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Analysis of DNA haplotypes suggests a genetic predisposition to trisomy 21 associated with DNA sequences on chromosome 21

(DNA polymorphisms/Down syndrome/nondisjunction/meiosis)

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ABSTRACT **To test the hypothesis that there is a genetic predisposition to nondisjunction and trisomy 21 associated with DNA sequences on chromosome 21, we used DNA polymorphism haplotypes for chromosomes 21 to examine the distribution of different chromosomes 21 in Down syndrome and control families from the same ethnic group. The chromosomes 21 from 20 Greek families with a Down syndrome child and 27 control Greek families have been examined for DNA polymorphism haplotypes by using four common polymorphic sites adjacent to two closely linked single-copy DNA sequences (namely pW228C and pW236B), which map somewhere near the proximal long arm of chromosome 21. Three haplotypes, ++++, +---, and −+++ with respective frequencies of 43/108, 24/108, and 23/108, account for the majority of chromosomes 21 in the control families. However, haplotype −+++ was found to be much more commonly associated with chromosomes 21 that underwent nondisjunction in the Down syndrome families (frequency of 21/50; χ^2 for the two distributions is 9.550; $P = 0.023$; degrees of freedom, 3). The two populations (control and trisomic families) did not differ in the distribution of haplotypes for two DNA polymorphisms on chromosome 17. The data from this initial study suggest that the chromosome 21, which is marked in Greeks with haplotype −+++ for the four above described polymorphic sites, is found more commonly in chromosomes that participate in nondisjunction than in controls. We propose an increased tendency for nondisjunction due to DNA sequences associated with a subset of chromosomes 21 bearing this haplotype.**

Down syndrome (DS) (trisomy 21) is the most frequently identified genetic cause of mental retardation with an incidence of approximately 1:600 to 1:1000 live births (1). In 1959 Lejeune demonstrated that patients with DS have an additional chromosome 21 (2). Primary trisomy 21 occurs in about 95% of DS; other chromosomal abnormalities resulting in triplication of the chromosome 21 include the robertsonian translocations D;21 or G;21 (3). DS is usually the result of meiotic nondisjunction (NDJ; see refs. 4 and 5 for reviews), and the effect of maternal age is well established (1). About 70% of the NDJ events occur in the maternal first meiotic division and 10% in each of the other meiotic divisions—namely, maternal second and paternal first and second (6, 7).

In this study we set out to test the hypothesis that a genetic predisposition to trisomy 21 is associated with DNA sequences on chromosome 21. We used DNA polymorphisms adjacent to DNA sequences on the proximal long arm of chromosome 21 and performed haplotype analysis on such chromosomes. The frequencies of different haplotypes of chromosomes 21 that participated in NDJ (in families with DS) and in control families of the same ethnic group were compared. Our initial observations suggest that a subpopulation of a particular chromosome 21 identified by a certain haplotype of linked DNA polymorphic sites has a greater tendency for NDJ and formation of trisomy 21. Further study is required to establish the true significance of this observation.

MATERIAL AND METHODS

Subjects. Our subjects were members of Greek families containing a child with trisomy 21. For each family, the parents, the affected individuals, and at least one normal child were examined for the DNA polymorphisms of interest. A total of 20 such families participated in the study. Seven mothers were greater than age 35 yr when the DS child was born and 13 were below the age of 35. The control population consisted of Greek families seen for prenatal diagnosis of hemoglobinopathies. A total of 27 such nuclear families were studied.

Restriction Endonuclease Analysis. Nuclear DNA was isolated from leukocytes of 10–15 ml of EDTA-anticoagulated peripheral blood as described (8). DNA (5–10 μg) was digested with one of various restriction endonucleases under conditions recommended by the commercial suppliers. Electrophoresis, transfer of DNA fragments to nitrocellulose filters, hybridization of genomic fragments with radioactive probes, washing of filters, and autoradiography were carried out as described (9, 10).

Probes and DNA Polymorphisms. The following probes were used: (*i*) genomic *Eco*RI fragments pW228C [1.5 kilobases (kb)], pW236B (1.85 kb), and pW231C (2.1 kb), which are single-copy fragments and map to the long arm of human chromosome 21 (11); (*ii*) genomic and cDNA fragments of the human superoxide dismutase gene *SOD1*, which map on 21p22.1 (12) [kindly supplied by Y. Groner of Weizmann Institute, Rehovot, Israel (13, 14)]; and (*iii*) cDNA fragment phGH800, which contains the human growth hormone gene (ref. 15; kindly provided by J. Phillips of Vanderbilt University). All fragments were radiolabeled with ^{32}[P]dATP and ^{32}[P]dCTP by the nick-translation function of *Escherichia coli* DNA polymerase (16).

The following DNA polymorphic sites were used in the study: *Bam*HI and *Msp* I sites adjacent to probe pW228C (9), *Eco*RI and *Taq* I sites adjacent to pW236B (9), *Taq* I polymorphic site adjacent to pW231C (17), *Bgl* II and *Msp* I sites in the *SOD1* gene sequence (17), and two *Msp* I sites in the human growth hormone gene cluster (18).

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Abbreviations: DS, Down syndrome; NDJ, nondisjunction; kb, kilobase(s).

RESULTS

We chose to use the *Bam*HI, *Msp* I, *Eco*RI, and *Taq* I polymorphic sites adjacent to probes pW228C and pW236B for the haplotype analysis of different chromosomes 21 for the following reasons. (*i*) All four polymorphic sites were highly informative in the ethnic group under study, with frequencies of the less common allele in the range of 0.3 for each polymorphic site (17). (*ii*) No crossing-over event between the two probes has been observed to date in about 30 chances for recombination ($\hat{\theta} = 0.00$, $\hat{z} = 6.91$ from ref. 17). In addition, the high degree of linkage disequilibrium that exists between the two polymorphic sites adjacent to one probe and those adjacent to the other suggests that these probes lie very close to each other (17). Therefore, haplotypes for the four polymorphic sites can be developed by using linkage analysis in nuclear families (19), and each chromosome 21 can be identified and marked with a specific haplotype. (*iii*) The construction of a linkage map for human chromosome 21 provided evidence that the two DNA sequences (pW228C and pW236B) lie in the proximal long arm of chromosome 21, relatively close to the centromere (17, 29), although current linkage data do not permit a more precise statement.

Haplotypes in the Control Population. In the 27 Greek families that were used as a control population, we were able to examine the polymorphism haplotypes in 108 chromosomes 21, using one or more offspring in each family to establish the linkage phase of polymorphic markers in the four parental chromosomes 21 (Table 1, group A). Ten of 16 possible haplotypes were observed, but the 3 most common (namely, ++++, +---, and -+++) accounted for 40%, 22%, and 21% of chromosomes 21, respectively. In the nomenclature of the haplotype analysis, the + and − refer to the presence or absence of a given polymorphic site. The order of the four polymorphic sites in chromosomes 21 with the above described haplotypes is *Bam*HI-pW228C, *Msp* I-pW228C, *Eco*RI-pW236B, and *Taq* I-pW236B, respectively. (The chromosomal order of these sites is unknown.) The observation of nonrandom association of these polymorphic sites and the extent of linkage disequilibrium between them have been discussed elsewhere (see ref. 17).

Haplotypes of Chromosomes 21 Involved in NDJ. The haplotypes of chromosomes 21 that were involved in the NDJ events were analyzed in the 20 Greek families with trisomy 21. First, the haplotypes of chromosomes 21 in the parents of these families were identified by using a normal child for the linkage analysis. The presence or absence of the polymorphic sites in the three chromosomes 21 in the trisomic individuals was determined by using the difference in the intensity of hybridization of the allelic fragments (20). Thus, when the intensity of the + fragment (presence of the polymorphic site) was twice that of the − fragment (absence of this site), the trisomy 21 child was scored as ++− at that polymorphic site. An example is given in Fig. 1, and similar results were obtained with both probes and all four polymorphic sites.

Our goal was to determine the haplotypes of the 2 chromosomes 21 that were involved in the NDJ in each trisomic child. This was possible in only 10 of the 20 families (see example A in Fig. 2). The origin of NDJ was determined not only with the four polymorphic sites that were used for the haplotype analysis but also with additional polymorphisms adjacent to other chromosome 21-specific probes (namely, cloned superoxide dismutase gene *SOD1* and pW231C). Table 1 shows the distribution of haplotypes in those 20 chromosomes (group B). In the other 10 families, all 3 chromosomes 21 were counted (see example B in Fig. 2). Table 1 and the Fig. 3 histogram show the distribution of haplotypes in those 50 chromosomes (group C) from all 20 families (20 haplotypes of group B and 30 haplotypes from the remaining 10 families). Because of the bias in the analysis of the data (in each of the latter 10 families, we count 1 chromosome 21 that did not participate in the NDJ), we then used the probability of a maternal or paternal meiotic NDJ from the literature (refs. 6, 7, and 21; see the introduction for frequencies) to count only 2 instead of 3 chromosomes 21 in the DS patients on a probabilistic basis. After this correction, which we believe provides a better figure for the frequency of the haplotypes in the chromosomes 21 involved in the NDJ, the distribution of these haplotypes is not different from that of group C of Table 1 (data not shown). It is clear that the haplotype −+++ is significantly more frequent in the chromosomes 21 that are involved in the NDJ than in the chromosomes 21 of the control group. In addition, haplotype ++++ is less frequent than expected in the NDJ event.

The distribution of haplotypes of the 80 chromosomes 21 from the parents of the trisomy 21 offspring is also presented in Table 1 (group D). The difference between this distribu-

Table 1. Haplotype analysis for linked polymorphic sites on human chromosome 21

	Haplotype*											
	++++		+---		-+++		Other		Total	Statistical data		
Group†	*n*	%	*n*	%	*n*	%	*n*	%	*n*	χ^2	*P*	df
A	43	40	24	22	23	21	18‡	17	108			
B	1	5	6	30	12	60	1	5	20	A,B = 17.180	0.0005	3
C	14	28	12	24	21	42	3	6	50	A,C = 9.556	0.023	3
D	27	33	23	28	25	31	5§	6	80	A,D = 7.097	0.069	3
E	14	43	10	31	5	16	3	9	32	A,E —	NS	
F	11	38	6	21	11	38	1	3	29			
G	3	18	6	35	7	41	1	6	17	F,G —	NS	

NS, not significant; df, degrees of freedom.

*For each haplotype the following linked polymorphic sites have been examined from left to right: *Bam*HI-pW228C, *Msp* I-pW228C, *Eco*RI-pW236B, *Taq* I-pW236B.

†A, chromosomes 21 from Greek families with a history of hemoglobinopathies (control population); B, chromosomes 21 from 10 Greek patients with trisomy 21 (only the chromosomes 21 that participated in the NDJ were counted); C, chromosomes 21 from 20 Greek patients with trisomy 21 [this category includes the chromosomes 21 of group B and all chromosomes 21 from 10 additional Greek patients in which it was not possible to distinguish the two chromosomes (out of 3) that participated in the NDJ]; D, chromosomes 21 from the parents of 20 Greek families with trisomic offspring; E, chromosomes 21 from parents of Greek families with trisomic offspring that certainly did not participate in the NDJ and, therefore, can serve as an internal control; F, chromosomes 21 from Greek patients with trisomy 21 as in group C, with maternal age <35 yr at the time of birth of the trisomic child; G, as in group C, with maternal age >35 yr at the time of birth of the trisomic child.

‡Haplotypes: +-++, 6; ++--, 4; -++-, 3; -+--, 2; +--+, 1; ++-+, 1; +++-, 1.

§Haplotypes: +-++, 3; +++-, 1; -+--, 1.

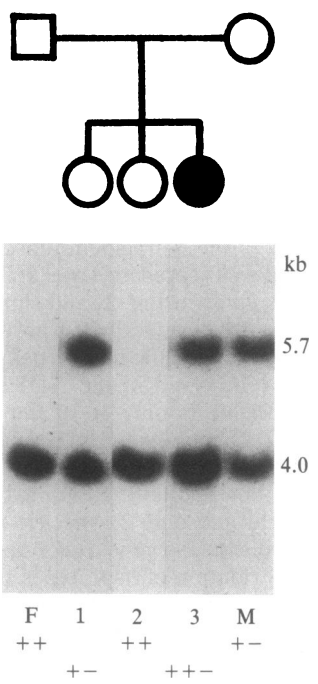

FIG. 1. The inheritance of the polymorphic *Msp* I site adjacent to probe pW228C in a family with trisomy 21 offspring. The pedigree is shown above the autoradiogram. The filled circle in the pedigree represents the female individual with trisomy 21. F, father; M, mother; 1, 2, 3, offspring as shown in the pedigree; + and −, presence or absence of the *Msp* I polymorphic site. Note that the trisomy 21 child contains two copies of the allele with the presence of the site and one copy of the allele with the absence of this site.

tion and the distribution of the haplotypes in the chromosomes 21 of the control population approaches statistical significance.

To examine whether the families in the control group and the group with the DS offspring were comparable and representative of the Greek population, we examined the polymorphism haplotypes for a control set of markers on chromosome 17, namely two *Msp* I DNA polymorphic sites adjacent to the human growth hormone gene cluster. Table 2 shows the results of the haplotype analysis for these two polymorphic sites in the two groups. No difference in the

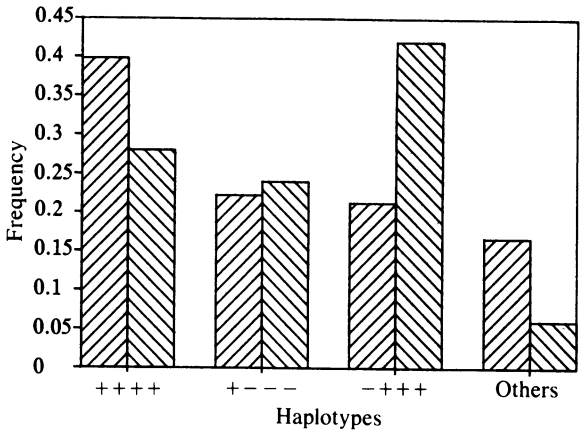

FIG. 3. Histogram of the frequency of the haplotypes for chromosome 21 in the control population and the chromosomes 21 that participated in the NDJ (groups A and C of Table 1). For each pair of bars, the first represents the chromosomes 21 of group A, and the second represents the chromosomes 21 of group C.

distribution of these haplotypes was found between the chromosomes 17 of the control group and those of the parents of trisomy 21 offspring.

An additional internal control was also used. It is clear that only one parent contributes to the NDJ, and the other parent contributes one additional chromosome 21 to the trisomic child. Therefore, we examined the distribution of haplotypes for the four polymorphic sites on the chromosomes 21 in the parent of a Down offspring who did not contribute to the NDJ phenomenon. This was not possible in every family, and only 32 chromosomes 21 could be identified as nonparticipating in the NDJ event. The distribution of the haplotypes for these 32 chromosomes also is presented in Table 1 (group E) and shows no difference from the haplotypes of chromosomes 21 in the control population.

Maternal Age and Haplotypes. Although the number of families with Down syndrome studied here is not extensive, no difference was found between the distribution of haplotypes for the chromosomes 21 that were involved in the NDJ in mothers less than or greater than age 35 yr at the time of birth of the trisomic child. The two distributions (groups F and G of Table 1) are not different from each other, but more families are needed for a definitive answer to this question.

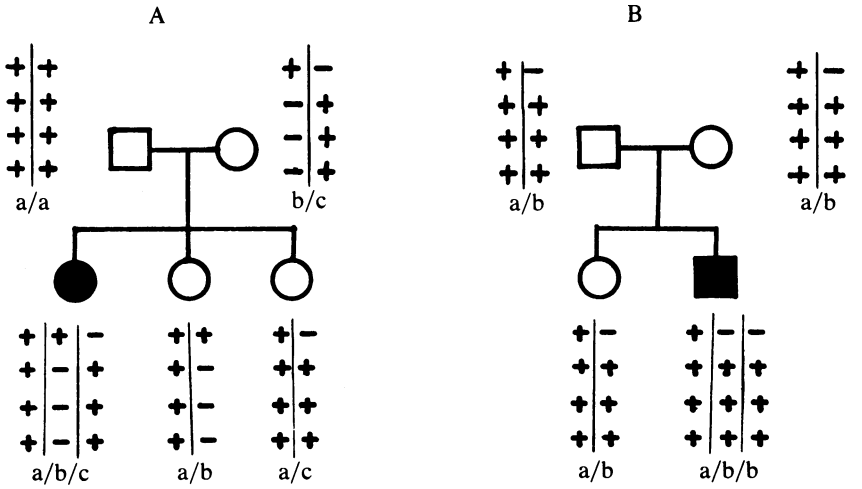

FIG. 2. Representative Greek families with trisomy 21 and the haplotypes of chromosome 21 for each individual. Filled symbols, the propositi; + and −, presence or absence of each polymorphic site. The four sites in each haplotype are from top to bottom: *Bam*HI–pW228C, *Msp* I–pW228C, *Eco*RI–pW236B, and *Taq* I–pW236B. For simplicity the haplotypes are also designated with letters. In family A the NDJ occurred in the maternal germ cells; in family B the origin of NDJ cannot be identified. In the 10 families in which the haplotype analysis provided information on the origin of NDJ, this origin was as follows: maternal meiotic divisions, 8; paternal meiotic divisions, 2.

Table 2. Haplotypes for *Msp* I polymorphic sites adjacent to the human growth hormone genes on chromosome 17

Group*	Haplotype ++	−−	+−	−+	Total *n*	$\chi^2_{A,B}$	*P*
A	46	29	1	3	79		
B	39	30	1	1	71	1.171	NS

NS, not significant.
*A, control Greek population; B, Greek families with trisomy 21.

DISCUSSION

In this study we used DNA polymorphism haplotype analysis to test the hypothesis that a chromosome 21-specific genetic predisposition to trisomy 21 exists in man. Our data suggest that in the Greek population, one particular chromosome 21, marked with the haplotype −+++ for the polymorphic sites *Bam*HI–pW228C, *Msp* I–pW228C, *Eco*RI–pW236B, and *Taq* I–pW236B, is found more frequently in the chromosomes 21 that are involved in NDJ; therefore, we propose the hypothesis that some fraction of this particular chromosome 21 population in Greeks contains DNA sequences that have a tendency for NDJ and predispose to trisomy 21.

There is very little evidence in the literature for a genetic predisposition to NDJ (2). Alfi *et al.* studied the occurrence of Down syndrome in a highly inbred population of Saudi Arabians in Kuwait (22). They found that the incidence of DS was significantly higher in consanguineous than in nonconsanguineous matings of the same population. Hook summarized all the other studies concerning consanguinity and inbreeding in parents of DS (1). The data available from these studies do not reject the hypothesis of a genetic predisposition to NDJ. The studies of Harlap are of particular interest (23–25). He conducted a perinatal study on DS in West Jerusalem and found that the rate of DS in non-European Jewish mothers was about 60% higher at all maternal ages than that of Jewish mothers of European origin; the data suggest a different frequency of a gene(s) for predisposition to NDJ.

In *Drosophila* females it appears that most mutants affecting meiosis disrupt the disjunction of more than one specific chromosome. There are, however, at least two mutants that increase NDJ in both sexes and affect only the chromosome on which they are located (26). Furthermore, there is a group of male-specific meiotic mutants affecting meiosis I in *Drosophila*; two loci on the second chromosome in particular affect chromosome 4 only (27). These data provide some plausible grounds for expecting mutations in humans that may predispose to chromosome-specific NDJ and, in particular, trisomy 21.

The genetic predisposition of DS may have several components, but our data point to a chromosome 21-specific predisposition as one of these components. This genetic component could be: (*i*) a single gene or genes on chromosome 21 that affect the disjunction mechanism of the same chromosome on which this gene is located or (*ii*) a structure of the centromere in some particular chromosomes 21 that may predispose to NDJ. The haplotype −+++ may mark these latter chromosomes and is present, therefore, more commonly in chromosomes 21 that are involved in the NDJ event. It is not known how far (in genetic linkage terms) the DNA polymorphisms for this haplotype are from the centromere; therefore, it is not known how efficient this haplotype is as a marker for centromeres potentially involved in NDJ. DNA polymorphic markers adjacent to centromeric single-copy sequences specific for chromosome 21 are needed to examine this point. It is also likely that only a subset of the −+++ chromosome has a tendency for NDJ. This can be better analyzed by using additional linked polymorphisms in families with DS.

Because our data comprise only an initial analysis of a few families, we have made no attempt to provide a relative risk figure for the occurrence of DS in mothers who have or lack the haplotype of concern here. Clearly, more families are required for any significant relative risk figure, and the basic observation has to be confirmed in at least one more ethnic group not genetically related to Greeks.

A possibility that cannot be excluded and also can explain our data is a preferential survival of trisomy 21 embryos with the −+++ chromosome 21. It is well documented that only about 20% of trisomy 21 conceptuses survive gestation (1, 5). It is possible, therefore, that every chromosome 21 has the same tendency for NDJ, but trisomy 21 embryos with the −+++ chromosome survive better; therefore, this chromosome 21 is more frequently observed in the families examined.

Very recently Jackson-Cook *et al.* (28) found a significantly higher incidence of double nucleolar-organizing regions in the acrocentric chromosomes of parents of DS offspring than in control individuals. The significance and the relationship of this finding to the chromosome 21 which is marked with haplotype −+++ are not yet known. It may represent the same predisposing factor or a completely different one.

We understand that, although the present observations are provocative and potentially important, further studies are needed to elucidate the different genetic factors predisposing to NDJ, to establish their true significance and to identify the DNA sequences that influence chromosomal disjunction during meiosis.

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