The Egyptian Journal of Medical Human Genetics (2016) **17**, 57–62

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The Egyptian Journal of Medical Human Genetics

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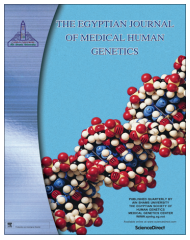

ORIGINAL ARTICLE

Chromosomal abnormalities and autism

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Received 19 April 2015; accepted 11 May 2015
Available online 19 June 2015

KEYWORDS

Autism;
Chromosomal abnormality;
High resolution karyotype

Abstract *Background:* Autism is a neurodevelopmental disorder characterized by clinical, etiologic and genetic heterogeneity. Many surveys revealed cytogenetically visible chromosomal abnormalities in 7.4% of autistic patients documented as well as several submicroscopic variants. This study had been conducted to identify some aspects that might be involved in the pathogenesis of autism which is necessary for offering proper genetic counseling to families of autistic patients and their role in the prenatal diagnosis of autism.

Methods: This cross sectional study was conducted at the Child Psychiatry Clinic, Pediatric Hospital, Ain Shams University on 30 autistic patients who were subjected to the following tools: Confirmation of diagnosis using DSM-IV-TR criteria, IQ assessment using Stanford-Binet intelligence scale and assessment of severity of autistic symptoms using childhood autism rating scale (CARS). Full clinical examination, neurological examination, EEG, audiological assessment were also done. High resolution karyotyping was done for detection of numerical or structural chromosomal abnormalities as deletion, duplication, translocation of chromosomes.

Results: All the results of cytogenetic analysis were normal with no detectable numerical or structural chromosomal abnormalities. Males are affected more than females, only one case had history of drug intake (progestin), two cases had history of anti-D injection and two cases had history of diabetes mellitus during pregnancy. Four cases had history of respiratory distress and seven cases had history of jaundice. Two cases had history of generalized tonic clonic convulsion and four cases had history of EEG abnormalities. Fifteen cases of our autistic patients had mild mental retardation and six cases had moderate mental retardation.

Conclusion: Chromosomal abnormalities were not detected in the studied autistic children, and so the relation between the genetics and autism still needs further work up with different study methods and techniques.

1. Introduction

Autism is a syndrome characterized by impairment in social communication, repetitive behavior, abnormal movement

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Peer review under responsibility of Ain Shams University.

http://dx.doi.org/10.1016/j.ejmhg.2015.05.002
1110-8630

and sensory dysfunction [1]. It is currently estimated that 3–6 children out of every 1000 worldwide have autism with three to four fold higher incidence in males than in females [2]. Autism is a neuro-developmental disorder characterized by clinical, etiologic and genetic heterogeneity, it is often associated with other conditions such as disorders of the CNS (tuberous sclerosis), developmental delay, attention deficit, epilepsy, anxiety and mood disorders [3]. Many surveys revealed cytogenetically visible chromosomal anomalies in 7.4% of autistic patients, among the most consistent cytogenetic findings are fragile-x and duplication of maternal chromosome 15q11–13 [4].

Environmental component is another important aspect of research in autism. Environmental factors such as mercury and radiation have been proposed as possible causes of autism [5]. Several studies provided strong evidence against the hypotheses that MMR vaccination causes autism [6]. The causes of autism are still unclear, although results from twin and family studies provide evidence for strong genetic contribution, with the probability of multiple genetic loci involved [7].

Despite significant research on prenatal, natal, neonatal and other risk factors in autism, the causal nature of these associations is still disputed due to several current methodological limitations of studies [8].

2. Patients and methods

The present study enrolled 30 cases with autism diagnosed with DSM-IV-TR criteria [9,10]. The patients were 23 males (76.7%) and 7 females (23.3%). Their age ranged from 2 to 9 years. They were recruited from the psychiatric clinic, pediatric hospital, Ain Shams University.

All cases were subjected to the following:

1. Detailed history taking with special emphasis on, onset, course, age, sex and consanguinity of the patients.
   - Antenatal history included history of threatened abortion, chronic illness as diabetes mellitus and hypertension, medications as antiepileptics, antithyroid, and progestin, and anti-D injection.
   - Natal and postnatal history including, gestational age, complications during delivery, history of prematurity, perinatal problems and postnatal course especially occurrence of neonatal hypoxia, respiratory distress and jaundice.
   - Developmental assessment included both motor and mental development: age of sitting without support, walking unassisted, first spoken word, combining words, accurate details of cognitive abilities, gross and fine motor functions and history of vaccination.
   - Past history including: major childhood illnesses, history of allergy and gastrointestinal disorders as diarrhea or constipation.
   - Family history for any similar condition, any genetic disease or other psychological or mental disorders in the family.
2. Through clinical examination
   - Laying stress on neurological examination.
3. Psychiatric evaluation:
   - Confirmation of diagnosis using DSM-IV-TR criteria of autism i.e., impairment in language, social skills and restricted stereotyped interests or activity.
   - Assessment of intelligence quotient (IQ) using Stanford-Binet intelligence scale (1986) [11], which is used to measure the cognitive abilities of children aging from 2 to 16 years. Abnormal intellectual function is diagnosed when IQ is below 70.
   - Assessment of severity of autistic symptoms using childhood autism rating scale (CARS) [12], which rates the child from one to four in each of fifteen areas (relating to people, emotional response, imitation, body use, object use, listening response, fear or nervousness, verbal and non verbal communication, activity level, consistency of intellectual response, adaptation to change, visual response, taste, smell, touch response and general impression).
4. Genetic evaluation:
   - Pedigree of family of autistic patients.
   - Cytogenetic analysis by high resolution banding techniques [13] which have facilitated the identification of chromosomes and major structural abnormalities by examining chromosomes with more number of bands by obtaining prophase and prometaphase chromosomes which are less condensed. High resolution banding facilitates better karyotyping analysis and more precise designation of breakpoints and subtle chromosome abnormalities [13]. Prophase and prometaphase cells can be obtained by harvesting the cultures without metaphase block, or by reducing the concentration of the blocking agent (colcemid) and the duration of treatment. An alternative approach is to accumulate the maximum number of cells at a particular stage of the mitotic cell cycle and release them synchronously to capture the early stages of mitosis, prophase and prometaphase. This approach with a brief exposure to low concentration of colcemid, is employed to obtain considerably long chromosomes.

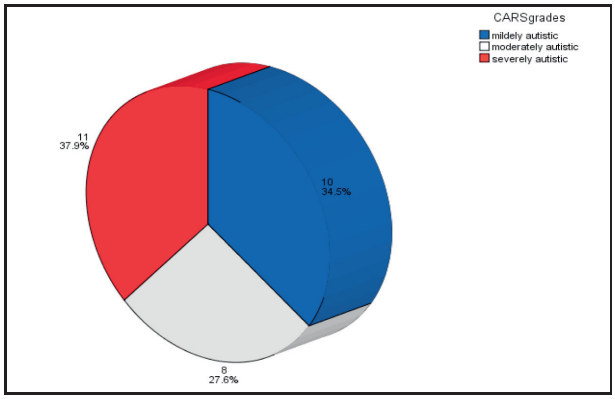

Figure 1 Degree of autism according to CARS in autistic patients.

3. Results

The present study included 30 cases with autism diagnosed with DSM-IV-TR criteria [9,10]. The patients were 23 males

(76.7%) and 7 females (23.3%). Their ages ranged from 2 to 9 years, with a mean age of 4.79 ± 1.9 SD. 16 patients (53.3%) of our autistic patients were diagnosed at age <2 years old and 14 patients (46.7%) were diagnosed with autism >2 years old (Table 1).

The first symptom of autism in our patients was delayed speech in 56.7%, followed by lack of eye contact in 30%, stereotyped movements in 3%, while mixed presentation was found in 10% (Table 2).

There was no history of prenatal complications in 76.7% of mothers of autistic children, but 3.3% had history of drug intake as progestin, 6.7% had history of anti-D injection, 6.7% had history of diabetes mellitus during pregnancy and 6.7% had history of fetal loss. 53.3% of autistic patients were delivered by cesarean section and 46.7% were delivered by normal vaginal delivery.

In our study 23.3% had jaundice, 13.3% had respiratory distress and 3.3% needed cardiopulmonary resuscitation after birth (Table 3). Fifty percent of our patients had mild mental retardation (IQ = 50–70%), 26.7% had below average mentality (IQ = 71–89) and 80% with moderate mental retardation (IQ = 31–49). Eleven cases of our autistic patients were severely autistic (36.7%), ten cases had history of mild degree of autism (33.3%) and nine cases were with moderate degree of autism (30%) (Table 4) (Fig. 1). 6.7% of our autistic patients had attacks of generalized tonic colonic convulsions and 93.3% were normal (Table 5).

Seventy-six percent of autistic patients were males with normal results of high resolution karyotyping and 23% were females with normal results of high resolution karyotyping (Figs. 2–4).

4. Discussion

This study was conducted on 30 autistic children, 53.3% of autistic children were diagnosed before the age of 2 years, while the remaining 46.6% were diagnosed after the age of 2 years. Autistic group's age ranged from 2 to 9 years with an average age of 5.68 years. These findings are against Jonson [14], who found that 75% of autistic children were diagnosed <3 years and the remaining 25% were diagnosed >3 years old. In the current study 76.7% of autistic children were males and 23.3% were females. This goes with other studies, one of them involved 2685 patients with autism, and the prevalence was more in males than females [15].

The goal of this study was to identify the role of chromosomal abnormalities in the etiology of autism and detect whether cytogenetic analysis including high resolution banding technique is useful to identify autistic children or not. Several investigations on autistic children have previously reported a remarkable variety of chromosomal aberrations which involved all the chromosomes [16].

In our study chromosomal abnormalities were negative. This result may be due to small number of the patients studied and low incidence of chromosomal abnormalities in autistic patients and so more advanced techniques as microarray and

Table 1 Patients' age, age of diagnosis and sex of autistic patients.

Age	2–9 years (4.7 ± 1.9)	
Age of diagnosis		
<2 years	16	53.3%
>2 years	14	46.7%
Sex		
Male	23	76.7%
Female	7	23.3%

Table 2 Specific symptoms of autism.

	Number	Percent
Delayed speech	17	56.7
Lack of eye contact	9	30.0
Stereotyped movement	1	3.3
Mixed (lack of eye contact and stereotyped movement)	3	10.0
Total	30	100.0

Table 3 Antenatal, natal and postnatal history in autistic patients.

	Number	Percent
Antenatal history		
−ve	23	76.7
Drug intake (progestin)	1	3.3
Anti-D injection during pregnancy	2	6.7
Chronic illness (DM)	2	6.7
Fetal loss	2	6.7
Natal history		
NVD	14	46.7
CS	16	53.3
Postnatal history		
−ve	14	46.7
Res. distress	4	13.3
Jaundice	7	23.3
Resuscitation required	1	3.3
ICU admission	1	3.3
Mixed	3	10.0

−ve = negative; DM = diabetes mellitus; NVD = normal vaginal delivery; CS = cesarean section; ICU = intensive care unit.

Table 4 The level of IQ and degree of autism according to CARS in autistic patients.

	Number	Percent
Level of IQ		
Moderate MR	6	20.0
Mild MR	15	50.0
Below average	8	26.7
Normal IQ	1	3.3
Total	30	100.0
Degree of autism (CARS)		
Mild	10	33.3
Moderate	9	30
Severe	11	36.7
Total	30	100.0

MR = mental retardation; IQ = intelligent quotient; CARS = childhood autism rating scale.

Table 5 Convulsion in autistic patients.

	Number	Percent (%)
Convulsion		
−ve	28	93.3
+ve	2	6.7
Total	30	100.0

+ve = positive; −ve = negative.

CGH (comparative genomic hybridization) must be used. These results go with the results of Selvi et al. [17] study which showed that no complex rearrangement or chromosomal abnormalities were detected as they performed high resolution karyotyping on 12 autistic patients, peripheral blood samples were taken, cultured and processed after which 25 metaphases from each sample were analyzed in 100 magnification to rule out the chromosomal abnormalities.

Zhong et al. [18] were against our results as they found that there are chromosomal abnormalities detected in four out of fifty cases and about 8% by high resolution banding which are confirmed by FISH. These changes were one case with translocation between chromosome 4 and 6 t (4,6) (q23–24,p21), one case with longer short arm of chromosome 21(21p+) and two cases with pericenteric inversion of chromosome 9.

The prevalence of chromosomal abnormalities was low in several studies including Tajeran et al. [19] who found that among fifty clinically autistic children, only one patient a 7 year old boy was with a ring chromosome 14 (4%). The karyotype of the patient was 46,xy,r (14). Chromosomal alterations which were observable by microscope have been reported in 3–5% of ASD cases [20]. The most frequent abnormalities are 15q11–13 duplications, 2q37 and 22q11 and 22q11.3 deletions [21].

Clinical genetic approach was done to identify genetic causes of autism and found positive genetic findings in about 5% with high resolution chromosomal abnormality, 5% with fragile x syndrome, 5% with Rett syndrome, 10% with other genetic syndromes (tuberous sclerosis) and 10% with structural genomic deletions or duplications using chromosomal microarray [22].

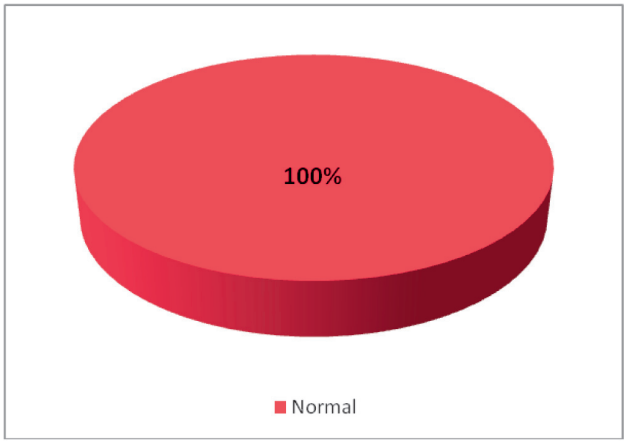

Figure 2 High resolution karyotyping in autistic children.

Shen et al. [23] performed a study on 993 patients with autism. They used high resolution karyotyping, fragile x testing and chromosomal microarray. They found abnormal karyotyping in 2.2%, abnormal fragile x testing in 0.5% and microdeletions and microduplications in 18.2% using chromosomal microarray.

Rare microscopic chromosomal abnormalities occur at a rate of 7.4% in autism. Moreover, multiple studies have converged on particular chromosomal abnormalities in autism, the most common abnormalities are maternally inherited duplications at 15q11–13. These duplications are found in 1–3% of patients diagnosed with idiopathic autism [24].

Several studies have originated from or been strongly supported by traditional cytogenetic evidence leading to a number of findings. For example, Thomas et al. [25] reported de novo deletions or translocations that affect the same region on X chromosome at Xp22.3 in three girls with autism.

This study revealed no significant contribution of chromosomal abnormalities to the etiology of autism. So it appears that to date, only a small percentage of autistic disorders have been associated with specific chromosomal abnormalities [26].

As a result there is no conclusive factor that can be elicited as being a risk for autism.

Chromosomal abnormality involvement in the pathogenesis of autism is not new, so many investigators focused on genetic pathogenesis of this neurodevelopmental disorder using different techniques such as array CGH and cytogenetic analysis [27]. Chromosomal rearrangement plays an important role in the etiology of autism, hence chromosomal abnormalities have provided considerable insight into the candidate gene and possible molecular pathway involved in autism [28]. Autism is not a single clinical disorder. The variable phenotype spectrum might be suspected as a polygenic and multifactorial

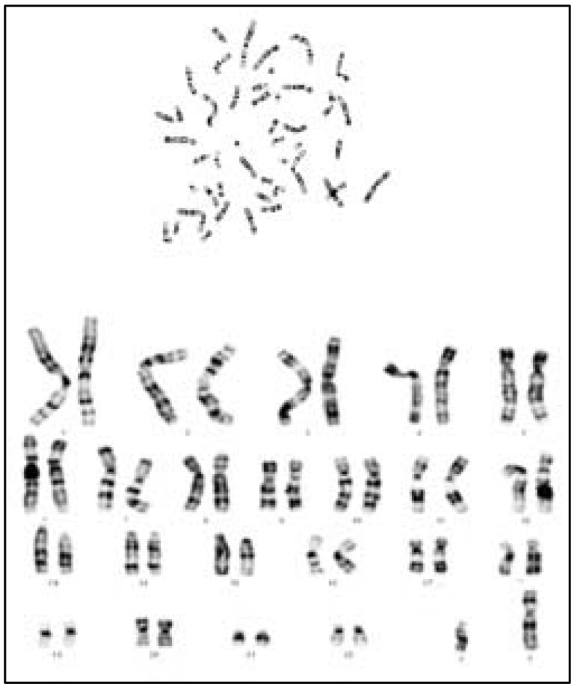

Figure 3 Karyotype – normal male high resolution karyotyping in a case of our autistic patients.

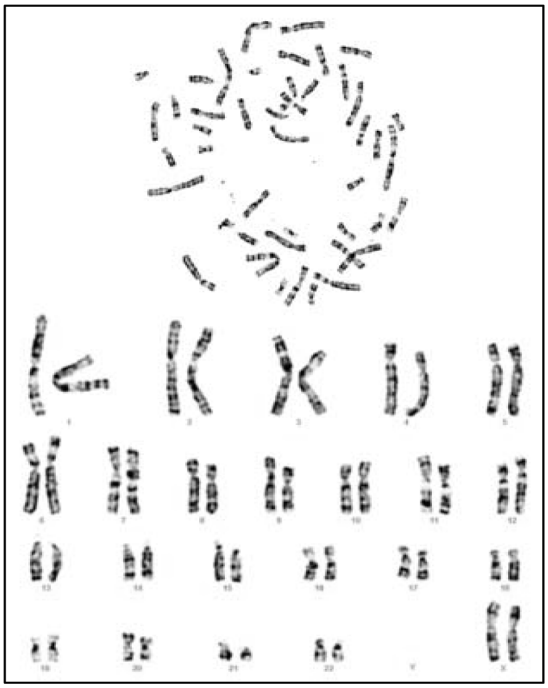

Figure 4 Karyotype – normal female high resolution karyotyping in a case of our autistic patients.

disorder, however many questions about autism remain poorly explained [29].

In our study 56.7% of our patients had delayed speech, and these results are in accordance with Zwavigendum et al. [30], who found that children with autism developed several specific behavioral markers including, lack of eye contact, loss of social smile, loss of social interest and delay in expressive and receptive language.

Dalton et al. [31] hypothesize that, the maternal antibodies immunoglobulin G (IgG) in the mothers' blood cross the placenta and enter into the fetus brain, react against fetal brain proteins and cause autism. In our study 6.7% of mothers of autistic children received anti-D injection as they were rhesus negative.

Bolten et al. [32] suggested additional differentiation between complications based on the severity as determined by the selection of obstetric complication known to be associated with autism.

More than half of the cases at about 53.3% were delivered by cesarean section, yet it seemed to have no impact on the etiology of autism. Other studies thought that cesarean section is a risk factor for autism [33]. Kolevzon and Reichenberg [34] disapproved that cesarean section can be accused as a risk factor for autism.

Only one case of mothers of autistic children received progestins during pregnancy, consistent with Lidisky and Schneider [35] who reported that there was no drug intake correlation to the autistic disorder as well.

Postnatal factors like hyper-bilirubinemia are also alarming and suspected in the etiology of autism. In the current study there was no significant correlation between neonatal jaundice and autism, supporting the belief of many researchers as Gardener et al. [36]. On the other hand, few suggested that hyperbilirubinemia occurs more frequently than expected among children later diagnosed with autism [37].

Hara [38] found that seizures that occur in autistic children are most commonly of the generalized type and said that epilepsy is one of the negative factors of cognitive, adaptive and behavioral outcomes for individuals with autism. In our study 6.7% of our autistic patients have generalized attacks of convulsion.

Kaplan et al. [39] stated that, mental retardation usually coexists with autistic disorder. About 40% of autistic children are moderately or severely retarded. In our study about 50% have mild mental and 20% were with moderate mental retardation.

5. Conclusion

Chromosomal abnormalities were not detected in the studied autistic children, and so the relation between chromosomal abnormalities and autism still needs further work up with different study methods and techniques.

But, no matter how rare the cause is or whether it is genetic or environmental, these factors have the potential to provide clues to the etiology and neurobiology of autism.

What is known about environmental agents does not account for many cases, but it is a source of information on the nature of autism.

6. Recommendations

Future studies using a larger case group with stress on the role of genetic susceptibility and the role of family studies to determine the susceptible genes are necessary. Advanced techniques should be used for detection of the genetic cause of autism as microarray.

Conflict of interest

We have no conflict of interest to declare.

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