Duplication 16p11.2 in a Child With Infantile Seizure Disorder

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Received 10 January 2010; Accepted 23 February 2010

Submicroscopic recurrent 16p11.2 rearrangements are associated with several neurodevelopmental disorders, including autism, mental retardation, and schizophrenia. The common 16p11.2 region includes 24 known genes, of which 22 are expressed in the developing human fetal nervous system. As yet, the mechanisms leading to neurodevelopmental abnormalities and the broader phenotypes associated with deletion or duplication of 16p11.2 have not been clarified. Here we report a child with spastic quadriaparesis, refractory infantile seizures, severe global developmental delay, hypotonia, and microcephaly, and a de novo 598 kb 16p11.2 microduplication. Family history is negative for any of these features in parents and immediate family members. Sequencing analyses showed no mutations in DOC2A, QPRT, and SEZ6L2, genes within the duplicated 16p11.2 region that have been implicated in neuronal function and/or seizure related phenotypes. The child’s clinical course is consistent with a rare seizure disorder called malignant migrating partial seizure disorder of infancy, raising the possibility that duplication or disruption of genes in the 16p11.2 interval may contribute to this severe disorder. © 2010 Wiley-Liss, Inc.

Key words: autism; seizure; 16p11.2; microarrays; DOC2A; QPRT; SEZ6L2

INTRODUCTION

Recurrent rearrangements at 1q21.1, 15q11.2, 15q13.3, 16p13.11, 17q21.31, and 22q11.2 have been associated with various neuropsychiatric disorders such as mental retardation and schizophrenia [Mefford, 2009; Mefford and Eichler, 2009]. Recently, recurrent microdeletions in 15q11.2 and 16p13.11 have also been implicated in certain idiopathic generalized epilepsies [de Kovel et al., 2010], raising the possibility that other recurrent microdeletions and their reciprocal microduplications may also be involved in epileptogenesis.
region a formidable challenge for genotype–phenotype correlation studies, and more detailed clinical assessment of patients with 16p11.2 rearrangements are needed.

A number of epileptic syndromes of infancy and childhood are known, with the genetic bases of some already elucidated [Nabbout and Dulac, 2008]. Malignant migrating partial seizures of infancy (MMPSI) is a rare, age-specific, epileptic encephalopathy; seizures are refractory to vigorous antiepileptic therapy and after seizure onset there is a profound loss or arrest of both cognitive and motor milestones in survivors [Gross-Tsur et al., 2004]. Mutational analysis of the KCNQ2, KCNQ3, SCN1A, SCN2A, CLCN2, and MECP2 genes in infants clinically described as having malignant migrating partial seizures have been carried out, but no pathogenic alterations were detected [Coppola et al., 2006]. The genetic basis of MMPSI remains as yet unknown.

Here, we report a patient with a de novo 16p11.2 microduplication and a history of severe static encephalopathy characterized by spastic quadriaparesis, severe global developmental delay, hypotonia, and microcephaly. He also had severe refractory infantile seizures, consistent with a diagnosis of MMPSI [Gross-Tsur et al., 2004].

**CLINICAL REPORT**

A boy presented at 4 months of age to the Genetics Clinic for evaluation of a seizure disorder. He was conceived by intrauterine sperm injection. His mother, 34 years old at time of conception, had a prior history of spontaneous abortion with the same partner; she was treated with Repronex (a fertility medication containing follicle stimulating hormone and luteinizing hormone which promotes egg release) and Ovidrel (a recombinant human stimulating hormone and luteinizing hormone which stimulates the ovaries to produce eggs) and exhibited myopathic facies. He also had severe refractory infantile seizures, consistent with a diagnosis of MMPSI [Gross-Tsur et al., 2004].

At 4 months of age, his weight, length, and head circumference were at the 60th (7.1 kg), 37th (62.9 cm), and 9th (40.5 cm) centiles, respectively. He exhibited esotropia, but no major craniofacial dysmorphisms. Muscle bulk appeared normal but tone was reduced in the truncal region and significant head lag was noted. His suck and grasp reflexes were intact bilaterally. No cutaneous pigmentary changes were noted by Wood’s lamp examination. Long-term video-EEG monitoring demonstrated frequent multifocal epileptiform discharges within right and left temporal regions, absence of normal sleep architecture, and numerous tonic seizures (many subclinical), which lateralized predominantly to the right temporal region, but were also present in the left temporal region.

Introduction of treatment with vigabatrin at about 5 months of age resulted in a substantial reduction in seizure frequency. Within about 2 months, clinically detected seizures ceased, and did not recur during treatment with vigabatrin. However, his neurodevelopment did not improve.

Repeat EEG at 9 months of age remained significantly abnormal, with predominant rhythms at 4–4.5 Hz over the central and occipital areas as well as intermittent faster (12–16 Hz) rhythms over both the frontal and central areas and persistent sharp spike waves at T4. EEG never demonstrated hypersynchrony. At 10 months of age, his weight, length, and head circumference were at the 32nd (9.2 kg), 37th (72.3 cm), and 1st (42.8 cm) centiles, respectively. He exhibited positional plagiocephaly, esotropia, minimal head control, poor trunk control, and hypertonia of the upper and lower extremities bilaterally. He intermittently focused on the examiner.

At 27 months of age, his weight, length, and head circumference were at the 46th (13 kg), 43rd (89.3 cm), and 1st (45.7 cm) centiles, respectively. An undescended right testicle was noted. He had spastic quadriplegia, with minimal head control and poor trunk control with intermittent extremity hypertonia on flexion and extension. Clonus was noted of the left lower extremity. He did not exhibit clear interest or awareness of his surroundings and appeared to have no purposeful movements. A repeat MRI showed mild hypoplasia of the corpus callosum but no degenerative changes. At 34 months of age, his weight, length, and head circumference were at the 61th (14.5 kg), 14th (90.3 cm), and 2nd (46.1 cm) centiles, respectively. He continued to have esotropia and exhibited myopathic facies.

Family history was negative for seizure, developmental delay, psychiatric disorders, or mental retardation. In addition to the mother, a maternal aunt was suspected to have adrenal hyperplasia. There were two early infant deaths in distant cousins on the maternal side of the family and a maternal second cousin with epilepsy. There was no consanguinity in the family.

Molecular genetic testing included normal blood karyotype, normal MECP2 sequencing and deletion and duplication analyses, normal methylation PCR for SNRPN, normal CDKL5 sequencing analysis, normal serum long chain fatty acids, acylcarnitines, trans-ferrin, peroxisomal and plasma amino acid profiles, normal urine organic acid profile, and negative urine S-sulfocysteine. Lactic acid was at the upper normal level of 2.2 MEq/L and creatine phosphokinase was normal at 145 IU/L (normal range 38–240 IU/L). Serum sialotransferrin testing for congenital disorders of glycosylation was normal. An EMArray Cyto6000 oligonucleotide-based chromosomal microarray analysis at the Michigan Medical Genetics Laboratories (MMGL) revealed a de novo 598 kb duplication of 16p11.2.

**MATERIALS AND METHODS**

DNA samples from the proband and his parents were obtained after acquiring informed consent approved by the Institutional Review Board for Human Subject Research at the University of Michigan.
Array Comparative Genomic Hybridization (CGH)

Array CGH was conducted in MMGL at the University of Michigan using the custom-designed EMArray Cyto6000 chip, implemented on the Agilent 44K platform [Baldwin et al., 2008] which contains 43,103 oligonucleotide probes spaced on average every 75 kb with whole genome coverage. DNA was isolated from blood samples using a standard, semi-automated method (Biorobot M48 workstation, Qiagen, Inc., Valencia, CA). The procedures for DNA digestion, labeling and comparative genomic hybridization were as described in Agilent Oligonucleotide-Based Array CGH for Genomic DNA Analysis, Protocol version 4.0 June 2006 (Agilent Technologies, Inc., Santa Clara, CA) with some modifications [Baldwin et al., 2008]. The fluorescent signals on the array slides were scanned into image files using GenePix 4200A scanner and GenePix-Pro 6.1 software (Axon Instruments/Molecular Devices Corp., Union City, CA). The array images were then imported and evaluated by Agilent Feature Extraction 9.5 software to determine copy number differences and/or aberrations between the patient DNA and the sex mismatched DNA [Baldwin et al., 2008]. Patient DNA was labeled with Cy3 and sex-mismatched pooled reference DNA was labeled with Cy5. All the labeled DNA samples were cleaned of reagents and unincorporated dyes by vacuum filtration. Purified fluorescently labeled patient DNA and reference DNA were mixed together, and hybridized to the EmArray Cyto6000 [Baldwin et al., 2008]. Data were analyzed by interpreting the resulting Cy3/Cy5 ratio. Numbering of the Cyto6000 44K EMArray was according to Genome Build UCSC hg 17 assembly (Build 35, May 2004). Chr16:29,500,284-30,098,069 coordinates in hg17 remain unchanged in hg18 (March 2006) and hg19 (GRCh37) with 100% of bases and 100% of span, as determined using the Convert function on the UCSC Genome Browser (http://genome.ucsc.edu/).

Expression in Fetal Nervous System

Search for genes expressed in the human fetal nervous system was carried out using the BGEE Gene Expression Evolution database (http://bgee.unil.ch/bgee/bgee) release 06 (September 17, 2009) based on Ensembl Genome Browser database release 55 (http://www.ensembl.org/).

RESULTS

Array CGH analysis showed a ~598 kb gain of genomic material at 16p11.2 (chr16:29,500,284–30,098,069; hg17), which contains 24 known genes (Fig. 1A,C and Table I). Array CGH analysis of both parents of the proband indicated no 16p11.2 gain (data not shown). The duplicated 16p11.2 region was flanked by segmental duplications (low copy repeats, LCRs) and adjacent areas of copy number polymorphisms as noted in Figure 1B. Genes coding for both known and hypothetical proteins within the duplicated region are shown in Figure 1C and those coding for known proteins are detailed in Table I. Microsatellite analysis was uninformative for the duplication (data not shown) but qPCR analysis in the proband and his parents confirmed the 16p11.2 microduplication results, and further demonstrated that this rearrangement occurred de novo (Fig. 2).

We sequenced the complete coding regions, associated splice sites, and 5` and 3` untranslated regions (UTRs) of QPRT, DOC2A, and SEZ6L2 in the proband and his parents (data not shown). Our sequence data did not identify any putative pathogenic alterations.

DISCUSSION

We report here on a boy with a common recurrent 16p11.2 duplication and infantile refractory seizures whose clinical course is consistent with MMPSI [Gross-Tsur et al., 2004]. The ~598 kb gain of genomic material in our patient is similar in size to previously reported recurrent 16p11.2 duplications (~600 kb) and has approximately the same chromosomal boundaries [McCarthey et al., 2009; Shinawi et al., 2009].

To date, there is minimal information about seizure phenotypes in individuals with 16p11.2 deletions or duplications. Shinawi et al. [2009] reported a series of 16p11.2 deletion and duplication patients and found 3/10 duplication patients have seizures, one confirmed as a de novo rearrangement. Bijlsma et al. [2009] reported three patients with 16p11.2 deletions and history of...
FIG. 1. Whole genome 44 K oligonucleotide-based microarray analysis. A: The ~598 kb duplication at 16p11.2; (B) the segmental duplications and copy number polymorphisms adjacent to the duplicated (597,786 bp) region (between the vertical red lines) as determined using the UCSC Genome Browser [http://genome.ucsc.edu/]; and (C) the genes within the ~598 kb region (within the red rectangle) as determined using the Ensembl Genome Browser [http://www.ensembl.org/].
<table>
<thead>
<tr>
<th>Gene</th>
<th>Entrez gene ID</th>
<th>Name</th>
<th>Expression in human fetal nervous system</th>
<th>Disorder/function</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALDOA</td>
<td>226</td>
<td>Fructose-bisphosphate aldolase A</td>
<td>Y</td>
<td>A glycolytic enzyme catalyzing the reversible conversion of fructose-1,6-bisphosphate to glyceraldehyde 3-phosphate and dihydroxyacetone phosphate</td>
</tr>
<tr>
<td>ASPHD1</td>
<td>253982</td>
<td>Aspartate beta-hydroxylase domain containing 1</td>
<td>Y</td>
<td>Catalyses oxidative reactions in a range of metabolic processes</td>
</tr>
<tr>
<td>C16orf53</td>
<td>79447</td>
<td>PTIP-associated 1 protein; PAXIP1-associated protein; PA1</td>
<td>Y</td>
<td>A component of a Set1-like multiprotein histone methyltransferase complex</td>
</tr>
<tr>
<td>CDIPT</td>
<td>10423</td>
<td>CDP-diacylglycerol-inositol 3-phosphatidyltransferase</td>
<td>Y</td>
<td>Phosphatidylinositol synthase</td>
</tr>
<tr>
<td>DOC2A</td>
<td>8448</td>
<td>Double C2-like domains, alpha</td>
<td>Y</td>
<td>Expressed mainly in the brain and suggested to be involved in Ca(2⁺)-dependent neurotransmitter release</td>
</tr>
<tr>
<td>FAM57B</td>
<td>83723</td>
<td>Protein FAM57B; L0C83723</td>
<td>Y</td>
<td>Member of the longevity-assurance (LAG1) protein family involved in determining life span</td>
</tr>
<tr>
<td>GDPD3</td>
<td>79153</td>
<td>Glycerophosphodiester phosphodiesterase domain containing 3; GDPD</td>
<td>Y</td>
<td>Involved in energy production and conversion</td>
</tr>
<tr>
<td>HIRIP3</td>
<td>8479</td>
<td>HIRA-interacting protein 3</td>
<td>Y</td>
<td>In vitro, the HIRIP3 gene product binds HIRA, as well as H2B and H3 core histones, indicating that a complex containing HIRA-HIRIP3 could function in some aspects of chromatin and histone metabolism</td>
</tr>
<tr>
<td>INO80E (CCDC95)</td>
<td>283899</td>
<td>INO80 complex subunit E</td>
<td>Y</td>
<td>Coiled-coil domain containing 95</td>
</tr>
<tr>
<td>KCTD13</td>
<td>253980</td>
<td>Potassium channel tetramerization domain-containing protein 13</td>
<td>Y</td>
<td>BTB/POZ domain-containing protein; polymerase delta-interacting protein 1; TNFAIP1-like protein</td>
</tr>
<tr>
<td>KIF22</td>
<td>3835</td>
<td>Kinesin-like DNA-binding protein</td>
<td>Y</td>
<td>Microtubule-dependent molecular motors that transport organelles within cells and move chromosomes during cell division</td>
</tr>
<tr>
<td>MAPK3</td>
<td>5595</td>
<td>Mitogen-activated protein kinase 3; ERK1; insulin-stimulated MAP2 kinase</td>
<td>Y</td>
<td>Involved in cell cycle progression</td>
</tr>
<tr>
<td>MAZ</td>
<td>4150</td>
<td>Myc-associated zinc finger protein</td>
<td>Y</td>
<td>Purine-binding transcription factor; serum amyloid A activating factor</td>
</tr>
<tr>
<td>MVP</td>
<td>9961</td>
<td>Major vault protein</td>
<td>N</td>
<td>Lung resistance-related protein</td>
</tr>
<tr>
<td>PPP4C</td>
<td>5531</td>
<td>Protein phosphatase 4 (formerly X), catalytic subunit; PP4, PPH3</td>
<td>Y</td>
<td>PP4 interacts with the survival of motor neurons complex and enhances the temporal localization of snRNPs</td>
</tr>
<tr>
<td>PRRT2</td>
<td>112476</td>
<td>Proline-rich transmembrane protein 2; CD225</td>
<td>Y</td>
<td>Interferon-induced transmembrane protein associated with cell growth suppression</td>
</tr>
<tr>
<td>QPRT</td>
<td>23475</td>
<td>Quinolinate phosphoribosyltransferase</td>
<td>Y</td>
<td>Quinolinate acts as a potent endogenous excitotoxin through hyperstimulation of N-methyl D-aspartate receptor in neurons</td>
</tr>
<tr>
<td>SEZ6L2</td>
<td>26470</td>
<td>Seizure related 6 homolog (mouse)-like 2</td>
<td>Y</td>
<td>Seizure related type I transmembrane receptor protein</td>
</tr>
</tbody>
</table>

(Continued)
developmental delay and seizures, one confirmed as a de novo rearrangement. Kumar et al. [2008] also reported one autism patient with a 16p11.2 deletion and history of seizures. Ghebranious et al. [2007] described a 16p11.2 microdeletion in monozygotic twins with complex phenotypes that include seizure disorder with onset at 11.5 and 13 years of age, along with mental retardation and heart defects. Although a strong association has been shown between 16p11.2 deletion/duplication and autism [Kumar et al., 2008; Marshall et al., 2008; Weiss et al., 2008] as well as autism and epilepsy (present in ~20% of autism cases) [Levisohn, 2007], more detailed clinical history and correlation with incidence of epilepsy (and/or autism) among patients with 16p11.2 chromosomal rearrangements is warranted.

Interestingly, microcephaly (circumference ≤ 5th centile) was found in 6/10 patients with 16p11.2 duplication by Shinawi et al. [2009] and in 1/9 patients with 16p11.2 duplication by McCarthy et al. [2009] and Weiss et al. [2008]. These observations are consistent with our patient’s microcephalic phenotype and highlight the phenotypic variability associated with increased 16p11.2 dosage.

The 16p11.2 duplicated region contains 24 known protein-coding genes, including QPRT, DOC2A, and SEZ6L2 (Table I) which are implicated in neuronal function and/or seizure-related phenotypes [Orita et al., 1995; Shimizu-Nishikawa et al., 1995; Groffen et al., 2006; Guillemin et al., 2007; Kumar et al., 2009; Vamos et al., 2009]. To our knowledge, no mutations in any of these genes have been identified in humans with seizure disorders.

QPRT (OMIM 606248) encodes the quinolinate phosphoribosyltransferase enzyme that uses quinolinate as its substrate. Quinolinate is an intermediate in the de novo synthesis pathway of nicotinamide adenine dinucleotide (NAD) from tryptophan (the kynurenine pathway) and acts as a potent endogenous excitotoxin through hyperstimulation of the N-methyl D-aspartate receptor in neurons [Guillemin et al., 2007]. Elevation of quinolinate levels in the human brain has been postulated to be involved in the pathogenesis of neurodegenerative and seizure disorders [Nemeth et al., 2005; Vamos et al., 2009]. It is possible that the 16p11.2 duplication in our patient negatively affects the expression of QPRT. An inverse correlation between copy number and gene expression has been reported in a minority (~10%) of copy number variants (CNVs) [Stranger et al., 2007]. If such an inverse correlation exists for the duplication and the abundance of QPRT product, an overall decreased amount of functional quinolinate phosphoribosyltransferase in our patient may lead to an increased level of quinolinate, and consequently, an increased propensity for seizure. Additional expression studies in our patient (or mouse models) would be needed in order to test this hypothesis.

The DOC2A (MIM 604567) gene product is mainly expressed in the brain, is suggested to be involved in Ca^{2+}-dependent neurotransmitter release, and is implicated in nervous system
development, synaptic transmission, exocytosis, and transport [Duncan et al., 2000; Groffen et al., 2006]. Although the role of DOC2A in epilepsy and human development is unclear, mice with Doc2a deletions show alterations in synaptic transmission and learning and behavioral deficits [Sakaguchi et al., 1999].

**SEZ6L2** is considered a seizure-related gene because a closely related ortholog, Sez-6, is upregulated in response to seizure-inducing reagents in mouse neurons [Shimizu-Nishikawa et al., 1995]. **SEZ6L2** is expressed in the human fetal brain, where expression is highest in post-mitotic cortical layers, hippocampus, amygdala, and thalamus [Kumar et al., 2009]. Although mice with Sez6l2 deletions do not show any obvious defects in development or behavior [Miyazaki et al., 2006], an association between a novel **SEZ6L2** coding variant R386H and autism has been proposed [Kumar et al., 2009]. The extent to which **DOC2A** and **SEZ6L2** are involved in infantile epilepsy remains to be determined.

Although our patient’s clinical phenotype may be attributable to the microduplication found in the 16p11.2 region (i.e., a gene-dosage effect), the duplicated 16p11.2 region might also contain mutations (such as a gain-of-function) in **QPRT**, **DOC2A**, or **SEZ6L2** that could contribute to his seizure phenotype. However, we detected no changes in the coding regions, splice sites, or untranslated regions of **QPRT**, **DOC2A**, and **SEZ6L2** in our patient.

Our patient’s phenotype might also be caused by an insertion of the duplicated region elsewhere in the genome, which could affect a seizure-related gene at that location. However, this seems less likely since common recurrent genomic rearrangements (such as recurrent 16p11.2 deletions and reciprocal duplications) often occur between LCRs and are commonly caused by NAHR events [Stankiewicz and Lupski, 2002; Mefford, 2009; Mefford and Eichler, 2009]. It remains possible that other protein(s) in the duplicated 16p11.2 region (Fig. 1C or Table 1) contribute to neuronal development and seizure disorders. Testing the expression levels of **QPRT**, **DOC2A**, **SEZ6L2**, and other genes in the duplicated region in patients with seizure disorder and 16p11.2 rearrangement may help determine the importance of these gene products in seizure risk. However, whether white blood cells are a suitable proxy for brain (or other nervous system) cells for such expression analyses is uncertain. Analysis of mutant mouse models will be helpful for further delineating the contributions of these genes to seizure phenotypes.

**ACKNOWLEDGMENTS**

We thank Dr. Jeffrey W. Innis for critical review of this manuscript and for support through MMGL. This study was also supported by funds from MMGL, the Department of Pediatrics, the Department of Human Genetics, and a CTSA pilot award (UL1RR024986) from the University of Michigan. RAK is supported by a postdoctoral fellowship award from Autism Speaks. DMM is supported by NIH grants DC009410 and NS054784.

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