

## Characterization of Chromosomal Abnormalities in Saudi Patients with Autism Syndrome

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### Abstract

Autism spectrum disorder (ASD) is a deficit that impairs the development of social skills, verbal and nonverbal communication, and repetitive patterns of behavior. This is the result of a neurological disorder that affects the manner in which information is collected and processed by the brain, causing problems in social skills. This study aimed 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 diagnosis of autism in AlMadinah Almonawwarah. A total of 30 mothers of children who filled a questionnaire were enrolled in this study; 27 of those mothers had only one autistic child, whereas 3 mothers of them have two autistic children. Chromosomal analysis was carried out for 18 patients participated including 9 autistic children and their mothers; all autistic children and their mothers showed normal karyotyping, only one autistic child (a sister twin) had heterochromatin in chromosome 16; actually, this child was not fully diagnosed ASD. This means that karyotyping is not enough for genetic diagnosis; but DNA sequencing may be more valuable. Furthermore, multidisciplinary approach that involves pediatrics, geneticists, neurologist and psychiatrist is required for diagnosis and management of autism cases.

**Keywords:** Autism spectrum disorder, Autism.

### Introduction

Autism is defined as a deficit that impairs the development of social skills, verbal and nonverbal communication, and repetitive patterns of behavior. This is the result of a neurological disorder that affects the manner in which information is collected and processed by the brain, causing problems in social skills.

The American Autism Society explains autism as a type of developmental disorder that occurs during the first three years of the child's life. This disorder results from a neurological disorder that affects brain function and thus affects various aspects of growth. The problems of

adaptation to the environment lie in the inability to carry out effective work and performance in the environment, and the inability to cope with and tolerate changes in the surroundings and deal with it. These children always respond to things more than their response to people and upset these children of any change happening in their environment and always repeat physical movements or passages of words. The prevalence of autism is increasing worldwide. In Saudi Arabia the incidence is around 1 in 400 children. In other words, there are about 53,000 diagnosed with ASD in the Kingdom of Saudi Arabia. Many studies reported the incidence of ASD in males 4 times more common than in females [1], [2].

Genetics researchers demonstrate that the females are more protected from the effects of heritable mutation and suggests that sex chromosomal genes and/or sex hormones, especially testosterone, may modulate the effects of genetic variation on the presentation of an autistic phenotype [3], [4].

Another concern is that, the consanguinity marriage rate is high among Saudi population, thus, it may increase the chances of inheriting an abnormal DNA which results in a birth defect such as Autism.

The present study aimed to study the new consistent chromosomes abnormalities in autism then compare them with other previous researches.

## Methodology

### **MATERIALS**

#### ***Ethical Consideration***

The study proposal was reviewed and ethically approved by the Scientific and the Ethical Committee (Institutional Review Board) at Taibah University. Written informed consents were obtained from all individuals participated in this study.

#### ***Study Design***

This study was a cross-sectional study. It was conducted at the Hanaif Medical Center for Autism in AL-Madinah, Saudi Arabia. Informed consents were obtained from all guardians of autistic children.

#### ***Study Population***

A cross-sectional study of 30 Saudi patients with autism participated in this study. Eighteen of them agreed to give a blood sample from mothers and their autistic children in this study.

#### ***Samples***

Blood samples (3 ml) were collected by experienced phlebotomist in lithium heparin tubes.

#### ***Data Collection Technique***

The individuals were interviewed. Demographic and clinical data collected in a specially designed questionnaire included number of autistic child in the family, pregnancy and labor complications (if there is any), mother's age during pregnancy with an autistic child, age of the child when diagnosed with ASD, consanguinity marriage, medication taken during pregnancy and treatment used for autistic child.

#### ***Cytogenetic Techniques***

##### ***Specimen Collection and Handling***

Peripheral blood samples were collected in sterile syringes containing lithium heparin. Specimens were immediately processed. Karyotyping was carried out to show the appearance of chromosomes in the nucleus of a cell. Chromosomes were individually distinguishable under the

light microscope during metaphase. Metaphase chromosomes obtained from specimens containing spontaneously divided cells or ones that cultured and chemically induced to divide in vitro [5].

##### ***Culture Initiation***

Eighteen culture flasks were labelled with specimen double ID, date of specimen inoculation, date of harvesting, the volume of the specimen was added [6]. In a biological safety cabinet, we transferred 10 ml of complete media, 0.5-0.8 ml of whole blood specimen, 1m of fetal bovine serum, and 200µl of Mitotic Stimulants Phytohemagglutinin (PHA) to both culture flasks. After that, Cultures were split and placed at 37°C for 72 hours [6].

##### ***Cell Harvest***

Flask for routine cytogenetics processing:

After 72 hours of incubation, both flasks were taken out of incubator, then 100µl of ethidium bromide was added and incubated for 1 hour at 37°C. 100µl of Mitotic Inhibitor; colcemid (colchicine) was added to all flasks and incubated at 37 OC in water bath for 30-40 min [6]. After incubation, the flasks were taken out and the materials were transferred into 15 ml falcon tubes, mixed by inversion; spun at 1500 RPM for 10 minutes. Supernatant was removed, pellet disrupted gently and 8ml of 0.075 M hypotonic potassium chloride (KCl) was added slowly into all tubes. The cells swollen (is critical for adequate spreading of chromosomes on the slide). Then after addition of KCl, tubes were incubated for 20 minutes at 37 OC in water bath. Then tubes were spinned at 1500 RPM for 10 minutes [6]. Supernatant was removed, pellet completely disrupted using vortex, then 1 ml of freshly prepared fixative was added slowly to one tube at a time, capped and well mixed using vortex at a medium speed. Then, more fresh fixative (5ml) were add and suspension was well agitated [6]. Three parts of absolute methanol and one part glacial acetic acid were used to stop the hypotonic solution action, to fix cells in the swollen state, and to lyse any red blood cells in the sample. Tubes Spun at 1500rpm for 10 minutes. These steps were repeated 3 times until the pellet looked clear and then proceeded to slide making [6].

##### ***SLIDE PREPARATION***

After the final fixative wash, supernatant was drawn off to just above the pellet. Pellet was suspended with enough fresh fixative to give a slightly cloudy suspension [6]. Slides were dropped from one culture tube at the same time to ensure that no labeling mix-ups occur. Two slides were cleaned with methanol and labeled with the case number slide and date [6]. The slide was held at a slight angle and with the help of jester; 30-50µl of suspension allowed to fall onto the slide. Slide dried by passing it over flame for few times. This procedure increase consistency with spreading and decrease watermarks. Then slides were aged in a 60±1°C oven overnight before banding [6].

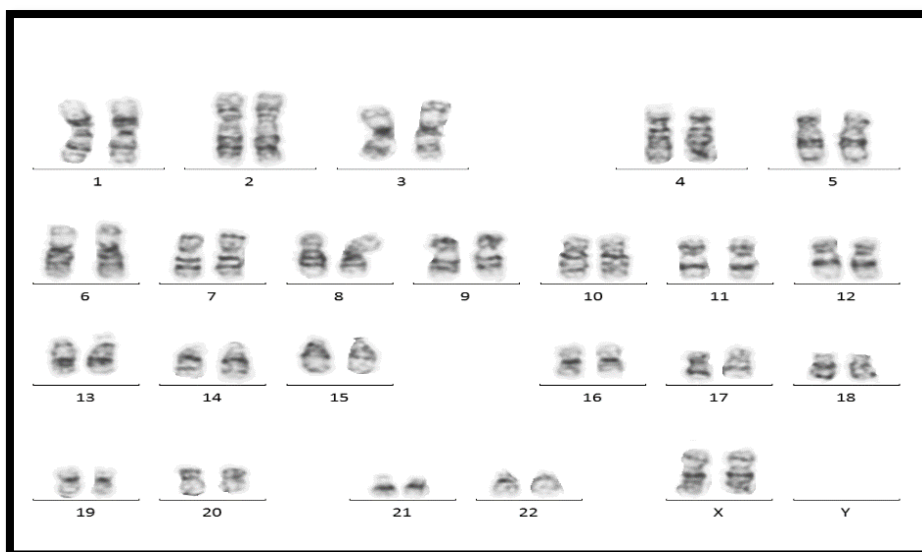
### **GTG BANDING Technique**

Three Coplin jars were prepared as follows:

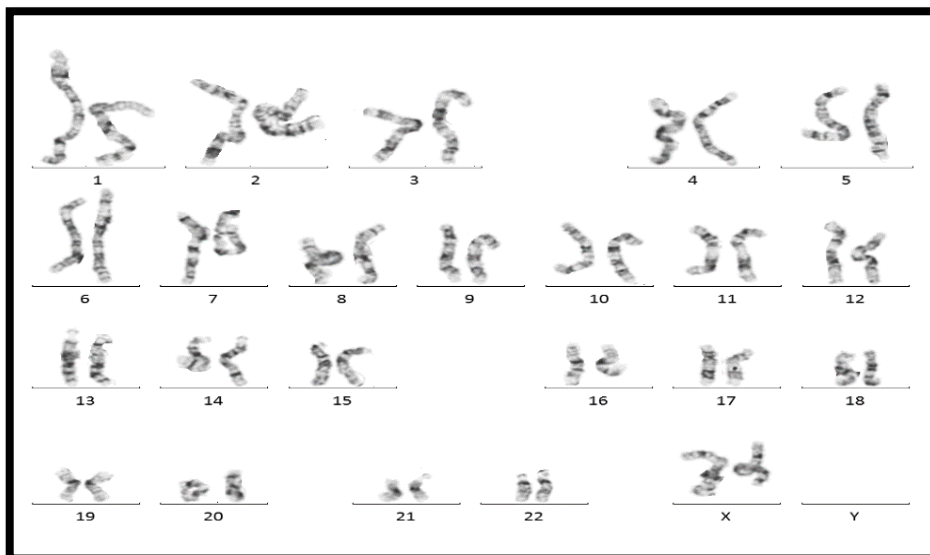
A-60 mL working 0.05% Trypsin into phosphate buffer solution. B-60 mL of pH 7.4 phosphate buffer solution (PBS). C-60 mL of 5% Giemsa solution. The slides were Placed in coplin jar (a) for 30 seconds. After that, it was briefly in a coplin jar (b) for 15 seconds. Then placed in coplin jar (c) for 4 minutes. Slides were rinsed up in tap water and allowed to dry [6]. After that, added 2-3 of distyrene plasticiser xylene (DPX) drops to each slide then cover slipped the slides with a glass coverslip and examined under microscope [6], [7].

### **Results**

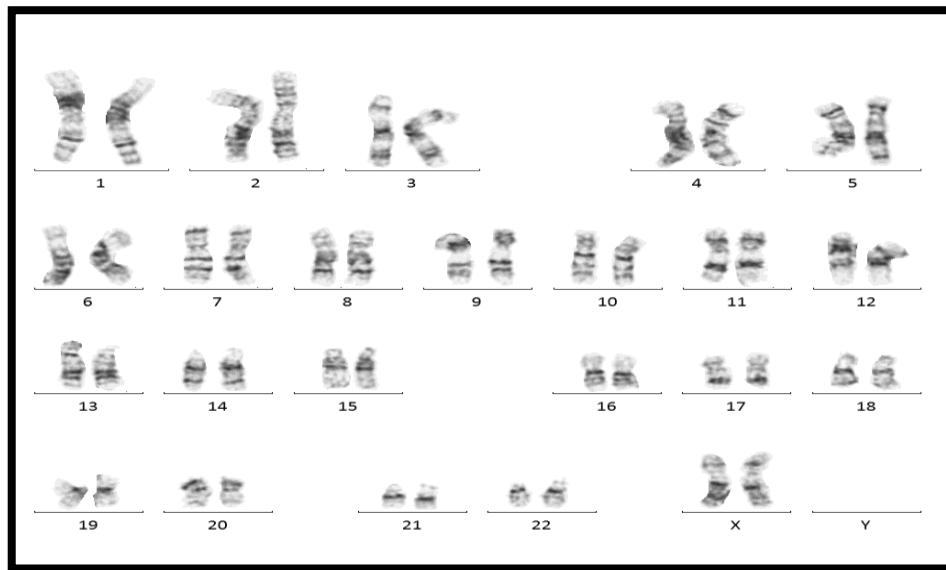
A total of (30) subjects enrolled in the present study, including mothers of children with autism. Chromosomal analysis was carried out in 18 patients participated, including 10 autistic children and their mothers. Of those children, 4 were males and 6 were females; all children were 5 -10 years old. Among those children there were twin girls. Chromosomal analysis was normal with no observable numerical or structural chromosomes abnormalities (figure 3), except one patient showed heterochromatin in chromosome 16.



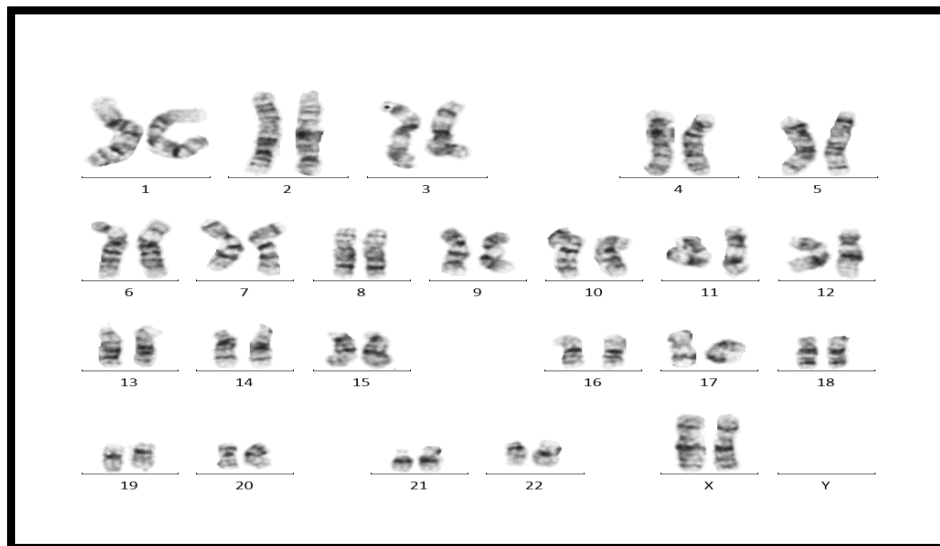
**Figure 1:** (46,XX) karyotype of case number 2.



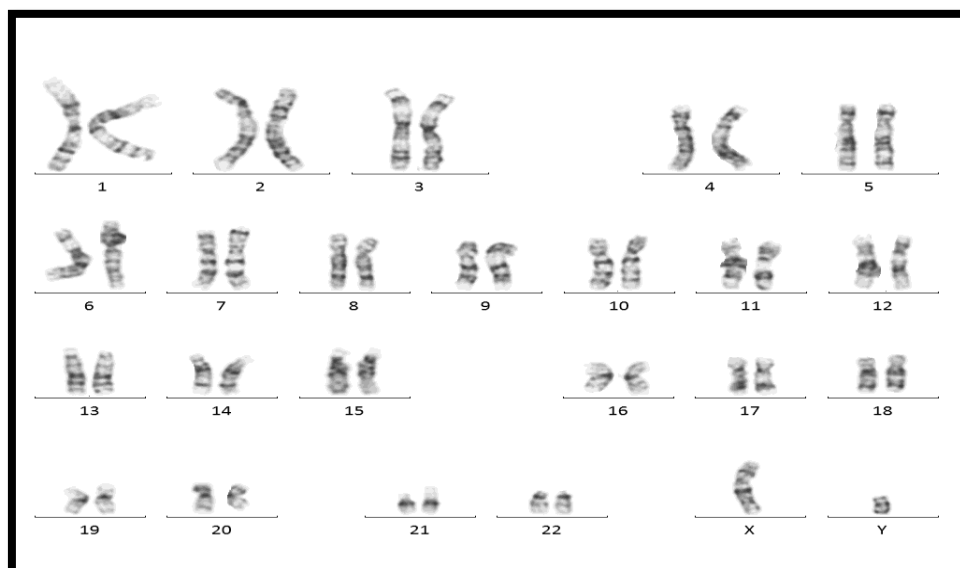
**Figure 2:** (46,XX ) karyotype of case number 3.



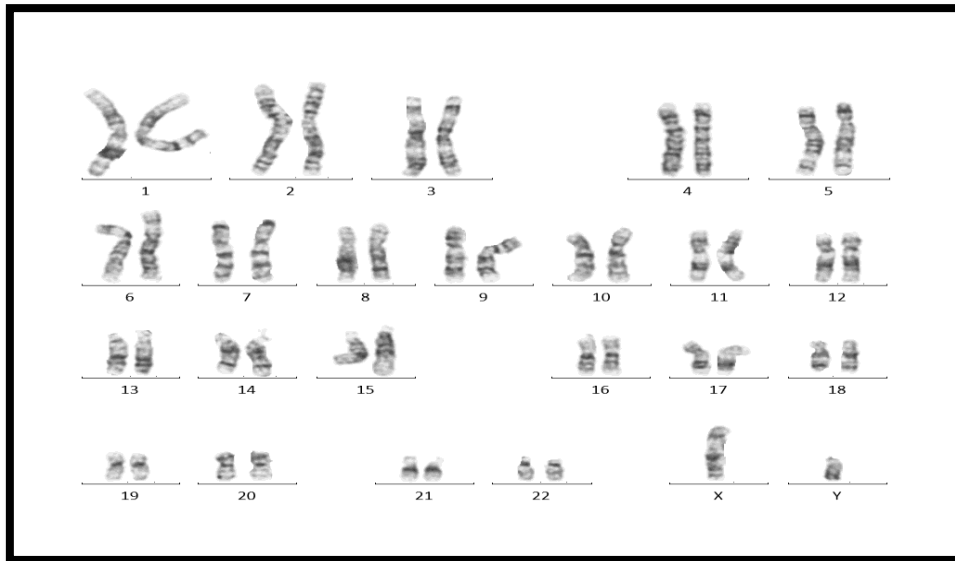
**Figure 3:** (46,XX) karyotype of case number 5.



**Figure 4:** (46,XX) karyotype of case number 6.



**Figure 5:** (46,XY) karyotype of case number 7.



**Figure 6:** (46,XY) karyotype of case number 8.



**Figure 7:** (46,XX) karyotype of case number 10; shows (16 hq+). heterochromatin in chromosome 16.

Regarding the number of autistic children in the families (27 families), every family had only one autistic child, whereas the other 3 families, everyone has more than one child (2 children) (Table 1).

**Table (1):** Number of autistic children in the family.

Number of autistic children in the family	Number of Cases	Percentage
One child	27	90%
Two children	3	10%

Pregnancy and labor complication showed that there was a history of pregnancy and labor complication in about 14 out of 30 (46.67%) of mothers of autistic children, but 18 out of 30 (53.33%) had no history of complication (Table 2):

**Table (2):** Pregnancy and labor complications.

Pregnancy and labor complication	Number of Cases	Percentage
Complication	<b>14</b>	<b>46.57%</b>
Do not have complications	<b>18</b>	<b>53.33%</b>

Consanguinity marriage shows that there were 14 families out of 30 (46%) had consanguineous marriage. Table (3).

**Table (3):** Consanguinity marriage.

	Number of Cases	Percentage
Consanguinity marriage	<b>14</b>	<b>46.57%</b>
No consanguinity marriage	<b>16</b>	<b>53.33%</b>

Concerning mothers ages during pregnancy as shown in table (4), 13 mothers (43.33%) aged 30-40 years old; whereas 11 mothers aged 25-30 years old (represented 36.67%).

**Table (4):** Mothers ages during pregnancy with autistic child.

Age	Number of Cases	Percentage
≤ 20 years	1	3.33%
20-25 years	4	13.33%
25-30 years	11	36.67%
30-40 years	13	43.33%
≤ 40 years	1	3.33%
Total	30	100%

Regarding the age of children when diagnosed with ASD, 14 autistic children (46.67%) were diagnosed at age ≥ 3 years old and 13 patients (43.33%) were diagnosed before 3 years old Table (5).

**Table (5):** Age of the child when diagnosed with ASD.

Age of the child when diagnosed with ASD	Number of Cases	Percentage
0 – 3 years	13	43.33%
3 – 6 years	14	46.67%
6-10 years	3	10%

**Table (6):** Twins case.

One family have twins	Sex	Diagnosis	Age of the child when diagnosed with ASD	Type of twins
Sister 1	Female	Full diagnosis	5 years	non-identical twins
Sister 2		Not fully diagnosed	6 years	

## Discussion

Throughout human history, researchers have tried to identify the reason for autism, but so far, the actual cause is not yet identified. Several recent studies revealed that, over 1000 genes change can be associated with autism. Variation in many genes can be combined with an environmental risk

factor, for example, a birth complication such as umbilical cord complication, parental age and other risks not been known. The chromosomal abnormalities usually occur spontaneously during conception. According to our knowledge, no previous study has been carried out to study chromosomal abnormalities in Almadinah Almonawarah; so, this study may be able to shed the light on the genetic

diagnosis and management of autism in Almadinah Almonawarah.

A total of (30) subjects participated in this study (including mothers of children with autism). Chromosomal analysis was carried out for the 18 patients participated, including 10 autistic children and their mothers. Of those children, 4 were males, and 6 were females. Among those children, there were twin girls. All autistic children and their mothers showed normal karyotyping, only one autistic child (a sister twin) had heterochromatin in chromosome 16, this child was not fully diagnosed with autism spectrum disorder figure 7.

Chromosome 16 exceeds more than 90 million DNA building blocks (base pairs) and represents almost 3 percent of the total DNA in cells. It has many functions which may be related to communication skills, but it was not proofed to have a relationship with ASD [8]. The structure and function of heterochromatic regions remain unstudied, as these areas of the genome have not been sequenced. However, there are indications that the extensive variability of the heterochromatin regions of the chromosomes, particularly those positioned close to the centromere, are characteristic for a number of pathological states, including undifferentiated mental retardation, which is often associated with autistic spectrum disorders in children [9], [10].

In the present study, questionnaires were distributed from (2<sup>nd</sup> March to 30 of March); 30 volunteers were contributed to this study. Although the time period of questionnaires distributed was very limited, we found a large number of autistic children in Almadinah Almonawarah. Thirty mothers of autistic children, filled a questionnaire, 27 of these families had only one autistic child, 3 families of them have two autistic children. Fourteen autistic children (46.67%) were diagnosed at age  $\geq 3$  years old and 13 patients (43.33%) were diagnosed before 3 years old. While the remaining were diagnosed after the age of 6 years. The difference in diagnosis and delayed diagnosis has led to difficulties in the child's condition. Therefore, most children have developed their degrees of autism due to lack of early diagnosis and lack of awareness of the community in this category [11].

Autistic mothers ages ranged from  $\leq 20$  years old to  $\geq 40$  years old. A high mother ages ranged from 30-40 years old were observed in 13 mothers (43.33%), and 11 mothers ages that presented 36.67% were ranged from 25-30. There was a history of pregnancy and labor complication in about 14 out of 30 (46.67%) such as bleeding and dysapontocytosis of mothers of autistic children, but 18 out of 30 (53.33%) had no history of complications. The consanguinity marriage rate was 14 out of 30 (46.57%). Many studies showed that inbreeding marriage does not cause birth defect, but it may increase the chances of inheriting an abnormal DNA which results in a birth defect. Autism may be considered as a one of inbreeding disorders such as schizophrenia, congestive heart failure, DM ...etc [12].

The majority of the autistic mothers (76.67%) did not receive medication during pregnancy, the others who used medication were 7 out of 30. In contrast, the majority of autistic children who used medication for treatment were 22 out of 30 (73.33%), while the remaining had not received any medication were 8 (26.67%).

## Conclusion

From the present study, we can conclude that only one autistic child showed chromosomal abnormality (heterochromatin of chromosome 16), this means that karyotyping alone is not enough regarding genetic diagnosis, but DNA sequencing may be more valuable. Moreover, multidisciplinary approach that involves pediatrics, geneticists, neurologist and psychiatrist is mandatory.

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