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CLINICAL REPORT

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Recurrent *KCNT2* missense variants affecting p.Arg190 result in a recognizable phenotype

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Abstract

KCNT2 variants resulting in substitutions affecting the Arg190 residue have been shown to cause epileptic encephalopathy and a recognizable facial gestalt. We report two additional individuals with intellectual disability, dysmorphic features, hypertrichosis, macrocephaly and the same de novo *KCNT2* missense variants affecting the Arg190 residue as previously described. Notably, neither patient has epilepsy. Homology modeling of these missense variants revealed that they are likely to disrupt the stabilization of a closed channel conformation of KCNT2 resulting in a constitutively open state. This is the first report of pathogenic variants in *KCNT2* causing a developmental phenotype without epilepsy.

KEYWORDS
dysmorphism, epileptic encephalopathy, intellectual disability, KCNT2, potassium channel, sequencing

1 | INTRODUCTION

Potassium (K^+) channels are a large family of pore-forming membrane proteins. They represent a heterogeneous group of voltage and ligand-gated ion channels with wide-ranging effects on many physiological processes including cell excitability (Jeevaratnam et al., 2018), hormone secretion (Ashcroft & Rorsman, 2013), and apoptosis (Szabò et al., 2010). In neurons, K^+ channels are essential for maintaining the inward-negative resting membrane potential. Their opening, which occurs in response to a range of signals, leads to K^+

efflux from the cell, resulting in the membrane potential becoming more negative and hence repolarization. The ability of K^+ channels to repolarize and hyperpolarize nerve and muscle cells helps to control action potential frequency and duration (Humphries & Dart, 2015). K^+ channels can be categorized by the stimulus to which they are activated and include voltage-gated (K_V), calcium-activated (K_{Ca}), inwardly rectifying (K_{IR}), ATP-sensitive (K_{ATP}), and sodium-activated (K_{Na}) channels. A recent systematic review identified 19 potassium channelopathies implicated in a variety of neurodevelopmental disorders (Kessi et al., 2020).

Humans have two $K_{(Na)}$ channel subunits, Slack and Slick, encoded by *KCNT1* (OMIM 608167) and *KCNT2* (OMIM 610044), respectively, that rectify outwardly. KCNT1 and KCNT2 share ~74% sequence identity (Bhattacharjee et al., 2003), show similar single-channel conductance, modulate the hyperpolarization that occurs following repetitive firing and form hetero-tetrameric channels in several brain regions such as the oculomotor nucleus and the medial nucleus of the trapezoid body (Chen, Kronengold, et al., 2009). A key difference between KCNT1 and KCNT2 is that KCNT1 channels have an absolute requirement for Na^+ for channel opening, whereas KCNT2 channels maintain a basal level of activity in the absence of Na^+ (Bhattacharjee et al., 2003).

Pathogenic variants in *KCNT1* have been recently identified to cause autosomal dominant nocturnal frontal lobe epilepsy (OMIM #615005) and epilepsy of infancy with migrating focal seizures (Barcia et al., 2019), as well as, early infantile epileptic encephalopathy with severe dystonia (OMIM #614959) (Gertler et al., 2019; Martin et al., 2014). The majority of cases are caused by gain-of-function variants, however, a single missense variant resulting in loss-of-function (p.Phe932Ile) has been described in a patient with epilepsy and leukoencephalopathy (Evely et al., 2017).

KCNT2 has recently been described as a human disorder gene (OMIM #617771) with only eight patients in total reported so far. Mao et al. reported two patients with epilepsy of infancy with migrating focal seizures and de novo truncating variants in *KCNT2* (p.Lys564* and p.Leu48Glufs*43) (Mao et al., 2020). Gururaj et al. reported a patient with a de novo p.Phe240Leu missense variant and epileptic encephalopathy with no dysmorphic features (Gururaj et al., 2017). The p.Phe240 residue is situated in the channel pore helix and the authors concluded that this particular variant causes a "change-in-function" by altering a K^+ channel that is usually upregulated by Cl^- to become a Na^+ channel down-regulated by Cl^-. Inuzuka et al. (2020) reported another patient with a de novo p.Thr242Asn variant, which lies in the same transmembrane domain, with a non-dysmorphic epileptic encephalopathy phenotype (Inuzuka et al., 2020). Alagoz et al. (2020) also reported two patients with de novo missense variants (p.Asn182Ile and p.Leu880Met, located in the S4 helical and C-terminal cytoplasmic domains, respectively) in *KCNT2* and epileptic encephalopathy without dysmorphic features.

Ambrosino et al. described two individuals with de novo missense variants in *KCNT2*, both affecting the arginine residue at position 190 (p.Arg190His and p.Arg190Pro) (Ambrosino et al., 2018). The probands had epilepsy, intellectual disability, hypertrichosis, and coarse facial features. Electrophysiological studies revealed a gain-of-function and constitutive activation for both variants. The gain-of-function effect was more pronounced with the substitution of positively charged arginine with neutral proline than with histidine. This suggested that substitution with partially protonated histidine may allow for the maintenance of some, but not all, charge interactions necessary for channel function. Prior to reports of pathogenic variants in *KCNT2* in humans, the mechanism of closure of the KCNT2 channel had been investigated by Dai et al. (2010) who performed electrophysiological studies on cRNA-transduced *Xenopus* oocytes. p.Arg190 was identified as an important candidate residue for channel gating due to its location in a transmembrane linker region. Mutation of p.Arg190 to Glu, Gln, and Ala showed that charge reversal or neutralization led to constitutive activation of the channel (Dai et al., 2010). Of note, substitution of p.Arg190 for another positively charged amino acid, lysine, resulted in channels with wild-type properties (i.e., normal function).

Given so few cases are reported, the mutational spectrum, clinical features, and the genotype–phenotype relations in KCNT2-related disorders remain undefined. We report two new individuals with a recognizable neurodevelopmental disorder without epilepsy and recurrent de novo *KCNT2* variants affecting the Arg190 residue. Using homology modeling, we advance the inferences made by Ambrosino et al. on models of the wild-type KCNT2 by showing that substitution of p.Arg190 with uncharged amino acids results in a likely constitutively open conformation.

2 | METHODS

2.1 | Case ascertainment

The index case was identified through a local re-analysis project of genome data from the 100,000 Genomes project via a previously described pipeline (Faundes et al., 2018; Vaz et al., 2019). A second unreported case was identified through a search of the DECIPHER database (DECIPHER 408952). Clinical features were compiled and compared with previously reported cases. Informed consent was obtained from the families of both individuals.

2.2 | Homology modeling

Human KCNT2 was modeled on chicken KCNT1 in open (PDB 5u70) and closed (PDB 5u76) conformations. Clustal Omega v1.2.3 (Sievers et al., 2011) was used to align the sequence of the chicken KCNT1 template PDB files (PDB 5u70 and PDB 5u76) to the human KCNT2 FASTA sequence. Homology models were subsequently generated using Modeler 9.24 (Eswar et al., 2006). Twenty models were built in each case and the model with the lowest Discrete Optimized Protein Energy score was chosen for visualization and analysis in KiNG 2.23 (Chen, Davis, & Richardson, 2009).

Mutant channels seen in patients (p.Arg190His and p.Arg190Pro), or created by Dai et al (p.Arg190Ala, p.Arg190Glu, p.Arg190Gln, and p.Arg190Lys) or present in the gnomAD database (Karczewski et al., 2020) (p.Arg190Cys) were created by amending the FASTA sequence for KCNT2 in the alignment file and running through Modeller9.24 separately.

3 | RESULTS

3.1 | Case reports

3.1.1 | Individual 1

The proband is the second child born to non-consanguineous Caucasian parents. Pregnancy was uncomplicated and she was born at term by normal vaginal delivery weighing 3.3 kg (+0.15 SD). Concerns regarding hypotonia and delay in her general development were raised during early infancy. She sat unsupported at 1 year of age and was walking unsteadily at the age of 2 years. She started using single words at the age of 2 years and three-word phrases at the age of 4 years 2 months. She attended school with a statement of educational needs due to severe learning difficulties. Currently, at the age of 32 years, she lives in residential care. She has never suffered from seizures.

She developed pubic and axillary hair at the age of 8 years before menarche aged 14 years. Her periods were irregular and a pelvic ultrasound scan which revealed polycystic ovaries. She had impaired fasting glucose at the age of 18 years and was treated with metformin until 21 years, when her fasting glucose had normalized. At 14 years, her height was 170 cm (+1.46 SD), her weight was 50.6 kg (+0.13 SD), and occipitofrontal circumference (OFC) was 58 cm (+3.35 SD). At her last clinic review, aged 27 years, her OFC was 60.5 cm (+5.12 SD). She has mild synophrys, epicanthic folds, large palpebral fissures, thick hair, long eyelashes, and diastema (Figure 1a). Her facial features have coarsened with time.

A CT brain aged 2 years was reported as normal. Her urine mucopolysaccharides, amino acids and organic acids profile, and plasma very long chain fatty acids were all within normal limits. Her thyroid function was also normal. Fragile X testing, karyotyping, and chromosomal microarray showed normal results.

The proband and her parents were recruited to the 100,000 Genomes Project (Caulfield et al., 2019) and analysis using Intellectual disability (v1.066), Mucopolysaccharidosis (v1.2), and Undiagnosed metabolic disorder (v1.397) panels did not reveal any pathogenic variants. Panel-agnostic re-analysis of the trio genome data (Faundes et al., 2018) (Vaz et al., 2019) revealed a de novo *KCNT2* NM_198503.3:c.569G > C (p.Arg190Pro) missense variant. The variant was classified as pathogenic according to the American College of Medical Genetics (ACMG) guidelines (PS2, PM2, PM5, PP3, PP2) (Richards et al., 2015). No other plausible candidates were identified during this re-analysis.

3.1.2 | Individual 2

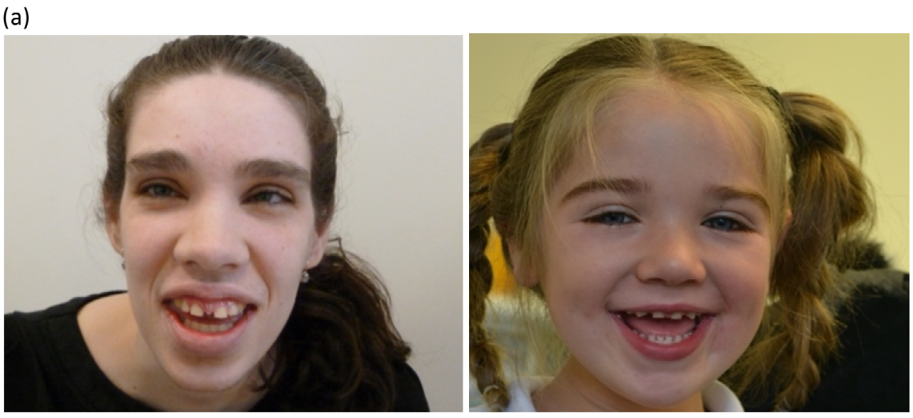
The proband was born to non-consanguineous Caucasian parents. She has an older maternal half-sister and a younger brother. Pregnancy had been unremarkable except for an iron transfusion at 32 weeks for anemia. She was born at 41 weeks, weighing 4.88 kg (+3.15 SD) with birth OFC 38 cm (+3.48 SD).

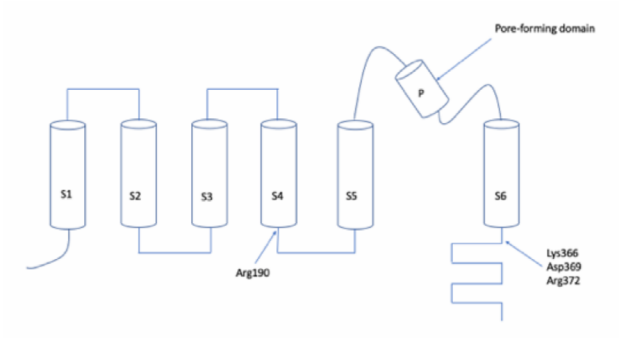
She first sat unsupported aged 6 months but delay in motor development was noted at 17 months as she was falling frequently and appeared to have poor leg coordination. She was walking independently by age 22 months. Her speech and language development was significantly delayed. Currently, aged 5 years 7 months, she speaks a few single words but also uses non-verbal gestures. She has moderate intellectual disability and with autistic traits. Her behavior has been described as hyperactive with a tendency for aggressive outbursts and she has difficulties with sleep requiring melatonin. She has never had seizures. She has slightly reduced tone in her legs but otherwise normal neurological examination. She suffers with constipation which has required regular Movicol and is also prone to wheezing episodes, for which she takes preventative inhalers.

At 4 years 8 months, her height was 108 cm (+0.57 SD) and OFC 54.4 cm (+3.00 SD). She has prominent eyebrows and long lashes, big ears, small square teeth, widely spaced teeth and hypertrichosis, mainly affecting her arms and back (Figure 1b). Her brain MRI scan at 18 months of age was reported to be normal. Gene-agnostic trio exome sequencing in the clinical setting revealed a de novo NM_198503.3:c.569G > A (p.Arg190His) missense variant. This variant was also classified as Pathogenic according to the American College of Medical Genetics (ACMG) guidelines (PS2, PM2, PM5, PP3, PP2) (Richards et al., 2015). No other likely causative rare de novo or biallelic variants were identified through the exome sequencing analysis.

3.2 | Homology modeling

K_{Na} channels resemble K_v channels in topography with six hydrophobic, transmembrane segments (S1-S6) along with a pore-lining loop found between S5 and S6 (Kaczmarek, 2013). Subunits assemble as tetramers to form a functional channel. The KCNT2 p.Arg190 residue is located within the S4 and S5 linker region and is evolutionary conserved among species (Ambrosino et al., 2018). p.Arg190 creates a constriction between the cytoplasmic domains immediately before (S5) and after (S6) the pore-forming domain. This constriction likely stabilizes the channel in closed state (Figure 1b). To understand the mechanistic basis of the condition in the cases presented here, we performed homology modeling. Modeling of the human wild-type KCNT2 revealed a network of charge interactions that we postulate to stabilize the channel in the closed state. These interactions occur between p.Arg190 and three other charged residues: p.Lys366, p.Asp369, and p.Arg372 (Figure 1c). These charge interactions are predicted to be lost in the open conformation (Figure 1c).





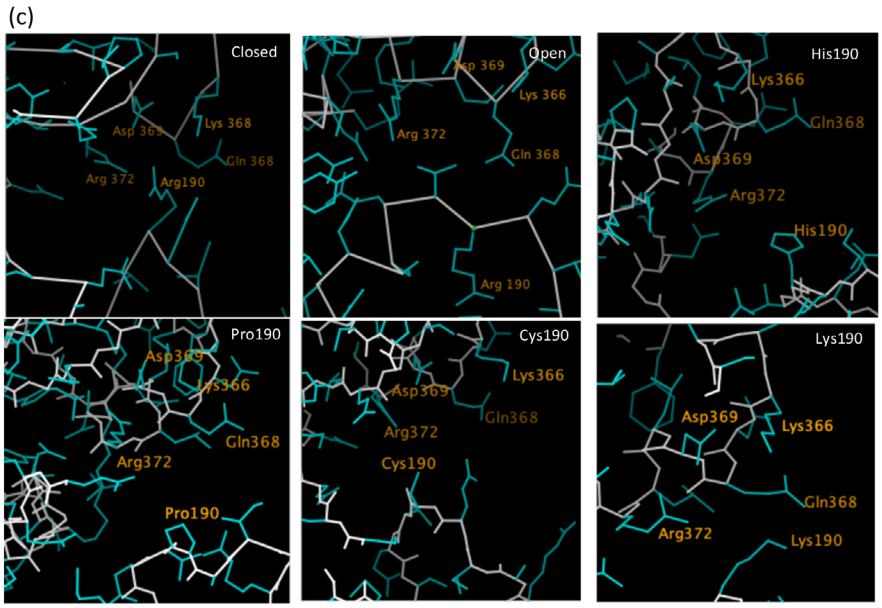


FIGURE 1 (a) Facial gestalt of cases with de novo KCNT2 variants. Individual #1 (left) and individual 2 (right). Note prominent eyebrows, thick hair, and diastema. (b) Schematic of KCNT2 (domains taken from Uniprot Q6UVM3). (c) Model of KCNT2 in open and closed state. Mutant models on the closed state show a reversion to an open state conformation

Modeling showed that the patient variants (p.Arg190His and p.Arg190Pro) abrogate the charge interactions and the channel is predicted to remain in a constitutively open conformation (Figure 1c). The distances between residue 190 and the other charged residues are increased in all mutated forms, including the p.Arg190Cys variant, which is present in 1/125,050 heterozygotes in gnomAD and comparable to the wild-type open conformation, with the exception of the p.Arg190Lys variant (Supplementary Table 1). The only mutant to retain wild-type electrophysiological properties, as reported by Dai et al., is the p.Arg190Lys substitution. Modeling this variant shows maintenance of the network of charge interactions with p.Lys366, p.Asp36,9 and p.Arg372 (Figure 1c) and the distances between these residues appear to be closer to the wild-type closed conformation.

4 | DISCUSSION

We describe two individuals with de novo missense *KCNT2* variants affecting the same residue at p.Arg190, which has been implicated in epileptic encephalopathy previously. Individual #1 presented with

hypotonia, developmental delay, severe intellectual disability, and macrocephaly. Individual #2 presented with developmental delay, moderate intellectual disability, and autistic traits. Both individuals share hirsutism, prominent eyebrows, long eyelashes, and diastema, all of which were also seen in the two previously reported p.Arg190 cases (Ambrosino et al., 2018). Their previous occurrence in affected individuals and absence of these variants from population databases lends weight to their pathogenicity.

Comparison of the clinical features of the two individuals described here with the eight previously described cases showed that all individuals were affected by intellectual disability. Epilepsy was seen in all mutation types although our two p.Arg190 cases did not have seizures, those reported by Ambrosino et al. did. Notably, intellectual disability, neonatal hypotonia, hirsutism, thick hair, prominent eyebrows, long eyelashes, and diastema were present in all cases with variants affecting p.Arg190 (Table 1). Both our cases had macrocephaly, which was not reported previously. Interestingly, the individuals with truncating variants reported by Mao et al and the individuals with other missense variants reported by Gururaj et al., Inuzuka et al., and Alagoz et al. all shared epilepsy and intellectual disability but no dysmorphic features nor macrocephaly (Table 1). KCNT2-associated neurodevelopmental disorders appear to show a genotype–phenotype correlation with missense variants affecting p.Arg190 causing a syndromic disorder with a recognizable facial gestalt. In these two cases, however, epilepsy was absent and we describe a new association with macrocephaly.

The occurrence of macrocephaly in these two individuals is noteworthy as constitutive activity of other ion channels, such as KCNB1, leading to cytoplasmic K+ loss have been linked with excessive neuronal apoptosis (Kondratskyi et al., 2015), although a decrease in cytoplasmic K+ is not obligatory for apoptosis (Börjesson et al., 2011). Interestingly, *KCNT2* expression, unlike that of *KCNT1*, has been found to be predominantly under control of NF-κβ, which is released during stressful stimuli such as hypoxia and injury (Tomasello et al., 2015). As a putative neuroprotective channel, constitutive activation of KCNT2 may not have the same effect on neuronal apoptosis as other previously studied ion channels.

The basis of the phenotypic differences between KCNT2 p.Arg190 variants and other mutations is likely due to the effect of the variants on the channel function. Patch-clamp experiments on the missense variant (p.Pro240Leu) described by Gururaj et al. showed a change in function, which was suggested to be the pathogenic mechanism. The missense variants reported by Inuzuka et al. (p.Thr242Asn) and Alagoz et al. (p.Asn182Ile and p.Leu880Met) were not investigated for their effect on channel function. In the case of the loss-of-function variants (p.Lys564* and p.Leu48Glufs*43) reported by Mao et al., whole-cell patch-clamp experiments showed a decrease in global current density in heteromeric mutant channels. Given that *KCNT2* appears to be tolerant of loss-of-function variants (pLi 0.04, pLEOUF 0.37), haploinsufficiency would appear unlikely to be the mechanism. mRNA or protein studies were not performed to prove these variants were truly loss-of-function and indeed, cells co-transfected with wild-type KCNT1, wild-type KCNT2 and also KCNT2$^{p.Leu48Glufs*43}$ showed currents similar to cells expressing KCNT1 alone, which may indicate a dominant negative effect for this variant.

Using homology modeling, we have shown that p.Arg190 participates in key interactions with neighboring charged residues in order to stabilize the closed channel state. Substitution of the charged arginine residue with uncharged amino acids results in a constitutive open state and recapitulates the gain-of-function effects seen in previous electrophysiological studies.

KCNT2 is highly expressed in the hippocampus and amygdala, where *KCNT1* is relatively less highly expressed (Human Protein Atlas available from http://www.proteinatlas.org) (Pontén et al., 2008; Uhlen et al., 2017). This difference in expression may suggest then KCNT2 functions independently from KCNT1 in these regions (Bhattacharjee et al., 2005). KCNT2 also has a consensus ATP binding site (amino acids 1032–1038), which, when occupied by ATP, inhibits activity of the channel. A unique attribute of KCNT2 is the requirement for ATP to dissociate from a site near the C-terminus, in the presence of elevated intracellular Na^+, to allow channel activation. During times of metabolic stress, such as hypoxia or even epileptiform activity, which both cause a reduction in ATP and elevation in intracellular Na^+, it is postulated that KCNT2 channels play a neuroprotective role by limiting excitability and maintaining a hyperpolarized membrane potential (Bhattacharjee et al., 2003). How a gain-of-function variant could bring about the phenotype observed in our cases is not currently understood. The occurrence of both loss-of-function and gain-of-function variants in the spectrum of KCNT2-associated developmental disorders indicates a delicate balance in maintaining membrane potential, which is disturbed in these conditions.

The occurrence of hypertrichosis in several K^+ channelopathies is also notable. The opening of intracellular K^+ channels has been suggested as a mechanism regulating hair growth. Several antihypertensive compounds, most notably minoxidil and diazoxide, have the known side effect of excessive hair growth (Suchonwanit et al., 2019; Uno et al., 1990). These have been found to induce hypertrichosis by enhancing the flux of potassium ions (Buhl et al., 1992). It is interesting that in the cases described here, and by Ambrosino et al., with gain-of-function variants, hypertrichosis is a common finding, whereas this was not reported by Gururaj et al. nor Mao et al. for those individuals with change-of-function or loss-of-function variants. In mice, KCNT2 channels are expressed in the dorsal root ganglia, specifically in Calcitonin gene-related peptide (CGRP)-containing neurons (Tomasello et al., 2017), which is interesting, given that CGRP, along with other neuropeptides such as substance P, is known to regulate hair growth (Samuelov et al., 2012). In a similar manner, gain-of-function variants in *ABCC9*, which contributes a subunit to SUR2, a K_{ATP} channel, cause Cantu syndrome (OMIM #239850). Cantu syndrome is another developmental disorder sharing phenotypic overlap, particularly hypertrichosis, with the KCNT2 p.Arg190Arg/His phenotype. SUR2 is also a known pharmacological target of minoxidil (Ohko et al., 2020).

Notably, the American College of Medical Genetics consensus guidelines (Richards et al., 2015) on variant interpretation place the

TABLE 1 Clinical characteristics of all reported cases of KCNT2-associated developmental disorders

Reference	(Inuzuka et al., 2020)	(Alagoz et al., 2020)		(Mao et al., 2020)		(Gururaj et al., 2017)	(Ambrosino et al., 2018)		This study	
Patient	**Patient 1**	**Patient 1**	**Patient 1**	**Patient 1**	**Patient 2**	**Patient 1**	**Patient 1**	**Patient 2**	**Patient 1**	**Patient 2**
Age	17 years	6 years	5 years	3 months	29 years	4 years	9 years	14 years	32 years	5 years
Sex	Male	Male	Male	Female	Female	Male	Female	Female	Female	Female
cDNA change (NM_198503.2)	p.Thr242Asn	c.545A > T	c.2838C > A	c.1690A > T	c.143_144delTA	c.720 T > A	c.569G > A	c.569G > C	c.569G > C	c.569G > A
Amino acid change	NS	p.Asn182Ile	p.Leu880Met	p.Lys564*	p.Leu48Glufs*43	p.Phe240Leu	p.Arg190His	p.Arg190Pro	p.Arg190Pro	p.Arg190His
Predicted functional consequence	–	NS	NS	LoF	LoF	"Change-of-function"	GoF	GoF	GoF	GoF
Neonatal hypotonia	Severe	+	+	+	–	–	+	+	+	+
Intellectual disability	+	+ (severity not stated)	+ (severity not stated)	Severe	Mild	Profound	Severe	Severe	Severe	Moderate
Epilepsy	5 months	+	+	+	+	+	+	+	–	–
Onset of seizures	Tonic motor seizures, hyperkinetic focal motor seizures	NS	NS	2 months	4 months	3 months	8 months	Birth	n/a	n/a
Seizure type	Levetiracetam and oxcarbazepine	NS	NS	Generalized tonic–clonic.	Focal and migrating.	Prolonged tonic as well as myoclonic jerks and atypical absences.	Generalized tonic–clonic. West syndrome evolving into Lennox-Gestaut syndrome.	Epilepsy of Infancy with Migrating Focal Seizures (EIMFS)	n/a	n/a
Epilepsy treatment	Normal	NS but "intractable"	NS but 'intractable'	Refractory to valproate, lamotrigine and levetiracetam.	NS	Refractory to topiramate, nitrazepam, levetiracetam, lamotrigine, vigabatrin, ethosuximide, pyridoxal phosphate and ketogenic diet.	Refractory to sultiame, valproate, vigabatrin, topiramate, levetiracetam, clobazam, gabapentin, lamotrigine, pyridoxal phosphate, rufinamide and pulsed methylprednisolone. Ketogenic diet.	Responded to phenobarbitone which had been discontinued with no further seizures at time of report.	n/a	n/a
MRI Brain	–	NS	Diffusely thin corpus callosum, dilated lateral ventricles and partial colpocephaly.	Normal	NS	Generalized reduction in white matter and thinning of corpus callosum.	Supratentorial mild volume loss and slightly delayed myelination.	Normal	Not performed – CT brain normal.	Normal

TABLE 1 (Continued)

Reference	(Inuzuka et al., 2020)	(Alagoz et al., 2020)		(Mao et al., 2020)		(Gururaj et al., 2017)	(Ambrosino et al., 2018)		This study	
Patient	Patient 1	Patient 1	Patient 1	Patient 1	Patient 2	Patient 1	Patient 1	Patient 2	Patient 1	Patient 2
Hypertrichosis	–	–	–	–	–	–	+	+	+	+
Prominent eyebrows	–	–	–	–	–	–	+	+	+	+
Long eyelashes	–	–	–	–	–	–	+	+	+	+
Diastema	–	–	–	–	–	–	+	+	+	+
Broad nasal tip	–	–	–	–	–	–	+	+	+	+
Other facial features	–	–	–	–	–	–	Short smooth philtrum with prominent upper lip.	Short smooth philtrum with prominent upper lip.	Coarse facial features, epicanthic folds, thickened lips and oral mucosa.	Large ears, small square teeth.
OFC (SDS)		'normal'	NS	NS	NS	NS	−0.22	+0.98	+5.12	+3.00

Abbreviations: GoF, gain-of-function; LoF, loss-of-function; NS, not stated; OFC, occipitofrontal circumference.

identification of a variant affecting the same amino acid residue as a known pathogenic variant in the "moderate" category as evidence for pathogenicity (PM5). It could be surmised that consideration should be given to the precise amino acid substitution and the effect this may have on macromolecular structure as we would strongly suspect that other uncharged substitutions affecting p.Arg190 in KCNT2 would be pathogenic. Our homology modeling of p.Arg190Cys seen in gnomAD would predict this to result in the same gain-of-function seen with the other charge-neutralizing substitutions, however, the distance between the charged residues is less affected than the other variants. In this case, most distances are shorter than those seen in the p.Arg190Ala substitution, which has the shortest distances for all experimentally proven gain-of-function variants (Supplementary Table 1). Of note, this variant does not occur in the gnomAD control group but rather the "non-cancer" group and hence some variants at this position may be either benign or have reduced penetrance.

In conclusion, we report two new cases of variants affecting the p.Arg190 residue in *KCNT2* causing a recognizable neurodevelopmental disorder. In our cases, epilepsy was not a feature and we also expand this phenotype to include macrocephaly. Differential diagnosis included metabolic storage disorders. We show that p.Arg190 is a critical charged residue and reversal or neutralization of this charge is predicted to result in constitutive channel activation.

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CONFLICT OF INTERESTS

All authors have no conflicts of interest to declare for this work.

AUTHOR CONTRIBUTION

Adam Jackson wrote the manuscript and performed re-analysis of genomic data for Individual #1. Homology modeling was completed by Adam Jackson under the guidance of Simon Lovell. Jill Clayton-Smith and Helen Stewart provided clinical details for both individuals. HR provided genetic insights from a clinical diagnostic perspective and analyzed the exome data for Individual #2. Genomics England Research Consortium performed genome sequencing in Individual #1.

Siddharth Banka, Jill Clayton-Smith and Simon Lovell supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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