FISH-mapping of translocation breakpoints associated with autism

M. Gilling1*, Z. Tümer1, I. Bache1, M. Bak1, E. Niebuhr1, M. Bugge1, R.M.J. Cotterill2, K. Brøndum-Nielsen3, U. Kristoffersson4, H-H. Ropers5, N. Tommerup1

1 Wilhelm Johannsen Centre for Functional Genome Research
2 Department of Molecular Genetics, Institute of Medical Biochemistry and Genomics, University of Copenhagen
3 The Technical University of Denmark
4 The J.F. Kennedy Institute, Glostrup, Denmark
5 The University Hospital of Lund, Sweden
6 Max Planck Institute of Molecular Genetics, Berlin, Germany
7 mette@medgen.ku.dk

Introduction

Autism (MIM 209980) is a Perseverative Development Disorder (PDD) considered to be neurodevelopmental in origin [1]. The phenotypic spectrum of autism is huge, which may be attributable to different etiologies or to a combination of factors, including genetic predisposition, teratogenic and environmental exposure. At present, no genetic, radiographic or metabolic marker for Autism has been identified. However the increased concordance rate in monozygotic (70%) vs. dizygotic (10%) twins [1] and the 50-75 times greater risk to siblings of probands (2-3%) compared to the general population prevalence (0.64%) indicates a strong genetic component in the etiology of Autism.

Genome scans have implied the existence of candidate loci in many chromosomal regions, including: 1p31.3; 2p12; 2q21; 2q23; 3p25.3; 3p25.1; 5p15.3; 5q13.1; 6q16.3; 7p15.2; 7q11.2; 7q13.3-36.2; 10p12; 10q22.3; 13q22.3; 15q12; 15q21.1; 16p13.12-13.2; 17p13.3; 18q21.1; 18q21.33; 19q13.12; 1q13.41; 22q11.21; 22q11.23; Xq11.33; Xq26.1, where 7q31-34 and 16p13.1-13.2 are the most consistent findings [1, 2, 3, 4].

Mendelian Cytogetic Network (MCN) is a collaborative study that aims at identifying disease-associated balanced chromosomal rearrangements. The MCN database (MCNdb) [http://www.mcndb.org] at present contains data on 51 patients with autism and a constitutional balanced chromosomal rearrangement. Approximately twenty of these patients have breakpoints coinciding with regions highlighted by the genome scans. Mapping of breakpoints of structural rearrangements by fluorescence in situ hybridization (FISH) has revealed genes associated with many other genetic disorders [5].

Materials and Methods

A: 46,XY, t(9;18)(p22;q21.1)pat, inv(10)(p11.2q21.2)mat

The above genotype was found in a Swedish twin-couple conceived by phenotypically normal parents. Both children were diagnosed with autism.

The breakpoint on chromosome 18q21 of these twins is of particular interest since genome scans have identified 18q21-33 as a region associated with autism [3, 4]. In addition, genome scans of both bipolar disorder and schizophrenia have shown an association to the same region of 18q, which strengthens the evidence that genes on chromosome 18q are important for normal psychiatric development [6, 7]. However, the finding of overlapping susceptibility regions for autism and schizophrenia could also indicate a diagnostic overlap between these disorders [8]. A BAC spanning the breakpoint on chromosome 18q21 in the Swedish twins has been identified. No gene has been disrupted by the translocation breakpoint on chromosome 18q, which is located proximal to the susceptibility region suggested by the genome scans. However, since the translocation can have caused changes in gene expression due to positional effect, this will be further investigated.

The chromosome 19 breakpoint was of no particular interest from the beginning as no studies so far indicate association between 9p and autism. However, for completeness the breakpoint has been mapped to one spanning BAC.

Even though the maternally inherited inversion 16 is probably a common polymorphism [9] and therefore may be of no interest in concern to autism, the inversion breakpoints are currently being FISH-mapped. This is done because the indicated breakpoints are close to the susceptibility regions on chromosome 10 that has been suggested by the genome scans: 1p21.2 [2] and 10p22.3 [10]. The breakpoints are by now narrowed down to a 1.7 Mb region on 10p and a 2 Mb region on 10q. Any disrupted genes will be further investigated in people diagnosed with autism.

B: 46,XX, t(5;18)(q34;q12.2)de novo

This female was diagnosed with Asperger syndrome. She is in many respects well functioning as she can speak, has near-normal intelligence and lives in a protected, shared home. She is severely myopic.

Even though the indicated breakpoint on chromosome 18q was proximal to the susceptibility region identified in genome scans, it nevertheless seemed interesting because chromosome 18, as mentioned above, has been associated with several psychiatric disorders [6, 7]. A deletion of approximately 3 Mb on chromosome 18q has been identified by FISH (Figure 1) and confirmed by high resolution CGH. Expression analysis is currently being performed to identify the most likely candidate gene(s) for autism, which will be further analysed in other patients with Asperger syndrome/autism.

The breakpoint on chromosome 5 was interesting because several neurotransmitter receptor genes are located at 5q. A BAC spanning the breakpoint on chromosome 5q has been identified (Figure 2), but no genes are disrupted.

C: 46,XY, t(4;16)(q24;p13.3)mat

This 18 years old man is diagnosed to be within the spectrum of autism disorders. Even though his cognitive functions are reduced, he is nevertheless able to speak. Although he is said to function poorly usually, supporting a relationship between the translocation and an autistic spectrum phenotype, the father too has difficulties in speech and reading.

The breakpoint on chromosome 16p13.3 is located very close to the autism susceptibility region identified in several genome scans: 16p13.3 - 16p13.2 [2]. As for chromosome 18, the genetic susceptibility region for autism that has been identified on chromosome 16 by genome scans, coincides with a susceptibility region for bipolar disorder [7]. Again, this could either suggest a common etiology for these psychiatric disorders or a diagnostic overlap. The mapping of the 16p13.3 breakpoint has shown that the breakpoint is outside, and distal to the autism-susceptibility region indicated by the genome scans, but the candidate genes identified at the 16p13.3 breakpoint will, nevertheless, be analysed in patients with autism.

The long arm of chromosome 4 has not been identified as a possible susceptibility region for autism, but it is currently being FISH-mapped for completeness. The breakpoint region is narrowed down to 1.2 Mb. If a likely candidate gene is disrupted it will be further analysed.

Figure 1. A chromosomal deletion is recognized as one fluorescent signal by FISH. An approximately 3 Mb deletion has been identified on chromosome 18 in patient B.

Figure 2. A BAC spanning a translocation breakpoint is recognized as three fluorescent signals by FISH. This FISH picture shows the BAC spanning the breakpoint on chromosome 5 of patient B.