$GABA_A$ Receptor Coupling Junction and Pore *GABRB3* Mutations are Linked to Early-Onset Epileptic Encephalopathy

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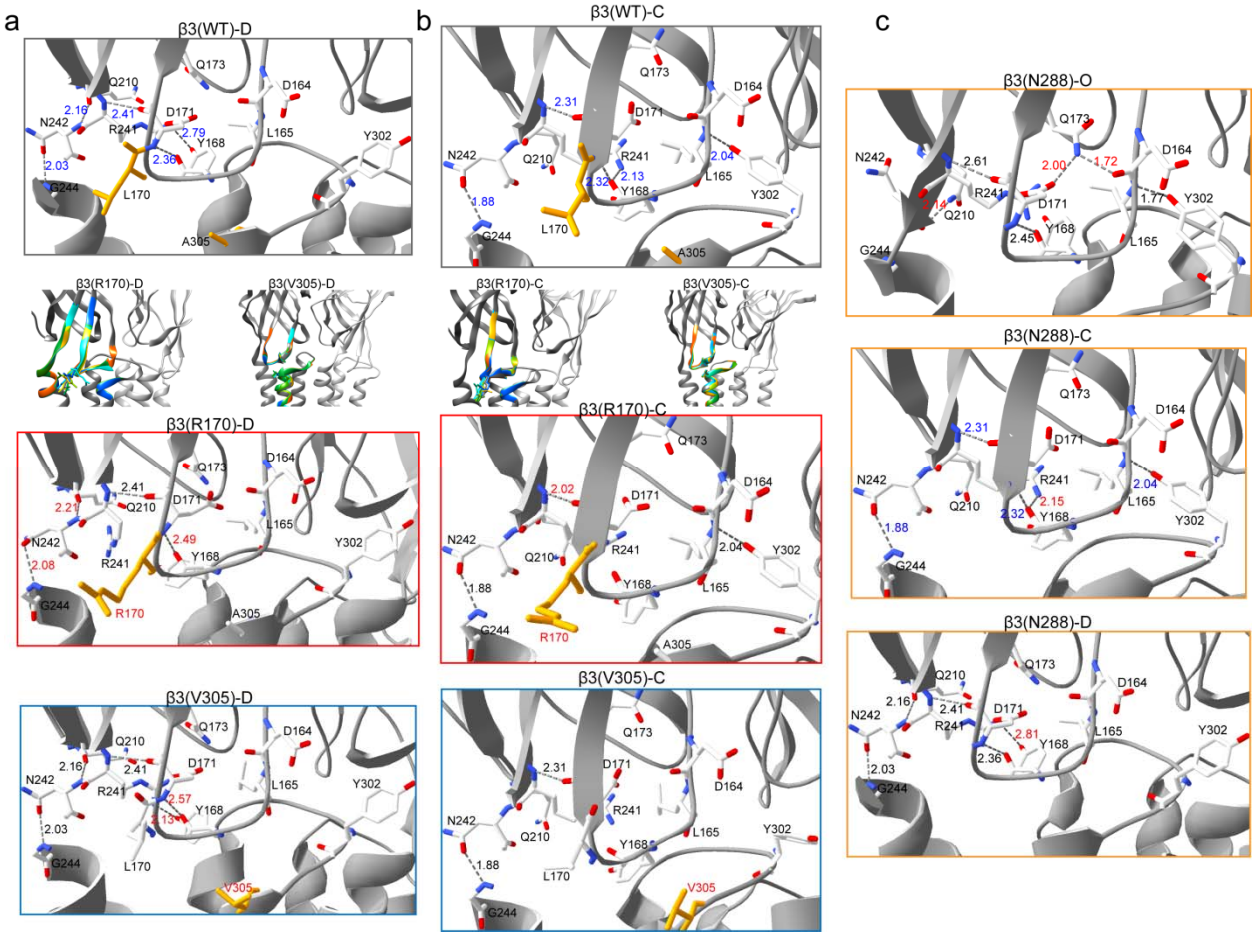
Supplementary information

Supplemental Figure 1

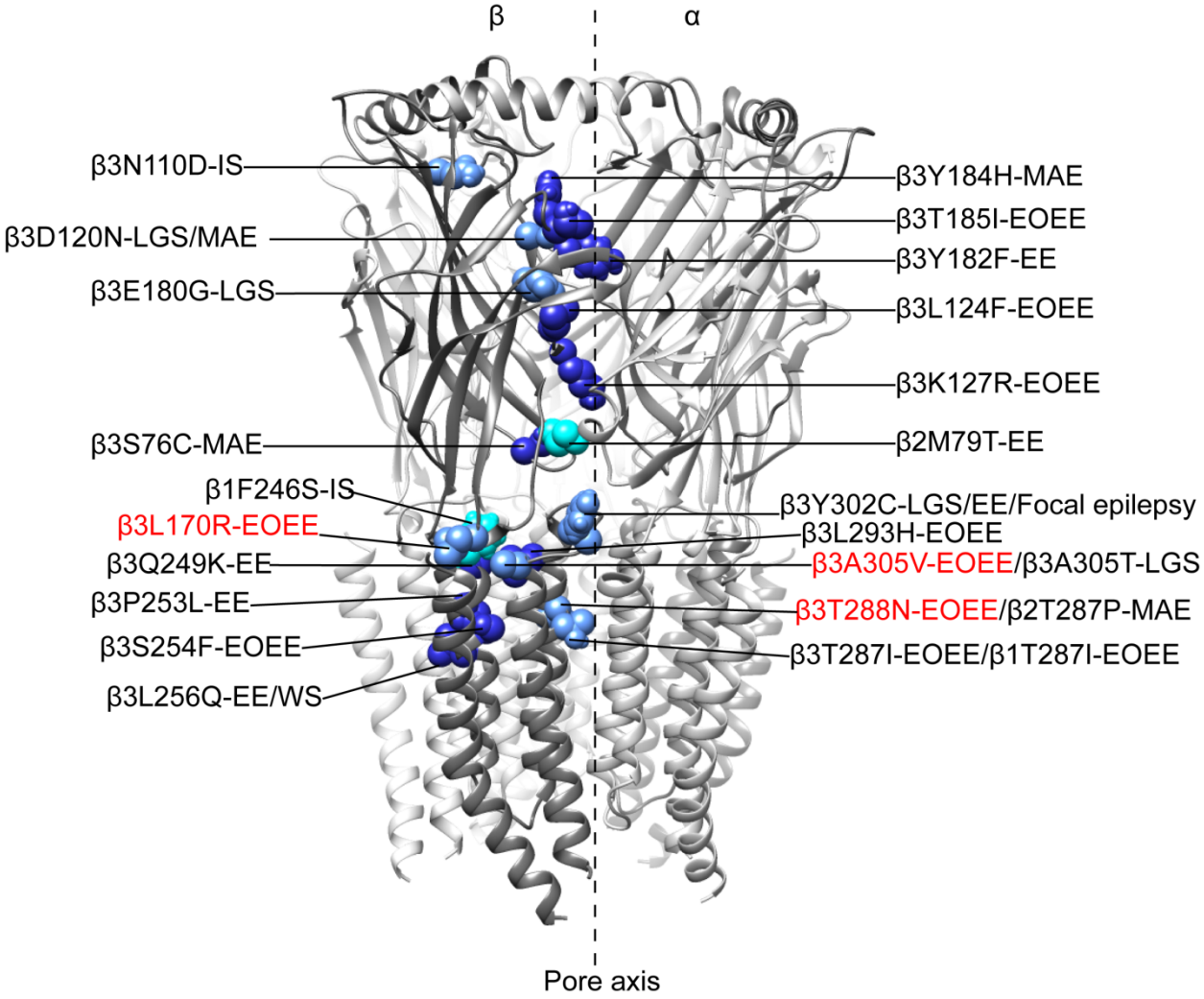
Supplemental Figure 1: Network of adjacent interactions among residues that were located in bordering regions at the coupling junction.



The predicted H-bonds for the L170R, A305V, and T288N models in the three states of the receptor were shown. The four domains and their amino acids were represented as in Figure 5. C, closed state. O, open state. D, desensitized state.

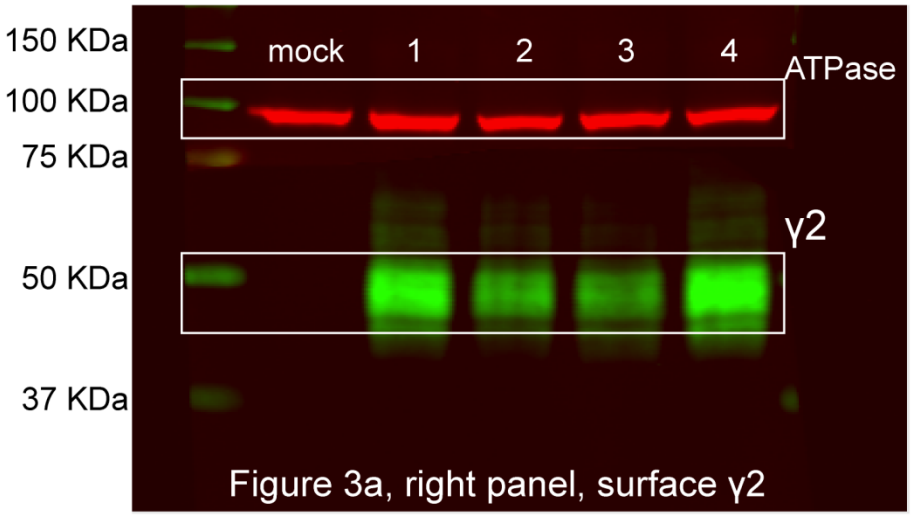
Supplemental Figure 2

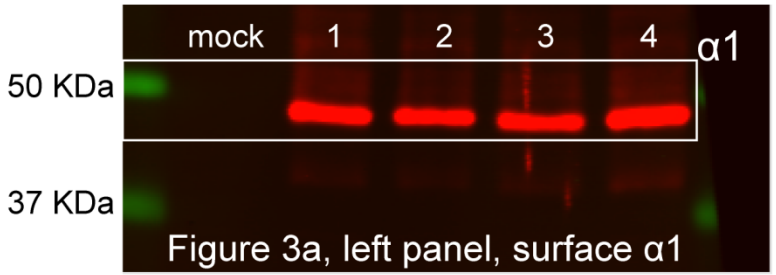
Supplemental Figure 2: Mapping of EOEE associated GABRB de novo mutations in the $GABA_A$ receptor

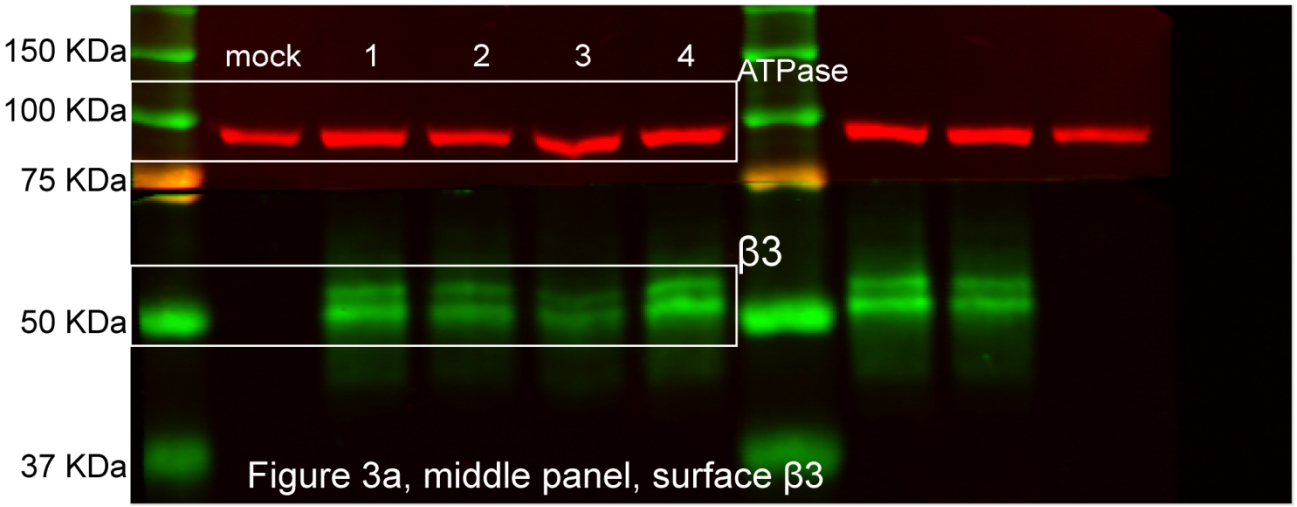


3-D structural model of the pentameric $GABA_A$ receptor structure at the β/α subunit interface mapping the *GABRB* mutations. The residues are shown is space filled representation and in different shades of blue for clarity (see details in the text).

35 ***Supplemental Figure 3***

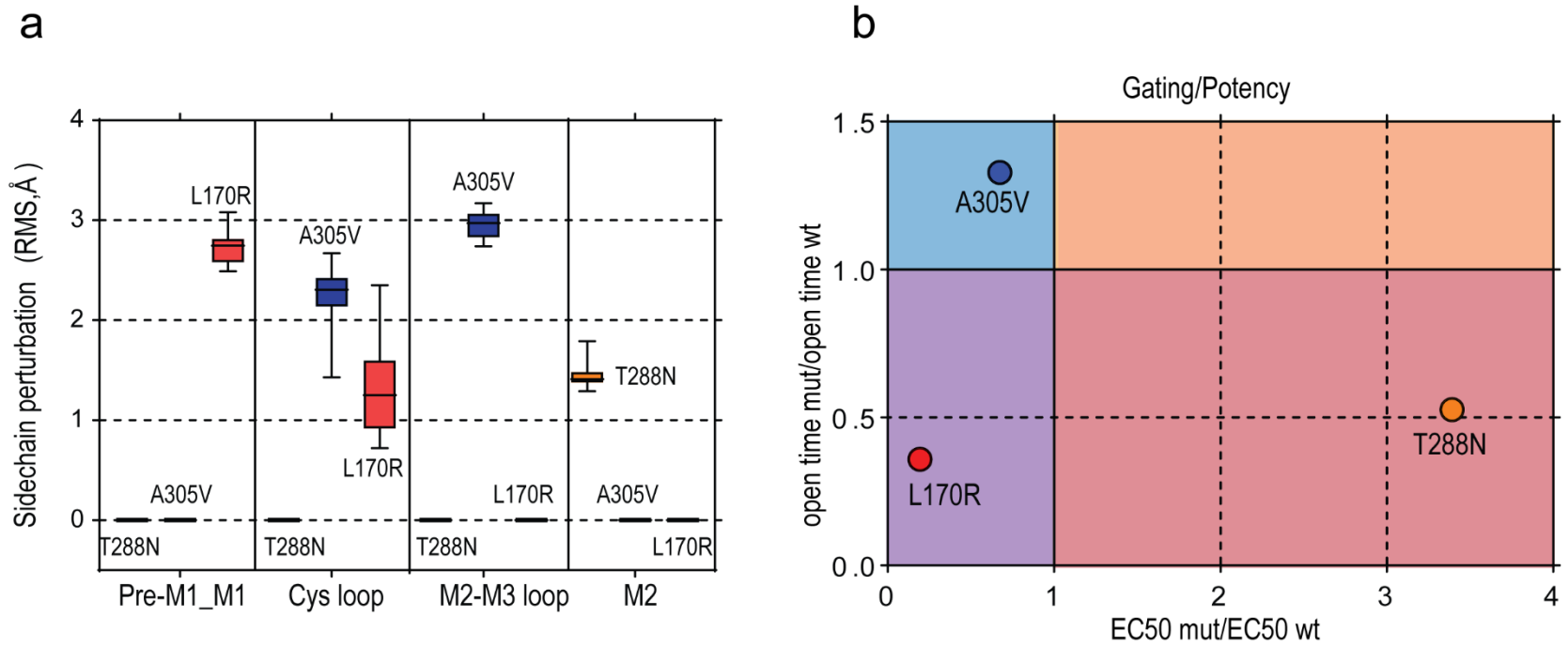






Supplemental Figure 3: Full-length gels for images shown in Figure 3a. The boxed region in each gel represents the cropped image shown in Figure 3a for each panel, respectively. Note that the loading control (ATPase) was the same for γ2 and α1 surface expression.

Supplemental Figure 4



Supplemental Figure 4: (**a**), box plots show predicted perturbations caused by the mutant sidechain residues L170R, A305V and T288N at Pre-M1_M1 (R241-Y245), Cys-loop (L165-Y168), M2-M3 loop (P298-Y302), and M2 (T281-E295) domains in the open conformation. RMS values for up to 10 simulations are represented as interleaved box and whiskers (25-75% percentile, median, and minimum and maximum). (**b**), Correlation of the potency and gating ratio plots for the coupling junction mutant residues L170R and A305V, and the pore mutant residue T288N. Ratios equal to 1 indicates no changes when compared to wild type (wt).

52 ***Table S1. Clinical Features in 3 Patients With EOEE and GABRB3 Missense Mutations.***

Case (Sex)	Age	Age at seizure onset	Diagnosis	Seizure types	Neurodevelopment delay and Cognitive outcome	EEG Finding	Brain imaging	Response to AED
PED 1, proband II-3 (M)	3 y	6 m	EOEE	Focal and secondary generalized tonic–clonic seizures (GTCS)	Severe delay at motor and language--delayed developmental milestones, and psychomotor regression, hypotonia and the rest was unremarkable. Poor coordination.	Generalized fast-wave in background activity; a large amount of widespread medium to high volatility multifocal sharp and spike discharges in anterior region during sleep (at 1 year).	Thin corpus callosum	Refractory to AEDs
PED 2, proband II-3 (M)	9 y	6 m	EOEE	Focal and secondary generalized tonic–clonic seizures (GTCS)	Severe delay at motor and language, unable to lift and hold the head upright or to sit, unable to recognize people and nonverbal at 1 year old. Limited comprehension. Very limited progression when seizures were overcome. Poor coordination.	Generalized multifocal low to high volatility sharp, spike, sharp-slow and spike-slow wave complexes in the left occipital and the posterior of temporal region, significant increased during sleep period, spread gradually to the opposite sides of same regions and same side of anterior regions (at 1 year).	Mild cortical atrophy (mainly frontal lobe).	Refractory to AEDs
PED 3, proband II-3 (M)	1 y 6 m (death)	3 m	EOEE	Partial seizures. Eyelid myoclonus with stiffening hands, or companied with eye deviation. The seizure onset usually lasted 10 sec. to 3 min., multiple times per day.	Severe delay at motor and language--unable to lift and hold his head upright or roll over at 6 months age, and unable to understand or express language at over 1 year old. Hypotonia and the rest were unremarkable. Poor coordination.	Bilateral discharges during inter-ictal period and focal spike wave discharges in the right temporal region during ictal period (at 5 months).	Mild cortical atrophy (mainly frontal lobe).	Refractory to AEDs

53 PED, pedigree; M, male; y, years; m, moths; EOEE, early onset epileptic encephalopathy; AEDs, antiepileptic drugs.
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Table S2. A list of 480 Candidate Genes Associated with Epileptic Encephalopathies

Gene Symbols													
ABAT	ASS1	CHRFAM7A	DDC	GABRA4	GPR98	IL6ST	KCNK1	MAP2	PAFAH1B1	PNKD	SCN2A	SLC6A13	TPP1
ABCC2	ATIC	CHRNA2	DEPDC5	GABRA5	GRIA1	IMPA2	KCNK3	MBD5	PAH	PNKP	SCN2B	SLC6A19	TPRXL
ABCC8	ATN1	CHRNA4	DGKD	GABRA6	GRIA2	INA	KCNK7	MBTPS2	PALLD	PNPO	SCN3A	SLC6A8	TREX1
ACADM	ATP1A2	CHRNA5	DHFR	GABRB1	GRIA3	IQSEC2	KCNK9	ME2	PARK2	POLG	SCN3B	SLC9A3	TRMT44
ACO2	ATP1A3	CHRNA7	DIAPH3	GABRB2	GRIA4	JRK	KCNMA1	MECP2	PC	POLG2	SCN4A	SLC9A6	TRPM6
ACOX1	ATP2A2	CHRNB2	DLX2	GABRB3	GRIK1	JUN	KCNMB2	MED17	PCDH19	PPP1R3C	SCN5A	SLC9A9	TSC1
ACP1	ATP6AP2	CLCN2	DLX5	GABRD	GRIK2	KARS	KCNMB3	MEF2C	PDCD10	PPT1	SCN7A	SMARCA2	TSC2
ACSF3	ATP6V0A2	CLCN4	DNM1	GABRE	GRIN1	KCNA1	KCNMB4	MFSD8	PDHA1	PRF1	SCN8A	SMARCA4	TSEN2
ACTA2	ATP7A	CLCNKB	DPM1	GABRG1	GRIN2A	KCNA2	KCNN2	MIB1	PDHX	PRICKLE1	SCN9A	SNIP1	TSEN34
ACTB	AUH	CLEC18A	DPYS	GABRG2	GRIN2B	KCNA6	KCNQ1	MMADHC	PDYN	PRICKLE2	SDHA	SOBP	TSEN54
ACY1	BCKDK	CLIC2	DYRK1A	GABRG3	GRIN2C	KCNAB1	KCNQ2	MOCS1	PEX1	PRNP	SEPSECS	SPAST	TSPO
ADAR	BDNF	CLN3	EFHC1	GABRP	GRIN2D	KCNAB2	KCNQ3	MOCS2	PEX10	PRODH	SERPINI1	SPR	TUBA1A
ADCK3	BRAT1	CLN5	EFHC2	GABRQ	GRIN3A	KCNAB3	KCNQ4	MOG	PEX12	PRRT2	SEZ6	SPTAN1	TUBA8
ADK	BRD2	CLN6	EGF	GABRR1	GRIN3B	KCNB1	KCNQ5	MSN	PEX13	PSAP	SHANK3	SRGAP2	TUBB2B
ADSL	BTD	CLN8	EHMT1	GABRR2	GRM1	KCNC1	KCNS2	MTHFR	PEX14	PSAT1	SLC12A1	SRPX2	TUBGCP6
AFG3L2	C10ORF2	CNP	EIF2AK3	GABRR3	GRN	KCNC2	KCNS3	MTMR9	PEX16	PSEN1	SLC12A5	ST3GAL3	U2AF1
AGTR2	CA1	CNR1	ELOVL4	GAD1	HAX1	KCNC4	KCNT1	MTR	PEX19	PTEN	SLC12A6	ST3GAL5	UBC
ALDH4A1	CACNA1A	CNTF	ELP4	GAMT	HCCS	KCND1	KCNV1	NAGA	PEX2	PTH	SLC16A1	STRADA	UBE2A
ALDH5A1	CACNA1E	CNTN2	EMX2	GATM	HCFC1	KCND2	KCNV2	NDE1	PEX26	PTS	SLC17A5	STXBP1	UBE3A
ALDH7A1	CACNA1G	CNTNAP2	EN2	GBA	HCN1	KCNE1L	KCTD7	NDP	PEX3	QDPR	SLC19A3	SUOX	VAMP2
ALG13	CACNA1H	COG6	EPM2A	GCDH	HCN2	KCNE2	KDM5C	NDUFV1	PEX5	RAB39B	SLC1A1	SV2A	VLDLR
AMACR	CACNA2D2	COG8	EPM2AIP1	GCH1	HCN4	KCNF1	KLK1	NF1	PEX6	RANBP2	SLC1A2	SYN1	VPS13A
AMT	CACNG2	COL4A1	FADD	GCM2	HDAC4	KCNG1	KRIT1	NGLY1	PFKL	RBFOX1	SLC1A3	SYNGAP1	WARS
ANK3	CALHM1	COQ2	FARS2	GCSH	HEPACAM	KCNG4	L1CAM	NHLRC1	PFKM	RBPJ	SLC20A2	SYP	WDR45
ANKRD11	CASK	COQ9	FKTN	GJA1	HEXA	KCNH2	LAMA2	NIPA2	PHF6	RELN	SLC25A12	SYT11	ZEB2
AP4E1	CASP9	CPA6	FLNA	GJD2	HEXB	KCNH3	LAMB1	NPY	PHGDH	RHAG	SLC25A13	SZT2	
ARG1	CASR	CPS1	FOLR1	GLB1	HLCS	KCNH8	LBR	NRXN1	PHOX2A	RHOA	SLC25A22	TBC1D24	
ARHGEF15	CBS	CPT2	FOS	GLDC	HNRNPU	KCNJ1	LGI1	NSDHL	PIGA	RMND1	SLC2A1	TBCE	
ARHGEF9	CCDC88C	CSTB	FOXG1	GLRA1	HOXA1	KCNJ10	LGI2	NSF	PIGL	RNASEH2A	SLC35A2	TBX1	
ARSA	CCL3	CTDP1	FUCA1	GLRB	HPD	KCNJ11	LGI4	NTRK1	PIGN	RNF213	SLC35A3	TCF4	
ARX	CCL4	CTSD	GABBR1	GLUD1	HSD17B10	KCNJ2	LIAS	NTRK2	PIGO	ROGDI	SLC35C1	TICAM1	
ASAH1	CCM2	CUL4B	GABBR2	GM2A	HTR1A	KCNJ3	LIFR	OPA1	PIGV	RPS6KA3	SLC46A1	TK2	

ASIC1	CDK5	CYP4F11	GABRA1	GOSR2	IDH2	KCNJ5	LMBRD1	OPHN1	PIR	SCARB2	SLC4A3	TLR3	
ASPA	CDKL5	D2HGDH	GABRA2	GPHN	IDS	KCNJ6	MANBA	OPRM1	PKHD1	SCN1A	SLC52A2	TMEM67	
ASPM	CHD2	DCX	GABRA3	GPR56	IER3IP1	KCNJ9	MAOB	OTX2	PLCB1	SCN1B	SLC6A11	TNF	