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A *de novo* 8.8-Mb Deletion of 21q21.1-q21.3 in an Autistic Male with a Complex Rearrangement Involving Chromosomes 6, 10, and 21

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Abstract

We report here on a normal-appearing male with pervasive developmental disorder who was found to have a *de novo*, apparently balanced complex rearrangement involving chromosomes 6, 10, and 21: 46,XY,ins(21;10)(q11.2;p11.2p13)t(6;21)(p23;q11.2). Further analysis by high-density oligonucleotide microarray was performed, showing an 8.8-Mb heterozygous deletion at 21q21.1-q21.3. Interestingly, the deletion is distal to the translocation breakpoint on chromosome 21. The deletion involves 19 genes, including *NCAM2* and *GRIK1*, both of which are associated with normal brain development and function, and have been considered as possible candidate genes in autism and other neurobehavioral disorders. This case underscores the utility of genomewide microarray analysis for the detection of copy number alterations in patients with apparently balanced complex rearrangements and abnormal phenotypes.

Keywords

complex chromosome rearrangement; microarray; microdeletion; pervasive developmental disorder-not otherwise specified

INTRODUCTION

Developmental abnormalities, congenital malformations, and mental retardation have been reported in patients thought to have an apparently balanced translocation or complex chromosomal rearrangement [Warburton 1991; Madan et al., 1997; Astbury et al., 2004]. These abnormal phenotypes may result from direct disruption of genes or their promoter regions [Kleczkowska et al., 1982; Fryns et al., 1986], or alternatively by submicroscopic deletions and duplications of one or more gene(s) near the chromosomal breakpoints [Gribble et al., 2005; Hayashi et al., 2005]. The availability and application of high-resolution microarrays has greatly improved the detection of microdeletions or

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microduplications in patients with abnormal clinical phenotypes such as multiple congenital anomalies or mental retardation [Ming et al., 2006; Stankiewicz and Beaudet, 2007; Shaffer et al., 2007; Weiss et al., 2008; Sharp et al., 2008]. In addition, there is an increasing body of literature in which the application of high-resolution microarrays has enabled the detection of cryptic deletions and duplications with apparently balanced translocations and complex chromosomal rearrangements [Li et al., 2008; Higgins et al., 2008; Fantes et al., 2008; Baptista et al., 2008; Sismani et al., 2008; Misceo et al., 2008]. As the utilization of microarrays in the clinical setting expands, there will likely be further reports of genomic abnormalities in patients with previously normal or “balanced” cytogenetic studies.

We report here on a normal-appearing male with pervasive developmental disorder-not otherwise specified (PDD-NOS) found to have a balanced complex chromosomal translocation by karyotype analysis. As the patient had an abnormal phenotype with an apparently balanced translocation, further examination by high-density oligonucleotide microarray found an 8.8-Mb deletion of 21q21.1-q21.3 containing 19 genes, including *NCAM2* and *GRIK1*, both of which are likely to be involved in the observed phenotype.

CLINICAL REPORT

The research study was approved by the Institutional Review Board of the Children's Hospital of Philadelphia. The patient is a 7-year-9-month-old male who was evaluated for autistic features at 4 years old, but was noted to have significant speech delay and poor social interactions as early as 2 years of age. He was born at 37 weeks to a 26-year-old, G2P0→1 mother via spontaneous vaginal delivery with a birth weight of 3.5 kg (90th centile), and a length of 48.3 cm (50th centile). He had an unremarkable early infancy history, and has been healthy except for the placement of tympanostomy tubes for ear infections as a young child, with subsequent normal hearing evaluations. He had no gross or fine motor delay but only began speaking at 4 years, when he was diagnosed with PDD-NOS via the Autism Diagnostic Observation Schedule (ADOS) and by criteria in the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV). He was also diagnosed with attention deficit hyperactivity disorder (ADHD) during this time. At his current age of 7 years and 9 months, he has a limited vocabulary (<500 words) with minimal spontaneous language, but is able to repeat words easily. He has no history of developmental regressions, and receives support for interpersonal relationships in school. The family history was noncontributory. On physical exam, he was a normal-appearing child with a height at the 20th centile and head circumference at the 90th centile. His eyes were slightly hypoteloric given his head circumference, with an interpupillary distance at the 20th centile. His palate was mildly high but intact. The rest of his physical exam was unremarkable. No specific syndromic diagnosis was made based on these findings, and a high-resolution karyotype and fluorescence *in situ* hybridization (FISH) of the subtelomeres were initially performed.

MATERIALS AND METHODS

Cytogenetic and fluorescence in situ hybridization (FISH) analysis

Metaphase chromosome analysis at a high-resolution banding level was performed on 72-hr PHA stimulated cultured peripheral blood lymphocytes using standard methods. Subtelomeric FISH and whole chromosome painting analyses were also performed on metaphase chromosome spreads using commercially available probes per the manufacturer's recommendations (Cytocell, Cambridge, UK).

Microarray experiments and data analysis

The microarray experiment was performed using the Affymetrix GeneChip 250K Nsp chip (Affymetrix, Santa Clara, CA, USA). 250 ng of genomic DNA from the subject was processed and labeled using reagents and protocols supplied by the manufacturer (Affymetrix, Santa Clara, CA, USA). Post-hybridization, the microarrays were processed in the Affymetrix GeneChip Fluidics Station 450 and resultant image (.CEL file) was analyzed with the Affymetrix GeneChip® Genotyping Analysis Software (GTTYPE). The .CEL files were further analyzed using the Copy Number Analyzer for GeneChip (CNAG, <http://www.genome.umin.jp/>) version 2.0 [Nannya et al., 2005], a publicly available software which allows the detection of copy number alteration using the signal intensities of the probes. The deletion was later confirmed by standard FISH techniques with a probe located in the deleted region (RP11-80N21).

RESULTS

Cytogenetic and FISH studies

Conventional cytogenetic studies revealed a complex chromosomal translocation between chromosomes 6, 10, and 21. Subtelomeric *in situ* hybridization analysis confirmed the 6;21 translocation. The additional inserted material on chromosome 21 was determined to be from chromosome 10 by chromosomal painting (Figure 1). Parental karyotypes as well as FISH with a probe located in the deleted region (RP11-80N21) were normal, demonstrating that this rearrangement is *de novo*.

Microarray analysis

Since the patient had an abnormal phenotype and a complex translocation involving several breakpoints, it was suspected that he might have loss of chromosomal material not visualized by routine cytogenetic analyses. Therefore, a high-density oligonucleotide SNP-based microarray analysis was performed with the Affymetrix 250k Nsp Array (Santa Clara, CA). Copy number analysis using CNAG detected an 8.8-Mb deletion on 21q21.1-q21.3 (chr21:21,379,642–30,108,064, hg18, NCBI build 36) (Figure 2). Following this data, his final karyotype is: 46,XY,ins(21;10)(q11.2;p11.2p13)t(6;21)(p23;q11.2).arr 21q21.1-q21.3(21,379,642–30,108,064)x1.

Based on analysis utilizing the UCSC Genome Browser (hg18, build 36), there are a total of 19 genes deleted (Table I). The proximal deletion breakpoint falls within the *NCAM2* gene, deleting 17 out of 18 exons, and the distal deletion involves 16 out of 17 exons of the *GRIK1* gene. There are no significant overlapping copy number variations in available CNV databases in this region (<http://projects.tcag.ca/variation/>), and no other relevant deletions or duplications were detected in the patient.

DISCUSSION

We have identified a *de novo* interstitial deletion of 21q21.1-q21.3 in a patient with an abnormal phenotype and an apparently balanced complex translocation. Initial comprehensive cytogenetic analysis revealed the complex translocation of chromosomes 6, 10, and 21 without any noticeable loss of genetic material. This is due to the fact that standard cytogenetic testing is limited in sensitivity to identifying chromosomal rearrangements smaller than 10-Mb. However, with the use of BAC-based array comparative genomic hybridization (CGH) and higher-density oligonucleotide microarrays, smaller deletions and duplications are being discovered and further characterized in patients with multiple congenital anomalies, developmental delays, mental retardation, and autism

spectrum disorders [Shaw-Smith et al., 2004; Rauch et al., 2004; Ming et al., 2006; Sebat et al., 2007; Marshall et al., 2008; Weiss et al., 2008; Christian et al., 2008].

We hypothesized that our patient's abnormal phenotype may have resulted from the disruption, loss or gain of an essential gene(s) near one or more of the rearrangement breakpoints, not detectable by the cytogenetic analysis. Therefore, a high-resolution oligonucleotide-based microarray was performed, revealing an 8.8-Mb deletion of 21q21.1-q21.3. The observation that the deletion in 21q21 is *de novo*, relatively large in size and contains 19 genes strongly supports a role for the deletion in the patient's phenotype. Although, it is equally possible that one or more of the complex rearrangement breakpoints may have directly disrupted a gene(s) relevant to the patient's phenotype, we were unable to address this possibility pending further analysis of the breakpoints.

Interestingly, this deletion is distal to the chromosome 21 translocation breakpoint at 21q11.2. FISH analysis in our patient using a BAC-clone approximately 1-Mb proximal to the deletion (RP11-49B5) showed the clone to be located on the derivative chromosome 6 (data not shown). Although the precise mechanisms are unclear, analyses in patients with apparently balanced translocations have previously identified similar findings, such as a deletion or duplication of different regions of the translocated chromosome or of a different chromosome not involved in the translocation [Ciccone et al., 2005; De Gregori et al., 2007; Sismani et al., 2008]. In another study, approximately 1/3 of the patients with *de novo* translocations had a copy number alteration unrelated to the translocation [Gribble et al., 2005]. At this time, it is difficult to determine if the deletion preceded, succeeded, or occurred during the translocation. Parental chromosome and FISH analyses were normal and did not reveal an insertion or detectable inversion that may have predisposed to the deletion in the patient.

Chromosomal rearrangements may occur via several mechanisms, which typically reflect the underlying architecture of the genome in the regions surrounding the breakpoints [Gu et al., 2008]. Non-allelic homologous recombination (NAHR) is the major mechanism underlying many of the recurrent genomic disorders (DiGeorge syndrome, Williams syndrome, etc) that are flanked by regions of segmental duplications or low-copy repeats (LCRs). These sequences with high sequence similarity may predispose to rearrangements by causing abnormal alignment of homologous chromosomes [Emanuel and Shaikh, 2001]. Rearrangements produced by nonhomologous end-joining (NHEJ) occur when double-strand DNA breaks are incorrectly repaired, leading to deleted or duplicated genomic segments. Although NHEJ does not involve larger regions of sequence identity such as LCRs, microhomology of the DNA may be present at the breakpoints. More recently, FoSTeS (Fork Stalling and Template Switching) has been described as a mechanism associated with complex rearrangements caused by abnormal DNA replication. In this instance, DNA strands from one replication fork may switch and become integrated into a downstream replication fork [Gu et al., 2008]. In the absence of precise mapping of the breakpoints, it is difficult to speculate which of these mechanisms may have played a role in the rearrangement observed in our patient. The lack of LCRs near the rearrangement breakpoints in our patient makes it highly unlikely that homology-based mechanisms like NAHR are involved.

To our knowledge, this particular 21q21.1-q21.3 deletion has not been described previously. However, there have been several reports of patients with overlapping deletions, most of whom have variable facial dysmorphism and clinical phenotypes, but commonly have mental retardation or developmental delays. A 12-year-old female with mild mental retardation and hypothyroidism was found to have a deletion of 21q11.1-q22.1 near marker D21S55 [Alhbob et al., 1996]. Two patients with developmental delay due to deletions of 21q11.2-

q21.1 and 21q21.1-q22.3 were previously described [Huret et al., 1995]. Additionally, two patients with mild mental retardation and short stature were shown to have deletions of 21q11.2-q21.1 and 21q11.2-q21.3, respectively [Roland et al., 1990]. Another child without mental retardation had small testes and was shown to have a deletion of 21q11.2-q21.3 [Korenberg et al., 1991]. A familial deletion of 21q11.2-q21.3 was described where one of two children had normal intelligence while the other sibling developed mental retardation and sensorial neural hearing loss [Wakui et al., 2004]. Another individual was reported with mental retardation, psychosis and dysmorphia and was shown to have a deletion at 21q21-q22.1 [Takhar et al., 2002]. In addition, a search of the DECIPHER database (<https://decipher.sanger.ac.uk/>) did not reveal any patients with significant overlapping regions and a similar clinical abnormality. It is worth noting that none of the other studies were performed using microarray analysis, and the breakpoint assignments are approximate, based upon cytogenetic analysis.

The region deleted in our patient leads to the loss or disruption of 19 genes, several of which may be involved in the phenotype based on the putative or known biological function (Table I). The genes that are expressed in the brain are more likely to be responsible for some or all of the clinical abnormalities, such as the speech delay and autistic features. The proximal and distal end-points of the deletion fall within two genes, *NCAM2* and *GRIK1*, respectively. Both of these genes are the good candidates in the etiology of the phenotype as they are expressed throughout the developing and adult brain, and appear to be important for proper brain maturation and function. *NCAM2* is part of the neural cell adhesion molecule family, which participates in many cellular processes, including neuronal migration, cell survival, outgrowth of neurites, and formation and plasticity of synapses [Kulahin and Walmod, 2008]. *NCAM2* has also been implicated as a candidate gene following analyses of autistic patients by linkage studies [Molloy et al., 2005]. *GRIK1* (*GluR5*) encodes a protein belonging to the kainate family of glutamate receptors that function as ligand-activated ion channels. Collectively, the glutamate receptors have been associated with various neurobehavioral phenotypes, including anxiety disorders, addictions, schizophrenia, epilepsy, learning, and cognition [Lipsky and Goldman, 2003; Pinheiro and Mülle, 2008]. *GRIK1* polymorphisms were shown to be associated with schizophrenia, bipolar disorder, and epilepsy in humans [Sander et al., 1997; Shibata et al., 2001; Lucarini et al., 2007; Woo et al., 2007; Dracheva et al., 2008], as well as anxiety-like behaviors in *GRIK1* knockout mice due to its regulation of inhibitory circuits in the amygdala [Wu et al., 2007]. The phenotype observed in our patient suggests that *NCAM2* and *GRIK1* may have a role in autism, mental retardation, and other neurobehavioral disorders.

In summary, we report here on a case of an almost 8-year-old male with PDD-NOS who was found to have an apparently balanced complex chromosomal rearrangement by classic cytogenetic techniques. However, further analysis by high-density oligonucleotide microarray showed loss of 21q21.1-q21.3, resulting in partial monosomy for this region. This report provides additional support for the use of high-density microarray analysis in the detection of various cryptic rearrangements in patients with apparently balanced chromosomal translocations and abnormal phenotypes. Furthermore, characterization of the deleted genes in this region may provide additional insight into their association with autism spectrum disorders.

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Figure 1. Chromosome paint and subtelomere analysis

The patient's metaphase chromosomes were analyzed using whole chromosome paint (WCP) and FISH with subtelomeric probes. Results from the normal 6, 10, and 21 chromosomes are shown on the left with the corresponding abnormal derivative chromosomes on the right. On the der(6), telomere analysis showed the 6q probe hybridized normally, but the 21q telomere was located on the short arm due to the t(6;21)(p23;q11.2). Telomere analysis on the der(10) was normal, but the karyotype and WCP demonstrated an interstitial del(10)(p11.2p13). On the der(21), the 6p telomere was located on the distal long arm. WCP showed minimal proximal 21q material, with 10p11.2-p13 (green) and 6p23-ter (red) chromosomal material also present.

Figure 2. Microarray Analysis

Affymetrix 250K array data for the patient's deleted chromosome 21. The data analysis and graphical output was carried out using the CNAG algorithm. The upper panel shows the Log_2Ratio of all probes on chromosome 21, where each dot represents a single SNP-based probe. The deleted probes are identified due to a decrease in the Log_2Ratio . The lower panel shows a smoothing curve over a 25-probe window. The ideogram for chromosome 21 is shown below the array data.

Table I

Selection of genes^a within the deleted region.

Gene	Function
NCAM2	This protein is a cell adhesion molecule involved in neuron-neuron adhesion, neurite fasciculation, and outgrowth of neurites.
JAM2	May play a role in the processes of lymphocyte homing to secondary lymphoid organs.
APP	Functions as a cell surface receptor and performs physiological functions on the surface of neurons relevant to neurite growth, neuronal adhesion and axonogenesis. Involved in cell mobility and transcription regulation through protein-protein interactions. Mutations of this gene have been associated with early-onset autosomal dominant Alzheimer's disease and cerebroarterial amyloidosis when cleavage products form amyloid plaques.
ADAMTS1	Cleaves aggrecan, a cartilage proteoglycan, and may be involved in its turnover (by similarity). Has angiogenic inhibitor activity. Active metalloprotease, which may be associated with various inflammatory processes as well as development of cancer cachexia. May play a critical role in follicular rupture.
ADAMTS5	Cleaves aggrecan, a cartilage proteoglycan, and may be involved in its turnover. May play an important role in the destruction of aggrecan in arthritic diseases.
CCT8	May deubiquitinate one or more critical proteins that are involved in the condensation of mitotic chromosomes, possibly acting selectively on histones H2A and H2B, the major ubiquitinated proteins of chromatin. It is able to deubiquitinate histone H2A in vitro, the phosphorylated form of the protein is also enzymatically active.
BACH1	Encodes a transcription factor that belongs to the cap'n'collar type of basic region leucine zipper factor family (CNC-bZip).
GRIK1	Receptor for glutamate. L-glutamate acts as an excitatory neurotransmitter at many synapses in the central nervous system.

^a A total of 19 genes were deleted, and those genes with known or putative biological function that may be involved in the patient's phenotype are listed here. Genes in bold-type are discussed further in the text. Information obtained from the UCSC Genome Browser: <http://genome.ucsc.edu>.