REPORT

Mutations in *UNC80*, Encoding Part of the UNC79-UNC80-NALCN Channel Complex, Cause Autosomal-Recessive Severe Infantile Encephalopathy

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Brain channelopathies represent a growing class of brain disorders that usually result in paroxysmal disorders, although their role in other neurological phenotypes, including the recently described *NALCN*-related infantile encephalopathy, is increasingly recognized. In three Saudi Arabian families and one Egyptian family all affected by a remarkably similar phenotype (infantile encephalopathy and largely normal brain MRI) to that of *NALCN*-related infantile encephalopathy, we identified a locus on 2q34 in which whole-exome sequencing revealed three, including two apparently loss-of-function, recessive mutations in *UNC80*. *UNC80* encodes a large protein that is necessary for the stability and function of NALCN and for bridging NALCN to UNC79 to form a functional complex. Our results expand the clinical relevance of the UNC79-UNC80-NALCN channel complex.

Neurotransmission is a complex process that involves propagation of action potentials along neurites and the release of synaptic vesicles at the synapse. Key to this process is the orchestrated action of numerous channels, both ion- and voltage-gated, which tightly control the amounts of ions across the nerve cell membrane during resting states but allow for a rapid change in the flow of ions during neuronal firing. Predictably, paroxysmal disorders (epilepsy and related disorders) are the most common clinical manifestation of perturbed physiology of these channels, both in Mendelian and non-Mendelian forms.[1–3] However, in the past few years, an expanding number of genes that code for various brain channel components have been found to be mutated in individuals with static encephalopathy that manifests as global developmental delay in early childhood and as intellectual disability in long-term survivors. For example, the Zimmermann-Laband (MIM: 135500) and Keppen-Lubinsky (MIM: 614098) dysmorphology and intellectual disability syndromes were recently found to be caused by mutations in the genes encoding potassium channels KCNH1 (*KCNH1* [MIM: 603305]) and KCNJ6 (*KCNJ6* [MIM: 600877]), respectively.[4,5] An X-linked intellectual disability and heart failure disorder (MIM: 300886) was found to be caused by a mutation in the gene encoding the calcium channel CLIC2 (*CLIC2* [MIM: 300138]).[6] Intellectual disability with no associated epilepsy has also been described in individuals with mutations in the genes encoding the calcium and sodium channels CACNA1G (*CACNA1G* [MIM: 604065]), CACNA1A (*CACNA1A* [MIM: 601011]), and SCN8A (*SCN8A* [MIM: 600702]).[7–10]

NALCN (MIM: 611549) encodes a brain-enriched, nonselective sodium leak channel that belongs to a family of more than 20 pore-forming alpha-1 subunits of voltage-gated calcium channels but is unique in that it forms a voltage-insensitive and non-selective cation channel.[11] Recessive loss-of-function mutations in this gene have been described in individuals with severe infantile encephalopathy and of Arab and Turkish descent.[12,13] NALCN was recently found to exist in a channel complex in which UNC80, encoded by *UNC80* (also known as *C2ORF21* [MIM: 612636]), bridges NALCN to UNC79, and the presence of UNC80 and UNC79 is necessary for the channel function.[14] The similarity of the neurological phenotypes of worms and fruit flies that have mutant versions of the corresponding orthologs provides in vivo evidence of the interdependency of the mammalian UNC79-UNC80-NALCN complex of proteins.[15,16] This is further supported by the available mouse knockout models for *NALCN* and *UNC79*; both fail to nurse and die shortly after birth.[11,17] The mammalian phenotype of *UNC80* loss of function remains unknown. In this report, we show that recessive loss-of-function mutations in *UNC80* result in severe infantile non-epileptic encephalopathy with minimal brain changes, a phenotype nearly identical to that described for *NALCN*-related encephalopathy.

The index individual in family 1 (F1_IV:5) is a 6.5-year-old boy who has been suffering from severe truncal hypotonia since birth, progressive peripheral spasticity, and profound global developmental delay. He is unable to sit or roll over and has no speech. His first-cousin parents had two sons who died at 4 and 6 years of age with a reportedly identical phenotype of profound static encephalopathy

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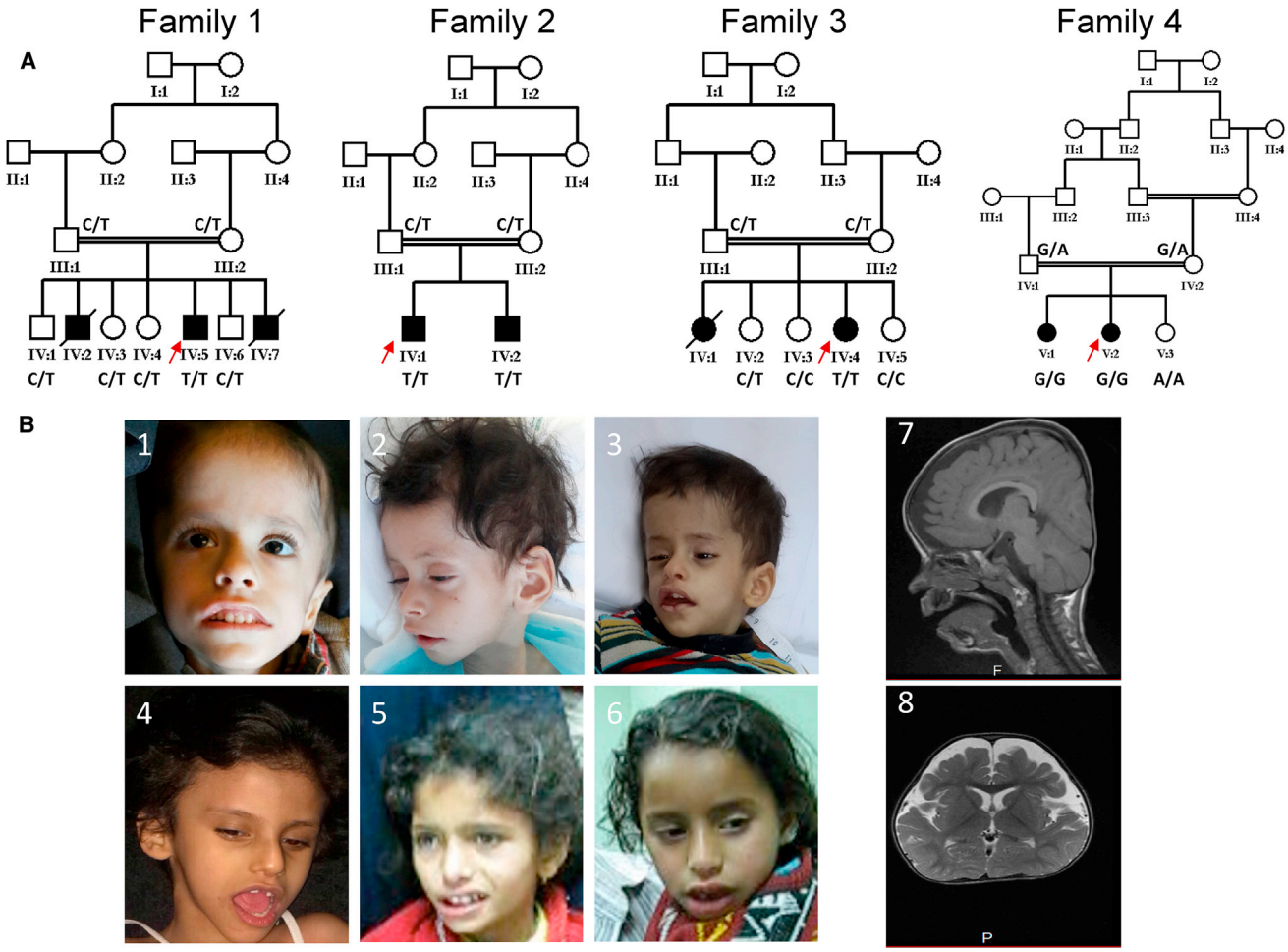

Figure 1. Characterization of Four Families with Severe Non-Epileptic Encephalopathy
(A) Pedigrees of the four study families.
(B1) Facial image of individual F1_IV:5 (6 years and 5 months), showing high forehead, prominent nasal bridge, and tented upper lip.
(B2) Facial image of individual F2_IV:1 (2 years).
(B3) Facial image of individual F2_IV:2 (11 months).
(B4) Facial image of individual F3_IV4 (8 years and 9 months), showing similar facial appearance to the other affected individuals. Note the additional presence of microcephaly, plagiocephaly, and brachycephaly.
(B5) Facial images of individual F4_V:1 and
(B6) of individual F4_V:2, showing similar but milder facial features.
(B7 and B8) Brain MRI of individual F1_IV:5, showing mild diffuse brain atrophy.

and no seizures. The index individual has two living brothers and two sisters who are all healthy (Figure 1). At 4.5 years of age, he was cachectic with a weight of 8.1 kg (−5.2 SD), short with a length of 89 cm (−3.7 SD), and microcephalic with a head circumference of 46 cm (−3.2 SD). He was inattentive and had no visual tracking ability as well as bilateral strabismus and nystagmus. He had severe axial hypotonia and appendicular hypertonia with spasticity. Plasma lactate, ammonia, uric acid, lipid profile, tandem mass spectrometry (MS), urine organic acids, plasma amino acids, and congenital disorder of glycosylation screenings did not reveal any abnormality. Chromosomal analysis and microarray were unremarkable. Glycosaminoglycans and oligosaccharides in the urine were normal. Lysosomal enzyme analysis was unremarkable. *HEXB* and *SMN1* mutation analysis did not reveal pathogenic mutations. Brain MRI showed mild diffuse brain atrophy, and MRS (magnetic resonance spectroscopy) showed a mild reduction of the N-acetylaspartate peak but a normal lactate peak. Skeletal survey showed diffuse osteopenia.

The index individual in family 2 (F2_IV:1) is a 2-year-old boy with a nearly identical phenotype of axial hypotonia, profound global developmental delay, failure to thrive, and no seizures. He also has a history of recurrent aspiration. Current weight was 4.8 kg (−6.5 SD), length was 79 cm (−2.4 SD), and head circumference was 43 cm (−4 SD). He had bilateral nystagmus and severe head lag but did not have contractures. Chromosomal analysis and microarray were normal. Tandem MS, creatine kinase, urine organic acids, plasma lactate, and ammonia were normal. Brain MRI showed normal

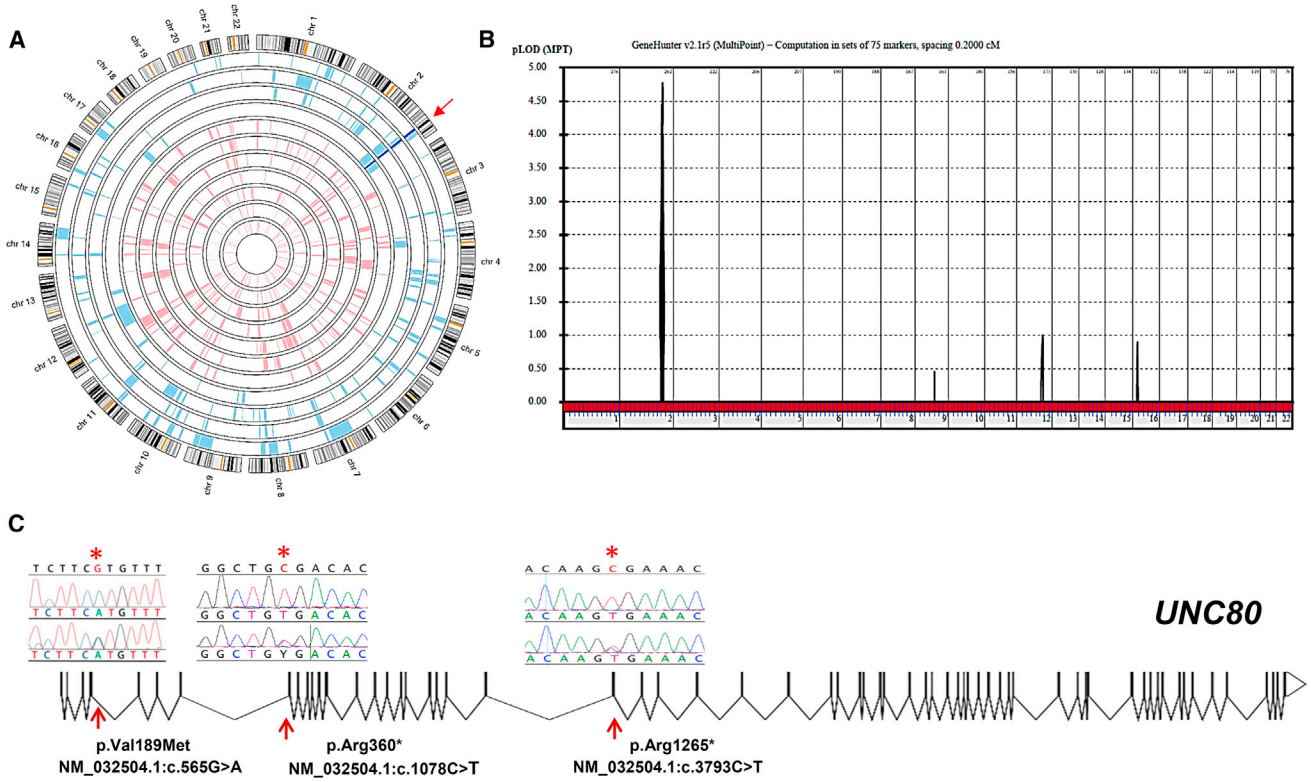

Figure 2. Identification of *UNC80* Mutations in Severe Non-Epileptic Encephalopathy
(A) Genome-wide autozygosity mapping combining families 1, 2, and 3 reveals a single interval that is exclusively shared by the affected members and is demarcated by three navy bars (red arrow).
(B) Linkage analysis shows a corresponding significant peak on chromosome 2.
(C) Cartoon of *UNC80* with the position of the two homozygous nonsense and one homozygous missense variants shown (in each chromatogram, the tracing of the affected individual is shown on top and that of the carrier parent is shown below with a red asterisk indicating the position of the variant).

parenchyma and myelination. Abdominal ultrasonography was normal.

He has a similarly affected older brother (F2_IV:2) whose evaluation at 13 months of age revealed severe hypotonia, global developmental delay, and failure to thrive (weight 5.13 kg [−5.1 SD], length 71 cm [−2 SD], and head circumference 43 cm [−2.9 SD]). Similar to that of his brother, his brain MRI showed normal parenchyma and myelination, and an electroencephalogram (EEG; not performed on F2_IV:1) revealed non-specific generalized slow-wave abnormalities. These are the only two children born to healthy first-cousin parents.

Although not directly related, the two families belong to the same tribe from Northern Saudi Arabia. This, together with their highly similar phenotype, prompted us to investigate the possibility that their condition is caused by autozygosity for a single founder mutation, so we recruited them after obtaining written informed consent (King Faisal Specialist Hospital and Research Center Institutional Review Board Research Advisory Committee no. 2121053).[18,19] Indeed, autozygosity mapping, performed as described before, revealed a single autozygous interval, on chromosome 2, that is exclusively shared by the affected members of the two families (205–210 Mb; UCSC Genome Browser hg19) (Figure 2). Exome sequencing of one of the three available affected members, followed by autozygome filtering performed as described before,[9,20] revealed a single novel coding variant, c.3793C>T (p.Arg1265*), within the critical autozygosity interval in *UNC80* (GenBank: NM_032504.1). This homozygous variant was confirmed by Sanger sequencing and segregated with the disease as predicted within the two families.

Independently, clinical exome sequencing (performed at Genome Diagnostics Nijmegen, the Netherlands) of the index individual (F3_IV:4) in an unrelated Saudi family (family 3) revealed a variant of unknown significance in *UNC80* (GenBank: NM_032504.1), c.1078C>T (p.Arg360*). This individual is currently 7 years old and has history of profound global developmental delay, central hypotonia, failure to thrive, and no seizures. Although there is a history of neonatal hypotonia, she was only formally evaluated at 5.5 months of age, at which point she was showed early signs of failure to thrive, decelerating head growth (weight 3.6 kg [−3.6 SD], length 59.3 cm [−2 SD], and head circumference 40.1 cm [5th percentile]), as well as generalized hypotonia, normal deep tendon reflexes, strabismus, and no distinctive facial dysmorphia. At her last evaluation, at the

Table 1. Clinical Summary of Affected Individuals

Individual	Age (years)	Consanguinity	Family History	Birth Weight (kg)	Birth Length (cm)	Birth Head Circumference (cm)	Cachexia	Hypotonia	Microcephaly	Seizures	Facial Dysmorphia	Brain MRI	EEG
F1_IV:5	6.5	+	+	3	46	NA	+	+++	+	–	minor	abnormal	ND
F2_IV:1	2	+	+	2.7	51	36	+	+++	+	–	minor	normal	abnormal
F2_IV:2	1	+	+	2.9	53	34	+	+++	–	–	minor	normal	ND
F3_IV:4	7	+	+	2.6	53	34	+	++	+	–	minor	normal	abnormal
F4_V:1	8	+	+	2.8	50	34.5	±	++	–	single	minor	ND	abnormal
F4_V:2	4	+	+	3	51	34	±	++	–	–	minor	ND	ND

+, present; –, absent; ±, borderline; ++, less severe; +++, more severe; NA, not available; ND, not done.

age of 7 years, she weighed 13.45 kg (−3.4 SD), was 109.5 cm long (−2.4 SD), and had a head circumference of 48.5 cm (−2.4 SD). She appeared cachectic with significant muscle wasting, especially in the face, giving rise to mild dysmorphism in the form of a triangular face, bitemporal narrowing, and an apparently large and persistently opened mouth. She was able to sit, but not stand, unsupported. There was virtually no expressive or receptive speech. She had bilateral strabismus, generalized hypotonia, and preserved deep tendon reflexes. Her first-cousin parents had a reportedly similarly affected daughter who died at 16 years of age and have three other healthy daughters. Brain MRI of the index individual revealed normal findings, whereas an EEG revealed non-specific generalized slow-wave abnormalities (continuous generalized delta slowing, disorganized and symmetric) suggestive of mild to moderate encephalopathy but with no epileptiform discharges. Both *UNC80* mutations are absent in an in-house database of 650 exomes from Saudi Arabian individuals and in the ExAC Browser and fully segregate with the phenotype in the respective families (Figure 1). Combination of this family with the other two confirmed the previously highlighted critical locus and increased the corresponding LOD score on linkage analysis to 4.7 (Figure 2).

In an attempt to identify additional indiviuduals with *UNC80*-related encephalopathy, we engaged in an international collaboration with the Laboratory for Pediatric Brain Diseases at the University of California San Diego, who had independently ascertained an Egyptian family with a similar phenotype after obtaining written informed consent with proper approvals from the institutional review board of the local institution. The index individual (F4_V:2) is a 4-year-old girl with signs of intellectual disability and failure to thrive. Her head circumference was 47.5 cm (9^{th} percentile) and her weight was 14 kg (14^{th} percentile). She showed signs of global developmental delay but had no history of seizures. An affected older sister (F4_V:1) in this family was 8 years old at examination and had a head circumference of 50 cm (5^{th} percentile). She also showed a failure to thrive and had a height of 112 cm (−2.8 SD) and a weight of 18.5 kg (−2.2 SD). She presented with profound global developmental delay, hyperactivity, very limited verbal ability, and irritability. Individual F4_V:1 had one seizure episode, and a sleep-deprived EEG revealed moderate dysrhythmia but no epileptiform discharges. Both affected siblings in family 4 had mild facial dysmorphia, including underdeveloped cheeks and relatively large mouth and ears (Figure 1). The clinical features of the three study families are summarized in Table 1.

Exome sequencing of individuals F4_V:1 and F4_V:2 and filtering of the variants via the same pipeline described above revealed a single homozygous surviving variant in *UNC80* (GenBank: XM_005246476.1): c.565G>A (p.Val189Met). This variant is absent in ExAC, has high in silico predicted pathogenicity scores (PolyPhen 0.991,

SIFT 0.00, and CADD 29.3), and is fully segregated with the disease within this family (Figure 2).

UNC80 encodes a large protein of unknown domain structure. It was identified in *C. elegans* in a genetic suppressor screen for mutants that reverse the phenotype observed in worms with gain-of-function mutations in *nca* (*NALCN* ortholog).[16] Unlike loss-of-function *nca* mutant worms that display the "fainter" phenotype (failure to initiate and/or maintain rhythmic locomotion), gain-of-function mutants display exaggerated body bends, or the "coiler" phenotype. This genetic suppressor screen identified two mutants that ameliorate the coiler phenotype: *hp424* and *hp369*, which were found to harbor recessive loss-of-function mutations in *unc-79* and *unc-80*, respectively. Subsequent analysis revealed recapitulation of the *nca*-related "fainter" phenotype in *unc-80* mutants as well as in nca and unc-80 double mutants, suggesting that the two proteins act in the same pathway. In addition, nca and unc-80 were found to be dependent on each other at the protein level, such that deficiency of one leads to deficiency of the other.[16] Similar results were generated from the study of the corresponding fruit fly orthologs.[15] It was later shown that NALCN and UNC80 physically interact and that this interaction is required to recruit UNC79, which is in turn required for NALCN to function as part of a trimeric channel complex.[14] These results are highly consistent with the remarkably similar phenotype observed in *NALCN*- and *UNC80*-related encephalopathy, as we report in this study. They also suggest that *UNC79* is an attractive candidate that should be considered in future genetic analysis of infantile encephalopathy.

Interestingly, Chong et al. recently suggested that de novo *NALCN* dominant mutations can result in a different phenotype consisting of congenital contractures of the face, hands, and feet.[21] Although the authors suggested these are dominant-negative mutations, Aoayagi et al. demonstrated empirically that at least one of these mutations results, in fact, in a coiler phenotype in *C. elegans*, consistent with it being a gain-of-function mutation.[22] These recent findings are relevant to the discussion of the *UNC80*-related phenotype because a single de novo nonsense *UNC80* mutation was previously reported in a large study of de novo mutations in individuals with autism spectrum disorder.[23] Under the assumption that variant was indeed causal, and given the complete lack of neurological abnormalities in the heterozygotes for the truncating mutations reported in our study, one might provoke a different mutational mechanism, e.g., dominant negative, to explain the different phenotype, although this will be difficult because the domain structure of UNC80 is unknown. Unfortunately, we could not test the effect of the mutations identified in this study in easily accessible materials, i.e., lymphoblastoid cells and fibroblasts, because *UNC80* does not appear to be expressed in these tissues. This prompted us to test the expression of its murine ortholog (*Unc80*) in various adult tissues, and we found expression to be nearly exclusive to the brain (Figure S1).

In summary, we show that biallelic mutations in *UNC80* are associated with a severe encephalopathy phenotype that is congenital in onset and lacks epilepsy and major brain malformations, in a pattern highly reminiscent of the *NALCN*-related encephalopathy phenotype. We note the relatively milder phenotype of individuals with the missense variant in comparison to the phenotype of those individuals with truncating variants, although future cases will be needed to properly assess potential genotype-phenotype correlation. With this discovery, *UNC79*, encoding the only component of the UNC80-UNC79-NALCN channel complex with no corresponding human phenotype as of yet, is an attractive candidate gene to be tested in individuals with infantile encephalopathy.

Supplemental Data

Supplemental Data include one figure and can be found with this article online at http://dx.doi.org/10.1016/j.ajhg.2015.11.013.

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Web Resources

The URLs for data presented herein are as follows:

CADD, http://cadd.gs.washington.edu/
ExAC Browser, http://exac.broadinstitute.org/
GenBank, http://www.ncbi.nlm.nih.gov/genbank/
OMIM, http://www.omim.org/
PolyPhen-2, http://genetics.bwh.harvard.edu/pph2/
SIFT, http://sift.bii.a-star.edu.sg/
UCSC Genome Browser, http://genome.ucsc.edu

References

1. Kasperavičiūtė, D., Catarino, C.B., Matarin, M., Leu, C., Novy, J., Tostevin, A., Leal, B., Hessel, E.V., Hallmann, K., Hildebrand, M.S., et al.; UK Brain Expression Consortium (2013). Epilepsy, hippocampal sclerosis and febrile seizures linked by common genetic variation around SCN1A. Brain *136*, 3140–3150.
2. Kullmann, D.M. (2010). Neurological channelopathies. Annu. Rev. Neurosci. *33*, 151–172.
3. Ryan, D.P., and Ptácek, L.J. (2010). Episodic neurological channelopathies. Neuron *68*, 282–292.

4. Kortüm, F., Caputo, V., Bauer, C.K., Stella, L., Ciolfi, A., Alawi, M., Bocchinfuso, G., Flex, E., Paolacci, S., Dentici, M.L., et al. (2015). Mutations in KCNH1 and ATP6V1B2 cause Zimmermann-Laband syndrome. Nat. Genet. *47*, 661–667.
5. Masotti, A., Uva, P., Davis-Keppen, L., Basel-Vanagaite, L., Cohen, L., Pisaneschi, E., Celluzzi, A., Bencivenga, P., Fang, M., Tian, M., et al. (2015). Keppen-Lubinsky syndrome is caused by mutations in the inwardly rectifying K+ channel encoded by KCNJ6. Am. J. Hum. Genet. *96*, 295–300.
6. Takano, K., Liu, D., Tarpey, P., Gallant, E., Lam, A., Witham, S., Alexov, E., Chaubey, A., Stevenson, R.E., Schwartz, C.E., et al. (2012). An X-linked channelopathy with cardiomegaly due to a CLIC2 mutation enhancing ryanodine receptor channel activity. Hum. Mol. Genet. *21*, 4497–4507.
7. Romaniello, R., Zucca, C., Tonelli, A., Bonato, S., Baschirotto, C., Zanotta, N., Epifanio, R., Righini, A., Bresolin, N., Bassi, M.T., and Borgatti, R. (2010). A wide spectrum of clinical, neurophysiological and neuroradiological abnormalities in a family with a novel CACNA1A mutation. J. Neurol. Neurosurg. Psychiatry *81*, 840–843.
8. Trudeau, M.M., Dalton, J.C., Day, J.W., Ranum, L.P., and Meisler, M.H. (2006). Heterozygosity for a protein truncation mutation of sodium channel SCN8A in a patient with cerebellar atrophy, ataxia, and mental retardation. J. Med. Genet. *43*, 527–530.
9. Alazami, A.M., Patel, N., Shamseldin, H.E., Anazi, S., Al-Dosari, M.S., Alzahrani, F., Hijazi, H., Alshammari, M., Aldahmesh, M.A., Salih, M.A., et al. (2015). Accelerating novel candidate gene discovery in neurogenetic disorders via whole-exome sequencing of prescreened multiplex consanguineous families. Cell Rep. *10*, 148–161.
10. Al-Owain, M., Alazami, A.M., and Alkuraya, F.S. (2011). An autosomal recessive syndrome of severe cognitive impairment, dysmorphic facies and skeletal abnormalities maps to the long arm of chromosome 17. Clin. Genet. *80*, 489–492.
11. Lu, B., Su, Y., Das, S., Liu, J., Xia, J., and Ren, D. (2007). The neuronal channel NALCN contributes resting sodium permeability and is required for normal respiratory rhythm. Cell *129*, 371–383.
12. Al-Sayed, M.D., Al-Zaidan, H., Albakheet, A., Hakami, H., Kenana, R., Al-Yafee, Y., Al-Dosary, M., Qari, A., Al-Sheddi, T., Al-Muheiza, M., et al. (2013). Mutations in NALCN cause an autosomal-recessive syndrome with severe hypotonia, speech impairment, and cognitive delay. Am. J. Hum. Genet. *93*, 721–726.
13. Köroğlu, Ç., Seven, M., and Tolun, A. (2013). Recessive truncating NALCN mutation in infantile neuroaxonal dystrophy with facial dysmorphism. J. Med. Genet. *50*, 515–520.
14. Lu, B., Zhang, Q., Wang, H., Wang, Y., Nakayama, M., and Ren, D. (2010). Extracellular calcium controls background current and neuronal excitability via an UNC79-UNC80-NALCN cation channel complex. Neuron *68*, 488–499.
15. Lear, B.C., Darrah, E.J., Aldrich, B.T., Gebre, S., Scott, R.L., Nash, H.A., and Allada, R. (2013). UNC79 and UNC80, putative auxiliary subunits of the NARROW ABDOMEN ion channel, are indispensable for robust circadian locomotor rhythms in Drosophila. PLoS ONE *8*, e78147.
16. Yeh, E., Ng, S., Zhang, M., Bouhours, M., Wang, Y., Wang, M., Hung, W., Aoyagi, K., Melnik-Martinez, K., Li, M., et al. (2008). A putative cation channel, NCA-1, and a novel protein, UNC-80, transmit neuronal activity in C. elegans. PLoS Biol. *6*, e55.
17. Speca, D.J., Chihara, D., Ashique, A.M., Bowers, M.S., Pierce-Shimomura, J.T., Lee, J., Rabbee, N., Speed, T.P., Gularte, R.J., Chitwood, J., et al. (2010). Conserved role of unc-79 in ethanol responses in lightweight mutant mice. PLoS Genet. *6*, e1001057.
18. Alkuraya, F.S. (2010). Autozygome decoded. Genet. Med. *12*, 765–771.
19. Alkuraya, F.S. (2012). Discovery of rare homozygous mutations from studies of consanguineous pedigrees. Current Protocols in Human Genetics (John Wiley & Sons).
20. Alkuraya, F.S. (2013). The application of next-generation sequencing in the autozygosity mapping of human recessive diseases. Hum. Genet. *132*, 1197–1211.
21. Chong, J.X., McMillin, M.J., Shively, K.M., Beck, A.E., Marvin, C.T., Armenteros, J.R., Buckingham, K.J., Nkinsi, N.T., Boyle, E.A., Berry, M.N., et al.; University of Washington Center for Mendelian Genomics (2015). De novo mutations in NALCN cause a syndrome characterized by congenital contractures of the limbs and face, hypotonia, and developmental delay. Am. J. Hum. Genet. *96*, 462–473.
22. Aoyagi, K., Rossignol, E., Hamdan, F.F., Mulcahy, B., Xie, L., Nagamatsu, S., Rouleau, G.A., Zhen, M., and Michaud, J.L. (2015). A gain-of-function mutation in NALCN in a child with intellectual disability, ataxia, and arthrogryposis. Hum. Mutat. *36*, 753–757.
23. Iossifov, I., Ronemus, M., Levy, D., Wang, Z., Hakker, I., Rosenbaum, J., Yamrom, B., Lee, Y.H., Narzisi, G., Leotta, A., et al. (2012). De novo gene disruptions in children on the autistic spectrum. Neuron *74*, 285–299.