

ELECTRONIC LETTER

FISH characterisation of an identical (16)(p11.2p12.2) tandem duplication in two unrelated patients with autistic behaviour

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Partial trisomy 16p is a rare chromosomal anomaly in newborns: of the fewer than 30 carrier patients so far reported, most were born to parents with a balanced translocation involving the p arm of chromosome 16.¹

Pure partial trisomy 16p has been reported in seven patients,²⁻⁶ three of whom (all showing behavioural problems with autistic traits) carried a tandem duplication of the (16)(p11.2-p12) region^{4,6}; minor dysmorphisms were reported in only one patient.⁴

Linkage studies indicated chromosome 16p as a major location for autism susceptibility genes,⁷ while association was reported between autistic traits and attention deficit or hyperactivity disorders mapping to the 16p13 band.⁸ In addition TSC2, one of the genes responsible for tuberous sclerosis, a syndrome often associated with autistic traits, maps to the same cytogenetic band.⁹

We report the clinical phenotype and refined molecular cytogenetic characterisation of a patient carrying a (16)(p11.2p12.2) duplication. By extending the FISH analysis to a previously described patient with an apparently similar chromosomal rearrangement,⁶ we found that low copy repeats map to the 16p11.2 and 16p12.2 duplication endpoints, suggesting non-allelic homologous recombination as the pathogenetic mechanism. This finding is consistent with the non-random occurrence of the observed chromosomal rearrangement and the high frequency of segmental duplications identified throughout chromosome 16.¹⁰⁻¹² We also inferred from genotype-phenotype correlation studies that genes involved in autism susceptibility are located within the duplicated region.

CASE REPORT

Patient 1 is a 25 year old man, the first son of unrelated parents. At the time of his birth, his mother was aged 30 and his father 29 years. He was born at term with a weight of 2.550 kg (3rd centile).

The father suffered from alcohol misuse and left the family when the patient was 12 years old. Because of behavioural problems shown after their father left home, his younger brother lived in a home until he completed his professional studies. His sister suffers from epileptic seizures, which appeared when she was 16 years old. Her brain magnetic resonance imaging scan was normal.

Psychomotor delays were noticed in the patient's first months of life. In infancy and childhood, he suffered from recurrent episodes of bronchitis, otitis, gastroenteritis, and febrile and epileptic seizures. At the age of three years, he was diagnosed as suffering from autism, severe mental retardation (25<IQ<39), and epilepsy. He could not walk unassisted until the age of eight years, and was 10 years old when he first used words and word approximations. He never achieved continence, and still needs help in eating and

Key points

- Refined molecular cytogenetic characterisation of unrelated patients with autistic behaviour carrying a familial (mother and daughter) or presumptive de novo duplication in chromosome 16p showed the same genomic region (16p11.2→16p12.2) involved in a direct duplication extending for about 8 Mb.
- The duplication endpoints were found to map within duplicons located at 16p11.2 and 16p12.2, suggesting that the pathogenetic mechanism leading to the duplication is non-allelic homologous recombination between low copy direct repeat elements.
- The clinical phenotypic spectrum of the reported patients was compared with the aim of revealing genotype-phenotype correlation. The only clinical feature shared by the 16p11.2→16p12.2 duplication carriers is autistic behaviour, although different grade impairments in the social interaction and communication domains characterised both the unrelated and the related patients. This finding suggests that dosage sensitive genes within 16p11.2→16p12.2 may be involved in the susceptibility to autism spectrum disorders.

complete assistance in dressing and self care. He has been living in a home for severely disabled children and young adults since the time of the diagnosis.

A clinical evaluation performed at the age of 25 years revealed a weight of 42 kg (<3rd centile), a height of 151 cm (<3rd centile) and an occipitofrontal head circumference of 54 cm (25th centile). The patient had an asymmetric and squared face, bitemporal constriction, hypertelorism, a broad nasal bridge and a broad nasal tip with a prominent columella, a short philtrum, long ears, a large mouth with thick lips, and large and irregular teeth.

A parallel psychiatric evaluation¹³ confirmed the previous diagnosis of autism. In relation to the qualitative impairment in social interaction and communication, his eye contact is poor, even when he is approaching people: he has to grasp people walking around him with a hand to embrace them with poorly modulated strength. When meeting new people, he predictably acts in a stereotyped manner: he throws away his shoes, seeks help in tying shoelaces, wants to play hide and seek or peekaboo, bites the other's clothes and repetitively vocalises the same word or word approximation

Abbreviation: FISH, fluorescence in situ hybridisation

in a very high pitched tone. He uses eight words or word approximations on a daily basis, without attaching any apparent predictable meaning to them. He understands simple and highly contextualised messages.

In terms of restricted, repetitive, or stereotyped behaviour and interests, when left alone, he engages in repetitive actions such as touching the inner side of his left cheek with the tip of his right index finger, and transforms everything (including food) into a marble shape. The physical contact with others suggests that he seeks out excessive tactile sensations. He likes to sit down on the floor with his legs crossed and rocks his body.

Neurological examination revealed that the patient had an ataxic gait and bilateral club foot. His fine motor skills were limited. His attention span was very short and he was hyperactive; but his memory was quite good. Seizures were successfully controlled by phenobarbital, which was interrupted when he was 20.

Instrumental examinations included a brain magnetic resonance imaging scan, which revealed slight ventricular dilatation and moderate diffuse cortical atrophy, and an electroencephalogram showing a diffuse epileptic pattern.

Patient 2 is a 5 year old girl affected by borderline cognitive impairment (IQ = 80) and behavioural problems, who has been previously reported (together with her mother) as also carrying a duplication of chromosome region 16p11.2→p12.1.⁶ As patient 2 does not show repetitive and stereotyped behavioural patterns, or restricted interests, she partially satisfies the DSM-IV criteria for autism (at least one other impairment in the social interaction domain would be required), which are conversely fulfilled by her mother. They are both verbal with echolalia. Of relevance to the autism diagnosis, the mother of patient 2 had difficulties in attention and concentration, non-verbal learning disabilities (weak visuospatial functioning), sustained by a verbal IQ (= 100) significantly higher than the performance one (= 64) and weakness in planning tasks. At school she experienced learning difficulties, while in her life she suffered from severe anxiety secondary to social adaptation problems and social incompetence (unable either to keep non-demanding jobs or to provide her daughter with essential daily care).

MATERIAL AND METHODS

Chromosomes were prepared from peripheral blood lymphocyte cultures following standard procedures. QFQ banded metaphases were analysed and the karyotype was described in accordance with the International System for Human Cytogenetic Nomenclature.¹⁴

Whole chromosome 16 specific painting was performed on the metaphase chromosomes according to the manufacturer's specifications (Oncor). Partial chromosome painting from a human/hamster hybrid (Hy57) was used following a previously described protocol.¹⁵ The BAC probes used in the FISH study are listed in table 1. The RPCI-11 BAC clones (P de Jong; <http://bacpac.chori.org/>) covering the genomic region between 16p12.3 and 16p11.2 were selected according to the UCSC Genome Browser (<http://genome.cse.ucsc.edu>, release July 2003), and provided by the YAC Screening Centre (Dibit, HSR, Milan, Italy) and Prof. Rocchi Mariano (University of Bari, Italy).

All of the probes were nick translation labelled with biotin or digoxigenin (La Roche, Switzerland). The FISH protocols of Lichter et al¹⁶ and Lichter and Cremer¹⁷ were followed, with minor modifications.

CYTOGENETIC AND FISH FINDINGS

Analysis of the QFQ banded metaphase chromosomes from case 1 revealed the presence of an aberrant 16 chromosome, with additional chromosomal material on the p arm, in all of

the analysed cells (fig 1A). The karyotypes of the proband's mother and sister were normal, while the father was not available for analysis. Characterisation of proband aberrant chromosome 16, using whole chromosome 16 painting and subsequent use of partial (16p11.2→16p12) chromosome painting, showed that the additional material is derived from chromosome 16 and established the karyotype as 46,XY,dup(16)(p11.2p12.1) (see fig 1A, pcp Hy57).

To define the 16p duplicated sequences, we used FISH with BAC clones mapping within the 16p12.3→16p11.2 genomic interval (table 1), thus allowing the extension of the duplicated region to be defined on the basis of the presence or absence of an additional hybridisation signal on the abnormal chromosome 16.

Most of the BAC clones comprised between clones 98D10 and 343H14 (16p12.1) and 368N21 (16p11.2) gave two distinct hybridisation signals on chromosome dup (16), as against the single signal observed on the normal homologue (fig 1B).

The clones telomeric and centromeric to these BACs gave a single hybridisation signal on chromosome dup(16), the intensity and size of which was comparable to that observed on normal chromosome 16 excluding the target region from the duplication.

Interestingly, the clones targeting the telomeric breakpoint (343H14, 98D10) and the centromeric breakpoint (368N21) showed a peculiar hybridisation pattern with two signals on normal chromosome 16p (at 16p12.2 and at 16p11.2) and three distinct signals on the p arm of the aberrant chromosome 16 (figs 1C–D); they also showed very small and faint signals at 16q chromosomal locations (16q22 and 16q24) in 50% of the analysed metaphases. Similarly, the probes mapping inside the duplicated region (322D12 and 24N18) gave two signals on normal chromosome 16p and four signals on dup(16).

To compare the extension of the chromosome 16 duplication, we also characterised metaphases from the previously reported case 2 using the same FISH. Using part of the same probe panel (62C15, 688G12, 98D10, 120F20, 141E3, 28A6, 368N21, 753J6, and 455F5), we found that the hybridisation pattern was identical (see figs 1E–G for 141E3, 98D10, and 368N21), thus demonstrating that this patient carries the same duplication.

BAC clones 98D10 and 368N21 were identified as encompassing the breakpoint regions involved in the duplication at respectively 16p12.1 and 16p11.2 in both patients. The duplication extends for 8 Mb according to the July 2003 UCSC draft (Human Genome Browser UCSC, <http://genome.cse.ucsc.edu>, release July 2003, for physical mapping)

To determine whether the duplication is direct or inverted, both patients underwent dual colour FISH using BACs 141O15 (16p12.2) and 151I10 (16p11.2). The position of the hybridisation signals on the duplicated chromosome demonstrated that the rearrangement is a direct duplication (see fig 1I for patient 1).

BIOINFORMATIC SEARCH

Following the finding that clones 368N21 and 98D10 encompassing the centromeric (16p11.2) and telomeric breakpoint (16p12.2) gave multiple signals on the normal 16 chromosome, with 98D10 also hybridising to the telomeric bkp and vice versa (figs 2A–B), we concluded that both breakpoints underlying the 16p11.2→16p12.1 duplication in two independent patients map within two low copy repeats on chromosome 16p.^{10–12 18 19}

The overall FISH results agree well with the data in the literature and those provided by a bioinformatic search using the USCS Genome Browser (<http://genome.cse.ucsc.edu>). As can be seen from the physical map shown in fig 2D, a number

Table 1 FISH results obtained using BAC clones targeting the 16p12.3→16p11.2 interval in two patients carrying 16p partial duplication

RPCI-11 clone name	Cytogenetic localisation	Other region recognised by the probe	FISH signal on normal 16p	FISH signal on dup(16)	
				Patient 1	Patient 2
167K14	16p12.3		+	+	
429K17	16p12.3		+	+	
444F15	16p12.3-12.2		+	+	
232I1	16p12.2		+	+	
62C15	16p12.2		+	+	+
688G12 [†]	16p12.2	16p12.2	++	++	++
343H14*	16p12.2	16p11.2	++	+++	+++
98D10*	16p12.2	16p11.2	++	+++	+++
120F20	16p12.2		+	++	++
338J22	16p12.2		+	++	
322D12	16p12.2		++	++++	
141O15	16p12.2		+	++	
21M24	16p12.2		+	++	
16A8	16p12.2		+	++	
141E3	16p12.2		+	++	++
13G4	16p12.2		+	++	
139E11	16p12.2		+	++	
450G5	16p12.2		+	++	
478K1	16p12.1		+	++	
451N20	16p12.1		+	++	
417E21	16p12.1		+	++	
62O3	16p12.1		+	++	
15I10	16p11.2		+	++	
24N18	16p11.2	16p12.2	++	++++	
28A6	16p11.2		+	++	++
368N21*	16p11.2	16p12.2	++	+++	+++
753J6	16p11.2			+	+
301D18	16p11.2			+	
279M12	16p11.2			+	
114A4	16p11.2			+	+

The BAC probes in bold target the duplication in both patients

*Additional small and faint signals at 16q22 and 16q24 chromosomal locations.

[†]additional small and faint signals at 16p13 and 16q22.

of STS markers (D16S2778, SHGC-102060, SHGC-58072, RH71359) map to both duplication breakpoints as well as the full length transcripts of the LAT1-3TM gene. These transcripts are gene expression products of truncated paralogues of the hLAT1 (SLCL7A5) gene (16q24.3) which map to 16q12.2 and 16p11.2 as low copy repeats.^{19, 20} NCBI BLAST homology search analysis revealed that the nucleotide sequence of clone 368N21 is very similar to, but distinct from, the 98D10 and CTA-61E3 sequences (our results and²⁰). More precisely, >96% similarity is found in sequence

stretches, sized from 200 bp to 3.4 Kb, which account for a total of 38 Kb out of the overall 77 Kb aligned sequence. Moreover, clone 98D10, which targets the telomeric duplication breakpoint, fully overlaps the CTA-61E3 clone which was found to contain a 110 kb insert spanning two chromosome 16 low copy repeats (LCR16u and LCR16v). Interestingly LCR16v, which contains a portion of the SLCL7A5 gene,¹⁹ maps to both duplication breakpoints (fig 2B) and may thus be a target of the recombination process which mediates the rearrangement in both patients (fig 2C).

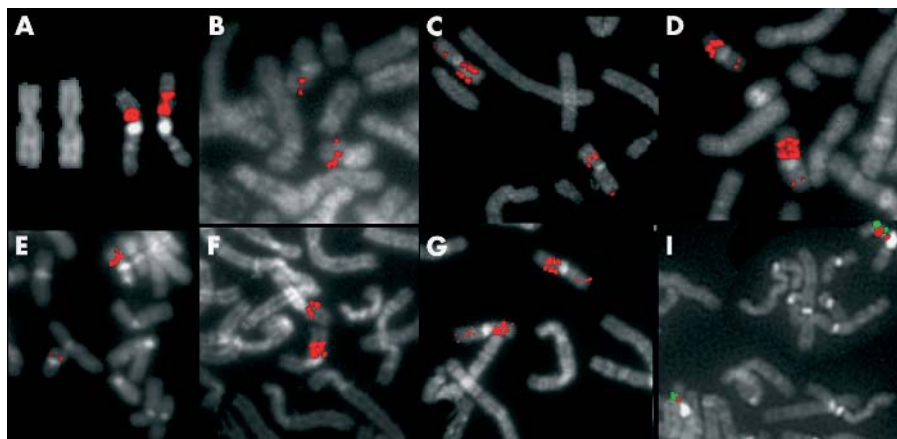


Figure 1 (A) 16 and dup(16) QFQ banded chromosomes (left), and chromosome 16 and dup(16) painted by means of partial chromosome painting Hy 57 (right). Partial metaphases of patient 1 hybridised with probes 141E3 (B), 98D10 (C) and 368N21 (D): 141E3 gives two distinct hybridisation signals on the dup(16p) chromosome, and 98D10 and 368N21 give three signals on the p arm of aberrant chromosome 16, which are thus identified as respectively targeting the tel and cen bkps. Partial metaphases of patient 2 hybridised with probes 141E3 (E), 98D10 (F) and 368N21 (G): all of the probes give the same hybridisation pattern as that observed in patient 1. (H) Partial metaphase of patient 1 cohybridised with BACs 141O15 (green) and 15I10 (red), both targeting duplicated regions, showing the presence of a direct duplication.

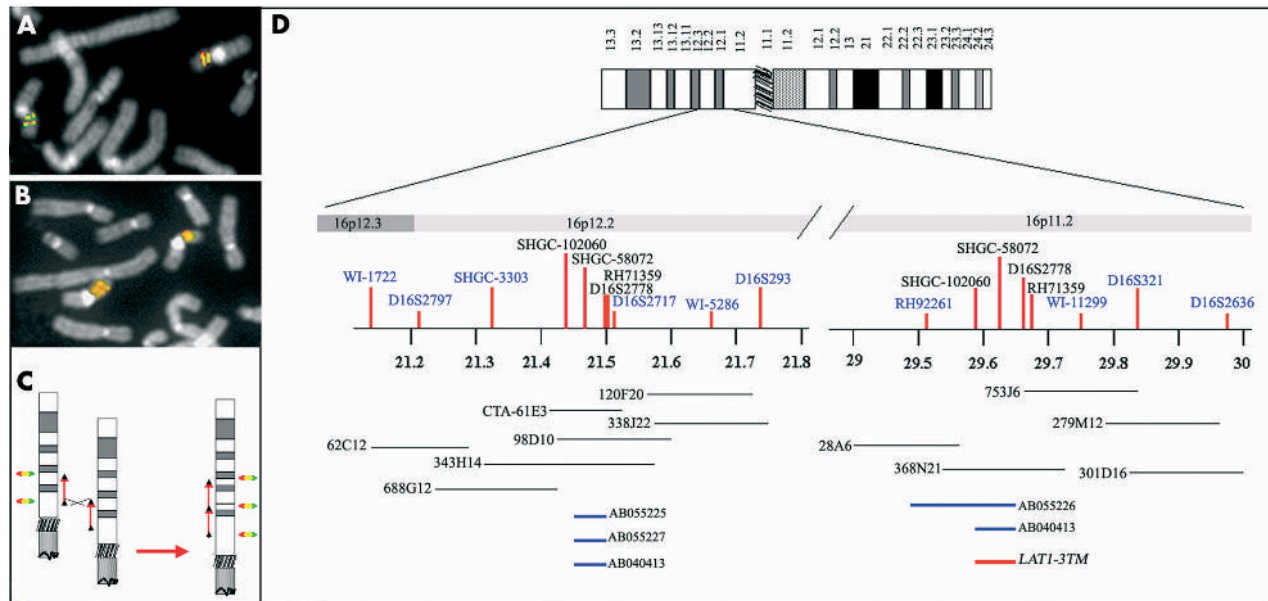


Figure 2 Dual colour FISH of 98D10 (red) and 368N21 (green), respectively targeting the tel and cen bkps, in normal control (A) and patient 1 metaphases (B) showing merged yellow signals (red+green) at both bkp regions on normal 16p and dup(16). (C) Putative mechanism leading to dup(16) as a result of non-allelic homologous recombination mediated by low copy repeat sequences: the red arrows on the idiogrammed chromosomes 16 point to the duplicated region, while the black arrowheads mark the low copy repeats mapping at duplication ends. The FISH patterns at the duplication endpoints are also given. (D) Physical map of the genomic region containing the 16p12.2 and 16p11.2 duplication bkps. The scale refers to the July 2003 human draft sequence from the UCSC genome browser <http://genome.cse.ucsc.edu>. The genomic clones covering the regions flanking the duplication bkps are indicated by black lines. The blue and red lines indicate respectively the LATI-3TM transcripts and gene. The markers clustering bkp regions are shown in black.

DISCUSSION

Looking at the overall clinical phenotype of the patients with dup(16p)(p11.2p12.2), the only shared features seem to be autistic behaviour and cognitive impairment. Neither the mother nor the daughter described by Engelen et al showed dysmorphism,⁶ whereas our patient has minor signs. Furthermore, the mother's height and occipitofrontal head circumference were below the 3rd centile, but her daughter's height fell in the normal range (her occipitofrontal head circumference is not known), whereas our patient's height and weight were below the 3rd centile, and his occipitofrontal head circumference was normal despite the relative macrocephaly.

In terms of autistic behaviour, each patient shows qualitative impairments in the social interaction and communication domains. However, our patient's phenotype is much more severe as his lack of social reciprocity is a consequence of his complete unawareness of the rules of human interaction: he is non-verbal and does not compensate for this with gestures of any kind that would demonstrate at least a basic interaction, and he is also incapable of any creative or symbolic play—that is, play that involves using toys and objects in a variety of conventional ways and pretending the objects or oneself are something or someone else. In the case of patient 2 and her mother, verbal language is poorly aimed at social interaction, and this goes together with their impaired reciprocity.

Patient 2 may belong to the "broader autistic phenotype"^{21,22}. Nevertheless, it must be pointed out that the combination of protocols eliciting autistic behaviours and a specifically structured questionnaire would be better than clinical history for the formulation of a final diagnosis in cases of less severe autism. Her mother shows hand and finger motor stereotypies, and repetitive behaviours

due to abnormal preoccupations. Our patient indulges in body rocking, and is highly interested in seeking tactile sensations.

It is known that individuals with an autistic syndrome follow different courses throughout life: some develop speech, one quarter to one third develop seizures, the majority are low-functioning, while others are so high functioning that it is hard to believe they have the same syndrome²³. A few improve, moving within the spectrum of autism, so that boys diagnosed with Asperger's syndrome received in their childhood the diagnosis of autism. Such a clinical dissimilarity is also ascertained in our patients, two of whom are related, carrying the same chromosome duplication. This observation supports the evidence that autism is a complex genetic disorder presenting with a true clinical spectrum. Nevertheless a few core autistic features are shared by the three patients and may thus be attributed to dosage sensitive genes in the 16p duplicated region. The observed gradient in the phenotypic severity may be caused by the interplay of genes within the duplicated region with other genes and environmental factors. Genes contributing to the more severe autistic phenotype in patient 1 are those responsible for the familial epilepsy, while the social adaptation problems might have been enhanced by the family context. In particular, the severe cognitive deficiency in our patient may be related to the presence of epileptic seizures, which were experienced in early infancy. Sex related factors may also play a role, as the male:female ratio reported in autism is 4:1.

There are few published reports of patients with mental retardation and autistic behaviour plus minor anomalies who carry a duplication of chromosome 16p.^{3,4,6} The patient described by Carrasco et al⁴ carried an apparently larger duplication than that characterised here, but it included the same region.

It has recently been reported that chromosome 16p has a duplison rich genomic organisation,^{12 18 19} but there is no mention of the involvement of 16p low copy repeats in gross chromosomal rearrangements. We have provided FISH evidence that the bkps underlying the (16)(p11.2→16p12.1) duplication in two independent patients fall within two of the low copy repeats mapped to chromosome 16. Taken together, these findings support the non-random occurrence of the rare rearrangements described here, suggesting that direct repeats within duplisons at 16p11.2 and 16p12.2 may be the targets of non-allelic homologous recombination leading to duplication of the comprised region.

More generally, our data are consistent with the rapidly growing evidence that low copy repeat elements, which play a well known role in mediating deletions or duplications in human genomic disorders, are also involved in non-recurrent chromosomal rearrangements.^{24 25} Given the genotype and phenotype of our three patients and the published reports concerning 16p duplication, chromosome interval 16p11.2→p12.2 emerges as a candidate region for autistic disorders. Comparison of unrelated patients with autistic traits who carry a duplication of the same chromosomal region is a powerful means of pinpointing a candidate region in which susceptibility to autism spectrum disorders can be identified.

More than 100 genes have been mapped in the approximately 8 Mb duplicated interval (Human Genome Browser UCSC, <http://genome.cse.ucsc.edu>, release July 2003), and so it is only possible to speculate about the potential role of specific genes in autism. However, on the basis of their functions and expression patterns, voltage ion channel subunit γ -1 (SCNN1G), sodium cotransporter (KST1), and voltage dependent calcium channel γ -3 (CACNG3) can be considered first choice candidates. Indeed members of voltage gated sodium channels such as SCN1A, SCN2A, and SCN3A map on chromosome 2 autism associated region, and sequence variants in these gene have also been reported in familial autism.^{7 26}

An increased dosage of one or more of the above genes (or others, the function of which is yet unknown) may cause specific tissue and developmental effects leading to autistic traits. Rare patients such as those described here are a valuable resource for pinpointing the chromosomal regions harbouring autism susceptibility genes, but targeted approaches such as transgenic mouse models or differential gene expression using micro-arrays are needed to identify the crucial 16p12.1-p11.2 genes contributing to autism.

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