Neuroscience and Behavioral Physiology, Vol. 40, No. 7, 2010

Cytogenetic, Molecular-Cytogenetic, and Clinical-Genealogical Studies of the Mothers of Children with Autism: A Search for Familial Genetic Markers for Autistic Disorders

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Translated from Zhurnal Nevrologii i Psikhatrii imeni S. S. Korsakova, Vol. 109, No. 6, pp, 54–64, June, 2009.

State-of-the-art cytogenetic and molecular-cytogenetic methods for studying human chromosomes were used to analyze chromosomal anomalies and variants in mothers of children with autistic disorders and the results were compared with clinical-genealogical data. These investigations showed that these mothers, as compared with a control group, showed increases in the frequencies of chromosomal anomalies (mainly mosaic forms involving chromosome X) and chromosomal heteromorphisms. Analysis of correlations of genotypes and phenotypes revealed increases in the frequencies of cognitive impairments and spontaneous abortions in the mothers of children with autism with chromosomal anomalies, as well as increases in the frequencies of mental retardation, death in childhood, and impairments to reproductive function in the pedigrees of these women. There was a high incidence of developmental anomalies in the pedigrees of mothers with chromosomal variants. These results lead to the conclusion that cytogenetic and molecular-cytogenetic studies of mothers and children with autism should be regarded as obligatory in terms of detecting possible genetic causes of autism and for genetic counseling of families with autistic children.

KEY WORDS: autism, genetics of autism, chromosomal anomalies, chromosomal variants.

Autism is clinically heterogeneous and is one of the most widespread pediatric mental disorders. Recent decades have seen an increase in the incidence of this condition. Thus, the total incidence of autism in 1986 was 1:5000, compared with 1:250 in 2000 [1, 4, 14, 20, 32]. For this reason, autism has in recent decades been subject to increased attention by psychiatrists, geneticists, and pediatricians. Autism, or autistic spectrum disorder, is a group of diseases characterized by impairments in form of significant difficulties in social interactions and communication, along with limited and stereotypical behavior, interests, and actions. The diagnostic criteria for autistic disorders are laid out in DSM-IV and ICD-10. In general, autistic disorders represent a generalized term including classical autism, Asperger's syndrome, and undifferentiated types of autism, as well as neuromental regression and Rett's syndrome [1, 32, 33]. These forms are discriminated by significant differences in symptom severity and the developmental characteristics of linguistic skills and cognitive and social behavior at the early stages of development. Thus, for example, children with "classical" autism are characterized by minor deviations in development at ages of up to three years; in Asperger's syndrome there are significant abnormalities in social interactions, with limited and stereotypical behavior, interests, and activity, with apparently normal linguistic and cognitive development at up to three years. Rett's syndrome is characterized by signs of severe inherited encephalopathy leading to profound disability in the first years of life. Rett's syndrome, which is due to a mutation in the regulatory gene MECP2, coding for methyl-CpG-binding protein 2 (MeCP2), also involves chromosomal anomalies and abnormalities. These include a particular type of late replicating chromosome X, the so-called type C, which is seen only in Rett's syndrome and is used as a diagnostic test during the preclinical phase of the disease [2, 11, 13, 36, 37].

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0097-0549/10/4007-0745

Gene mutations in this syndrome were seen in 82% of children in a Russian cohort [13] and the type C chromosome was seen in 95% [2, 4, 37].

Autism was first described by the American physician Kanner [30]. He described children with delayed psycholinguistic development and marked social isolation which was inexplicable in relation to the level of the children's development; he termed this syndrome "infantile autism," working partly from the criteria for schizophrenia [18], where loss of social interest is also a feature. At the same time, the Austrian psychiatrist Asperger [16] described similar patients with "autistic psychopathy" and normal intellectual capacities. He also noted that the fathers of these patients were withdrawn and socially isolated. These two investigators independently proposed a genetic basis for the disease. We have demonstrated [5, 38] increases in the incidence of chromosomal variants (or chromosomal heteromorphism) in the mothers of children with the idiopathic form of autism. The inherited nature of autism is no longer doubted. Among the genetic factors determining the pathogenesis of autism are mutations in genes on chromosome X, a number of specific chromosomal anomalies in chromosomes 7 and 15, and an increased frequency of variability in the heterochromatin regions of chromosomes [4, 6, 11, 15, 19, 27]. Overall, chromosomal anomalies are seen in 10–14% of cases of autism, while chromosomal heteromorphism (chromosomal variants) occurs in 60% [5, 6, 19, 25, 27, 38]. However, most of the cases described lack epidemiological data on chromosomal anomalies and variants. Many authors [5, 19, 38] have discussed the question of the significance of chromosomal anomalies and abnormalities in the pathogenesis of autism. Given that chromosomal variants are seen in 60% of cases and chromosomal anomalies and rearrangements in 5–14% of children with autism and have been described in many chromosomes, special studies have been performed to identify candidate genes for autism by detailed analysis of the gene composition of regions with chromosomal abnormalities [19, 20]. Chromosomal rearrangements most commonly (in about 1% of cases) affect region 15q11-13. In most cases, this is a duplication of maternal origin or an additional chromosome with an inverted duplication [31, 34]. The phenotype in the presence of this duplication or inversion is characterized by frequent epileptic attacks in childhood, muscle hypotonia, and impairments to motor coordination combined with mental retardation (moderate to severe) and delayed psycholinguistic development or a lack of speech. Additional abnormalities often include high levels of hyperactivity [34].

Deletions in region q11-13 of the paternal or maternal chromosome 15 are known to be associated with Angelman syndrome, Prader-Willi syndrome, and genomic imprinting. As deletions of region 15q11-13 of maternal origin in Angelman syndrome are clinically more similar to autistic disorders, this suggested the possible involvement of maternally inherited genes in region 15q11-13 in the etiology of autism. Deletions in regions 2q37, 7q31, and 22q11 have also been addressed in terms of their possible involvement in the etiology of autism. The results of studies on the linkage of 2q37 deletions accompanying developmental anomalies, hypotonia, kidney diseases, brachydactyly, and autism suggested possible linkage with areas of 2q1-q33 [17, 20, 23, 34, 40]. Deletions of chromosome 7 did not identify any autism-associated candidate genes. Signs of autism have been described in microdeletion syndromes associated with the 22q11.2 region (velocardiofacial syndrome and Di Giorgio syndrome) [22]. On the other hand, studies of more than 100 patients with classical autism found that not one single case showed a 22q11.2 deletion. Some reports have now shown that the cause of autism may consist of deletions in region 22q13.3. Apart from the chromosomes identified above, anomalies of chromosomes 6, 17, and X have been studied in relation to links with autism. Studies of candidate genes potentially associated with these chromosomes have not produce any unambiguous results [19, 20, 27, 29, 40, 41]. The high frequency of autism among the pediatric population and the severe clinical pictures seen in many forms of autism have led investigators to identify and study cytogenetic, genetic, and neurobiological markers of this disease, both in children and their parents. High incidences of variations of the heterochromatin regions of individual chromosomes have been demonstrated in children with autistic disorders (59.4%) and their parents (61.1%) [6]. Chromosomal variants were found much more frequently in mothers than in fathers, by factors of 2–2.5. Furthermore, it has been demonstrated that chromosomal anomalies potentially associated with autistic disorders in children are often seen in their mothers [38]. It should be noted that analysis of chromosomal anomalies and variations in the juxtacentromeric areas of individual chromosomes in the mothers of children with autism has not yet been performed.

The aim of the present work was to identify possible associations between chromosomal anomalies, chromosomal variants, and clinical-genealogical data in mothers with autistic disorders and autism in their children using state-of-the-art cytogenetic and molecular-cytogenetic approaches to the analysis of human chromosomes.

MATERIALS AND METHODS

A representative group of 96 mothers of children with autism was studied. Apart from autism, the children of these mothers had mental retardation of different severities, delayed psychomotor development and/or psycholinguistic development (DPMD and/or DPLD). The age at which mothers received genetic consultations varied from 24 to 60 years (mean 33.5 years). Mean age at the time of birth of the autistic child was 27.4 years (from 17 to 45 years). Cells were analyzed only from mothers of children with unknown genetic causes of illness, including Asperger's syndrome.

The mothers of children with known genetic causes, i.e., with diagnoses of Rett's syndrome, fragile-X chromosome-linked mental retardation (FRAXA syndrome), tuberous sclerosis, Lemli–Opitz, Williams, Angelman syndromes, and other known single-gene syndromes, were excluded from the present study.

The control group consisted of 59 women who had not had children with autistic disorders, aged from 24 to 48 years (mean 33.2 years).

Diagnoses of autism were made in all children in terms of the DSM-IV criteria [21]. Correlations between genotypes and phenotypic features were studied by analyzing 96 genealogies of mothers of children with autism and the health of 1991 of their relatives.

Cytogenetic and molecular-cytogenetic studies were performed using methods described previously [3, 4, 10, 24, 36, 39]. Metaphase chromosome preparations were made from peripheral blood lymphocytes cultured in vitro using a standard protocol. Cytogenetic analysis was performed on chromosome preparations under a light microscope at a magnification of ×1125. At least 20 cells were analyzed in each case using differential chromosome staining for length (the G and, obligatorily, the C method) [4]. Apart from chromosomal anomalies, variations in heterochromatin regions of individual chromosomes were assessed in the karyotype, i.e., so-called chromosome variants, with increases and decreases in the juxtacentromeric heterochromatin regions of one homolog, as well as inversions of these regions (chromosomal heteromorphism). Chromosomal variants were assessed using a standard method [6]. Molecular cytogenetic analysis was performed by fluorescent in situ hybridization (FISH) with original DNA probes specific for the variable heterochromatin regions of chromosomes 1, 9, and 16 [10, 24, 35, 42]. Fluorescent signals were detected using a fluorescence microscope fitted with the appropriate set of filters and a computerized image analysis system. Quantitative analysis of hybridization signals and comparison of chromosomal variants in the mothers of children with autism and the control group were performed using an original rapid quantitative FISH protocol [24, 42, 43].

Data were analyzed statistically using the Pearson χ^2 and Student's *t* tests, which are standard in medical-biological investigations.

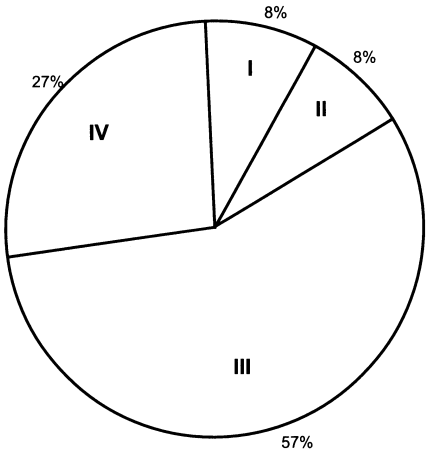

Fig. 1. Incidences of chromosomal anomalies, variants, and their combinations in the mothers of children with autism. I) Karyotypes with chromosomal anomalies without chromosomal variants; II) karyotypes with chromosomal anomalies and variants; III) karyotypes with chromosomal variants; IV) normal karyotypes (without chromosomal anomalies and variants).

RESULTS AND DISCUSSION

Cytogenetic and molecular-cytogenetic investigations identified 16 (17.8%) mothers of children with autism who had chromosomal anomalies, as shown in Table 1.

These results show that 15 mothers had mosaic forms of syndromes associated with anomalies in the sex chromosomes, including Shereshevskii–Turner syndrome, trisomy of chromosome X, and additional marker chromosomes also originating from the X chromosome. In addition, one mother had a chromosome 21 duplication in the q21 region present in all cells (see Table 1, No. 3), while another had monosomy of chromosome 18 in one out of five mosaic clones (see Table 1, No. 13). Anomalous clones were present in small proportions of cells – from 4% to 25%. In one case, a chromosome anomaly (see Table 1, No. 4) present in both the mother and the child showed chromosomal instability, apparent as additional nonspecific translocations and microdeletions of different chromosomes. It should be noted that in half of the 16 cases of chromosomal anomalies also showed chromosomal variants (see Table 1, Nos. 2, 8, 9, 10, 13, 14, 15, 16; Fig. 1), while chromosomal anomalies in three cases were identical in the mothers and children (see Table 1, Nos. 7, 13, 16). Chromosomal instability identical in mothers and children in the form of nonspecific monosomies, translocations, and microdeletions were seen in two cases, one of which had a normal karyotype. Changes in the sizes of heterochromatin juxtacentromeric regions of chromosomes (extreme increases or decreases, as well as inversions) were found in 62 (64.6%) mothers (including eight mothers with chromosome anomalies). Combinations of variants were seen in 22 cases and the maximum number of variants in an individual was five. The mean number of variants per individual was 1.58. The numbers of and types of variants of individual chromosomes and groups are shown in Table 2.

The data presented in Table 2 and Fig. 2 show that the largest proportion of variants was associated with chromosome 9 (17%, or, including combined variants, 37.8%) and chromosome 1 (12%, or, including combined variants, 25.5%). In combined form, chromosomal variants were seen in 35% of cases. Chromosomal anomalies were not

TABLE 1. Results of Cytogenetic and Molecular Cytogenetic Studies of Mothers of Children with Autism

No.	Age, years	Indications for investigation	Karyotype
		Mothers of children chromosomal anomalies and chromosomal variants	
1	32 (25*)	mother of child with autism	47,XXX/46,XX
2	47 (35*)	mother of child with an early form of autism	45,X,1qh-,9phqh,16qh-/46,XX,1qh-,9phqh,16qh-
3	29 (21*)	mother of child with an early form of autism	46,XX,dup(21)(q21q21)
4	27 (23*)	mother of child with speech delay to 4 years, autism, and preserved intellect; the mother had dysfunctional ovaries	45,X/46,XX chromosomal instability observed
5	32 (28*)	mother of child with an early form of autism	45,X/47,XX,+mar(der(X)/46,XX
6	42 (26*)	mother of child with autism and mental retardation	47,XXX/45,X/46,XX
7	35 (31*)	mother of child with DPLD** and autism	45,X/46,XX
8	31 (22*)	mother of child with autism	45,X,9phqh/ 47,XXX,9phqh/46,XX,9phqh
9	32 (28*)	mother of child with autism	45,X,9ph/47,XXX,9ph/48,XXXX,9ph/46,XX,9ph
10	29 (25*)	mother of child with an early form of autism; the mother was tall, had microcephaly, and a small mandible	45,X,1phqh/46,XX,1phqh
11	35 (27*)	mother of child with an early form of autism	47,XXX/46,XX
12	33 (29*)	mother of child with autism	45,X/46,XX
13	35 (29*)	mother of child with an early form of autism	45,X,22pstk+/47,XX,+18,22pstk+/ 47,XXX,22pstk+/45,XX,-18, 22pstk+/46,XX,22pstk+
14	27 (22*)	mother of child with DPLD and autism	45,X,1phqh/46,XX,1phqh
15	29 (25*)	mother of child with an atypical form of autism	45,X,21pss/46,XX,21pss
16	37 (33*)	mother of child with DPLD and autism	45,X,9qh-/47,XXX,9qh-/46,XX,9qh-
		Mothers of children with chromosomal variants but no chromosomal anomalies	
17	30 (28*)	mother of child with autism and DMA***	46,XX,1qh-
18	27 (21*)	mother of child with DPLD and autism	46,XX,1phqh
19	27 (23*)	mother of child with DPLD and early autism	46,XX,1phqh
20	38 (29*)	mother of child with DPLD and autism	46,XX,1phqh
21	34 (29*)	mother of child with DPLD and autism	46,XX,1phqh
22	27 (22*)	mother of child with an early form of autism	46,XX,1phqh
23	34 (27*)	mother of child with mental retardation and autistic features	46,XX,1phqh
24	33 (27*)	mother of child with autism, DPLD, and DMA	46,XX,1phqh
25	29 (22*)	mother of child with autism	46,XX,1phqh
26	30 (22*)	mother of child with autism	46,XX,9qh+
27	40 (35*)	mother of child with autism	46,XX,9ph

TABLE 1. Continued

No.	Age, years	Indications for investigation	Karyotype
28	30 (25*)	mother of child with autism	46,XX,9qh-
29	47 (23*)	mother of child with Asperger's syndrome	46,XX,9qh-
30	31 (27*)	mother of child with autism	46,XX,9phqh
31	37 (30*)	mother of child with autism	46,XX,9phqh
32	35 (27*)	mother of child with Asperger's syndrome and anomalies of the hands	46,XX,9qh-
33	32 (25*)	mother of child with DMD**** and autism	46,XX,9qh+
34	30 (27*)	mother of child with DMD and autism	46,XX,9phqh
35	30 (26*)	mother of child with DMD and autism	46,XX,9phqhqh+
36	31 (26*)	mother of child with DMD and autism	46,XX,9phqh
37	26 (23*)	mother of child with an early form of autism	46,XX,9phqh
38	27 (22*)	mother of child with autism and a brain abnormality	46,XX,9qh+
39	43 (36*)	mother of child with autism and mental retardation	46,XX,9phqh
40	40 (34*)	mother of child with DPLD and autism	46,XX,16qh-
41	41 (33*)	mother of child with DPLD and autism	46,XX,16qh-
42	28 (25*)	mother of child with DPLD and autism	46,XX,16qh-
43	24 (21*)	mother of child with autism and DMA	46,XX,16qh-
44	35 (30*)	mother of child with autism	46,XX,13pstk+
45	32 (26*)	mother of child with DPLD and autism	46,XX,13pstk+
46	40 (38*)	mother of child with autism	46,XX,15cenh+
47	25 (21*)	mother of child with an early form of autism	46,XX,15phqh
48	25 (19*)	mother of child with behavioral abnormalities	46,XX,20ph+
49	26 (17*)	mother of child with autism	46,XX,21ps+
50	35 (32*)	mother of child with mental retardation and autism	46,XX,1phqh,9phqh
51	24 (20*)	mother of child with autism	46,XX,1phqh,9phqh,16qh-
52	48 (45*)	mother of child with DPLD and autism	46,XX,1phqh,9phqh,9phqh
53	38 (33*)	mother of child with an early form of autism	46,XX,1phqhqh-,9qh-,21pstk+
54	35 (29*)	mother of child with autism	46,XX,1qh-,9qh-,15pss,16qh-
55	32 (23*)	mother of child with autism; the mother had chronic pyelonephritis, squint, long-sightedness, obesity, microcephaly (studying at special school for disabled children)	46,XX,1phqh,16qh-

TABLE 1. Continued

No.	Age, years	Indications for investigation	Karyotype
56	39 (36*)	mother of child with an early form of autism, hydronephrosis, delayed physical development; the mother had squint, signs of premature aging, hirsutism on the hands, and congenital heart disease	46,XX,1phqh,15cenh+
57	39 (34*)	mother of child with an early form of autism	46,XX,1qh-,22cenh+
58	36 (25*)	mother of child with an early form of autism, facial abnormalities, and microcephaly	46,XX,1phqh,12cenh+
59	33 (27*)	mother of child with DPLD and autism	46,XY,1phqh,9phqh,13pss, 14pss,17ph+
60	29 (23*)	mother of child with autism	46,XY,1phqh,14pstk+,15pss
61	31 (26*)	mother of child with an early form of autism	46,XX,1phqh,9phqh
62	34 (27*)	mother of child with DPLD and autism	46,XX,9phqh,16qh+
63	34 (27*)	mother of child with an early form of autism	46,XX,9phqh,9qh+,16qh-
64	34 (29*)	mother of child with an early form of autism	46,XX,9qh+,22pstk+
65	30 (23*)	mother of child with autism	46,XX,9ph,9phqh
66	31 (27*)	mother of child with an early form of autism and DMA	46,XX,9phqh,9qh+
67	37 (27*)	mother of child autism and DMA; the mother had decreased intellect and asymmetrical occlusion	46,XX,9phqh,13pstk+ps+
68	33 (29*)	mother of child with an early form of autism	46,XX,9qh-,16qh-
69	36 (32*)	mother of child with an early form of autism	46,XX,14pss,15pstk+
70	29 (25*)	mother of child with autism and DMA	46,XX,15phqhcenh+

Notes. *mother's age at birth of child; **DPLD is delayed psycholinguistic development; ***DMA is developmental microanomalies; ****DMD is delayed mental development.

seen in the control group, while chromosomal variants in the 1qh+ and 9qh+ forms were encountered in three (5.1%) of the 59 cases. The total incidences of chromosomal variants in the control and study groups were significantly different $p < 0.05$).

Molecular-cytogenetic studies of chromosome variants using chromosome 1 as an example (Fig. 3) completely supported the data obtained during the cytogenetic analysis and also allowed DNA copy numbers to be assessed when the juxtacentromeric block of chromosome 1 was enlarged. DNA probes for α and classical satellite DNA showed that increases in juxtacentromeric heterochromatin were associated with the latter, while variations in the copy numbers of the two types of DNA were independent of each other.

The incidences of mental and other diseases were analyzed in the mothers of children with autism (Table 3). This revealed CNS pathology in 27 (28%) of the 96 mothers. Mental pathology was detected in 19 (20%) of mothers. Autistic personality traits were found in five (5%) and cognitive impairments in nine (8%) mothers. Furthermore, neurotic reactions were seen in six (6%) cases, migraine in three (3%), and multiple sclerosis, sensorineural deafness, and retinal dystrophy each in one (1%) case. There was a tendency to an increase in the incidence of spontaneous abortions, which were seen in 23 (24%) women. It should be noted that 33 of 96 mothers had had contact with mutagenic factors (chemical mutagens, radionuclides, UHF fields).

Analysis of diseases in the genealogies of all the mothers of children with autism demonstrated that 1991 relatives had various degrees of cognitive impairments, schizophrenia, autism, and undifferentiated mental disorders, though the incidence was not significantly different from that in the overall population, at 3% (see Table 3). Relatives had a high incidence of oncological disease – 45 (2.3%) relatives of mothers of children with autism had oncological disease, while WHO figures indicate that the average European incidence of oncological pathology is five times lower.

Studies of correlations between genotypes and phenotypic signs were performed by analysis of health in 96

TABLE 2. Chromosomal Variants (including combined) Detected in the Mothers of Children with Autism and Their Incidences

Chromosome group	Chromosome	Chromosomal variants		
		Types	total number by individual chromosomes* (frequency, %)	total number by group* (frequency, %)
A	1	qh-; qh+; phqh	25 (25.5)	25 (25.5)
C	9	qh-; qh+; phqh; ph	37 (37.8)	38 (38.8)
	12	cenh+	1 (1)	
D	13	pstk+; ps+; pss	5 (5.1)	16 (16.3)
	14	pstk+; pss	3 (3.1)	
	15	pstk+; pss; cenh+; phqh	8 (8.1)	
E	16	qh-; qh+	11 (11.2)	12 (12.2)
	17	ph+	1 (1)	
F	20	ph+	1 (1)	1 (1.0)
G	21	pstk+; ps+; pss	3 (3.1)	6 (6.2)
	22	pstk+; cenh+	3 (3.1)	
Total	11	26	98 (100)	98(100)

Note. *Counting of the frequencies of heteromorphism of individual chromosomes also included combined chromosomal variants.

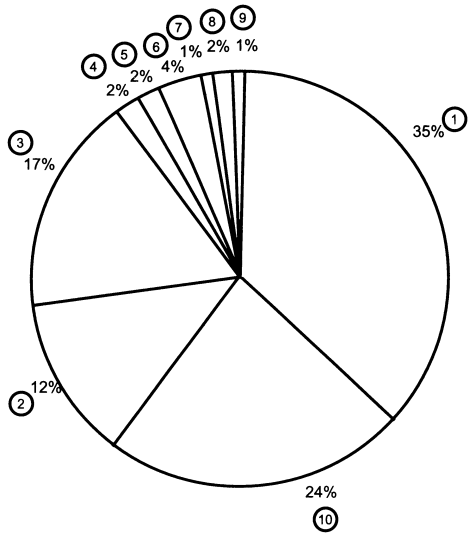

Fig. 2. Proportions of each chromosomal variants in the mother of children with autism. *1*) Karyotypes (without chromosomal variants); *2*) chromosome 1; *3*) chromosome 9; *4*) chromosome 13; *5*) chromosome 15; *6*) chromosome 16; *7*) chromosome 20; *8*) chromosome 21; *9*) chromosome 22; *10*) combined variants.

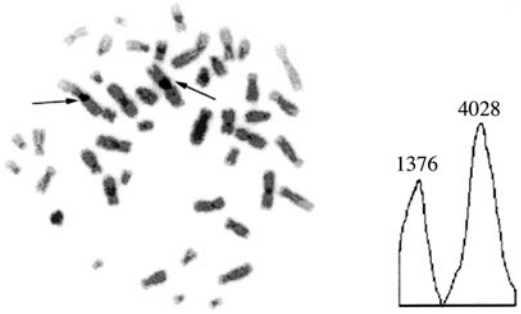

Fig. 3. Quantitative FISH in heteromorphism of the juxtacentromeric heterochromatin region of chromosome 1 (1qh+). Arrows show homologous chromosomes 1, one of which (right) has an enlarged block of pericentromeric heterochromatin (a chromosomal variant). The ratio of the intensities of the two signals (1376 and 4028 pixels) provides evidence of an approximately three-fold difference in the DNA contents of these regions of chromosome 1.

mothers of children with autism and health data from 1991 of their relatives. The mothers were divided into three groups: those with chromosomal variants (n = 54), those with chromosomal anomalies (n = 16), and those with normal karyotypes without chromosomal variants (n = 26). Relatives of mothers with chromosomal variants included 1115 with normal karyotypes, 527 with normal karyotypes without chromosomal variants, and 349 with chromosomal anomalies.

Analysis of correlations between the genotypes of mothers of children with autism and their phenotypic traits showed that mothers with chromosomal anomalies had a three-fold greater incidence of cognitive impairments, a 1.5-fold higher incidence of spontaneous abortions, and an almost four-fold greater incidence of endocrine abnormalities (for example, obesity) as compared with women of other groups. Twice as many mothers with chromosomal anomalies had been exposed to harmful factors such as UHF fields and hyperthermia as in other groups (Table 4).

The relatives of mothers with chromosomal anomalies, as compared with the other groups, had a five-fold higher incidence of intellectual impairments, while the incidence

TABLE 3. Analysis of Diseases in the Relatives (n = 1991) of Mothers of Children with Autism

Pathological states	Number of cases	
	abs.	%
CNS pathology:	**60**	**3.15**
mental retardation	8	0.4
autism	6	0.3
schizophrenia	4	0.2
epilepsy	5	0.3
undifferentiated mental illness	8	0.4
stammering	5	0.3
migraine	2	0.1
delayed speech development	4	0.2
limited intellect	11	0.6
asocial behavior	3	0.15
suicides	4	0.2
Other pathology:	**73**	**11.85**
oncological diseases	45	2.3
death in childhood	15	0.8
sudden death	2	0.1
multiple developmental anomalies	3	0.15
cardiac abnormalities	2	0.1
testicular feminization syndrome	1	0.05
Stein–Leventhal disease	1	0.05
thyroid disease	4	0.2
Impairments to reproductive functions:	**17**	**10.8**
spontaneous abortions in grandmothers in the maternal line (n = 96)	10	10.4
stillbirths	7	0.4

of death in childhood was 4–5 times higher and that of spontaneous abortions among grandmothers in the maternal line was twice as high. However, developmental abnormalities were most frequently found in the pedigrees of mothers with chromosomal variants. At the same time, undifferentiated mental illness occurred twice as frequently in the group of mothers with the normal karyotype (Table 5).

Data showing increases in the incidences of cognitive impairments and spontaneous abortions in mothers of children with autism and chromosomal anomalies supported the occurrence of true mosaicism with respect to chromosome X aneuploidies in these mothers. Increases in the incidence of mental retardation, death in childhood, and impairments of reproductive functions (spontaneous abortions in the mothers of the women studied, infertility) in the pedigrees of mothers of children with autism and chromosomal anomalies may provide evidence of the segregation of cases of chromosomal pathologies in these families.

Published data indicate that the parents of children with autism have increased levels of anxiety states, including social phobias, depression, and manias, this applying to both mothers and fathers. The parents of children with autism and low levels of functioning were found to have higher levels of mental illnesses than the parents of children with autism and high levels of functioning. These results support the hypothesis that there are borderline autistic states in the parents and siblings of children with autism [1, 31, 33, 34].

The heritability of autistic disorders is not doubted; on the other hand, the genes responsible for idiopathic autism have not been identified. Despite the non-uniform gender ratio (males:females = 4:1), no data have been obtained supporting X-linked loci or gender-restricted inheritance [29]. It should also be noted that in studies of twins and nuclear families, the well studied marker BAP (the BAP marker is associated with language abilities) showed no differences between adults of both genders. As high phenotypic variability and genetic heterogeneity are seen in this disease, the following are needed: 1) accurate identification of the phenotype with consideration of the wide spectrum of autistic disorders, with differential diagnosis against other diseases in this spectrum (for example, Rett's syndrome); 2) detailed cytogenetic analysis of each individual with autistic disorders, including analysis of juxtacentromeric heterochromatin.

On assessment of the cytogenetic findings, it should be noted that heterochromatin juxtacentromeric regions of chromosomes consist of blocks of highly repeated DNA sequences whose major property is complete transcriptional inactivation. These chromosomal regions are believed to be inherited codominantly and not to contain active genes. Nonetheless, the presence of chromosomal variants with

TABLE 4. Analysis of Diseases in the Mothers (n = 96) of Children with Autism in Relation to Cytogenetic Abnormalities

Pathological state	Group of mothers					
	with chromosomal variants (n = 54)		with normal karyotypes without chromosomal variants (n = 26)		with chromosomal anomalies (n = 16)	
	abs.	%	abs.	%	abs.	%
CNS pathology:						
cognitive impairments	3	5.5	2	8	4	25
autistic personality features	3	5.5	1	4	1	6
neurotic reactions	4	7	2	8	0	—
migraine	1	2	1	4	1	6
multiple sclerosis	1	2	0	—	0	—
sensorineural deafness	0	—	1	—	—	—
Other pathology:						
DMA	5	9	4	15	2	12.5
developmental anomalies	1	2	—	—	—	
thyroid disease	7	13	2	8	—	—
obesity	3	5.5	0	—	3	19
Reproductive impairments:						
spontaneous abortions	11	20	6	23	5	31
Stein–Leventhal disease	0	—	0	—	1	6
Mutagenic factors:						
physical mutagens*	14	26	4	15	9	56
chemical mutagens	5	9	0	—	1	6.5

Note. * UHF fields and hyperthermia.

increased and decreased juxtacentromeric heterochromatin regions in the chromosomes of patients with autism may be linked with particular impairments to the functional activity of genes located in the immediate vicinity of the variable heterochromatin regions of the chromosomes (the so-called "gene position effect") [6, 9]. Our data show that about 70% of the chromosomal variants detected in children with autism were inherited from their mothers. This indicates that the chromosomal variant in the mother can be regarded as a marker for a group of parents with a risk of having children with autism. It should be noted that our studies showed an increase in the incidence of cases with combined chromosomal variants in the mothers of children with autism (24% of all mothers of children with autism, compared with 35.5% in mothers with chromosomal variants). This is evidence that in children, unlike the situation in mothers, complex derangements of the functional activity of genes located close to the heterochromatin regions of different chromosomes may be associated with pathological processes underlying the phenotypic manifestations of autism. Current molecular genetic studies of autism have developed a range of high-resolution techniques, and use of large cohorts may produce stable results in relation to gene activity.

Analysis of the relationship between the severity of phenotypic manifestations and the frequency and type of chromosomal variants in children showed that variability of the juxtacentromeric heterochromatin was characteristic of the milder forms of illness [5]. This is probably evidence that severe forms of autism in children are associated with more serious genetic lesions [6, 11, 12, 14, 15, 25–28, 41, 43] or the adaptive role of chromosomal variants in different diseases [3, 8]. This latter may also apply to the mothers of children with autism, where chromosomal variants were seen without defining clinical signs.

In conclusion, it should be noted that detailed cytogenetic studies are recommended for all children with autistic disorders, particularly if they also have mental retardation, EEG anomalies, or convulsions, muscle hypotonia, severe motor disorders, and signs of minor developmental anomalies [7]; the mothers should also be studied. As autistic conditions are largely genetically determined, the detection of chromosomal anomalies as a probable cause of autistic disorders is very significant in genetic consultations.

The detection of cytogenetic anomalies and the monogenic nature of diseases associated with autism demonstrate their genetic heterogeneity and different patterns of inheritance in individual families. In the case of idiopathic autism, i.e., cases with unknown causes, monogenic, polygenic, and multifactorial mechanisms of inheritance have been proposed. We suggest that studies of autistic disorders require autistic conditions to be addressed both in the context of gene interactions and epigenetic mechanisms [4, 13, 28].

As the incidence of autistic disorders in the population is high and the genetic mechanisms and the mechanisms by which the phenotype forms are very diverse, genetic consultation for medical geneticists is a very complex process in

TABLE 5. Analysis of Diseases in Relatives (n = 1991) of Mothers of Children with Autism in Relation to Cytogenetic Changes Detected in Mothers

Pathological state	Group of mothers					
	with chromosomal variants (n = 1115)		with normal karyotypes and no variants (n = 527)		with chromosomal anomalies (n = 349)	
	abs.	%	abs.	%	abs.	%
CNS pathology:						
schizophrenia	3	0.3	1	0.2	—	
autism	2	0.2	3	0.6	1	0.3
mental retardation	2	0.2	1	0.2	4	1.1
"limited" intellect	3	0.3	2	0.4	7	2
delayed speech development	2	0.2	1	0.2	1	0.3
asocial behavior	2	0.2	1	0.2	0	—
suicides	2	0.2	2	0.4	0	—
undifferentiated mental illness	3	0.3	4	0.7	1	0.3
other CNS diseases	6	0.7	5	1	3	0.8
Other pathology:						
oncological diseases	27	2.4	10	1.9	8	2.3
developmental anomalies	4	0.4	1	0.2	0	—
death in childhood	6	0.5	2	0.4	7	2
Reproductive impairments:						
spontaneous abortions in maternal grandmothers (n = 96)	4	0.4	2	0.4	4	1.1
stillbirths	5	0.4	1	0.2	1	0.3
infertility	0	—	0	—	2	0.7

autism. Detailed investigations of children and their families are required in order to exclude all known inherited syndromes. The aim of genetic consultation in autism, as in other diseases, is to provide families and children with complete information and to establish the risk of further disease. While some diseases have known genetic causes, measures of further risk may be different for each individual autistic condition. For dominant monogenic diseases with full penetrance (for example, tuberous sclerosis), the risk for siblings is 50%. In the case of recessive monogenic disease, such as Lemli–Opitz syndrome, the risk for siblings is 25%. If a child has FRAXA syndrome, the risk of disease for his or her brother is 50%, while 50% of sisters will be carriers and/or have subtle clinical manifestations of the disease. Results obtained from studies of families with idiopathic autistic disorders establish the risk of disease in siblings in 5% (from 2–8%) of cases [14, 20]. On the other hand, when chromosomal anomalies such as duplications or duplicated inversions of chromosome 15 in region q11-q13 are present, the risk of further disease essentially coincides with the population incidence, as most duplications and inversions arise de novo during meiosis [41]. There is no doubt that our data on the presence of chromosomal anomalies and variants in the mothers of children with autism show that they can be regarded as genetic markers of disease and demonstrate the need for more detailed genetic studies of a whole family [11, 15, 28]. Help in adapting families and lives to the disease are also important in genetic consultation.

The following conclusions arise from these results and discussion:

1) there was a high incidence of chromosomal anomalies in the mothers of children with autism, mainly as mosaic forms involving the X chromosome;

2) there was a higher incidence of chromosomal heteromorphism (chromosomal variants) in the mothers of children with autistic disorders than in the control group;

3) identification of genomic changes in affected children and their mothers is important for identifying genetic and neurobiological markers for autistic disorders;

4) analysis of correlations between phenotypes and genotypes revealed an increased incidence of cognitive impairments and spontaneous abortions in the mothers of children with autism with chromosomal anomalies, along with an increased incidence of mental retardation, death in childhood, and impaired reproductive function in pedigrees of these women as compared with the other groups of mothers. At the same time, there was a high incidence of developmental abnormalities in the pedigrees of mothers with chromosomal variants; and

5) cytogenetic and molecular cytogenetic studies of mothers and children with autism should be regarded as obligatory for detecting possible genetic causes of autism

and for genetic counseling of families including children with autistic disorders.

This study was supported by the Russian Humanities Scientific Foundation (Project No. 060600639a, RF).

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