HUMAN MUTATION 28(7), 674–682, 2007

RAPID COMMUNICATION

Array CGH Identifies Reciprocal 16p13.1 Duplications and Deletions That Predispose to Autism and/or Mental Retardation

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Communicated by Haig Kazazian

Autism and mental retardation (MR) are often associated, suggesting that these conditions are etiologically related. Recently, array-based comparative genomic hybridization (array CGH) has identified submicroscopic deletions and duplications as a common cause of MR, prompting us to search for such genomic imbalances in autism. Here we describe a 1.5-Mb duplication on chromosome 16p13.1 that was found by high-resolution array CGH in four severe autistic male patients from three unrelated families. The same duplication was identified in several variably affected and unaffected relatives. A deletion of the same interval was detected in three unrelated patients with MR and other clinical abnormalities. In one patient we revealed a further rearrangement of the 16p13 imbalance that was not present in his unaffected mother. Duplications and deletions of this 1.5-Mb interval have not been described as copy number variants in the Database of Genomic Variants and have not been identified in >600 individuals from other cohorts examined by high-resolution array CGH in our laboratory. Thus we conclude that these aberrations represent recurrent genomic imbalances which predispose to autism and/or MR. Hum Mutat 28(7), 674–682, 2007.

KEY WORDS: autism; mental retardation; array CGH; copy number variant

INTRODUCTION

Autism is a descriptive term referring to a neurodevelopmental disorder that begins in early childhood. It is characterized by social and communicative impairment accompanied by repetitive and stereotypic behavior and narrow interests. Autism is currently estimated to have a prevalence of five to 16 cases per 10,000 [Fombonne, 2003], with a male to female ratio of about four to one or even higher for mild cases. Heritability of autism and Autistic Spectrum Disorder (ASD) is high, with concordance rates of 60 to 90% in monozygotic twins and recurrence risks for siblings of affected probands of 3 to 5% [Bacchelli and Maestrini, 2006; Bailey et al., 1995; Steffenburg et al., 1989]. It is widely believed that autism is a multifactorial disorder, and numerous linkage and association studies have been performed to map and identify the underlying susceptibility genes. So far, these studies have provided evidence for susceptibility loci on 12 different chromosomes including 16p [reviewed by Battaglia and Bonaglia, 2006]. Autistic behavior is often recognized in individuals with mental retardation (MR), and conversely, between 65% and 85% of autistic patients are mentally retarded [Chudley et al., 1998; Gillberg, 1998; Sponheim and Skjeldal, 1998], suggesting that MR and autism are caused by related pathogenetic mechanisms (e.g. [Laumonnier et al., 2004; Belmonte and Bourgeron, 2006]).

Fluorescence in situ hybridization (FISH) studies have revealed subtelomeric deletions in 2 to 3% of unselected patients with MR [Ravnan et al., 2006]. More recently, several groups have employed CGH arrays comprising several thousand equally-spaced bacterial artificial chromosome probes (BACs) and a mean resolution of about 1 Mb to identify interstitial submicroscopic deletions and duplications in patients with mental retardation and autism [Jacquemont et al., 2006; Menten et al., 2006; Miyake

The Supplementary Material referred to in this article can be accessed at http://www.interscience.wiley.com/jpages/1059-7794/suppmat.

Received 8 November 2006; accepted revised manuscript 28 March 2007.

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Grant sponsors: Max Planck Innovation Funds; Deutsche Forschungsgemeinschaft; Grant number: SFB577.

Reinhard Ullmann and Gillian Turner contributed equally to this work.

DOI 10.1002/humu.20546
Published online 4 May 2007 in Wiley InterScience (www.interscience.wiley.com).

et al., 2006; Rosenberg et al., 2006; Schoumans et al., 2005; Shaw-Smith et al., 2004; Vissers et al., 2003]. Tiling path BAC arrays, typically consisting of >32,000 overlapping BAC clones, are not yet widely available, but there is evidence that their 10-fold higher resolution will lead to a substantial further increase in the number of clinically relevant genomic imbalances detected [de Vries et al., 2005; Ishkanian et al., 2004].

Here we report on a recurrent 1.5-Mb duplication on chromosome 16p13.1, which was detected by means of sub-megabase-resolution whole-genome tiling path BAC arrays in four severe autistic males and several variably affected and unaffected relatives from three unrelated Australian families. A reciprocal deletion of the same segment was found in three unrelated mentally retarded patients.

MATERIALS AND METHODS

Array CGH

Array CGH [Pinkel et al., 1998; Solinas-Toldo et al., 1997] was performed as described previously [Erdogan et al., 2006]. In brief, sonicated patient and reference DNA was labeled by random priming (Bioprime Array CGH, Invitrogen, Carlsbad, CA) with Cy3 and Cy5 (Amersham Biosciences, Piscataway, NJ), respectively, and hybridized onto a tiling path BAC array, consisting of the human 32-k BAC Re-Array Set (BACPAC Resources Center; http://bacpac.chori.org/pHumanMinSet.htm), DNA kindly provided by Pieter de Jong [Krzywinski et al., 2004; Osoegawa et al., 2001; Ishkanian et al., 2004], a 1-Mb Resolution BAC set (clones kindly provided by Nigel Carter, Wellcome Trust Sanger Centre) [Fiegler et al., 2003], and a set of subtelomeric clones (assembled by members of the COSTB19 Action: Molecular cytogenetics of solid tumors). All protocols are provided in detail on our website (www.molgen.mpg.de/~abt_rop/molecular_cytogenetics) and details concerning this platform have been submitted to the Gene Expression Omnibus (GEO; www.ncbi.nlm.nih.gov/geo; GLP: 5000 and 5114) [Barrett and Edgar, 2006; Edgar et al., 2002]. For the analysis and visualization of array CGH data, our own software-package CGHPRO [Chen et al., 2005] was employed. No background subtraction was applied. Raw data were normalized by "Subgrid LOWESS." For the assessment of copy number gains and losses, we employed conservative $\log_2$ ratio thresholds of 0.3 and −0.3, respectively. Deviant signal intensity ratios involving three or more neighboring BAC clones were considered as genomic aberrations unless they were fully covered by a known DNA copy number variant, as listed in the Database of Genomic Variants (http://projects.tcag.ca/variation) [Zhang et al., 2006]. CGH on 244-k oligonucleotide arrays was performed according to the protocol provided by the manufacturer and analyzed using the company's software CGH Analytics (Agilent, Santa Clara, CA).

Quantitative PCR and FISH

Primer pairs for quantitative PCR (qPCR) were selected using the Primer3 software [Rozen and Skaletsky, 2000]. Mfold [Zuker, 2003] was employed to rule out primers forming secondary structures. For the initial verification, two primer pairs mapping to the aberrant segment were used (primer pairs 7 and 8). In addition, six primer pairs were designed to fine-map the telomeric breakpoint of the aberrant segment on chromosome 16p13.1, which is extremely rich in low copy repeats. PCR reactions were done in a 7900HT Fast Real Time PCR System (Applied Biosystems) using SYBR Green PCR Master Mix (Applied Biosystems) and the following conditions: 95°C for 10 min/(95° for 10 sec/58°C for 1 min) × 40/95°C for 10 sec/58°C for 15 sec/95°C for 15 sec.

In Families D and E, patients and parents were tested with three additional primer pairs (9–11). In Family F, chromosomal breakpoints were initially determined by FISH, using BAC clones RP11-680G24 (chr16: 14906038-15040525) and RP11-958N24 (chr16: 16304614-16390764) as probes (NCBI35; HG17). A list of primer pairs is given in Supplementary Table S1 (available online at http://www.interscience.wiley.com/jpages/1059-7794/suppmat).

RESULTS

Array CGH

Whole genome array CGH screening of 70 autistic individuals from an Australian cohort revealed apparently identical submicroscopic 16p13.1 duplications in two unrelated patients. The duplication encompassed an interval of about 1.5 Mb, ranging from 14.89 to 16.39 Mb (NCBI35; HG17; BAC clone RP11-566I03 to RP11-413I10). A third duplication of this interval was identified by quantitative PCR in a second Australian cohort comprising 112 patients. Array CGH, in combination with qPCR, revealed that this duplication was of the same size. Diagnosis of autism was made after comprehensive assessments including medical/pediatric, psychometric/psychologist, speech and language, occupational therapist, with standardized autism rating scales; all patients fulfilled the DSM-IV-TR criteria [APA, 2000]. Pedigrees of all patients are depicted in Figure 1 and results of array CGH and qPCR are summarized in Table 1.

Investigation of the parents and other relatives showed that at least two of these duplications were familial. In one of these (Family A; Fig. 1), a brother (Patient III-2), a sister (Patient III-3), the mother (Patient II-2), and the maternal grandmother of the index patient (Patient III-1) also carried the duplication. Apart from the apparently healthy grandmother, all of these were also affected, albeit to a different degree. Both brothers are profoundly handicapped adults with no speech. Autism and developmental delay was diagnosed in early childhood, but their behavior was markedly different. The index patient (Patient III-1) was continuously hyperactive, destructive, and aggressive toward others such that for much of the time he was physically restrained. By contrast, his younger brother (Patient III-2) was passive, tractable, and easy to manage. Both are of normal physical

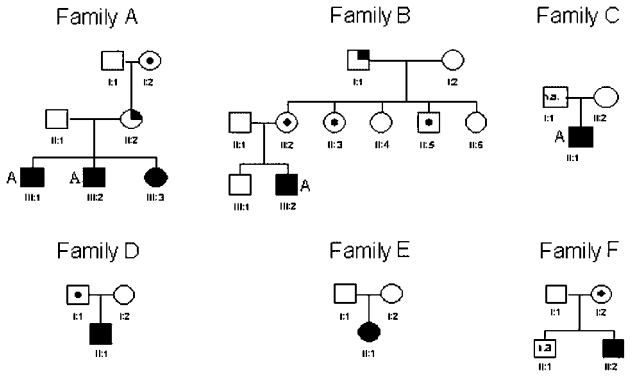


FIGURE 1. **Pedigrees of all families analyzed in this study. Duplications were found in Families A to C, while deletions occurred in Families D to F. All affected patients were carriers of the duplication or deletion, respectively. Autistic patients are indicated by an "A" beside the symbol. ■, affected; □, unaffected; a dot in the symbol highlights unaffected carriers; n.a., not analyzed. Partially filled symbols indicate mildly affected patients.**

TABLE 1. Array CGH and qPCR Results*

	DNA copy number of 16p13.1	Breakpoint mapping by qPCR	
		Primers 1–2	Primers 3–6
Family A			
I-2	dup	bal	dup
II-1	bal	–	–
II-2	dup	bal	dup
III-1	dup	bal	dup
III-2	dup	bal	dup
III-3	dup	bal	dup
Family B			
I-1	dup	bal	dup
I-2	bal	–	–
II-1	bal	–	–
II-2	dup	bal	dup
II-3	dup	bal	dup
II-4	bal	–	–
II-5	dup	bal	dup
II-6	bal	–	–
III-1	bal	–	–
III-2	dup	bal	dup
Family C			
I-1	–	–	–
I-2	bal	–	–
II-1	dup	bal	dup
Family D			
I-1	del	bal	del
I-2	bal[a]	–	–
II-1	del	bal	del
Family E			
I-1	bal[a]	–	–
I-2	bal[a]	–	–
II-1	del	del	del
Family F			
I-1	bal	–	–
I-2	del	bal	del
II-2	del	bal	del

*DNA copy number was tested by submegabase resolution array CGH and aberrations were confirmed by qPCR using primer pairs 3–8. In Family D and E additional independent confirmation was performed with primer pairs 9–11; bal, dup, and, del: present in 2, 3, and 1 copies, respectively; –, not analyzed.

[a]Tested only with qPCR and not by array CGH. In Family F, DNA copy number changes were also verified by means of 1 Mb resolution array CGH and FISH.

appearance with head circumferences (HC) of 58 and 59 cm, respectively (90–97th percentile). Their sister (Patient III-3) had learning problems at school consistent with her IQ of 54, but she was described as an affectionate, delightful infant by her grandmother. More recently, she developed a talent for swimming and reached State level in competitions for handicapped individuals. She is of normal appearance with a HC on the 50th percentile. Her mother (Patient II-2) had an IQ of 72, delayed speech development, and learning problems. As an adult she has obsessive compulsive disorder and is under the care of a psychiatrist.

In Family B, the diagnosis of autism in the index patient (Patient III-2) was never in doubt. From the age of 9 months, when he started to walk, he became hyperactive and aggressive with much disruptive behavior, hand flapping, no speech, and no functional play. He is of normal physical appearance with an HC at age 3.5 years of 51 cm (60th percentile). His older brother (III-1), who does not carry the duplication, had delayed speech development, but at the age of 5.5 years, he had no features of autism and attended a normal class. The duplication was also found in their healthy mother (II-2) who is a health professional, her normal sister (II-3) and brother (II-5), and in their maternal grandfather (I-1). He underwent detailed examination using a self-administered questionnaire for symptoms of autism and was identified as having borderline symptoms.

In Family C, the proband (Patient II-1) had normal motor development but speech was delayed with prominent early echolalia. He was an aloof child with much societal anxiety, often misinterpreting the behaviors of others. He had narrow preoccupations such as cartoon characters about which he would talk at length. At age 18 years, he was attending a work and living skills program and had a part-time job at a bakery. His HC was 59.5 cm. His healthy mother did not carry the duplication, and his apparently healthy father could not be investigated.

The reciprocal change, a deletion spanning the same 1.5-Mb interval on chromosome 16p13.1, was observed in two unrelated mentally retarded patients (see Families D and E in Fig. 1) in a Danish cohort comprising 95 patients [Kirchhoff et al., 2005, 2004] and in a retarded patient from Brazil (Family F).

The index patient in Family D (Patient II-1) was delivered by Cesarean section at 36 weeks of gestation because of polyhydramnios. The mother of the proband had developed diabetes mellitus during a former pregnancy. The birth weight of the boy was 4,960 g (>97th centile) and the HC was 37.5 cm (>97th centile). During the first days of life he needed mechanical ventilation due to pulmonary hypertension; a hypertrophic cardiomyopathy was diagnosed, which remained stationary over time and did not necessitate any further intervention. At 7 months of age, MRI showed no gross anatomical changes of the brain. Psychomotor development was retarded; at age 4 years, his motor development corresponded to a 3-year-old child, and his language corresponded to an 18-month-old child. He had developed autistic traits, which became less apparent when demands were lowered to match his intelligence. Facial dysmorphism was most noticeable in the first 2 years. It included frontal bossing, low-set ears with prominent anthelix, a broad nasal bridge, and a thin upper lip with the shape of cupid's bow. His HC remained increased at the 97th centile (52 cm at age 2 years). His growth was normal at the 75th centile. The deletion was inherited from the father, who had learning disabilities and left school after 7 years to be a fisherman.

The other Danish patient (Family E, Patient II-1) was a 19-year-old woman with psychomotor retardation and echolalia, a slightly dysmorphic appearance including downslanting palpebral fissures, epicanthic folds, ptosis, and small hands and feet. She was short and overweight; at age 17 years, her height was 153.5 cm (<3rd centile) and her weight was 72 kg (weight-for-height >97th centile). Her development had been delayed; at the age of 10 years, it corresponded to that of a 6-year-old child, and she had problems socializing. She attended a school for children with special needs. CT of the brain showed no gross anatomical changes. Prior to the identification of the 16p13.1 deletion by array CGH, conventional karyotype analysis and investigations to rule out Prader-Willi syndrome and a 22q11 deletion had given normal results. Analysis of the parents by qPCR revealed that the 16p13.1 deletion occurred de novo.

In the Brazilian patient (Family F, Patient II-2), the 16p13.1 deletion was originally detected with a 1-Mb CGH BAC array [Rosenberg et al., 2006]. FISH confirmed the deletion and showed that the breakpoints mapped within BACs RP11-680G24 (chr16: 14906038-15040525) and RP11-958N24 (chr16: 16304614-16390764; NCBI35; HG17). Later on, the deletion was reanalyzed with the whole genome tiling path BAC array. The patient was a 12-year-old boy, the second child of nonconsanguineous parents. Apart from a brother of the maternal grandmother, who was said to be mentally retarded (not examined), all family members including his elder brother were considered clinically normal.

Congenital cataracts were diagnosed just after birth and were operated at 1.5 years of age. He was hypotonic, and holding up his head, sitting up without support, and walking were delayed. He was able to speak only isolated words, and attended a school for mentally impaired children. He was an affectionate and kind child, who did not show antisocial or aggressive behavior. Craniofacial features included narrow frontal bone, frontal hirsutism, synophris, small, low-set and prominent ears, bulbous nose, thin upper lip and protruding lower lip, high palate, dental malocclusion, and prognathism. He had severe visual impairment, and presented convergent strabismus and circular nystagmus. Joint hyperextensibility was noted. Hand calluses were secondary to hand biting. Height (1.52 m), weight (42 kg), and HC (54 cm) were in the normal range for his age. The same deletion was detected in his mother, who had a similar facial appearance, but was not mentally retarded. CGH analysis using a 244-k Agilent oligonucleotide array revealed a further rearrangement at the proximal breakpoint in Patient F II-2, leading to an additional loss of about 40 kb that was not present in his healthy mother (chr16: 14.95-14.99; HG17; Fig. 2). A comparison of affected and unaffected carriers in

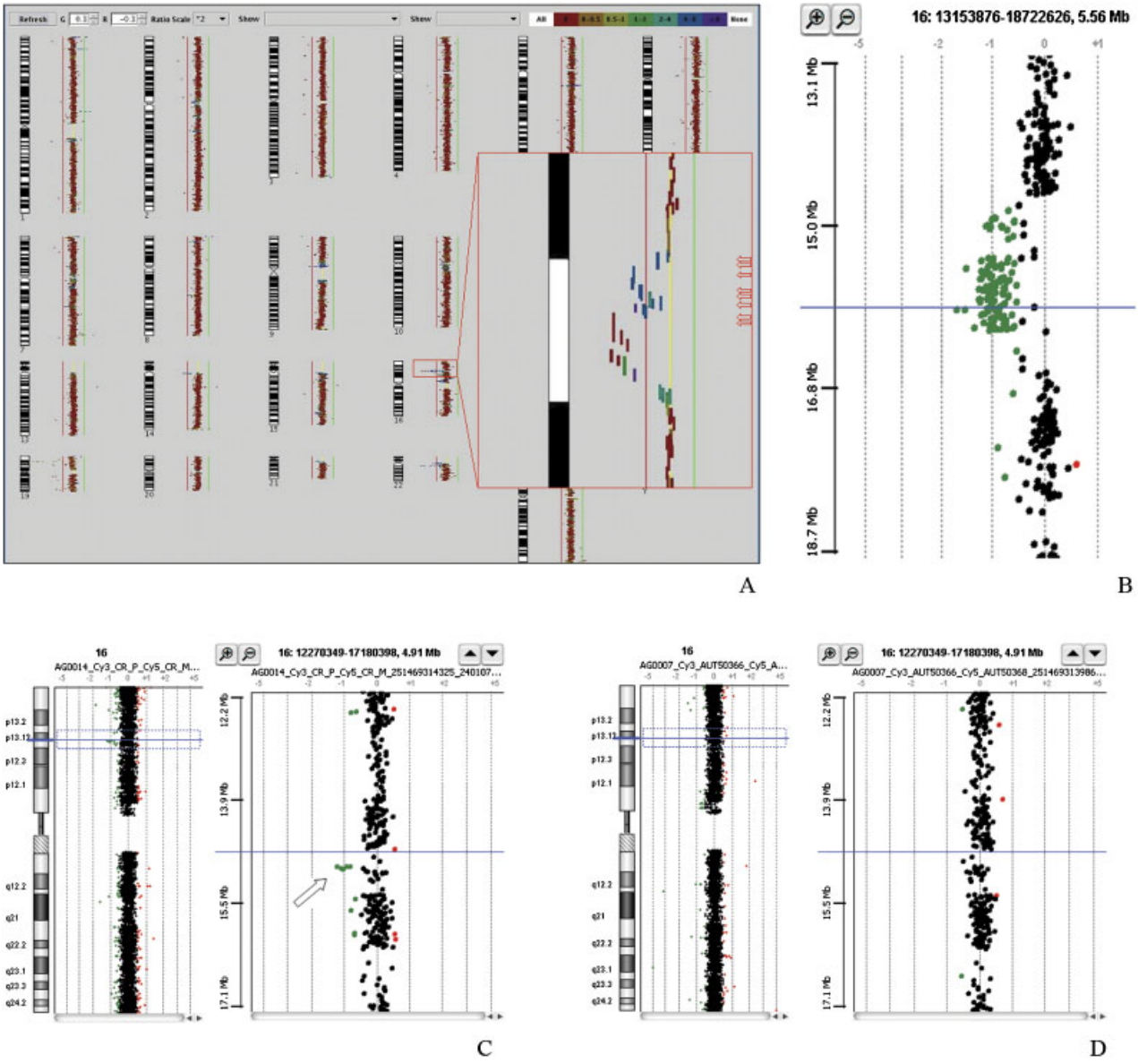


FIGURE 2. **Array CGH results. A:** Array CGH result for Patient D II-1 using a whole genome tiling path BAC array. Data analysis and visualization was performed by CGHPRO. For each BAC clone, Cy3/Cy5 signal intensity ratios are given and plotted alongside the relevant chromosomes. Red and green lines correspond to $\log_2$ ratios −0.3 (loss) and 0.3 (gain), respectively. Red to blue color codes indicate BACs with increasing percentage of LCRs, as described previously [Chen et al, 2005]. Inset: closer view of a deletion involving 16p13.1, which is flanked by LCRs (green and blue clones). Note that due to the high content of LCRs (blue clones), the aberration appears discontinuous. Red arrows indicate the positions of qPCR primer pairs 1–8, which were used to verify DNA copy numbers and to assess the particularly LCR-rich distal boundaries of deletions and duplications. BAC array CGH results for the other families are given in Supplementary Figure S1. **B:** Array CGH result obtained with the same DNA sample (Patient D II-1) using a 244-k oligonucleotide array. The regions of low oligonucleotide density mainly correspond to sites rich in LCRs. **C:** In Family F, the cohybridization of Patient I-2 vs. Patient II-2 on a 244-k oligonucleotide array revealed a further rearrangement. Separate array CGH analysis of patient and both parents demonstrated that this change occurred de novo in the affected son (data not shown). The blue line and rectangle in the whole chromosome 16 view to the left indicate the region that is zoomed in. **D:** Cohybridization of probands, Patient II-2 vs. Patient III-2 of Family B, on a 244-k oligonucleotide array.

Families A, B, and D (Patients A I-2 vs. III-1; B II-2 vs. III-2; and D I-1 vs. II-1) on the same 244-k oligonucleotide array platform did not reveal any differences.

Array CGH data discussed in this work have been deposited in NCBI's Gene Expression Omnibus [Barrett and Edgar, 2006; Edgar et al., 2002] and are accessible through GEO Series accession number GSE 6225.

Verification of DNA Copy Number Changes by FISH and qPCR

In Families A, B, and C, initial verification of array CGH results was done by qPCR using primer pairs 7 and 8. Deletions in the index patients of Families D and E were confirmed by qPCR with primer pairs 9, 10, and 11, which were also used to test the parents of these two families; subsequently, D I-1 was also analyzed by array CGH. In Family F, the deletion was initially identified by array CGH using a 1-MB array and verified by FISH.

The distal part of the aberration is located in an island of low copy repeats (LCRs; Figs. 2 and 3a), which complicates the interpretation of array CGH results in this region. Detailed analysis of the distal breakpoint region by qPCR with primer pairs 1–6 revealed that in all carriers but Patient E II-1, in whom the aberration occurred de novo, the distal breakpoint was located between primers 2 and 3 (chr.16: 14.64 and 14.89 Mb; NCBI35; HG17). Table 1 summarizes qPCR and array CGH results for all cases analyzed. All qPCR primer pairs and their genomic location are listed in Supplementary Table S1.

LCRs and DNA Copy Number Variants

Both endpoints of this 16p13.1 aberration are located in areas with high LCR content. There are several paralogous repeats at the proximal and distal breakpoint, the longest of which is segmental duplication no. 11712 (Chr16: 14817732-14956148; Chr16: 16216473-16354758; NCBI35; HG17), with an alignment length of 138,286 bp. The repeats are in direct orientation and nonallelic homologous recombination (NAHR) between these LCRs seems to be a likely explanation for the recurrence of these 16p13 rearrangements and for their identical size. NAHR between LCRs located in 16p11.2 and 16p12.2, respectively, has been implicated previously in the generation of two identical 8-Mb duplications in two unrelated autistic patients [Finelli et al., 2004].

Several other balanced and unbalanced DNA copy number variants have been reported for 16p13.1 that also attest to the genomic instability of this region. In the vicinity of the breakpoint interval determined by qPCR in this study, three inversion polymorphisms have been described ([Tuzun et al., 2005]; Database of Genomic Variants [Zhang et al., 2006]; Locus 1753). In addition, the same database lists a number of DNA copy number variants (Locus 1753–1755, Fig. 3a) [Fredman et al., 2004; Locke et al., 2006; Redon et al., 2006]. However, up to now, all copy number variants found in this region are much smaller than the aberrant interval described in the six index patients of this study (Fig. 3a).

We screened for duplications or deletions of this 1.5-Mb interval in more than 600 normal individuals and patients from other cohorts that have been analyzed using the same high-resolution BAC array platform and data analysis parameters, but no similar or identical DNA copy number changes were identified in any of these individuals (Fig. 3b).

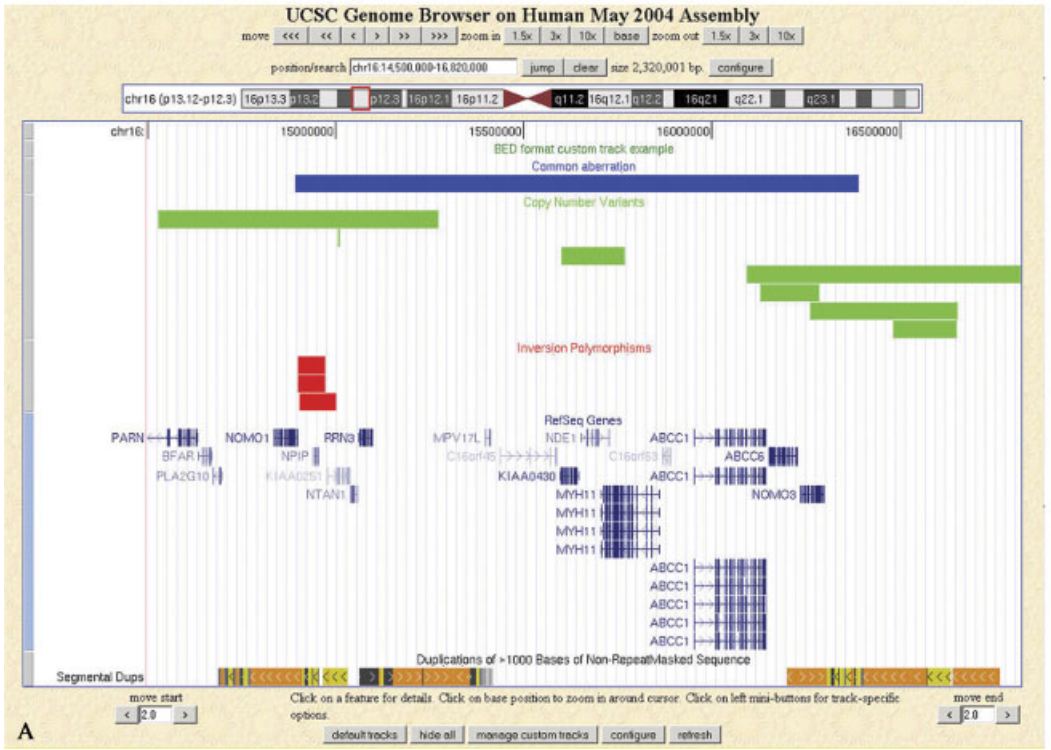
DISCUSSION

In this study we have identified a 1.5-Mb duplication at 16p13 in four severe autistic males from three unrelated families and some of their variably affected and unaffected relatives. A reciprocal deletion of the same segment was found in three mentally retarded patients, two from Denmark and one from Brazil, all of whom had dysmorphic features. The aberrant chromosomal region is flanked by LCRs. NAHR between neighboring LCRs frequently leads to genomic disorders, which are characterized by either gain or loss of the intervening sequence [Shaw and Lupski, 2004]. The paradigm for a microdeletion syndrome and its corresponding microduplication counterpart is Charcot Marie Tooth (CMT1A) and hereditary neuropathy and liability to pressure palsy (HNPP) [Chance and Lupski, 1994], but there are also numerous other examples, like Smith Magenis syndrome [Potocki et al., 2000], the 22q11.2 deletion/duplication syndrome [Ensenauer et al., 2003], and Williams Beuren syndrome [Somerville et al., 2005; Kriek et al., 2006]. In mentally retarded patients, numerous different microdeletions and duplications have been identified [de Vries et al., 2005], including a recurrent one on chromosome 17q21 [Koolen et al., 2006; Sharp et al., 2006; Shaw-Smith et al., 2006]. These findings have corroborated previous reports indicating that mental dysfunction can result from defects of many different genes, and that the complexity of (severe) MR is largely due to genetic heterogeneity [Inlow and Restifo, 2004; Ropers, 2006].

Several observations strongly argue against the possibility that the genomic imbalances described here represent neutral DNA copy number variants. In at least one of our patients, the aberration appeared de novo, and a de novo duplication involving a very similar or identical interval has been reported in a patient with mild developmental delay and learning disabilities [Kriek et al., 2006]. Another 16p13.1 duplication, but with unknown inheritance, has been identified recently by array CGH in a patient with MR [Sharp et al., 2006] and further de novo duplications of 16p13 in autistic patients have been detected by conventional karyotyping (Autism Chromosome Rearrangement Database; http://projects.tcag.ca/autism) [Gillberg, 1998; Hebebrand et al., 1994]. Evidence for the involvement of genes on 16p13 has also come from linkage and association studies that have identified autism susceptibility loci in this region [Lamb et al., 2005; Philippe et al., 1999]. It is of note in this context that in our study, only the carriers of deletions showed additional dysmorphic features.

In the families described here, 16p13.1 duplications and deletions are present in all affected patients, but several of the duplication carriers are only mildly affected and some are completely unaffected; likewise, some carriers of the reciprocal deletion are also of normal intelligence. These findings indicate that duplications and deletions of this 1.5-Mb segment strongly predispose for, but do not suffice to cause autism and MR and suggest that the manifestation of this disorder may be dependent on other factors.

One of these factors in duplication carriers may be the gender of the transmitting parent. From the limited family data available, it appears that if the duplication is transmitted by the father, the offspring is unaffected, whereas maternal transmission leads to clinical manifestation. This is reminiscent of 15q duplications, which are usually associated with autism only if they are carried on a maternally-derived chromosome 15. Moreover, all four severely autistic carriers of the duplication are males. In five females with this duplication, two had mild MR, one with psychiatric problems but without autistic features; whereas in the other three, intelligence and behavior were normal. Similar sex-limited and



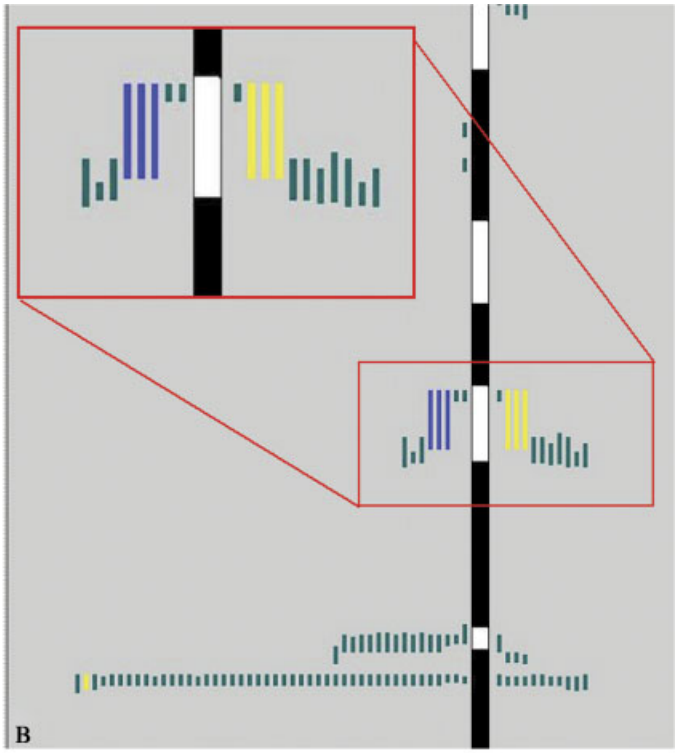


FIGURE 3. **DNA copy number variants and LCRs in the 16p13 region. A:** Screenshot from the University of California, Santa Cruz (UCSC) Genome Browser showing that the 16p13.1 region is rich in LCRs (segmental duplications; orange, yellow and black bars at the bottom). Inversion polymorphisms (red bars) and DNA copy number variants (green bars) have been reported, but none of them encompass the entire interval described in this work (blue bar). The distal endpoint of this interval is defined by primer pair 3, the first primer pair showing a DNA copy number change in all cases except Patient E II-1. For more details, see Results. **B:** DNA copy number variants in the 16p13 region detected by high-resolution array CGH in patients with autism and MR (duplications: yellow bars, deletions: blue bars) and in 600 normal individuals and patients from other cohorts (dark green; manuscripts and submission to public databases in preparation). All experiments were performed with the same BAC array platform, employing standardized protocols as well as identical parameters for the analysis of the data. To increase the sensitivity of this comparative study, circular binary segmentation in combination with a $\log_2$ threshold of ± 0.2 were used for the identification of aberrations.

parent-of-origin–specific effects related to 16p have been observed in a previous linkage study [Lamb et al., 2005]. Epigenetic differences would be an obvious explanation for these findings. We have looked for differences in methylation between affected and unaffected carriers by means of methylated DNA immunoprecipitation (MeDIP) [Weber et al., 2005], but at least at the level of resolution this technique can provide on our BAC array platform, all corresponding duos showed similar methylation patterns for this region (data not shown).

Phenotypic variability might also be due to subtle differences of the size and internal organization of the unbalanced fragment. As suggested by BAC array CGH and qPCR, all 16p13 changes share the same proximal and distal breakpoints. However, as indicated by the high frequency of balanced and unbalanced variants, this genomic interval seems to be rather unstable, and an existing imbalance might even predispose to further (e.g., meiotic) rearrangements. Indeed, in Family F, CGH on a 244-k oligonucleotide array revealed a further rearrangement of this region in the patient that was not seen in his unaffected mother. Interestingly, this de novo change largely overlaps with an inversion polymorphism that has been detected recently by fosmid end sequencing [Tuzun et al., 2005].

From a functional point of view, both *NDE1* and *NTAN1* appear to be plausible candidate genes. NDE1 [Luttik et al., 1998] is predominantly expressed in the brain. Its gene product interacts with PAFAF1B1, the product of the lissencephaly gene *LIS* [Reiner and Sapir, 1998], and MAPK1, which plays a role in synaptic plasticity [Ratto and Pizzorusso, 2006]. Homozygous Nde1 knockout mice have significantly reduced brain sizes [Feng and Walsh, 2004], which is interesting given the fact that in humans, 20 to 30% of autistic individuals have enlarged brains, and in 2 out of 3 index cases with 16p duplications and autism, significantly enlarged HCs were observed.

NTAN1, another attractive candidate gene, which encodes an N-terminal asparagine amidase [Grigoryev et al., 1996], has been implicated in social behavior, learning, and memory [Balogh et al., 2000, 2001; Kwon et al., 2000]. *NTAN1* is located in a small island of single copy sequence embedded in a large cluster of highly conserved LCRs (Fig. 3a). This may render this gene particularly vulnerable to further rearrangements, which may distinguish individuals with normal and aberrant phenotypes. There is evidence that both *NDE1* and *NTAN1* are involved in copy number variants. *NDE1* was found to be deleted in three independent normal individuals [Locke et al., 2006] (Database of Genomic Variants), and DNA copy number variants have also been described for the telomeric LCR-rich region, one of which covers *NTAN1* (Database of Genomic Variants) (Fig. 3a) [Redon et al., 2006]. However, genomic imbalances involving the entire 1.5-Mb interval have not been reported before. Moreover, we have not observed them in more than 600 normal individuals and patients that were analyzed on the same array platform. Noteworthy, in this reference set we have also failed to identify any variant that encompasses the LCR-free stretch of sequence harboring *NDE1* (Fig. 3a and b).

In summary, we have described a submicroscopic duplication encompassing a 1.5-Mb segment on chromosome 16p13.1, which seems to predispose for autism in males, and a deletion of the same interval that is associated with MR and other clinical abnormalities. However, the phenotypic distinction between these two genomic disorders is not sharp, as several of the duplication carriers have also MR without autism. This observation supports the concept that the pathogenetic mechanisms leading to autism and MR are causally related and it should pave the way for their identification.

ACKNOWLEDGMENTS

We sincerely thank the patients and parents for participation in this study and the NSW Health Department for their support; Melanie Wendehack, Vivien Boldt, and Murat Sari for technical assistance; and the reviewers for their helpful comments. We also thank Pieter de Jong and the BACPAC Resources Centre for providing DNA and clones of the human "32k" BAC Re-Array set, Nigel Carter and the Mapping Core and Map Finishing groups of the Wellcome Trust Sanger Institute for the initial clone supply of the 1-Mb Sanger set, and the COST B19 Action "Molecular Cytogenetics of Solid Tumours" for the assembly of the subtelomeric clone set.

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