

Supplemental Note: Case Reports

Proband 1

KCNB2 variant: c.1141A>G, p.Thr381Ala, *de novo*

Method of Identification: Trio exome and sanger sequencing

Patient information: The patient exhibited global developmental delay, hypotonia and intellectual disability but no autistic traits. The disease phenotype overlapped partially with Zimmerman-Laband and DOORS syndromes, which are neurodevelopmental disorders with epilepsy and hypoplasia of the terminal phalanges and nails. She also exhibited dysmorphisms that included abnormal nails, mild blepharoptosis, beaked nose, flat midface, open mouth, drooling, full lower lip. She has no family history. She was born at term by C-section. Birth weight was 2.79 kg and length was 48 cm. The patient age at last visit was 5 years with a height of 107.5 cm and a weight of 19.7 kg. She exhibited epileptic seizures with effective treatment with Levetiracetam. Her MRI exhibited prominence of the anterior horn of lateral ventricle and 3rd ventricle and decreased bilateral hippocampus. She exhibited aortic dilation and neurogenic bladder. Other clinical features include diabetes, cataract, gingival fibromatosis, cortical vision impairment, Duane syndrome, hyperopia, astigmatism, low bone density, oropharyngeal dysphagia, cataract, and low bone density.

Proband 2

KCNB2 variant: c.281G>A, p.Gly94Glu, *de novo*

Method of Identification: Whole exome and Trio whole genome sequencing

Patient information: The patient, at two years of age, exhibited developmental delay but was making slow progress. He rolled at 6 months, sat at 2 years (propped), and could sit independently (but not stand unsupported) at 2.5 years. He exhibited language delay but made sounds and coos at 2.5 years. At this age, the patient is too young to assess for intellectual disability and autistic traits and no seizures were noted. Neurological assessment revealed Hypotonic/ataxic cerebral palsy per neurology. He also exhibited dysmorphisms that included synophrys and full lashes, single transverse palmar crease on one side, high palate and clinodactyly. The patient had no immediate family history, but there are some still births in extended family (maternal side) and a maternal cousin with possible autism spectrum disorder. The patient was born at 38 5/7 weeks to a G1 P0->1 mother with appropriate prenatal care and no exposures reported. Fetal ultrasound was normal except for concern for small cerebellum. Birth weight was 3.152 kg, length was 50.8 cm, head circumference was 32.3 cm and APGAR score of 7/8. Age at last visit was 2.5 years with weight of 14.8 kg (77th percentile based on CDC boys 2-20 years), height of 98 cm (94th percentile), and head circumference of 48.7 cm (33rd percentile). Other anomalies include abnormal trabeculation of left ventricular myocardium and slight

shawl scrotum. Other clinical features include delayed visual maturation and bilateral sensorineural hearing loss.

Proband 3

KCNB2 variant: c.1937C>T, p.Ala646Val, Inherited

Method of Identification: Trio whole exome and whole genome sequencing

Patient information: The patient exhibited delayed motor milestones, speech and language delay, apraxia, weakness, easily fatigue, irritability, regression, intellectual disability, and other neurodevelopmental disorders but no autistic traits. No seizures were noted. She also exhibited macrocephaly, deep set eyes, broad forehead, horizontal palpebral fissures, thin upper lips, tapered fingers and puffy feet. The patient inherited the mutation from her father in an autosomal dominant manner who was symptomatic with pneumothorax, pain, hypotonia, joint pain, weakness, fatigue and clumsiness. Her brother was also symptomatic who was positive for neurodevelopmental disorder, abnormal EEG, hypotonia, pain, and apraxia. The patient was born at term by C-section. Her birth growth parameters are unavailable. Her age at last visit was 3 years (in 2020) with a head circumference of 52 cm (98% percentile). Other clinical features include microscopic hematuria, episodic edema, and rashes.

Proband 4

KCNB2 variant: c.641C>T, p.Thr214Met, *de novo*

Method of Identification: Trio whole exome sequencing

Patient information: The patient was born at term with a weight of 4.24 kg, length of 53 cm and head circumference of 37 cm. He started walking at 17 months and exhibited language delay, intellectual disability, and mild autistic traits in infancy. No seizures were noted. He also exhibited synophrys, nail hypoplasia and myopia. The patient had no family history, and the age of last visit was 18 years. His growth parameters at this stage included a weight of 64 kg, height of 183 cm and head circumference of 59.5 cm.

Proband 5

KCNB2 variant: c.994T>G, p.Tyr332Asp, *de novo*

Method of Identification: Trio whole exome sequencing

Patient information: The patient exhibited severe global developmental delay and intellectual disability. According to BSID-III, she exhibited motor development p1.8, cognitive development index of 64% (cognitive age of 9 months at age 15.7 months) and language index of 72%. She couldn't walk independently yet with no speech and proximal hypertonia. Slow progress was observed with no regression. . Patient exhibited dysmorphisms in the hands and in the head. The latter included frontal bossing, suggestion of macrocephaly (but not on measurement) and broad forehead. She was born at term by C-section and had normal birth weight (3.335 kg). Her age at last visit

was 21 months with head circumference of 49.5 cm (+1SD). She has a family history of hypertension and obesity. Brain anomalies include delayed myelination, T2 abnormalities in basal ganglia (hyperintensity in globus pallida or hypo-intensity of other ganglia), small volume of thalamus, subtle increased volume of lateral and 3rd ventricles and peripheral CSF, small corpus callosum (especially the posterior part), subtle small volume of adenohypophysis, small cerebellar hemisphere on the left side and cyst in the left temporal side. Other neurological features include tongue protrusion, severe strabismus and retinal 'bear tracks'.

Proband 6

KCNB2 variant: c.911G>A, p.Arg304Gln, *de novo*

Method of Identification: Trio whole exome sequencing

Patient information: The patient exhibited delayed motor milestones, speech and language delay with intellectual disability and autistic traits. Other neurological features include ADHD, mood disorder and aggressive behavior. Facial dysmorphisms include double hair whorl, malar flattening, long philtrum, up-slanting palpebral fissures, epicanthal folds and broad nasal tip. He also had tapered fingers and short toes. He was born at term by vaginal delivery and had a birth weight of 3.28 kg. His age and height at last visit were 9 years and 135.9 cm, respectively. Family history included mental health issues in paternal uncle and paternal grandmother.

Proband 7

KCNB2 variant: c.827C>T, p.Pro276Leu, mosaic in proband (47T, 72C)

Method of Identification: Trio whole exome sequencing

Patient information: The patient exhibited normal motor development with language delay and moderate intellectual disability. He had behavioural issues that were treated with Risperidone, Fluoxetine, Guanfacine and methylphenidate. He suffers from drug-resistant focal and generalized seizures (Age of onset: 3 years and 9 months) that show multifocal abnormalities with frontal predominance on EEG. Previous anti-seizure medications (ASM) include Ethosuximide, VPA, perampanel, topiramate, clobazam, rufinamide, memantine, levetiracetam, IVIG; Current ASM include Lamotrigine, Oxcarbazepine, Sulthiame, Vagal Nerve Stimulator and Diazepam *prn*. Other neurological features include diadochokiensia with monopodal stand more insecure on the left. No brain abnormalities were detected except for non-specific prominent perivascular spaces. He was born at term, Apgar score of 2-6-9 with no perinatal complications. His age at last visit was 15 years with the head circumference of 51.7 cm, height of 150.6 cm and weight at 52 kg. Family history was unremarkable; the father was mosaic with sequencing reads for both T and C nucleotides (17T, 192C).

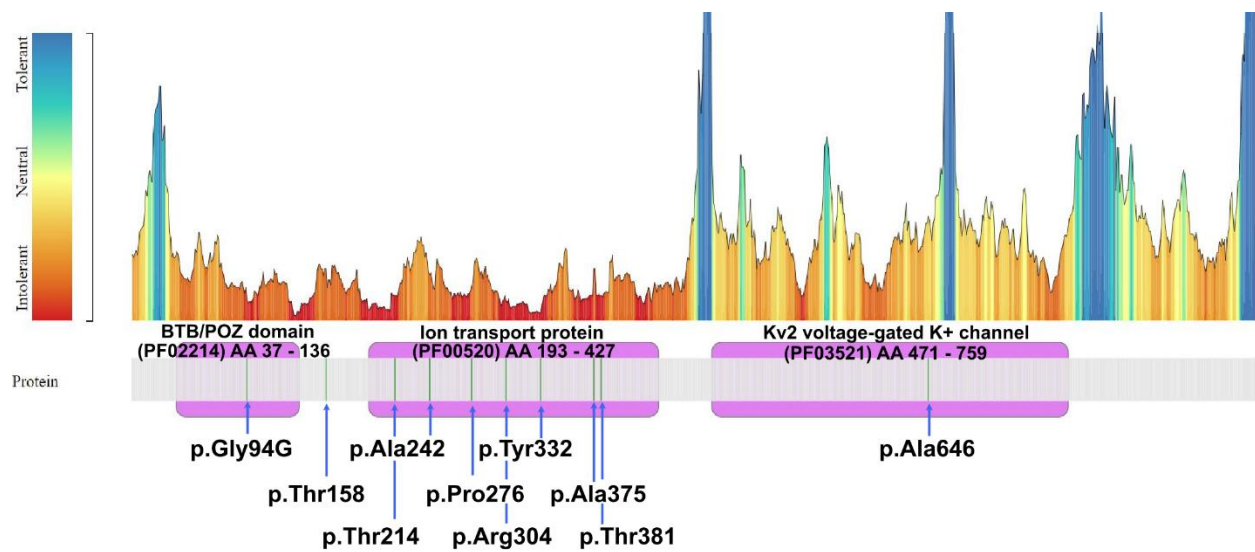


Figure S1. Metadome's missense tolerance landscape for KCNB2 (NM_004770.2). All amino acids are intolerant to missense variants (scores in Table S2).

Table S1. Primers used for mutagenesis.

| <i>KCNB2</i> (NM_004770.3) variants | Forward primer | Reverse primer |
|-------------------------------------|------------------------------------|-------------------------------------|
| c.281G>A, p.Gly94Glu | aaatggaagtgaaggctctggatgccgatcaaag | cttgatcgcatccagaagccttcactccattt |
| c.472A>G, p.Thr158Ala | cgctctcgcatagcctctgcctctcgc | gcgagaggcagaggctatgagagagcg |
| c.641C>T, p.Thr214Met | ccattgcttctctcaatatgctgccggagc | gctccggcagcatattgagagacaaagcaatgg |
| c.724G>A, p.Ala242Thr | tccatggtaaacctgaatacacacagcctccac | gtggaggctgtgtgtattacatggtttaccatgga |
| c.911G>A, p.Arg304Gln | gaggatgcgcatgattggaagatctggaccac | gtggtccagatcttccaaatcatgcgcatcctc |
| c994T>G, p.Tyr332Asp | ttcacccttagcgaggatgacaatgaattgggct | agcccaattcattgtcactccgcctaagggtgaaa |
| c.1141A>G, p.Thr381Ala | tcacatagccaacagcggatcatggtgatggtg | caccatcaccatgaccgctgttggtatggtga |
| c.1124C>T, p.Ala375Val | ggatcatggtgatggtgaccacaaaatgatgc | gcatcatttgggggtcaccatcaccatgacc |
| c.1937C>T, p.Ala646Val | ggggggcccctaactcttgggtgctcttct | agaagagcaccaagagtaggggcccccc |

Table S2. Summary of *in-silico* predictions for all *KCNB2* variants reported in our study

ACMG classification of variants (not taking into account functional data presented here), metadome amino acid missense tolerance scores and pathogenicity prediction and conservation scores obtained using Ensembl's VEP NB. Definition of acronyms and details on the scores with links and references for the tools can be found in the dbNSFP v4 "read me" file at <https://usf.app.box.com/s/6yi6huheisol3bhml8bitcon1oi53pm>

See excel file for data.

Table S3. Reversal potential of Kv2.2 variants

| Variants | Without WT | With WT | Without WT | With WT |
|----------|---|------------------------|---|------------------------|
| | ES: 110 NMDG ⁺ , 5 K ⁺ ; IS: 110 K ⁺ , 5 NMDG ⁺ | | ES: 110 Na ⁺ , 5 K ⁺ ; IS: 110 K ⁺ , 5 Na ⁺ | |
| | V _{rev} in mV (95% C.I.) | | | |
| WT | -60.8 (-59.2 to -62.6) | | -63.8 (-61.7 to -65.9) | |
| G94E | -65.3 (-60.8 to -70.1) | -64.3 (-61.1 to -67.6) | -67.6 (-62.8 to -72.8) | -68.1 (-62.1 to -74.8) |
| T158A | -64.8 (-59.1 to -71.2) | -62.9 (-58.3 to -68.0) | -67.4 (-61.5 to -74.1) | -70.0 (-61.3 to -80.5) |
| T214M | n.a. | -58.0 (-52.9 to -63.5) | n.a. | -68.3 (-63.6 to -73.4) |
| A242T | -68.0 (-63.6 to -72.8) | -72.9 (-66.7 to -79.9) | -70.1 (-65.7 to -75.0) | -70.7 (-62.0 to -81.1) |
| R304Q | -67.6 (-63.5 to -72.0) | -65.0 (-58.6 to -72.1) | -65.0 (-58.0 to -73.0) | -70.7 (-65.5 to -76.4) |
| Y332D | -60.4 (-56.6 to -64.4) | -56.6 (-53.4 to -60.0) | -64.2 (-58.2 to -70.9) | -64.0 (-57.9 to -70.7) |
| A375V | -71.2 (-64.9 to -78.3) | -62.0 (-57.6 to -66.7) | -81.6 (-75.5 to -88.6) | -75.2 (-68.9 to -82.4) |
| T381A | -60.0 (-54.0 to -66.7) | -70.4 (-65.3 to -75.9) | -60.7 (-55.9 to -66.0) | -68.8 (-63.3 to -75.0) |
| A646V | -66.5 (-60.9 to -72.6) | -66.7 (-62.9 to -70.8) | -61.8 (-57.8 to -66.0) | -69.9 (-64.0 to -76.5) |

n.a. - not analysed; the amplitudes were too low for determination of reversal potential.

C.I. - Confidence Intervals