or unexcluded (LOD scores above -2) loci were found in the family, including six putatively linked loci with multipoint LOD scores reaching the maximal expected values of this pedigree (from +1.579 to +2.279) and various uninformative regions. **(B)** Close-up view of the linkage analysis results for chromosome 17. A significant multipoint LOD score (Zmax = +2.001) was found between markers rs2058237 and rs792786, in a region encompassing the *CACNA1G* gene.

Supplemental Figure S3

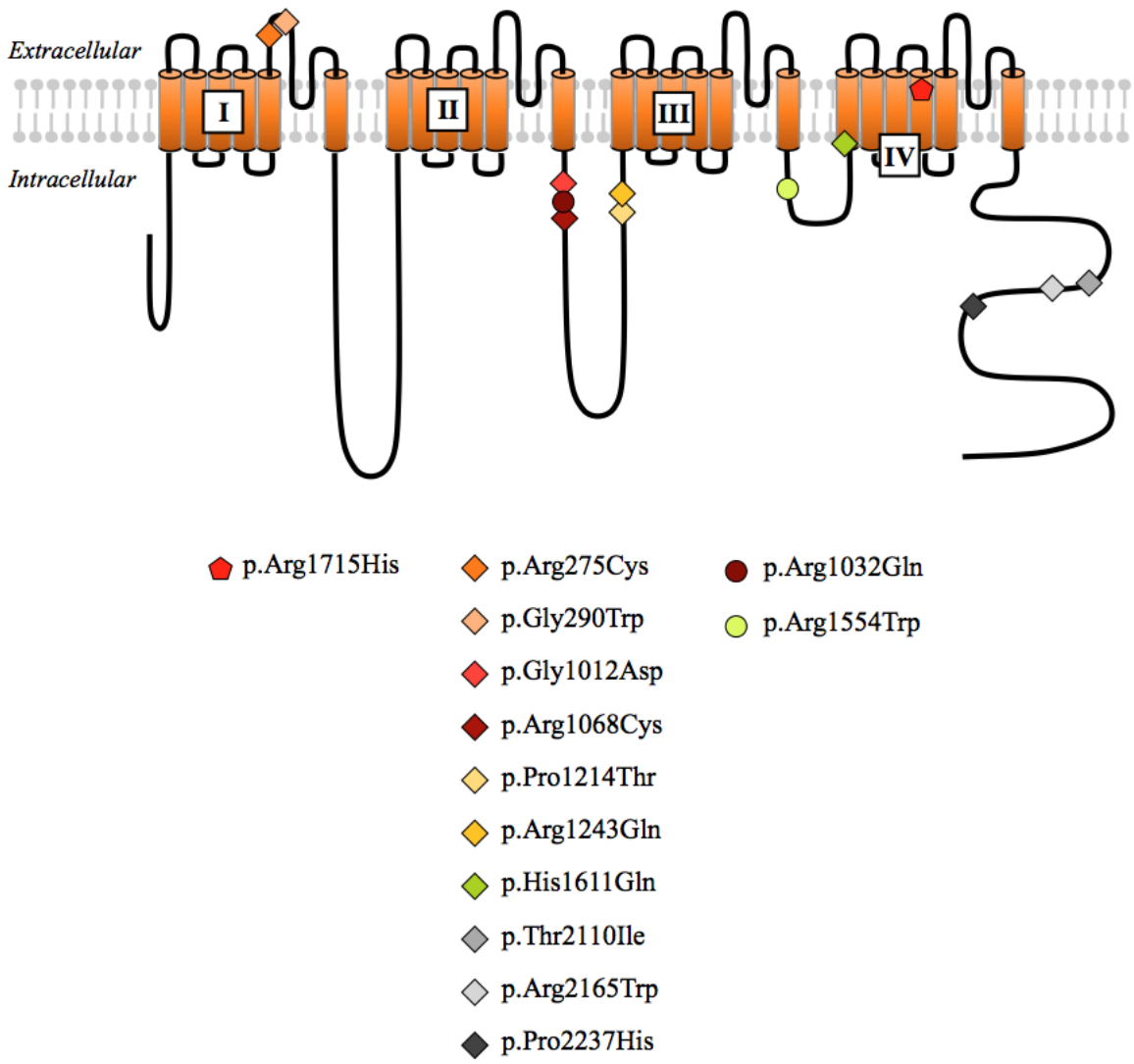

Figure S3. Schematics of Cav3.1 with other variants found by panel sequencing.

Schematics of Cav3.1 with all changes linked to *CACNA1G* variants encompassed by panel sequencing. Genetic characteristics of the variants are summarized in Table S4. The deleterious p.Arg1715His change is shown with a bright red pentagon; Variants of Unknown Significance are represented with diamonds, Variants of Unknown Significance found in individuals otherwise presenting a *CACNA1A* frameshift are indicated with circles.

Supplemental Table S1

Individual	AAD-SAL-233-15 (III-10)	AAD-SAL-233-25 (IV-16)
Number of reads	292635836	270474232
Aligned reads	291158538 (99.50%)	268296715 (99.19%)
Aligned but not duplicated reads	91598083 (31.30%)	95951959 (35.48%)
Percentage of target with >30x coverage	86.57%	85.47%
Number of variants	34226	36501
Number of variants in exons +/-200bp	31133	33083

Table S1. Whole exome sequencing statistics.

The BWA software was used in order to map reads on the reference genome: Human reference hg19. Reads related to PCR duplicates were removed from the alignment using Samtools.

Mutations were called according to the following criteria: (i) position coverage greater or equal to 20, ii) mutation proportion greater or equal to 25%, iii) mutated bases called at least 10% on each strand.

Supplemental Table S2

Chromosome	Position	Reference	Alternative	Gene	Segregation	Aminoacid change	SIFT score	Polyphen2_HDIV score	Polyphen2_HVAR score	MutationTaster score	GERP++	PhyloP 46 way	Maximal Population Frequency	1000G ALL	ExAC All	ExAC East Asian	ExAC South Asian	EVS All	EVS African American	EVS European American
10	95372766	G	A	*PDE6C*	present in all affected and healthy individual 19 (IV-1)	NM_006204.2: c.284G>A: p.Arg95His	0 (D)	0.987 (D)	0.573 (P)	D (1)	4.5	1.527	0.0003	.	0	0.0001	0.0002	0.0002	.	0.0003
17	48694921	G	A	*CACNA1G*	present in all affected, absent from all unaffected	NM_018896.4: c.5144G>A: p.Arg1715His	0 (D)	1 (D)	1 (D)	D (1)	5.02	2.348	.	.	.	.	.	.	.	.

Table S2. Characteristics of AAD-SAL-233 variants.

Summary of characteristics for both variants segregating in family AAD-SAL-233. Annotation was performed using Annovar (www.openbioinformatics.org/annovar/).[2] The database frequencies originate from 1000 genomes (www.1000genomes.org/), Exome Variant Server (EVS, http://evs.gs.washington.edu/EVS) and Exome Aggregation Consortium (ExAC, http://exac.broadinstitute.org/). Pathogenicity scores are evaluated as follows: SIFT (http://sift.jcvi.org/) predicts deleteriousness (D) under 0.05; Polyphen2 (http://genetics.bwh.harvard.edu/pph2/) classifies SNPs as probably damaging (D; HDIV>=0.957, HVAR>=0.909), possibly damaging (P; 0.453<=HDIV<=0.956, 0.447<=pp2_hdiv<=0.908), or benign (B; HDIV<=0.452, HVAR <=0.446); LRT differentiates variants with deleterious (D), neutral (N) or unknown (U) effect;[3] MutationTaster (http://www.mutationtaster.org/) classifies them as "disease_causing_automatic" (A), "disease_causing" (D), "polymorphism" (N) or "polymorphism_automatic" (P) with a given probability value, 1 being the most probable. For both GERP++ (http://mendel.stanford.edu/SidowLab/downloads/gerp/) and PhyloP (http://compgen.bscb.cornell.edu/phast/help-pages/phyloP.txt), higher scores indicate better residue conservation.

Supplemental Table S3

	PCRs / GSJunior (n=96)					Fluidigm / MiSeq (n=384)				
Exon	**Mean coverage of exon (all individuals)**	**Mean percentage of exon covered <10x**	**Number of individuals with >=10% of exon covered <10x**	**Mean percentage of exon covered >=30x**	**Number of individuals with >=90% of exon covered >=30x**	**Mean coverage of exon (all individuals)**	**Mean percentage of exon covered <10x**	**Number of individuals with >=10% of exon covered <10x**	**Mean percentage of exon covered >=30x**	**Number of individuals with >=90% of exon covered >=30x**
1	70.1	23.9	23	38.3	36	0.1	99.9	384	0	0
2	67.8	37.9	36	43.2	41	458.4	0.7	5	99.1580729	379
3	75.2	0.0	0	93.7	89					
4	75.1	3.2	3	93.7	89	637.2	0.4	2	99.47916667	382
5	52.7	17.9	17	80.0	76	670.6	0.7	3	99.05442708	380
6	109.0	0.5	1	98.8	92	611.6	1.2	6	98.7223958	378
7	83.6	1.1	1	97.9	93					
8	64.6	10.5	60	77.0	3	473.9	1.1	7	98.74505208	374
9	111.1	3.3	7	90.1	72	880.6	0.5	3	99.37604167	381
10	62.4	11.6	11	88.4	84	497.9	1.5	5	97.62005208	379
11	77.0	0.8	1	93.5	87	798.8	0.8	5	99.1302083	379
12	86.0	0.7	1	90.7	76					
13	65.3	0.0	0	86.3	82					
14	47.3	3.2	3	64.2	61	53.1	6.5	53	70.33203125	217
15	73.3	0.0	0	94.9	89	553.9	9.8	119	82.3257812	177
16	75.3	4.2	4	71.6	68					
17	59.2	7.8	16	72.9	39	625.9	0.8	4	98.94192708	378
18	53.6	1.1	1	91.6	87	711.4	1.1	6	98.8440104	378
19	41.3	20.0	19	71.4	66					
20	93.4	0.0	0	100.0	95	270.1	4.1	54	94.625	313
21	40.6	7.4	7	51.9	49					
22	66.9	2.1	2	93.7	89	0.6	99.7	384	0	0
23	53.6	0.0	0	84.9	80	379.7	0.4	2	99.47916667	382

24	71.6	0.0	0	64.2	61	568.6	0.4	2	99.47916667	382
25	68.5	0.0	0	92.9	87	165.5	1.3	5	97.76302083	374
26	46.9	5.3	5	65.3	62	263.1	1.3	5	98.69791667	379
27	30.5	12.6	12	32.8	31	74.0	1.8	7	93.37760417	349
28	55.2	0.0	0	82.1	78	709.7	0.6	2	98.75260417	382
29	76.4	0.0	0	93.7	89					
30	90.0	1.1	1	95.8	91	518.0	1.1	7	98.5570313	373
31	93.5	1.0	2	81.2	55					
32	72.2	0.0	0	92.6	88					
33	80.8	1.4	3	79.4	52	559.5	0.6	3	99.32447917	380
34	62.8	0.0	0	93.7	89	475.3	0.4	2	99.20208333	381
35	75.4	0.0	0	94.7	90	675.6	0.3	2	99.47916667	382
36	59.1	0.0	0	93.4	88	353.3	1.0	4	98.95833333	380
37	36.4	3.1	4	44.3	35	0.7	99.3	383	0.312760417	0
38	74.1	7.9	49	80.8	24	316.4	0.7	4	99.20859375	379

Table S3. Coverage statistics for both amplicon panels.

For the PCRs / GSJunior panel (n=96), PCRs were performed using specific primers (available upon request), with Fast Start High Fidelity Taq (Roche Life Science) following the manufacturer's protocol. Sequencing was performed with the Roche GS Junior 454 sequencing system, following the manufacturer's protocol.

For the Fluidigm / MiSeq panel (n=384), PCRs were performed using specific primers (Fluidigm D3 Assay Design), with Fast Start High Fidelity Taq (Roche Life Science), using the Fluidigm Access Array, following the manufacturer's protocol. Sequencing was performed with the MiSeq sequencing system (2x300bp, V5), following the manufacturer's protocol.

Supplemental Table S4

Comment	Chromosome	Position	Reference	Alternative	Aminoacid change	SIFT score	Polyphen2_HDIV score	Polyphen2_HVAR score	MutationTaster score	GERP ++	PhyloP 46 way	EVS chromosome count	ExAC chromosome count
	10	95381695	A	G	NM_006204.2:c.730A>G:p.Met244Val	0 (D)	0.046 (B)	0.023 (B)	0.846 (D)	3.64	2.166	G=1/A=13005	G=1/A=121365
	10	95386461	C	T	NM_006204.2:c.1004C>T:p.Pro335Leu	0.02 (D)	0.996 (D)	0.732 (P)	1 (D)	4.58	2.89	T=0	T=8/C=118458
Causative *FA2H* homozygous variant validated in the family	10	95399849	G	A	NM_006204.2:c.1505G>A:p.Arg502His	0.08 (T)	0.76 (P)	0.136 (B)	1 (N)	-2.12	-0.561	A=0	A=4/G=121342

Table S4. Characteristics of other *PDE6C* variants found by panel sequencing.

Summary of characteristics for all *PDE6C* variants found by panel sequencing in 384 individuals. Annotation was performed using Annovar (www.openbioinformatics.org/annovar/).[2] The database frequencies originate from 1000 genomes (www.1000genomes.org/), Exome Variant Server (EVS, http://evs.gs.washington.edu/EVS) and Exome Aggregation Consortium (ExAC, http://exac.broadinstitute.org/). Pathogenicity scores are evaluated as follows: SIFT (http://sift.jcvi.org/) predicts deleteriousness (D) under 0.05; the variant was else considered tolerated (T); Polyphen2 (http://genetics.bwh.harvard.edu/pph2/) classifies SNPs as probably damaging (D; HDIV>=0.957, HVAR>=0.909), possibly damaging (P; 0.453<=HDIV<=0.956, 0.447<=pp2_hdiv<=0.908), or benign (B; HDIV<=0.452, HVAR <=0.446); MutationTaster (http://www.mutationtaster.org/) classifies them as "disease_causing_automatic" (A), "disease_causing" (D), "polymorphism" (N) or "polymorphism_automatic" (P) with a given probability value, 1 being the most probable. For both GERP++ (http://mendel.stanford.edu/SidowLab/downloads/gerp/) and PhyloP (http://compgen.bscb.cornell.edu/phast/help-pages/phyloP.txt), higher scores indicate better residue conservation.

Supplemental Table S5

Individual	Chr	Position	Ref	Alt	Amino acid change	Variant(s) in other genes involved in ADCA	Sanger confirmation	Segregation within family	Electrophysiological evidences	SIFT score	Polyphen2_HDIV score	Polyphen2_HVAR score	MutationTaster score	GERP++	phyloP 46way	Maximal Population Frequency	1000G ALL	ExAC ALL	EVS ALL
Pathogenic variant (3 Strong PS1 according to ACMG guidelines, Richard et al., 2015)																			
recurrent : AAD-SAL-233, AAD-GRE-319, AAD-SAL-454	17	48694921	G	A	NM_018896:c.5144G>A:pArg1715His	No	Yes	6 affected in AAD-SAL-233, 3 affected in AAD-GRE-319	statistically significant alterations of activation and inactivation curves	0 (D)	1 (D)	1 (D)	D (1)	5.02	2.348	.	.	.	.
Variants of Unknown Significance (VUS)																			
H202	17	48674228	C	T	NM_018896:c.3202C>T:p.Arg1068Cys	NA	Yes	NA	no statistically significant difference	0.03 (D)	1 (D)	0.999 (D)	D (1)	2.78	1.052	0.0002	.	0	.
H202	17	48703688	C	A	NM_018896:c.6710C>A:p.Pro2237His	NA	Yes	NA	no statistically significant difference	0.02 (D)	0.994 (D)	0.974 (D)	N (1)	4.32	2.484	0.0023	0.0002	0.0005	0.0003
K087	17	48692795	C	A	NM_018896:c.4833C>A:p.His1611Gln	NA	Yes	NA	no statistically significant difference	0.79 (T)	0.999 (D)	0.993 (D)	D (1)	4.75	2.17	.	.	.	.
7909	17	48677170	C	A	NM_018896:c.3640C>A:p.Pro1214Thr	NA	Yes	NA	NT	0.4 (T)	0.042 (B)	0.07 (B)	N (1)	0.264	-0.055	.	.	.	.
SAL-REB-399-400	17	48701820	C	T	NM_018896:c.6329C>T:p.Thr2110Ile	NA	Yes	NA	NT	0.22 (T)	0.43 (B)	0.24 (B)	N (1)	2.88	1.338	.	.	.	.
AAD-BOR-SAR-565-012	17	48678124	G	A	NM_018896:c.3728G>A:p.Arg1243Gln	NA	Yes	NA	NT	0.06 (T)	0.999 (D)	0.98 (D)	N (1)	5.46	2.569	0.005	0.001	0.0001	.
AAD-SAL-MAR-931-001	17	48650036	G	T	NM_018896:c.868G>T:p.Gly290Trp	No	Yes	NA	NT	0.02 (D)	1 (D)	1 (D)	D (1)	5.36	2.541	.	.	.	.
AAD-LIL-SEP-246-001	17	48673978	G	A	NM_018896:c.3035G>A:p.Gly1012Asp	No	Yes	NA	NT	0.48 (T)	1 (D)	0.999 (D)	D (0.869)	4.14	2.375	0.0003	.	0.0001	0.0002
AAD-STR-ALB-916-001	17	48703471	C	T	NM_018896:c.6493C>T:p.Arg2165Trp	Missense monoallelic VUS in *POLG*	Yes	NA	NT	0.02 (D)	1 (D)	0.958 (D)	N (0.996)	4.3	1.217	0	.	0	.
AAD-GRE-CUI-568-014	17	48649991	C	T	NM_018896:c.823C>T:p.Arg275Cys	Missense VUS in *REEP1*	Yes	Present in unaffected mother	NT	0.01 (D)	1 (D)	0.999 (D)	N (0.99)	4.37	1.234	0.0004	.	0.0001	.
Variants of Unknown Significance AND pathogenic variant in another gene involved in ADCA																			
AAD-BOR-BAR-414-003	17	48685335	C	T	NM_018896:c.4660C>T:p.Arg1554Trp	Frameshift in *CACNA1A* (to confirm)	Yes	NA	NT	0.01 (D)	1 (D)	0.997 (D)	D (1)	3.71	2.196	0.0001	.	0	.
AAD-SAL-JAC-070-007	17	48674121	G	A	NM_018896:c.3095G>A:p.Arg1032Gln	Frameshift in *CACNA1A*, missense monoallelic VUS in *POLG* (to confirm)	Yes	NA	NT	0.27 (T)	0.88 (P)	0.329 (B)	N (0.867)	0.624	-0.086	.	.	.	.

Table S5. Characteristics of other *CACNA1G* variants found by panel sequencing.

Summary of characteristics and pathogenicity evidences for all *CACNA1G* variants encompassed by panel sequencing. All scores are described in Table S2. No other variant than p.Arg1715His could lead to a definite conclusion regarding its pathogenicity, due to lack of other affected for segregation confirmation, or lack of functional proof. Only one other variant, p.Gly290Trp, is predicted pathogenic by all *in silico* softwares and is never present in public databases. No variant is located nearby p.Arg1715His, nor in S4 segments, regardless of the domain. All variants were therefore classified as variant of unknown significance (VUS).[4]

Chr: Chromosome
NA: not available
NT: not tested
Ref / Alt: reference and alternative base
VUS: variant of unknown significance (ACMG class 3)

Supplemental Table S6

	WT		His1611Gln		Arg1068Cys		Pro2273His	
	Mean±SEM	n	Mean±SEM	n	Mean±SEM	n	Mean±SEM	n
Current density (pA/pF)	-74.58±11.18	19	-43.47±13.35	4	-92.3±22.12	9	-68.76±16.79	6
$V_{1/2}$ act (mV)	-47.2±0.65	18	-45.61±0.27	4	-47.01±0.63	8	-44.89±0.88	6
K $_{Vact}$ (mV)	4.761±0.14	18	5.08±0.17	4	4.87±0.26	8	4.911±0.19	6
$V_{1/2}$inact (mV)	-70.91±0.48	15	-70.07±0.39	4	-72.61±1.19	9	-69.54±0.46	6
K $_{Vinac}$ (mV)	4.29±0.07	15	3.96±0.03	4	4.50±0.19	9	4.7±0.22	6
τ_{act} at -40 mV (ms)	4.38±0.39	10	4.15±0.28	4	3.59±0.19	4	3.94±0.28	5
τ_{inact} at -40 mV (ms)	16.27±0.77	10	17.03±1.59	4	13.8±0.96	4	15.45±0.53	5
recovery (ms)	105.1±9.48	12	103.5±7.08	4	81.45±3.18	4	91.69±3.41	5
τ_{deact} at -70mv (ms)	3.87±0.25	8	3.65±0.33	3	3.18±0.148	4	3.44±0.29	6

Table S6. Electrophysiological properties of additional Cav3.1 variants.

V1/2 represents the half-activation and respectively half inactivation potential, K the slope factor, τact τinact and τdeact the activation, inactivation and respectively deactivation kinetics. n is the number of cells. No statistically significant differences were seen between the WT and the different variants presented in this table.

Supplemental References

1 Abecasis, G.R., Cherny, S.S., Cookson, W.O., Cardon, L.R. (2002). Merlin-rapid analysis of dense genetic maps using sparse gene flow trees. Nat. Genet. *1*, 97-101.

2 Wang, K., Li, M., Hakonarson, H. (2010). ANNOVAR: Functional annotation of genetic variants from next-generation sequencing data. Nucleic Acids Research. *38*, e164.

3 Chun, S., Fay, J.C. (2009). Identification of deleterious mutations within three human genomes. Genome Res. *19*:1553-1561.

4 Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., Grody, W., Hegde, M., Lyon, E., Spector, E., et al., on behalf of the ACMG Laboratory Quality Assurance Committee (2015). Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet. Med. *17*, 405-423.