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ORIGINAL ARTICLE

De novo KCNH1 mutations in four patients with syndromic developmental delay, hypotonia and seizures

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The voltage-gated Kv10.1 potassium channel, also known as *ether-a-go-go*-related gene 1, encoded by *KCNH1* (potassium voltage-gated channel, subfamily H (eag related), member 1) is predominantly expressed in the central nervous system. Recently, *de novo* missense *KCNH1* mutations have been identified in six patients with Zimmermann–Laband syndrome and in four patients with Temple–Baraitser syndrome. These syndromes were historically considered distinct. Here we report three *de novo* missense *KCNH1* mutations in four patients with syndromic developmental delay and epilepsy. Two novel *KCNH1* mutations (p.R357Q and p.R357P), found in three patients, were located at the evolutionally highly conserved arginine in the channel voltage-sensor domain (S4). Another mutation (p.G496E) was found in the channel pore domain (S6) helix, which acts as a hinge in activation gating and mainly conducts non-inactivating outward potassium current. A previously reported p.G496R mutation was shown to produce no voltage-dependent outward current in CHO cells, suggesting that p.G496E may also disrupt the proper function of the Kv channel pore. Our report confirms that *KCNH1* mutations are associated with syndromic neurodevelopmental disorder, and also support the functional importance of the S4 domain.
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INTRODUCTION

In humans, at least 12 voltage-gated potassium channel (Kv) subfamilies (Kv1 to Kv12) contribute to neuronal signaling in the nervous system.[1,2] KCNH channels are important regulators of cellular excitability and have been associated with cancer,[3] cardiac long QT syndrome type 2, epilepsy and schizophrenia.[4] Heterozygous mutations in Kv are involved in epileptic disorders: *KCNA1* (Kv1.1), *KCNA2* (Kv1.2), *KCNC1* (Kv3.1), *KCNQ2* (Kv7.2), *KCNQ3* (Kv7.3) and *KCNH5* (Kv10.2).[2,5–12] The Kv10.1 channel encoded by *KCNH1* belongs to the *ether-a-go-go* family within the Kv family.[1] *KCNH1* is expressed in diverse regions of the central nervous system[13] including the hippocampus,[14] and consists of tetrameric α-subunits, with each subunit containing six membrane-spanning α-helices (S1–S6).[15,16] Of these transmembrane α-helices, S1 – S4 segments act as voltage-sensor domains, and S5 and S6 form a pore-lining loop.[15,16] Although severe neuronal developmental impairment was observed in the zebrafish knockdown of *kcnh1*,[17] the role of *KCNH1* in human diseases is under investigation.

Previously, heterozygous *KCNH1* (Kv10.1) mutations have been reported in six patients with Zimmermann–Laband syndrome (ZLS), and in four patients with Temple–Baraitser syndrome (TBS).[10,15] These patients showed developmental delay, various types of infantile-onset seizures, some had dysmorphic faces, abnormal muscle tone, aplastic/hypoplastic thumb or toe nails and thick and broad toes,[10,15] suggesting that *KCNH1* mutations may cause a phenotypic continuum of neurodevelopmental disorders with some distinctive dysmorphic features. Functional analysis showed that all the *de novo* mutations, except for p.Gly496Arg causing outward rectifying currents,[15] resulted in decreasing the activation threshold to the negative potentials.[10,15] Therefore, mutations in *KCNH1* show hyperactive effects may disrupt cell proliferation and neuronal activity.[10,15]

In this study, we identified three *de novo* heterozygous *KCNH1* mutations in four different patients with syndromic developmental delay and infantile epilepsy. The nature of the mutations and the clinical features of the patients are described and discussed.

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MATERIALS AND METHODS

Case reports

Patient 1. This 8-year-old boy was born, without asphyxia, to healthy, non-consanguineous parents after an uneventful pregnancy (40 gestational weeks). His birth weight, height and occipitofrontal circumference (OFC) were 3446 g (+0.6 s.d.), 49.0 cm (+0.5 s.d.) and 35.0 cm (+1 s.d.), respectively. His first seizure, a generalized tonic–clonic seizure, occurred at 6 months of age. On examination, he had moderate developmental delay (developmental quotient: 50) and hypotonia with minor anomalies including a long philtrum and thick upper lip. He also had long eyelashes and broad thumbs of his hand and first toes of his feet. The electroencephalograph (EEG) showed spikes. Routine biochemical analyses were all normal and chromosomal G-banding was 46,XY (normal). Brain magnetic resonance imaging (MRI) at age 11 months showed no abnormality. He underwent physiotherapy and his idiopathic epilepsy was controlled with valproic acid and zonisamide. He achieved head control at 5 months, sitting alone at 1 year and self-supported standing at 3 years. An EEG at 1 year showed several small spikes in the right frontal and central areas. On examination at 7 years, he could sit alone, roll over and crawl, but speak no meaningful words. His body weight, height and OFC were 21.0 kg (−0.9 s.d.), 121.8 cm (−0.5 s.d.) and 49.5 cm (−1.6 s.d.).

Patient 2. This 6-year-old boy was born without asphyxia to healthy parents after an uneventful pregnancy (40 gestational weeks). There was no family history of epilepsy, but his father was deaf. His birth weight was 4000 g (90 – 95 percentile). His first seizure was at 1 month, and the EEG showed temporal focus spikes. His seizures were controlled by combination therapy with frisium, trileptin and risperdal. On examination, he showed severe developmental delay and hypertonia. He had a coarse face with thick eyebrows, long eyelashes, epicanthic folds, a broad nasal bridge, full cheeks, a long philtrum, thick lips, an open mouth, gingival enlargement, and abnormal and delayed dentition (Figure 1; Table 1). Bilateral broad and long first toes and clinodactyly of his fifth toes are shown in Figure 1. Laboratory tests including urinary amino acids, blood lactate, pyruvate, thyroid stimulating hormone and routine biochemical analyses were all normal.

Patient 3. This 7-year-old boy was born without asphyxia to healthy, non-consanguineous parents after an uneventful pregnancy (40 gestational weeks). There was no family history of epilepsy. His birth weight, length and OFC were 2872 g (−0.3 s.d.), 48 cm (−0.5 s.d.) and 33 cm (−0.2 s.d.), respectively. His first seizure was at 4 months, when he had a generalized tonic–clonic seizure that lasted 2 min. An EEG showed rare spikes in the right central area, and carbamazepine was started. Since then, he has had five focal seizures of his right upper extremities with secondary generalization. He was then referred to our hospital for evaluation of developmental delay and epilepsy at 9 months. On examination, he had moderate developmental delay (developmental quotient: 45) and hypotonia with minor anomalies including mild hypertelorism, bilateral ptosis, a broad nasal bridge, loss of nasolabial fold, anteverted nostrils, a long philtrum, an open mouth, thick lips, downturned corners of a triangular mouth and a right simian crease. He had broad toes, but no hypoplasia of his toenails. He had a pseudomyopathic face. Laboratory tests, including urinary amino acids, blood lactate, pyruvate, thyroid stimulating hormone and routine biochemical analyses, were all normal. Chromosomal G-banding was normal as was tandem mass screening of the urine. Brain MRI at 11 months was also normal. He was diagnosed with intellectual disability with idiopathic epilepsy, and treated with physiotherapy and carbamazepine. He achieved head control at 6 months, sitting alone at 13 months and standing at 2.5 years. An EEG at 2 years and 4 months showed several small spikes in the right central area.

On examination at 6 years, he could sit alone, roll over and crawl, but had no meaningful words. His developmental quotient was 13, and his body weight, height and OFC were 15.4 kg (−1.5 s.d.), 106.5 cm (−1.3 s.d.) and 49.7 cm (−1.1 s.d.), respectively. He had pes planovalgus and hypotonia. He also had constipation, gingival hypertrophy and delayed dentition. He had mild choreoathetotic movement of his extremities, which was exaggerated with febrile illness. Brain MRI was normal, and an EEG showed disorganized waking background for his age, and high-voltage slow waves and equivocal spikes were noted in the bilateral frontal areas during sleep and wakefulness. He had eight seizures during the previous year, which were focal clonic seizures of his right arm and face, and automatic seizures of his right arm, which sometimes developed to generalized tonic–clonic convulsions. The seizure duration was always within 3 min without status epilepticus. He was treated with carbamazepine and clobazam, which showed relative effectiveness. Other anticonvulsants, including levetiracetam, topiramate, zonisamide, valproic acid and lamotrigine, were ineffective.

Patient 4. This 3-year-old boy was born without asphyxia, the third child to healthy non-consanguineous parents after a 38-week uneventful pregnancy. His two older sisters were healthy. His birth weight, length and OFC were 2334 g (−1.6 s.d.), 46.5 cm (−1.2 s.d.) and 32 cm (mean), respectively. He presented with generalized convulsions within 24 h of his birth, and was admitted to the neonatal care unit for 4 days. An EEG did not detect paroxysmal activity and there was no recurrence of seizures until he was 43 days old. He fed well with milk, but failed to gain body weight as expected during his first month.

On days 43 and 46, he stopped breathing and moving for 7–10 s, which was accompanied by cyanosis and general tonic–clonic seizures lasting for 1 – 2 min for each episode. He was readmitted to the hospital on day 50, presenting with continual hyperirritability, myoclonus and intermittent oculogyric movements. He was referred to our department at age 56 days. On admission, he was 55.5 cm in length (−1.9 s.d.), weighed 3.8 kg (−3.5 s.d.) and had an OFC of 38.8 cm (−0.7 s.d.). Interictal EEG recordings revealed sporadic, multifocal spikes and sharp waves. Brain MRI at 61 days was normal. He had multiple minor anomalies including large ears, a high-arched palate and bilateral ptosis (Table 1). Neurologically, muscular hypotonia was remarkable, whereas deep tendon reflexes were brisk. Routine biochemical analyses were all normal, and G-band karyotyping was 46, XY.

For epileptic seizures, valproic acid, clobazam and levetiracetam were effective in combination. Meaningful words have never been acquired. At age 12 months, he was 77.8 cm in height (+0.9 s.d.), he weighed 8.41 kg (−1.1 s.d.) and his OFC was 47.0 cm (+0.5 s.d.). Brain MRI at 1 year 2 months showed no brain malformation but a moderately decreased brain volume was noticed. Photographs in Figure 1 were taken at 1 year and 11 months. At 3 years, his height and body weight are 11.6 kg (−1.3 s.d.) and 96 cm (+1.1 s.d.), respectively. He can not control his head or sit alone.

Whole-exome sequencing

Genomic DNA was extracted from the peripheral blood of patient and parents. Approximately, 3-μg DNA was sheared and used for a SureSelect Human All Exon V4 or V5 library (Agilent Technologies, Santa Clara, CA) according to the manufacturer's instructions. Captured DNA was sequenced on an Illumina HiSeq2000 (Illumina, San Diego, CA) with 101-bp paired-end reads. Image analysis and base calling were performed by sequence control software real-time analysis and CASAVA software v1.8 (Illumina). The quality controlled (Path Filter) reads were mapped to the human reference genome (UCSC hg19, NCBI build 37), using Novoalign (http://www.novocraft.com/). After the removal of PCR duplication by Picard 1.55 (http://broadinstitute.github.io/picard/), single-nucleotide variants, and short insertions and deletions were identified using Genome Analysis Toolkit (http://www.broadinstitute.org/gatk/). These single-nucleotide variants, and insertions and deletion were annotated using ANNOVAR (http://www.openbioinformatics.org/annovar/). This allowed the removal of common variants registered in dbSNP137 (minor allele frequency $\geqslant 0.01$). All variants within exons or ± 30 bp from exon–intron boundaries, those registered in dbSNP137, the National Heart, Lung and Blood Institute Exome Sequencing Project Exome Variant Server (NHLBI-ESP 6500, http://evs.gs.washington.edu/EVS/), or our in-house database (exome data from 575 Japanese individuals) were removed. Variants were confirmed by Sanger sequencing using an ABI PRISM 3500xl autosequencer (Life Technologies, Carlsbad, CA). In this analysis, mutations were annotated based on *KCNH1* isoform1, NM_172362.2, NP_758872. This study was approved by the Institutional Review Board of Yokohama City University School of Medicine. Written informed consent was obtained from patients or parents.

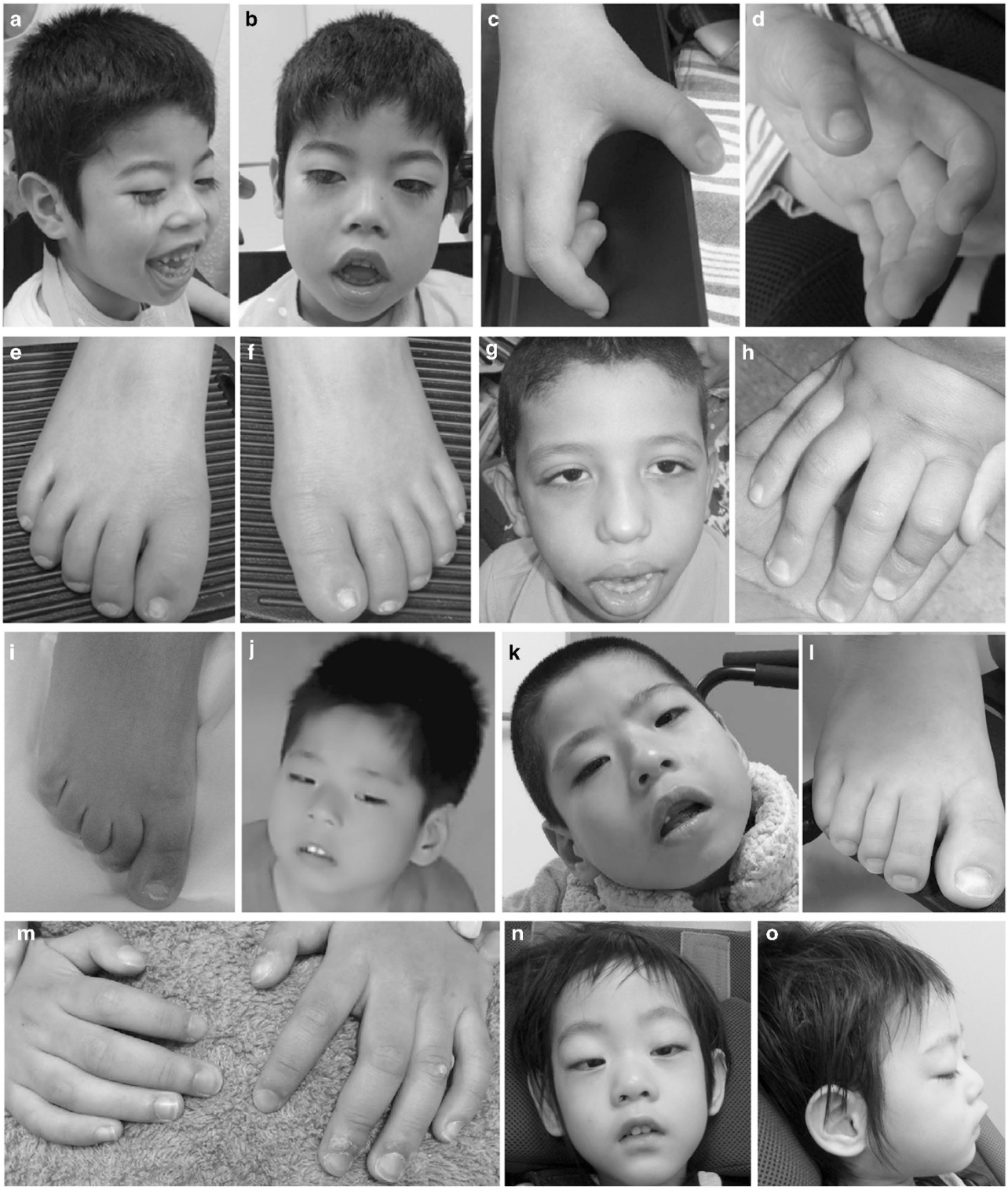

Figure 1 Clinical features of four patients with a *KCNH1* mutation. Facial appearance (**a**, **b**), right hand (**c**), left hand (**d**), right foot (**e**) and left foot (**f**) of patient 1. Long eyelashes, hypoplastic toe nails and broad thumbs and toes are evident. Facial appearance (**g**), right hand (**h**) and right foot (**i**) of patient 2. Hypoplastic toe nails and anonychia of the toes are apparent. Facial appearance at 4 years old (**j**) and 6 years old (**k**), right foot, showing broad first toe (**l**), right and left hands (**m**) of patient 3. Facial appearance (**n**, **o**) of patient 4. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

RESULTS

Identification of *de novo KCNH1* mutations

We identified three novel missense *KCNH1* mutations by whole-exome sequencing (WES) in four unrelated patients with developmental delay and epilepsy. Trio-based WES was performed in two patients, and the other two were analyzed by proband-only WES. Each WES performance is shown in Supplementary Table 1. We focused on rare nonsynonymous variants that were absent in

Table 1 Genetic and clinical features in patients with KCNH1 mutations

	This study					*ZLS*	*TBS*
Patient	*Patient 1*	*Patient 2*	*Patient 3*	*Patient 4*	*Positive/ all*	*Kortum* et al.[15]	Simons et al.[10]
Diagnosis	Developmental delay	Epilepsy	Epilepsy	Developmental delay, hypotonus			
Mutation (KCNH1 NM_172362)	c.1070G>A	c.1070G>A	c.1070G>C	c.1487G>A			
Aminoacid change	p.Arg357Gln	p.Arg357Gln	p.Arg357Pro	p.Gly496Glu			
Sex	Male	Male	Male	Male			
Birth (week)	40w1d	40w	40w	38w			
Birth weight	3446 g (+0.6 s.d.)	4000 g (90–95 percentile)	2872 g (–0.3 s.d.)	2334 g (–1.6 s.d.)			
Birth length	49.0 cm (+0.5 s.d.)	unknown	48 cm (–0.5 s.d.)	46.5 cm (–1.2 s.d.)			
Head circumference at birth	35.0 cm (+1 s.d.)	unknown	33 cm (–0.2 s.d.)	32 cm (–0.1 s.d.)			
Neurodevelopment							
Intellectual disability	Severely affected	Severely affected	Severely affected	Severely affected	4/4	6/6	5/5
Developmental delay	Severely affected	Severely affected	Severely affected (DQ 13 at 6 years old)	Severely affected	4/4	6/6	5/5
Muscle tension	Hypotonia	Hypertonia	Hypotonia	Hypotonia	4/4	4/6	6/6
Epilepsy (seizures)	+	+	+	+	4/4	6/6	6/6
Micro or macrocephaly	–	–	–	–	0/4	1/6 Macrocephaly	4/6 (<50 percentile)
Facial features							
Coarse face	+	+	–	+	3/4	6/6	6/6
Hypertelorism	–	–	+	–	1/4	1/6	5/6
Thick eyebrow	+	–	–	+	2/4	2/6	NA
Epicanthic folds	–	+	+	+	3/4	1/6	3/6
Bilateral ptosis	–	–	+	+	2/4	1/6	NA
Flat nasal bridge	+	–	+	+	3/4	NA	6/6
Large ear	+	–	+	+	3/4	1/6	NA
Wide nose	+	+	+	+	4/4	3/6	6/6
Anteverted nares	+	–	+	+	3/4	NA	1/6
Wide mouth with downturned corners	+	+	+	–	3/4	1/6	6/6
Full cheeks	+	+	+	+	4/4	NA	1/6
Open mouth	+	+	+	–	3/4	NA	1/6
Thick lips	+	+	–	–	2/4	2/6	6/6
Gingival enlargement	–	+ (unknown before anticonvulsant treatment)	–	–	1/4	5/6 (4/5 before anticonvulsant treatment)	NA
High palate	–	–	–	+	1/4	NA	NA
Long eyelashes	+	+	+	+	4/4	1/6	NA
Abnormal/delayed dentition	+	+	+	–	3/4	NA	NA
Limbs							
Absence/hypoplasia of thumb nail	+	–	–	–	1/4	4/6	6/6
Absence/hypoplasia of great toe nail	+	+	+	–	3/4	4/6	6/6
Broad thumbs or toes	+	–	+	+	3/4	NA	5/6
Long great toes	+	+	+	–	3/4	NA	6/6
Short stature	–	–	+	+	2/4	1/6	NA
Scoliosis	–	–	–	–	0/4	5/6	NA
Hypertrichosis	–	–	–	–	0/4	3/6	NA
Gastrointestinal							
Constipation	–	–	+	+	2/4	1/6	2/4
Gastro–esophageal reflex	–	NA	–	–	0/3	2/6	1/4
Examination							
EEG	Abnormal spike+	Spikes in the temporal region	Rare spikes in the right central area	Multifocal spikes and sharp waves	4/4	6/6	6/6
Abnormal brain MRI	–	–	–	–	0/4	2/4	1/6
Abnormal blood workup	–	–	–	–	0/4	NA	NA

Abbreviations: +, present; –, absent; DQ, developmental quotient; EEG, electroencephalograph; MRI, magnetic resonance imaging; NA, not available; TBS, Temple–Baraitser syndrome; ZLS, Zimmermann–Laband syndrome.

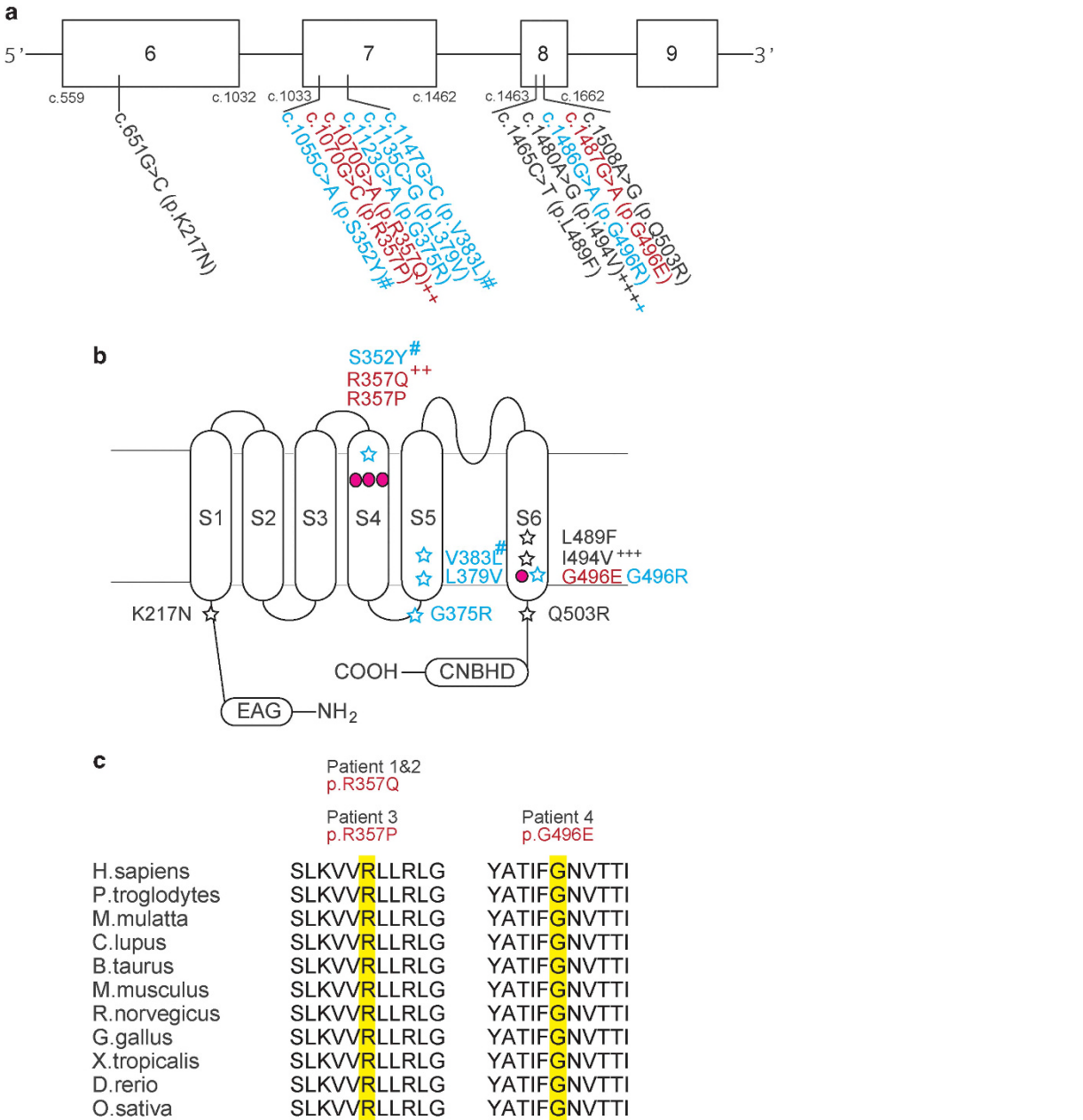

Figure 2 *KCNH1* mutations in patients. (**a**) Genomic structure of exons 6–9 of *KCNH1*. Colored annotations indicate mutations identified in this study (red), in TBS[10] (black) and in ZLS patients[15] (light blue). ++ and ++++ indicate two and four patients had mutations. # indicates mutations found simultaneously in one patient. (**b**) Schematic presentation of the KCNH1 (Kv10.1) channel and the location of mutated amino-acid residues found in this (red) and two previous.[10,15] (black and light blue). (**c**). The three mutations found in this study occur at evolutionally highly conserved amino acids.

dbSNP137, our in-house 575 control exomes and the Exome Variant Server database. Trio-based WES analysis revealed *de novo*, autosomal recessive and X-linked recessive candidate variants in two families (Supplementary Table 2). *KCNH1* mutations found in four patients were predicted as disease-causing based on by three computational programs sorting Intolerant from Tolerant (SIFT, http://sift.jcvi.org/www/SIFT_enst_submit.html), polymorphism phenotype (PolyPhen-2, http://genetics.bwh.harvard.edu/pph2/), and mutation prediction for the deep-sequencing age (Mutation Taster, http://www.mutationtaster.org/) (Supplementary Table 2). *De novo* occurrences of all the mutations were confirmed by Sanger sequencing (Supplementary Figure 1). Locations of the three *KCNH1* mutations along with 10 previously reported mutations are illustrated in Figure 2. The c.1070G>A (p.R357Q) was observed in two independent patients and c.1070G>C (p.R357P) was observed in one patient; both were located at an evolutionally highly conserved arginine in the channel voltage-sensor (S4) domain (Figure 2; Table 1). The other mutation, c.1487G>A (p.G496E), was located at an evolutionally highly conserved glycine in the channel pore domain (S6). A similar mutation (p.G496R) at the same position has been reported previously[15] (Figure 2; Table 1).

DISCUSSION

In this study, we identified three novel missense *KCNH1* mutations occurring *de novo* in four patients with severe developmental delay and infantile epilepsy. As shown in Figure 2a, published data have revealed one ZLS patient with a mutation in exon 6, three with mutations in exon 7, and five ZLS and two TBS patients with mutations in exon 8. Our series had mutations in exon 7 and 8. Exon 7 mainly encodes domains S4 and S5, whereas exon 8 encodes the S6 domain. Combining the four new patients with the 10 previously reported patients, 15/16 patients had mutations in either exon 7 or 8, equivalent to domains S4–S6, suggesting these exons are the hotspots for *KCNH1*-mutant disorders.

The two novel *KCNH1* mutations found in three patients described here are located in the channel voltage-sensor domain (S4). The positively charged residues (arginine and lysine) are responsible for voltage sensing in the S4 domain in Kv channel.[2,12,17,18] Interestingly, the two detected mutations (p.R357Q and p.R357P) both substituted a positively charged amino-acid residue at Arg357. The repeated arginine motif is important for maintaining the voltage-sensor domain; thus, change from arginine to an uncharged residue (p.R357Q and p.R357P) is highly likely to disrupt the sensitivity and cooperativity of the sensor of the Kv channel. Although one ZLS patient carried two mutations, one in S4 (p.S352Y) and one in S5 (p.V383L) simultaneously,[15] the impact of the p.S352Y mutation was not determined. Our reports emphasize the importance of the S4 domain of KCNH1 in humans.

In this report, we have also presented a patient with a mutation, p.G496E, located in the channel pore domain (S6).[19] This mutation has been previously reported in a patient with ZLS.[15] Glycine residues in Kv channels are known to confer flexibility to protein structures[20–22] so that they act as movable hinges.[23] Compared with glycine, which is nonpolar and a small amino acid, arginine and glutamate belong to positively and negatively charged groups, respectively, and they are both larger in molecular mass. Therefore, it can be postulated that the molecular size and charge changes of this component of the channel pore would affect the proper functioning of the gate. Mutant *KCNH1* (p.G496R) channels expressed in CHO cells have shown no voltage-dependent K current,[15] suggesting that the G496 residue was essential for proper channel gating.

In the four patients described here, the initial symptom was developmental delay, followed by various types of infantile-onset seizures. Some patients have dysmorphic faces, hypotonia and thick broad toes. In addition, three of the four patients had long eyelashes and an open mouth (Figure 1; Table 1). Pathogenic mutations in *KCNH1* have been found in ZLS and TBS patients[10,15] who also display intellectual disability, epilepsy, hypoplasia or aplasia of nails, abnormal muscle tone and craniofacial dysmorphologies (Table 1). It is not easy to clearly differentiate these two syndromes based on clinical phenotypes, and the four patients in this study could not be assigned to either of these syndromes (Table 1). Therefore, we conclude that the mutations in *KCNH1* likely cause a phenotypic continuum of neurodevelopmental disorders covering ZLS and TBS. *KCNH1* mutations found in syndromic developmental delay and infantile seizures cluster in S4 − S6 domains and highlight the functional importance of those domains in Kv10.1.

In conclusion, we report three *de novo* missense *KCNH1* mutations in four patients with syndromic developmental delay and epilepsy. More information of *KCNH1* mutations and their clinical consequences are absolutely needed to delineate the clear phenotype–genotype correlation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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