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POSITIONAL CLONING OF A COMPLEX GENETIC ILLNESS: AUTISM

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ABSTRACT

Positional cloning is the approach of choice for the identification of genetic mutations underlying the pathological development of complex genetic diseases like autism. Such genes can be identified using carriers of a chromosomal rearrangement which goes along with the sequencing of novel candidate genes. We report here the positional cloning of a reciprocal translocation associated with an autistic phenotype. We mapped the translocation breakpoints by fluorescence in situ hybridization (FISH). The characterization of the chromosomal breakpoints helped us to identify new candidate regions containing some novel candidate genes.

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INTRODUCTION

Positional cloning is one of the most important molecular approaches to genetic disorders. Since its introduction in research practice in the early 1980s, it has been widely utilised for defining the molecular origin of genetic diseases and still represents a necessary research step in this area (Castermans *et al*., 2004). The term positional cloning identifies a series of molecular techniques (not a single one) that usually lead, during successive steps, to the definition of causative genes. Overall, the strategy of positional cloning starts with patient recruitment and chromosome collection. Both constitute critical steps to the overall process. A genetic analysis of the enrolled patients and their families is also performed. The identification and characterisation of chromosome abnormalities as translocations, deletions and duplications by classical cytogenetic methods or by the newly developed microarray-based comparative genomic hybridisation (array CGH) technique may define extensions and borders of the genomic regions involved (Shaffer and Bejjani, 2006). The step following the definition of a critical genomic region is the identification of candidate genes that is based on the analysis of available databases from genome browsers. Positional cloning culminates in the identification of the causative gene mutations and the definition of its functional role in the pathogenesis of the disorder, by the use of cell based or animal-based experiments (Fantes *et al*., 2008). These steps constitute the so-called inverse genetics, a term that indicates how genotype identification is followed by phenotype characterisation, in contrast to "forward genetics", in which the study of phenotype leads to genotype identification (Gajecka *et al*., 2006). Inverse genetics is gaining increasing popularity, thanks to the completion of genome sequencing, the improvement of databases, and the use of modern proteomic techniques that allow the detection of even very tiny differences in the proteomes of patients with respect to control samples. There is a long list of genetic diseases characterised, over years, by traditional positional

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cloning including the causative genes of schizophrenia (DISC1 gene) (Millar *et al*., 2000), of X linked mental retardation (NLGN genes) (Thomas *et al*., 1999) and cystic diseases (ADPKD1 gene) (Reeders *et al*., 1985).

MATERIALS AND METHODS

Cytogenetics and FISH analyses

Karyotypes were determined by analysis of G-banded chromosomes on the patient with the translocation and his parents. To map the two breakpoints, we used FISH on metaphase chromosomes of the patient. Bacterial Artificial Chromosomes (BAC) clones are circular DNA molecules which contain a host DNA used in sequencing and hybridizations strategies. BACs covering genomic regions on both chromosomes were selected according to the UCSC Genome Browser (www. genome.ucsc.edu), and provided by BACPAC Resource Centre (BPRC) (www. and Prof. Mariano Rocchi (University of Bari, Italy). BAC clones were biotinylated with biotin-11-dUTP (Sigma) by nick translation using the Bio Nick Labelling System (Invitrogen Life Technologies) according to the instructions of the manufacturers.

RESULTS

Cytogenetics and FISH analyses

Routine and high-resolution chromosome studies revealed a de novo translocation carried by the proband. The G-band pattern suggested a balanced translocation involving 7p22 and 16p11.2 and established the karyotype as 46, XY, t(7;16) (p22; p11).

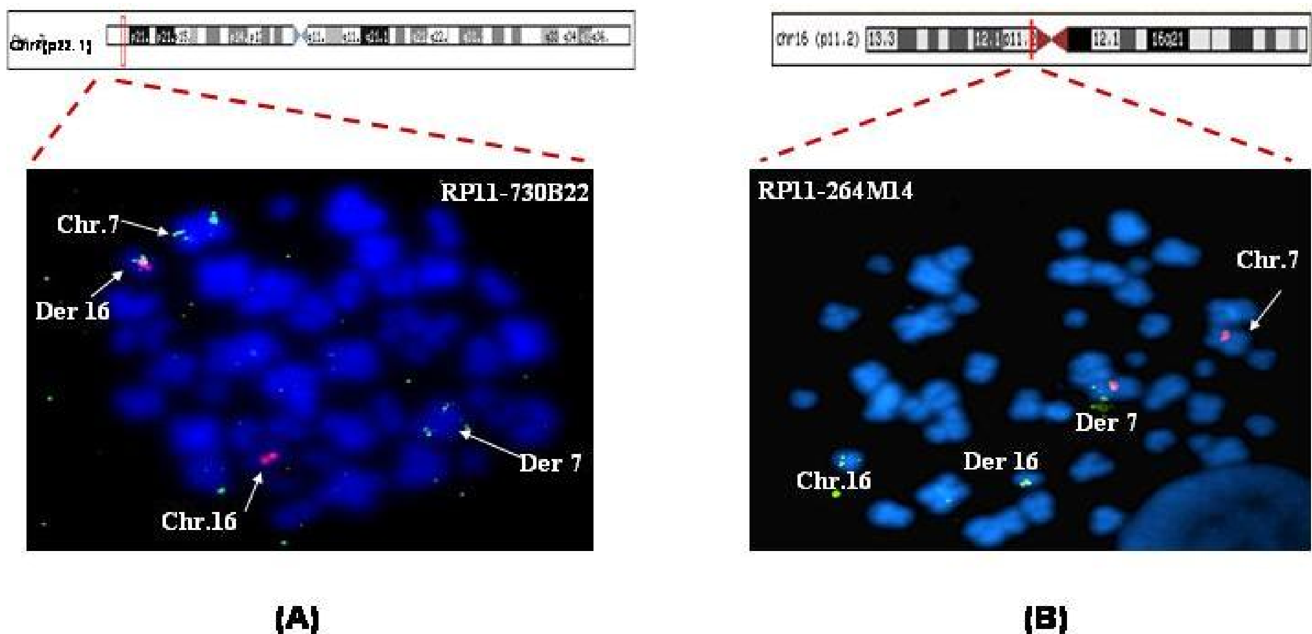
The parent karyotypes were normal. In order to fine map the two breakpoints, we performed FISH using BAC clones. After several rounds of hybridization, we identified two genomic clones spanning each breakpoint of the translocation. The BAC clone RP11-730B22 is specific of band 7p22.1 and spans the breakpoint on chromosome 7 (Fig. 1.a). BAC RP11-264M14 spans the chromosome 16 breakpoint in 16p11.2 (Fig. 1.b).

Genes in the vicinity of the breakpoints

According to the UCSC Genome Browser and Ensembl databases (www.ensembl.org), no known gene is localized in the genomic region corresponding to the BAC spanning the 16p11.2 breakpoint. We found a candidate predicted gene, called Q6NUR6, localized in the 7p22.1 breakpoint region but not interrupted by the breakpoint. The Q6NUR6 is predicted as an E3 ubiquitin ligase.

DISCUSSION

A major contribution to the identification of disease genes comes from studies in patients with chromosome anomalies that, when present, suggest a direct causative link with the disease (Veenstra-Vander Weele and Cook, 2004). Balanced translocations or inversions are of particular interest, since, in principle, they are not associated with loss or gain of DNA material. Therefore, the association of an “apparently” balanced translocation/inversion with an abnormal phenotype may indicate that the aberration is not really balanced and that a small quantity of DNA, undetectable by traditional cytogenetic analysis, has been lost during rearrangement. Alternatively, a balanced translocation may have interrupted the disease gene or separated it from an expression regulatory



(A) Physical map of genomic region 7p22.1 derived from the May 2006 version of the Ensembl Genome Browser (http://www.ensembl.org/Homo_sapiens/index.html).
FISH analysis with the BAC RP11-730B22 (green) located in 7p22.1 and a centromeric probe of chromosome 16 (red), shows that this BAC is spanning the breakpoint on chromosome 7.
(B) FISH analysis with the BAC RP11-264M14B22 (green) located in 16p11.2 and a telomeric probe of chromosome 7 (red), shows that this BAC is spanning the breakpoint on chromosome 7.

Fig.1. Physical mapping of the translocation breakpoints

region. In these cases, cloning of chromosome breakpoints leads to the identification of the disease-associated gene (Vorstman *et al*., 2006). The identification of the gene for polycystic kidney disease (PKD1) is a good example of this approach (Connors *et al*., 1997). In our study, the characterization of the chromosomal breakpoints helped us identify new candidate regions for autism (7p22.1 and 16p11.2). In the best of our knowledge this is the first report of autism with these specific chromosomal regions (Bayou *et al*., 2008).

The step that follows the definition of a critical region for a given disease is the identification of candidate genes. It is based on the analysis of available databases from genome browsers such as Ensembl (http://www.ensembl.org) or Santa Cruz Genome Browser UCSC (http://genome.ucsc.edu) that contain large numbers of data covering the whole genome but few areas that are still to be characterised. In such cases, alternative strategies should be designed. Databases contain information on gene maps and specific expression profiles and, in some cases, provide relevant hints on functions. For genes not yet cloned, or in the case of limited expression data in specific tissues, hypothetical transcripts are defined, as deduced from bio-informatic tools. Genome browsers provide a considerable number of data on expression and functions of paralogous and orthologous genes in different animal species, including data on mouse mutants that may be essential in the strategy (Matuszekand Talebizadeh, 2009). Once the list is available, it is necessary to rank genes endowed in the critical zone on the basis of expression and/or function consistent with disease development. One practical criterion is to consider those genes that are expressed in organs and tissues involved in the disease phenotype, either during adulthood or embryonic development (Stamova *et al*., 2009).

Conclusion

Positional cloning based on genome-wide mapping and candidate gene analysis will probably remain the method of choice for defining genetic traits in the near future. Among basic requirements that have to be fulfilled prior to the start of the molecular approach, the availability of informative families with clear clinical phenotypes still remains a challenge for researchers. Owing to technology advancements, an increasing number of molecular tools, such as adequate DNA markers, information on gene sequences and expression profiles and functions, will allow the extension of positional cloning to those conditions still lacking molecular characterisation. Multifactorial diseases represent the new frontier that has to be rapidly faced. The definition of regulatory mechanisms for gene expression (transcriptome), a better knowledge of protein structure and functions (proteome) and new animal models of the disease are essential topics for future studies.

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