***Supplementary Information.*** **Malerba et al. Genotype-phenotype correlations in patients with *de novo KCNQ2* pathogenic variants**

**Table e-1.** Clinical data and epilepsy features of the patients.

| Pt | Gender/ Last evaluation | *KCNQ2* (ENSG00000075043) change | Epilepsy | | | | Current therapy | EEG examinations at onset | MRIs (age) | ID (age) | Neurological examination | Other features |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Onset | | Follow-up | | | | | | | |
| | | | Age | Sz types (frequency) | Sz types | Sz freedom (age) | | | | | | |
| 1 | M/3y 3m | c.560C>T, p.Ser187Phe | 1 day | Tonic sz with head deviation, gaze's fixity, oral automatisms, tachycardia, tachypnea (multiple or in clusters) | Tonic sz with opisthotonus, head deviation, gaze's fixity, oral automatisms, tachypnea, tachycardia; generalized tonic-clonic sz with sialorrhea and gaze's fixity | No | CBZ | Multifocal epileptic discharges, disorganized, slowed background | Unremarkable (8d) | Severe (3y 3m) | Hypotonia, good head control, poor eye contact, assisted walking, ataxic, no speech (few words) | Self-injuring behaviour, intermittent exotropia, ataxia |
| 2 | M/2y 6m | c.973A>G, p.Arg325Gly | 1 day | Tonic sz with apnea, desaturation, oral automatisms, sialorrhea (multiple or clusters) | Tonic sz; focal clonic sz; tonic spasms with apnea, desaturation, bradycardia | No | CBZ, LEV | Burst-suppression, focal abnormalities in R occipital, asynchrony | Asymmetry, L ventricle >R (14d); mild cerebral atrophy (>F lobes), wide ventricles (1y); delayed myelination (2y) | Profound (2y 6m) | Hypotonia, no eye contact, no walking, no speech | Sleep disorders |
| 3 | F/2y 11m | c.1657C>T, p.Arg553Trp | 2 days | Tonic sz; clonic sz (febrile, sporadic) | Tonic sz; tonic-clonic sz (febrile) | Yes (10m) | VPA | Multifocal epileptic discharges (>F-T regions) | Unremarkable (6d); mild cerebral atrophy (WM), mild CC hypoplasia (18m) | Moderate (2y 11m) | Mild hypotonia, good head control, good eye contact, independent walking, poor speech | ASD, hyperkinesia |

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| 4 | M/1y 3m | c.845A>T, p.Asp282Val | 1 day | Tonic sz with perioral cyanosis, head deviation, gaze's fixity (multiple or clusters) | Tonic sz; focal sz; parcel myoclonus; clusters of tonic sz with head deviation, regurgitation, vomiting and reflex audiogenic sz; myoclonic status epilepticus | No | TPM, VPA, ETS | Burst-suppression, disorganized, slowed background, theta activity | CC hypoplasia (6d); mild cerebral atrophy, mild T2/FLAIR hyperintensity of periventricular WM, wide ventricles, CC hypoplasia, severe bilateral optic nerves atrophy (15m) | Profound (1y 3m) | Severe hypotonia, no eye contact, no head control, no walking, continuous parcel myoclonus | Cortical blindness |
| 5 | F/1y 9m | c.1655A>C, p.Lys552Thr | 3 days | Tonic sz; hemi-clonic sz; eyelid myoclonia; asymmetric tonic sz upon awakening (multiple or clusters) | Clonic sz; tonic sz | No | CBZ, CBD | Burst-suppression, multifocal discharges | Unremarkable (5d); CC hypoplasia (1y 9m) | Severe (1y 9m) | Hypotonia, peripheral hypertonia, delayed head control, good eye contact, no walking | Motor stereotypies, intermittent exotropia |
| 6 | F/1y | c.812G>A, p.Gly271Asp | 1 day | Tonic sz with perioral cyanosis, laryngeal stridor, hyperactivity, crying, autonomic features (multiple or clusters) | Clonic sz | Yes (4m) | B6, TPM | Burst-suppression, multifocal epileptic discharges, discontinuous background | Unremarkable (1m); mild CC hypoplasia, pale nuclei hyperintensity, median-cross fibres of pons (1y) | Profound (1y) | Severe global hypotonia, poor eye contact, no head control, no walking | Laryngomalacia, GERD |
| 7 | F/2y | c.569A>T, p.Gly189Ile | 1 day | Tonic sz with apnea; focal clonic sz (multiple or in clusters) | Tonic sz; focal clonic sz | No | LEV | Burst-suppression | Diffuse cerebral atrophy (>F lobes), CC hypoplasia (2y) | Profound (2y) | Severe axial hypotonia, no eye contact, no walking, no speech | GERD, sleep disorders |
| 8 | F/4m | c.587C>T, p.Ala196Val | 3 days | Focal tonic sz, clonic jerks with desaturation, cyanosis (multiple or in clusters) | Tonic sz with gaze’s fixity, head deviation, clonic jerks (febrile); clonic sz with severe desaturation | No | PB, LEV | Slow background | Unremarkable (2m) | Not applicable (4m) | Mild global hypotonia, good eye contact, good head control | No |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 9 | M/4y 6m | c.1687G>A, p.Asp563Asn | 1 day | Focal clonic sz with cyanosis (multiple or clusters) | Tonic sz with head deviation, gaxe's fixity, perioral cyanosis, also in sleep; tonic-clonic sz (sporadic) | Yes (4y) | VPA, CBZ | Focal (frontal) sporadic discharges (>R) in sleep (non-REM) | Unremarkable, cyst of cavum septi (5d); mild CC hypoplasia, cyst of cavum septi (4y 6m) | Severe (4y 6m) | Hypotonia, good eye contact, no walking, no speech | Hyperkinesia, motor stereotypies, sleep disorders |
| 10 | M/3y | c.830C>T, p.Thr277Ile | 2 days | Tonic sz with cyanosis, then focal clonic sz (multiple) | One febrile sz at 1y | Yes (1.5m) | VPA, CBZ | Burst-suppression | Unremarkable (1y) | Severe (3y) | Hypotonia, poor head control, no walking, no speech | ASD |
| 11 | F/7y 10m | c.740C>T, p.Ser247Leu | 2 days | Tonic sz (multiple) | Tonic sz; myoclonic; tonic spasms | No | VGB, CLN | Burst-suppression, abnormal background | Wide ventricles (2y) | Severe (7y 10m) | Severe hypotonia, dystonic, no head control, no walking, no speech | GERD, sleep disorders |
| 12 | F/12y 1m | c.881C>T, p.Ala294Val | 1 day | Eyelid myoclonia (multiple) | Absences | Yes (6y) | CBZ | NA | Pituitary enlargement (9y) | Severe (11y 8m) | Hypotonia, no walking, poor speech | Precocious puberty, strabismus, hyperkinesia |
| 13 | F/1y 1m | c.1665C>G, p.Phe555Leu | 2 days | Focal tonic sz (multiple) | None | Yes (1m) | CBZ | Burst-suppression, abnormal background | Unremarkable (2m) | Mild (1y) | Axial hypotonia, distal hypertonia, good head control, no walking | GERD |
| 14 | F/1y 4m | c.826A>C, p.Thr276Pro | 1 day | Focal tonic sz (multiple) | None | Yes (1m) | CBZ | Burst-suppression, multifocal abnormalities | Unremarkable (1m) | Severe (1y 4m) | Hypotonia, no head control, no walking | No |
| 15 | F/9y 9m | c.873G>T, p.Arg291Ser | 1 day | Tonic sz with perioral cyanosis, head deviation, gaze's fixity (multiple or clusters) | None | Yes (1y) | CBZ, LEV | Burst-suppression, multifocal abnormalities | Cerebral atrophy >F lobes (5y) | Severe (9y 5m) | Hypotonia, dystonic, no walking, no speech | No |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 16 | M/12y | c.365C>T, p.Ser122Leu | 2 days | Focal tonic sz (multiple) | Infrequent tonic sz (only 3 sz at 10y) | Yes (3d-10y; 10y-12y). Relapsed | OXC | Multifocal epileptic abnormalities (spike, slow wave, polySpike predominant over posterior regions and left P-T region) | Unremarkable (1m); dysplasia (11y) | Normal (12y) | Normal | No |
| 17 | F/10y | c.1118+1G>A | 2 days | Tonic or clonic sz with cyanosis (clusters lasting 3 days) | None | Yes (3d) | PB | Spike and spike-waves in C-T regions (>R) in sleep | Unremarkable (2y) | Normal (10y) | Normal | No |
| 18 | F/4y 3m | c.1642G>C, p.Asp548His | 3 days | Focal motor sz with secondary generalization (multiple or clusters) | Tonic sz; focal motor sz; spasms (multiple or clusters) | No | CBZ | Burst-suppression | Unremarkable (6y) | Severe (6y) | Good eye contact, independent walking, no speech | ASD, sleep disorders |
| 19 | F/4y | c.901G>A, p.Gly301Ser | 1 day | Focal motor sz with autonomic features (multiple or clusters) | Tonic sz; focal clonic sz with or without eyes deviation (daily and then sporadic) | Yes (6m) | None | Burst-suppression pattern, multifocal abnormalities, discontinuous background | Unremarkable (3d; 2m) | Mild (4y) | Mild global hypotonia, good head control, good eye contact, delayed independent walking, poor speech | No |
| 20 | M/5m | c.927+5G>C | 10 days | Focal clonic sz (multiple) | None | Yes (20d) | None | Multifocal epileptic abnormalities, abnormal background, slow discharges | Unremarkable (21d) | Not applicable (5m) | Good head control, good eye contact, no walking | No |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 21 | M/3y 1m | c.637C>T, p.Arg213Trp | 2 days | Tonic sz; clonic sz (febrile, sporadic) | Tonic-clonic sz (febrile, sporadic) | Yes (2y 7m) | CBZ | Normal | CC hypoplasia, secondary enlargement of cisterna magna due to hypoplastic cerebellar vermian, hippocampal malrotation and L temporal lobe atrophy (2m) | Mild (3y) | Hypotonia, good head control, poor eye contact, delayed walking, poor speech | ASD |
| 22 | M/3y 1m | c.812G>A, p.Gly271Asp | 15 days | Focal tonic sz with clonic jerkings and desaturation; clonic sz (multiple) | None | Yes (1y) | None | Multifocal epileptic abnormalities | Unremarkable (2m) | Mild (3y) | Good head control, clumsy walking, poor speech (language disorder) | Strabismus, GERD |
| 23 | F/11y | c.637C>T, p.Arg213Trp | 1 day | Tonic sz with head deviation and cyanosis (multiple until 6m) | Tonic sz with cyanosis and bradycardia, head and eyes deviation (febrile and afebrile) wakefulness and in sleep | Yes (4y) | VPA | Multifocal epileptic abnormalities | Unremarkable (4y) | Moderate (11y) | Ataxic, assisted walking, poor speech, learning disorders | Behavioral disorders (mild irritability), ataxia |
| 24 | F/2y 6m | c.593G>A, p.Arg198Gln | 6 months | Infantile spasms (multiple) | Tonic sz | No | LTG, RUF, CBD | Multifocal epileptic abnormalities, hypsarrhythmia | Delayed WM myelination, CC hypoplasia, enlargement of subarachnoid spaces (11m) | Severe (2y 6m) | Hypotonia, no walking, no speech, non-epileptic myoclonus | Non-epileptic myoclonus |
| 25 | F/13y | c.587C>T, p.Ala196Val | 2 days | Tonic sz; clonic sz (infrequent) | Tonic-clonic sz; tonic sz (sporadic at 4m, 30m, 4y, 12y) | Yes (4m). Relapsed | None | Burst-suppression pattern, discontinuous background, multifocal abnormalities | Unremarkable (2y) | Normal (13y) | Normal, good head control, independent walking, normal speech | No |

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| 26 | F/12m | c.901G>A, p.Gly301Ser | 2 days | Focal tonic asymmetric sz with head deviation, apnea, cyanosis (multiple) | None | Yes (24d) | CBZ | Burst-suppression, multifocal abnormalities, discontinuous background | Unremarkable (1m) | Mild (12m) | Mild axial hypotonia, good head control, good eye contact, no walking | No |
| 27 | F/7y | c.629G>A, p.Arg210His | 1 day | Tonic sz; focal sz (multiple) | Focal sz | Yes (4y) | CBZ | Burst-suppression | CC hypoplasia (1m) | Mild (5y) | Normal | No |
| 28 | M/6y 8m | c.629G>A, p.Arg210His | 2 days | Focal sz (multiple or clusters) | Focal sz | No | CBZ | Burst-suppression | Delayed myelination (1y 4m) | Moderate (3y) | Independent walking (4y) with clumsiness, macrocephaly, ASD | ASD, macrocephaly, syringomyelia |
| 29 | F/3y 9m | c.798T>A, p.Asp266Glu | 18 days | Focal sz with head deviation (multiple or clusters) | Spasms; focal sz | Yes (2m) | VPA | Burst-suppression | Hypomyelination, CC hypoplasia (9m) | Severe (3y 9m) | Spastic-dystonic tetraplegia, axial hypotonia, distal hypertonia, movement disorder (peripheral and oro-lingual dyskinesia), no head control, no walking, no speech | Convergent strabismus (R>L) |
| 30 | M/4y 4m | c.802C>T, p.Leu268Phe | 1 day | Focal sz (multiple) | Focal sz; myoclonic absences | No | VPA, ETS | Burst-suppression | Unremarkable (1m) | Moderate (4y 4m) | Good eye contact, independent walking (>17 m), poor speech (vocalizations, single words) | Motor stereotypies, repetitive behaviour |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 31 | F/3y | c.590T>C, p.Leu197Pro | 1 day | Tonic sz (multiple) | Tonic sz; clonic sz | Yes (5m) | CBZ | NA | Subarachnoid bleeding (3d) | Mild (3y) | Good head control, no walking, language delay (small sentences) | No |
| 32 | F/9y | c.910_912TTC, p.Phe305del | 1 day | Tonic sz; clonic sz (multiple) | Generalized tonic-clonic sz | No | VPA | NA | Wide ventricles, CC hypoplasia, wide supratentorial sulci (2y) | Severe (9y) | Hypotonia, spasticity, cerebral paresis, nystagmus, no speech, no walking | Cortical blindness |
| 33 | F/5y | c.881C>T, p.Ala294Val | 1 day | Tonic sz with head deviation, apnea, bradycardia (multiple) | Tonic-clonic sz (2 nocturnal episodes at 4y 3m) | Yes (3m-4y3m). Relapsed | CBZ | Burst suppression | Delayed bifrontal myelination, CC hypoplasia (7m) | Profound (4y) | Axial hypotonia, dystonic quadriparesis, episodic dystonic hyperextension, delayed head control, no eye contact, no speech, no walking | Irritability, dysphagia (PEG), laryngomalacia, OSAS |
| 34 | F/3y 11m | c.1639C>T, p.Arg547Trp | 3 days | Tonic sz with apnea, desaturation, hyporeactivity, clonic jerks (multiple) | None | Yes (9d) | None | Unremarkable | Unremarkable (3m) | Normal (3y 11m) | Normal | No |

Pt: patient(s); M: male; F: female; y: year(s); m: month(s); d: day(s); sz: seizure(s); EEG: electroencephalogram; MRI: magnetic resonance imaging; CBZ: carbamazepine; LEV: levetiracetam; VPA: valproate; TPM: topiramate; ETS: ethosuximide; OXC: oxcarbazepine; VGB: vigabatrin; PB: phenobarbital; CLN: clonazepam; RUF: rufinamide; CBD: cannabidiol; LTG: lamotrigine; B6: pyridoxine; GERD: gastro-esophageal reflux disease; ASD: autism spectrum disorder; ID: intellectual disability; OSAS: obstructive sleep apnea syndrome; PEG: *percutaneous endoscopic gastrostomy;* NA: not available; F: frontal; T: temporal; O: occipital; C: central; P: parietal; L: left; R: right; CC: corpus callosum; WM: white matter

**Table e-2**. List of mutations obtained in the two partitions.

| Transmembrane (TM) MMs | Other MMs | VSD MMs | Pore MMs |
|---|---|---|---|
| c.587C>T, p.Ala196Val | c.365C>T, p.Ser122Leu | c.560C>T, p.Ser187Phe | c.740C>T, p.Ser247Leu |
| c.590T>C, p.Leu197Pro | c.560C>T, p.Ser187Phe | c.569A>T, p.Gly189Ile | c.798T>A, p.Asp266Glu |
| c.593G>A, p.Arg198Gln | c.569A>T, p.Gly189Ile | c.587C>T, p.Ala196Val | c.802C>T, p.Leu268Phe |
| c.629G>A, p.Arg210His | c.873G>T, p.Arg291Ser | c.590T>C, p.Leu197Pro | c.812G>A, p.Gly271Asp |
| c.637C>T, p.Arg213Trp | c.973A>G, p.Arg325Gly | c.593G>A, p.Arg198Gln | c.826A>C, p.Thr276Pro |
| c.740C>T, p.Ser247Leu | c.1639C>T, p.Arg547Trp | c.629G>A, p.Arg210His | c.830C>T, p.Thr277Ile |
| c.798T>A, p.Asp266Glu | c.1642G>C, p.Asp548His | c.637C>T, p.Arg213Trp | c.845A>T, p.Asp282Val |
| c.802C>T, p.Leu268Phe | c.1655A>C, p.Lys552Thr | | c.873G>T, p.Arg291Ser |
| c.812G>A, p.Gly271Asp | c.1657C>T, p.Arg553Trp | | c.881C>T, p.Ala294Val |
| c.826A>C, p.Thr276Pro | c.1665C>G, p.Phe555Leu | | c.901G>A, p.Gly301Ser |
| c.830C>T, p.Thr277Ile | c.1687G>A, p.Asp563Asn | | |
| c.845A>T, p.Asp282Val | | | |
| c.881C>T, p.Ala294Val | | | |
| c.901G>A, p.Gly301Ser | | | |

Partition 1 includes MMs located in the transmembrane environment (TM MMs) and all the others in the extracellular and intracellular coils (Other MMs). Partition 2 incorporates the MMs belonging to the VSD, VSD MMs (S3-S4 linker and S4 helix, only) and the pore region (Pore MMs).

**Table e-3.** Genotype-phenotype correlations for the two partitions of the missense variants.

| | | COGNITIVE OUTCOME | | SEIZURE FREEDOM | |
|---|---|---|---|---|---|
| | | **Normal** | **Abnormal** | **≤ 1 year** | **> 1 year** |
| **MODEL 1** | **Transmembrane** | 1 | 19 | 10 | 4 |
| | **Others** | 2 | 9 | 5 | 1 |
| **MODEL 2** | **VSD** | 1 | 9 | 2 | 3 |
| | **Pore** | 0 | 13 | 9 | 1 |

Model 1: in the first column, we compared cognitive outcome (normal/abnormal) between missense mutations (MMs) located in the transmembrane domain and all the others located in the coils (*p=0.28*). In the second column, time to seizure offset (≤1 year/>1 year) analyzed between the same subgroups of MMs (*p=0.43*).

Model 2: in the first column cognitive outcome stratified for MMs located in the voltage sensor domain (VSD) and the pore region (pore) (*p=1.00*); time to seizure offset is reported in the second column (*p=0.08*).

**Structural Modeling.** We used comparative (homology) modeling techniques to produce a 3D structural model of the human Kv7.2 (hKv7.2) TM domain. First, following [e1], we generated a structure of hKv7.2 using the model of the open hKv7.1 channel described in [e2], which is based on a refined version [e3] of the X-ray structure of the rat $K_V1.2$ (PDB ID: 2A79, [e4]). Kv7.1 shares about 60% sequence identity with Kv7.2. The single Kv7.2 subunit, comprising residues 92 to 324, was built with the SWISS MODEL web server [e5]. Then, the full channel was assembled from four copies of the single subunit using the MatchMaker tool of the UCSF Chimera suite [e6]. Finally, the protein complex was refined at the atomic level by the fragment-guided Molecular Dynamics simulation program FG-MD [e7], which identifies analogous fragments from the PDB server and then uses spatial restraints to guide the conformational sampling. The obtained configuration is used for all the figures in this work. Additionally, the positions of the mutated residues were mapped in a second model built using as template the recent CryoEM structure of Xenopus Kv7.1 (PDB ID: 5VMS, [e8]), where the channel is in a so-called decoupled state, with an activated VSD and a closed pore. This structure was identified as the best template using HHPred ([e9]), and the model was obtained following the homology procedure described above. Despite conformational differences between the two models, the mutated residues map in the same locations.

**Figure e-1.** Structural modeling of the Kv7.2 VSD (helices S1-S4). The residues involved in mutations are highlighted with Cα spheres. The structure is colored by conservation grades according to the ConSurf software (highly conserved residues are shown in maroon, average ones in white, and highly variable ones in turquoise).

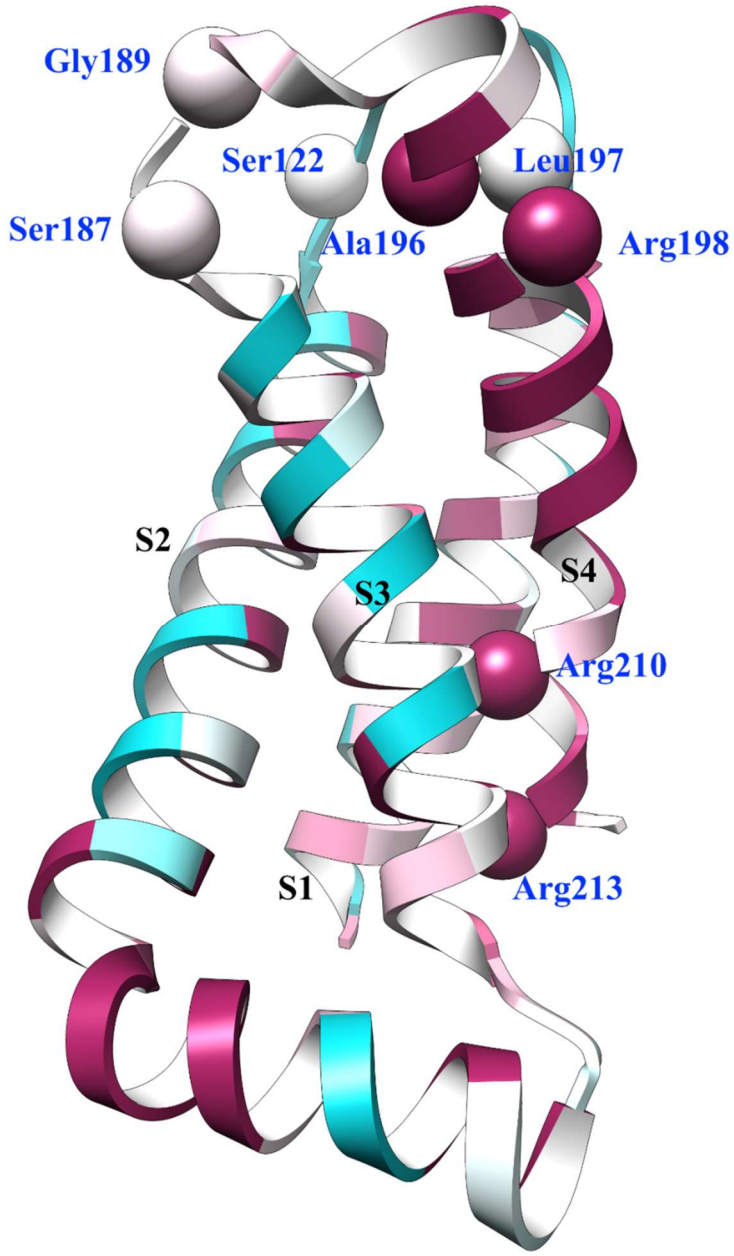
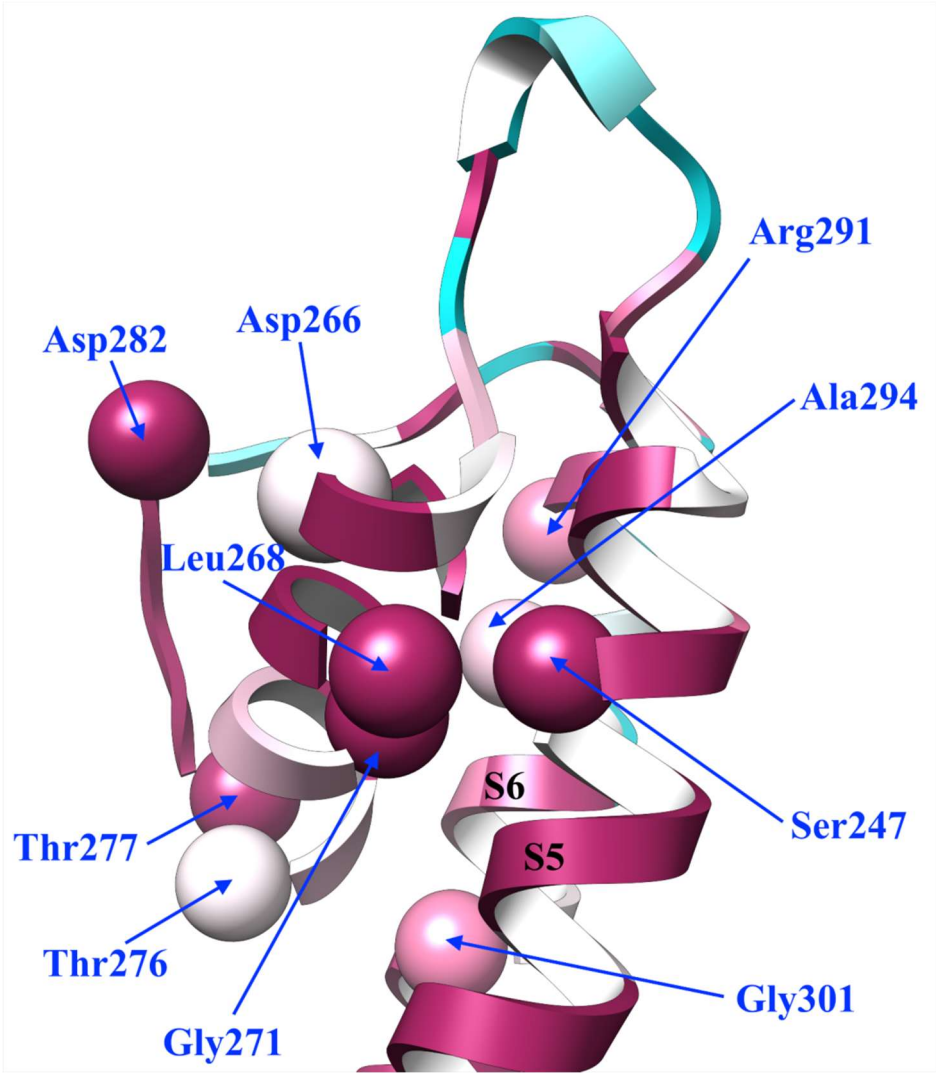


**Figure e-2.** Structural modeling of the Kv7.2 pore domain (helices S5-S6). The residues involved in mutations are highlighted with Cα spheres.



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