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NON-DISJUNCTION OF CHROMOSOME 21, ALPHOID DNA VARIATION, AND SOCIOGENETIC FEATURES OF DOWN SYNDROME

The analysis of non-disjunction of chromosome 21 and alphoid DNA variation by using cytogenetic and molecular cytogenetic techniques (quantitative fluorescence in situ hybridization) in 74 nuclear families was performed. The establishment of possible correlation between alphoid DNA variation, parental age, environmental effects, and non-disjunction of chromosome 21 was made. The efficiency of techniques applied was found to be 92 % (68 from 74 cases). Maternal non-disjunction was found in 58 cases (86 %) and paternal non-disjunction — in 7 cases (10 %). Post-zygotic mitotic non-disjunction was determined in 2 cases (3 %) and one case was associated with Robertsonian translocation 46,XX,der(21;21)(q10;q10),+21. Maternal meiosis I errors were found in 43 cases (64 %) and maternal meiosis II errors — in 15 cases (22 %). Paternal meiosis I errors occurred in 2 cases (3 %) and paternal meiosis I errors — in 5 cases (7 %). The lack of the correlation between alphoid DNA variation and non-disjunction of chromosome 21 was established. Sociogenetic analysis revealed the association of intensive drug therapy of infectious diseases during the periconceptual period and maternal meiotic non-disjunction of chromosome 21. The correlation between non-disjunction of chromosome 21 and increased parental age as well as exposure to irradiation, alcohol, tobacco, mutagenic substances was not found. The possible relevance of data obtained to the subsequent studies of chromosome 21 non-disjunction is discussed.

Introduction. Trisomy of chromosome 21 is a leading cause of mental retardation and congenital malformations in humans occurring in about 1 in 800 live births [1]. The aetiology of clinical entity referred as Down syndrome was found to be trisomy 21 more than 45 years ago [2]. The current trends in genetics of Down syndrome are focused on the investigation of parental origin of trisomy 21 and its possible association with the environmental effects such as pollution, exposure to alcohol, tobacco, and other mutagens as well as parental age. The recognized mechanism of trisomy formation associated with clinically distinct trisomy syndromes (such as Down syndrome) is non-disjunction of chromosomes or sister chromatids due to the errors occurring during meiosis. To date non-disjunction of chromosome 21 in Down syndrome was studied more than in 1500 families [3—5]. Although these studies have brought new insights in our comprehension of molecular mechanisms underlying Down syndrome there is still no clear understanding concerning genetic and environmental causes of trisomy 21.

Human chromosome 21 is characterized by increased variability of DNA content within short arm as well as centromeric heterochromatin; it is the special case for alphoid satellite DNA [6—9]. This fact was used as the basis of the hypothesis suggesting that variation of alphoid DNA may be related to non-disjunction of chromosome 21 [10]. Subsequent studies of the correlation between these two phenomena have yielded conflicting results [11, 12]. Therefore, it is still of great interest to test this hypothesis in a larger cohort of families with Down syndrome offspring.

Here, we have studied the alphoid DNA variation and non-disjunction of chromosome 21 by means of cytogenetic technique and qualitative as well as quantitative fluorescence *in situ* hybridization (FISH) in 74 families with children affected by Down syndrome. A study for establishing the correlation between non-disjunction of chromosome 21, environmental effects, and parental age was made as well.

Materials and methods. Seventy four nuclear families with children affected by Down syndrome were subjected to cytogenetic, molecular cytogenetic, and sociogenetic analysis. All the families originated from different regions of Russian Federation. Written informed consent was obtained from the family members (mothers and fathers) for whom the studies were carried out.

Chromosomal preparations were obtained from blood lymphocytes of mothers, fathers, and Down syndrome affected children followed by metaphase chromosome preparations and routine cytogenetic analysis. All the procedures mentioned were performed according to standard protocols [13] with some modifications [14]. The morphological peculiarities of chromosome 21 were studied (specificity or presence of satellites, short arm (p) morphology) in order to determine the parental origin of chromosome 21 as described before [6, 12].

FISH was performed according to the previously described protocols of hybridization and detection [8, 9, 15—17]. The probe from the original collection of DNA probes marking alphoid DNA of chromosomes 13 and 21 were applied [8, 17, 18]. In order to exclude or confirm mosaicism for trisomy 21, less than 100 metaphase spreads and 300 interphase nuclei were scored. Semi-quantitative assessment of alphoid DNA variation of chromosome 21 was performed as described by Verma et al. [19]. Quantitative FISH assessment of chromosome 21 heteromorphisms for identification of non-disjunction origin and meiotic stage was performed according to the protocol previously described in details [20].

The sociogenetic features were assessed by interviewing the parents of Down syndrome affected children using the extended questionnaire including the questions concerning periconceptional period (infectious diseases; drug therapy; exposure to alcohol, tobacco, and substances with known mutagenic effect). The special attention was paid to occasional or professional exposure to irradiation (Sperling K. and Pelz J., written communication, 2003).

Results and discussion. Routine cytogenetic analysis has indicated 71 children to have an additional chromosome 21. The parents of children with Down syndrome except one mother were characterized by normal karyotype. One family was characterized by 45,XX,der(13;14)(q10;q10) karyotype in mother and 46,XX,der(13;14)(q10;q10),+21 karyotype in daughter. Case report was described in one of the previous issues of this journal [21]. In one infant with Down syndrome cytogenetic analysis revealed the combined trisomy of chromosome 21 and disomy of chromosome Y (48, XYY,+21). In another Down syndrome affected child the 46,XX,der(21;21)(q10;q10),+21 karyotype was found. FISH studies have confirmed the data of cytogenetic analysis in 72 children and their parents and have indicated the occurrence of mosaicism for trisomy of chromosome 21 in two infants with 86 and 75 % of trisomic clone content.

Cytogenetic analysis of chromosome 21 heteromorphism was found to be effective in 47 (64 %) of the 74 cases for identification of non-disjunction parental and meiotic origin. Fig. 1 demonstrates the

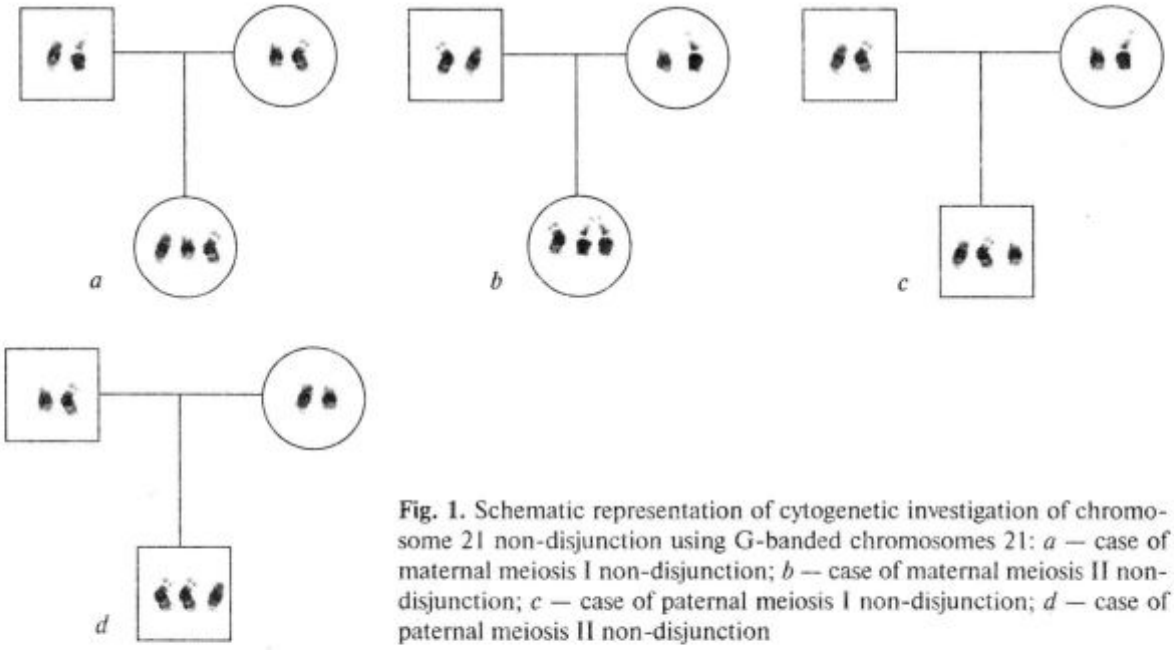

Fig. 1. Schematic representation of cytogenetic investigation of chromosome 21 non-disjunction using G-banded chromosomes 21: *a* — case of maternal meiosis I non-disjunction; *b* — case of maternal meiosis II non-disjunction; *c* — case of paternal meiosis I non-disjunction; *d* — case of paternal meiosis II non-disjunction

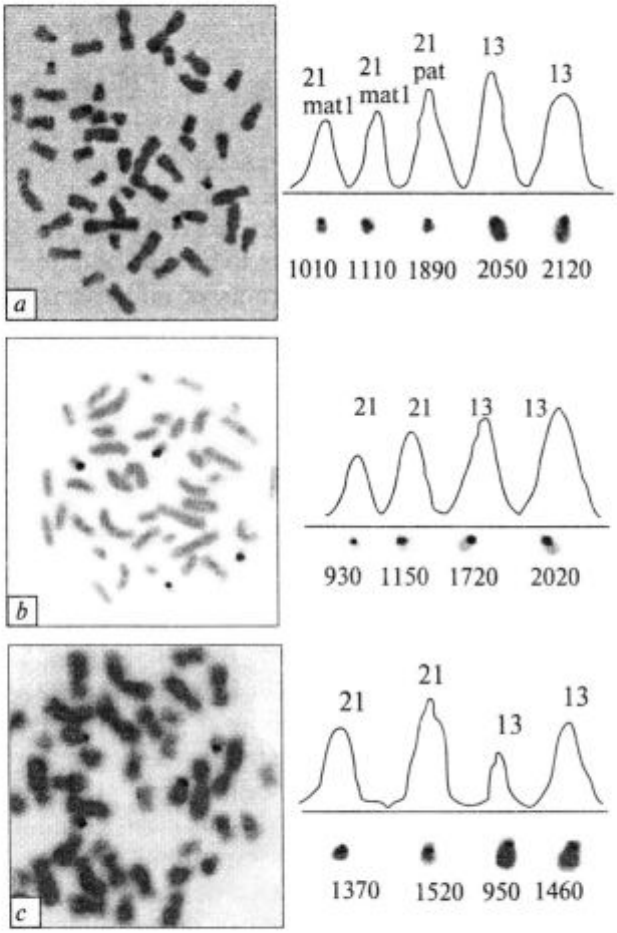

Fig. 2. Identification of chromosome 21 non-disjunction in a family with Down syndrome affected child by quantitative FISH: *a* — quantification of FISH signal intensities for chromosomes 13 and 21 in affected child; *b* — quantification of FISH signal intensities for chromosomes 13 and 21 in mother; *c* — quantification of FISH signal intensities for chromosomes 13 and 21 in father. The comparative analysis of the FISH signal intensity ratios allowed us to conclude the non-disjunction of chromosome 21 to occur in maternal meiosis I as two additional chromosomes 21 in child have practically the same signal intensity

schematic representation of this analysis. Among informative cases, 37 (79 %) were found to be due to the non-disjunction of maternal origin and 10 (21 %) cases — of paternal origin. The assessment of meiotic non-disjunction origin have indicated errors to occur in 24 (51 %) cases in maternal meiosis I, 6 (13 %) cases — maternal meiosis II, 5 (11 %) cases — paternal meiosis I, 2 (4 %) cases — paternal meiosis II. Seven (15 %) cases of maternal non-disjunction and 3 (6 %) cases of paternal non-disjunction were uninformative for determining the meiotic origin.

For qualitative assessment of alphoid DNA variation FISH signals for chromosome 21 were divided into five classes according to their size compared with the length of short arm of chromosome 18. The lack of signal for chromosome 21 corresponded to 0 point, very small signal — 1 point, small signal — 2 points, medium signal — 3 points, large signal — 4 points, very large — 5 points [19]. This approach allowed identifying of alphoid DNA variation in all the members of nuclear families. Among 222 individuals studied the lack of signal for chromosome 21 was observed only in one mother. Chromosomes 21 with very large FISH signal corresponding to the length of the chromosome 18 short arm were not detected at all. For the evaluation of possible alphoid DNA contribution to non-disjunction of chromosome 21 the mean values of alphoid DNA sizes in three groups (affected children, mothers, and fathers) were defined. Surprisingly, all three mean values of alphoid DNA sizes in these groups (children, mothers and fathers) were equal (numerical value — 2.2). These data were then reconfirmed by quantitative FISH assay using digital analysis of microscopic image. The data obtained indicates that not only there is any correlation between chromosome 21 non-disjunction and alphoid DNA size variation but also that the variation of chromosome 21 alphoid DNA is generally the same for Down syndrome affected as well as unaffected population.

Qualitative FISH assessment of alphoid DNA variation was found to be effective in 41 (55 %) of 74 cases for identification of parental and meiotic origin of chromosome 21 non-disjunction. Among informative cases, 34 (83 %) were found to be due to the non-disjunction of maternal origin and in 7 (17 %) cases the non-disjunction was of paternal origin. Non-disjunction in maternal meiosis I was observed in 23 (56 %) cases and maternal meiosis — in 7 (17 %) cases. In 4 (10 %) cases of maternal non-disjunction the analysis has not provided us with the information about the meiotic origin of non-disjunction (semi-informative cases). In cases of trisomy 21 found to originate due to errors in paternal meiosis one (2 %) case was found to be due to the error occurred in meiosis I and four (10 %) cases — in meiosis II. In two (5 %) cases of paternal non-disjunction the analysis was semi-informative (the non-disjunction meiotic origin was undeterminable). As it was mentioned above two cases

of Down syndrome were found to be mosaic for trisomy 21. Therefore, these two cases were attributed to post-zygotic mitotic non-disjunction.

Quantitative FISH analysis performed by means of digital capturing of microscopic pictures followed by quantification of relative intensities of FISH signals and its ratio comparison [20] was used for confirmation of cytogenetic and qualitative FISH studies of chromosome 21 non-disjunction (Fig. 2). The efficiency of this technique was found to be 80 % (59 of 74 cases). In major part of families studied quantitative FISH confirmed previous data. However, in a number of cases the quantitative FISH approach has provided different data concerning meiotic origin of non-disjunction from cytogenetic and qualitative FISH analyses. Due to the highest efficiency of quantitative FISH detected the results obtained by this technique were considered as ultimate. In addition quantitative FISH allowed the determination of non-disjunction origin and meiotic stage in 12 cases previously classified as uninformative or semi-informative. The data obtained have led us to conclude that quantitative FISH application for identification of parental and meiotic origin of trisomy 21 can be applied directly after cytogenetic study skipping the qualitative FISH analysis.

Taking into account the data obtained the overall efficiency of the techniques applied for the study of chromosome 21 non-disjunction was as effective as 92 % (68 of 74 cases). The combined data on the non-disjunction of maternal origin was indicated the errors to occur during maternal meiosis I in 43 (64 %) of 68 informative cases and maternal meiosis II — in 15 cases (22 %). In 7 (10 %) of 68 informative cases the non-disjunction was of paternal origin identified to occur during paternal meiosis I in 2 (3 %) cases and paternal meiosis II in 5 (7 %) cases. Two cases (3 %) were characterized by post-zygotic mitotic non-disjunction. One case (1 %) was found to be caused by Robertsonian translocation 46,XX,der(21;21)(q10;q10),+21. Quantitative FISH analysis allowed us to conclude that two chromosomes 21 involved in Robertsonian translocation were of maternal origin. The summary of chromosome 21 non-disjunction analysis performed in the present study is shown in Fig. 3. It should be noted that independent quantitative fluorescent PCR study of non-disjunction of chromosome 21 in 31 families from the group

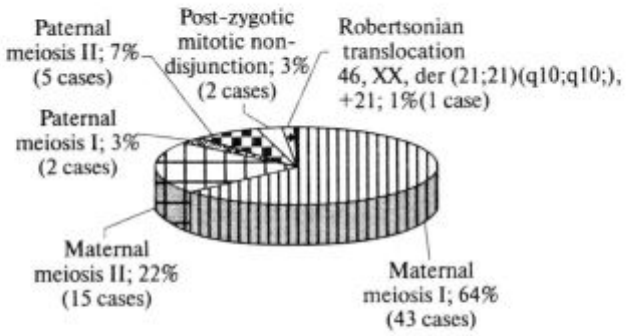

Fig. 3. Meiotic and parental origin of trisomy 21 in the nuclear families studied

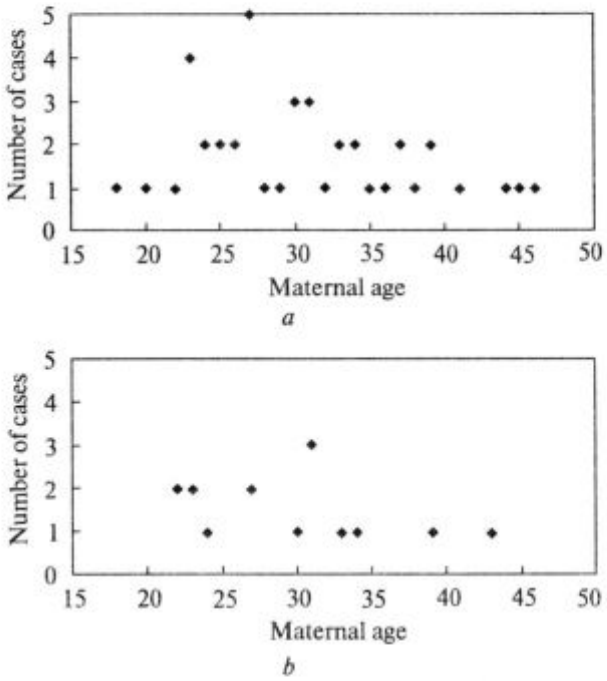

Fig. 4. Maternal age and trisomy 21 due to non-disjunction in maternal meiosis I (*a*) and II (*b*). This demonstrates the lack of the correlation between meiotic maternal non-disjunction of chromosome 21 and maternal age

presently studied have completely confirmed our results obtained by means of cytogenetic and molecular cytogenetic techniques [22]. The data on non-disjunction of chromosome 21 obtained in the present study have been found to be in accordance with previous comprehensive studies of chromosome 21 non-disjunction [3—5, 22].

The meiotic process is very sensitive to endogenous and exogenous factors. Parental age; exposure to alcohol, tobacco, and other mutagenic substances; exposure to irradiation are among the

recognized effects associated with non-disjunction of chromosome 21 [5, 22, 23]. In the present study the sociogenetic features of Down syndrome were assessed in order to establish the correlation between parental age, environmental effects, and non-disjunction of chromosome 21. The analysis of maternal age effect is shown in Fig. 4. Our data indicate that maternal age varied from 18 to 46 years (mean age 30 years) in families with children having trisomy 21 due to maternal non-disjunction. The analysis has indicated the lack of correlation between maternal age and non-disjunction due to errors in maternal meiosis I and II. However, a large proportion of previous studies stated the strict correlation between increase of chromosomal trisomy frequency and maternal age [5]. The lack of this correlation may be attributed to the specifity of the group analyzed. However, the possibility of determination of such correlation in a larger group of families with Down syndrome offspring from a smaller geographic area should not be excluded. Among the cases of paternal non-disjunction of chromosome 21 the paternal age varied from 23 to 54 years (mean age 36 years). The correlation between paternal age and non-disjunction of chromosome 21 was not revealed as well. This is probably due to the lower amount of cases of paternal non-disjunction.

As it was mentioned a large number of environmental risk factors are supposed to have aetiological significance for Down syndrome [5, 22]. Occasional or professional exposure to irradiation is considered as one of the most important factors that might contribute to chromosomal non-disjunction. We have investigated the rate of occasional or professional exposure to irradiation of mothers in cases of maternal non-disjunction as well as fathers in cases of paternal non-disjunction. Interviewing of the parents has shown that there was not any professional exposure to the irradiaton. All the subjects informative for non-disjunction analysis have indicated that they were subjected to irradiation no more than five times in medical purposes only. This represents routine medical procedures known to be performed for general population of Russia. Therefore, our data indicate that there is no correlation between non-disjunction of chromosome 21 and exposure to irradiation.

Additional environmental effects supposed to make a possible contribution to trisomy formation are exposure to alcohol, tobacco, and substances with known mutagenic effects [5]. None of the members of the studied families was found to have alcoholic abuse. The majority of respondents have indicated the lack of alcohol exposure during periconceptual period and poor history of alcohol consumption. In 12 cases fathers have provided the information that they have frequently consumed alcohol before the conception. However, all these cases were characterized by trisomy 21 occurring due to maternal non-disjunction. Only in 4 (7 %) of 58 cases of maternal non-disjunction mothers recognized that their alcohol consumption during periconceptual period was more intensive than usually. This data indicates that the alcohol consumption may have only occasional effect on non-disjunction of chromosome 21. The smoking history of mothers of children with trisomy 21 due to maternal non-disjunction was estimated as poor. This data allowed us to conclude the lack of significant contribution of maternal tobacco smoking to chromosome 21 non-disjunction. Among cases of paternal chromosome 21 non-disjunction 4 of 7 fathers have classified themselves as regular smokers. However, due to low amount of cases the association between paternal non-disjunction and tobacco exposure could not be recognized as significant. The analysis of exposure to substances with known mutagenic effects in Down syndrome children parents revealed that only 3 (5 %) of 58 cases related to maternal non-disjunction may be associated with long-term history of subjection to mutagenic substance effect. All additional positive answers concerning exposure to mutagenic substances were obtained from fathers of children affected by trisomy 21 found to be associated with maternal non-disjunction. Therefore, neither professional nor occasional exposure to substances with mutagenic effects was detected to be related to trisomy 21 formation.

The analysis of effect of contraceptives as well as infectious disease drug therapy was carried out as well. All the respondents have not indicated the use of contraceptives during the periconceptual period. Among the cases of maternal non-disjunction 25 (43 %) of 58 respondents (mothers) have indicated to be affected by diagnosed infectious diseases during the periconceptual period and, therefore, all of them were subjected to intensive drug therapy (primarily antibiotics). The distribution of meiotic origin in this group was as follows: 2 cases

associated with maternal meiosis II non-disjunction and 23 cases — with maternal meiosis I non-disjunction. The data obtained indicate the possibility of intensive drug therapy of infectious diseases during the periconceptual period in mothers to be related aetiologically to non-disjunction of chromosome 21.

In conclusion of the study performed it should be noted that alphoid DNA variation is not associated with non-disjunction of chromosome 21. The combined application of cytogenetic and molecular cytogenetic techniques for chromosome 21 non-disjunction analyses was found to be as effective as 92 %. Taking into account the complete accordance of the analysis with independent quantitative fluorescent PCR study we conclude the combined use of cytogenetic and quantitative FISH non-disjunction assays to be suitable for identification of parental and meiotic origin of trisomy 21. Routine cytogenetic and molecular cytogenetic methods are considered as indispensable for postnatal diagnosis of chromosome abnormality. Therefore, the possibility of direct identification of chromosome 21 non-disjunction origin during routine cytogenetic and molecular cytogenetic diagnosis of Down syndrome can be considered as an advantage of the techniques proposed. It is noteworthy that it may be of additional significance for the case-control surveillance of factors supposed to contribute to trisomy 21 formation performed in laboratories aimed to diagnose chromosomal abnormalities. The examination of parental and meiotic origin of trisomy 21 carried out was found to be in accordance with most comprehensive studies targeting to bringing together the extended data on chromosome 21 non-disjunction [3—5]. The sociogenetic analyses have shown that strict correlation between exogenous as well as endogenous effects and trisomy 21 formation is only the case for intensive drug therapy of infectious diseases during the periconceptual period in mothers. Surprisingly, to the best of our knowledge this correlation was not previously described. Finally it should be emphasized that although we are still far from determination of molecular mechanisms underlying the formation of trisomy 21 the analysis of chromosome 21 non-disjunction and its association with environmental effects should not be abandoned in order to uncover the mystery of genesis of one of the most common human genetic disorder.

РЕЗЮМЕ. Проведен анализ нерасхождения хромосомы 21 и вариации альфоидной ДНК в 74 ядерных семьях с детьми, страдающими синдромом Дауна, с помощью цитогенетических и молекулярно-цитогенетических (количественная флюоресцентная гибидизация in situ) методов. Помимо этого, был также проведен анализ корреляции между вариацией альфоидной ДНК, возрастом родителей, факторами окружающей среды и нерасхождением хромосомы 21. Эффективность использованных методов составила 92 % (68 из 74 случаев). Материнское нерасхождение было обнаружено в 58 случаях (86 %), отцовское — в 7 случаях (10 %). Постзиготическое митотическое нерасхождение было определено в 2 случаях (3 %) и в одном случае — робертсоновская транслокация 46,XX,der (21;21)(q10;q10),+21. Ошибки в материнском мейозе I были обнаружены в 43 случаях (64 %), в материнском мейозе II — в 15 случаях (22 %). Ошибки в отцовском мейозе I были определены в 2 случаях (2 %), в отцовском мейозе II — в 5 случаях (7 %). Было установлено отсутствие корреляции между вариацией альфоидной ДНК и нерасхождением хромосомы 21. Социогенетический анализ показал наличие корреляции между интенсивной лекарственной терапией инфекционных заболеваний в периконцепционном периоде и нерасхождением хромосомы 21 в материнском мейозе. Показано также, что нерасхождение хромосомы 21 нельзя определить достоверно связанным с большим возрастом родителей, воздействием радиации, употреблением алкоголя, курением табака, а также воздействием химических соединений с мутагенным эффектом. Обсуждается значимость полученных результатов для последующих исследований нерасхождения хромосомы 21.

РЕЗЮМЕ. За допомогою цитогенетичних і молекулярно-генетичних (кількісна флуоресцентна гібридизація in situ) методів проведено аналіз нерозходження хромосоми 21 та варіації альфоїдної ДНК у 74 сім'ях з дітьми, що страждають на синдром Дауна. Крім того, проведено аналіз кореляції між варіацією альфоїдної ДНК, віком батьків, факторами оточуючого середовища та нерозходженням хромосоми 21. Ефективність використаних методів склала 92 % (68 з 74 випадків). Материнське нерозходження було виявлено у 58 випадках (86 %), батьківське нерозходження — у 7 випадках (10 %). Постзиготичне мітотичне нерозходження визначено у 2 випадках (3 %) і в одному — робертсонівська транслокація 46, XX, der(21;21)(q10; q10),+21. Помилки в материнському мейозі I виявлені у 43 випадках (64 %), в материнському мейозі II — у 15 випадках (21 %). Помилки в батьківському мейозі I були визначені у 2 випадках (2 %), у батьківському мейозі II — у 5 випадках (7 %). Встановлено відсутність кореляції між варіацією альфоїдної ДНК та нерозходженням хромосоми 21. Соціогенетичний аналіз показав наявність кореляції між інтенсивною лікарською

терапією інфекційних хвороб у періконцепційному періоді та нерозходженням хромосоми 21 в материнському мейозі. Показано також, що нерозходження хромосоми 21 не можна визначати достовірно зв'язаним з великим віком батьків, дією радіації, вживанням алкоголю, палінням, а також впливом хімічних сполук з мутагенним ефектом. Обговорюється значення отриманих результатів для наступних досліджень нерозходження хромосоми 21.

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