Subtelomeric region of chromosome 2 in patients with autism spectrum disorders

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ABSTRACT. Autism spectrum disorders are severe psychiatric diseases commonly identified in the population. They are diagnosed during childhood and the etiology has been much debated due to their variations and complexity. Onset is early and characterized as communication and social interaction disorders and as repetitive and stereotyped behavior. Austistic disorders may occur together with various genetic and chromosomal diseases. Several chromosomal regions and genes are implicated in the predisposition for these diseases, in particular those with products expressed in the central nervous system. There are reports of autistic and mentally handicapped patients with submicroscopic subtelomeric alterations at the distal end of the long arm of chromosome 2. Additionally, there is evidence that alterations at 2q37 cause brain malformations that result in the autistic phenotype. These alterations are very small and not identified by routine cytogenetics to which patients are normally submitted, which may result in an underestimation of the diagnosis. This study aimed at evaluating the 2q37 region in patients with autistic disorders. Twenty patients were studied utilizing the
fluorescence in situ hybridization technique with a specific probe for 2q37. All of them were also studied by the GTC banding technique to identify possible chromosomal diseases. No alterations were observed in the 2q37 region of the individuals studied, and no patient presented chromosomal diseases. This result may be due to the small sample size analyzed. The introduction of routine analysis of the 2q37 region for patients with autistic disorders depends on further studies.

Key words: Autism spectrum disorders; Chromosome 2; Fluorescence in situ hybridization; FISH technique

INTRODUCTION

Due to the difficulty in establishing diagnostic boundaries between diseases considered to be invasive development disorders (CID-10; OMS, 1993), such as Asperger’s syndrome, non-specified pervasive development disorders and autism, they are referred to as autistic spectrum disorders (ASDs; CDC, 2006). These are severe psychiatric diseases which are characterized by multiple neurological manifestations that have a heterogeneous etiology which is difficult to elucidate and poorly understood (Bailey et al., 1996; Steiner et al., 2003). These diseases are present at birth, become apparent before 30 months of life and are characterized by abnormal responses to audible and visual stimuli as well as lack of or underdeveloped speech. Individuals have severe problems in social relationships, ritualistic behavior associated with abnormal routines and resistance to change, reduced capacity for abstract and symbolic thoughts or for imaginative games, with intelligence being subnormal, normal or above normal (ABRA, 1994). Most cases (75%) have mental deficiency (Rutgers et al., 2004), 15 to 40% convulsions and 20 to 25% electroencephalographic alterations (Smalley, 1997; Gabis et al., 2005). However, with the exception of Rett syndrome which shows a genetic etiology and in many cases new mutations of the MECP2 gene mapped on Xq28 (Tejada, 2006), the etiology of other pervasive development disorders remains unclear.

ASDs are very complex with participation of genetic factors in their etiology and, in many cases, the influence of environmental factors. The prevalence is estimated at 1-4 cases for every 1000 children with a male to female ratio of 2-4. These numbers have been increasing since the introduction of molecular methods of diagnostic testing and the establishment of more adequate strategies for clinical evaluation (APA, 1994; Smalley, 1997; Gillberg and Wing, 1999; Bertrand et al., 2001). According to some authors, these diseases seem to be more frequent with estimates of up to 1:500 live newborns (Levy et al., 2003; Castermans et al., 2004).

Genetic investigations are essential to understand the etiology and physiopathology of the disease, considered the consequence of some and cause of others (Veenstra-Vanderweele et al., 2004). Concordance in homozygotic twins is 95.7% and the probability of family recurrence is 75 times greater than in the general population, which implicates a risk of recurrence in cases not associated with a specific etiology estimated at 2.9% (Ritvo et al., 1985; Hallmayer et al., 2002). Additionally, ASDs, especially autism, which has been the most studied, have also been associated with chromosomal anomalies and with several genetic diseases, in particular with fragile X syndrome (Challman et al., 2003; Muhle et al., 2004; Polleux and Lauder, 2004; Cohen et al., 2005).
Everything suggests that predisposition to autism involves allelic heterogeneity and heterogeneity of the locus, which may also explain the endophenotypic variability, that is, the measurable components of the disease that are important elements in diagnosis (Gottesman and Gould, 2003; Veenstra-Vanderweele et al., 2004).

Despite the numerous studies, few candidate genes have been identified with certain involvement in the etiopathogenesis of ASDs (Jamain et al., 2003; Hebert et al., 2006). Some potential genes for predisposition are mapped to 3q, 4p, 7q, 13q, 15q, 16p, and chromosome X. Other possible loci that have been suggested are: 2q31, 2q37, 5p15, 11q25, 16q22.3, 17p11.2, 18q21.1, 22q11.2, 22q13.3, and Xp22.2-p22.3 (Junaid and Pullarkat, 2001; Suresh et al., 2006; Vostman et al., 2006).

Many of these genes seem to confer from minor physical malformations to malformations of the central nervous system, frequently resulting in genetic anticipation due to the broad phenotype highlighted by many authors (Veenstra-Vanderweele et al., 2004; Bartlett et al., 2005; Philippi et al., 2005; Sutcliffe et al., 2005; Bishop et al., 2006; Trikalinos et al., 2006; OMIM, 2007).

Interestingly, submicroscopic subtelomeric alterations have been reported in autism. The distal end of the long arm of chromosome 2 is one of the regions that is possibly altered in some cases (Wolff et al., 2002; Lukusa et al., 2005; Segurado et al., 2005). Alterations at the 2q terminal have also been described in patients who have mental deficiency and major and minor malformations but are considered to be karyotypically normal using conventional cytogenetic techniques, with these alterations only being detected using fluorescent in situ hybridization (FISH) (Riegel and Schinzel, 2002). Detection of these alterations may, apart from contributing to the understanding of biological mechanisms involved in ASDs, help genetic counseling of the families.

The aim of this study was to investigate the subtelomeric region of the long arm of chromosome 2 (2q37) in patients with ASDs.

**PATIENTS AND METHODS**

After approval of the Ethics Committee, written informed consent was obtained from the 20 individuals with ASDs according to the criteria of the DSM-IV. These subjects attend the Municipal School of Autism in São José do Rio Preto “Maria Lúcia de Oliveira”, AMA in Ribeirão Preto and other specialized schools and clinics. Among the participants, 12 were autistic, two had been diagnosed with Asperger’s syndrome and six with non-specified invasive developmental disorder. The diagnoses were made by psychiatrists and psychologists, members of multidisciplinary teams and specialists in ASDs. Patients who presented comorbidities with genetic features or probable environmental factors involved in the etiology of their disease were not enrolled in this study.

Individuals without prior karyotypic investigations were assessed using the GTG banding technique (Grouchy and Turleau, 1977) to test for chromosomal diseases. All participants were submitted to a genetic-clinical investigation and were photographed.

Metaphases for study were obtained from lymphocytes from 5 mL peripheral blood samples cultured for 72 h according to the standard protocol utilized by the Genetics Laboratory of the Medicine School in São José do Rio Preto, FAMERP (Moorhead et al., 1960).

The FISH technique was performed using a specific probe for 2q37 and for the centromeric region of chromosome 2 (control identification). Denaturation of the chromosomes,
preparation of the hybridization mixture and post-hydridization rinsing were performed according to the guidelines for use of the probes (Vysis). All experiments were performed utilizing a slide to control the efficiency of hybridization (of lymphocytes from normal individuals) and three or more slides of the study cases.

Analyses were carried out by two researchers who independently analyzed around ten metaphases of each case using an Olympus BX60 fluorescent microscope with filters for DAPI, FITC, rhodamine and a triple filter with an HBO 100 W light source. The criteria for analysis and description of the results were those reported by Harris et al. (1995) and Eastmond et al. (1995) and contained in ISCN (Schaffer and Tommerup, 2005).

RESULTS

No alterations of the 2q37 region were observed in this series. None of the individuals presented chromosomal diseases.

Figure 1 demonstrates a metaphase submitted to the FISH technique, which shows the normal expected result, that is, two centromeric signals which identify chromosome 2 and two subtelomeric signals that specifically identify the 2q37 region.

Figure 1. Metaphase submitted to the fluorescence in situ hybridization technique demonstrating the four normal signals, two centromeric (large arrows) and two subtelomeric (small arrows), of the 2q37 region.

DISCUSSION

Some individuals with autism have been described with alterations at 2q37, which implicates 2q37 as a region of interest for susceptibility to ASDs, with possible candidate genes, in particular CENTG2 (centaurin gamma 2 gene) (Vorstman et al., 2006; Zafeiriou et al., 2007).
In a pilot study, Wolff et al. (2002) studied ten autistic children and ten controls evaluating the subtelomeric regions of all chromosomes using a multiprobe system. Among the participants, the only alteration detected was the deletion at 2q37 in one of the patients. Alterations in this region have been observed by other authors in patients with ASD and dysmorphic clinical signs (Ghaziuddin and Burmeister, 1999; Smith et al., 2001; Lukusa et al., 2004; Sherr et al., 2005; Wassink et al., 2005).

These findings suggest that in 2q there are genes involved in the etiology of ASDs and that some patients may have submicroscopic subtelomeric alterations that explain the cause of the disorder (Riegel and Schinzel, 2002; Sherr et al., 2005). The alterations described at 2q37 are very slight and may be underestimated in the ASD population, as they cannot be detected by cytogenetic analysis using routine GTG banding, to which the patients are generally submitted in their clinical genetic evaluation. Thus, studies that utilize the FISH technique may be more enlightening.

In our study, however, of the 20 patients assessed by the most adequate technique to investigate 2q37, none showed alterations. This result may have been due to the number of patients analyzed. A possible proposal to introduce the analysis of the 2q37 region in the protocol of genetic investigation of patients with ASDs depends on further studies with larger sample sizes.

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REFERENCES


Steiner CE, Guerreiro MM and Marques-de-Faria AP (2003). Genetic and neurological evaluation in a sample of


Riegel M and Schinzel A (2002). Duplication of (2)(q11.1-q13.2) in a boy with mental retardation and cleft lip and palate:


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