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Chromosome 21 Disomy in the Spermatozoa of the Fathers of Children with Trisomy 21, in a Population with a High Prevalence of Down Syndrome: Increased Incidence in Cases of Paternal Origin

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Summary

Between April 1991 and December 1994, epidemiological studies detected a population with a high prevalence of Down syndrome in El Vallès, Spain. Parallel double studies were carried out to determine the parental and the meiotic origins of the trisomy 21, by use of DNA polymorphisms, and to establish the incidence of disomy 21 in the spermatozoa of the fathers of affected children, by use of multicolor FISH. Results show that the overall incidence of chromosome 21 disomy in the fathers of affected children was not significantly different from that in the control population (0.31% vs. 0.37%). However, analysis of individual data demonstrates that two cases (DP-4 and DP-5) with significant increases of disomy 21 (0.75% and 0.78% vs. 0.37%) correspond to the fathers of the two individuals with Down syndrome of paternal origin. DP-5 also had a significant increase of sex-chromosome disomies (0.69% vs. 0.37%) and of diploid spermatozoa (1.13% vs. 0.24%).

Introduction

Down syndrome (DS) is the most frequent chromosome abnormality in newborns (1/650–1/700) and the most common cause of mental retardation. Studies of the parental origin of the syndrome have shown that in 90% of cases the trisomy is of maternal origin, in 5% of cases it is paternal, and in the remaining 5% the origin of the trisomy is mitotic (i.e., postzygotic) (Antonarakis 1993). A correlation between reduced recombination and nondisjunction has been established for several autosomal trisomies, such as trisomy 16 (Hassold et al. 1991*a*) and trisomy 21 (Warren et al. 1987; Sherman et al. 1991), as well as for some numerical anomalies of the sex chromosomes (Hassold et al. 1991*b*; MacDonald et al. 1994).

Studies of unfertilized human oocytes have shown an increase of G-group disomies in aneuploid female gametes (Pellestor 1991; Benkhalifa et al. 1996). FISH studies of spermatozoa have also demonstrated a tendency to nondisjunction for chromosomes 21 (Spriggs and Martin 1995; Blanco et al. 1996) and 16 (Williams et al. 1993) and for the sex chromosomes (Martin et al. 1996). On the other hand, disomy studies carried out on other chromosome pairs have produced much lower figures (Williams et al. 1993; Spriggs and Martin 1995; Blanco et al. 1996; Martin et al. 1996).

The area of El Vallès, Spain, an industrial region close to Barcelona, with a mean of 7,250 births per year, registered between April 1991 and December 1994 a prevalence of 23.8/10,000 live or dead births affected by DS. According to the European Registry of Congenital Anomalies and Twins (EUROCAT), this is a high-prevalence population. This led the Fundació Catalana per la Síndrome de Down to support a study on the origin and mechanisms of production of trisomy 21 in this high-prevalence population. Molecular studies of the parental and meiotic origin of the trisomy and FISH studies on the incidence of disomy 21 in the fathers of affected children were carried out blindly and in parallel. We report herein our results on the incidence of chromosome 21 disomy in spermatozoa as related to the parental origin of Down syndrome.

Material and Methods

Semen samples from 15 fathers of children affected by DS in the high-prevalence population described (23.8/10,000) were obtained between January 1994 and March 1997. The fathers were aged 28–44 years, and the sperm concentrations were >20 M/ml in all cases. As a control, we used nine individuals, 23–37 years old, without children and with normal karyotypes and nor-

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mal semen parameters (Blanco et al. 1996). The protocol of study was approved by our institutional ethics committee, and written informed consent was obtained from all individuals involved.

Semen samples were obtained by masturbation. The samples were fixed in methanol:acetic acid (3:1) and processed for FISH analysis. Sperm nuclei were decondensed by slide incubation in 5 mM DTT and 1% Triton X-100. Details of sperm fixation, nuclear decondensation, and FISH processing have been described elsewhere (Vidal et al. 1993).

A locus-specific probe for chromosome 21, spanning the 21q22.14–q22.3.region, directly labeled with Spectrum Orange (LSI 21, Spectrum Orange, Vysis) and a centromeric probe for chromosome 6, directly labeled with Spectrum Green (CEP 6, Spectrum Green, Vysis) were used for the FISH study. The protocol of denaturation, incubation, and detection was performed according to the manufacturer's instructions.

Analyses were done by use of an Olympus BX60 epifluorescence microscope equipped with filter sets for FITC, Texas Red, and DAPI/Texas Red/FITC according to the following criteria: (1) overlapped spermatozoa or sperm heads without well-defined boundaries were not evaluated; (2) in cases of disomy or diploidy, all signals should have the same intensity and be separated from each other by a distance longer than the diameter of each signal; and (3) nullisomies were not directly scored and were conservatively considered to be equivalent to the incidence of disomies (Blanco et al. 1996). According to our criteria, the efficiency of hybridization was calculated as the number of haploid spermatozoa plus twice the percentage of disomic spermatozoa plus the percentage of diploid sperm (Blanco et al. 1996).

In the samples from the fathers of children affected by DS, we analyzed ~10,000 spermatozoa per individual, determining for each case the percentages of haploid sperm, sperm disomic for chromosome 21, sperm disomic for chromosome 6, and diploid spermatozoa. The assessment of nullisomic sperm has not been performed because of the impossibility of discriminating between nullisomic sperm and hybridization failures (Blanco et al. 1996). Partial results from anomalies of chromosomes 6 and 21 in the control population have been published elsewhere (Blanco et al. 1996) and now include some further studies (table 1). Data were analyzed statistically with an InStat 2.01 program (Graph Pad).

In the DS population, at the same time as the determination of disomy 21 in human sperm, we assessed the parental origin of the extra chromosome 21 in the affected offspring. Thus, high–molecular weight genomic DNA was isolated from blood tissues from the father, mother, and affected child of each family in the sample. The parental origin and the meiotic stage of nondisjunction were determined by polymorphism analysis using chromosome 20 microsatellites from the centromere to qter (Antonarakis et al. 1992) (D21S120, D21S258, D21S13E, D21S172, D21S1410, D21S11, D21S1436, D21S1437, D21S1268, APP, D21S1435, D21S1442, D21S1270, IFNAR, D21S267, D21S1444, D21S268, D21S1446, PFKL, D21S171). Pericentromeric markers were used to detect the parental origin and to distinguish between meiosis I (MI) errors and meiosis II (MII) or mitotic errors. Nonpericentromeric markers were used to discriminate between MII and mitotic errors. Each study was done independently, and results were compared only at the end of the whole experiment.

Table 1

Results of Dual FISH on Sperm Nuclei

Sample	Haploid (*n*[%])	Disomy 21 (*n*[%])	Disomy 6 (*n*[%])	Diploid (*n*[%])
DP-1	10,055 (98.98)	26 (.25)	2 (.02)[a]	9 (.09)[a]
DP-2	10,056 (98.53)	23 (.22)	11 (.11)	21 (.20)
DP-3	10,009 (98.78)	17 (.17)	10 (.10)	20 (.20)
DP-4	10,000 (98.36)	76 (.75)[b]	6 (.06)	19 (.19)
DP-5	9,736 (94.39)	81 (.78)[b]	16 (.15)	137 (1.33)[d]
DP-6	10,029 (99.36)	24 (.24)	3 (.03)	13 (.13)
DP-7	10,030 (98.28)	42 (.41)	4 (.04)	40 (.39)
DP-8	9,451 (96.96)	16 (.16)	5 (.05)	30 (.31)
DP-9	10,000 (99.21)	25 (.25)	6 (.06)	25 (.25)
DP-11	10,004 (98.98)	18 (.18)	6 (.06)	17 (.17)
DP-13	10,051 (98.21)	40 (.39)	7 (.07)	22 (.22)
DP-14	10,150 (97.89)	34 (.33)	10 (.10)	23 (.22)
DP-15	10,251 (98.30)	13 (.12)	5 (.05)	24 (.23)
DP-16	10,040 (98.34)	17 (.17)	3 (.03)	20 (.19)
DP-17	10,082 (98.86)	17 (.17)	8 (.08)	69 (.68)[b]
Total	149,944	469	100	489
$x \pm$ SD	98.23 ± 1.21	.31 ± .20	.07 ± .03[c]	.32 ± .31
Control	27,563	91	29	77
$x \pm$ SD	98.05 ± .63	.37 ± .11	.13 ± .12	.27 ± .10

NOTE.—The table shows partial and total results from the fathers of DS patients. Total numbers differ from numbers in text because nullisomic and nonhybridized spermatozoa have not been included in the table. At the bottom, total results from the control population are shown.

[a] Percentage of chromosome 18 disomy; probe for chromosome 18 also used as ploidy control (see text).

[b] Significant differences when compared to controls ($P < .05$).

[c] Percentage of chromosome 6 disomy excluding case DP-1.

[d] Highly significant differences when compared to controls ($P < .0001$).

Results

Overall, we analyzed 173,249 spermatozoa from the fathers of children with DS and 28,044 sperm from the control individuals (table 1). The numbers in table 1 are lower because nullisomic sperm and hybridization failures have not been included.

The mean efficiency of hybridization was 98.5% (range 94.33%–100%). Sperm without hybridization signals could be hybridization failures for both probes,

Table 2

Percentages of Sperm Chromosome 21 Disomies in Fathers of DS Children and Trisomy Origin of the Extra Chromosome 21 in the Affected Children

Sample	Disomy 21 (%)	Origin
DP-1	.25	Maternal MI
DP-2	.22	Mitotic
DP-3	.17	Maternal MI
DP-4	.75	Paternal MI
DP-5	.78	Paternal MII
DP-6	.24	Maternal MI
DP-7	.41	Maternal MI
DP-8	.16	Maternal MI
DP-9	.25	Maternal MI
DP-11	.18	Mitotic
DP-13	.39	Maternal MI
DP-14	.33	Maternal MI
DP-15	.12	Maternal MI
DP-16	.17	Maternal MI
DP-17	.17	Translocation

nullisomy for both chromosomes, or decondensation errors.

In patient DP-1, the initial assessment of disomy 21 was done by use of the locus-specific probe for chromosome 21 plus the centromeric probe for chromosome 6. The observation of FISH results showed an extra, unexpected signal for the centromere of chromosome 6 in ~75% of the nuclei (data not shown in detail). As a result, the discrimination between disomic and diploid sperm was difficult. This is why, in this patient, we used a probe for chromosome 18 as a control of ploidy. This particular case has been analyzed in depth elsewhere (Egozcue et al. 1997); results showed that, in DP-1, the centromeric probe for chromosome 6 gave an extra, smaller signal localized in the centromere of chromosome 10 after a study carried out in banded lymphocyte metaphases. The percentages of disomy for chromosomes 6 and 21 and the frequencies of diploidy of DS fathers and controls are shown in table 1.

Statistical analysis of the frequencies of disomy for chromosomes 6 and 21 by use of a nonpaired Student's *t* test demonstrated that the incidence of disomy 21 was significantly higher ($P < .005$) than the frequency of disomy 6, both in control individuals and in the fathers of children with DS. On the other hand, by use of the χ^2 test, the frequency of disomy for each of these chromosomes was similar in the fathers of children with DS and in the controls ($P > .05$)

However, in comparison of each individual with the controls, it became evident that samples DP-4 and DP-5 had a highly significant increase in the frequency of disomy 21 ($P < .0001$). This increase was also detected ($P < .05$) in comparison of DP-4 and DP-5 with the remaining fathers of DS offspring. Sample DP-5 also had a highly significant increase in the frequency of diploidy ($P < .0001$), whereas sample DP-17 had a significantly higher frequency of diploidy ($P < .005$).

Results on the origin of the trisomy (table 2) demonstrated that in 12 cases (80%) the error was meiotic. Two cases (13.3%) were postzygotic and one case (6.7%) resulted from a de novo translocation t(14;21). Of the cases of meiotic origin, 10 cases (83.30%) were maternal and the other 2 (16.7%) resulted from paternal nondisjunction. These two cases corresponded to the patients with highly significant increases in the frequency of sperm disomic for chromosome 21 (table 2).

Discussion

Chromosome 21 Disomy

In our study, the frequency of disomy for chromosomes 6 and 21 in the DS population was similar to that found in control individuals. However, in both groups, the frequency of disomy for chromosome 21 was significantly higher than the frequency of disomy for chromosome 6. This supports the existence of a considerable interchromosomal variability already detected for chromosome 16 (Williams et al. 1993), for chromosome 21 (Spriggs and Martin 1995; Blanco et al. 1996), and for the sex chromosomes (Martin et al. 1996).

On the other hand, when each case was analyzed individually, it also became evident that two patients had highly significant increases in the frequency of chromosome 21 disomy, compared with both the mean frequency found in the control population and the mean frequency found in the fathers of children affected by DS. These two cases were those in which the nondisjunction event was paternal.

To explain the increase in the frequency of disomy for chromosome 21, we had to consider the possibility that these two fathers had disruptions of meiosis. To test this hypothesis, we evaluated the frequency of sex-chromosome disomies and of disomy 18 in both fathers (table 3). The results in DP-4 were within normal limits. This means that, in this case, if meiosis was disrupted, the

Table 3

Results of Triple-Color FISH for DP-4, DP-5, and Control Men

Sperm	DP-4 (*n*[%])	DP-5 (*n*[%])	Control (*n*[%])
23,X	5,086 (50.42)	4,808 (47.24)	25,432 (50.03 ± .80)
23,Y	4,876 (48.34)	5,036 (49.48)	24,778 (48.70 ± 1.18)
24,XY	11 (.11)	58 (.57)[a]	56 (.11 ± .05)
24,XX	3 (.03)	1 (.01)	49 (.10 ± .04)
24,YY	16 (.16)	11 (.11)	83 (.16 ± .06)
24X/Y,+18	6 (.06)	10 (.10)	48 (.10 ± .03)
Diploid	14 (.14)	92 (.90)[a]	126 (.24 ± .15)

NOTE.—Nullisomic and nonhybridized spermatozoa have not been included.

[a] Significant differences when compared to controls ($P < .05$).

anomaly should affect only pair 21. This possibility cannot be discarded, because Templado et al. (1981) demonstrated that synapsis may be regulated at the level of individual bivalents. In case DP-5, the frequency of sex-chromosome disomies was significantly increased (0.69% vs. 0.37%; $P < .05$). This increase resulted mainly from first-meiotic errors, producing XY sperm (0.57% vs. 0.11%). Furthermore, this individual also had a highly significant increase in the frequency of diploid sperm (1.33% vs. 0.24%). These data could indicate the existence of a generalized disruption of the synaptic process that could lead to an increased incidence of chromosomal anomalies. In DP-5, trisomy 21 was the product of an error in MII and, thus, probably was not related to a failure of the mechanisms of chromosome pairing and recombination. Nevertheless, it has been suggested that maternal nondisjunction in MII could be the result of meiotic disruptions in MI (Lamb et al. 1996), although, to date, there are no data supporting the presence of this phenomenon in male meiosis. However, in both cases, a random error of meiosis as the cause of the anomaly could not be discarded.

Increased Incidence of Disomy 21 and Paternal Origin

When trying to determine whether the parental origin of the trisomy in these two cases could be related to the twofold increase in the frequency of chromosome 21 disomy or was random, and taking into account the low incidence of paternal cases in DS, we had to consider several possibilities. First, although we followed generally accepted criteria to evaluate the frequency of disomy (Blanco et al. 1996), we are also convinced that the frequency of disomies resulting from MII errors may be underestimated because the two signals corresponding to the chromatids of the nondisjoined chromosome may be closer than accepted by general standards (Egozcue et al. 1997). Taking this into account, we reevaluated the data in samples DP-4 (MI error) and DP-5 (MII error), disregarding the distance and intensity criteria. Thus, we made a double assessment at the same time: first, we analyzed the spermatozoa according to the distance and intensity criteria, and, in the second analyses, those spermatozoa with two spots for the probe of chromosome 21 were assessed as disomic sperm, although they did not comply with our third assessment criteria. The results (not shown in detail) confirmed the frequency of disomy in DP-4 (0.75% vs. 0.78%), but the frequency increased slightly in DP-5 (0.78% vs. 1.05%). Even when these results are taken into consideration, the incidence is not sufficient to fully justify the paternal origin of the trisomy.

Second, the increased incidence of chromosome-21 and sex-chromosomes disomy and of diploid spermatozoa in DP-5 could be a consequence of a more severe disruption of meiosis (Bernardini et al. 1997; Van Hummelen et al. 1997; Pieters et al. 1998). In our work, we have analyzed only five chromosomes (6, 18, 21, X, and Y), but it is possible that other chromosomes could be affected in the same way. Thus, the percentage of sperm nuclei that could produce viable offspring could be significantly lowered because of the decreased viability of most chromosomal anomalies (i.e., monosomies, trisomies, and triploids); however, for the same reason, the risk of producing viable chromosomally unbalanced offspring could be increased.

An increased incidence of diploid spermatozoa was detected in two patients. As indicated above, the increased incidence of diploidy in DP-5 could be explained by a severe disruption of the meiotic process. FISH studies in sperm nuclei from infertile patients have shown a significant increase of diploid sperm (Pang et al. 1995; Bernardini et al. 1997; Egozcue et al. 1997; In't Veld et al. 1997), and it is known that infertile patients have, in some cases, disruptions of the synaptic process (Egozcue et al. 1983; Speed and Chandley 1990; Lange et al. 1997; Pieters et al. 1998). Thus, there could be a correlation between abnormal pairing and the incidence of diploid sperm, although the mechanisms involved are unknown.

Clinical Significance

The clinical significance of the results obtained by analysis of the frequency of disomy in sperm nuclei is still a matter of discussion for most authors working in this field. Recently, some laboratories have incorporated FISH analysis of human sperm nuclei into the workup of oligoasthenozoospermic patients, to better evaluate the very heterogeneous intracytoplasmic sperm injection (ICSI) population and to try to prevent the increased incidence of sex-chromosome abnormalities in children conceived by ICSI (In't Veld et al. 1995; Liebaers et al. 1995). Some of these studies have shown an increased incidence of sex disomies and diploid sperm, but at a level that is moderate and similar to that described in this article (reviewed in Egozcue et al. 1997). Thus, it seems that a moderate increase in the frequency of disomy in a particular population could have a direct influence on the incidence of the expected trisomy. Obviously, additional cases are needed to determine whether there is a clear correlation between the origin of trisomies and a given increase of disomy in spermatozoa.

Remarks and Conclusions

So far, FISH studies on the frequency of disomy for different chromosomes in decondensed sperm heads have provided some data that have been confirmed by different authors and can be summarized as follows: (1) some chromosomes, such as 16 (Williams et al. 1996), 21 (Spriggs and Martin 1995; Blanco et al. 1996), and

the sex chromosomes (Martin et al. 1996), have special tendencies to nondisjunction; (2) disomies are more frequent in some individuals than they are in others (reviewed in Downie et al. 1997 and Egozcue et al. 1997); (3) the increase in the incidence of disomies in cases in which it is possible to establish a cause-and-effect relationship (47,XXY; 47,XYY) (Chevret et al. 1996, 1997; Blanco et al. 1997) is moderate and is similar to that found in the two fathers described in this paper; and (4) a generalized disruption of meiosis could be related to an increase of diploid sperm.

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