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BRIEF COMMUNICATION

Linkage analysis and exome sequencing identify a novel mutation in *KCTD7* in patients with progressive myoclonus epilepsy with ataxia

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Summary

Epilepsy affects approximately 1% of the world's population. Genetic factors and acquired etiologies, as well as a range of environmental triggers, together contribute to epileptogenesis. We have identified a family with three daughters affected with progressive myoclonus epilepsy with ataxia. Clinical details of the onset and progression of the neurologic presentation, epileptic seizures, and the natural history of progression over a 10-year period are described. Using autozygosity genetic mapping, we identified a high likelihood homozygous region on chromosome 7p12.1-7q11.22. We subsequently applied whole-exome sequencing and employed a rare variant prioritization analysis within the homozygous region. We identified p.Tyr276Cys in the potassium channel tetramerization domain–containing seven gene, *KCTD7*, which is expressed predominantly in the brain. Mutations in this gene have been implicated previously in epileptic phenotypes due to disturbances in potassium channel conductance. Pathogenicity of the mutation was supported by bioinformatic predictive analyses and variant cosegregation within the family. Further biologic validation is necessary to fully characterize the pathogenic mechanisms that explain the phenotypic causes of epilepsy with ataxia in these patients.

KEY WORDS: **Whole-exome sequencing, Epilepsy, KCTD7.**

Globally, 70 million people are living with some form of epilepsy, a neurologic condition characterized by recurrent seizures due to abnormal excessive or synchronous neuronal activity in the brain.[1] Epilepsy is multifactorial in its causation, and its chronic nature results in a considerable economic burden estimated at around $100 million per year in North America.[2] Common forms of epilepsy tend to be complex disorders in which a number of genes and environmental factors together contribute to epileptogenesis.[3] Conversely, some relatively rare forms of epilepsy are monogenic.[4]

Next-generation sequencing (NGS) technologies have successfully identified the cause of nearly 200 disorders since 2009.[5] Briefly, NGS is a rapid and cost-effective sequencing approach that can be applied to study any genetic disease.[5] One subtype of NGS, whole-exome sequencing (WES), sequences only coding variants and has been a particularly robust diagnostic tool for monogenic disorders.[5] Herein we describe our methods of identifying the probable cause of a rare disease designated as epilepsy with ataxia using genetic mapping and WES.

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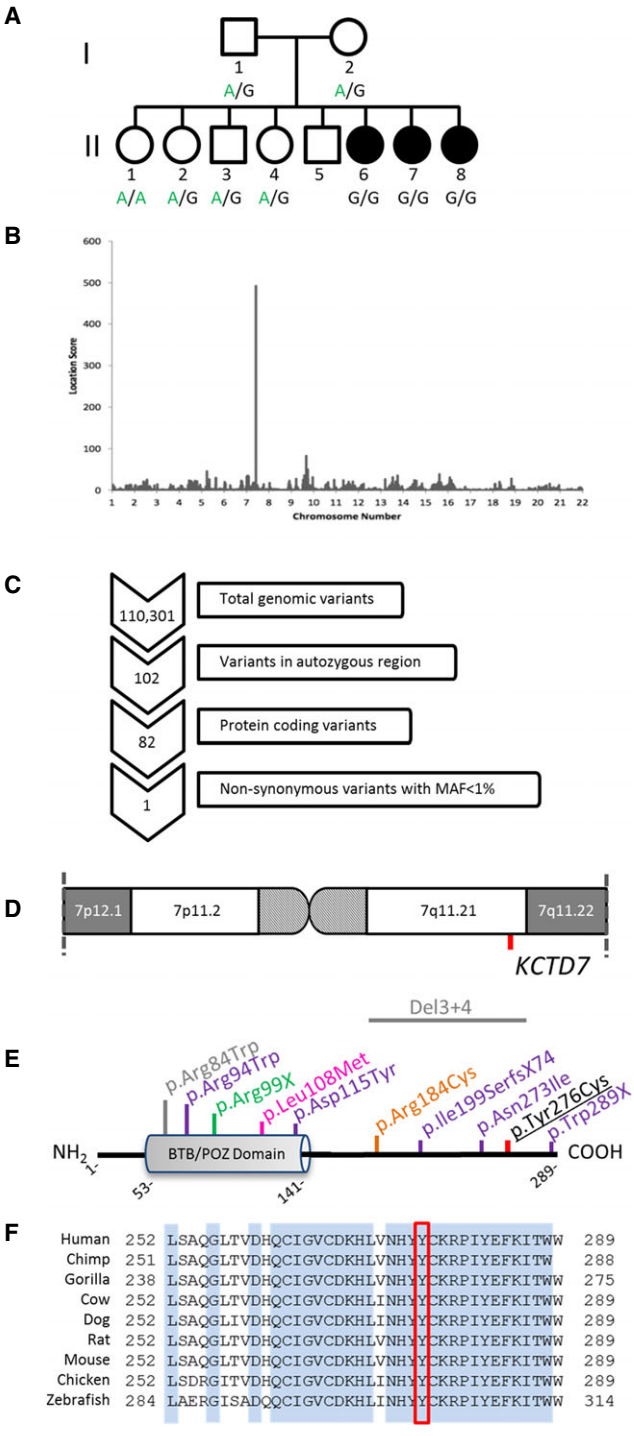

Figure 1.
(**A**) Family pedigree with three daughters affected with epilepsy with ataxia. Squares depict males and circles depict females. Affected individuals are shaded. (**B**) Genome-wide autozygosity mapping generated a high priority region on chromosome 7 with a high location score. (**C**) Whole-exome sequencing produced one rare, nonsynonymous variant (minor allele frequency [MAF] <1%). (**D**) KCTD7 is located on chromosome 7q11.21 within the autozygous region. (**E**) BTB/POZ domain is the only known functional domain in the KCTD7 protein. Several mutations have been reported in KCTD7: mutations in green, purple, pink, orange, and black were observed in a Moroccan, Turkish, Pakistani, Mexican, and Mennonite subjects, respectively. Gray represents unknown ethnicity. Del3+4 refers to the complete deletion of exons three and four (**F**) Multiple protein alignments of KCTD7 demonstrate high homology of amino acid residue p.Tyr276 (shown in red box) across a set of divergent species. The residues shaded in blue indicate fully conserved residues.

Methods

Detailed methods are available online (Data S1).

Results

Clinical description of epilepsy with ataxia

The three sisters are the youngest of eight children born to a nonconsanguineous Low German–speaking Mennonite couple originally from Durango, Mexico (Fig. 1A, Table S1).

Case I

The oldest of three siblings, this patient became symptomatic at age 2 years when she first presented with seizures. Prior to the onset of seizures, signs of developmental delay, such as walking at 19 months and speech limited to single words, were evident. The initial seizures were associated with febrile events, and later afebrile generalized clonic seizures were reported without any obvious provocative triggers. The generalized seizures were associated with uprolling of the eyeballs and clonic activity in all four limbs; the seizures were varied in duration but were self-limited. Postictal hemibody weakness was reported for variable periods. Additional seizure types including sudden head and shoulder drops associated with loss of posture and falls as well as episodes of staring and unresponsiveness were also reported after the onset of afebrile seizures. Following the onset of seizures, developmental regression was noted, with loss of expressive language skills and eventual progression to a nonverbal state, with limited comprehension of spoken language. The first neurologic assessment disclosed the presence of significant hypotonia, motor incoordination, and gait ataxia. There were no signs of ocular movements, nystagmus, or cranial nerve deficits. By age 7 years, seizure control was vastly improved on a combination of lamotrigine (6 mg/kg/day) and clonazepam (0.02–0.05 mg/kg/day). However, neurologic examination confirmed the presence of multifocal myoclonus and development of pyramidal signs (hyperreflexia, extensor plantar responses). A mild right hemiparesis was also noted and was explained by identification of a small epidural hematoma sustained during a fall associated with myoclonic astatic seizure. Addition of levetiracetam (35 mg/kg/day) significantly improved control of myoclonus and drop attacks. Between age 9 and 14 years, there were additional signs of progression, with

loss of independent ambulation, incontinence, with inability to feed independently and manage activities of independent living.

Case II

During her first evaluation at age 3 years, this patient was reported to have had normal development of milestones until age 2 years. She then showed generalized clonic seizure activity, head and shoulder drops, and staring spells associated with eyelid fluttering. She began to show developmental regression after the onset of seizures, and lost expressive speech and had significant sialorrhea (drooling). Neurologic examination disclosed findings similar to her elder sibling: moderately increased tone in lower extremities, hyperreflexia, and gait ataxia. Her seizures were well controlled on a combination of valproic acid (15–20 mg/kg) and clonazepam (0.03–0.05 mg/kg). Since the age of 5 years her seizures have remained well controlled, but her gait and myoclonus have continued to worsen. However, she retains the ability to walk and feed herself.

Case III

The youngest of three affected siblings, this patient presented with a generalized seizure at 15 months. Prior to this, she showed signs of developmental delay in walking but had a vocabulary of several words. She has continued to experience both febrile and afebrile events and occasional head drops, but no observed staring spells. Her seizures have remained relatively easy to control with monotherapy (valproic acid) initially, and with the appearance of myoclonic jerks clonazepam was added. The patient is currently 12 years of age, with mild neurologic symptoms compared to her sisters with respect to gait and coordination. She is capable of independent ambulation, and she can feed and dress herself. She is cognitively delayed, and has limited expressive speech, but unlike her older sibling she has yet to show further decline in her functioning.

Electroencephalography (EEG) features

All three siblings share similarities in their seizure phenotype and electroencephalographic findings (Figs. S1–S4). Background rhythms show poor organization and diffuse nonspecific slowing. In the early stages, abundant paroxysmal bursts of generalized spike wave discharges were prominent, however, with improving seizure control and increasing serial recordings showed multiple independent spike foci and regional expression of epileptiform activity distributed over bilateral central, temporal and parietal electrode chains, often with shifting asymmetry. In the instance of a single staring spell associated with behavioral arrest captured during a recording, the EEG showed focal spikes, with a posterior distribution and ictal rhythms that spread progressively from the occipital regions to become generalized with an abrupt onset and offset. All three siblings showed a photoparoxysmal response on photic stimulation, which was self-limited in nature (Figs. S1–S4).

Gene and mutation mapping

Although the family is unaware of any consanguinity, we modeled an autosomal recessive disease inheritance given the known founder effect within the Mennonite community. Genome-wide autozygosity mapping generated a high priority locus on chromosome 7p12.1-7q11.22, ~14.3 Mb in length with a significant location score of 492 (Fig. 1B). Next, we performed WES and employed a nonsynonymous rare variant filtering analysis (Fig. 1C). We identified a novel variant within the autozygous region: g.661041-76A>G, c.827A>G, p.Tyr276Cys in *KCTD7* (NM_153033.4), the potassium channel tetramerization domain–containing seven gene, which is expressed predominantly in the brain, specifically in cortical neurons, in granular and pyramidal cell layers of the hippocampus, and in cerebellar Purkinje cells, and is involved in hyperpolarization of the cell membrane via interaction with a component of the ubiquitin ligase complex (Fig. 1D).[6–8] It is notable that 11 other pathogenic variants have been reported in *KCTD7,* all of which have been implicated in progressive myoclonus epilepsy (Fig. 1E, Table 1).[6,8–11] Recently, this variant was misreported as p.Thr276Cys in a consanguineous family; herein, we rectify the misannotation of the mutation in *KCTD7* and the family kinship.[12]

We performed subsequent variant validation analysis and observed *KCTD7* p.Tyr276Cys to cosegregate with disease status in the family (Fig. 1A). All three affected individuals are homozygous for the variant and unaffected siblings are either heterozygous or homozygous for the wild-type allele. As expected, both parents are heterozygous. Furthermore, *KCTD7* p.Tyr276Cys was consistently predicted to be damaging by multiple in silico analyses, suggesting that deviation from the wild-type amino acid residue may disrupt protein function (Fig. 1F). The NHLBI ESP Exome Variant Server, which is a publicly available repository of >6,500 individuals, reported the minor allele frequency of p.Tyr276Cys in *KCTD7* to be 0.008% in the general population, a very low carrier frequency, as expected, given the few reports of patients presenting with epilepsy with ataxia.

Discussion

The epilepsy phenotype in the three siblings followed over 10 years shows a distinctive natural history. Features of the first phase include motor and speech delay prior to onset of seizures. In the second phase, initial complex febrile seizures evolve into multiple seizures types including generalized clonic seizures, atypical absences, and myoclonic head and shoulder drops. Developmental regression involving loss of language is accompanied by prominent ataxia, incoordination, and nonepileptic myoclonus. There appears to be a plateau in the third phase lasting several

Table 1. Key features of all reported patients with *KCTD7* mutations

Study	Blumkin et al. (2012)[11]	Kousi et al. (2012)[8]	Krabichler et al. (2012)[9]	Van Bogaert et al. (2007)[10]	Kousi et al. (2012)[8]	Kousi et al. (2012)[8]	Staropoli et al. (2012)[6]	Kousi et al. (2012)[8]	Kousi et al. (2012)[8]	Current report	Kousi et al. (2012)[8]
No. of patients carrying same listed mutation in study	1	1	1	3	2	1	2	2 (from two separate families)	1	3	2
Sex	M	M	M	F, F, F	F, M	M	F, M	F, M	M	F, F, F	F, M
Age of onset (months)	10	10	10	16–24	12–24	12–24	8–9	6–12	12–24	15-24	12–24
Ethnicity	Unknown	Turkish	Turkish	Moroccan	Pakistani	Turkish	Mexican	Turkish	Turkish	Mennonite	Turkish
KCTD7 mutation	1) p.Arg84Trp 2) Del exons 3+4	p.Arg94Trp	p.Arg94Trp	p.Arg99X	p.Leu108Met	1) p.Asp115Tyr 2) p.Asn273Ile	p.Arg184Cys	p.Ile199SerfsX74	p.Asn273Ile	p.Tyr276Cys	p.Trp289X
Zygosity of patient(s)	Compound heterozygous	Homozygous	Homozygous	Homozygous	Homozygous	Compound heterozygous	Homozygous	Homozygous	Homozygous	Homozygous	Homozygous
Consanguineous marriage of parent	No	No	Yes	Yes	Yes	No	No	First family: yes; second family: no	No	No	Yes
Presenting symptoms	Myoclonic seizures, oral automatisms, severe dysarthria, opsoclonus-like eye movements	Myoclonic atonic	Myoclonic seizures, tonic episodes, hypotonia, dystonia, dyskinesia	Myoclonic seizures, hypotonic	Myoclonic and atonic seizures	Myoclonic seizures, generalized tonic–clonic seizures	Myoclonic seizures, spasticity and vision loss	Myoclonic seizures, generalized tonic–clonic seizures	Myoclonic seizures	Myoclonic seizures, tonic episodes, dysarthria	Myoclonic, generalized tonic–clonic seizures
Ataxia	Yes	No	No	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes
Cognitive functions	Impaired	Impaired	Impaired	Impaired	Impaired	Impaired	Impaired	Impaired	Impaired	Impaired	Impaired

years, wherein seizure control improves. Late deterioration was seen in the most severely affected child, involving loss of ambulation and incontinence. There is considerable variability in the severity of symptoms across the three siblings, with the youngest being most mildly affected and not yet having experienced significant deterioration in function.

The EEG features again suggest an evolution that mirrors the clinical neurologic progression, with abundant multifocal spikes as well as generalized epileptiform activity in the initial stages, followed in later years by a more regional expression of spike wave activity. Photic stimulation is associated with the development of photoparoxysmal responses that are self-limited and do not outlast the stimulus train. The seizures appear to respond particularly well to treatment with several agents alone or in combination, including valproic acid, lamotrigine, clonazepam, and levetiracetam. The latter has been particularly helpful in reducing myoclonic seizures and falls. The clinical presentation and progression share some features with other progressive myoclonus epilepsy syndromes such as neuronal ceroid lipofuscinoses.[6]

KCTD7 is a member of the KCTD gene family.[4] The family of proteins shares an N-terminal BTB/POZ domain that demonstrates sequence homology to the TI domain in voltage-gated potassium channels.[6] To date, there have been 11 *KCTD7* mutations identified in 19 different patients presenting with some form of progressive myoclonus epilepsy (Table 1). These patients have similar clinical presentations including myoclonic seizures and cognitive impairment; however, only a subset of these patients have ataxia and/or dystonia, which may suggest that that disorder is more severe depending on the type of mutation and its genomic location (Table 1). Of interest, all 11 mutations identified are within the coding region of *KCTD7*, with 5 in its known functional domain, BTB/POZ; however, mutations within the BTB/POZ domain do not necessarily result in a clinically different phenotype. Given the genetic heterogeneity of progressive myoclonus epilepsy, there are 11 commercially available gene tests that screen for mutations in *KCTD7* among other genes, in many childhood-onset epilepsy-associated disorders, which act as rapid and economical diagnostic tools for the practicing clinician (Table S2).

In summary, we have identified a novel homozygous variant in *KCTD7*, which is predominantly expressed in the brain. Mutations in this gene have been implicated previously in epileptic phenotypes due to disturbances in potassium channel conductance; therefore, our results further support the role of KCTD7 in epileptic seizures.

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Disclosure

The authors have no conflicts of interest to declare. We also confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Patient 1. A snapshot of the EEG recording at age 11 years of age, abundant multifocal independent spikes, as well as synchronized, bihemispheric generalized

spike waves superimposed on slow background rhythms with superimposed fast rhythms (beta frequencies) attributable to a benzodiazepine effect.

Figure S2. Patient 1. At 14 years of age, on a coronal montage, generalized 2–3 Hz multiphasic spike and wave discharges with a predominant distribution over midline parasagittal and bitemporal regions.

Figure S3. Patient 3. Excess fast background rhythms are seen, with focal repetitive spike and waves in the right hemisphere, and wave maxima over C4, P4, and T4–T6 electrodes.

Figure S4. Patient 3. At 6 Hz flash frequency a generalized and self-limited photoparoxysmal response is seen.

Table S1. Clinical description of patients with epilepsy with ataxia.

Table S2. Available gene tests for mutations in *KCTD7*.

Data S1. Patients and methods.