CLINICAL REPORT

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Interstitial Deletion 5q14.3q21.3 With MEF2C Haploinsufficiency and Mild Phenotype: When More Is Less

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An 18-year-old female with mild mental disability (global IQ 69), febrile seizures with subsequent myoclonic/grand mal epilepsy, and subtle morphologic changes is described with del 5(q14.3q21.3) by karyotype and minimal DNA deletion of 21.08 Mb by array comparative genomic hybridization microarray analysis (arr chr5:83,592,798-104,671,993 X1) that encompasses at least 50 genes. Included in the deletion interval is the *MEF2C* gene that usually causes severe mental disability when haploinsufficient, illustrating the complexity of clinic–cytogenetic correlation even with defined segmental aneuploidy. Interaction of MEF2C with the deleted febrile seizure (*FEB4*) and juveline myoclonic epilepsy (*EJM4*) loci plus the G-protein receptor (GPR98/MASS1/Usher syndrome) gene may moderate the phenotype, perhaps through common regulation by calcium.

Key words: clinic–cytogenetic correlation; array comparative genomic hybridization; deletion 5q14.3q21.3; epilepsy; *MEF2C* gene; *FEB4* locus; *GPR98* gene; Usher syndrome

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INTRODUCTION

Microarray comparative genomic hybridization (aCGH) provides new opportunities for clinic–cytogenetic correlation through its ability to specify aneuploid segment length and, by reference to genome coordinates or secondary fluorescent in situ hybridization (FISH) studies, those genes that are triplicated or haploinsufficient [Stankiewicz and Beaudet, 2007; Baldwin et al., 2008]. However, variable phenotypes produced by identical aneuploid segments in different patients or family members illustrate the multifactorial nature of dysmorphogenesis induced by chromosome imbalance and emphasize persisting biologic questions despite the 100-fold increase in cytogenetic resolution. A patient with mild phenotype despite significant chromosome 5q deletion encompassing the neuropathic *MEF2C* gene exemplifies the precision and interpretative challenges presented by aCGH.

CLINICAL REPORT

An 18-year-old female presented for genetic evaluation prompted by parental concern for her reproductive risks. She had a birth weight of 2,778 g after a 36-week gestation complicated by a maternal allergic reaction to Compazine. Mother was 19 and father 21, and the patient had a normal nursery stay after delivery with Apgar scores of 8 and 10. The parents became concerned about her development at age 6–7 months, with milestones at the upper range of normal (sitting at 7 months, walking at 14 months, single words at 10–12 months, 3-word phrases at age 4 years). The face at age 6 months (Fig. 1) shows slight variations compatible with early hypotonia as the cause of late-normal motor development (narrowing at the temples, lateral extension of the superior ear helices, U-shaped upper lip vermilion). She had two febrile seizures with presumed viral illness during infancy and by age 18 months was diagnosed with myoclonic epilepsy. Treatment with Depakote and Clonezapam was instituted and discontinued after she was seizure-free at age 4 years. She had no further medical problems and had menarche at age 12 with regular cycles. She has progressed in regular classes at school with some special education and tutoring.

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FIG. 1. The patient at age 6 months (left); 6 years (center), and 14 years (right).

Her global IQ was measured at 69 with slightly lower scores in language.

About 6 months prior to genetic evaluation she had a grand mal seizure that prompted an EEG showing focal activity and treatment with Depakote 250 mg three times a day. She remains on anticonvulsant therapy with no seizures and is on course to graduate from high school. Normal routine chromosome analysis and thyroid functions were performed at age 3–4 years; head MRI was normal then and again after her recent seizure. Her past medical history is negative for operations, chronic illnesses or signs of autism, and her review of systems showed no sleep, gastrointestinal, or skin problems.

The family history is essentially benign with no consanguinity; the parents and 2 half-sisters through the patient's mother are healthy and no other relatives among 6 parental sibs and 12 nieces or nephews had seizures or motor delays.

On physical examination, height, weight, and head circumference were proportionate at the 25th centile for age. The patient was interactive during the visit with appropriate reciprocal speech and social skills. The hair pattern and texture, craniofacies, chest, heart, back, and extremities (including palmar/plantar creases) were normal, and the genitalia were mature (Tanner V). She had normal strength and muscle mass with no cranial deficits.

CYTOGENETIC AND ACGH FINDINGS

Standard PHA stimulated chromosome studies revealed a deletion between bands q14.3 and q21.3 of chromosome 5 (Fig. 2A) that was not observed in the prior analysis. To quantify the extent of deletion, DNA was extracted using the *DNeasy Blood & Tissue Kit* (Qiagen Inc., Valencia, CA) and microarray analysis was performed using the *EmArray Cyto6000* (Agilent Technologies Inc., Santa Clara, CA), a genome-wide plus targeted array [Baldwin et al., 2008]. Hybridization was performed according to the manufacturer's protocol without modification, and the array was scanned using an Agilent B scanner. The resulting data were analyzed using *DNA Analytics v4.0.81*, and a deletion spanning bands q14.3 to 21.3 was visualized on chromosome 5 (Fig. 2A). The maximum deletion size was determined to be around 21.22 Mb, starting after base pair 83,541,273 and ending before 104,761,701; the minimal deletion size was 21.08 Mb spanning base pairs 83,592,798–104,671,993. Standard PHA stimulated chromosome studies on the parents were normal.

DISCUSSION

We describe a patient with mild motor delays, subsequent learning differences, seizures, and deletion 5q14.3q21.3 demonstrated by routine chromosome analysis and quantified as minimally deleting 21.08 Mb by aCGH (83,592,798–104,671,993 bp). The deletion was missed by routine chromosome analysis in 1993 but is evident on high-resolution chromosome analysis (Fig. 2A) and quantified by microarray analysis (Fig. 2B). Contiguous oligonucleotides showing decreased signal by aCGH along with selected genes in the deleted interval are shown in Table I.

Several large chromosome 5 deletions overlap with that in our patient, all with significant dysmorphology and mental disability [Silengo et al., 1981; Ohdo et al., 1982; Rodewald et al., 1982; Harprecht-Beato et al., 1983]. More relevant to our patient are the smaller, overlapping deletions of 5q14.3 [Le Meur et al., 2010], 5q13q15.1 [Krishna et al., 1997], 5q14.3q15 [Cardoso et al., 2009], and 5q13q15 [Stoll et al., 1980], respectively associated with severe epilepsy-mental disability, growth hormone deficiency, periventricular heterotopia with epilepsy, and altered morphology with severe mental disability. Normal growth in our patient might position the putative growth-regulating gene upstream of her deletion at 5q13 [Krishna et al., 1997], but evident gene interaction in the region suggests a multifactorial cause for growth delay.

Two epilepsy-associated loci and two candidate genes lie within the 5q14.3q21.3 region deleted in our patient. Febrile seizures confer a 5- to 7-fold increased risk for epilepsy, and one of several loci (FEB4-MIM60453) was defined by linkage studies of 48 families [Nakayama et al., 2000, 2002]. Nearby is a locus for autosomal dominant juvenile myoclonic epilepsy (EJM4-MIM611364) linked to region 5q12q14 in a Southern Indian family with teenage onset of myoclonic jerks and generalized epilepsy without febrile seizures

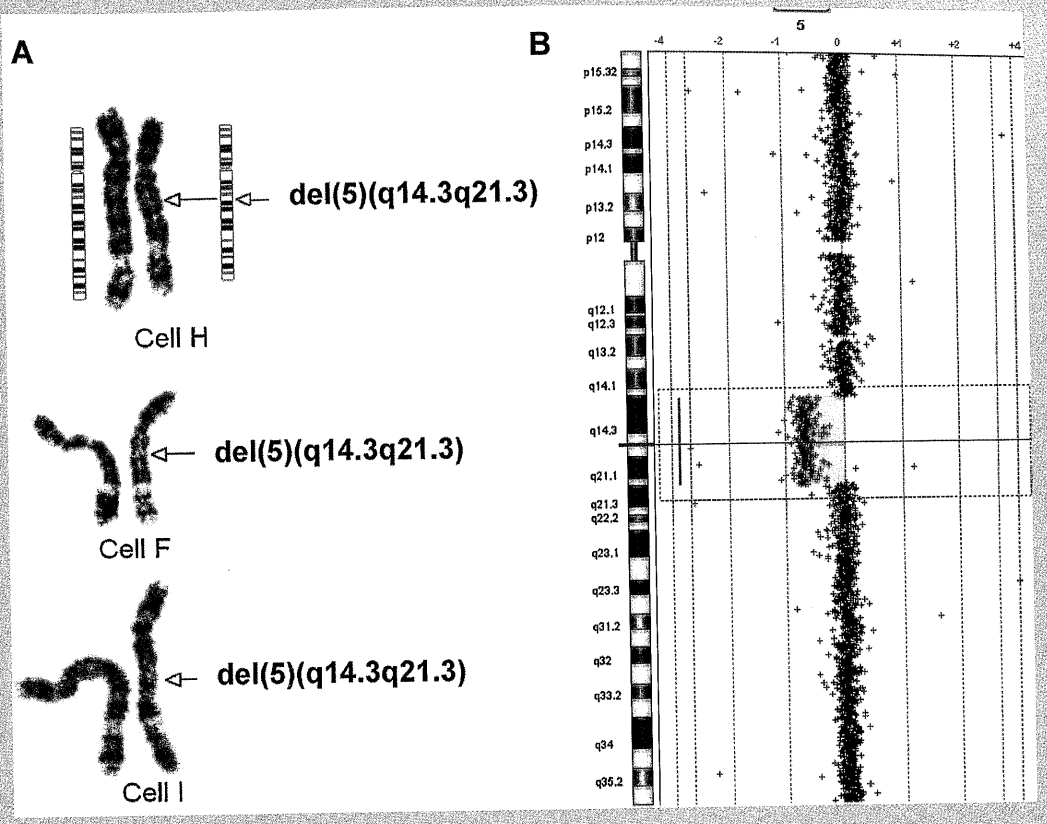

FIG. 2. A: Routine chromosome analysis (500 bands) from three patients cells showing deletion 5q14.3q21.3; B: comparative genomic hybridization along chromosome 5 showing a decrease in patient hybridization between bands 5q14.3 and 5q21.3 (83,592,798–104,671,993 bp). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com]

[Kapoor et al., 2007]. Differentiation of epilepsy loci is also suggested by the one family among those studied by Nakayama et al. [2002] who had both febrile and afebrile seizures due to a nonsense mutation in a G-protein-coupled receptor 98 (GPR98) gene at 5q14.3 (89,890,373–90,495,789 bp). This gene, also known as the very large G-protein-coupled receptor 1 (VLGR1) or monogenic audiogenic seizure susceptibility 1 (MASS1) gene (MIM602851), is mutated in patients with type 2C Usher syndrome [MIM 605472—Kent et al., 2002] but not in febrile seizure families linked to FEB4 by Deprez et al. [2006]. Also relevant is the MEF2C gene (MADS box transcription enhancer factor 2, polypeptide C, MIM600662) at 5q14.3 (88,051,922–88,214,780 bp) that is clearly within our patient's deleted region—either mutated or haploinsufficient in patients with severe intellectual disability, epilepsy, autism, movement disorders, and cerebral malformations [Le Meur et al., 2010; Nowakowska et al., 2010].

Le Meur et al. [2010] reported five patients with severe epilepsy and disability who had a minimal common deleted region that encompassed only the *MEF2C* gene, a gene encoding a calcium-regulated transcription factor that acts during neurogenesis to augment excitatory synapses. The latter patients and the severely disabled patient of Nowakowska et al. [2010] with abnormal corpus callosum, occipital demyelination, and partial *MEF2C* gene deletion are very different from our patient with her larger 5q deletion that encompasses the GPR 98 and *MEF2C* genes. Interaction among these or other genes must moderate our patient's MEF2C deletion, indicating haploinsufficiency of MEF2C with potential half-normal expression may sometimes have milder effects on distal pathways (including *MECP2* genes) than the disruptive mutations reported by Zweier et al. [2010].

Mice with selective knock-out of MEF2C expression in brain do have milder learning and memory deficits in contrast to the severe craniofacial, neuronal, and heart defects that occur with other MEF2C deletions [Barbosa et al., 2008]. Regulation of the MEF2C gene includes enhancement of DNA-binding activity by phosphorylation on serine-59, acetylation on lysine-4, inhibition by phosphorylation on serine-396, and at least two alternative transcripts [Barbosa et al., 2008]. Equally complex splicing of VLGR1 and its component *GPR 98* genes with an epilepsy-associated repeat (EAR), also influenced by calcium [Scheel et al., 2002], add to the gene–environmental interactions that can explain variable consequences of 5q/MEF2C deletion.

Our patient and others with *MEF2C* gene haploinsufficiency illustrate the difficulty of interpreting aneuploid mechanisms even when the deleted segments and their component genes are precisely defined. These variable phenotypes may require complementary SNP arrays that define particular alleles/parental origins and eventual correlation with CSF expression or neuroimaging techniques

TABLE I. Patient aCGH Results for Selected Oligonucleotide Probes and Genes Compared to Cases 1–6 of Le Meur et al. [2010]

Oligonucleotide probe number(s) or gene	Signal	Coordinates	Expression	1	2	3	4	5
#17192050	Normal	083,541,214-083,541,273						
#17192212 to #17197292 (29 oligos)	↓	083,592,798-085,871,711						
COX7C (No oligo within gene region)	None	085,913,784-085,916,581	Ganglia, AV node, liver					
#17197574 to #17198782 (8 oligos)	↓	085,990,184-086,523,471						
RASA1 #17198894 to #17199431 (5 oligos within gene)	↓	086,564,151-086,687,732	Brain, heart, lung, muscle			↓		
		086,587,461-086,832,400						
#17199525-#17202047 (17 oligos)	↓	086,879,253-087,993,498		↓		↓		
MEF2C #37164849, #17202661 (2 oligos within gene)	↓	088,014,058-088,179,302	Brain, heart, lung, kidney	↓	↓	↓	↓	↓
		088,098,249-088,177,097						
#17202380 to #17206081 (15 oligos)	↓	88229048-89505967		↓	↓	↓	↓	
CETN3 (no oligo within gene region)	None	089,689,531-89,705,603)	Brain, lung		↓	↓		
#01280554 to #37269187 (4 oligos)	↓	89559719-89791124			↓	↓		
GPR98 #17206983 to #17208453 (8 oligos within gene)	↓	089,854,617-090,460,032	Brain, lung, smooth muscle		↓	↓		
		089,891,166-090,448,165						
#17208613 to #37271411 (3 oligos)	↓	090,502,299-090,619,361			↓	↓		
ARRDC3 #01282269 (1 oligo within gene)	↓	090,664,541-090,679,149	Brain, ganglia			↓		
		090,672,075-090,672,134						
#17209125 to #17215732 (37 oligos)	↓	090,723,925-093,456,106				↓		
C5orf36 #17215848 to #01287108 (7 oligos within gene)	↓	(93,486,557-93,954,309)	Brain, prostate			↓		
		093,531,105 093,924,160						
#17216995 to #37282050 (14 oligos)	↓	093,987,828-094,890,478				↓		
ARSK (no oligo within gene region)		094,890,825-094,940,805	Brain			↓		
#17219472 to #17222418 (15 oligos)	↓	094,952,872-096,091,247				↓		
CAST #17222418 (1 oligo within gene region)	↓	095,997,941-096,110,383	Brain, ovary			↓		
		096,091,188-096,091,247						
#1722723 to #17231260 (53 oligos)	↓	096,183,439-100,109,665						
ST8SIA4 #17231540 (1 oligo within gene region)	↓	100,142,640-100,238,987	Ganglia, skin, adipocytes					
		100,204,233-100,204,292						
#17231777 to #17236049 (28 oligos)	↓	100,277,629-102,361,504						
GIN1 #37299259 (1 oligo within gene region)	↓	102,421,704-102,455,842	Brain, ganglia, adrenal					
		102,432,136-102,432,195						
#17236414 to #37304762 (31 oligos)	↓	102,531,814-104,671,993						
#37304903	Normal	104,761,701-104,761,760						

Oligonucleotide numbers following A-16-P prefix as designated by Affymetrix, all 60 bp in length; gene names, coordinates, descriptions, and tissue expression taken from UCSC genome browser [Kent et al., 2002]; genes with significant neural expression were selected for display—COX7C, cytochrome c oxidase subunit VIIc precursor, nuclear-encoded; RASA1, RAS p21 protein activator 1 isoform 1—cytoplasmic GTPase-activating protein; MEF2C, myocyte-specific enhancer factor 2C binding to MEF2 regulatory region of muscle-specific genes; CETN3, Centrin 3 centrosomic protein with 4 calcium-binding domains; GPR98 G-protein-coupled receptor 98 precursor calcium-binding 7-transmembrane receptor; ARRDC3, arrestin domain-containing 3; C5orf36, hypothetical protein; ARSK, arylsulfatase K precursor; CAST, calpastatin isoform h—endogenous calpain [calcium-dependent cysteine protease inhibitor]; ST8SIA4, ST8 alpha-*N*-acetyl-neuraminide—protein synthesizing polysialic acid that modulates neural cell adhesion molecule [NCAM1]; GIN1, zinc finger, H2C2 domain containing. The numbered right panel represent patients from the study by Le Meur et al. [2010] with] indicating the genes deleted in these patients.

[Nordin et al., 1998; Stanfield et al., 2008]. They emphasize the need for large aCGH databases in the public domain and forecast difficulties of interpretation that will attend use of aCGH for prenatal diagnosis.

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