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

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Abstract

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 quadriparesis, 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.

Keywords

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.

Submicroscopic chromosomal rearrangements involving 16p11.2 have been associated with a number of clinical conditions over the last several years. Microdeletions and duplications of 16p11.2 were first reported to have a significant association with autism (OMIM 209850), a childhood neurodevelopmental disorder that emerges in the first three years of life and is

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characterized by impairments in verbal and non-verbal communication, social reciprocity, restricted interests, and repetitive behavior [Kumar et al., 2008; Marshall et al., 2008; Weiss et al., 2008]. Microdeletions and/or duplications in 16p11.2 have also been identified in patients with mental retardation [Bijlsma et al., 2009], schizophrenia [McCarthy et al., 2009], obesity [Bochukova et al., 2009], and in normal individuals [Bijlsma et al., 2009]. The highly recurrent nature of 16p11.2 rearrangements is thought to be mediated by flanking segmental duplications or low copy repeats (LCRs) that predispose this locus to non-allelic homologous recombination [Kumar et al., 2008]. The wide range of phenotypes associated with 16p11.2 deletions and duplications make this 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 quadriparesis, 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 stimulates the ovaries to produce eggs) and Ovidrel (a recombinant human chorionic gonadotropin which promotes egg release) and was on dexamethasone (a pregnancy category C medication) throughout her pregnancy due to a history of congenital adrenal hyperplasia. The patient was born at 38 weeks gestational age via augmented vaginal delivery with a birth weight at the 40th centile (3.4 kg). Apgar scores were 6 and 8 at 1 and 5 minutes, respectively. He exhibited seizure activity starting at one day of life, characterized by generalized stiffening, rolling back of the head, and high pitched crying which lasted 30 seconds to 1 min. His initial electroencephalogram (EEG) showed frequent multifocal independent epileptiform discharges, most frequently involving the temporal regions. Treatment with phenobarbital, topiramate, and oxcarbazepine in the first four months of life had no impact on seizure frequency. He continued to have about four clinically recognized seizures daily, and made no developmental progress. Magnetic resonance imaging (MRI) of the brain with spectroscopy at 2 months of age was unremarkable.

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 had bilateral shifting 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 two 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 hypsarrhythmia. 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), 43th (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, transferrin, 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., 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. Data were analyzed by Agilent's CGH Analytics 3.5 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 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/).

Microsatellite analysis and quantitative-PCR (qPCR)

Confirmation studies using microsatellite analysis and qPCR were performed as previously described [Kumar et al., 2008].

DNA sequencing

We sequenced three candidate genes (*QPRT*, *DOC2A*, and *SEZ6L2*) in the patient and his parents. Primer sequences for *DOC2A* and *SEZ6L2* have been previously reported [Kumar et al., 2009]. Primer sequences for *QPRT* were as follows: Exon 1F (5′-GCTTCTGAGTTCCCCATCAG-3′) and Exon 1R (5′-CCAGAGGAGGCAACAAGG-3′); Exon 2F (5′-GGCCAGTTCCCAGTTTCACT-3′) and Exon 2R (5′-CTGTTCACCCGGTCATGG-3′); Exon 3F (5′-GTGCTGGGCCCTATCGTC-3′) and Exon 3R (5′-ACAAGCCAAGGGGAGGTAAG-3′); Exon 4.1F (5′-GGACAACTTCAAGCCAGAGG-5′) and Exon 4.1R (5′-ACATTTGCTGACCCCTCACT-3′); and Exon 4.2F (5′-GGCACATTTGGCACTAGCTT-3′) and Exon 4.2R (5′-AAGGTTTTGGCCTGTCTGG-3′). The following sequences were added to each primer to facilitate sequencing: forward (F) sequencing tail (5′-TGTAAAACGACGGCCAGT-3′) and reverse (R) sequencing tails (5′-CAGGAAACAGCTATGAC-3′). DNA was amplified in a reaction comprised of: 20 ng genomic DNA, 1x buffer I (1.5 mM MgCl2, Applied Biosystems, Foster City, CA), 1 mM dNTPs (Applied Biosystems), 0.4 μM primer (each of forward and reverse; IDT, Coralville, IA), and 0.25 units AmpliTaq Gold (Applied Biosystems) in a total volume of 10 μl. Thermocycling conditions used 'Touchdown' PCR [Korbie and Mattick 2008]. PCR products were purified in a 10 μl reaction comprised of 6.6 units Exonuclease I and 0.66 units shrimp alkaline phosphatase that were incubated at 37°C for 30 min followed by 80°C for 15 minutes. Sequencing reactions and data analysis were carried out as previously described [Kumar et al., 2009].

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 (17 Sept. 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 (Figs. 1A and 1C, and Table 1). 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 Fig. 1B. Genes coding for both known and hypothetical proteins within the duplicated region are shown in Fig. 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 [McCarthy 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 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 ≤ 5^{th} 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 sequence 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 nonallelic homologous recombination (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 I) 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.

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REFERENCES

Baldwin EL, Lee JY, Blake DM, Bunke BP, Alexander CR, Kogan AL, Ledbetter DH, Martin CL. Enhanced detection of clinically relevant genomic imbalances using a targeted plus whole genome oligonucleotide microarray. Genet Med. 2008; 10:415–429. [PubMed: 18496225]

Bijlsma EK, Gijsbers AC, Schuurs-Hoeijmakers JH, van Haeringen A, van de Putte DE Fransen, Anderlid BM, Lundin J, Lapunzina P, Jurado LA Perez, Chiaie B Delle, Loeys B, Menten B, Oostra A, Verhelst H, Amor DJ, Bruno DL, van Essen AJ, Hordijk R, Sikkema-Raddatz B, Verbruggen KT, Jongmans MC, Pfundt R, Reeser HM, Breuning MH, Ruivenkamp CA. Extending the phenotype of recurrent rearrangements of 16p11.2: deletions in mentally retarded patients without autism and in normal individuals. Eur J Med Genet. 2009; 52:77–87. [PubMed: 19306953]

Bochukova EG, Huang N, Keogh J, Henning E, Purmann C, Blaszczyk K, Saeed S, Hamilton-Shield J, Clayton-Smith J, O'Rahilly S, Hurles ME, Farooqi IS. Large, rare chromosomal deletions associated with severe early-onset obesity. Nature. 2009

Coppola G, Veggiotti P, Del Giudice EM, Bellini G, Longaretti F, Taglialatela M, Pascotto A. Mutational scanning of potassium, sodium and chloride ion channels in malignant migrating partial seizures in infancy. Brain Dev. 2006; 28:76–79. [PubMed: 16168594]

de Kovel CG, Trucks H, Helbig I, Mefford HC, Baker C, Leu C, Kluck C, Muhle H, von Spiczak S, Ostertag P, Obermeier T, Kleefuss-Lie AA, Hallmann K, Steffens M, Gaus V, Klein KM, Hamer HM, Rosenow F, Brilstra EH, Trenite DK, Swinkels ME, Weber YG, Unterberger I, Zimprich F, Urak L, Feucht M, Fuchs K, Moller RS, Hjalgrim H, De Jonghe P, Suls A, Ruckert IM, Wichmann HE, Franke A, Schreiber S, Nurnberg P, Elger CE, Lerche H, Stephani U, Koeleman BP, Lindhout D, Eichler EE, Sander T. Recurrent microdeletions at 15q11.2 and 16p13.11 predispose to idiopathic generalized epilepsies. Brain. 2010; 133:23–32. [PubMed: 19843651]

Duncan RR, Shipston MJ, Chow RH. Double C2 protein. A review. Biochimie. 2000; 82:421–426. [PubMed: 10865129]

Ghebranious N, Giampietro PF, Wesbrook FP, Rezkalla SH. A novel microdeletion at 16p11.2 harbors candidate genes for aortic valve development, seizure disorder, and mild mental retardation. Am J Med Genet A. 2007; 143A:1462–1471. [PubMed: 17568417]

Groffen AJ, Friedrich R, Brian EC, Ashery U, Verhage M. DOC2A and DOC2B are sensors for neuronal activity with unique calcium-dependent and kinetic properties. J Neurochem. 2006; 97:818–833. [PubMed: 16515538]

Gross-Tsur V, Ben-Zeev B, Shalev RS. Malignant migrating partial seizures in infancy. Pediatr Neurol. 2004; 31:287–290. [PubMed: 15464643]

Guillemin GJ, Cullen KM, Lim CK, Smythe GA, Garner B, Kapoor V, Takikawa O, Brew BJ. Characterization of the kynurenine pathway in human neurons. J Neurosci. 2007; 27:12884–12892. [PubMed: 18032661]

Korbie DJ, Mattick JS. Touchdown PCR for increased specificity and sensitivity in PCR amplification. Nat Protoc. 2008; 3:1452–1456. [PubMed: 18772872]

Kumar RA, KaraMohamed S, Sudi J, Conrad DF, Brune C, Badner JA, Gilliam TC, Nowak NJ, Cook EH Jr. Dobyns WB, Christian SL. Recurrent 16p11.2 microdeletions in autism. Hum Mol Genet. 2008; 17:628–638. [PubMed: 18156158]

Kumar RA, Marshall CR, Badner JA, Babatz TD, Mukamel Z, Aldinger KA, Sudi J, Brune CW, Goh G, Karamohamed S, Sutcliffe JS, Cook EH, Geschwind DH, Dobyns WB, Scherer SW, Christian

SL. Association and mutation analyses of 16p11.2 autism candidate genes. PLoS ONE. 2009; 4:e4582. [PubMed: 19242545]

Levisohn PM. The autism-epilepsy connection. Epilepsia. 2007; 48(Suppl 9):33–35. [PubMed: 18047599]

Marshall CR, Noor A, Vincent JB, Lionel AC, Feuk L, Skaug J, Shago M, Moessner R, Pinto D, Ren Y, Thiruvahindrapduram B, Fiebig A, Schreiber S, Friedman J, Ketelaars CE, Vos YJ, Ficicioglu C, Kirkpatrick S, Nicolson R, Sloman L, Summers A, Gibbons CA, Teebi A, Chitayat D, Weksberg R, Thompson A, Vardy C, Crosbie V, Luscombe S, Baatjes R, Zwaigenbaum L, Roberts W, Fernandez B, Szatmari P, Scherer SW. Structural variation of chromosomes in autism spectrum disorder. Am J Hum Genet. 2008; 82:477–488. [PubMed: 18252227]

McCarthy SE, Makarov V, Kirov G, Addington AM, McClellan J, Yoon S, Perkins DO, Dickel DE, Kusenda M, Krastoshevsky O, Krause V, Kumar RA, Grozeva D, Malhotra D, Walsh T, Zackai EH, Kaplan P, Ganesh J, Krantz ID, Spinner NB, Roccanova P, Bhandari A, Pavon K, Lakshmi B, Leotta A, Kendall J, Lee YH, Vacic V, Gary S, Iakoucheva LM, Crow TJ, Christian SL, Lieberman JA, Stroup TS, Lehtimaki T, Puura K, Haldeman-Englert C, Pearl J, Goodell M, Willour VL, Derosse P, Steele J, Kassem L, Wolff J, Chitkara N, McMahon FJ, Malhotra AK, Potash JB, Schulze TG, Nothen MM, Cichon S, Rietschel M, Leibenluft E, Kustanovich V, Lajonchere CM, Sutcliffe JS, Skuse D, Gill M, Gallagher L, Mendell NR, Craddock N, Owen MJ, O'Donovan MC, Shaikh TH, Susser E, Delisi LE, Sullivan PF, Deutsch CK, Rapoport J, Levy DL, King MC, Sebat J. Microduplications of 16p11.2 are associated with schizophrenia. Nat Genet. 2009; 41:1223–1227. [PubMed: 19855392]

Mefford HC. Genotype to phenotype-discovery and characterization of novel genomic disorders in a "genotype-first" era. Genet Med. 2009; 11:836–842. [PubMed: 20010361]

Mefford HC, Eichler EE. Duplication hotspots, rare genomic disorders, and common disease. Curr Opin Genet Dev. 2009; 19:196–204. [PubMed: 19477115]

Miyazaki T, Hashimoto K, Uda A, Sakagami H, Nakamura Y, Saito SY, Nishi M, Kume H, Tohgo A, Kaneko I, Kondo H, Fukunaga K, Kano M, Watanabe M, Takeshima H. Disturbance of cerebellar synaptic maturation in mutant mice lacking BSRPs, a novel brain-specific receptor-like protein family. FEBS Lett. 2006; 580:4057–4064. [PubMed: 16814779]

Nabbout R, Dulac O. Epileptic syndromes in infancy and childhood. Curr Opin Neurol. 2008; 21:161–166. [PubMed: 18317274]

Nemeth H, Toldi J, Vecsei L. Role of kynurenines in the central and peripheral nervous systems. Curr Neurovasc Res. 2005; 2:249–260. [PubMed: 16181118]

Orita S, Sasaki T, Naito A, Komuro R, Ohtsuka T, Maeda M, Suzuki H, Igarashi H, Takai Y. Doc2: a novel brain protein having two repeated C2-like domains. Biochem Biophys Res Commun. 1995; 206:439–448. [PubMed: 7826360]

Sakaguchi G, Manabe T, Kobayashi K, Orita S, Sasaki T, Naito A, Maeda M, Igarashi H, Katsuura G, Nishioka H, Mizoguchi A, Itohara S, Takahashi T, Takai Y. Doc2alpha is an activity-dependent modulator of excitatory synaptic transmission. Eur J Neurosci. 1999; 11:4262–4268. [PubMed: 10594652]

Shimizu-Nishikawa K, Kajiwara K, Sugaya E. Cloning and characterization of seizure-related gene, SEZ-6. Biochem Biophys Res Commun. 1995; 216:382–389. [PubMed: 7488116]

Shinawi M, Liu P, Kang SH, Shen J, Belmont JW, Scott DA, Probst FJ, Craigen WJ, Graham B, Pursley A, Clark G, Lee J, Proud M, Stocco A, Rodriguez D, Kozel B, Sparagana S, Roeder E, McGrew S, Kurczynski T, Allison L, Amato S, Savage S, Patel A, Stankiewicz P, Beaudet A, Cheung SW, Lupski JR. Recurrent reciprocal 16p11.2 rearrangements associated with global developmental delay, behavioral problems, dysmorphism, epilepsy, and abnormal head size. J Med Genet. 2009

Stankiewicz P, Lupski JR. Genome architecture, rearrangements and genomic disorders. Trends Genet. 2002; 18:74–82. [PubMed: 11818139]

Stranger BE, Forrest MS, Dunning M, Ingle CE, Beazley C, Thorne N, Redon R, Bird CP, de Grassi A, Lee C, Tyler-Smith C, Carter N, Scherer SW, Tavare S, Deloukas P, Hurles ME, Dermitzakis ET. Relative impact of nucleotide and copy number variation on gene expression phenotypes. Science. 2007; 315:848–853. [PubMed: 17289997]

Vamos E, Pardutz A, Klivenyi P, Toldi J, Vecsei L. The role of kynurenines in disorders of the central nervous system: Possibilities for neuroprotection. J Neurol Sci. 2009

Weiss LA, Shen Y, Korn JM, Arking DE, Miller DT, Fossdal R, Saemundsen E, Stefansson H, Ferreira MA, Green T, Platt OS, Ruderfer DM, Walsh CA, Altshuler D, Chakravarti A, Tanzi RE, Stefansson K, Santangelo SL, Gusella JF, Sklar P, Wu BL, Daly MJ. Association between microdeletion and microduplication at 16p11.2 and autism. N Engl J Med. 2008; 358:667–675. [PubMed: 18184952]

Fig. 1.

Whole genome 44K 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/).

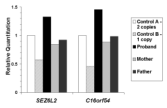

Fig. 2.

16p11.2 Microduplication confirmation by qPCR. Probes for *SEZ6L2* and *C16orf54* were as reported in Kumar et al. [2008]. Controls A and B are known to have two copies and one copy, respectively, of *SEZ6L2* and *C16orf54*.

Table 1

Known Protein Coding Genes and Related Disorders Contained in the 16p11.2 Duplication Region

Gene	Entrez Gene ID	Name	Expression in Human Fetal Nervous System*	Disorder/Function
ALDOA	226	Fructose-bisphosphate aldolase A	Y	A glycolytic enzyme catalyzing the reversible conversion of fructose-1,6-bisphosphate to glyceraldehyde 3-phosphate and dihydroxyacetone phosphate.
ASPHD1	253982	Aspartate beta-hydroxylase domain containing 1	Y	Catalyses oxidative reactions in a range of metabolic processes.
C16ORF53	79447	PTIP-associated 1 protein; PAXIP1-associated protein; PA1	Y	A component of a Set1-like multiprotein histone methyltransferase complex.
CDIPT	10423	CDP-diacylglycerol--inositol 3-phosphatidyltransferase	Y	Phosphatidylinositol synthase.
DOC2A	8448	Double C2-like domains, alpha	Y	Expressed mainly in the brain and suggested to be involved in Ca(2+)-dependent neurotransmitter release.
FAM57B	83723	Protein FAM57B; LOC83723	Y	Member of the Longevity-assurance (LAG1) protein family involved in determining life span.
GDPD3	79153	Glycerophosphodiester phosphodiesterase domain containing 3; GDPD	Y	Involved in energy production and conversion.
HIRIP3	8479	HIRA-interacting protein 3	Y	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.
INO80E (CCDC95)	283899	INO80 complex subunit E	Y	Coiled-coil domain containing 95.
KCTD13	253980	Potassium channel tetramerization domain-containing protein 13	Y	BTB/POZ domain-containing protein; Polymerase delta-interacting protein 1; TNFAIP1-like protein.
KIF22	3835	Kinesin-like DNA-binding protein	Y	Microtubule-dependent molecular motors that transport organelles within cells and move chromosomes during cell division.
MAPK3	5595	Mitogen-activated protein kinase 3; ERK1; Insulin-stimulated MAP2 kinase	Y	Involved in cell cycle progression.
MAZ	4150	Myc-associated zinc finger protein	Y	Purine-binding transcription factor; serum amyloid A activating factor.
MVP	9961	Major vault protein	N	Lung resistance-related protein.
PPP4C	5531	Protein phosphatase 4 (formerly X), catalytic subunit; PP4, PPH3	Y	PP4 interacts with the Survival of Motor Neurons complex and enhances the temporal localization of snRNPs.
PRRT2	112476	Proline-rich transmembrane protein 2; CD225	Y	Interferon-induced transmembrane protein associated with cell growth suppression.

Gene	Entrez Gene ID	Name	Expression in Human Fetal Nervous System*	Disorder/Function
QPRT	23475	Quinolinate phosphoribosyltransferase	Y	Quinolinate acts as a potent endogenous excitotoxin through hyperstimulation of N-methyl D-aspartate receptor in neurons.
SEZ6L2	26470	Seizure related 6 homolog (mouse)-like 2	Y	Seizure related type I transmembrane receptor protein.
SPN	6693	Sialophorin; CD43; GPL115	Y	Important for immune function and T-cell activation.
TAOK2	9344	Serine/threonine-protein kinase TAO2	Y	Prostate-derived STE20-like kinase 1.
TBX6	6911	T-box 6	Y	Anatomical structure morphogenesis and mesoderm development.
TMEM219	124446	Transmembrane protein 219	Y	Conserved in chimpanzee, dog, cow, mouse, and rat; function unkown.
YPEL3	83719	Yippee-like 3	Y	Conserved in chimpanzee, mouse, rat, zebrafish, A.thaliana, and rice; function unknown.
ZG16 (AC009133.7)	653808	Zymogen granule protein 16 homolog	N	Jacalin-like lectin domain containing; JCLN.

* Based on BGEE Gene Expression Evolution database (http://bgee.unil.ch/bgee/bgee) release 06 (Sept.2009) which is based on Ensembl release 55; N, no; and Y, yes.