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Two patients with balanced translocations and autistic disorder: *CSMD3* as a candidate gene for autism found in their common 8q23 breakpoint area

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Recent studies estimated a rate of 3–5% of cytogenetic abnormalities involving many different chromosomes in autistic spectrum disorders (ASDs). Here, we report on two unrelated male patients with *de novo* translocations, autistic behaviour and psychomotor delay. These two patients carry a balanced chromosome translocation t(5;8)(q14.3;q23.3) and t(6;8)(q13;q23.2), respectively. A detailed physical map covering the regions involved in the translocations was constructed using BAC clones mapping on chromosomes 5q14.3, 6q13 and 8q23. Fluorescence *in situ* hybridisation (FISH) analyses were carried out using these genomic clones. We fine mapped the two translocation breakpoints on chromosomes 8 identifying their position within a short 5 Mb genomic region. Breakpoints on chromosomes 8 in both patients do not interrupt any known gene but both map in a region containing the *CSMD3* gene, which thereby can be considered as a candidate for ASDs.

Introduction

Autistic spectrum disorders (ASDs; MIM 209850) are a group of neurodevelopmental disorders characterised by qualitative impairments in a triad of behavioural domains: (1) social interactions; (2) development of language, communication and imaginative play; (3) restricted, stereotypical and repetitive patterns of interest, behaviour and activities (DMSIV; ICD10).[1,2] ASDs are relatively common conditions that manifest in early childhood with onset before 36 months of age and are frequently accompanied by mental retardation (75%) and epilepsy (25%).[3] Individuals with ASDs are often divided into two groups such as a syndromic or complex group in whom autism is associated by malformations or dysmorphic features and a non-syndromic group where the patients have a normal appearance.[4]

Prevalence of autism estimates in the general population has gradually increased over the last 20 years;[5] epidemiological studies in North America and Europe report an incidence of about 1 in 150 children with male/female ratio of approximately 4:1.[4,6] Current evidence suggests that ASDs are predominantly hereditable disorders; heritability is estimated to be more than 90% genetic and several genes are thought to contribute to the disorder, although the mode of inheritance is unknown.[5] The rate of recurrence in siblings of affected children is estimated to be

2–8%, much greater than the prevalence rate in the general population.[1] Moreover, twin studies report a rate of concordance of 60–91% in monozygotic twins (MZ).[5] Surprising disparity in some MZ twins indicates that other factors can modify these phenotypes. The study of several cases shows that susceptible genetic background and random environmental events may be necessary for the full expression of the disorder. Viral infections, for example, rubella and cytomegalovirus, during or after pregnancy have been associated with ASDs in some infants. Another possible mechanism is a random epigenetic mutation in early embryonic life that altered the expression of the genetic trait.[7,8]

Despite the abundance of investigations into the genetics of ASDs, the identity and number of genes involved are not yet known. The wide phenotypic variability of the ASDs most likely reflects the interaction of multiple genes within an individual's genome and the existence of distinct genes and gene combinations among those affected.

Until recently, only 5–10% of autism cases were distinguishable with an underlying genetic cause. Two recent studies of Jacquemont *et al*[9] and Sebat *et al*[6] suggest that this number is actually 10–20% and that it may grow to 30–40% with further research.[4]

The identification of the candidate genes for autism through linkage and association studies is very difficult towing to the considerable genetic and phenotypical heterogeneity. Many whole-genome analyses, linkage and association studies in multiplex families have identified genomic regions most likely to contain ASDs susceptibility loci on 20 different chromosomes; regions implicated in different studies include chromosomes 1q, 2q, 4q, 5q, 6q, 7q, 8q22-24, 10q, 13q, 15q, 17q, 18q, 22q and X.[10–13]

Several candidate gene studies have been carried out, suggesting the involvement of genes essential in neurodevelopment, synaptic function, language and metabolism (*PIK3CG*,[5,14] *FOXP*,[1,15] *GABA-A* receptor,[1,5,16] *UBE3A*,[5] etc). More recently, mutations in *MECP2, PTEN, SHANK* and *NLGN4* have been reported to be involved in a small percentage of autism cases.[17]

An alternative and potentially successful approach aimed at eliciting candidate genes or candidate regions is the detailed analysis of the boundaries of the cytogenetic abnormalities found in individuals with autism.[18] Recent studies estimated a rate >5% of cytogenetic abnormalities (including unbalanced translocations, inversions, rings and interstitial deletions and duplications) in ASDs, and a high number of such cases have been described in the literature with regard to most chromosomes.[5,19,20] Two studies[6,9] greatly strengthen the growing awareness that a substantial fraction of ASDs is caused by genomic rearrangements.

We have identified two male patients with autism and psychomotor delay, each associated with a *de novo* balanced translocation, respectively t(5;8)(q14.3;q23.3)

and t(6;8)(q14;q23.2). We report here the fine physical mapping of the breakpoints involved in the two translocations by fluorescence *in situ* hybridisation (FISH) analysis. This analysis focused on a common area of breakage on the two translocated chromosomes 8, in the region q23, already observed as a potential susceptibility locus.[21] The purpose of this study is to search for a new potential susceptibility gene for ASDs in these patients.

Materials/subjects and methods
Cytogenetic analysis
Metaphase slides were prepared from peripheral blood lymphocyte cultures from the two patients, obtained after informed consent, using standard methods. Chromosome analysis was performed by routine QFQ-banding (approximately 550 band resolution). The investigation was extended to their parents who were reported to be normal.

FISH analysis
FISH experiments with different BAC clones from each rearranged chromosomal region were performed. BAC clones from human contig RPCI-11 were used. BAC DNA was labelled with digoxigenin-11-dUTP using nick translation kit (Roche, Basel, Switzerland). The labelling procedure was carried out according to the manufacturer's instructions. The DNA probe was dissolved in $10 \mu l$ of hybridisation solution according to the procedure described by Rocchi *et al* (http://www.biologia.uniba.it/rmc). Digoxigenin-labelled probes were visualised with antibodies anti-mouse and anti-rabbit IgG conjugate. Chromosomes were counterstained with DAPI or propidium Iodide. The slides were analysed in an Olympus fluorescence microscope (BX60), and images were captured by a cooled CCD camera (Sensys) and analysed using an imaging system with MacProbe software v. 4.2.3 (PSI).

Contigs
We have used the NCBI (http://www.ncbi.nlm.nih.gov/; updated 17 April, 2007), ENSEMBL (http://www.ensembl.org/index.html; Ensembl release 44 – April 2007) and UCSC (http://genome.ucsc.edu; update March 2006) data sets to construct the map, which contains the translocation breakpoints, to search for candidate genes and to assemble figures for this paper.

BAC clones from library RPCI-11 were donated by the Italian Telethon service (San Raffaele, Milano) and by Professor M Rocchi, DI.GE.MI. – Department of Genetics and Microbiology, University of Bari.

Expression analysis
Total RNA was extracted from the peripheral blood leukocyte of a control sample using the Trizol Reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's recommendations.

RT-PCR was carried out with the First-Strand cDNA Synthesis kit for RT-PCR (AMV; Roche) according to the manufacturer's instructions. Specific primers for *CSMD3* with forward (5′-TGGTCATGAAGTATTTCTTCAGTG-3′) and reverse (5′-CATTGATATACAACTGTGTCTCTAT-3′) directions were designed according to the sequence of the gene (http://www.ncbi.nlm.nih.gov/). First round RT-PCR was performed in $20\,\mu l$ volumes on $800\,ng$ of total RNA. A second round PCR was carried out using $2\,\mu l$ of the first PCR product as template and the specific primers. The amplified products were separated on 2% agarose gel and visualised by ethidium bromide staining.

Results

We report here on two unrelated male patients, each with a *de novo* balanced translocation. The first patient carries t(5;8)(q14.3;q23.3) and the second t(6;8)(q13;q23.2). Both are affected by autistic disorder, developmental delay and have a common area of breakage in 8q23. In addition, the first patient presents with epilepsy also. We initially focused our attention on this common area of breakage in 8q23 to determine if a single gene, possibly interrupted by the translocation, could be the cause of both phenotypes.

Cases report

Patient 1 (M.L.) A 5.5-year-old Sardinian boy was born to a 31-year-old mother and 36-year-old father, both healthy and nonconsanguineous. There was a history of complicated pregnancy – a funicular knot at the third month, oligohydramnios since the fifth month and fetal growth retardation at the 32nd week of pregnancy. He was delivered by caesarean section at the 34th week of pregnancy. Birth weight was $1540\,g$ (3rd centile), length was 42.5 (3rd centile) and CC was $29\,cm$ (3th centile). APGAR scores were 9 and 9 after $1\,min$ and $5\,min$, respectively. Brain ultrasonography in the neonatal period showed calcifications in the thalamus and nucleus dentatus bilaterally. The patient showed delayed psychomotor development with an IQ level of 38, severe speech delay, short attention span and epilepsy. The autism evaluation using CARS and ABC manuals met the DMS-IV criteria for autistic disorder. The physical examination at 5.5 years of age showed: height $97\,cm$ (<3rd centile), weight $15\,400\,kg$ (3rd centile) and CC $48.5\,cm$ (3rd centile). He presented with a wide-based gait, right hemiparesis, stereotypic hand movements and no social interest. Metabolic screening, lysosomal enzyme analysis, fragile X and Rett DNA testing, ocular and cardiac evaluations as well as PEV, PAE and EMG were normal. A brain MRI showed periventricular leukomalacia more prevalent in the left cerebral hemisphere and the EEG showed multifocal, parossistic and polymorphic anomalies, especially in the anterior cerebral area. The karyotype was 46,XY, t(5;8)(q14.3;q23.3) *de novo*.

Patient 2 (A.A.)
A 3.3-year-old Sardinian boy was born at term after an uncomplicated pregnancy. The mother was 22 years old at the time of delivery and the father was 51 years. Consanguinity of IV degrees was noted. His younger sister was healthy. Birth weight was $4200\,g$ (90th centile), length $52\,cm$ (90th centile), CC $36.5\,cm$ (75th centile). He showed early hypotonia and development delay. He walked at 16 months and presented with severe speech delay. At present, he does not talk and he has an IQ score within the moderate range of mental retardation. Autistic behaviour was recognised during childhood with complete avoidance of eye contact and no interest in social relations. He had a short attention span and did not exhibit imaginative play. He met the DSM-IV criteria for autistic disorder using CARS and ABC manuals for autism evaluation. At physical evaluation, at the age of 3.3 years, he was $99\,cm$ tall (50th centile) and weighed $17\,kg$ (75th centile) with a CC of $50\,cm$ (40th centile). He showed joint hypermobility, and high-arched palate but did not display dimorphic features. Metabolic screening, fragile X and Rett DNA testing, EEG, brain MRI scan, ocular and cardiac evaluations and PAE were normal. The karyotype was 46,XY,t(6;8)(q13;q23.2) *de novo*.

Mapping of the 8q23 translocation breakpoints
To localise the breakpoints of both translocations t(5;8) and t(6;8), first on chromosomes 8, we performed a systematic series of FISH analyses using BAC clones from the RPCI-11 human genomic BAC library mapping to the 8q23 region. We constructed a BAC contig map with ENSEMBLE and UCSC databases. The BAC clones RP11-536K17 and RP11-118J9 in the patient t(5;8) (Figure 1a and c) and the BAC clone RP11-946L20 in the patient t(6;8) (Figure 1b and c), respectively, spanned the chromosome 8 breakpoint. To identify a candidate gene mapping to the two common breakpoint regions, a genomic sequence analysis was performed by searching the NCBI, ENSEMBLE and UCSC databases.

In both patients, no known gene is interrupted by the translocation breakpoints on chromosomes 8. In patient 1, the breakpoint occurs $\sim 2.8\,Mb$ distal to the 5′ of the *CSMD3* gene and $\sim 0.5\,Mb$ distal to the 5′ of the *TRPS1* gene; in patient 2, the breakpoint is located $\sim 1.1\,Mb$ distal to the 3′ end of the *CSMD3* gene (Figure 1c). Both chromosomes 8 breakpoints are close to *CSMD3* (CUB and Sushi multiple domains 3), a gene spanning on $1.2\,Mb$ in 8q23 consisting of 73 exons.[22,23] This gene encodes a transmembrane protein composed of a long N-terminal polypeptide with CUB and Sushi multiple domains exposed outside the cell, a single transmembrane domain and a C-terminal domain of 55 amino-acid residues located in the cytoplasm. Northern blot hybridisation using human adult and fetal multiple-tissue blots and PCR amplification of different cDNA fragments using a
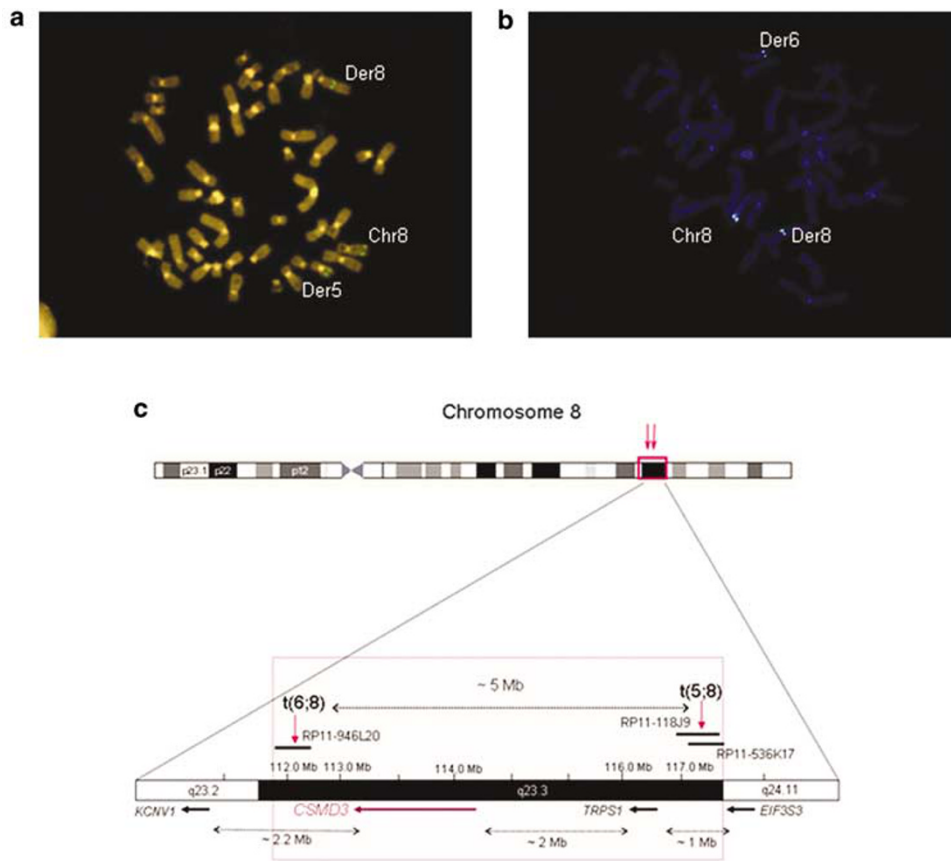
Figure 1 Mapping of the 8q breakpoint regions involved in the t(5;8) and t(6;8) translocations using chromosome 8 BAC clones. (**a**) Fluorescent *in situ* hybridisation (FISH) analysis with the clone RP11-536K17 on metaphase chromosomes of patient 1. Hybridisation signals (green dots) can be detected on normal chromosome 8 (chr 8) and the two derivate chromosomes der(5) and der(8). (**b**) FISH analysis with the clone RP11-946L20 on metaphase chromosomes of patient 2. Hybridisation signals (white dots) can be detected on the normal chromosome 8 (chr 8) and the two derivate chromosomes der(6) and der(8). (**c**) Physical map of the 8q23 region with corresponding genomic BAC clones used for FISH and map of the region surrounding the two breakpoints on chromosome 8. Arrows indicate the transcription orientation of the genes CSMD3 and TRPS1 relative to the centromere of chromosome 8 and the location of the breakpoints (red). The diagram is not drawn to scale.

multiple-tissue cDNA panel were performed by Shimizu et al[22] and the analyses indicated that the *CSMD3* gene is weakly expressed in most tissues, while it is expressed mainly in the adult and fetal brain and testis as a transcript of about 13 kb. Some shorter transcripts were also found in the testis and fetal brain. Any *CSMD3* transcript was detected in leukocytes.[22] To verify the expression of the gene on peripheral blood, we performed an RT-PCR on three control samples but never detected any transcript (data not shown). To exclude the possibility of an over-expression of *CSMD3* led by the rearrangements, we verified its expression in the leukocytes of translocated patient 2. No transcript was detected (data not shown). Comparative genomic analysis revealed the presence of a member belonging to the conserved family of *CSMD3* in the mouse and fugu genomes.

The other gene closer to the translocation breakpoint in patient 1 is *TRPS1* (trichorhinophalangeal syndrome I), a gene that encodes for a 1281 amino-acid-specific

zinc-finger protein, which is a putative transcription factor.[24,25] Mutations in this gene lead to autosomal dominant disorders characterised by craniofacial and skeletal anomalies – the trichorhinophalangeal syndromes (TRPS). Three variants of this syndrome have been described in the literature: TRPSI (MIM 190350) and TRPSII, also known as Langer–Giedion syndrome (MIM 150230) and TRPSIII (MIM 190351). The hallmarks found in all three types are sparse scalp hair, a bulbous tip of the nose, bushy eyebrows, a long flat philtrum, a thin upper vermilion border, protruding ears and short stature. Skeletal abnormalities include cone-shaped epiphyses at the phalanges, hip malformations and short stature. TRPSII is an example of contiguous gene syndromes: patients with TRPSII, or Langer–Giedion syndrome, have also multiple cartilaginous exostoses caused by mutations in the *EXT1* gene, which is mutated in a subgroup of patients with multiple cartilaginous exostoses and deleted in all patients with TRPS type II.

Analysis of the 8q23 breakpoints area by the Ensemble database DECIPHER (http://decipher.sanger.ac.uk/) and by the Autism chromosomal rearrangements database (http://projects.tcag.ca/autism/; update 18 May 2007) showed that there are no other chromosomal rearrangements or segmental duplications reported so far associated with ASDs.

Sebat *et al*[6] showed that copy number variations (CNVs) are more frequent in patients with ASDs than in unaffected individuals and more frequent in sporadic cases than in 'multiplex' families. We carried out a more detailed analysis of this region using the database of the Autism CNV Project (http://projects.tcag.ca/cgi-bin/naar10k/cnvHome). This database reports the results of a recent study on autism genetics performed using an analysis of CNV in families with ASDs (Autism Genome Project Consortium, 2007). This analysis showed the presence of 8 CNVs (all gain) associated with autism overlapping the 8q23 breakpoint region harbouring the *CSMD3* gene. We performed the same analysis using the database of genomic variants (http://projects.tcag.ca/variation) and ascertained that in the same region, there are 20 genomic variations reported in the normal population.

Furthermore, a detailed analysis of the 8q23 breakpoints area by the Ensemble database showed that noncoding RNAs are not located in this ∼5 Mb region and that, in addition, there are LINE and SINE transposones and recombination hot spot.
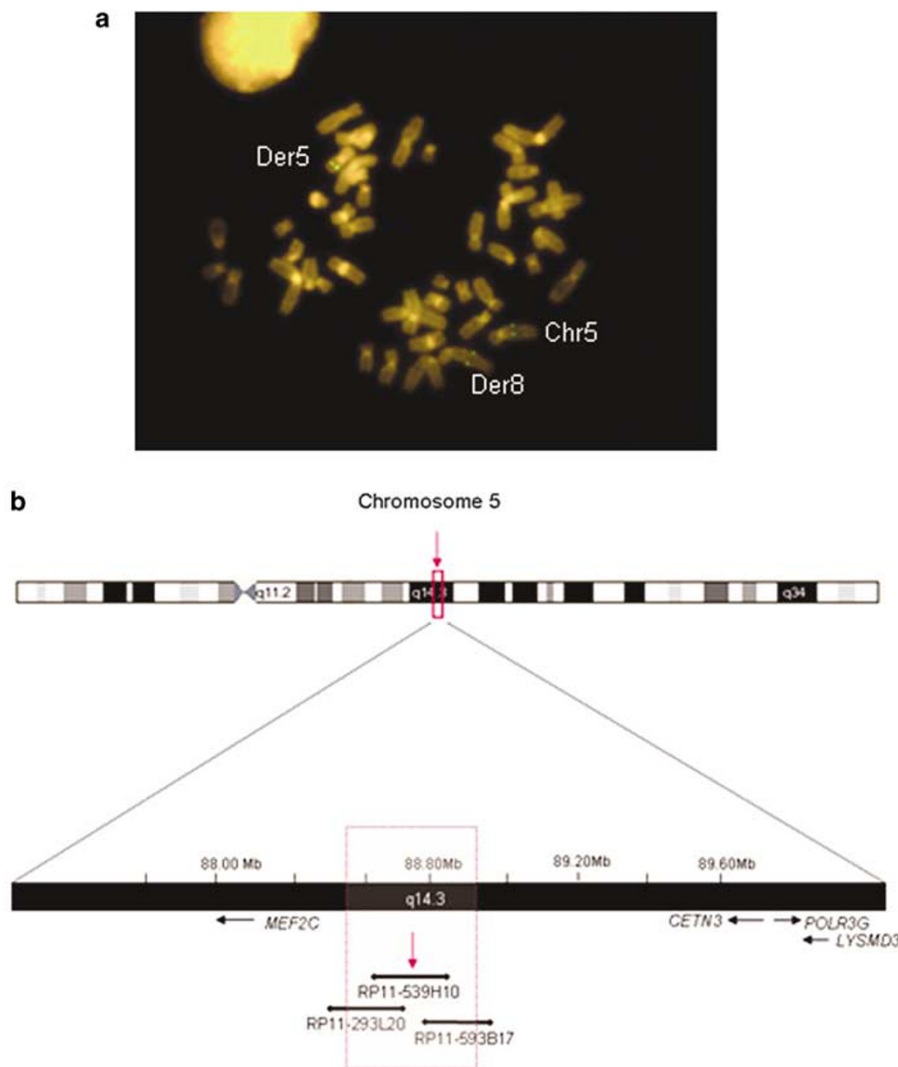

Figure 2 Mapping of the 5q breakpoint region involved in the t(5;8) translocation using chromosome 5 BAC clones. (**a**) FISH analysis with the clone RP11-539H10 on metaphase chromosomes. Hybridisation signals (green dots) can be detected on normal chromosome 5 (chr 5) and the two derivate chromosomes der(5) and der(8). (**b**) Physical map of the 5q14.3 region with corresponding genomic BAC clones used for FISH and map of the region surrounding the breakpoint on chromosome 5. Arrows indicate the transcription orientation of the genes relative to the centromere of chromosome 5 and the location of the BAC clones. The diagram is not drawn to scale.

Mapping of the 5q14.3 breakpoint

The breakpoint on chromosome 5 was also characterised by FISH experiments using clones from the RPCI-11 human genomic BAC library. The BAC clone RP11-539H10 spanned the chromosome 5 breakpoint (Figure 2a and b). A genomic sequence analysis was performed by searching the public databases: also, in this case, no known gene was interrupted by the chromosome rearrangement.

The gene found proximal to the translocation is *MEF2C* (myocyte enhancer factor 2C): this regulatory protein is, like the myogenic basic helix–loop–helix proteins, involved in myogenesis. The KO mouse for this gene showed that its transcript is an essential regulator of cardiac morphogenesis and right ventricular development.[26] Variants of *MEF2C* were found in the skeletal muscle and brain. Leifer *et al*[27] found that the brain form was expressed by neurons in particular layers of the cerebral cortex and that expression declined during post-natal development. The gene distal to the breakpoint is *CETN3* (centrin, EF-hand protein, 3): the protein encoded by this gene contains four EF-hand calcium-binding domains, and it is a member of the centrin protein family; this was highly enriched in the centrosome fraction of cell extracts and appears to represent the most abundant centrosomal isoform.[28]

In this chromosomal region, the analysis by DECIPHER showed that there are three other chromosomal rearrangements associated with mental retardation/developmental delay and some other minor features:

- 46, XY, del(5)(q14.3;q14.3);
- 46, XY, dup(5)(q13.3;q15).

Furthermore, the Ensemble database shows that non-coding RNAs are not located in this area. We carried out a more detailed analysis using the database of the Autism CNV Project that showed the presence of five CNVs overlapping this region between the *MEF2C* and the *CETN3* genes and we found out that there are three genomic variants also reported in the normal population (http://projects.tcag.ca/variation).

Mapping of the 6q13 breakpoint

Breakpoint mapping was carried out by FISH analysis with BAC probes from the chromosomal region 6q13. The BAC clones delimiting the chromosomes 6 breakpoint were RP11-678H04 and RP11-453A07. It was not possible to locate the fine position of the breakpoint because we no longer have the availability of the blood sample for patient 2. The genomic region included between the telomeric and centromeric BAC clones is ~0.7 Mb and there is no known gene interrupted by the translocation breakpoint (Figure 3).

The gene closer to the translocation is *CD109* (a GPI-linked cell-surface antigen)[29] and the distally located gene is *COL12A1* (collagen, type XII, α 1; this gene encodes the α chain of type XII collagen, a member of the FACIT – fibril-associated collagens with interrupted triple helices – collagen family).[30] Another gene next to the translocation breakpoint is *SLC17A5* (solute carrier family 17, member 5): mutations of this gene cause Salla disease (adult form of sialuria, also called sialic acid storage disease) characterised by progressive psychomotor retardation of early onset, lysosomal storage and increased urinary excretion of free sialic acid.[31] Analysis by using the Ensemble database
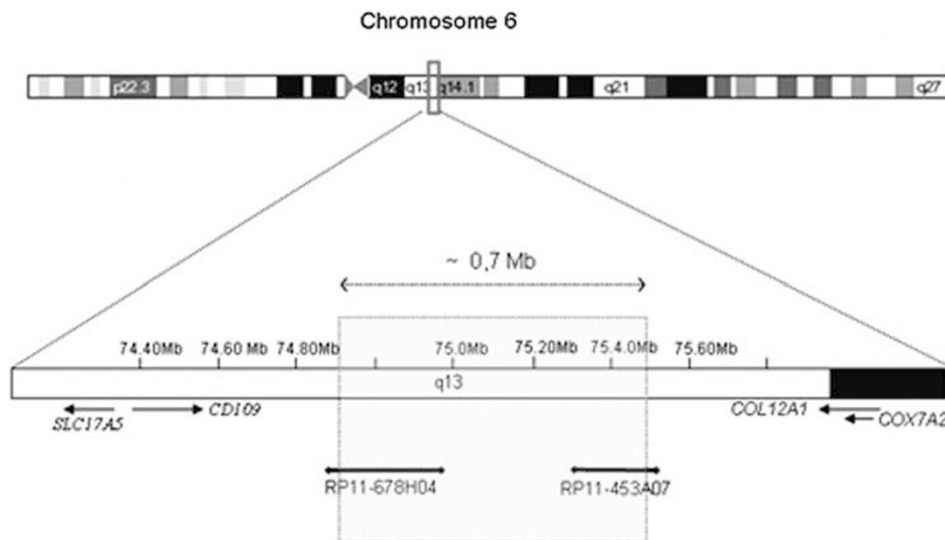

Figure 3 Physical map of the 6q13 region with corresponding genomic BAC clones used for FISH and map of the region surrounding the breakpoint on chromosome 6. Arrows indicate the transcription orientation of the genes relative to the centromere of chromosome 6 and the location of the 2 BAC clones. The diagram is not drawn to scale.

(DECIPHER) and the Autism Chromosome Rearrangement Database of this chromosomal region revealed other more chromosomal rearrangements characterised by mental retardation/developmental delay, autism and some other minor features:

- del(6)(q13;q14.1);
- del(6)(q13;q16.1);
- t(6;14)(q13;q21).

Detailed analysis of this 6q13 breakpoint region performed with both the database of the Autism CNV Project and the database of genomic variants showed the presence of 7 CNVs and 6 genomic variations, respectively. A detailed analysis of this area by the Ensemble database pointed out that noncoding RNAs are not located in this region.

Discussion

The study of chromosomal rearrangements associated with abnormal phenotypes has proven to be a powerful method for the identification of disease-related genes. Indeed, there are numerous examples of human disorders in which analysis of chromosomal abnormalities has subsequently led to the discovery of critical disease intervals and eventually to the identification of genes involved in the aetiology of a disease.[32–34] In this paper, we report two unrelated male patients with balanced reciprocal translocations t(5;8)(q13;q23.3) and t(6;8)(q13;q23.2), associated with ASDs and developmental delay. Both patients were reported to have a common breakage area in 8q23.

FISH analyses with BAC clones were carried out to determine precisely the position of each chromosomal breakpoint to identify candidate gene(s) implicated in the pathogenesis of autistic disorder. To find the candidate gene, a genomic sequence analysis was performed by searching the public databases (NCBI, ENSEMBL and UCSC). This analysis excluded the presence of known genes interrupted by these breakpoints and, moreover, showed that on chromosomes 8 both breakpoints are located in a 5 Mb genomic region between BAC RP11-946L20 and RP11-536K17, region that contains the *CSMD3* gene.

Although the hypothesis of a 'disease gene at breakpoint' would be the simplest one, more and more evidence is being accumulated to support the existence of different mechanism underlying disease with chromosomal rearrangements outside genes and their regulatory regions known as 'position effect'.[35,36] The mechanism that may cause such effects are: (1) separation of the gene from its enhancer or promoter region, (2) removal of the long-range insulator or boundary element, (3) competition with another enhancer, (4) juxtaposition with an enhancer element from another gene and (5) position–effect variegation, that is the insertion of the gene in a new heterochromatin environment.

Considering the positional effect, our observations suggest that *CSMD3* is a positional candidate gene for ASDs. The literature reports that this chromosomal region harbours a genetic locus for the benign adult familiar myoclonic epilepsy type 1 (BAFME1/GFAME; MIM 601068).[37] Mutational analysis of the *CSMD3* gene in patients with BAFME1/GFAME identified three single nucleotide changes that are not responsible for the pathogenesis of this syndrome.[22]

The expression of *CSMD3* on the fetal and adult brain suggests that this gene is a good candidate for the pathogenesis of ASDs. There is little information about the function of CUB – containing proteins, although it has been postulated that they would be mainly involved in the developmental process and that CUB and Sushi domains are thought to be sites of protein–protein or protein–ligand interactions, suggesting that CSMD3 proteins are either transmembrane receptors or adhesion proteins. A recent study shows that SEZ-6 containing both CUB and Sushi domains is involved in signalling and cell–cell adhesion. *SEZ-6* is strongly expressed in the developing forebrain and is necessary for the formation of the neural network.[23]

CSMD1 (CUB and Sushi multiple domain 1), the founding member of the gene family containing multiple CUB and Sushi multiple domain, has been cloned as a candidate tumour-suppressor or tumour-progression gene associated with head and neck squamous cell carcinomas and in prostate cancers. Richter *et al*[38] showed that promoter methylation of *CSMD1* might epigenetically silence the gene; particularly, they suggest that methylation of the DNA preceding *CSMD1*'s first exon is correlated with the downregulation of its level of expression in the squamous cell carcinoma cell lines; whereas in the normal tissue, there is little promoter methylation. In the light of these observations, we think that it would be interesting to also evaluate the methylation status for *CSMD3*.

These findings should encourage further investigations, including the mutational screening of *CSMD3* in a large group of patients with ASDs, to clarify an association with this pathology and its potential role in cognitive function.

The other gene closer to the breakpoint region on chromosomes 8 in patient 1 is *TRPS1*. Mutations of this gene lead to an autosomal dominant disorder, the trichorhinophalangeal syndromes (TRPS), characterised by craniofacial and skeletal anomalies,[24] which were not observed in our patients. Although the 8q23 region is the most likely location for an ASDs autism candidate gene for each of the cases described here, the possibility that the phenotype observed in the patients is a consequence of a gene defect occurring at the breakpoint on chromosomes 5 and 6, respectively, cannot be ruled out. It is generally

accepted that most neuropsychiatric development disorders like ASDs are polygenic or multifactorial; that is, several synergistically acting genes in an affected individual's genome may be required to produce the full autistic phenotype. Furthermore, brain development and complex behaviours are multidetermined, with genes turning cascades of proteins on or off, while they influence one another. A specific set of genetic polymorphisms may determine one's susceptibility to ASDs, yet, even then, environmental triggers may modify the phenotypic expression of the disorder. In these patients, we suppose that changes in the dosage of genes could have quantitative effects on gene functions, including haploinsufficiency or altered transcription patterns; instead, we cannot exclude the involvement of epigenetic regulatory mechanisms in the aetiology of the autism, as suggested in other cases.[7]

Our observations regarding the expression of *CSMD3* in fetal and adult brain suggest that this gene may be involved in the pathogenesis of ASDs. However, future studies, including mutational analysis of *CSMD3* in a large number of patients affected by autism and behavioural studies on a *Csmd3* knockout mouse, are necessary to confirm or reject this assumption.

Acknowledgements

References

1 Muhle R, Trentacoste SV, Rapin I: The genetics of autism. *Pediatrics* 2004; **113**: 472–486.
2 American Psychiatric Association: *DSM-IV – Diagnosis and Statistical Manual of Mental Disorders* 1994, (4th edn) American Psychiatric Publishing: Washington DC.
3 Ishikawa-Brush Y, Powell JF, Bolton P: Autism and multiple exostoses associated with an X;8 translocation occurring within the *GRPR* gene and 3′ to the SDC2 gene. *Hum Mol Genet* 1997; **6**: 1241–1250.
4 Beaudet AL: Autism: highly heritable but not inherited. *Nat Med* 2007; **13**: 534–536.
5 Vorstman J, Staal WG, van Daalen E, van Engeland H, Hochstenbach PFR, Franke L: Identification of novel autism candidate regions through analysis of reported cytogenetic abnormalities associated with autism. *Mol Psychiatry* 2006; **11**: 18–28.
6 Sebat J, Lakshmi B, Malhotra D *et al*: Strong association of *de novo* copy number mutations with autism. *Science* 2007; **316**: 445–449.
7 Schanen NC: Epigenetics of autism spectrum disorders. *Hum Mol Genet* 2006; **15**: 138–150.
8 Badcock C, Crespi B: Imbalanced genomic imprinting in brain development: an evolutionary basis for the aetiology of autism. *J Evol Biol* 2006; **19**: 1007–1032.
9 Jacquemont ML, Sanlaville D, Redon R *et al*: Array-based comparative genomic hybridisation identifies high frequency of cryptic chromosomal rearrangements in patients with syndromic autism spectrum disorders. *J Med Genet* 2006; **43**: 843–849.
10 Yonan AL, Alarcòn M, Cheng R *et al*: A genomewide screen of 345 families for autism-susceptibility loci. *Am J Hum Genet* 2003; **73**: 886–897.
11 Freitag CM: The genetics of autistic disorders and its clinical relevance: a review of the literature. *Mol Psychiatry* 2007; **12**: 2–22.
12 Herbert MR, Russo JP, Yang S *et al*: Autism and environmental genomics. *Neurotoxicology* 2006; **27**: 671–684.
13 Philippe A, Martinez M, Guilloud-Bataille M *et al*: Genome-wide scan for autism susceptibility genes. *Hum Mol Genet* 1999; **8**: 805–812.
14 Serajee FJ, Nabi R, Zhong H, Mahbubul Huq AH: Association of *INPP1*, *PIK3CG*, and *TSC2* gene variants with autistic disorder: implications for phosphatidylinositol signalling in autism. *J Med Genet* 2003; **40**: 119.
15 Gong X, Jia M, Ruan Y *et al*: Association between the *FOXP2* gene and autistic disorder in Chinese population. *Am J Med Genet B Neuropsychiatr Genet* 2004; **127**: 113–116.
16 McCauley JL, Olson LM, Delahanty R *et al*: A linkage disequilibrium map of the 1-Mb 15q12 *GABA(A)* receptor subunit cluster and association to autism. *Am J Med Genet B Neuropsychiatr Genet* 2004; **13**: 51–59.
17 Persico AM, Bourgeron T: Searching for ways out of the autism maze: genetic, epigenetic and environmental clues. *Trends Neurosci* 2006; **29**: 349–358.
18 Castermans D, Vermeesch JR, Fryns JP *et al*: Identification and characterization of the *TRIP8* and *REEP3* genes on chromosome 10q21.3 as novel candidate genes for autism. *Eur J Hum Genet* 2007; **15**: 422–431.
19 Castermans D, Wilquet V, Steyaert J, Van De Ven W, Fryns JP, Devriendt K: Chromosomal anomalies in individuals with autism. *Autism* 2004; **8**: 141–161.
20 Reddy KS: Cytogenetic abnormalities and fragile-X syndrome in Autism Spectrum Disorder. *BMC Med Genet* 2005; **6**: 1–16.
21 Ylisaukko-oja T, Alarcón M, Cantor RM, Auranen M, Vanhala R, Kempas E: Search for autism loci by combined analysis of Autism genetic resource exchange and Finnish families. *Ann Neurol* 2006; **59**: 145–155.
22 Shimizu A, Asakawa S, Sasaki T *et al*: A novel giant gene *CSMD3* encoding a protein with CUB and sushi multiple domains: a candidate gene for benign adult familial myoclonic epilepsy on human chromosome 8q23.3–q24.1. *Biochem Biophys Res Commun* 2003; **309**: 143–154.
23 Ling Lau W, Scholnick SB: Identification of two new members of the CSMD gene family. *Genomics* 2003; **82**: 412–415.
24 Momeni P, Glockner G, Schmidt O *et al*: Mutations in a new gene, encoding a zinc-finger protein, cause tricho-rhino-phalangeal syndrome type I. *Nat Genetic* 2000; **24**: 71–74.
25 Ludecke HJ, Wagner MJ, Nardmann J *et al*: Molecular dissection of a contiguous gene syndrome: localization of the genes involved in the Langer-Giedion syndrome. *Hum Mol Genet* 1995; **4**: 31–36.
26 Lin Q, Schwarz J, Bucana C, Olson EN: Control of mouse cardiac morphogenesis and myogenesis by transcription factor MEF2C. *Science* 1997; **276**: 1404–1407.
27 Leifer D, Krainc D, Yu YT *et al*: MEF2C, a MADS/MEF2-family transcription factor expressed in a laminar distribution in cerebral cortex. *Proc Natl Acad Sci USA* 1993; **90**: 1546–1550.
28 Middendorp S, Paoletti A, Schiebel E, Bornens M: Identification of a new mammalian centrin gene, more closely related to Saccharomyces cerevisiae CDC31 gene. *Proc Natl Acad Sci USA* 1997; **94**: 9141–9146.
29 Lin M, Sutherland DR, Horsfall W *et al*: Cell surface antigen *CD109* is a novel member of the alpha(2) macroglobulin/C3, C4, C5 family of thioester-containing proteins. *Blood* 2002; **99**: 1683–1691.
30 Gerecke DR, Olson PF, Koch M *et al*: Complete primary structure of two splice variants of collagen XII, and assignment of alpha 1(XII) collagen (*COL12A1*), alpha 1(IX) collagen (*COL9A1*), and alpha 1(XIX) collagen (*COL19A1*) to human chromosome 6q12-q13. *Genomics* 1997; **41**: 236–242.

31 Verheijen FW, Verbeek E, Aula N, Beerens CEMT, Havelaar AC, Joosse M: New gene, encoding an anion transporter, is mutated in sialic acid storage diseases. *Nat Genet* 1999; **23**: 462–465.

32 Crisponi L, Deiana M, Loi A *et al*: The putative forkhead transcription factor FOXL2 is mutated in blefarophimosis/ptosis/epicanthus inversus syndrome. *Nat genetics* 2001; **27**: 159–166.

33 Srivastava AK, Montoen O, Saarialho-Kere U *et al*: Fine mapping of the *EDA* gene: a traslocation breakpoint is associated with a CpG island that is transcribed. *Am J Hum Genet* 1996; **1**: 126–132.

34 Tadin-Strapps M, Warburton D, Salas-Alanis JC, Lopez-Cepeda LD, Christiano AM: Fishing for new genes in skin biology: impact of cytogenetics on gene discovery. *Clin Genet* 2004; **66**: 94–106.

35 Kleinjan DJ, van Heyningen V: Position effect in human genetic disease. *Hum Mol Genet* 1998; **7**: 1611–1618.

36 Velagaletti GVN, Bien-Willner GA, Northup JK *et al*: Position effects due to chromosome breakpoints that map ∼900 Kb upstream and ∼1.3 Mb downstream of SOX9 in two patients with campomelic dysplasia. *Am J Hum Genet* 2005; **76**: 652–662.

37 Mikami M, Yasuda T, Terao A: Localization of a gene for benign adult familiar myoclonic epilepsy to chromosome 8q23.3-q24.1. *Am J Hum Genet* 1999; **65**: 745–751.

38 Richter MT, Tong BD, Scholnick SB: Epigenetic inactivation and aberrant transcription of CSMD1 in squamous cell carcinoma cell lines. *Cancer Cell Int* 2005; **5**: 29.