REPORT

Monoallelic and Biallelic Variants in *EMC1* Identified in Individuals with Global Developmental Delay, Hypotonia, Scoliosis, and Cerebellar Atrophy

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The paradigm of a single gene associated with one specific phenotype and mode of inheritance has been repeatedly challenged. Genotype-phenotype correlations can often be traced to different mutation types, localization of the variants in distinct protein domains, or the trigger of or escape from nonsense-mediated decay. Using whole-exome sequencing, we identified homozygous variants in *EMC1* that segregated with a phenotype of developmental delay, hypotonia, scoliosis, and cerebellar atrophy in three families. In addition, a de novo heterozygous *EMC1* variant was seen in an individual with a similar clinical and MRI imaging phenotype. *EMC1* encodes a member of the endoplasmic reticulum (ER)-membrane protein complex (EMC), an evolutionarily conserved complex that has been proposed to have multiple roles in ER-associated degradation, ER-mitochondria tethering, and proper assembly of multi-pass transmembrane proteins. Perturbations of protein folding and organelle crosstalk have been implicated in neurodegenerative processes including cerebellar atrophy. We propose *EMC1* as a gene in which either biallelic or monoallelic variants might lead to a syndrome including intellectual disability and preferential degeneration of the cerebellum.

The endoplasmic reticulum (ER)-membrane protein complex (EMC) family was first identified in yeast as a multiprotein transmembrane complex containing six subunits, with a possible function in elimination of misfolded membrane proteins in the ER.[1] Studies in *C. elegans* suggest that emc-6 is required for protein folding and that it possibly modulates ER-associated degradation (ERAD) of specific proteins.[2] More recent studies have implicated the EMC in facilitating contacts between the ER and mitochondria, perhaps by modulating the folding or processing of proteins directly involved in such tethering.[3] The EMC is required for the stable expression of other multi-pass transmembrane proteins such as rhodopsin, and its loss has been associated with retinal degeneration in *Drosophila*.[4] Despite these advances in understanding, the function of the EMC and its relevance to human disease remain to be elucidated.

Aberrations of protein folding, the unfolded protein response, ERAD, and glial lipid droplet accumulation and reactive oxygen species induced by mitochondrial defects have been associated with neurodegeneration.[5–7] Aberrant communication between mitochondria and the ER has been implicated in Alzheimer disease (MIM: 104300) (*PSEN1* [MIM: 104311], *PSEN2* [MIM: 600759]), amyotrophic lateral sclerosis (ALS) type 8 (MIM: 608627) (*VAPB* [MIM: 605704]), ALS type 16 (MIM: 614373) (*SIGMAR1* [MIM: 601978]), Charcot-Marie-Tooth disease (CMT) type 2A (MIM: 609260) (*MFN2* [MIM: 608507]), and Parkinson disease (MIM: 168601) (*SNCA* [MIM: 163890]).[8] Specifically, upregulation of mitochondria-associated ER membrane (MAM) function with increased ER-mitochondrial communication has been detected in presenilin mutant cells as well as in fibroblasts from individuals with both familial and sporadic forms of Alzheimer disease.[9] α-synuclein, encoded by *SNCA* and implicated in Parkinson disease (PD), has recently been shown to localize to MAM rather than to the mitochondria, and MAM function is downregulated in cell and mouse models of PD expressing pathogenic variants of α-synuclein. Early events occurring in dopaminergic neurons at the level of MAM are hypothesized to possibly cause long-term disturbances that might lead to PD.[10,11]

In this study, we report four different families affected by developmental delay, hypotonia, scoliosis, and cerebellar atrophy. All affected individuals had potential pathogenic *EMC1* variants, including a homozygous frameshift variant, two homozygous missense variants, and a de novo heterozygous variant, that were identified by whole-exome

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http://dx.doi.org/10.1016/j.ajhg.2016.01.011.

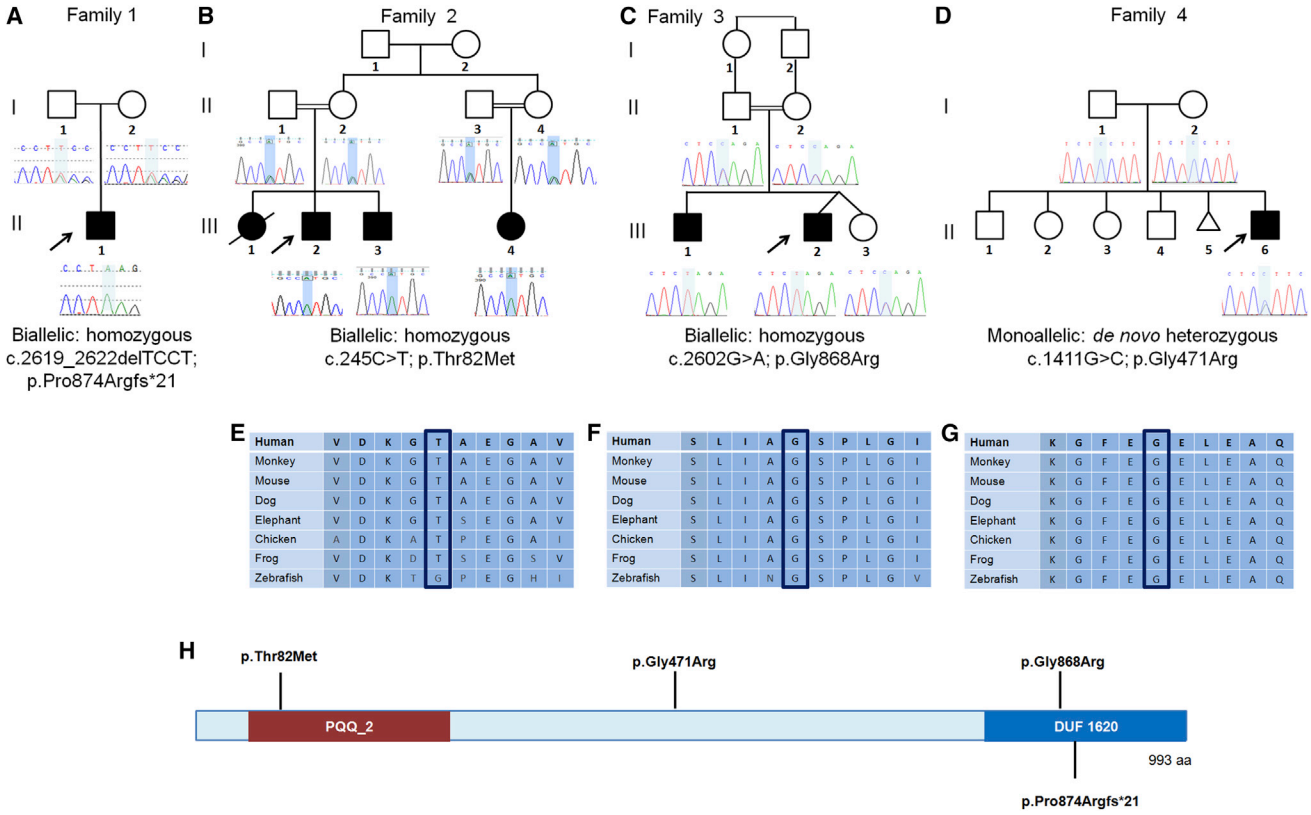

Figure 1. Pedigrees and Sanger Validation and Segregation of the Identified Variants
(A) Family 1.
(B) Family 2. Note the consanguinity.
(C) Family 3. Note the consanguinity.
(D) Family 4. Segregation of the monoallelic variant in *EMC1* in individual II-6 demonstrates the variant in the proband but not in his parents, consistent with a de novo event.
(E–G) Evolutionary conservation of the altered amino acid residue at positions 82 (E), 868 (F), and 471 (G).
(H) Depiction of the conserved domains of EMC1 and location of the variants identified in the four families.

sequencing (WES) in the respective families. We characterize the phenotypic features of this syndrome and propose *EMC1* as a gene in which either biallelic (homozygous) or monoallelic (heterozygous) variants might lead to neurodegenerative disease.

After informed consent was given, family 1 was sequenced by a trio approach (proband A3, II-1 in Figure 1A and parents) with an Agilent SureSelect Human All Exon V4 (50 Mb) kit (Agilent Technologies). The individuals in families 2 and 3 provided consent according to the Baylor-Hopkins Center for Mendelian Genomics (BHCMG) research protocol, and WES was performed on two individuals in family 2 (BAB3445, III-2 and BAB4742, III- 4 in Figure 1B) and two individuals in family 3 (BAB6897, III-1 and BAB6896, III-2 in Figure 1C), according to previously described protocols.[12] Individual BH4387_1 (family 4, II-6 in Figure 1D) underwent clinical WES at Baylor-Miraca Genetics Laboratories (Baylor College of Medicine [BCM]), according to the protocol described in Yang et al.[13] Because this did not reveal a predicted pathogenic variant in any known gene associated with the phenotype, the family gave consent for research studies under a protocol approved by the BCM institutional review board. Parental exome sequencing was obtained through the BHCMG research initiative. In brief, for families 2–4, DNA samples were prepared into Illumina paired-end libraries and underwent whole-exome capture via the BCM-HGSC core design (52 Mb, Roche NimbleGen), followed by sequencing on the Illumina HiSeq 2000 platform (Illumina). Average depth of coverage on WES for all affected individuals ranged from ~81× to ~201×. Specifically, in individual BH4387_1 (family 4, II-6 in Figure 1D), all exons of *EMC1* were covered at an average of 64× or greater and copy-number variants (CNVs) were analyzed with the CoNVex and CoNIFER tools. Data produced were aligned and mapped to the human genome reference sequence (Genome Reference Consortium GRCh37) with the Mercury pipeline.[14] Single-nucleotide variants (SNVs) were called with the ATLAS (an integrative variant analysis pipeline optimized for variant discovery) variant calling method and SAMtools (the Sequence Alignment/Map) and annotated with the in-house-developed "Cassandra" annotation pipeline that uses ANNOVAR (annotation of genetic variants) and additional tools and databases.[15–17] The GeneMatcher tool[18] facilitated identification of family 1, which has a homozygous frameshift *EMC1* variant (see Supplemental Note for detailed downstream WES analysis).

Analysis of families 1 and 4 assumed either de novo or recessive inheritance. Analysis of family 2 assumed a recessive inheritance pattern on the basis of consanguinity, unaffected parents, and multiple affected members, both females and males. Family 3 was analyzed for recessive or X-linked models. We calculated de novo variants by in silico subtraction of parental variants from the proband's variants in VCF files and by using the read depth-of-coverage information extracted from BAM files. Bioinformatics tools were used to predict conservation and pathogenicity of candidate variants, and variants were compared to an internal database (~5,000 exomes) and publicly available databases such as the 1000 Genomes Project, the NHLBI GO Exome Sequencing Project (ESP) Exome Variant Server, the Atherosclerosis Risk in Communities Study (ARIC) Database, and the Exome Aggregation Consortium (ExAC) database. Amplicons containing the candidate variants identified by WES were amplified from genomic DNA via conventional PCR. The promoter region of *EMC1* was amplified in individual BH4387_1 (II-6, Figure 1D) to assess for intronic variation not detected by WES. Primers are listed in Table S1. PCR products were purified with ExoSAP-IT (Affymetrix) and analyzed by standard Sanger di-deoxy nucleotide sequencing (DNA Sequencing Core Facility, BCM).

The clinical findings for seven affected individuals from four families are summarized in Table 1; detailed case reports can be found in the Supplemental Note and photos are available in Figure 2. Two families reported known consanguinity and included multiple affected individuals; the other two families each had a single affected individual (Figure 1). Ancestries were diverse and included European, Turkish, Saudi Arabian, and Hispanic. All affected individuals shared global developmental delay, ranging from severe (families 1, 2, and 4) to mild-moderate (family 3) (see Supplemental Note for details). Ophthalmology exam was abnormal in all families, and included cortical visual impairment (family 1), abnormal visual evoked potentials and abnormal electroretinogram (family 2), esotropia, hyperopia, and astigmatism (family 3), and myopia and optic atrophy (family 4). Additional shared features in three of the families (families 1, 2, and 4) were scoliosis and diminished deep tendon reflexes (DTRs). Dystonic posturing and increased tone in the extremities were found in two families (families 1 and 2). Birth and serial postnatal head circumference measurements in affected individuals in family 2 indicated an acquired microcephaly, with normal head circumferences at birth (~50th percentile) decreasing to *Z* scores ranging from −3.3 to −4 in childhood. Features unique to family 1 included subclinical multifocal electrographic seizures, laryngotracheomalacia, and imperforate anus.

Brain MRI studies (Figure 2) showed cerebellar atrophy and a foreshortened corpus callosum in all four families. Affected individuals in families 1, 2, and 4 all had varying degrees of cerebral atrophy, and affected individuals in family 1 also had small hippocampi. Sequential MRI studies at two different ages were available for individuals in families 1, 2, and 4. These indicated a progression of brain atrophy, most pronounced in the cerebellum, implicating a neurodegenerative process (Figures 2K and 2L and 2R and 2S).

WES identified a homozygous frameshift variant (chr1:g.19547308_19547311delAGGA [c.2619_2622delTCCT; p.Pro874Argfs*21], UCSC Browser hg19) in *EMC1* (GenBank: NM_015047.2) in family 1 (Figure 1A). This variant is located in exon 21 of 23, and thus might trigger nonsense-mediated decay (NMD) and cause a loss-of-function allele. RNA and protein from the affected individual were not available for study. Although consanguinity was not known in this family, WES data indicate that this variant lies in a small stretch of absence of heterozygosity (AOH) spanning ~100 kb (see Supplemental Note). A recent study that identified a small shared region of AOH spanning ~140 kb between families of common ethnicity estimated a common ancestor approximately 16.2 generations in the past (± 8.7 generations).[19] Thus, it might be expected that this *EMC1* frameshift variant could be encountered in the future in additional families with global developmental delay and progressive cerebral and cerebellar atrophy.

Homozygous missense variants in *EMC1* were identified in families 2 and 3 (Supplemental Note, Table S2). The three available affected individuals in family 2 shared a homozygous variant (chr1:g.19570485G>A [c.245C>T; p.Thr82Met], hg19), located within a shared AOH region of ~9.26 Mb (Figure S1). This *EMC1* variant alters a relatively conserved amino acid (Figure 1E) from a polar to a nonpolar residue, has a CADD phred-like score of 29.8, and is predicted to be deleterious by four of four additional bioinformatics prediction programs (Table S2). This variant has not been observed in publicly available databases and was seen only once in a heterozygous state in our internal database. In family 3, homozygous variants in three potential candidate genes segregated with the phenotype in the family, including a homozygous missense variant in *EMC1* (chr1:g.19547328C>T [c.2602G>A; p.Gly868Arg], hg19) with a CADD phred-like score of 33 and deleterious predictions in three of four other prediction programs (Table S2). Notably, a homozygous loss-of-function variant in *SMCO2* was also identified, suggesting the potential contribution of variants to the manifested disease trait at more than one locus. However, given the involvement of the cerebellum on neuroimaging and the foreshortened corpus callosum, findings that are consistent with (yet milder than) the findings in the other three families affected by *EMC1* variants described in this study, we cannot rule out the contribution of a mild *EMC1* allele to the phenotype. This can be viewed in accordance with recent publications describing dual diagnoses in individuals[13] and particularly the contribution of multiple loci for recessive disease diagnoses in children born to consanguineous parents (unpublished data).

Analysis of trio WES in family 4 yielded a number of rare variants (Table S2), including a single nonsynonymous heterozygous de novo variant (chr1:g.19561645C>G

Table 1. Clinical Features of Individuals with *EMC1* Variants

	Family 1	Family 2			Family 3		Family 4	No. of Families Affected
	A3	BAB3445	BAB3446	BAB4742	BAB6896	BAB6897	BH4387_1	
Age at last exam	4 years	13 years	5 years	3 years	10 years	12 years	12 years	NA
Global developmental delay	+	+	+	+	+	+	+	4/4
Speech delay	+	+	+	+	+	+	+	4/4
Seizures	+ (subclinical)	–	–	–	–	–	–	1/4
Scoliosis	+	+	+	+	–	–	+	3/4
Head circumference: approximate Z score[a]	−1.6 (2 years 9 months)	−4	−3.8	−3.3	0	0	−0.34	NA
Dysmorphic features	short upper lip, mild hypertelorism, retrognathia	deep-set eyes, gingival hyperplasia, short philtrum, retrognathia, persistent fetal fingerpads	deep-set eyes, gingival hyperplasia, short philtrum, retrognathia, persistent fetal fingerpads	deep-set eyes, gingival hyperplasia, short philtrum, retrognathia, persistent fetal fingerpads	deep-set eyes, retrognathia	deep-set eyes	low anterior hairline, dysplastic ears, gingival hyperplasia, micrognathia	NA
Truncal hypotonia	+	+	+	+	+	–	+	4/4
Increased tone in extremities	+	+	+	+	–	–	–	2/4
Diminished DTRs	+	+	+	+	NR	–	+	3/4
Dystonic posturing	+	+	+	+	–	–	–	2/4
Abnormal ophthalmology exam	cortical visual impairment	abnormal VEP and ERG	abnormal VEP and ERG	abnormal VEP and ERG	esotropia, hyperopia, astigmatism	NR	myopia, optic atrophy	4/4
Cerebellar atrophy	+	+	+	+	+	NR	+	4/4
Cerebral atrophy	+	+	+	+	–	NR	+ (mild)	3/4
Foreshortened or atrophic corpus callosum	+	+	+	+	+	NR	+	4/4
Other clinical findings	laryngotracheomalacia, imperforate anus	NR	NR	NR	NR	NR	vitiligo	NA
Variants in *EMC1*	c.2619_2622delTCCT (p.Pro874Argfs*21), hom.	c.245C>T (p.Thr82Met), hom.	c.245C>T (p.Thr82Met), hom.	c.245C>T (p.Thr82Met), hom.	c.2602G>A (p.Gly868Arg), hom.		c.1411G>C (p.Gly471Arg), de novo	NA

Abbreviations are as follows: DTRs, deep tendon reflexes; ERG, electroretinogram; hom., homozygous; NA, not applicable; NR, not reported; VEP, visual evoked potential.
[a]The age in parentheses indicates the age at which head circumference was measured, if not at last exam.

[c.1411G>C; p.Gly471Arg], hg19) in *EMC1*. The p.Gly471Arg variant alters a conserved amino acid (Figures 1D and 1G) from a nonpolar neutral residue to a polar basic residue, has a CADD phred-like score of 28.2, and is predicted to be deleterious by MutationTaster and LRT (Table S2). The variant was not observed in any publicly available database nor in our internal database. All exons of *EMC1* were covered at an average of 64× or greater in this individual, and no other rare missense variants in *EMC1* were observed. In search of a possible second hit in this gene, we evaluated the exome read depth data for a CNV and did not identify a deletion within or near *EMC1*. Moreover, we sequenced the promoter region of *EMC1*, but no additional rare variant was identified (data not shown). Using a combination of in silico tools and literature search, we were unable to identify any published interactions between EMC1 and other proteins implicated in numerous types of cerebellar atrophy or pontocerebellar hypoplasia (Table S3).

The summation of the molecular data in these four families with varying degrees of developmental delay and cerebellar atrophy includes homozygous variants in

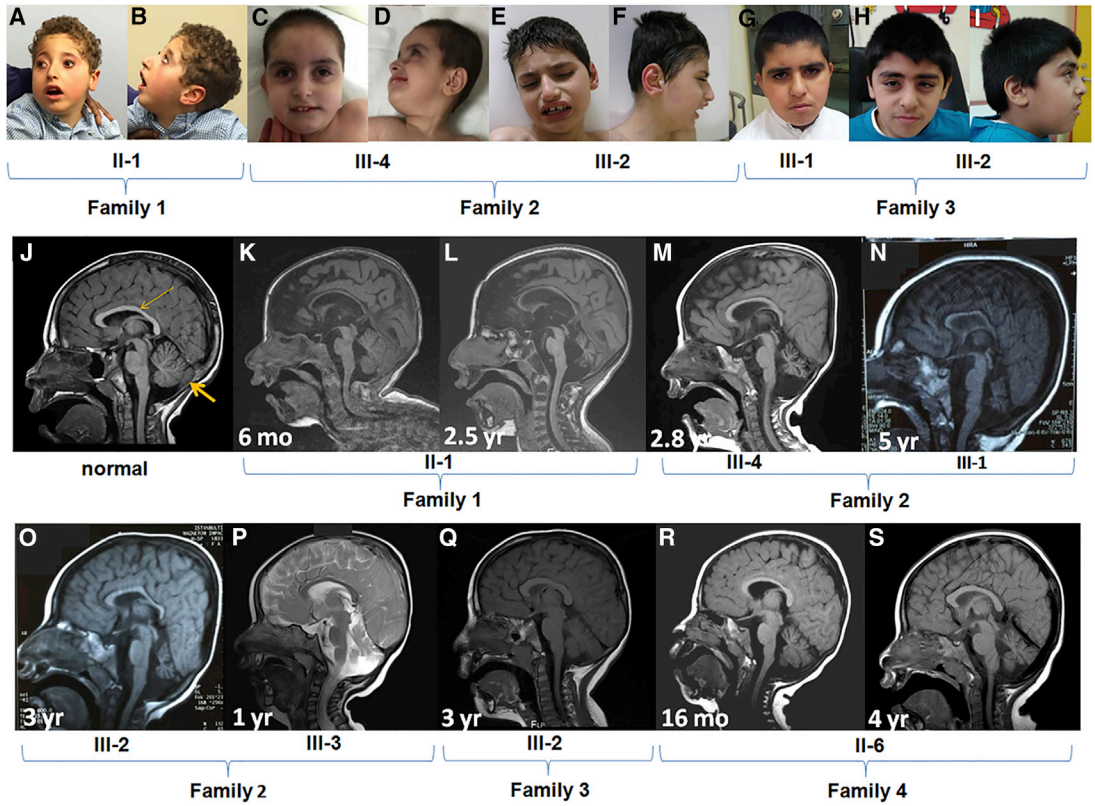

Figure 2. Facial Features and Midsagittal Brain MRI Sections of Affected Individuals
(A–L) Front and profile photographs of affected individuals from families 1–3 (A–I) and T1-weighted sagittal images (K–L) indicate progressive cerebellar atrophy, atrophic corpus callosum, and diminished cerebral white matter in family 1, as compared to the control individual (J). Thick arrow points to cerebellum vermis; thin arrow indicates corpus callosum.
(M–P) T1-weighted (M–O) and T2-weighted (P) sagittal images indicate cerebellar atrophy, cerebral atrophy, and a thin foreshortened corpus callosum in affected individuals of family 2.
(Q) Atrophy of the superior cerebellar vermis and a foreshortened corpus callosum in family 3.
(R and S) Progressive cerebellar atrophy, mild cerebral atrophy, and foreshortened corpus callosum in family 4.
Identifiers refer to pedigree numbers in Figure 1.

EMC1 in three families (c.2619_2622delTCCT, c.245C>T, and c.2602G>A) and a de novo heterozygous variant (c.1411G>C) in a fourth family (Figure 1). Common phenotypic features included progressive neurodegeneration with a predilection for the cerebellum (4/4 families), global developmental delay with marked speech delay (4/4 families), scoliosis (3/4 families), truncal hypotonia (4/4 families), diminished DTRs (4/4 families), dystonic posturing (2/4 families), and an abnormal ophthalmological exam (4/4 families) (Table 1). Serial brain MRI results from individuals in families 1, 2, and 4 indicated interval development of cerebellar atrophy, suggesting a progressive neurodegenerative process. Only a single MRI (at the age of 3 years) was available for an affected individual from family 3, so we could not evaluate progression of the atrophy in that family.

Cerebellar atrophy, implying irreversible and progressive loss of tissue, is typically more pronounced in the cerebellar vermis than in the hemispheres[20] and can be subgrouped into categories based on associated neuroimaging findings in an effort to direct diagnosis.[21] Molecularly, cerebellar atrophy can involve various mechanisms, including defective DNA damage response (i.e., *ATM* [MIM: 607585] in ataxia telangiectasia [MIM: 208900]), abnormal DNA single-strand break repair (i.e., *TDP1* [MIM: 607198] in spinocerebellar ataxia with axonal neuropathy-1 [MIM: 607250]),[22] aberrant DNA repair and/or RNA processing (i.e., *APTX* [MIM: 606350] and *SETX* [MIM: 608465] in ataxia with oculomotor apraxia, types 1 [MIM: 208920] and 2 [MIM: 606002], respectively),[23] or protein folding (*SACS* [MIM: 604490] in Charlevoix-Saguenay spastic ataxia [MIM: 270550]).[23,24] Pontocerebellar hypoplasia, of which some subtypes represent neurodegeneration starting in utero, is often associated with defective tRNA splicing.[19,25,26]

EMC1 is located on chromosome 1p36.13, consists of 23 exons, and encodes a 993 amino acid protein that is one of ten unique EMC subunits in humans.[27] The EMC has been implicated in ER-mitochondria crosstalk, protein folding, and possibly in ERAD of specific proteins,[1,2] aberrations of which have all been associated with neurodegeneration.[5,6,24,28] Impaired intracellular communication was recently shown with mutations in *COPA* (MIM: 601924), whereby impaired inter-organellar transport, retrograde

Golgi-to-ER transport, results in increased ER stress and generation of cytokines and is associated with hereditary autoimmune-mediated lung disease and arthritis.[29] Similarly, perturbed vesicular Golgi-ER transport and resultant increased ER stress was hypothesized to underlie a phenotype of rhabdomyolysis, metabolic crises, and cardiac arrhythmia associated with mutations in *TANGO2*,[30] highlighting the importance of maintaining proper intracellular organelle crosstalk.

EMC1 is ubiquitously expressed and includes two conserved domains: a quinoprotein alcohol dehydrogenase-like domain (PQQ_2) spanning residues 28–242 and an as-yet-uncharacterized domain of unknown function 1620 (DUF1620) including residues 786–992. The homozygous p.Thr82Met and p.Gly868Arg variants lie in the PQQ_2 and DUF1620 domains, respectively, whereas the de novo p.Gly471Arg variant is not located in either domain (Figure 1H). There has been a single previous report of a putative association between an *EMC1* variant and human disease. In a family with non-syndromic retinitis pigmentosa, Abu-Safieh et al.[31] identified a homozygous missense *EMC1* variant (c.430G>A [p.Ala144Thr]) not found in 380 ethnically-matched control individuals. Given the variable clinical severity in the families reported herein, in conjunction with support from the literature regarding the role of EMC1 in rhodopsin biosynthesis,[4] it seems plausible to hypothesize that the p.Ala144Thr variant identified by Abu-Safieh et al.[31] might represent a mild allele with an isolated ophthalmological phenotype. Alternatively, the energy demands of the visual system could make it particularly susceptible to variant alleles affecting mitochondrial function.

The clinical features encountered in families 3 and 4 were less severe than those seen in families 1 and 2. Nonetheless, all individuals for whom serial brain MRIs were available had evidence of a progressive neurodegenerative process primarily affecting the cerebellum, suggesting a unifying molecular pathogenesis in these cases. We were not able to identify a second mutation in *trans* with the de novo c.1411G>C variant in family 4, either by analysis of the WES data or by Sanger sequencing of the putative promoter region. Moreover, no CNV was identified within or in proximity to *EMC1*. A deep intronic variant could not be ruled out. We thus propose that *EMC1* might ultimately be added to the growing list of genes in which both autosomal-recessive and sporadic de novo mutations might lead to human disease.

Multiple genes exist for which both monoallelic and biallelic inheritance have been demonstrated and published simultaneously[32] or in a sequential fashion (Table S4). Classic Mendelian medical genetics implies that a specific gene is associated with a phenotype that shows a consistent pattern of inheritance, and indeed this holds true in the majority of recognizable gene-disease associations. Laminopathies, a broad range of phenotypically distinct disorders associated with mutations in *LMNA* (MIM: 150330), could be considered the prototype of genes that harbor variant alleles that could lead to either dominant or recessive disease traits. The diversity of the laminopathies, which include premature aging syndromes, lipodystrophies, cardiomyopathies, muscular dystrophies, and neuropathies (reviewed in Chojnowski et al.),[33] has been explained by a paralleled diversity of hypotheses. Differential allelic expression,[34] haploinsufficiency for late-onset phenotypes versus dominant-negative or toxic gain-of-function in early phenotypes,[35] and digenic inheritance[36,37] have all been considered.

Additional examples of genes with both dominant and recessive forms of inheritance are listed in Table S4. The phenotype caused by de novo mutations can be more severe than the recessive phenotype, as with *GJB2* ([MIM: 121011] keratitis-icthyosis-deafness versus nonsyndromic deafness),[38] *KIF1A* ([MIM: 601255] severe intellectual disability with progressive cerebral and cerebellar atrophy versus hereditary spastic paraplegia),[39] and *MAB21L2* ([MIM: 604357] microphthalmia, coloboma, and skeletal dysplasia versus isolated eye findings).[32] Alternatively, the recessive phenotype can be more severe than the dominant phenotype, as with *AARS* ([MIM: 601065] epileptic encephalopathy versus axonal CMT),[40] *DEAF1* ([MIM: 602635] epilepsy, autism, and intellectual disability versus nonsyndromic intellectual disability),[41–43] and *EGR2* ([MIM: 129010] CMT versus congenital hypomyelinating neuropathy).[44,45] Notably, the dominant and recessive forms of disease caused by a particular gene primarily affect the same organ system(s), with the difference being severity or additional associated features. Thus, caution must be exercised in interpretation of novel variants that do not conform to the expected inheritance pattern of either a specific gene or a specific disorder (i.e., monoallelic variants in *GMNN* [MIM: 602842] associated with Meier-Gorlin syndrome, for which all other genes follow autosomal-recessive inheritance).[46]

Genotype-phenotype correlations can be associated with different mutation types (missense versus nonsense, i.e., *RARB* [MIM: 180220] and microphthalmia),[47] mild versus severe alleles (i.e., *PMP22* [MIM: 601097] SNVs and CMT),[48] or localization of the mutations in specific protein domains.[49,50] Mutations in *EGR2* (MIM: 129010) affecting the zinc finger DNA-binding domain lead to a severe, autosomal-dominant congenital hypomyelinating neuropathy, whereas mutations outside this functional region lead to an autosomal-recessive, less severe CMT.[45] Similarly, heterozygous mutations in *NALCN* (MIM: 611549) found in specific pore-forming segments of the encoded sodium channel lead to congenital contractures of limbs and face, hypotonia, and developmental delay (CLIFAHDD) syndrome, presumably via a dominant-negative effect, whereas homozygous variants in other regions lead to a less severe autosomal-recessive condition characterized by hypotonia and intellectual disability.[49] Allelic truncating mutations triggering or escaping NMD can also modulate inheritance patterns.[51–53] Classic recessive β-thalassemia can be associated with premature

termination codons (PTCs) in the 5′ region of the β-globin gene, which trigger NMD, whereas mutations in the 3′ region escape NMD and give rise to an atypical dominant form of disease.[52] Likewise, retinitis pigmentosa nonsense mutations in the rhodopsin gene that trigger NMD result in mild recessive alleles, whereas those that escape NMD result in severe dominant alleles, presumably due to toxicity of the truncated protein.[54] In the case of *EMC1*, one hypothesis might be that there is an important functional domain yet to be identified in the vicinity of the highly conserved amino acid altered by the de novo p.Gly471Arg variant, rendering this variant more severe, perhaps by perturbing the function of a complex via a dominant-negative interaction. Identification and study of additional families with disease-associated *EMC1* variants, as well as functional studies, will help clarify the genotype-phenotype correlation.

In conclusion, we define the phenotypic spectrum of *EMC1*-associated disease, which includes global developmental delay with marked speech delay, truncal hypotonia with peripheral dystonic posturing and diminished DTRs, scoliosis, abnormal ophthalmologic exam, and atrophy of the brain primarily affecting the cerebellum. We hypothesize that *EMC1* variants lead to a neurodegenerative process by an as-yet-uncovered mechanism, possibly involving protein folding or dysregulation of ER-mitochondria tethers, and we provide evidence that *EMC1* might lead to disease via either a biallelic or monoallelic mechanism.

Accession Numbers

The accession numbers for the DNA variant data reported in this paper are ClinVar: SCV000258465, SCV000258466, SCV000258467, and SCV000258468.

Supplemental Data

Supplemental Data include a Supplemental Note, one figure, and four tables and can be found with this article online at http://dx.doi.org/10.1016/j.ajhg.2016.01.011.

Acknowledgments

We thank the families for participation in this study. This work was supported in part by a US National Human Genome Research Institute and National Heart Lung and Blood Institute grant (U54HG006542) to the Baylor-Hopkins Center for Mendelian Genomics and a National Institute of Neurological Disorders and Stroke grant (RO1 NS058529) to J.R.L. T.H. is supported by the NIH Medical Genetics Research Fellowship Program (T32 GM07526). W.-L. C. is supported by the Cancer Prevention & Research Institute of Texas Training Program (RP140102). We thank Dr. Hugo Bellen and Dr. Hector Sandoval for critical review of the manuscript. J.R.L. has stock ownership in 23andMe, is a paid consultant for Regeneron Pharmaceuticals, has stock options in Lasergen, is a member of the Scientific Advisory Board of Baylor Miraca Genetics Laboratories, and is a co-inventor on multiple United States and European patents related to molecular diagnostics for inherited neuropathies, eye diseases, and bacterial genomic fingerprinting. The Department of Molecular and Human Genetics at Baylor College of Medicine derives revenue from the chromosomal microarray analysis and clinical exome sequencing offered in the Baylor Miraca Genetics Laboratory (http://www.bmgl.com). W.K.C. is a paid consultant to BioReference Laboratories and for Regeneron Pharmaceuticals. J.W.B. is an employee of Illumina.

Received: December 17, 2015
Accepted: January 19, 2016
Published: March 3, 2016

Web Resources

The URLs for data presented herein are as follows:

1000 Genomes, http://browser.1000genomes.org
Atherosclerosis Risk in Communities Study (ARIC) Database, http://drupal.cscc.unc.edu/aric/
CADD, http://cadd.gs.washington.edu/
ClinVar, https://www.ncbi.nlm.nih.gov/clinvar/
ExAC Browser, http://exac.broadinstitute.org/
GenBank, http://www.ncbi.nlm.nih.gov/genbank/
GeneMatcher, https://genematcher.org/
Genome Reference Consortium, www.ncbi.nlm.nih.gov/projects/genome/assembly/grc/human
NHLBI Exome Sequencing Project (ESP) Exome Variant Server, http://evs.gs.washington.edu/EVS/
OMIM, http://www.omim.org/
The Human Protein Atlas, http://www.proteinatlas.org/
UCSC Human Genome Browser, http://genome.ucsc.edu/cgi-bin/hgGateway

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