

ARHGEF9 disease

Phenotype clarification and genotype-phenotype correlation

OPEN

Michael Alber, MD
Vera M. Kalscheuer, PhD
Elysa Marco, MD
Elliott Sherr, MD, PhD
Gaetan Lesca, MD
Marianne Till, MD
Gyri Gradek, MD
Antje Wiesener, MD
Christoph Korenke, MD,
PhD
Sandra Mercier, MD,
PhD
Felicita Becker, MD
Toshiyuki Yamamoto,
MD, PhD
Stephen W. Scherer, PhD
Christian R. Marshall,
PhD
Susan Walker, PhD
Usha R. Dutta, PhD
Ashwin B. Dalal, MD
Vanessa Suckow
Payman Jamali, MD
Kimia Kahrizi, MD
Hossein Najmabadi, PhD
Berge A. Minassian, MD

Correspondence to
Dr. Alber:
michael.alber@med.uni-tuebingen.de

ABSTRACT

Objective: We aimed to generate a review and description of the phenotypic and genotypic spectra of *ARHGEF9* mutations.

Methods: Patients with mutations or chromosomal disruptions affecting *ARHGEF9* were identified through our clinics and review of the literature. Detailed medical history and examination findings were obtained via a standardized questionnaire, or if this was not possible by reviewing the published phenotypic features.

Results: A total of 18 patients (including 5 females) were identified. Six had de novo, 5 had maternally inherited mutations, and 7 had chromosomal disruptions. All females had strongly skewed X-inactivation in favor of the abnormal X-chromosome. Symptoms presented in early childhood with delayed motor development alone or in combination with seizures. Intellectual disability was severe in most and moderate in patients with milder mutations. Males with severe intellectual disability had severe, often intractable, epilepsy and exhibited a particular facial dysmorphism. Patients with mutations in exon 9 affecting the protein's PH domain did not develop epilepsy.

Conclusions: *ARHGEF9* encodes a crucial neuronal synaptic protein; loss of function of which results in severe intellectual disability, epilepsy, and a particular facial dysmorphism. Loss of only the protein's PH domain function is associated with the absence of epilepsy. *Neurol Genet* 2017;3:e148; doi: 10.1212/NXG.000000000000148

GLOSSARY

AED = antiepileptic drug; **Cb** = collybistin; **NGS** = next-generation sequencing; **XLID** = X-linked intellectual disability.

X-chromosomal mutations are a common cause of intellectual disability (X-linked intellectual disability [XLID]) in males accounting for 10%–12% of ID.^{1,2} XLID is highly heterogeneous and is usually divided into syndromic and nonsyndromic forms, depending on the association with particular clinical findings.^{1,2} Epileptic seizures accompany XLID in almost half the disorders, in some beginning in infancy prior to the developmental delay being evident. Recent next-generation sequencing (NGS) approaches have expedited the identification of XLID genes, including those associated with epilepsy,^{1–3} many of which encode proteins involved in synaptic function.⁴ *ARHGEF9* is one such gene,^{5–9} encoding collybistin (Cb), a brain-specific guanine nucleotide exchange factor with an essential role in inhibitory synaptic transmission.⁵ Cb interacts with the inhibitory receptor–anchoring protein gephyrin and is required for the formation of gephyrin and gephyrin-dependent

See editorial

From the Department of Paediatrics (Neurology) (M.A., B.A.M.), Program in Genetics and Genome Biology (S.W.S., C.R.M., S.W., B.A.M.), The Hospital for Sick Children and University of Toronto, Canada; Research Group Development and Disease (V.M.K., V.S.), Max Planck Institute for Molecular Genetics, Berlin, Germany; Department of Neurology (E.M., E.S.), University of California, San Francisco; Service de Cytogenetique Constitutionnelle (G.L., M.T.), Hospice Civils de Lyon, France; Center for Medical Genetics and Molecular Medicine (G.G.), Haukeland University Hospital, Bergen, Norway; Humangenetisches Institut (A.W.), Universitaetsklinikum Erlangen, Germany; Zentrum für Kinder- und Jugendmedizin (C.K.), Elisabeth Kinderkrankenhaus, Oldenburg, Germany; Service de Genetique Medicale (S.M.), CHU Hotel Dieu, Nantes, France; Neurologische Universitätsklinik (F.B.), Tübingen, Germany; Institute of Medical Genetics (T.Y.), Tokyo Women's Medical University, Japan; Diagnostics Division (U.R.D., A.B.D.), Center for DNA Fingerprinting and Diagnostics, Telangana, India; Shahrood Welfare Organization (P.J.), Shahrood, Iran; and Genetics Research Center (K.K., H.N.), University of Social Welfare and Rehabilitation Sciences, Tehran, Iran.

Funding information and disclosures are provided at the end of the article. Go to Neurology.org/ng for full disclosure forms. The Article Processing Charge was paid by the authors.

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 (CC BY-NC-ND), which permits downloading and sharing the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

GABA_A clusters in the postsynaptic membrane (figure 1).^{5,8,10} Cb knockout mice exhibit increased anxiety, impaired spatial learning, and convulsions.^{10,11}

Over 10 patients with *ARHGEF9* mutations have been reported,^{5-9,12-15} indicating that this will likely be one of the more common XLIDs. Descriptions of the published cases vary from scant (in large NGS projects) to case reports with substantial detail. In the present study, we combine detailed descriptions of the phenotypes of 18 patients with *ARFGH9* mutations. In 4 cases, adequate detail was present at first published description. In 6 cases, we went back to the patients mentioned in tables of NGS results and worked with their physicians to obtain clinical details. The remaining are new unpublished patients.

METHODS Two brothers (patients P10 and P11) with ID and epilepsy and unremarkable MRIs were tested by clinical whole-exome sequencing and found to have mutations in the *ARHGEF9* gene. Further 16 patients (including 5 females) with mutations or chromosomal disruptions affecting *ARHGEF9* were identified through a literature review and by contacting genetic centers in North America, Asia, and Europe.

Standard protocol approvals, registrations, and patient consents. A detailed developmental epilepsy and general medical history together with examination findings were obtained for each patient via a standardized questionnaire filled by their treating physician, or if this was not possible by a review of the described phenotypic features in publications. No samples were obtained

from patients for research purposes. Written consent was obtained from patients' parents for the publication of recognizable photographs.

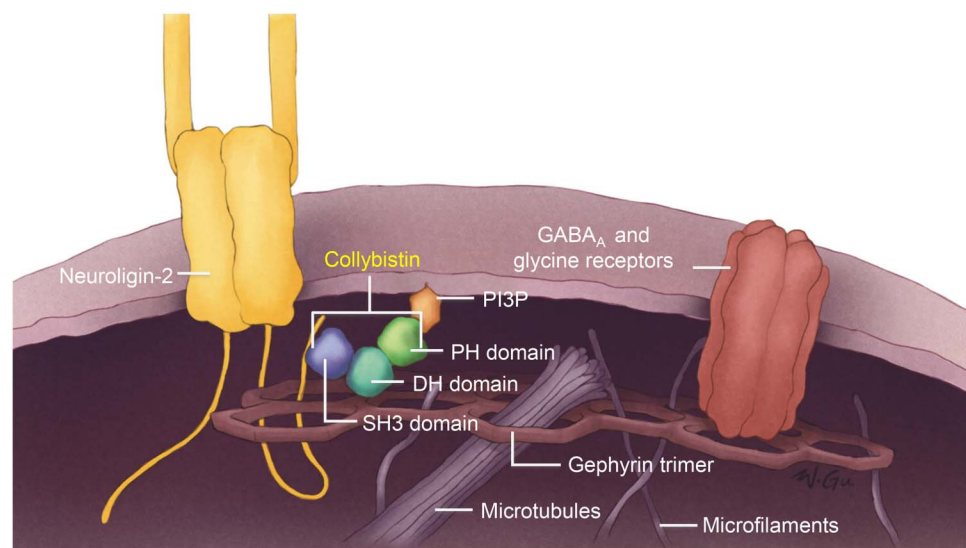
X-chromosome inactivation analysis. In the unpublished cases (patients P1, P3, and P4) X-chromosome inactivation analysis was performed with the human androgen receptor assay: amplification of the CAG repeat in exon 1 of the androgen receptor gene was performed by PCR with fluorescence-tagged primers. Subsequently, digestion with the methylation-sensitive enzyme HpaII was performed, and fragments were analyzed with an automated capillary sequencer (ABI 3100; Applied Biosystems, Foster City, CA). Genescan and Genotyper Software (Applied Biosystems) were used to determine fragment sizes and intensities, and the degree of X-inactivation was calculated.

RESULTS Genetic findings. Tables 1 and 2 summarize the genetic findings of the 18 patients. Eleven had hemizygous *ARHGEF9* mutations, including premature stop codon, splicing, and point mutations. Six were de novo mutations; the 2 above brothers (P10 and 11) and patients P8, P15, and P18 showed a maternal inheritance, the latter with 3 affected brothers with a similar phenotype. Seven had chromosomal disruptions. The 11 mutations were in exons 8 (3), 9 (2), 1 (1), 3 (1), 4 (1), 5 (1), and 7 (1), and in intron 9 (1) (exon numbering based on the longest *ARHGEF9* transcript NM_015185.2). The patients with chromosomal disruptions had deletions (4), translocations (2), and a paracentric inversion (1). The 5 female patients all showed skewed X-inactivation of the normal chromosome.

Clinical features. Tables 1 and 2 summarize the clinical features of the 18 patients.

Demographics. Current age or age at last examination ranged from 4 to 57 years. Presenting symptoms: onset

Figure 1 Interaction of collybistin in the postsynaptic membrane



Schematic representation of the interactions of collybistin in the formation of gephyrin and gephyrin-dependent GABA_A clusters in the postsynaptic membrane.

Table 1 Genetic findings and phenotypic features of patients with *ARHGEF9* mutations or disruptions (females)

Genetic findings and clinical features (reference)	P1	P2 ^a	P3	P4	P5 ⁹
Chromosomal disruption	Balanced translocation (46,XX,t(X;20)(q12;p13))	Balanced translocation (46,X,t(X;18)(q11.1;q11.21))	27 kb Xq11.1 deletion: arrXq11.1(62,838,630-62,865,334)	7.5 kb Xq11.1 deletion: arrXq11.1(62,854,862-62,862,403)x1	Paracentric inversion (46,X,inv(X)(q11.1q27.3))
Inheritance	De novo	De novo	De novo	De novo	De novo
X-inactivation (testing)	Skewed 100 (RT-PCR with <i>ARHGEF9</i> primers vs controls)	Skewed 100 (RT-PCR with <i>ARHGEF9</i> primers vs controls)	Skewed 95:5 (RT-PCR with <i>ARHGEF9</i> primers vs controls)	Skewed 90:10 (Mercier)	Skewed 100 (replication studies on phytohemagglutinin-stimulated T lymphocytes after BrdU incorporation)
Functional study	N/A	Loss of gephyrin + GABA _A R clusters	N/A	N/A	N/A
Current age/age of examination (AE)	10	15 (AE)	9	4	25
Presenting age and symptoms	10 mo; SZ, DD	7 mo; DD	12 mo; DD	31 mo; SZ, DD	18 mo; HA, DD
Dysmorphic features	CD	Facial	Facial	Facial	Facial
Somatic parameter, percentile	C N/A; H <3; W 3	C <3; H <3; W <3	C 50; H 3; W 90	C 90; H 90; W 90	C N/A; H 65; W 60
Developmental milestones, mo	S: 12; W: 16	S: 21	S: 15; W: 22	S: 11; W: 30; Wo: 42	W: 13; Wo: 20
Intellectual disability (IQ testing)	Severe	Severe	Moderate	Moderate	Moderate (48)
Autistic features	Yes	NR	No	No	No
Hyperactivity	Yes	Yes	Yes	No	No
Hyperekplexia	No	No	No	No	No
Hyperarousal	No	No	No	No	Yes
MRI	Normal	Normal	Normal	N/A	UF
Epilepsy	Yes	Yes	No	Yes	No
Seizure types	GTC	GTC	—	F, GTC	—
Seizure onset	10 mo	7 y	—	31 mo	—
EEG findings	Gen. SW	Slow BG	—	Normal	—
AEDs	VPA, PHT, PGB	VPA, CLB	—	None	—
Response to AED	<50% red	NR	—	Seizure free	—
Other features	Nonverbal, hyperpigmentation	Nonverbal (auto-) aggressive behavior, sleep problems, OSA, insensitivity to thermal pain	Hypotonia, pes adductus, sleep problems	PE, enhanced tendon reflexes, mild joint hypermobility	Sensory hyperarousal

Abbreviations: AED = antiepileptic drug; BG = background; BrdU = bromodeoxyuridine; C = head circumference; CD = clinodactyly; CLB = clobazam; DD = developmental delay; GTC = generalized tonic-clonic seizures; H = height; HA = hyperammonemia; N/A = not applicable; NR = not reported; OSA = obstructive sleep apnea; PE = pectus excavatum; PGB = pregabalin; PHT = phenytoin; RT = real time; S = sitting; SW = spike and wave; SZ = seizures; UF = unspecific findings; VPA = valproate; W = body weight; W = independent walking.

of symptoms occurred at a mean age of 15 months (median 9 months, range 1 day–7 years). Presenting symptoms varied from seizures (5), developmental delay in combination with seizures (4), and developmental delay/intellectual disability alone (6). One patient presented with hyperekplexia and seizures shortly after birth and 1 patient with hyperarousal and developmental delay (with no seizures).

Intellectual disability. The majority of patients (13) showed delayed early developmental milestones. All patients had ID ranging from mild (1) to moderate

(7) to severe (10). Of the 10 patients with severe ID, 7 were nonverbal.

Neuropsychiatric features. Autistic features were reported in 4 patients. Five patients had hyperactivity.

Dysmorphic features. Photographs for review of dysmorphic features were available for 9 of the 18 patients. Eight patients showed mild facial dysmorphic features. Out of this group, it appears that the male patients who are most severely affected with the overall neurologic syndrome (i.e., most severe ID and seizures) show more distinctive and consistent facial features, including enlarged, fleshy earlobes, a sunken

Table 2 Genetic findings and phenotypic features of patients with *ARHGEF9* mutations or disruptions (males)

Genetic findings and clinical features (reference)	P6 ⁷	P7 ⁶	P8 ⁶	P9 ⁵	P10	P11	P12
Chromosomal disruption/ NM number	1.29 Mb Xq11.11 deletion: arrXq11.1 (61,848,414-63,138,698) [Hg18]x0	737 kb Xq11.11 deletion: arrXq11.1 (62,321,746-63,058,548) x.dn	NM_001173479	NM_015185.2	NM_015185.2	NM_015185.2	NM_015185.2
Complementary DNA level	N/A	N/A	c.4C>T	c.164G>A	c.950C>G	c.950C>G	c.530T>C
Protein level	N/A	N/A	p.Q2 ^a	p.G55A	p.S317W	p.S317W	p.L177P
Genomic DNA	N/A	N/A	63005022G>A	62944437C>T	62885872G>C	62885872G>C	62917036A>G
Exon	N/A	N/A	1	3	8	8	5
Domain	N/A	N/A	None	SH3	None	None	DH
Functional study	N/A	N/A	N/A	Loss of gephyrin + GABA _A R clusters	N/A	N/A	N/A
Inheritance	De novo	De novo	Maternally	De novo	Maternally	Maternally	De novo
Current age/age of examination (AE)	11	5 (AE)	5 (AE)	D (4)	23	31	4
Presenting age and symptoms	5 mo; SZ	9 mo; DD	6 mo; DD	Birth; HE; SZ	13 mo; SZ	30 mo; SZ	4 mo; SZ
Dysmorphic features	Facial	No	No	NR	Facial	Facial	No
Somatic parameter, percentile	C 97; H 97; W 97	C 90-97; H 75-90; W >97	C 75-90; H 50-75; W 10-25	NR	C 50; H 25; W 10-25	C P50; H 97; W >97	C 25-50; H 90-97; W 50-75
Developmental milestones, mo	S: 12; W: 23	S: 12; W: no	S: 12; W: 24	NR	S: 12; W: 18	S: 6; W: 15	W: 20
Intellectual disability (IQ testing)	Severe	Severe	Severe	Severe	Severe	Severe	Severe
Autistic features	No	NR	NR	NR	Yes	No	Yes
Hyperactivity	Yes	NR	NR	NR	No	No	No
HE	No	NR	NR	Yes	No	No	No
Hyperarousal	No	NR	NR	NR	No	No	No
MRI	Normal	HFL	PMG	BA	UF	Normal	Normal
Epilepsy	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Seizure types	FD, GTC	FD	GTC	T (startle)	GTC	F, FD, GTC	FC, FD, GTC
Seizure onset	5 mo	24 mo	20 mo	Birth	13 mo	30 mo	4 mo
EEG findings	Normal	Bilateral temporal SW	CSWS	NR	Gen. 2 Hz SW, slow BG	Gen. 2 Hz SW, slow BG	Slow BG, 4 Hz rhythm
AEDs	CBZ, PB, TPM, LEV, OXC	VPA	Multiple AEDs	PB, LTG	VPA, CBZ, CLB	CBZ, CLB	VPA, LEV, LTG

Continued

Table 2 Continued

Genetic findings and clinical features (reference)	P6 ⁷	P7 ⁶	P8 ⁶	P9 ⁵	P10	P11	P12
Response to AED	50% red	Seizure free	Refractory	Refractory	50% red	50% red	50% red
Other features	Nonverbal, PE, aggressive behavior	Nonverbal, trigonocephalic head	Nonverbal, ataxic gait	HE	—	Nonverbal	Nonverbal, hypospadias nevus flammeus, sleep problems
Genetic findings and clinical features (reference)	P13	P14 ¹⁵	P15 ^{12,13}	P16 ¹⁴	P17 ¹⁴	P18	
Chromosomal disruption/NM number	NM_015185.2	NM_015185.2	NM_015185.2	NM_015185.2	NM_015185.2	NM_015185.2	
Complementary DNA level	c.311G>A	c.869G>A	c.1012C>T	c.1198G>A	c.1300+2T>C	c.1067G>A	
Protein level	p.R104Q	p.R290H	p.R338W	p.E400K	p. ? Exon skipping	p.R356Q	
Genomic DNA	62926208C>T	62893973C>T	62885810G>A	62875476C>T	62875372A>G	62875607C>T	
Exon	4	7	8	9	Intron 9	9	
Domain	DH	DH	PH	PH	None	PH	
Functional study	N/A	Reduced PI3P-binding + loss of gephyrin clusters	Reduced PI3P-binding + loss of gephyrin clusters	N/A	N/A	N/A	
Inheritance	De novo	De novo	Maternally	De novo	De novo	Maternally	
Current age/age of examination (AE)	15	57	26 (AE)	2 (AE)	3 (AE)	28	
Presenting age and symptoms	12 mo; SZ, DD	6 mo; SZ, DD	N/A	6 mo; DD	2 mo; SZ	7 y; ID	
Dysmorphic features	No	No	Facial	Facial	Facial, CD	No	
Somatic parameter, percentile	C 60; H <3; W <3	N/A	C 90; H 70	C 10-25; H 25-50; W 10-25	C 97; H 50; W N/A	C 97; H 75; W 75	
Developmental milestones, mo	S: 18; W: no	N/A	NR	S: 12; W: 29	S:12; W: no	S: 6; W: 12; Wo: 12	
Intellectual disability (IQ testing)	Severe	Moderate (40-50)	Moderate (43)	Moderate	Moderate	Mild (50-70)	
Autistic features	Yes	No	No	NR	NR	No	
Hyperactivity	Yes	No	NR	NR	NR	No	
HE	No	No	NR	NR	NR	No	
Hyperarousal	No	No	NR	NR	NR	No	
MRI	UF	HS	N/A	Normal	MD	N/A	
Epilepsy	Yes	Yes	Yes	No	No	No	
Seizure types	F, GTC, M, T	FD, GTC	NR	—	—	—	
Seizure onset	12 mo	6 mo	NR	—	—	—	
EEG findings	MISF, 4 Hz rhythm, slow BG	Temporal SW	NR	—	—	—	

Table 2 Continued

Genetic findings and clinical features (reference)	P13	P14 ¹⁵	P15 ^{12,13}	P16 ¹⁴	P17 ¹⁴	P18
AEDs	CBZ, VPA, TPM, LEV, LTG, VGB, Br, STM, CLB, LCS, KD	VPA, TPM, LEV, PRM, LCS, ESL	NR	—	—	—
Response to AED	Refractory	Refractory	NR	—	—	—
Other features	Nonverbal, episodic tachypnea	—	Macro-orchidism	Fetal finger and toe pads, sleep problems	Fetal finger pads, short fifth finger	—

Abbreviations: AED = antiepileptic drug; BA = brain atrophy; BG = background; Br = bromide; C = head circumference; CBZ = carbamazepine; CD = clinodactyly; CLB = clobazam; CSWS = continuous spikes and waves during slow-wave sleep; D = deceased; DD = developmental delay; ESL = eslicarbapazine; F = febrile seizures; FC = focal clonic seizures; FD = focal dyscognitive seizures; GTC = generalized tonic-clonic seizures; H = height; HE = hyperreflexia; HFL = hypoplastic frontal lobe; HS = hippocampal sclerosis; KD = ketogenic diet; LCS = lacosamide; LEV = levetiracetam; LTG = lamotrigine; M = myoclonic seizures; MD = myelination delay; MISF = multiple independent spike foci; N/A = not applicable; NR = not reported; OXC = oxcarbazepine; PB = phenobarbital; PE = pectus excavatum; PMG = polymicrogyria; PRM = primidone; S = sitting; STM = sulthiame; SW = spike and wave; SZ = seizures; T = tonic seizures; TPM = topiramate; UF = unspecified findings; VGB = vigabatrin; VPA = valproate; W = body weight; W = independent walking; Wo = first words.

^aLifted over from Hg18 to Hg19.

appearance of the middle face (midface hypoplasia) in combination with a protruding of the jaw (prognathism) (figure 2). Two patients showed 5-digit clinodactyly.

Neuroimaging findings. MRI data were available in 15 patients. Findings were none (7), hippocampal sclerosis (1), hypoplastic frontal lobe (1), brain atrophy (1), polymicrogyria (1), delayed myelination (1), or nonspecific (T2 hyperintensities, accentuated perivascular spaces) (3).

Seizure/epilepsy details. Thirteen patients had epilepsy with onset at a mean age of 20 months (median 12 months, range 1 week–7 years). Seizure types were variable, including generalized tonic-clonic (9), focal dyscognitive (5), focal (4), myoclonic (1), and tonic (2) seizures. EEG findings were available for 11 of the 13 patients and showed generalized (3), bilateral (1), multifocal (1), and focal (1) epileptiform discharges and were normal in 2 patients. One patient with polymicrogyria showed continuous spike-and-wave discharges in slow-wave sleep. Two patients had only moderate background slowing.

Epilepsy treatment. Four patients had medically refractory epilepsy, 4 showed 50% seizure reduction with antiepileptic drugs (AEDs), 1 showed seizure reduction of less than 50%, and 2 became completely seizure free without further AED treatment. AEDs consisted mainly of combination therapy (10); only 1 patient was on monotherapy (1).

Additional features included sleep disorder (4), ataxic gait (1), aggressive behavior (2), fetal finger and toe pads (2), pigmentation abnormalities (2), pectus excavatum (2), hyperreflexia (1), tachypnea (1), macro-orchidism (1), and insensitivity to thermal pain (1).

DISCUSSION We describe and review the phenotypes of 18 patients with *ARHGEF9* mutations and chromosomal disruptions. Symptoms usually present in early childhood with delayed motor development alone or in combination with seizures. ID later on is usually moderate to severe with the majority of severe cases being nonverbal.

Epilepsy is common (13/18), starting usually at an early age (median 12 months). Seizure semiology is variable with a majority of patients showing generalized tonic-clonic seizures and many showing multiple seizure types. Most of the epilepsies were difficult to treat with 10 patients on combination AED therapy.

Facial dysmorphism, in particular, enlarged earlobes, midface hypoplasia, and prognathism appear to be a constant feature of the neurologically severely affected male patients.

The overall picture, therefore, is one of a moderate-to-severe XLID (with severe cases nonverbal), moderate-to-severe epilepsy (with severe cases poorly

Figure 2 Facial dysmorphism of 2 severely affected male patients



Patients P6 (A) and P11 (B) from tables 1 and 2. Note enlarged fleshy earlobes, midface hypoplasia, and prognathism.

responsive or intractable to AEDs), and a facial dysmorphism in the severe cases.

Mothers of the various male patients in our study do not have XLID, indicating that random skewing of X-inactivation protects female carriers of *ARHGEF9* mutations. We did, however, find 5 female patients. X-inactivation studies in all 5 showed skewing in favor of the abnormal X-chromosome (table 1), rendering them equivalent to male counterparts with hemizygous mutations. The female phenotype would invariably be affected by remaining expression from the normal allele in cells in which it is active, and by differences in the extent of X-inactivation skewing in the brain compared to leucocytes.

Close analysis of males in the genotype and phenotype tables (1 and 2, respectively) suggests a tentative genotype—epilepsy phenotype correlation related to the region of the protein affected by the

mutation. To clarify this, we briefly review some aspects of Cb structure and function. Inhibitory neurotransmitter receptor channels (GABA_A and glycine) imbedded in the postsynaptic membrane are anchored and clustered therein by a submembrane lattice composed of the gephyrin protein. The gephyrin lattice itself binds the plasma membrane via intermediary proteins critical among which is Cb. Cb binds gephyrin through its DH domain and attaches to the membrane through both its termini as follows: the N-terminal SH3 domain binds the membrane-integral neuroligin-2 protein, and the C-terminal PH domain binds the phosphoinositol 3-phosphate (PI₃P) constituent of the plasma membrane (figure 1).^{5,8,10} In male patients P6 and P7, the entire *ARHGEF9* gene is deleted, and in patient P8, there is a premature stop in the second codon (table 1). These 3 patients therefore lack Cb altogether, and their phenotypes would be representative of the complete loss of Cb function. Table 2 shows that all 3 have severe ID and epilepsy. Patients 9 through 15 have various missense mutations affecting different regions of the protein, but not the C-terminal PI₃P-interacting PH domain. All have epilepsy. Where functional experiments were performed (patients 9, 14, and 15), it was shown that the mutations result in loss of gephyrin clusters.^{5,12,16} The 3 remaining patients (16 through 18) all have mutations that affect the PH domain. None of these patients have epilepsy (and all have mild-to-moderate ID). Therefore, tentatively, mutations affecting only the PI₃P-interacting C-terminus of the protein are not associated with epilepsy. Future functional experiments for these and subsequent PH domain mutations may uncover differences from the effects of the epileptogenic mutations and a possible understanding of epileptogenesis in this disease.

An additional important observation gleaned from our summary tables is comparing male patients P8 and P9 (table 2). Patient P8 has a stop mutation, p.Q2*, in the very first exon, and his symptom constellation is very severe and similar to patients P6 and P7 in whom the entire gene is deleted. This first exon is coding, and therefore mutation p.Q2* truncating, in a shorter transcript (NM_001173479) that skips over the next 2 exons utilized by the longest transcript (NM_015185.2) of the gene. The shorter transcript, NM_001173479, was confirmed to be highly expressed in both the developing and adult human brain and may be the dominant transcript.⁶ Patient P9 is the very first *ARHGEF9* patient described, and a striking feature of his presentation was hyperreflexia. His mutation is in exon 2, of the longer transcript, which is not utilized by the shorter transcript. Hyperreflexia is not found in any of the patients described

after that first patient (tables 1 and 2), which suggests that this symptom may result from a particular function of the slightly longer protein encoded by the longer transcript.

As in so many other neurogenetic disorders, our review reveals that the *ARHGEF9* disease is not uniform and varies with the gene mutation and likely other genetic and extragenetic factors. Therefore, the diagnostic approach in candidate patients still requires chromosomal microarray testing as the first-line genetic testing because of the substantial diagnostic yield and low relative cost, followed by a gene panel or whole-exome sequencing approach as second tier.

There is, however, a core phenotype of moderate-to-severe ID and epilepsy with a facial dysmorphism (large earlobes, midface hypoplasia, and prognathism) in patients with complete loss of the gene's function.

This phenotype is moderated with lesser mutations—less severe intellectual disability and seizures, and absence of facial dysmorphism. As mentioned, mutations affecting the last exon alone appear to be nonpilotogenic.

AUTHOR CONTRIBUTIONS

Michael Alber: acquisition of data, analysis and interpretation of data, and study supervision. Vera M. Kalscheuer, Elysa Marco, Elliott Sherr, Gaetan Lesca, Marianne Till, Gyri Gradek, Antje Wiesener, Christoph Korenke, Sandra Mercier, Felicitas Becker, Toshiyuki Yamamoto, Stephen W. Scherer, and Christian R. Marshall: acquisition of data and critical revision of manuscript for intellectual content. Susan Walker: acquisition of data, analysis of data, and critical revision of manuscript for intellectual content. Usha R. Dutta, Ashwin B. Dalal, Vanessa Suckow, Payman Jamali, Kimia Kahrizi, and Hossein Najmabadi: acquisition of data and critical revision of manuscript for intellectual content. Berge A. Minassian: acquisition of data, analysis and interpretation of data, and critical revision of manuscript for intellectual content.

ACKNOWLEDGMENT

The authors thank the patients and their families. They thank Ms. Wendy Gu (University Toronto) for generating the graphic of figure 1.

STUDY FUNDING

Study funded by Genome Canada and the Ontario Brain Institute. This work was supported by the Ontario Brain Institute, Genome Canada, The McLaughlin Foundation, The Centre for Applied Genomics and the EU FP7 project GENCODYS, grant number 241995 (to V.M.K., H.N., and K.K.). B.A.M. holds the University of Toronto Michael Bahen chair in Epilepsy Research.

DISCLOSURE

Dr. Alber has received travel funding/speaker honoraria from AES Fellows Program 2015. Dr. Kalscheuer reports no disclosures. Dr. Marco has been a consultant for Grand Rounds Second Opinions (Clinical opinions through UCSF) and has received research support from the Wallace Research Foundation. Dr. Sherr has served on the scientific advisory board of InVita; holds a patent for Blood-based diagnostic for autism; has been a consultant for Personalis; has received research support from National Institute of Neurological Disorders and Stroke, Simons Foundation, CURE Foundation, and the John and Marsha Goldman Foundation; holds stock/stock options in InVita and ChemoCentryx; has worked with the following law firms: Sheuerman, Martini & Tabari; Galloway and Lucchese; Zuger Kirmis & Smith; Donahoe & Kearney;

LaFollette, Johnson; Huff Powell & Bailey; and Bell, Roper and Kohlmyer; and has served on the Board of Directors of the National Organization of Disorders of the Corpus Callosum. Dr. Lesca, Dr. Till, Dr. Gradek, Dr. Wiesener, Dr. Korenke, Dr. Mercier, and Dr. Becker report no disclosures. Dr. Yamamoto has received research support from the Japan Ministry of Education, Science, Sports and Culture. Dr. Scherer has served on the scientific advisory board of Population Diagnostics; has served on the editorial boards of *Genomic Medicine*, *Genes*, *Genomes*, *Genetics*, *Journal of Personalized Medicine*, *The Open Genomics Journal*, *The Hugo Journal*, *Genome Medicine*, the *Journal of Neurodevelopmental Disorders*, *Autism Research*, *PathoGenetics*, *Comparative and Functional Genomics*, *BMC Medical Genomics*, and *Cytogenetics and Genome Research*; and has received research support from Genome Canada/Ontario Genomics Institute, Canadian Institutes of Health Research, Canadian Institute for Advanced Research, McLaughlin Centre, Canada Foundation for Innovation, the government of Ontario, Autism Speaks, and SickKids Foundation. Dr. Marshall has received travel funding from Affymetrix and Life Technologies and serves on the editorial board of *Genes*, *Genomes*, *Genetics*. Dr. Walker reports no disclosures. Dr. Dutta has received research support from the Department of Biotechnology—India (University of Mysore) and SERB. Dr. Dalal has served on the editorial board of *Official Publication of Society for Indian Academy of Medical Genetics* and has received research support from the Department of Science and Technology (Govt. of India) and the Indian Council of Medical Research (Govt. of India). Dr. Suckow, Dr. Jamali, Dr. Kahrizi, and Dr. Najmabadi report no disclosures. Dr. Minassian holds patents for diagnostic testing of the following genes: EPM2A, EPM2B, MECP2, VMA21; has received research support from the National Institute of Neurological Disorders and Stroke of the NIH under award number P01 NS097197; and receives license fee payments/royalty payments from patents for diagnostic testing of the following genes: EPM2A, EPM2B, MECP2, VMA21. Go to Neurology.org/ng for full disclosure forms.

Received November 17, 2016. Accepted in final form March 14, 2017.

REFERENCES

1. Ropers HH Genetics of early onset cognitive impairment. *Annu Rev Genomics Hum Genet* 2010;11:167–187.
2. Stevenson RE, Charles E, Schwartz R, Rogers RC. Atlas of X-linked Intellectual Disability Syndromes, 2nd ed. New York: Oxford University Press; 2012.
3. Hu H, Haas SA, Chelly J, et al. X-exome sequencing of 405 unresolved families identifies seven novel intellectual disability genes. *Mol Psychiatry* 2016;21:133–148.
4. Humeau Y, Gambino F, Chelly J, Vitale N. X-linked mental retardation: focus on synaptic function and plasticity. *J Neurochem* 2009;109:1–14.
5. Harvey K, Duguid IC, Alldred MJ, et al. The GDP-GTP exchange factor collybistin: an essential determinant of neuronal gephyrin clustering. *J Neurosci* 2004;24:5816–5826.
6. Shimojima K, Sugawara M, Shichiji M, et al. Loss-of-function mutation of collybistin is responsible for X-linked mental retardation associated with epilepsy. *J Hum Genet* 2011;56:561–565.
7. Lesca G, Till M, Labalme A, et al. De novo Xq11.11 microdeletion including ARHGEF9 in a boy with mental retardation, epilepsy, macrosomia, and dysmorphic features. *Am J Med Genet A* 2011;155A:1706–1711.
8. Kalscheuer VM, Musante L, Fang C, et al. A balanced chromosomal translocation disrupting ARHGEF9 is associated with epilepsy, anxiety, aggression, and mental retardation. *Hum Mutat* 2009;30:61–68.
9. Marco EJ, Abidi FE, Bristow J, et al. ARHGEF9 disruption in a female patient is associated with X linked mental retardation and sensory hyperarousal. *J Med Genet* 2008;45:100–105.

10. Papadopoulos T, Soykan T. The role of collybistin in gephyrin clustering at inhibitory synapses: facts and open questions. *Front Cell Neurosci* 2011;24:5–11.
11. Papadopoulos T, Korte M, Eulenburg V, et al. Impaired GABAergic transmission and altered hippocampal synaptic plasticity in collybistin-deficient mice. *EMBO J* 2007;26:3888–3899.
12. Long P, May MM, James VM, et al. Missense mutation R338W in ARHGEF9 in a family with X-linked intellectual disability with variable macrocephaly and macro-orchidism. *Front Mol Neurosci* 2016;8:83.
13. Johnson JP, Nelson R, Schwartz CE. A family with mental retardation, variable macrocephaly and macro-orchidism, and linkage to Xq12-q21. *J Med Genet* 1998;35:1026–1030.
14. de Ligt J, Willemsen MH, van Bon BW, et al. Diagnostic exome sequencing in persons with severe intellectual disability. *N Engl J Med* 2012;367:1921–1929.
15. Lemke JR, Riesch E, Scheurenbrand T, et al. Targeted next generation sequencing as a diagnostic tool in epileptic disorders. *Epilepsia* 2012;53:1387–1398.
16. Papadopoulos T, Schemm R, Grubmüller H, Brose N. Lipid binding defects and perturbed synaptogenic activity of a collybistin R290H mutant that causes epilepsy and intellectual disability. *J Biol Chem* 2015;290:8256–8270.