RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART A

CLTC as a Clinically Novel Gene Associated With Multiple Malformations and Developmental Delay

Joseph DeMari,[1]* Cameron Mroske,[2] Sha Tang,[2] Joseph Nimeh,[1] Ryan Miller,[1] and Robert R. Lebel[1]

[1]Department of Pediatrics, Section of Medical Genetics, SUNY Upstate Medical University, Syracuse, New York

[2]Ambry Genetics Corporation, Aliso Viejo, California

Manuscript Received: 12 June 2015; Manuscript Accepted: 20 November 2015

Diagnostic exome sequencing has recently emerged as an invaluable tool in determining the molecular etiology of cases involving dysmorphism and developmental delay that are otherwise unexplained by more traditional methods of genetic testing. Our patient was large for gestational age at 35 weeks, delivered to a 27-year-old primigravid Caucasian whose pregnancy was complicated by preeclampsia. Neonatal period was notable for hypoglycemia, apnea, bradycardia, hyperbilirubinemia, grade I intraventricular hemorrhage, subdural hematoma, laryngomalacia, hypotonia, and feeding difficulties. The patient had numerous minor dysmorphic features. At three and a half years of age, she has global developmental delays and nystagmus, and is being followed for a mediastinal neuroblastoma that is currently in remission. Karyotype and oligo-microarray were normal. Whole-exome, next generation sequencing (NGS) coupled to bioinformatic filtering and expert medical review at Ambry Genetics revealed 14 mutations in 9 genes, and these genes underwent medical review. A heterozygous de novo frameshift mutation, c.2737_2738dupGA p.D913Efs*59, in which two nucleotides are duplicated in exon 17 of the CLTC gene, results in substitution of glutamic acid for aspartic acid at position 913 of the protein, as well as a frame shift that results in a premature termination codon situated 58 amino acids downstream. Clathrin Heavy Chain 1 (CHC1) has been shown to play an important role in the brain for vesicle recycling and neurotransmitter release at pre-synaptic nerve terminals. There is also evidence implicating it in the proper development of the placenta during the early stages of pregnancy. The *CLTC* alteration identified herein is likely to provide an explanation for the patient's adverse phenotype. Ongoing functional studies will further define the impact of this alteration on CHC1 function and consequently, human disease.

Key words: *CLTC*; clathrin; developmental delay; whole exome sequencing

How to Cite this Article:
DeMari J, Mroske C, Tang S, Nimeh J, Miller R, Lebel RR. 2016. *CLTC* as a clinically novel gene associated with multiple malformations and developmental delay.
Am J Med Genet Part A 9999A:1–9.

INTRODUCTION

The number of patients diagnosed through exome sequencing has grown exponentially since the inception of the technology in 2008, and its introduction as a clinical test in 2011 [National Institutes of Health Consensus Development Conference Statement: phenylketonuria: screening and management, October 16–18, 2000, 2001; Worthey et al., 2011; Dixon-Salazar et al., 2012; Need et al., 2012; Sailer et al., 2012; Hanchard et al., 2013; Shamseldin et al., 2013]. Herein, we describe the application of diagnostic exome sequencing (DES) for determining the molecular etiology of an infant girl's dysmorphism and developmental delay. The family trio (healthy parents and affected proband) was referred to Ambry Genetics (Aliso Viejo, CA) for DES because the proband had previously undergone extensive biochemical and molecular testing that was uninformative.

CLTC encodes clathrin heavy chain 1 (CHC1), one of the components of clathrin, which serves as the vesicle coat protein involved in intracellular trafficking and endocytosis (The UniProt Consortium: Q00610, 2014) [UniProt, 2014]. As of September 1, 2015, a thorough examination of three human gene-disease databases (ClinVar, DECIPHER, and HGMD) revealed a single instance of a de novo constitutive CLTC missense alteration being associated with human neurological/developmental disease: CLTC NM_004859 c.2669 C > T, p.P890L (DECIPHER v9.1: GRCh37/hg19 Chr17:57754422 C > T). The patient with this alteration is listed as having clinical features that include abnormalities of the head, neck, and nervous system (DECIPHER v9.1). The three

Conflict of interest: none

*Correspondence to:
Joseph DeMari, 5059 Peck Hill Rd, Jamesville, NY 13078.
E-mail: demarij@upstate.edu

Article first published online in Wiley Online Library (wileyonlinelibrary.com): 00 Month 2016
DOI 10.1002/ajmg.a.37506

website addresses which we employ for human gene-disease associations are: ClinVar: http://www.ncbi.nlm.nih.gov/clinvar/, DECIPHER: https://decipher.sanger.ac.uk/, and HGMD: https://portal.biobase-international.com/hgmd/pro/start.php.

Additional phenomena make *CLTC* a candidate for the etiology of human neurological and developmental disease: (i) *CLTC* is highly expressed in brain, as well as in other tissues related to human growth and development (Unigene: Hs.491351), and (ii) inactivation of CHC1 in rat and fruit fly models prevents the recycling or release of vesicles at the pre-synaptic termini of neurons [Bazinet et al., 1993; Miesenbock et al., 1998; Kasprowicz et al., 2008]. Furthermore, mutations in *CLTCL1*, the second member of the Clathrin Heavy Chain family, have been associated with neurological diseases including seizures, intellectual disability, autism and schizophrenia[Holmes et al., 1997; Chahrour et al., 2012; Gulsuner et al., 2013]. The gene products of *CLTC* and *CLTCL1* exhibit 89.2% amino acid similarity (AlignX, a component of Vector NTI Advance 11.5.1 ©2011 Invitrogen, part of Life Technologies, Carlsbad, CA). Recently, a patient with a deletion of 17q23.1 including *CLTC* has been reported to have microcephaly, developmental delay, and growth retardation [Ballif et al., 2010]. A single patient with non-syndromic hydrocephalus has been reported with a duplication overlapping the *CLTC* gene [Landrum et al., 2014].

CLTC is also highly expressed in invasive extravillous trophoblasts [Majeed et al., 2014], which are cells of fetal origin that play a key role in remodeling the placental vasculature during the earliest stages of pregnancy [Uzan et al., 2011]. Recent work has shown that clathrin plays an integral role in cell migration through its activity in facilitating the endocytosis and recycling of integrins at focal adhesion plaques [Ezratty et al., 2009; Majeed et al., 2014].

CLINICAL REPORT AND METHODS

The patient was conceived through in vitro fertilization (IVF) and born to non-consanguineous parents who were both healthy, save for the decreased fertility of unknown etiology. The pregnancy was complicated by preeclampsia. A maternal cousin was noted to display 3–4 syndactyly of all four extremities, while a distant paternal cousin was born with cleft palate and was marked by an unknown syndrome.

The proband was born at 35 and 1/7 weeks gestation with a birth weight of 3,244 g (90–95th centile), length 50 cm (90th centile), and head circumference 36 cm (>97th centile). She was delivered by cesarean-section due to breech presentation and premature maternal contractions. APGAR scores were five (1 min) and nine (5 min). Her neonatal period was notable for hypoglycemia, apnea, bradycardia, hyperbilirubinemia, grade I intraventricular hemorrhage, subdural hematoma, laryngomalacia, hypotonia, and feeding difficulties. Upon discharge from the NICU at age 1 month 4 days, pertinent physical findings included "sunset" eyes, mild frontal bossing, prominent jaw, large anterior fontanel, significant global hypotonia but with good extremity movement, weak suck, weak cry, bilateral hip laxity, and jaundice. EEG and brain MRI prior to discharge were interpreted as normal.

In the following months, her head circumference increased to above the 97th centile, and she presented to the emergency department at 5 months of age with lethargy and emesis. An MRI revealed progressive ventricular enlargement with cerebral atrophy, and a ventriculoperitoneal shunt was placed promptly. Etiology of the hydrocephaly was unknown. During that hospital admission, her first genetics evaluation revealed abnormal physical features including apparently low-set ears, depressed nasal bridge, anteverted nares, apparently widely set involuted nipples, hypotonia, hyporeflexia, and poor social interactions for age.

The patient has central apnea (resolving) and hypothyroidism. Vitamin K dependent clotting factor deficiency (VKCFD1) was diagnosed and is now under good control with high-dose vitamin K supplementation. At 18 months of age, surgical resection of a left posterior mediastinal mass revealed a ganglioneuroblastoma of intermixed (Schwannian stroma-rich) type. Pathologic analysis showed an encapsulated, differentiated tumor with a low mitotic-karyorrhectic index and calcifications. Tumor genetic analysis failed to discover an amplified MYCN gene and did not show a LOH at 1p and 11q. Her numerous minor dysmorphic features persist and at 3 years 6 months of age, she continues to exhibit global developmental delays and nystagmus. Weight has trended steadily along the 50th centile, height at the 90th centile, and head circumference has stabilized at the 75th centile with her VP shunt in place. Hypotonia is improving slowly with therapy, and she can now roll and is starting to bear weight. Similarly, her social skills are improving slowly with socialization and speech therapy, and she has acquired monosyllabic babbling, a few signs using American Sign Language, and is much more socially alert and interactive. The following tests were performed and the results shown to be normal: karyotype, SNP microarray, EMG, and metabolic workup including fatty acid profile, plasma amino acids (AA), glycosylation studies, urine organic acids (UOA) and acylglycines, plasma acylcarnitines, and very long chain fatty acids. Genomic DNA (gDNA) was isolated from whole blood extracted from each trio member (proband and parents) utilizing the QIAsymphony gDNA extraction system (Qiagen, Hilden, Germany).

Samples were prepared using either the SureSelect Target Enrichment System (Agilent Technologies, Santa Clara, CA) [Gnirke et al., 2009] or the SeqCap EZ VCRome 2.0 system (Roche NimbleGen, Madison, WI) [Bainbridge et al., 2010]. Briefly, each DNA sample was sheared, blunt-end repaired, and ligated to indexed adapters. Utilizing solution-based hybridization with oligonucleotide probes, the coding exons and neighboring intronic sequence of each genome were enriched, while off-target sequences were washed away. The resultant enriched exome libraries were applied to the solid surface flow cell of an Illumina HiSeq 2000 sequencer (Illumina, San Diego, CA) for clonal amplification and sequencing using paired-end, 100-cycle chemistry.

The acquisition and processing of data, alignment of sequence to GRCh37, and bioinformatic filtering were performed as described in Farwell et al. [2014]. Initial data processing/base calling, including extraction of cluster intensities, was performed using RTA 1.12.4 (HiSeq Control Software 1.4.5). The sequence quality filtering script was executed using Illumina CASAVA software (ver 1.8.2, Illumina). Sequencing run metrics, including data yield (Mbases), %PF (pass-filter), # of reads, % of raw clusters per lane, and quality

scores, were recorded in the Demultiplex_Stats.htm file and verified to surpass quality thresholds. Sequences were aligned to the human genome reference sequence GRCh37 and variant calls generated using CASAVA and Pindel [Ye et al., 2009]. All exonic sequence and a minimum of two bases of flanking intronic sequence were analyzed. Data analysis focused on nonsense variants, small insertions and deletions, canonical splice site alterations, and non-synonymous missense alterations. Previously described gene mutations and polymorphisms were screened using resources that included The Human Gene Mutation Database (HGMD)[Stenson et al., 2009], the Single Nucleotide Polymorphism database (dbSNP) [Sherry et al., 2001], 1,000 genomes, HapMap data [International HapMap, 2003] and NCBI resources such as PubMed. Importantly, the in silico filtering pipeline protected all variants annotated within the Human Gene Mutation Database (HGMD) [Stenson et al., 2009] and/or the Online Mendelian Inheritance in Man (OMIM) database. Stepwise filtering, included the removal of common SNPs, intergenic and 3′/5′ UTR variants, non-splice-related intronic variants, and synonymous variants. Remaining variants were then filtered for family history using models of inheritance. For every filtered variant, Ambry Variant Analyzer (AVA) was utilized to annotate the data, which included nucleotide and amino acid conservation; biochemical nature of the amino acid substitution; population frequency as tabulated in the ESP and 1,000 genomes databases; and finally, the impact of the nucleotide substitution on gene product function, as predicted by the in silico modeling algorithms PolyPhen [Adzhubei et al., 2010] and SIFT [Ng and Henikoff, 2001, 2002, 2003, 2006; Kumar et al., 2009]. Importantly, each candidate mutation was reviewed by a molecular geneticist in order to assess the likelihood of pathogenicity. NGS alignments were viewed using the Integrative Genomics Viewer (IGV) software (Broad Institute, Cambridge, MA) [Robinson et al., 2011].

Candidate alterations identified as being causative were confirmed using automated fluorescence dideoxy Sanger sequencing. Co-segregation analysis was performed on each available family member. Target specific primers were designed to include 5′ terminal sense and antisense sequencing tags. The gene-specific moieties of the primers were designed with the aid of PrimerZ; [Tsai et al., 2007] oligonucleotides were synthesized by Integrated DNA Technologies (Coralville, IA) and sequencing was performed on an automated ABI3730 DNA sequencer (Life Technologies) according to standard procedures (Fig. 1).

RESULTS

NGS Run Metrics and Bioinformatic Analysis

Exome sequencing of the family trio (proband, mother, and father) resulted in an average of approximately 11 Gb of sequence per sample. The mean coverage of captured regions was 99.97% per sample, with >93% of bases covered at least 10-fold. On average, >92% of bases had a quality score of Q30 or higher. Overall, the average base-call mean quality score for the family trio exceeded Q36 (Table I). The removal of common SNPs, intergenic and 3′/5′ UTR variants, non-splice-related intronic variants, and synonymous variants through stepwise filtering resulted in approximately 12,000 variants being associated with each trio member (Table II).

Subsequent filtering based on family history and inheritance modeling (autosomal dominant/recessive and X-linked dominant/recessive) within the trio narrowed the list of candidates to 17 genes and 24 unique alterations (Table III). After manual review of each alteration in order to rule out sequencing artifacts and polymorphisms, followed by expert medical interpretation to rule out genes lacking clinical overlap, the list of candidates was narrowed to six clinically novel genes and eight unique alterations. Of these, we deemed that the *CLTC* gene (NM_004859), which harbored an apparently de novo alteration, c.2737_2738dupGA p.D913Efs*59, warranted further investigation/confirmation because it (i) displayed sufficient clinical overlap with the patient's phenotype, and (ii) harbored a mutation that would clearly result in LOF of the *CLTC* gene product.

Sanger Sequencing and Co-Segregation Analysis

As indicated by the red arrow in Figure 2A, automated bi-directional fluorescence dideoxy sequencing confirmed (i) the presence of *CLTC* c.2737_2738dupGA, p.D913Efs*59 in the proband, and (ii) the de novo origin of the alteration.

The de novo D913Efs*59 alteration results from the duplication of contiguous nucleotides G and A at positions 2,737 and 2,738 of the *CLTC* coding sequence. This duplication, which maps to exon 17, results in the substitution of glutamic acid for aspartic acid at position 913 of the protein; moreover, it causes a frame shift that results in a premature termination codon (PTC) situated 58 amino acids[1] downstream of codon 913 (Fig. 2A). As this PTC is located approximately 2 kb upstream of the final mRNA exon/exon junction, the majority of newly synthesized PTC-containing transcript will be degraded via nonsense-mediated mRNA decay (NMD) [Holbrook et al., 2004]. As depicted in Figure 2A, any residual mutated mRNA that escapes NMD will generate a severely truncated protein; thus, we predict that D913Efs*59 exerts its pathological effects in the proband due to haploinsufficiency of full-length CLTC [Mort et al., 2008]. To our knowledge, D913Efs*59 has not been previously reported in dbSNP, nor is it listed in the NHLBI Exome Sequencing Project (ESP) or the 1,000 genome databases [Abecasis et al., 2010].

DISCUSSION

CLTC Is a Novel Gene Associated With Multiple Malformations and Developmental Delay

Considering the integral role of synaptic vesicle recycling in neurologic function, and the central role of the kinetochore in cell division and migration, clathrin can be expected to have a high level of evolutionary conservation. This has been appreciated by others working in this area [Brodsky, 2012].

Through the application of DES coupled to bioinformatic filtering and expert medical review, we have determined that a de novo *CLTC* alteration c.2737_2738dupGA, p.D913Efs*59 is the likely causative lesion that underlies the abnormal phenotype of an

[1]Human genome variation society (HGVS) nomenclature includes the first mutated codon in its numbering scheme, hence p.D913Efs*59.

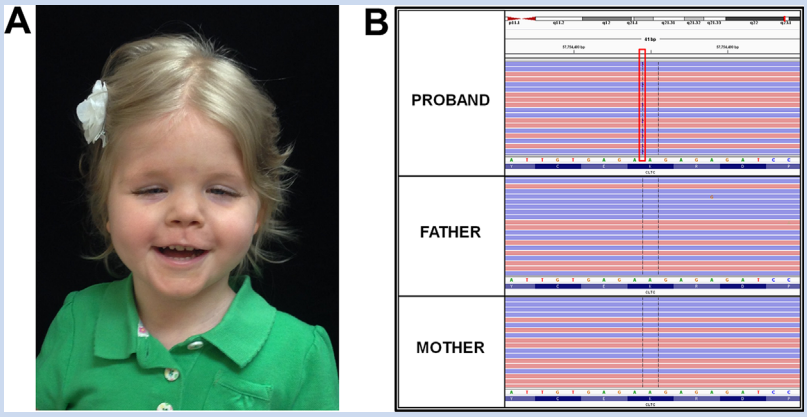

FIG. 1. The proband possesses a de novo duplication in her CLTC gene. A: Clinical photograph of the proband at 24 months of age demonstrating low set ears, ptosis, and facial dysmorphism. B: Whole exome trio-NGS identifies the alteration c.2737_2738dupGA in the affected patient (upper panel, red box) but not in the parents (lower panels). Approximately half the reads (54/104) carry the duplication, indicating the heterozygous nature of the mutation. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/ajmga].

infant girl who has developmental delays and multiple malformations. Clathrin heavy chain 1 (CHC1), which is encoded by *CLTC*, is an integral component of clathrin, the prototypical vesicle coat protein involved in intracellular trafficking and endocytosis. Highly expressed in brain as well as in other tissues related to growth and development, clathrin plays a specialized role in neuronal transmission by facilitating the recycling of synaptic vesicles in pre-synaptic nerve terminals. In experiments utilizing both rat and fruit fly models, knockdown of chc activity resulted in critical impairment of vesicle recycling at the pre-synaptic cleft of neurons and consequently, disruption of neurotransmission during episodes of intense synaptic activity [Bazinet et al., 1993; Miesenbock et al., 1998; Kasprowicz et al., 2008]. These experimental findings have implications for human neurological disease because contemporary evidence indicates that within the developing mammalian brain, synaptic burst activity is temporally linked to the establishment of discrete neuronal circuitry [Anastasiades and Butt, 2012].

Additional evidence for *CLTC* involvement in this case comes from human copy number variation (CNV) studies. A 2010 report documented a 5-year-old girl who harbored a 2.8 Mb microdeletion spanning *CLTC* and who displayed a number of symptoms consistent with those of our proband, including developmental delay, left-sided esotropia, and abnormal facial features such as a flattened nasal bridge and a simple right ear [Ballif et al., 2010]. Notably, the ESP database, which contains the exome data of over 6,000 healthy individuals, includes no report of *CLTC* gene-disruption alterations (nonsense, frameshift, and canonical splice site); the lack of such mutations in ESP supports the notion that *CLTC* haploinsufficiency is not tolerated in phenotypically normal

TABLE I. Hiseq Sequencing Run Metrics

	Proband	Father	Mother	Average
Yield (Gb)	14.205	10.140	9.691	11.345
Quality				
% of ≧Q30 bases (PF)	89.100	94.400	94.500	92.667
Mean quality score (PF)	35.100	36.600	36.700	36.133
Base coverage				
%Base_1x (%)	96.14	96.71	96.83	96.56
%Base_5x (%)	94.41	95.52	95.64	95.19
%Base_7x (%)	93.65	95.11	95.22	94.66
%Base_10x (%)	92.60	94.51	94.58	93.90
%Base_20x (%)	89.40	91.81	91.38	90.86
%Base_50x (%)	79.82	72.43	67.88	73.38
%Base_100x (%)	57.64	33.54	27.48	39.55
Mean_Coverage (%)	131.99	87.98	79.95	99.97

TABLE II. Bioinformatics Variant Filtering

Stepwise filtering[a]	Proband	Mother	Father	Average
No. of variants in coding regions[b]	116,339	116,905	118,264	117,169
No.post-removal of intergenic and 3'/5' UTR variants	87,010	88,704	89,327	88,347
No. post-removal of non-splice-related intronic[c] variants	22,884	22,586	22,768	22,746
No. post-removal of synonymous variants	12,042	11,798	11,898	11,913

[a]Stepwise filtering protects variants annotated within the human gene mutation database (HGMD) and/or the online mendelian inheritance in man (OMIM) databases.
[b]Variants refers to single nucleotide alterations, insertions, deletions, and indels with at least 10x base pair coverage.
[c]Intronic refers to >3 bp into the introns.

TABLE III. Variant Filtering Based on Inheritance Model & Interpretation

		Manual review[a]		
	Inheritance model filtering	Characterized genes	Novel genes	Notable candidate genes[b]
Autosomal dominant genes (alterations)	9 (9)	0 (0)	4 (4)	1 (1)
Autosomal recessive genes (alterations)	8 (15)	0 (0)	2 (4)	0 (0)
X-linked recessive genes (alterations)	0 (0)	0 (0)	0 (0)	0 (0)
X-linked dominant genes (alterations)	0 (0)	0 (0)	0 (0)	0 (0)
Y-linked genes (alterations)	–	–	–	–
Total genes (alterations)	17 (24)	0 (0)	6 (8)	1 (1)

[a]Manual filtering involves the removal of genes unrelated to the patient's evaluated phenotype and alterations considered benign.
[b]Notable candidate genes: Genes with disease phenotype association overlapping that of the proband.

individuals.[2] A separate report (ClinVar: RCV000050957.1) describes a single patient presenting with non-syndromic hydrocephalus who possesses a duplication that overlaps the *CLTC* locus [Landrum et al., 2014] (Table IV).

Finally, evidence supporting *CLTC* disruption as the underlying cause of the patient's phenotype can be gleaned from reports that detail mutations in *CLTCL1* which are associated with clinical disease. *CLTCL1* is the second member of the clathrin heavy chain family. A male patient in whom *CLTCL1* was disrupted due to a balanced (21;22)(p12;q11) translocation (HGMD CP973585) developed seizures at 2 years of age, and at 5.5 years displayed features considered reminiscent of 22q11 deletion syndrome; these included a Stanford Binet IQ of 44, dysmorphic facial features, genital anomalies, and hypermobility of the finger and wrist joints. Upon re-evaluation at age 23, this subject had a low IQ (WAIS-R), macrocephaly, dysmorphic facial features, long slender fingers, and hypotonia [Holmes et al., 1997]. It follows that a disruption in clathrin, and therefore, kinetochore stabilization, affects cell division in multiple tissue types and can, therefore, be implicated in dysmorphic features. In fact, it is documented that *CLTC* is expressed in multiple tissue types involved in human growth and development [Unigene: Hs.491351; Kim et al., 2014]. In an autism study, whole exome sequencing was employed to identify a family in whom a recessive *CLTCL1* missense mutation (c.3493C>T, HGMD CM124319) segregated perfectly with autism presentation. The mutation, which was predicted to be damaging, was homozygous in both affected children; in contrast, it was heterozygous in the unaffected parents and sibling [Chahrour et al., 2012]. In a recent effort to identify candidate genes involved in the etiology of schizophrenia, a de novo missense mutation (c.1921G > C, HGMD CM138084) was detected in *CLTCL1* in a schizophrenic patient who was from an otherwise healthy family. Bioinformatic analysis predicted that the mutation was deleterious to normal protein function. Moreover, when the expression profile of *CLTCL1* was mapped onto the transcriptome of normal human brain tissue, it formed a network that was significantly enriched for transcriptional co-expression and protein interaction between itself and other schizophrenia genes. This network localized spatially to the dorsolateral and prefrontal cortices of the developing human brain. Such data are highly suggestive that damaging de novo mutations in the clathrin heavy chain isoforms can cause neurological dysfunction [Gulsuner et al., 2013].

Ongoing biochemical analysis and in vitro studies will further define the impact of *CLTC* haploisufficiency on neurological dysfunction and dysmorphism in humans; nevertheless, the results of this unique case highlight the power of whole exome sequencing as a clinical diagnostic tool that possesses the requisite sensitivity and specificity to identify causative genetic lesions in patients. While one can speculate that the mutation herin described could have been involved with the development of the neuroblastoma in our patient, no satisfying hypothesis can be reached to explain why this particular tumor was associated with haploinsufficiency of CHC1. In summary, while more patients are needed to definitively establish this

[2]ESP describes a single individual who possesses a heterozygous frameshift mutation in CLTC; however, because the alteration (c.5022_5023insA p.M1675Nfs*4) occurs in the last exon and penultimate codon of the gene, it is not expected to trigger NMD.

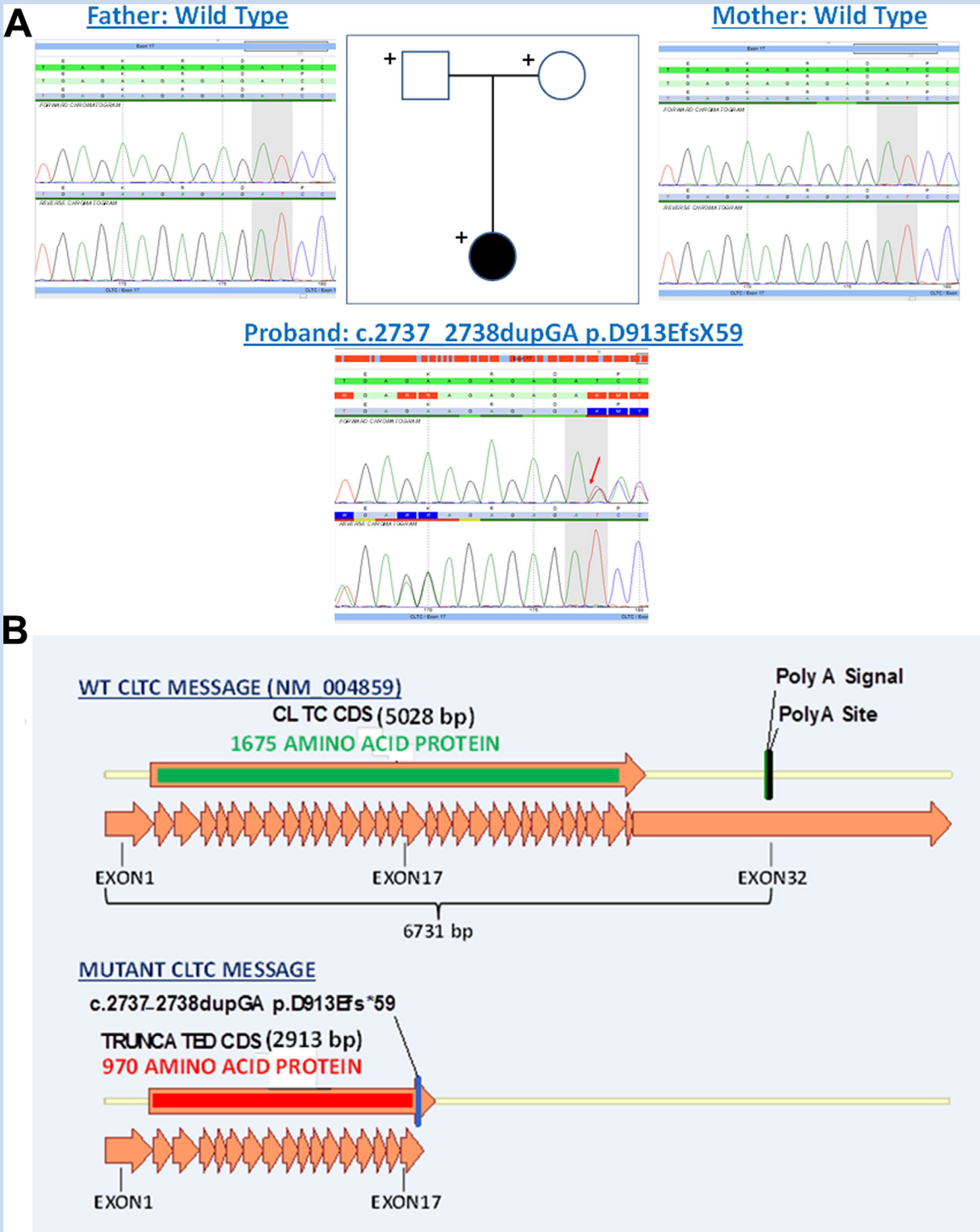

FIG. 2. A: Familial co-segregation/Sanger sequencing analyses confirm the de novo and heterozygous nature of the mutation. Forward and reverse chromatograms are depicted adjacent to each family member (pedigree, center panel). Note that "+" indicates whole exome sequencing was performed, while open shapes represent unaffected family members and the black circle represents the affected proband. The red arrow indicates the heterozygous c.2727_2738dupGA alteration in the proband's CLTC gene (lower two chromatograms). B: Nature of the mutation suggests a haploinsufficiency model of disease. CLTC c.2737_2738dupGA, p.D913Efs*59 creates a frameshift followed by a premature stop codon that results in a severely truncated message and protein that are subject to Nonsense Mediated Decay (NMD); this effectively reduces the dose of CHC17 activity by one half. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/ajmga].

TABLE IV. Familial Co-Segregation Analysis Results

Gene	Protein	RefSeq ID	Alteration	Proband	Father	Mother
CLTC	Clathrin heavy chain 1	NM_004859	c.2737_2738dupGA (p.D913Efs*59)	+/−	−/−	−/−

gene-phenotype relationship, diagnostic exome sequencing established a likely molecular diagnosis for a patient in whom traditional testing methods were uninformative; moreover, DES identified *CLTC* as a novel gene associated with neurological disease and dysmorphism.

Areas for Further Research

Preeclampsia is a multi-systemic disorder that complicates 3–8% of pregnancies in Western countries, and constitutes a major source of morbidity and mortality worldwide [Duley, 2009; Carty et al., 2010].

Although it is well known that preeclampsia is characterized by abnormal vascularization of the placenta, the actual pathophysiology of the disease remains unclear [Uzan et al., 2011]. Despite this paucity of knowledge, several facts have emerged to support the involvement of fetal genes in the etiology of this disease. Specifically, it has been revealed that (i) cells of fetal origin contribute to the development of the placenta,[Cross, 2003; Laivuori, 2007] (ii) the risk of preeclampsia is increased in cases where a single male has fathered several pre-eclamptic pregnancies with different partners,[Lie et al., 1998] and (iii) the risk of fathering a pre-eclamptic pregnancy is increased among males who were themselves born from a pre-eclamptic pregnancy [Esplin et al., 2001].

At the earliest stages of pregnancy, extravillous trophoblasts migrate to the lining of the uterus, where they invade and remodel the tissue in a process that establishes a normal placental vasculature [Uzan et al., 2011]. In the case of pre-eclampsia, trophoblasts fail to migrate to a sufficient depth into the maternal spiral arteries, with the result that the vasculature never undergoes sufficient transformation. The abnormal tissue is characterized by reduced blood flow to the feto-placental unit and by ischemia; consequently, fetal growth can be impaired and preeclampsia will eventually manifest in the mother (hypertension, proteinuria, etc.) [Moffett-King, 2002; Uzan et al., 2011].

Recent evidence suggests that clathrin disruption exerts a negative impact on trophoblast migration; in normal human placental tissue, both the clathrin heavy and light chains (CHC1 and CLCb) are highly expressed in invasive extravillous trophoblasts [Majeed et al., 2014]. Moreover, CLC depletion impairs cellular migration because β1-integrin cannot be recycled following endocytosis of its inactive form; consequently, CLC knock down leads to an overall reduction in β1-integrin surface expression [Majeed et al., 2014]. Such data support the notion that the dual processes of focal adhesion formation and disassembly, which occur during cellular migration, are innately coupled to the recycling of integrins via endocytosis; siRNA induced knock down of CHC1 expression caused clathrin depletion and inhibited the disassembly of focal adhesions, significantly decreasing the rate of cellular migration [Ezratty et al., 2009]. Follow-up functional studies concerning this pathophysiologic pathway of preeclampsia are legitimate avenues for further research in this area

ACKNOWLEDGMENTS

The DES for this patient was preformed as part of her clinical diagnostic workup. We are grateful to the patient and her family for permission to report those results here.

WEB RESOURCES

Accession numbers and URLs for data in this article are as follows:

ESP [Internet]: Exome Variant Server, NHLBI GO Exome Sequencing Project (ESP), Seattle, WA (URL: http://evs.gs.washington.edu/EVS/) May, 2014.

OMIM [Internet]: Online Mendelian Inheritance in Man, OMIM®. Johns Hopkins University, Baltimore, MD. MIM Number: 1189 55: 09/15/2011: World Wide Web URL:http://omim.org/

RefSeq (Accession NM_004859): The NCBI handbook [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2002 Oct. Chapter 18, The Reference Sequence (RefSeq) Project (see ref guidelines: http://www.ncbi.nlm.nih.gov/books/NBK50679/).

REFERENCES

2001. National Institutes of Health Consensus Development Conference Statement: Phenylketonuria: Screening and management, October 16–18, 2000. Pediatrics 108:972–982.

Abecasis GR, Altshuler D, Auton A, Brooks LD, Durbin RM, Gibbs RA, Hurles ME, McVean GA. 2010. A map of human genome variation from population-scale sequencing. Nature 467:1061–1073.

Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS, Sunyaev SR. 2010. A method and server for predicting damaging missense mutations. Nat Methods 7:248–249.

Anastasiades PG, Butt SJB. 2012. A role for silent synapses in the development of the pathway from layer 2/3 to 5 pyramidal cells in the neocortex. J Neurosci 32:13085–13099.

Bainbridge MN, Wang M, Burgess DL, Kovar C, Rodesch MJ, D'Ascenzo M, Kitzman J, Wu YQ, Newsham I, Richmond TA, Jeddeloh JA, Muzny D, Albert TJ, Gibbs RA. 2010. Whole exome capture in solution with 3 Gbp of data. Genome Biol 11:62.

Ballif BC, Theisen A, Rosenfeld JA, Traylor RN, Gastier-Foster J, Thrush DL, Astbury C, Bartholomew D, McBride KL, Pyatt RE, Shane K, Smith WE, Banks V, Gallentine WB, Brock P, Rudd MK, Adam MP, Keene JA, Phillips JA 3rd, Gowans GC, Stankiewicz P, Bejjani BA, Shaffer LG. 2010. Identification of a recurrent microdeletion at 17q23. 1q23. 2 flanked by segmental duplications associated with heart defects and limb abnormalities. Am J Hum Genet 86:454–461.

Bazinet C, Katzen AL, Morgan M, Mahowald AP, Lemmon SK. 1993. The Drosophila clathrin heavy chain gene: Clathrin function is essential in a multicellular organism. Genetics 134:1119–1134.

Brodsky FM. 2012. Diversity of clathrin function: New tricks for an old protein. Annu Rev Cell Dev Biol 28:309–336.

Carty DM, Delles C, Dominiczak AF. 2010. Preeclampsia and future maternal health. J Hypertens 28:1349–1355.

Chahrour MH, Yu TW, Lim ET, Ataman B, Coulter ME, Hill RS, Stevens CR, Schubert CR, Collaboration AAS, Greenberg ME, Gabriel SB, Walsh CA. 2012. Whole-exome sequencing and homozygosity analysis implicate depolarization-regulated neuronal genes in autism. PLoS Genet 8: e1002635.

Cross JC. 2003. The genetics of pre-eclampsia: A feto-placental or maternal problem? Clin Genet 64:96–103.

Dixon-Salazar TJ, Silhavy JL, Udpa N, Schroth J, Bielas S, Schaffer AE, Olvera J, Bafna V, Zaki MS, Abdel-Salam GH, Mansour LA, Selim L, Abdel-Hadi S, Marzouki N, Ben-Omran T, Al-Saana NA, Sonmez FM, Celep F, Azam M, Hill KJ, Collazo A, Fenstermaker AG, Novarino G, Akizu N, Garimella KV, Sougnez C, Russ C, Gabriel SB, Gleeson JG. 2012. Exome sequencing can improve diagnosis and alter patient management. Sci Transl Med 4:138–178.

Duley L. 2009. The global impact of pre-eclampsia and eclampsia. Semin Perinatol 33:130–137.

Esplin MS, Fausett MB, Fraser A, Kerber R, Mineau G, Carrillo J, Varner MW. 2001. Paternal and maternal components of the predisposition to preeclampsia. N Engl J Med 344:867–872.

Ezratty EJ, Bertaux C, Marcantonio EE, Gundersen GG. 2009. Clathrin mediates integrin endocytosis for focal adhesion disassembly in migrating cells. J Cell Biol 187:733–747.

Farwell KD, Shahmirzadi L, El-Khechen D, Powis Z, Chao EC, Davis BT, Baxter RM, Zeng W, Mroske C, Parra MC, Gandomi SK, Lu I, Li X, Lu H, Lu HM, Salvador D, Ruble D, Lao M, Fischbach S, Wen J, Lee S, Elliott A, Dunlop CL, Tang S. 2014. Enhanced utility of family-centered diagnostic exome sequencing with inheritance model-based analysis: Results from 500 unselected families with undiagnosed genetic conditions. Genet Med 17:578–586.

Gnirke A, Melnikov A, Maguire J, Rogov P, LeProust EM, Brockman W, Fennell T, Giannoukos G, Fisher S, Russ C, Gabriel S, Jaffe DB, Lander ES, Nusbaum C. 2009. Solution hybrid selection with ultra-long oligonucleotides for massively parallel targeted sequencing. Nat Biotechnol 27:182–189.

Gulsuner S, Walsh T, Watts AC, Lee MK, Thornton AM, Casadei S, Rippey C, Shahin H, Consortium on the Genetics of S, Group PS, Nimgaonkar VL, Go RC, Savage RM, Swerdlow NR, Gur RE, Braff DL, King MC, McClellan JM. 2013. Spatial and temporal mapping of de novo mutations in schizophrenia to a fetal prefrontal cortical network. Cell 154:518–529.

Hanchard NA, Murdock DR, Magoulas PL, Bainbridge M, Muzny D, Wu YQ, Wang M, McGuire AL, Lupski JR, Gibbs RA, Brown CW. 2013. Exploring the utility of whole-exome sequencing as a diagnostic tool in a child with atypical episodic muscle weakness. Clin Genet 83:457–461.

Holbrook JA, Neu-Yilik G, Hentze MW, Kulozik AE. 2004. Nonsense-mediated decay approaches the clinic. Nat Genet 36:801–808.

Holmes SE, Riazi MA, Gong W, McDermid HE, Sellinger BT, Hua A, Chen F, Wang Z, Zhang G, Roe B, Gonzalez I, McDonald-McGinn DM, Zackai E, Emanuel BS, Budarf ML. 1997. Disruption of the clathrin heavy chain-like gene (CLTCL) associated with features of DGS/VCFS: A balanced (21;22)(p12;q11) translocation. Hum Mol Genet 6:357–367.

International HapMap C. 2003. The international HapMap project. Nature 426:789–796.

Kasprowicz J, Kuenen S, Miskiewicz K, Habets RL, Smitz L, Verstreken P. 2008. Inactivation of clathrin heavy chain inhibits synaptic recycling but allows bulk membrane uptake. J Cell Biol 182:1007–1016.

Kumar P, Henikoff S, Ng PC. 2009. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat Protoc 4:1073–1081.

Laivuori H. 2007. Genetic aspects of preeclampsia. Front Biosci 12:2372–2382.

Landrum MJ, Lee JM, Riley GR, Jang W, Rubinstein WS, Church DM, Maglott DR. 2014. ClinVar: Public archive of relationships among sequence variation and human phenotype. Nucleic Acids Res 42: D980–D985.

Lie RT, Rasmussen S, Brunborg H, Gjessing HK, Lie-Nielsen E, Irgens LM. 1998. Fetal and maternal contributions to risk of pre-eclampsia: Population based study. BMJ 316:1343–1347.

Majeed SR, Vasudevan L, Chen CY, Luo Y, Torres JA, Evans TM, Sharkey A, Foraker AB, Wong NM, Esk C, Freeman TA, Moffett A, Keen JH, Brodsky FM. 2014. Clathrin light chains are required for the gyrating-clathrin recycling pathway and thereby promote cell migration. Nat Commun 5:3891.

Miesenbock G, De Angelis DA, Rothman JE. 1998. Visualizing secretion and synaptic transmission with pH-sensitive green fluorescent proteins. Nature 394:192–195.

Moffett-King A. 2002. Natural killer cells and pregnancy. Nat Rev Immunol 2:656–663.

Mort M, Ivanov D, Cooper DN, Chuzhanova NA. 2008. A meta-analysis of nonsense mutations causing human genetic disease. Hum Mutat 29:1037–1047.

Need AC, Shashi V, Hitomi Y, Schoch K, Shianna KV, McDonald MT, Meisler MH, Goldstein DB. 2012. Clinical application of exome sequencing in undiagnosed genetic conditions. J Med Genet 49:353–361.

Ng PC, Henikoff S. 2001. Predicting deleterious amino acid substitutions. Genome Res 11:863–874.

Ng PC, Henikoff S. 2002. Accounting for human polymorphisms predicted to affect protein function. Genome Res 12:436–446.

Ng PC, Henikoff S. 2003. SIFT: Predicting amino acid changes that affect protein function. Nucleic Acids Res 31:3812–3814.

Ng PC, Henikoff S. 2006. Predicting the effects of amino acid substitutions on protein function. Annu Rev Genomics Hum Genet 7:61–80.

Robinson JT, Thorvaldsdottir H, Winckler W, Guttman M, Lander ES, Getz G, Mesirov JP. 2011. Integrative genomics viewer. Nat Biotechnol 29:24–26.

Sailer A, Scholz SW, Gibbs JR, Tucci A, Johnson JO, Wood NW, Plagnol V, Hummerich H, Ding J, Hernandez D, Hardy J, Federoff HJ, Traynor BJ, Singleton AB, Houlden H. 2012. Exome sequencing in an SCA14 family demonstrates its utility in diagnosing heterogeneous diseases. Neurology 79:127–131.

Shamseldin HE, Swaid A, Alkuraya FS. 2013. Lifting the lid on unborn lethal Mendelian phenotypes through exome sequencing. Genet Med 15:307–309.

Sherry ST, Ward MH, Kholodov M, Baker J, Phan L, Smigielski EM, Sirotkin K. 2001. DbSNP: The NCBI database of genetic variation. Nucleic Acids Res 29:308–311.

Stenson PD, Mort M, Ball EV, Howells K, Phillips AD, Thomas NS, Cooper DN. 2009. The Human Gene Mutation Database: 2008 update. Genome Med 1:13.

Tsai MF, Lin YJ, Cheng YC, Lee KH, Huang CC, Chen YT, Yao A. 2007. PrimerZ: Streamlined primer design for promoters, exons and human SNPs. Nucleic Acids Res 35:W63–W65.

UniProt C. 2014. Activities at the universal protein resource (UniProt). Nucleic Acids Res 42:D191–D198.

Uzan J, Carbonnel M, Piconne O, Asmar R, Ayoubi JM. 2011. Pre-eclampsia: Pathophysiology, diagnosis, and management. Vasc Health Risk Manag 7:467–474.

Worthey EA, Mayer AN, Syverson GD, Helbling D, Bonacci BB, Decker B, Serpe JM, Dasu T, Tschannen MR, Veith RL, Basehore MJ, Broeckel U, Tomita-Mitchell A, Arca MJ, Casper JT, Margolis DA, Bick DP, Hessner MJ, Routes JM, Verbsky JW, Jacob HJ, Dimmock DP. 2011. Making a definitive diagnosis: Successful clinical application of whole exome sequencing in a child with intractable inflammatory bowel disease. Genet Med 13:255–262.

Ye K, Schulz MH, Long Q, Apweiler R, Ning Z. 2009. Pindel: A pattern growth approach to detect break points of large deletions and medium sized insertions from paired-end short reads. Bioinformatics 25:2865–2871.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site.