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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; Vorstman 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 hydridization (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.

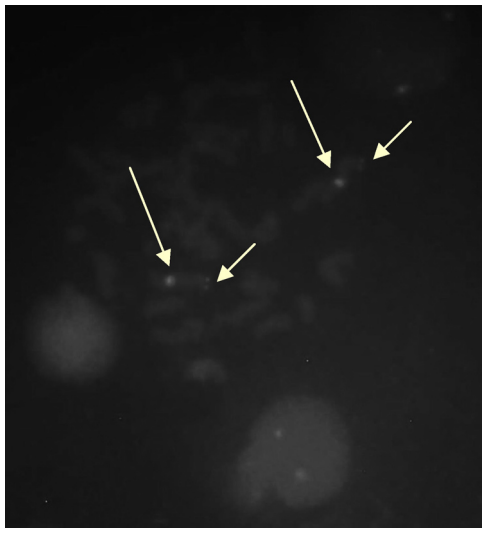

Figure 1. Metaphase submitted to the fluorescence *in situ* hybridization technique demonstrating the four normal signals, two centromeric (large arrows) and two subtelomeric (small arrows), of the 2q37 region.

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 routing 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

ABRA (Associação Brasileira de Autismo) (1994). Política de Atenção à Pessoa Portadora da Síndrome. ABRA, Brasília.

APA (American Psychiatric Association) (1994). Diagnostic and statistical manual of mental disorders (DSM-IV). American Psychiatric Press, Washington DC.

Bailey A, Phillips W and Rutter M (1996). Autism: towards an integration of clinical, genetic, neuropsychological, and neurobiological perspectives. *J. Child Psychol. Psychiatry* 37: 89-126.

Bartlett CW, Gharani N, Millonig JH and Brzustowicz LM (2005). Three autism candidate genes: a synthesis of human genetic analysis with other disciplines. *Int. J. Dev. Neurosci.* 23: 221-234.

Bertrand J, Mars A, Boyle C, Bove F, et al. (2001). Prevalence of autism in a United States population: the Brick Township, New Jersey, investigation. *Pediatrics* 108: 1155-1161.

Bishop DV, Maybery M, Wong D, Maley A, et al. (2006). Characteristics of the broader phenotype in autism: a study of siblings using the children's communication checklist-2. *Am. J. Med. Genet. B. Neuropsychiatr. Genet.* 141: 117-122.

Castermans D, Wilquet V, Steyaert J, Van de Ven W, et al. (2004). Chromosomal anomalies in individuals with autism: a strategy towards the identification of genes involved in autism. *Autism* 8: 141-161.

CDC (Centers for Disease Control and Prevention) (2006). Mental health in the United States: Parental report of diagnosed autism in children aged 4-17 years - United States, 2003-2004. *MMWR Morb. Mortal. Wkly. Rep.* 55: 481-486.

Challman TD, Barbaresi WJ, Katusic SK and Weaver A (2003). The yield of the medical evaluation of children with pervasive developmental disorders. *J. Autism Dev. Disord.* 33: 187-192.

Cohen D, Pichard N, Tordjman S, Baumann C, et al. (2005). Specific genetic disorders and autism: clinical contribution towards their identification. *J. Autism Dev. Disord.* 35: 103-116.

Eastmond DA, Schuler M and Rupa DS (1995). Advantages and limitations of using fluorescence *in situ* hybridization for the detection of aneuploidy in interphase human cells. *Mutat. Res.* 348: 153-162.

Gabis L, Pomeroy J and Andriola MR (2005). Autism and epilepsy: cause, consequence, comorbidity, or coincidence?

Epilepsy Behav. 7: 652-656.
Ghaziuddin M and Burmeister M (1999). Deletion of chromosome 2q37 and autism: a distinct subtype? *J. Autism Dev. Disord.* 29: 259-263.
Gillberg C and Wing L (1999). Autism: not an extremely rare disorder. *Acta Psychiatr. Scand.* 99: 399-406.
Gottesman II and Gould TD (2003). The endophenotype concept in psychiatry: etymology and strategic intentions. *Am. J. Psychiatry* 160: 636-645.
Grouchy J and Turleau C (1977). Atlas des Maladies Chromosomiques. Expansion Scientifique Française, Paris.
Hallmayer J, Glasson EJ, Bower C, Petterson B, et al. (2002). On the twin risk in autism. *Am. J. Hum. Genet.* 71: 941-946.
Harris C, Robinson T, Michalski K and Meisner L (1995). Specimen salvage with FISH. *App. Cytogenet.* 21: 66-70.
Herbert MR, Russo JP, Yang S, Roohi J, et al. (2006). Autism and environmental genomics. *Neurotoxicology* 27: 671-684.
Jamain S, Quach H, Betancur C, Rastam M, et al. (2003). Mutations of the X-linked genes encoding neuroligins NLGN3 and NLGN4 are associated with autism. *Nat. Genet.* 34: 27-29.
Junaid MA and Pullarkat RK (2001). Proteomic approach for the elucidation of biological defects in autism. *J. Autism Dev. Disord.* 31: 557-560.
Levy SE, Souders MC, Wray J, Jawad AF, et al. (2003). Children with autistic spectrum disorders. I: Comparison of placebo and single dose of human synthetic secretin. *Arch. Dis. Child* 88: 731-736.
Lukusa T, Vermeesch JR, Holvoet M, Fryns JP, et al. (2004). Deletion 2q37.3 and autism: molecular cytogenetic mapping of the candidate region for autistic disorder. *Genet. Couns.* 15: 293-301.
Lukusa T, Smeets E, Vogels A, Vermeesch JR, et al. (2005). Terminal 2q37 deletion and autistic behaviour. *Genet. Couns.* 16: 179-180.
Moorhead PS, Nowell PC, Mellman WJ, Battips DM, et al. (1960). Chromosome preparations of leukocytes cultured from human peripheral blood. *Exp. Cell Res.* 20: 613-616.
Muhle R, Trentacoste SV and Rapin I (2004). The genetics of autism. *Pediatrics* 113: e472-e486.
OMIM 209850 (Online Mendelian Inheritance in Man) (2007). http:www.ncbi.nlm.nih.gov.
OMS (Organização Mundial da Saúde) (1993). Classificação de transtornos mentais e de comportamento da CID-10: descrições clínicas e diretrizes diagnósticas. ARTMED, Porto Alegre.
Philippi A, Roschmann E, Tores F, Lindenbaum P, et al. (2005). Haplotypes in the gene encoding protein kinase c-beta (PRKCB1) on chromosome 16 are associated with autism. *Mol. Psychiatry* 10: 950-960.
Polleux F and Lauder JM (2004). Toward a developmental neurobiology of autism. *Ment. Retard. Dev. Disabil. Res. Rev.* 10: 303-317.
Riegel M and Schinzel A (2002). Duplication of (2)(q11.1-q13.2) in a boy with mental retardation and cleft lip and palate: another clefting gene locus on proximal 2q? *Am. J. Med. Genet.* 111: 76-80.
Ritvo ER, Freeman BJ, Mason-Brothers A, Mo A, et al. (1985). Concordance for the syndrome of autism in 40 pairs of afflicted twins. *Am. J. Psychiatry* 142: 74-77.
Rutgers AH, Bakermans-Kranenburg MJ, van Ijzendoorn MH and van Berckelaer-Onnes IA (2004). Autism and attachment: a meta-analytic review. *J. Child Psychol. Psychiatry* 45: 1123-1134.
Schaffer LG and Tommerup N (2005). An International System for Human Cytogenetic Nomenclature. S. Karger, Basel.
Segurado R, Conroy J, Meally E, Fitzgerald M, et al. (2005). Confirmation of association between autism and the mitochondrial aspartate/glutamate carrier SLC25A12 gene on chromosome 2q31. *Am. J. Psychiatry* 162: 2182-2184.
Sherr EH, Owen R, Albertson DG, Pinkel D, et al. (2005). Genomic microarray analysis identifies candidate loci in patients with corpus callosum anomalies. *Neurology* 65: 1496-1498.
Smalley SL (1997). Genetic influences in childhood-onset psychiatric disorders: autism and attention-deficit/hyperactivity disorder. *Am. J. Hum. Genet.* 60: 1276-1282.
Smith M, Escamilla JR, Filipek P, Bocian ME, et al. (2001). Molecular genetic delineation of 2q37.3 deletion in autism and osteodystrophy: report of a case and of new markers for deletion screening by PCR. *Cytogenet. Cell Genet.* 94: 15-22.
Steiner CE, Guerreiro MM and Marques-de-Faria AP (2003). Genetic and neurological evaluation in a sample of individuals with pervasive developmental disorders. *Arq. Neuropsiquiatr.* 61: 176-180.
Suresh R, Ambrose N, Roe C, Pluzhnikov A, et al. (2006). New complexities in the genetics of stuttering: significant sex-specific linkage signals. *Am. J. Hum. Genet.* 78: 554-563.
Sutcliffe JS, Delahanty RJ, Prasad HC, McCauley JL, et al. (2005). Allelic heterogeneity at the serotonin transporter locus (SLC6A4) confers susceptibility to autism and rigid-compulsive behaviors. *Am. J. Hum. Genet.* 77: 265-279.
Tejada MI (2006). Rett syndrome: a diagnostic, clinical and molecular update. *Rev. Neurol.* 42 (Suppl. 1): S55-S59.
Trikalinos TA, Karvouni A, Zintzaras E, Ylisaukko-oja T, et al. (2006). A heterogeneity-based genome search meta-analysis for autism-spectrum disorders. *Mol. Psychiatry* 11: 29-36.

Veenstra-Vanderweele J, Christian SL and Cook EH Jr (2004). Autism as a paradigmatic complex genetic disorder. *Annu. Rev. Genomics Hum. Genet.* 5: 379-405.

Vorstman JA, Staal WG, van Daalen E, van Engeland H, et al. (2006). Identification of novel autism candidate regions through analysis of reported cytogenetic abnormalities associated with autism. *Mol. Psychiatry* 11: I-18- I-28.

Wassink TH, Piven J, Vieland VJ, Jenkins L, et al. (2005). Evaluation of the chromosome 2q37.3 gene CENTG2 as an autism susceptibility gene. *Am. J. Med. Genet. B. Neuropsychiatr. Genet.* 136: 36-44.

Wolff DJ, Clifton K, Karr C and Charles J (2002). Pilot assessment of the subtelomeric regions of children with autism: detection of a 2q deletion. *Genet. Med.* 4: 10-14.

Zafeiriou DI, Ververi A and Vargiami E (2007). Childhood autism and associated comorbidities. *Brain Dev.* 29: 257-272.

