RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART A

Clinically Relevant Single Gene or Intragenic Deletions Encompassing Critical Neurodevelopmental Genes in Patients With Developmental Delay, Mental Retardation, and/or Autism Spectrum Disorders

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Received 19 January 2011; Accepted 19 May 2011

Recent studies suggest that copy number variations (CNVs) encompassing several genes involved in neurodevelopmental pathways are associated with a variety of neuropsychiatric phenotypes, including developmental delay (DD), mental retardation (MR), and autism spectrum disorders (ASDs). Here we present eight patients in a cohort of ~1,200 patients referred for clinical array CGH testing for various neurodevelopmental phenotypes, who were identified to carry small (<1.0 Mb with the majority <500 kb) either total gene or intragenic deletions encompassing critical synaptic and other neurodevelopmental genes. The presentations of these patients included variable degrees of DD, speech problems, learning disabilities, MR, autistic-like features, and mild non-specific dysmorphic features. These genes belong to four functional categories, including neuronal transcription factor genes (*NFIA* at 1p31.3, *MEF2C* at 5q14.3, and *CAMAT1* at 1p36.23p36.31), neuron-specific splicing factor genes (*RBFOX1* at 16p13.2p13.3), genes involved in synapse formation and maintenance (*CNTNAP2* at 7q35 and *LRFN5* at 14q21.2), and genes involved in neurotransmission (*CHRNA7* at 15q13.3 and *IL1RAPL1* at Xp21.2p21.3). Our report expands the list of neurodevelopmental genes deleted in various neurobehavioral phenotypes, expands the phenotypes caused by haploinsufficiency of previously reported critical neurodevelopmental genes, and elucidates the clinical relevance and need for careful clinical interpretation of some small CNVs <500 kb. This report also suggests that small clinically relevant deletions encompassing critical synaptic and other neurodevelopmental genes can present clinically with various neurobehavioral phenotypes, which implies the existence of overlapping neuronal pathways in the pathogenesis of these phenotypes.

Key words: neurodevelopmental disorders; developmental delay; mental retardation; autism; array CGH; CNV

How to Cite this Article:
Mikhail FM, Lose EJ, Robin NH, Descartes MD, Rutledge KD, Rutledge SL, Korf BR, Carroll AJ. 2011. Clinically relevant single gene or intragenic deletions encompassing critical neurodevelopmental genes in patients with developmental delay, mental retardation, and/or autism spectrum disorders.
Am J Med Genet Part A 155:2386–2396.

INTRODUCTION

The use of high-resolution array comparative genomic hybridization (array CGH) in a clinical setting has enabled the detection of numerous clinically relevant submicroscopic copy number gains or losses throughout the human genome. This technology has been widely used in the routine workup of neuropsychiatric phenotypes, including developmental delay (DD), mental retardation (MR), and autism spectrum disorders (ASD). Recent studies suggest that

Additional supporting information may be found in the online version of this article.

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Published online 16 August 2011 in Wiley Online Library (wileyonlinelibrary.com).
DOI 10.1002/ajmg.a.34177

copy number variations (CNVs) encompassing several genes involved in neurodevelopmental pathways may be associated with these conditions [Friedman et al., 2006; Menten et al., 2006; Froyen et al., 2007; Sebat et al., 2007; Szatmari et al., 2007; Christian et al., 2008; Marshall et al., 2008]. These CNVs include recurrent microdeletions and microduplications flanked by segmental duplication and mediated by non-allelic homologous recombination (NAHR), as well as non-recurrent deletions and duplications, varying in size from a few hundred kilobases (kb) to a few megabases (Mb), and mediated by other molecular mechanisms [Stankiewicz and Lupski, 2010]. The latter category includes a very interesting group of small CNVs that encompass either a single gene or part of a gene (intragenic), and involves critical synaptic and other neurodevelopmental genes [Guilmatre et al., 2009]. Recently, a theme has emerged suggesting that normal cognition and behavior depend on tight neuronal homeostatic control mechanisms. Altered dosage of genes involved in neuronal homeostasis leads to dysfunctional neuronal networks, failure of normal brain development, and results in different neurodevelopmental phenotypes [Ramocki and Zoghbi, 2008]. Functionally, most of these genes belong to five main categories, including neuronal transcription factor genes and genes that encode proteins involved in synapse formation and maintenance, protein ubiquitination, chromatin remodeling, and neurotransmission [Ramocki and Zoghbi, 2008; Guilmatre et al., 2009].

Here we report on eight patients referred for clinical array CGH analysis, who presented with variable degrees of DD, speech problems, learning disabilities, MR, autistic-like features, and mild non-specific dysmorphic features, and in which array CGH revealed small total gene or intragenic deletions involving several critical neurodevelopmental genes. This report expands the list of neurodevelopmental genes deleted in various neurobehavioral phenotypes, expands the phenotypes caused by haploinsufficiency of previously reported critical neurodevelopmental genes, and finally underscores the need for careful clinical interpretation of small CNVs <500 kb.

PATIENTS AND METHODS

Patients

During the period between January 2009 and December 2010 approximately 1,200 patients were referred to our cytogenetic lab for clinical array CGH testing mostly because of DD, intellectual disability, ASD, and/or multiple congenital anomalies. Excluding all benign CNVs and recurrent microdeletions, 59 patients (4.91%) were identified to carry deletions smaller than 1.0 Mb. Out of these 59 cases, the 8 cases reported here carried small (<1.0 Mb with the majority <500 kb) either total gene or intragenic deletions involving critical neurodevelopmental genes. All eight patients were examined by a clinical geneticist, and both G-banded and array CGH analyses were performed for diagnostic purposes. All deletions reported here were confirmed by fluorescence in situ hybridization (FISH) analyses. When parents and other family members were available, the de novo or inherited nature of the deletion was investigated using FISH.

Cytogenetic and Fluorescence In Situ Hybridization (FISH) Analyses

Routine as well as high-resolution G-banded chromosome analyses and FISH analyses were performed on metaphase preparations of peripheral blood lymphocytes from the patients and other family members using standard techniques. The FISH analyses were performed using probes from the RPCI-11 human genomic library (Empire Genomics, Buffalo, NY), and the clones identities were confirmed both by FISH analyses on normal metaphase spreads as well as end sequencing. The chromosomes were analyzed and the karyotype described according to the International System for Cytogenetic Nomenclature (ISCN 2009) [Shaffer et al., 2009].

Array Comparative Genomic Hybridization (Array CGH) Analysis

High-resolution whole-genome array CGH was performed using the 4 × 44k and/or 2 × 105k Agilent oligo-arrays (Agilent Technologies, Santa Clara, CA). These are custom-designed arrays that are based on the ISCA (International Standard Cytogenomic Array) consortium design. DNA was extracted from the patients' peripheral blood using the Qiagen blood mini kit (Qiagen, Valencia, CA). DNA labeling, slide hybridization, washing, and scanning were performed following the manufacturer's protocol. The arrays were scanned using the GenePix 4000B scanner (Molecular Devices, Sunnyvale, CA). The scanned arrays were analyzed using the "Feature Extraction v9.5" and "DNA Analytics v4.0" software (Agilent Technologies). All genomic breakpoints were mapped using the UCSC genome browser using human genome build 36 (NCBI36/hg18).

RESULTS

Table I summarizes the clinical features, growth parameters, inheritance, and cytogenetic findings of our patients. Array CGH plots aligned with a genomic map of the same region are shown in Figures 1 and 2. Confirmatory metaphase FISH images are shown in Supplementary Figure 1.

Patient 1

Patient 1 is a 25-year-old Caucasian female who was referred for a genetic consult because of dysmorphic features and cognitive delay. Her past medical history included cognitive delay, bipolar disorder/depression, incapability of making her own decisions, scoliosis, hypothyroidism secondary to partial thyroidectomy, late menarche, and delayed development of secondary sexual characteristics. The indication for partial thyroidectomy was not clear from the patient's history. A brain MRI performed at 22 years of age showed a diffusely decreased volume of white matter with hypoplasia of the corpus callosum, mild tonsillar ectopia without Chiari I malformation, and mild hydrocephalus. On physical exam she presented with macrocephaly, scarce hair, high forehead, hypotelorism, high palate, left low-set ear, pointed chin, webbed neck, and scoliosis. G-banded chromosome analysis revealed a 46,XX normal female chromosome complement. A subsequent renal ultrasound

TABLE I. Clinical Features, Inheritance, and Cytogenetic Findings of our Patients

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8
Gender	F	M	M	M	F	F	F	M
Age	25 y	2.5 y	14 y	3.0 y	16 y	10 y	4 y	6.0 y
Height	155 cm (5–10th centile)	89 cm (25th centile)	158 cm (25th centile)	92 cm (10–25th centile)	153 cm (5–10th centile)	136 cm (25–50th centile)	97 cm (10–25th centile)	109 cm (10th centile)
Weight	60 kg (25–50th centile)	12.8 kg (25–50th centile)	35 kg (<5th centile)	12.8 kg (10–25th centile)	52.5 kg (25–50th centile)	35.1 kg (50–75th centile)	15.0 kg (25–50th centile)	19 kg (25th centile)
Head circumference	58.1 cm (>97th centile)	50.5 cm (90–95th centile)	54.2 cm (25–50th centile)	46 cm (<5th centile)	54.5 cm (50th centile)	48.2 cm (<2nd centile)	49.4 cm (25–50th centile)	48.8 cm (2nd centile)
Inheritance	Mother is normal; father NA	De novo	De novo	NA	Mother is normal; father NA	Father and maternal grandmother are normal; mother NA	NA	Maternally inherited
DD	NA	+	+	+	+	+	+	+
Intellectual disability	+	NA	+	NA	+	+	+	+
Behavioral problems	Bipolar disorder, depression, incapable of making her own decisions	–	ADHD, PDD	–	–	–	Autistic-features	–
Seizures	–	–	–	–	–	+ (Grand-mal)	–	–
Other features	Macrocephaly, high-forehead, hypotelorism, high palate, pointed chin, webbed neck, scoliosis	Expressive and receptive language delay, relative macrocephaly, epicanthic folds, flat nasal bridge, posteriorly rotated ears, hyperkinesis with stereotypic movement of hands and feet	VPI, speech problems, abnormal ataxic gait, no dysmorphic features	Language delay, microcephaly	VPI, speech problems, long narrow face, upslanted palpebral fissures, small ears, broad nasal bridge, high palate, pointed chin, low posterior hairline	Microcephaly, receding forehead, upslanted palpebral fissures	Mild hypotonia, mild hypertelorism, upslanted palpebral fissures	Language delay, microcephaly, triangular shaped face, mild hypertelorism, upslanted palpebral fissures, diminished tone and strength
Brain MRI/CT	Hydrocephalus, hypoplasia of CC	Normal MRI	NA	NA	NA	NA	NA	NA
Gene	*NFIA*	*MEF2C*	*CAMTA1*	*RBFOX1* (*A2BP1*)	*CNTNAP2*	*LRFN5*	*CHRNA7*	*IL1RAPL1*
Cytogenetic locus	1p31.3	5q14.3	1p36.31	16p13.2	7q35	14q21.2	15q13.3	Xp21.2p21.3
Size of deletion (kb)	254	412	305	353	246	890	537	1,070

NA, information not available; ADHD, attention deficit hyperactivity disorder; PDD, developmental pervasive disorder; VPI, velopharyngeal insufficiency; CC, corpus callosum; y, years.

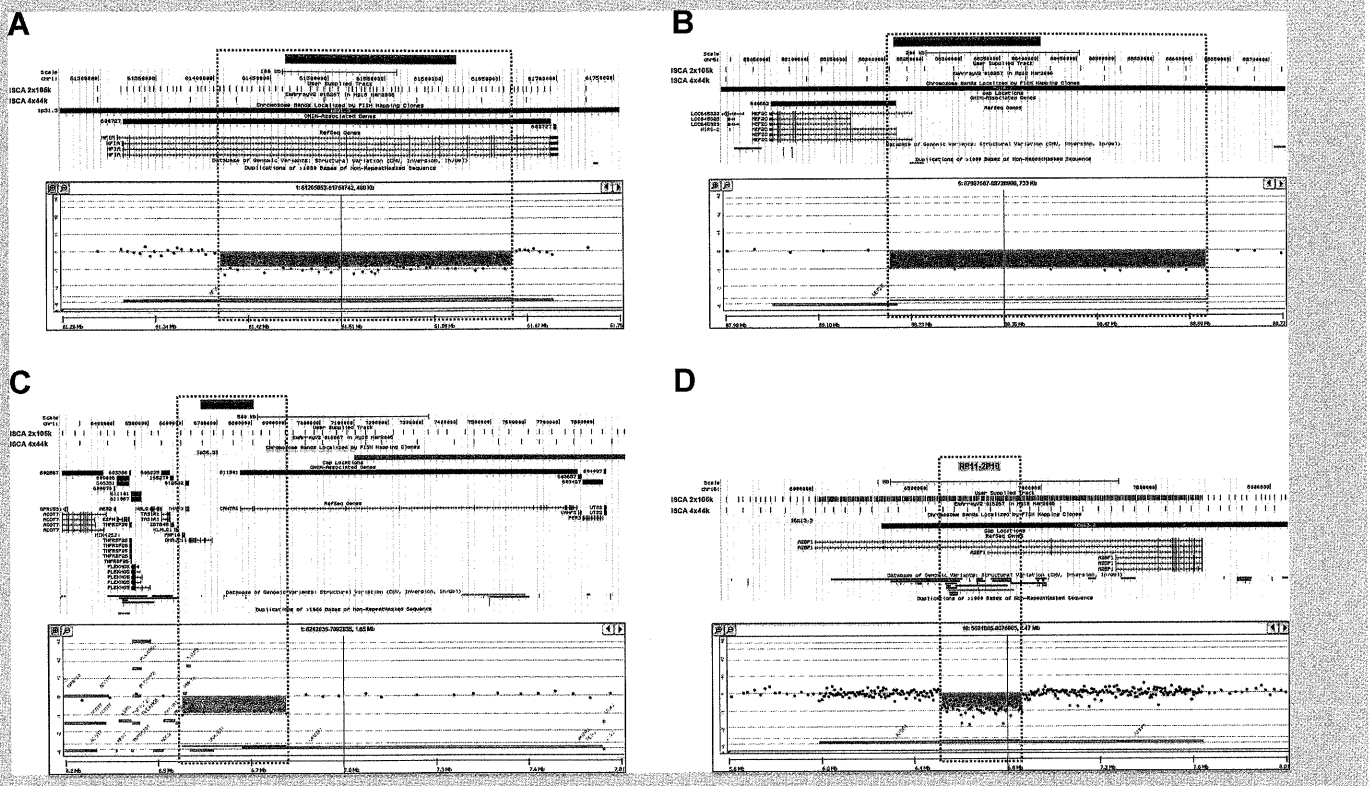

FIG. 1. Oligo-array CGH plots aligned with a genomic map of the same region generated using the UCSC genome browser [NCBI36/hg18], and showing the deleted region [dashed line], confirmatory FISH probes, genomic ruler, ISCA 2 × 105k and 4 × 44k array probes, cytogenetic band, annotated RefSeq genes, and benign CNVs reported in the DGVs. A: Patient 1 run on the ISCA 2 × 105k array. B: Patient 2 run on the ISCA 2 × 105k array. C: Patient 3 run on the ISCA 4 × 44k array. D: Patient 4 run on the ISCA 2 × 105k array. [Color figure can be seen in the online version of this article, available at http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1552-4833].

showed no evidence of major anomalies. Array CGH analysis demonstrated the presence of an ~254 kb intragenic deletion in the *NFIA* gene at 1p31.3 with breakpoints at genomic positions 61,405,254 and 61,659,346 bp. Metaphase FISH analysis using the RP11-1123B24 probe confirmed this deletion (Supplementary Fig. 1A). Using the UCSC genome browser, this deletion was shown to encompass seven exons (exon 5 through 11) of *NFIA* (Fig. 1A). Maternal FISH testing demonstrated that the mother is normal, whereas the father was unavailable for testing.

Patient 2

Patient 2 is a 2.5-year-old boy born at term after a normal pregnancy with birth weight of 2,920 g (10–25th centile). He has history of global DD with language (expressive and receptive) more affected than motor skills. He cannot walk independently, uses no words consistently and appropriately, and does not follow any spoken commands. On physical exam he presented with relative macrocephaly, epicanthic folds, depressed nasal bridge, slightly posteriorly rotated ears, and hyperkinesis with constant movement of his hands and feet. He had no verbal communication during the entire examination. Brain MRI was normal. G-banded chromosome analysis revealed a 46,XY normal male chromosome complement. Array CGH analysis demonstrated the presence of an ~412 kb deletion at 5q14.3 with breakpoints at genomic positions 88,205,506 and 88,618,256 bp, which encompasses the promoter region and the first three exons of the *MEF2C* gene (Fig. 1B). Metaphase FISH analysis using the RP11-690G22 probe confirmed this deletion (Supplementary Fig. 1B). Parental FISH analyses showed that they did not carry the deletion indicating a de novo event.

Patient 3

Patient 3 is a 14-year-old boy born at term after a normal pregnancy. He has history of global DD, learning disability, speech problems, abnormal ataxic gait, and velopharyngeal insufficiency. He has been diagnosed with attention deficit hyperactivity disorder (ADHD) and pervasive developmental disorder (PDD) and is currently receiving special education services through his school system. His physical exam showed a normal craniofacial appearance with no dysmorphic features. G-banded chromosome analysis revealed a 46,XY normal male chromosome complement. Array CGH analysis demonstrated the presence of an ~305 kb deletion at

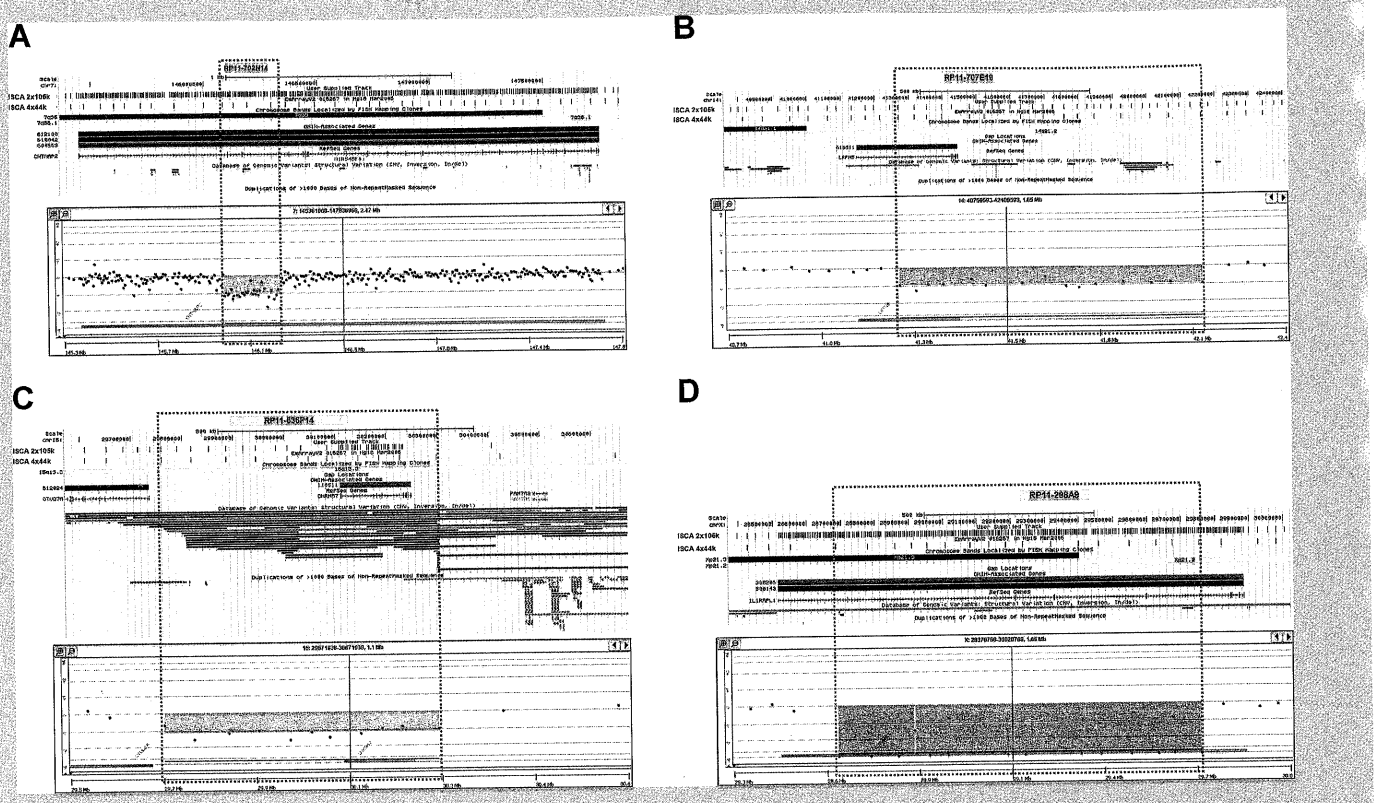

FIG. 2. Oligo-array CGH plots aligned with a genomic map of the same region generated using the UCSC genome browser [NCBI36/hg18], and showing the deleted region [dashed line], confirmatory FISH probes, genomic ruler, ISCA 2 × 105k and 4 × 44k array probes, cytogenetic band, annotated RefSeq genes, and benign CNVs reported in the DGVs. A: Patient 5 run on the ISCA 2 × 105k array. B: Patient 6 run on the ISCA 4 × 44k array. C: Patient 7 run on the ISCA 4 × 44k array. D: Patient 8 run on the ISCA 4 × 44k array. [Color figure can be seen in the online version of this article, available at http://onlinelibrary.wiley.com/journal/10.1002/[ISSN]1552-4833].

1p36.31 with breakpoints at genomic positions 6,594,333 and 6,899,375 bp. Metaphase FISH analysis using the RP11-242F24 probe confirmed this deletion (Supplementary Fig. 1C). Using the UCSC genome browser, this deletion was shown to encompass the promoter region and the first three exons of the *CAMTA1* gene, as well as three other RefSeq genes, including *PHF13*, *THAP3*, and *DNAJC11* (Fig. 1C). Parental FISH analyses showed that they did not carry the deletion indicating a de novo event.

Patient 4

Patient 4 is a 3-year-old boy delivered at term by cesarean section with a birth weight of 3,370 g (25–50th centile). The pregnancy was complicated by viral illness at 2 months of gestation, but ultrasounds were normal and no growth problems were noted in utero. He has history of failure to thrive (FTT), walked at 13 months of age, and experienced language delay. Physical exam revealed no dysmorphic features but he had microcephaly with a head circumference <5th centile; however, his height and weight were at the 10–25th centile consistent with continuing growth. He is currently receiving both speech and occupational therapy. G-banded chromosome analysis revealed a 46,XY normal male chromosome complement. Array CGH analysis demonstrated the presence of an ~353 kb intragenic deletion in the *RBFOX1* gene (also known as *A2BP1*) at 16p13.2 with breakpoints at genomic positions 6,550,763 and 6,904,158 bp. Metaphase FISH analysis using the RP11-2P10 probe confirmed this deletion (Supplementary Fig. 1D). Using the UCSC genome browser, this deletion was shown to encompass two exons (exons 3 and 4) of the *RBFOX1* gene, as well as the promoter region of transcript variant 6 (Fig. 1D). Neither parent was available for testing.

Patient 5

Patient 5 is a 16-year-old girl born at 40 weeks gestation with a birth weight of 3,200 g (25th centile). She walked at 14 months and was late talking. She has history of learning disability, speech problems, and velopharyngeal incompetence (VPI). She is currently in ninth grade with poor grades (C–F). She receives special services at school as well as speech therapy. On physical exam she presented with long narrow face, bifrontal orbital hypoplasia, upslanted palpebral fissures, small ears, broad nasal bridge, high-arched palate, pointed chin, and low posterior hairline. G-banded chromosome analysis revealed a 46,XX normal female chromosome complement. Array

CGH analysis demonstrated the presence of an ~246 kb intragenic deletion in the *CNTNAP2* gene at 7q35 with breakpoints at genomic positions 146,076,724 and 146,322,943 bp. Metaphase FISH analysis using the RP11-702N14 probe confirmed this deletion (Supplementary Fig. 1E). Using the UCSC genome browser, this deletion was shown to encompass two exons (exons 2 and 3) of the *CNTNAP2* gene (Fig. 2A). Maternal FISH testing demonstrated that the mother is normal, whereas the father was unavailable for testing.

Patient 6

Patient 6 is a 10-year-old girl born by cesarean section at 34 weeks gestation. The pregnancy was complicated by maternal hypertension. She was initially treated in the neonatal intensive care unit for breathing problems, but recovered quickly and was discharged at 8 days of age. Early developmental milestones were reported to be delayed. At 5 months of age, she was evaluated because of seizures and was found to have a 47,XXX karyotype. She continues to have grand mal seizures, and she has long-standing history of DD, learning problems, and narcolepsy. She receives special education services and her school performance has fallen off to the point where she is failing most courses. The patient's mother who is deceased had a history of DD and seizures attributed to severe prematurity, brain hemorrhage, and hyperbilirubinemia. On physical exam she presented with microcephaly, receding forehead, upslanted palpebral fissures, but otherwise no dysmorphic features. G-banded chromosome analysis confirmed the 47,XXX karyotype. Array CGH analysis demonstrated the presence of an ~890 kb deletion at 14q21.2 with breakpoints at genomic positions 41,269,035 and 42,160,020 bp, which encompasses the last five exons (exons 2 through 6) of the *LRFN5* gene (Fig. 2B). Metaphase FISH analysis using the RP11-707E19 probe confirmed this deletion (Supplementary Fig. 1F). Paternal and grand-maternal FISH analyses showed that neither carries this 14q deletion.

Patient 7

Patient 7 is a 4-year-old girl born at term after a normal pregnancy with a birth weight of 3,740 g (50–75th centile). She did not walk until 2.5 years old and her early milestones were delayed. She has limited speech, learning disability, and autistic features. On physical exam she presented with mild hypertelorism, upslanted palpebral fissures, and slightly diminished tone. G-banded chromosome analysis revealed a 46,XX normal female chromosome complement. Array CGH analysis demonstrated the presence of an ~537 kb deletion than spans the entire *CHRNA7* gene at 15q13.3 with breakpoints at genomic positions 29,759,738 and 30,297,359 bp (Fig. 2C). Metaphase FISH analysis using the RP11-636P14 probe confirmed this deletion (Supplementary Fig. 1G). Neither parent was available for testing.

Patient 8

Patient 8 is a 6-year-old boy born at term by cesarean section after a normal pregnancy with a birth weight of 3,060 g (10–25th centile). Early milestones were felt by the family to be appropriate, but they became concerned at 4 years of age when he was experiencing language delay. History since is one of global DD and learning disability but no autistic features. Currently, he has short words, short sentences, echolalia, and is in special education classes. On physical exam he presented with microcephaly, triangular shaped face, mild hypertelorism, upslanted palpebral fissures, and diminished tone and strength. G-banded chromosome analysis revealed a 46,XY normal male chromosome complement. Array CGH analysis demonstrated the presence of an ~1.07 Mb intragenic deletion in the *IL1RAPL1* gene at Xp21.2p21.3 with breakpoints at genomic positions 28,683,919 and 29,753,365 bp, which encompasses five exons (exons 2 through 6) of the *IL1RAPL1* gene (Fig. 2D). Metaphase FISH analysis using the RP11-298A9 probe confirmed this deletion (Supplementary Fig. 1H). Maternal FISH testing showed that the mother is a carrier for this deletion. She was apparently of normal intelligence possibly due to skewed X-chromosome inactivation.

DISCUSSION

High-resolution genomic microarray analysis allows the detection of pathogenic CNVs in ~17–19% of patients with DD/MR who had a normal G-banded chromosome analysis [Miller et al., 2010]. Recent studies suggest that CNVs encompassing several genes involved in neurodevelopmental pathways may be associated with various neurobehavioral disorders [Friedman et al., 2006; Menten et al., 2006; Froyen et al., 2007; Sebat et al., 2007; Szatmari et al., 2007; Marshall et al., 2008; Christian et al., 2008]. Interpreting the clinical significance of small CNVs, especially those <500 kb, is particularly challenging and their pathogenicity is determined by many factors. Adding to the complexity of this interpretation are several recent reports on many of the newly described recurrent microdeletions/microduplications with incomplete penetrance and variable expressivity. This is particularly true for the 1q21.1, 15q13.3, 16p11.2, 16p12.1, and 16p13.11 microdeletions/microduplications, which have been found in affected probands as well as unaffected parents [Brunetti-Pierri et al., 2008; Weiss et al., 2008; Hannes et al., 2009; van Bon et al., 2009; Girirajan et al., 2010]. These genomic regions have been hypothesized to be susceptibility loci for neurobehavioral disease, and CNVs involving these regions may not be sufficient to cause a phenotype. The incomplete penetrance and variable expressivity have been explained by the requirement for additional genetic, epigenetic, or environmental hits.

To elucidate the clinical relevance of some small CNVs, here we present 8 patients in a cohort of ~1,200 patients (0.66%) referred for clinical array CGH testing for various neurodevelopmental phenotypes, who were identified to carry small (<1.0 Mb with the majority <500 kb) either total gene or intragenic deletions encompassing critical synaptic and other neurodevelopmental genes. No other clinically relevant CNVs were noted in these eight patients. Also, no benign CNVs spanning these deleted regions have been reported in the Database of Genomic Variants (DGVs). From the functional point of view, these genes belong to four categories, including neuronal transcription factor genes, neuron-specific splicing factor genes, genes that encode proteins involved in synapse formation and maintenance, and in neurotransmission.

Transcription factors play a crucial role in regulating every stage f brain development and function. Improper dosage of a tran-ription factor essential for the regulation of gene expression leads detrimental consequences for neuronal networks in a developing rain. The first three patients (Patients 1, 2, and 3) described here rry deletions encompassing neuronal transcription factor genes, cluding *NFIA* (1p31.3), *MEF2C* (5q14.3), and *CAMTA1* p36.23p36.31). *NFIA* encodes a member of the Nuclear Factor family of transcription factors (OMIM 600727) that is critical for ormal brain development and function [Nagase et al., 2000; Zheng al., 2010]. Lu et al. reported five patients with haploinsufficiency f *NFIA* who showed a similar phenotype characterized by hypo-lastic or absent corpus callosum, hydrocephalus, and DD. Four atients had a tethered spinal cord, three had Chiari type I mal-rmation, three had seizures, and three had urinary tract anomalies the form of vesicoureteral reflux [Lu et al., 2007]. Koehler et al. 2010] recently reported a de novo ~4.9 Mb deletion at 1p31.3p32.2 hat encompasses the *NFIA* gene in a patient with hypoplasia of the orpus callosum, ventriculomegaly, and dysmorphic features. All x cases reported to date with haploinsufficiency of *NFIA* had nvolvement of one or more additional genes, which may have ontributed to the phenotype. To our knowledge, Patient 1 in our eport (who carried an ~254 kb intragenic deletion in *NFIA*) is the rst case described to date with *NFIA* haploinsufficiency with no nvolvement of other genes. Her clinical presentation is consistent ith the phenotype reported by Lu et al. and Koehler et al. including ognitive delay, macrocephaly, hydrocephalus, and hypoplasia of he corpus callosum. She also had history of bipolar disorder/ epression, but she did not have seizures, Chiari I malformation, or enal anomalies. However, a definitive evaluation for urinary reflux as not performed.

The second neuronal transcription gene reported here is *MEF2C*. *MEF2C* encodes a member of the Myocyte Enhancer Factor 2 MEF2) family of transcription factors, which act in the brain as ffectors of neurogenesis (OMIM 600662) [Flavell et al., 2006; halizi et al., 2006]. MEF2C is the predominant isoform in the eveloping cerebrocortex and is highly expressed in frontal cortex, erebellum, dentate gyrus, and amygdate [Leifer et al., 1994; Lyons t al., 1995]. Heterozygous mutations or deletions involving *MEF2C* have been reported to be associated with severe MR, absent peech, poor eye contact, stereotypic movements, and seizures [Le Meur et al., 2010; Zweier et al., 2010]. Patient 2 in our report carried de novo ~412 kb deletion that encompasses the promoter region nd first three exons of *MEF2C*. Similar to the cases reported by Le Meur et al. and Zweier et al., our patient presented with DD, xpressive and receptive language delay, and hyperkinesis with tereotypic constant movement of his hands and feet.

Patient 3 in this report carried a de novo ~305 kb deletion at p36.31 that encompasses the promoter region and first three exons f the *CAMTA1* gene, as well as three other RefSeq genes, including *PHF13*, *THAP3*, and *DNAJC11*. Given the current knowledge about he tissue-specific expression and function of the four genes, *CAMTA1* haploinsufficiency emerges as the most logical explan-tion for the patient's phenotype. *CAMTA1* encodes a member of he Calmodulin-binding transcription activator (CAMTA) family OMIM 611501) [Finkler et al., 2007]. It is expressed predomi-antly in all regions of the brain with little or no expression in other tissues [Huentelman et al., 2007]. Investigations of CAMTAs in various organisms imply a broad range of functions from sensory mechanisms to embryo development and growth control [Finkler et al., 2007]. *CAMTA1* has been reported recently to play a role in human memory performance and its expression to be enriched in memory-related human brain regions [Huentelman et al., 2007]. Patient 3 presented with global DD, speech problems, learning disability, ADHD, and PDD, but with no dysmorphic features. All of these are neurobehavioral features observed in the monosomy 1p36 syndrome (OMIM 607872), suggesting that *CAMTA1* might be a candidate for the neurobehavioral finding in monosomy 1p36 syndrome.

Multiple lines of evidence, including linkage studies and cytogenetic deletions, suggest a strong role for aberrant RNA processing in neurodevelopmental disorders. Patient 4 in this report carried an ~353 kb intragenic deletion in the *RBFOX1* (*A2BP1*) gene at 16p13.2, which encompasses exons 3 and 4, as well as the promoter region of transcript variant 6, the most abundant transcript in the brain. *RBFOX1* is one of the largest genes in the human genome (~1.7 Mb), and has six alternatively spliced variants that introduce alternative promoters (OMIM 605104) [Shibata et al., 2000]. It encodes an RNA-binding protein that binds to the C-terminus of ataxin-2 [Shibata et al., 2000], regulates alternative splicing of tissue-specific exons by binding to the hexanucleotide UGCAUG through its RNA-recognition motif, and is considered a neuron-specific splicing factor [Underwood et al., 2005]. Several patients have been described in the literature with disrupting translocations involving *RBFOX1*, and presenting with DD, MR, seizures, autistic-features, and non-specific dysmorphic features [Bhalla et al., 2004; Martin et al., 2007]. Our patient presented with similar features, including DD, language delay, and FTT. Also recently, *RBFOX1* has been identified in structural variation studies carried out on autism and schizophrenia cohorts [Sebat et al., 2007; Xu et al., 2008; Elia et al., 2010]. This supports the emerging evidence suggesting that *RBFOX1* is a candidate gene for neurobehavioral disorders.

Cell adhesion molecules (CAMs) facilitate both the organization and adhesion of the synapses. They help to recruit and organize key components such as synaptic vesicles at the presynaptic terminal and neurotransmitter receptors in the postsynaptic specialization [Tallafuss et al., 2010]. There is accumulating evidence that synaptic pathways, including those involving synaptic CAMs, are disrupted in some patients with neurobehavioral disorders [Betancur et al., 2009]. Patients 5 and 6 reported here carried deletions encompass-ing genes involved in synapse formation and maintenance, includ-ing *CNTNAP2* (7q35) and *LRFN5* (14q21.2). *CNTNAP2* encodes a member of the neurexin family of synaptic CAMs (OMIM 604569). *CNTNAP2* haploinsufficiency has been reported recently in a variety of neurodevelopmental disorders, including MR, ADHD, ASD, schizophrenia, and epilepsy [Bakkaloglu et al., 2008; Burbach and van der Zwaag, 2009; Mefford et al., 2010]. Patient 5 carried an ~246 kb intragenic deletion encompassing exons 2 and 3 of the *CNTNAP2* gene, which is predicted to disrupt the reading frame. She presented with learning disability, speech problems, VPI, and non-specific dysmorphic features; a phenotype consistent with previous *CNTNAP2* haploinsufficiency reports described in the literature and the DECIPHER database [Tallafuss et al., 2010].

The second synaptic gene reported here is *LRFN5*, which encodes a brain-specific member of the leucine-rich repeat and fibronectin type III domain-containing protein (LRFN) family (OMIM 612811). This family includes five members that are found only in vertebrates and code for transmembrane proteins that, like neuroligins, associate with the synapses. LRFNs are also called synaptic adhesion-like molecules because of their involvement in synaptic development and plasticity [Morimura et al., 2006; Ko and Kim, 2007]. Several members of this family have a PSD95 binding domain in the cytoplasmic C-terminus [Morimura et al., 2006]. Proteins with such domain are known to influence the postsynaptic density (PSD), a scaffold of proteins important for synaptic differentiation, maintenance, and plasticity [Ko and Kim, 2007]. Mutations in three other PSD-associated genes have been reported as a rare cause of ASD [Jamain et al., 2003; Durand et al., 2007]. Patient 6 reported here had a 47,XXX chromosome complement, however her clinical presentation could not be explained entirely by this karyotype. Array CGH demonstrated that she carries an ~890 kb deletion that encompasses the last five exons of *LRFN5*. She presented with DD, learning disability, seizures, microcephaly, and receding forehead. Although the inheritance of this deletion could not be established, neither the father nor maternal grandmother carried the deletion. de Bruijn et al., reported a girl with MR and severe autism, who carried a balanced de novo t(14;21) (q21.1;p11.2) and a de novo 2.6 Mb 2q31.1 deletion. The authors showed that the t(14;21) resulted in reduced expression of *LRFN5* by long-range epigenetic silencing, and speculated that the dysregulation of *LRFN5* in this patient may have contributed to the patient's autism [de Bruijn et al., 2010]. Taken together, this strongly suggests that *LRFN5* haploinsufficiency can increase the susceptibility to neurodevelopmental problems.

The final group of genes reported here are genes involved in neurotransmission, including *CHRNA7* (15q13.3) and *IL1RAPL1* (Xp21.2p21.3). *CHRNA7* encodes the alpha-7 subunit of the neuronal nicotinic acetylcholine receptor, which is a homopentameric synaptic ion channel protein that is highly expressed in the brain (OMIM 118511). It is identified as a major susceptibility locus for juvenile myoclonic epilepsy and schizophrenia. Patient 7 reported here carried an ~537 kb deletion that encompasses the entire *CHRNA7* gene and is flanked by segmental duplications. She presented with DD, limited speech, learning disability, mild hypotonia, autistic-features, and mild non-specific dysmorphic features. Shinawi et al. reported on 10 patients from four families with the same *CHRNA7* deletion. This is a recurrent microdeletion that maps to the distal region of the recurrent larger ~1.5 Mb 15q13.3 microdeletion (OMIM 612001), and is mediated by NAHR [Shinawi et al., 2009]. The presenting features of these patients included DD, MR, seizures, ADHD, and mild dysmorphic features, a phenotype similar to our patient [Shinawi et al., 2009].

Patient 8 described here carried an ~1.07 Mb maternally inherited deletion encompassing exons 2 through 6 of the *IL1RAPL1* gene. He presented with DD, language delay, learning disability, microcephaly, triangular shaped face, mild hypertelorism, and diminished tone and strength. *IL1RAPL1* (Interleukin-1 Receptor Accessory Protein-like 1) encodes a transmembrane protein that shows homology with interleukin-1 receptor accessory protein family, but does not seem to be involved in the interleukin-1 pathway (OMIM 300206). It is expressed at high-level in postnatal brain structures involved in the hippocampal memory system. It plays a role in the down-regulation of voltage-dependent calcium channel activity, calcium-dependent exocytosis, and neurite outgrowth. Non-overlapping deletions and nonsense mutations in *IL1RAPL1* have been reported in patients with X-linked MR (OMIM 300143). More recent reports also suggest a role for *IL1RAPL1* in ASD [Piton et al., 2008, 2010; Guilmatre et al., 2009].

The deletions reported here are expected to result in either loss of expression of one allele of the involved genes (i.e., total gene or promoter deletions) or severely compromise the function of the encoded proteins (i.e., intragenic multi-exon deletions that either disrupt the reading frame or span critical functional protein domains). A search in the DECIPHER database demonstrated that all deletions reported here overlap with larger deletions that involve other flanking genes, with the exception of DECIPHER patients [#248604 (*MEF2C*)], [#250901 (*RBFOX1*)], [#250286 (*CNTNAP2*)], [#2398 (*LRFN5*)], and [#252408 and #253957 (*IL1RAPL1*)], in whom only a single gene was involved and presented with various neurodevelopmental phenotypes. The presumed clinical significance of all deletions reported here is based on several factors, including lack of previously reported benign losses spanning these genomic regions (as shown in the DGVs), gene function and tissue-specific expression, previously reported patients with overlapping deletions (as reported in the literature and the DECIPHER database), and the de novo nature of two of the deletions. This report underscores the need for careful clinical interpretation of small CNVs (especially deletions <500 kb in size) encompassing either a single gene or part of a gene. This is particularly important for CNVs involving critical neurodevelopmental genes. The influence of rare CNVs either alone or combined with other genetic interactions on various neurobehavioral phenotypes has been documented for several genomic regions in patients with intellectual disability, ASDs, schizophrenia, and epilepsy [Walsh et al., 2008; Girirajan and Eichler, 2010; Pinto et al., 2010]. Based on the body of knowledge about the involved genes and the lack of benign copy number losses spanning those genes as reported in the DGVs, we suggest that the deletions noted in Patients 1, 2, 3, 7, and 8 are thought to be pathogenic involving either clearly haploinsufficient genes (i.e., *NFIA*, *MEF2C*, *CAMTA1*, *CHRNA7*, respectively) or X-linked genes (i.e., *IL1RAPL1*), whereas those noted in Patients 4, 5, and 6 (involving *RBFOX1*, *CNTNAP2*, *LRFN5*, respectively) represent most likely risk factors for neurobehavioral disease that require additional hits to manifest a phenotype. With the exception of the *CHRNA7* gene deletion, all other deletions reported here are in regions devoid of segmental duplications, and therefore are mediated by other mechanisms distinct from NAHR like either nonhomologous end joining (NHEJ) or fork stalling and template switching (FoSTeS) [Stankiewicz and Lupski, 2010]. Our report suggests that small clinically relevant deletions involving critical synaptic and other neurodevelopmental genes can present clinically with various neurobehavioral phenotypes. It is very likely that the dysfunction of specific neuronal pathways underlying each clinical condition depends on additional genetic and/or epigenetic hits in order to manifest a particular phenotype.

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