Numerical and structural chromosomal changes in a case of autism

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Abstract

Of all the chronic mental disorders the cause of autism is the least understood. Structural and numerical chromosomal aberrations have been seen in many patients with autism. Our paper is meant to present the results of cytogenetic and genealogical investigations in an autism case. ***Material and methods.*** *Cytogenetic analysis has been performed by standard karyotype and kinetochores were emphasized by modification of fixation stage of chromosomes preparation after the method described by Rooney and Czepulkowski. The information acquired from subject's parents and brother underlie of pedigree construction.* ***Results.*** *Cytogenetic analysis revealed normal and aberrant karyotypes (76% vs. 24%) with affecting chromosomes 2, 3, 14, 15, 17, 20, X and Y. We identified trisomy 3, and gonosomal trisomy (XXY), tetrasomy 14 and 15, isochromosome 2q, translocation (17;20) and duplication Yq. Treatment of cells in cultures with bleomicin led to a higher frequency of chromosomal aberrations than in case of patient's parents and brother. Kinetochores analysis exhibits the uni- and bilateral lack of kinetochores on some big and middle chromosomes. Study of pedigree showed that the autism has a multifactor heredity.* ***Conclusions.*** *Reported aneuploidy can be caused by centromere dysfunction raised from mutations of genes involved in structural and functional control of centromeres.*

Keywords: autism, aneuploidies, chromosomal rearrangements, centromere disfunction

Introduction

Autism is a dysfunction of the brain that appears during the first three years of life and continues in the adulthood. It has a prevalence index of 2: 10.000 individuals, and the ratio between men and women affected by it is 4:1 [5]. Autism affects three important areas of development: communication, social relations and behavior.

Of all the chronic mental disorders the cause of autism is the least understood. The identification of its genetic and environment factors is a high priority in this field. The cytogenetic studies made on autistic patients showed aberrations in the number and the structure of many chromosomes, including sex chromosomes [4]. The incidence of the chromosomal aberrations in autistic patients has an average of 5-10 % [12].

The inheritance of autism is also poor understood. The study of autism in monozygotic twins shows that there is no 100% correspondence between them, and thus suggesting an interaction of the genetic factors with the environmental factors [3].

In this paper we present the results of the cytogenetic and genealogical investigations in an autism case.

Material and methods

A. The presentation of the case. The subject studied, being 16 years old, male Arabian origin, showed an atypical behaviour. The autism was diagnosed in this origin country. Regarding The communication, the subject manifested a deviation of the expressive and perceptive language, stammering, and the IQ was 64, indicating a mild mental retardation.

B. Cytogenetic analysis. Lymphocytes cultures were performed and grown for 72 hours in a RMPI-1640 medium supplemented with 15% fetal calf serum and with 1% penicillin and streptomycin. The lymphocytes were stimulated to divide with 1% PHA. The chromosomal slides were obtained according to standard method and were stained by G-banding. 30 metaphases were examined, of which 10 were karyotyped.

C. The visualization of the kinetochores. The kinetochores were emphasized using the same technique of chromosomal preparation, but the fixation step was modified. It was made in three steps: the first fixation contained

methanol and acetic acid in the ratio 9:1. A second fixation reduced proportions of methanol and acetic acid to 5:1 and a third fixation consisted of 3:1 methanol – acetic acid. Slides were aged for 1 week at room temperature. The Cd banding (pairs of dots at the centromeres) was induced by incubating the slides in Earle saline solution (pH 8.5 – 9.0) at 85ºC for 10 minutes [7].

D. The treatment of cells cultures with bleomicine. The bleomicine, a well known clastogen, was administered to leucocytes cultures for 48 hours in a final concentation of 50 mcg/mL.

E. The genealogical study. Construction of pedigree was made according to information received from proband's parents and brother.

Results

Cytogenetic investigations were made to the subject and his parents and brother. The parents and the brother showed normal karyotypes. In contrast, the cytogenetic analysis of the subject points out a mosaicism of several cell lines: 46,XY/ 47,XY,+3/ 48,XY,+15,+15/ 47,XY,+14/47,XXY.

The normal chromosomal complement represented 76%. The trisomy 3 was present in 8%, the tetrasomy 15 appeared in 6% (figure 1) and the trisomy 14 was seen in 4% of the examined metaphases. 6% of the metaphases showed both numerical and structural aberrations. Among the chromosomal structure aberrations we identified the 2q isochromosome together with the trisomy 3 and the sex chromosomes trisomy (figure 2), but also duplications of the chromosome Y and translocation (17;20) were seen (figure 3). The secondary constrictions of the chromosomes 9 were diminished in all the examined metaphases (9qh-). The significance of this reduction is not obviously understood, although there is much works that reported this feature.

Exposing the cells to bleomicine, a well known clastogen, in a final concentration of 100 μg/ml led to the increasing of the chromosomal aberrations, reaching a value of 68% in 48 hours of treatment in the subject's case, and a value of 15-18 in the case of the subject's parents and brother. The most frequent aberrations were related to the big chromosomes (2, 3 and 5) and medium chromosomes (8, 9 and 15). The increased frequency of the induced chromosomal aberrations evidently suggests a deficiency of the enzymes involved in the repair processes of the lesions induced by bleomicine.

The genealogical analysis shows that two members of the mother line (8-II and 14-111) and one of the father line (4-III) presented behaviour disorders. This observation provides that autism has a complex inheritance, several genetic mutations being involved in the process. (figure 4). But the environmental factor which is not well understood in this case must not be excluded.

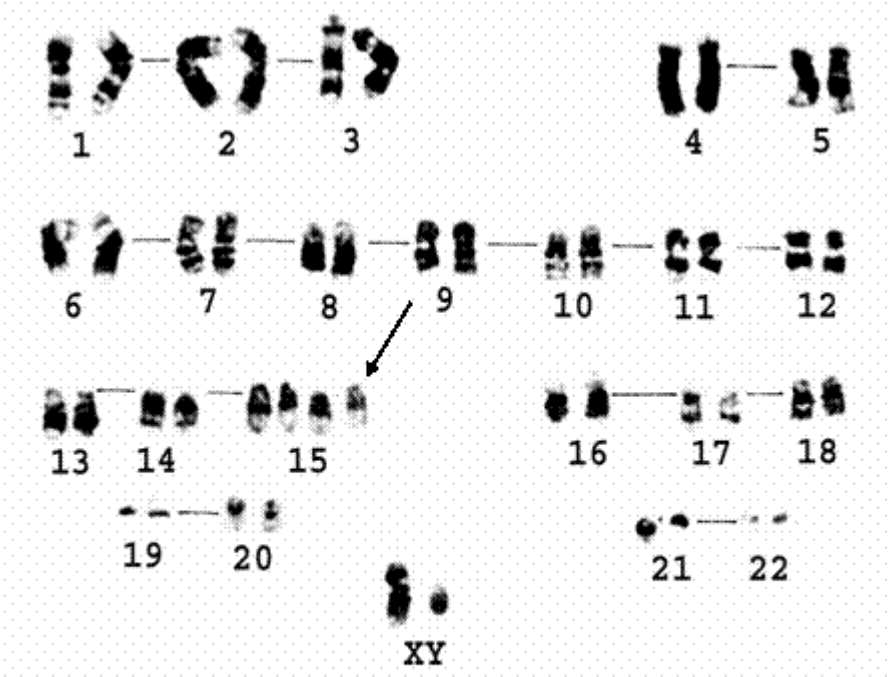

Figure 1. Karyotype 48,XY,+15+15

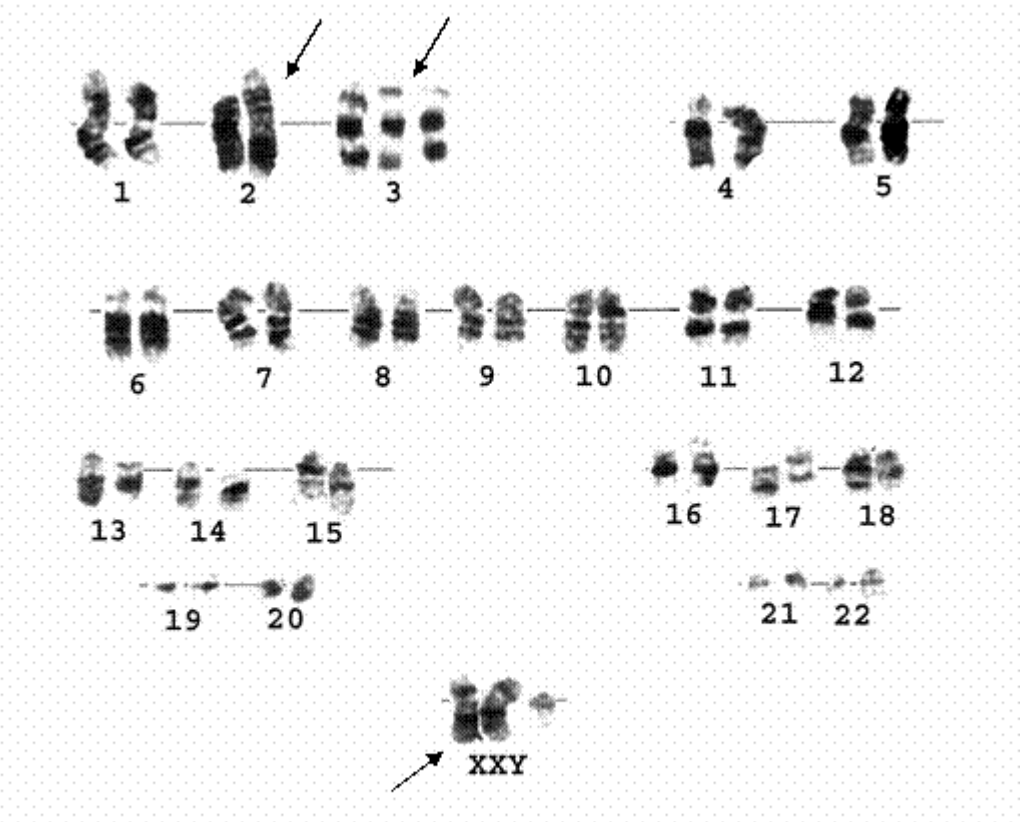

Figure 2. Karyotype 48,XXY,i(2q),+3,9qh-

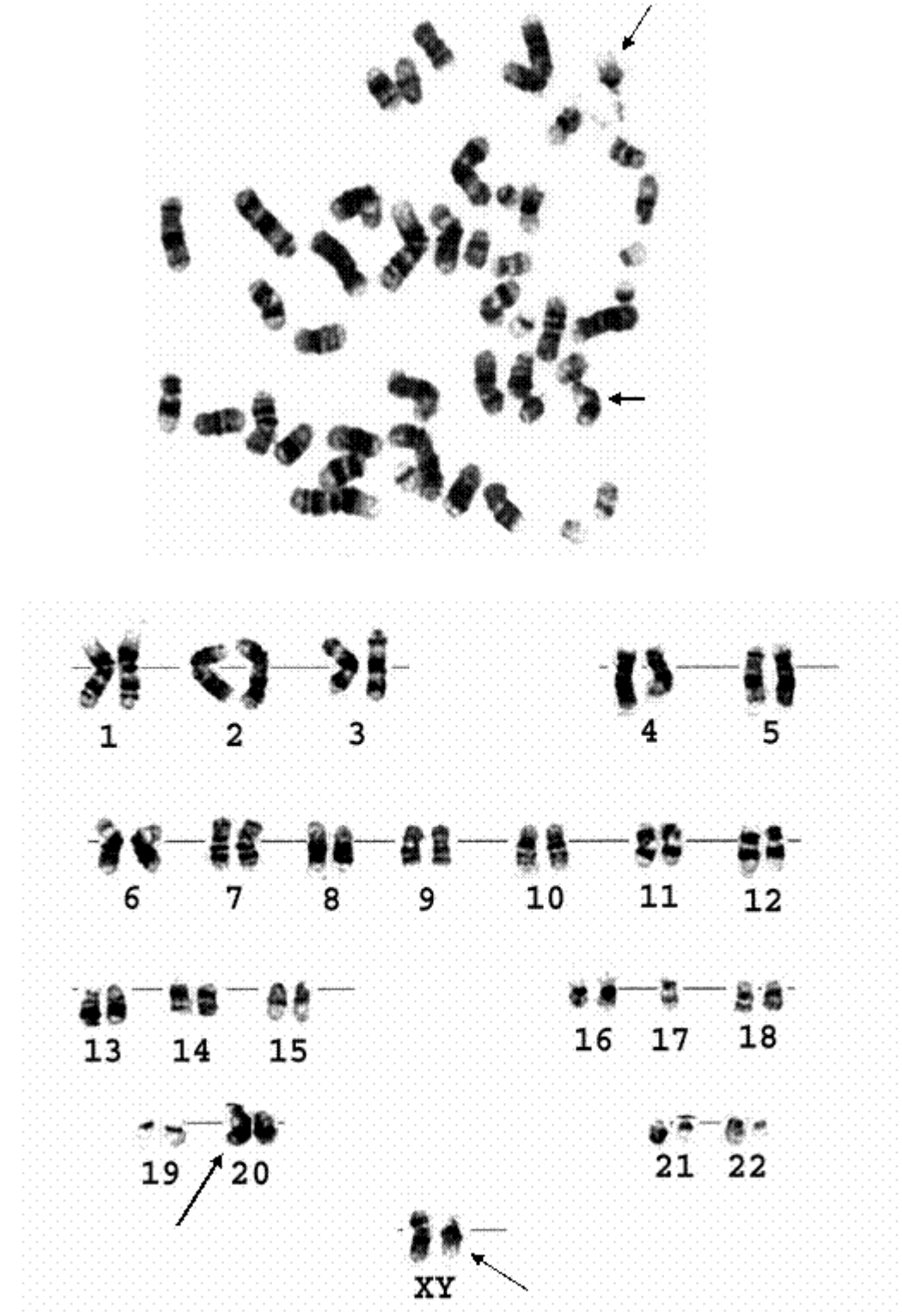

Figure 3. Karyotype 45,XY,t(17;20),Yq+

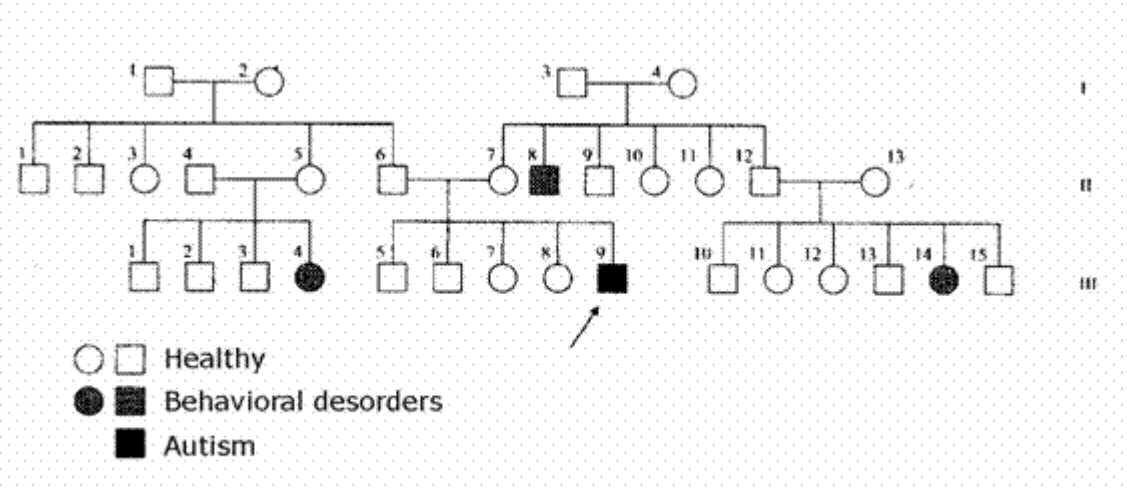

Figure 4. Subject's pedigree

Discussions

The evidence shows that 10-15% of the autistic subjects were diagnosed with genetic syndromes. There is an association between autism and Fra-X syndrome, autism and the tuberous sclerosis, autism and neurofibromatosis (NF 1), but also an association with other genetic syndromes. Many of autism cases display aberrations of the chromosome 15 that are characteristic for the Prader-Willi/Angelman syndrome. The most frequent aberrations were the deletions and the proximal duplications of the 15q that were inherited on the mother line [8]. The candidate genes for autism, associated to the chromosome 15, are the gene UBE3A (ubiquitin-protein-ligase) and the genes for the three subunits of the $GABA_A$ receptor [8]. The linkage technique indicates that the region 15ql 1-13 was identified as being a critical one because various scientific papers show that this chromosomal region is associated with the Prader-Willi/ Angelman syndrome [11]. Indeed, using of five satellites markers spread in PWACR (Prader-Willi/ Angelman critical region) led to the hypothesis that this region is involved in autism, although it is very limited [2]. The region 15ql 1-13 surrounds the GABRB3 locus (gamma aminobutyric acid-receptor) that has an crucial role in the development of the nervous system [9]. We have identified the tetrasomy 15, an aberration that has not been reported by other studies. Moreover, not even the tetrasomy 14 and the trisomy 3 were mentioned in the literature of specialty. Associations of the autism with the trisomy 21/monosomy X are mentioned. There are still studies revealing partial trisomies; thus, the partial trisomy 8p21-23 [6] was found in an autistic girl's case.

Recent genetic analysis show a suggestive binding between the anomalies of the chromosomes X and autism, the most common regions involved being Xql3-q21 and Xp22. A pericentromeric inversion was identified on X(p22.3;ql3.2) in the cases of two boys that were diagnosed with autism and manifested mental retardation and whose mothers were carriers of mutations in the genes KIAA 2022 located in the Xql3.2 and P2RY8 located in the Xp22.3 [1]. Similarly, structural aberrations of the chromosome Y were associated with autism [4]. Because the chromosome Y hosts more genes expressing in the brain (some of them having a critical role in the development of the nervous system), this may suggest that they have a role in the predisposition to autism.

Another chromosome involved in autism is chromosome 7, its modifications also included duplications, deletions and translocations. By using the linkage technique it was established that the locus 7q22 is susceptible to autism [5].

The mechanism of the tetrasomies and the trisomies production in mitosis is bound to the disfunctionality of the centromeres. The mechanism the isochromosomes are formed by is also related to the disfunctionality of the centromeres. But the centromeres region is involved in the organization of the kinetochores that have the main role in the attachment of the chromosomes to the fibers of the mitotic spindle and that function as real control points of the cell transition from metaphase to anaphase. A single unattached kinetochore to the spindle fibers will block the mitosis until the kinetochore will perform the linkage to the mitotic spindle. On the basis of evidence, we propose that the aneuploidies mentioned above are caused by the organization defects of the kinetochores. Our study shows that some kinetochores, that are visible as pair dots at centromeres of the chromosomes, were absent either unilateral or bilateral. This observation can be explained (i) by a deficiency in the centromeric proteins that associate with satellite alpha DNA, (ii) of some modifications of the control proteins of the mitosis, (iii) or the asynchronic replication of the satellite DNA. A proteins crowd (CENP-A…..CENP-I) are present in the structure of centromeric heterochromatin, many other proteins having a regulator role, interacting in a complex

network of signaling. It is easy to understand that the genes that encode these proteins can suffer mutations at a certain point, that are responsible for the default in the organization of the kinetochores or the malfunctions of the centromeres – kinetochores complexes. By homologue and non-homologue recombination of the genes during the mitosis, the different tetrasomies and trisomies can be explained in the examined metaphases. The small rate of aneuploidic cells demonstrates that the mutational event occurred later during the embryogenesis.

The formation of the centromeres – kinetochores complexes and the function of the cemtromeric heterochromatin are unclear. There are multiple chromosomal regions that have a cemtromeric potential. Recently, a neocentromere was noticed in the chromosome 3q26 in a father and his daughter, the centromeric region being deleted. The locus 3q26 was deprived of the satellite DNA, but it was vaguely marked by against centromeric antibodies (CREST). The electronic microscopy showed that the neocentromere formed kinetochores presenting a normal morphology and with the same size as those of the big chromosomes. The deleted centromere formed a small linear marker that reacted strongly with the CREST antibodies, but exhibited a reduced size of the kinetochores [10]

As to the inheritance of the autism, the studies made on monozygotic and dizygotic twins indicate the implication of the genetic factor in the etiology of the autism. As no study reported a 100 % concordance of autism in the monozygotic twins (65% for the monozygotic twins and only 15-18 % for the dizygotic twins), it means that the genetic factor interact with the environment factor. As a consequence, the pattern of inheritance is multifactorial, thus suggesting that the autism is the result of the interaction of several genetic mutations with the environmental factor [3].

It is difficult to measure to what extent these aberrations are responsible for autism, but it is obvious that the subject who exhibits these chromosomal abnormalities is the carrier of the mutations of some genes that regulate the structure and the function of the centromeres and also the repair of the chromosomal lesions.

Further research regarding this field will surely bring more light in the mechanism of the nondisjunction of the sister chromatids and of the isochromosome and bring further evidence regarding the role of the centromeric dysfunction in the occurrence of some diseases.

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