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Genetics

Lack of evidence for association of meiotic nondisjunction with particular DNA haplotypes on chromosome 21

(Down syndrome)

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ABSTRACT **The hypothesis of a predisposition to meiotic nondisjunction for chromosome 21 carrying a specific molecular haplotype has been tested. The haplotype in question is defined by the restriction fragment length polymorphisms for the *D21S1/D21S11* loci. Our results obtained on a sample of Northern Italian families with the occurrence of trisomy 21 (Down syndrome) failed to support this hypothesis, contradicting a previous study [Antonarakis, S. E., Kittur, S. D., Metaxotou, C., Watkins, P. C. & Patel, A. S. (1985) *Proc. Natl. Acad. Sci. USA* 82, 3360–3364]. These findings rule out an association between any specific *D21S1/D21S11* haplotype (as well as other haplotypes for the *D21S13*, *ETS2*, and *D21S23* loci) and a putative cis-acting genetic element favoring the meiotic missegregation of chromosome 21. For this reason, no preventive screening for couples at risk for trisomy 21 may be based on any of the haplotypes tested.**

Errors in the transmission of genetic material—a process that must occur with a high degree of precision—lead to aneuploidy in eukaryotic organisms. Little is known about the causes for incorrect pairing and aberrant segregation of chromosomes, but these mechanisms are likely to be influenced by both genetic and environmental factors (1). It has been proposed that correct chromosome pairing or synapsis plays a critical role in ensuring appropriate segregation, although the evidence to date is not conclusive. Therefore, genes encoding proteins involved in controlling or carrying out chromosome pairing and segregation might be involved in abnormal meioses. Studies of mutants in *Drosophila* point to a large number of loci that can affect segregation of chromosome pairs and suggest an inverse relationship between recombination and nondisjunction (NDJ) (2, 3). Genetic effects on meiosis might also be expected to operate through particular DNA sequences that eukaryotes have evolved to fulfill specific meiotic functions. In the yeast *Saccharomyces cerevisiae*, centromeric DNA sequences essential for both reductional and equational meiotic divisions have been defined (4). In *Lilium*, DNA sequences have been found that may play a role in the chromosome pairing or synapsis (5). No specific gene loci or structural DNA segments in man have yet been implicated in chromosome NDJ, although aneuploidy is the basis of many severe human disorders.

The most common human disorder resulting from aberrant chromosome segregation is Down syndrome (DS) or trisomy 21, affecting ≈1 birth in 1000 (6). Recently, an association has been reported between NDJ of chromosome 21 and a haplotype defined by restriction fragment length polymorphism (RFLP) at an anonymous DNA locus on this autosome (7). In view of the profound impact of such a finding for genetic counseling and prevention of DS, we investigated the general validity of this observation. In contrast to the previous study, we did not observe any particular haplotype on chromosome 21 associated with NDJ chromosomes—i.e., chromosomes that did not undergo disjunction (DJ).

MATERIALS AND METHODS

Family Samples. We examined 23 Italian families (group A) as a control group and 37 families with a DS child (group B). All of the control families consisted of mother, father, and one normal child (NC). Of 37 families with a DS child, 23 consisted of mother, father, and the DS child, and the remaining 14 consisted of the parents and two children, one NC and one with DS; of the 37 families, 35 were informative for the origin of nondisjunction (8), as described in detail elsewhere. Our DS families were not selected for advanced maternal age, having 5 mothers ≤ 25 years, 16 between 25 and 35 yr, and 6 ≥ 35 yr.

Cytogenetic Analysis. The parental origin and stage of NDJ were determined by evaluating the chromosome 21 satellite polymorphisms (8). Double-blind microscope scoring was performed in all cases, and the origin of NDJ attributed only in case of concordance (8).

Southern Blot Analysis. DNA was obtained from 20-ml samples of blood with EDTA as anticoagulant. Erythrocytes were lysed in 20 mM Tris chloride, pH 8.0/10 mM EDTA. The pellet was washed three times with the same solution, resuspended in 5 ml of 150 mM NaCl/2 mM EDTA/10 mM Tris chloride, pH 7.5/0.05% $NaDodSO_4$ and incubated overnight at 55°C in the presence of proteinase K at 100 μg/ml. DNA was extracted three times with phenol/chloroform, 24:1 (vol/vol) and once with chloroform. The last upper phase was precipitated with 2.5 vol of absolute ethanol in the presence of 0.3 M $NaC_2H_3O_2$. DNA was recovered by spooling and was dissolved in 1 ml of 10 mM Tris chloride/1 mM EDTA, pH 8.0. DNAs were digested with several restriction enzymes under conditions recommended by the suppliers (Boehringer Mannheim), electrophoresed in 0.8% agarose gel, and blotted onto a nylon membrane (Nytran; Schleicher & Schuell). Prehybridization was performed at 42°C for at least 2 hr in 1 M NaCl/50 mM Tris chloride, pH 7.5/1% $NaDodSO_4$/50 μg of salmon sperm DNA per ml/10× Denhardt's solution (0.02% polyvinylpyrrolidone/0.02% Ficoll/0.02% bovine serum albumin). The hybridization was overnight at 42°C in 1 M NaCl/50 mM Tris chloride, pH 7.5/1% $NaDodSO_4$/50% formamide/50 μg of salmon sperm DNA per ml. The ^{32}P-labeled probes (specific activity, 2 ×

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Abbreviations: DS, Down syndrome; RFLP, restriction fragment length polymorphism; NC, normal child; NDJ, nondisjunction; DJ, disjunction.

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10^9 cpm/μg) were added to 5–10 ng/ml of the hybridization solution.

Filters were washed twice for 15 min at room temperature in 6× SSC (1× SSC = 0.15 M NaCl/0.015 M sodium citrate)/0.1% $NaDodSO_4$, twice for 15 min at 37°C in 1× SSC/1% $NaDodSO_4$, and finally for 1 hr at 65°C in 0.1× SSC/1% $NaDodSO_4$. The membranes then were exposed to Kodak XAR-2 films at −70°C for 24 hr.

Chromosome 21 Sequences. The probes used for the haplotype analysis were single-copy chromosome 21 sequences chosen for mapping in different regions of the chromosome (Fig. 1). With the exclusion of H33 *ETS2* (9), which is part of a transcriptionally active gene, *ETS2*, all the others are anonymous sequences (ref. 10; R. E. Tanzi, personal communication). *D21S13*, a 7-kilobase (kb) *Eco*RI fragment located closest to the centromere, identifies a *Taq* I polymorphic site. *D21S1*, a 1.5-kb *Eco*RI fragment, recognizes two polymorphic sites (*Bam*HI and *Msp* I). *D21S11*, a 1.85-kb *Eco*RI fragment, identifies an *Eco*RI and a *Taq* I polymorphic site. *D21S1* and *D21S11* map in close proximity in the q21 region of chromosome 21. H33 *ETS2*, a 1-kb *Eco*RI fragment mapping to the q22.3 region of chromosome 21, identifies two polymorphic sites (*Msp* I and *Taq*I). *D21S23*, an *Eco*RI fragment of 0.95-kb located in the q22.3 region of the chromosome, recognizes a polymorphism with either of two enzymes (*Eco*RI or *Msp* I).

Statistical Methods. The difference between comparable haplotype distributions was tested by the χ^2 test for independence. When 2 × 2 tables were being compared, the continuity correction was applied (11); when expected frequencies in these tables were small, the Fisher exact test was used in lieu of the χ^2 test.

RESULTS

The total number of chromosomes 21 taken into account in the 23 control families (group A) was 92. In the second group of families with a DS child (group B), the total number of chromosomes 21 analyzed was 140. The origin, whether paternal or maternal, of the extra chromosome 21 was attributed in 35 DS children out of 37 on the basis of both cytogenetic and molecular data that were either informative additively or concordant (8). Maternal NDJ was the cause of trisomy in 25 (72%) of the cases, occurring in the great majority at the first meiotic division (22 cases). The origin of NDJ was paternal in 10 (28%) of the cases. Therefore, as far as the origin of NDJ is concerned, the families of group B are sufficiently representative of the values in the literature (12, 13), having a 3:1 ratio of maternal versus paternal. The chromosomes 21 within group B could be allocated to several subgroups: 62 NDJ chromosomes [NDJ at the first meiotic division (NDJI) in 54 and at the second meiotic division (NDJII) in 8] and 78 DJ chromosomes (chromosomes that underwent DJ).

The DNA sequences used to identify specific chromosome 21 haplotypes are reported in Fig. 1. Notably, *D21S13* is an anonymous sequence on the proximal long arm. *D21S1* and *D21S11* are the two closely associated sequences about 15 centimorgans from the centromere (R. E. Tanzi, personal communication) that were used in the previously mentioned study (7). RFLPs at *D21S1* and *D21S11* display linkage disequilibrium and can be considered as a single haplotype, since we have observed no recombination between the two loci in 145 informative meiotic events. *D21S23* and the genomic sequence for the H33 *ETS2* gene both map to the q22.3 region. The haplotypes identified by the RFLPs of the different sequences, together with their distribution in the control group A of chromosomes 21 were as follows. Two haplotypes + and −, with a frequency of 0.58 and 0.42, were identified by the *Taq* I RFLP for the *D21S13* sequence; the *Bam*HI and *Msp* I RFLPs for the *D21S1* sequence and the *Eco*RI and *Taq* I RFLPs for the *D21S11* sequence identify mainly three haplotypes, + + + + (0.40), + − − − (0.26), and − + + + (0.25), out of the 16 expected haplotypes. Three, − − (0.63), + + (0.21), and + − (0.15), were the most recognizable haplotypes of the four expected for the H33 *ETS2 Msp* I and *Taq* I RFLPs. Finally, the *Eco*RI, *Msp* I RFLPs for the *D21S23* sequence recognize two haplotypes,

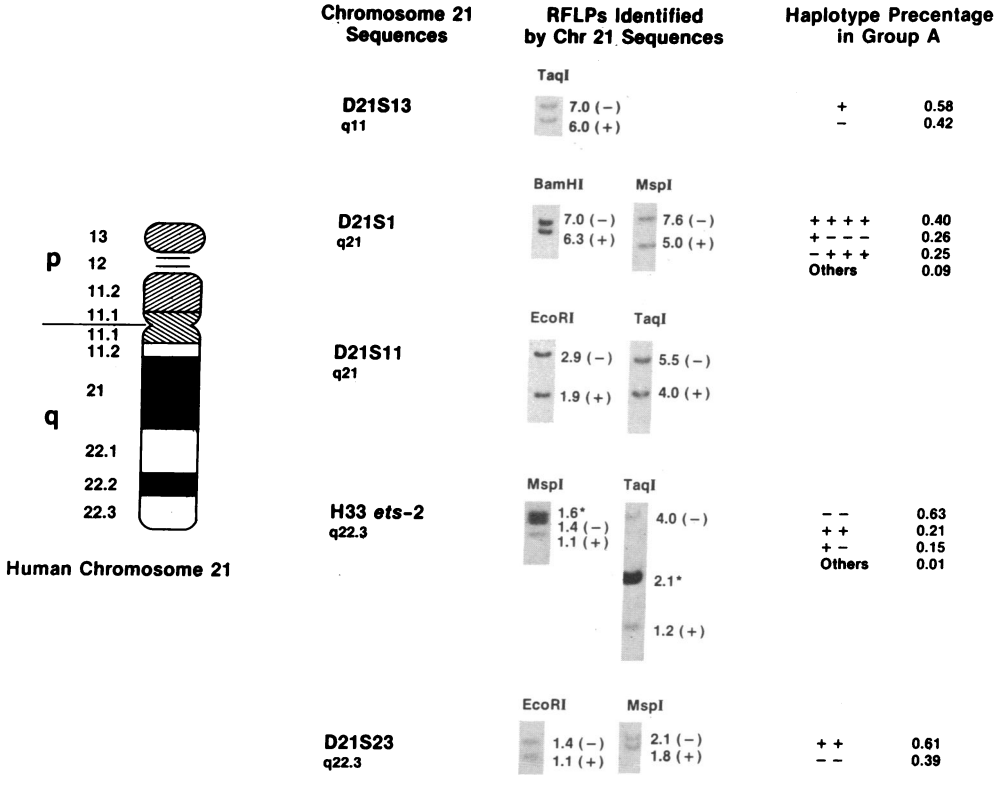

FIG. 1. RFLPs of the arbitrary chromosome 21 sequences used in this study. Their location is indicated. The + and − indicate the presence or the absence of a polymorphic fragment. (The asterisk refers to an invariant fragment.) Not all potential haplotypes were observed. For the *D21S1/D21S11*, mainly 3 haplotypes occurred of the 16 expected, and for the *H33-ETS2*, only three occurred of four expected.

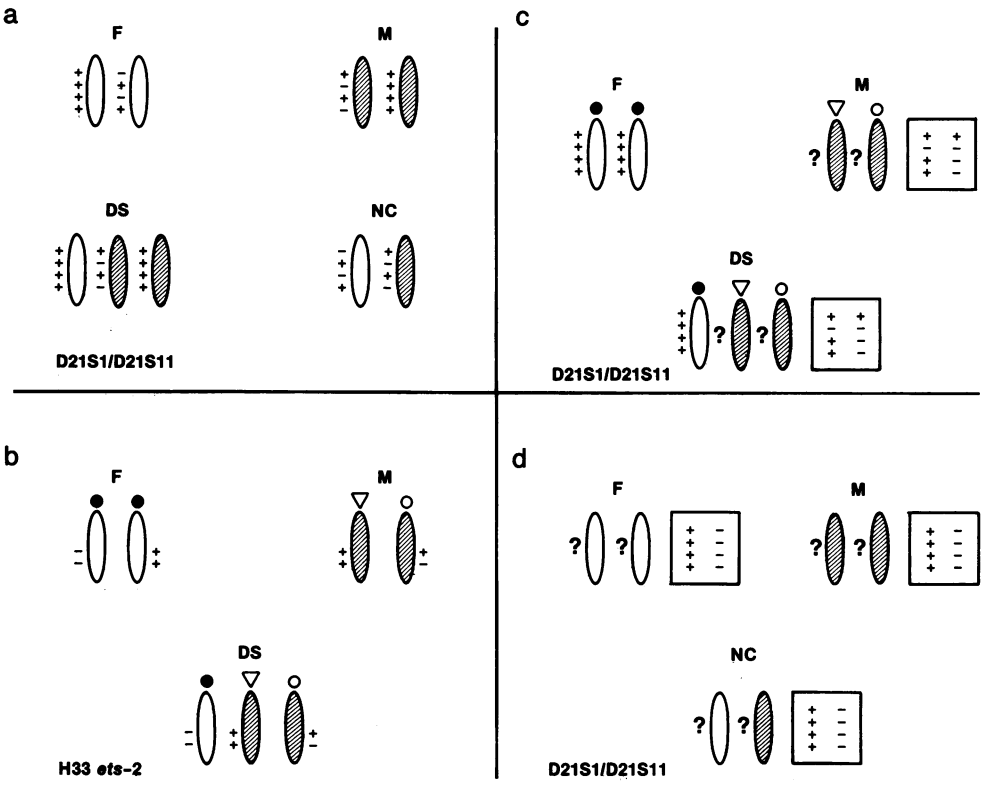

FIG. 2. The assignment of the haplotype for each individual chromosome 21 was possible in group B on the basis of knowledge of the haplotype of the NC (*a*) and knowledge of the parental origin of NDJ (morphologic and molecular polymorphism) coupled to a suitable pattern of RFLPs (*b*). The assignment was not possible when the information was represented only by the NDJ data (*c*) or when both parents and NC in group A were heterozygous for one or more sites (*d*).

++ and −−, with frequencies of 0.61 and 0.39, respectively (Fig. 1).

The assignment of the haplotypes was possible in the majority of cases for groups A and B, combining both the cytogenetic and molecular data of the father (F), mother (M), and children (DS or NC) (8). The criteria used are exemplified in Fig. 2. In group B, either the knowledge of the haplotype of the NC (Fig. 2*a*) or the knowledge of the cytogenetic origin of NDJ (morphology of satellites) coupled to a suitable pattern of RFLPs (Fig. 2*b*) was helpful in determining the linkage phase. A few recombinational events preceding the NDJs at meiosis I and II were recognized by combining both the cytogenetic and the molecular analysis (8). In all cases, these recombinations had an effect on the repositioning of the more distal haplotypes for the *ETS2* and *D21S23* loci. In theory, the repositioning of a given haplotype occurring in prophase I might affect the subsequent segregational phases. For this reason, we took into account the effect of recombinations. In these cases, the haplotypes were assigned to the recombinant chromosomes. The recombinant chromosomes were defined by their centromere and accordingly distributed in the proper grouping. The assignment of the haplotype was impossible either when the information represented by the NDJ data was insufficient (Fig. 2*c*) or when (in group A) the heterozygosity for one or more polymorphic sites was present in both the parents and the child (Fig. 2*d*). For these

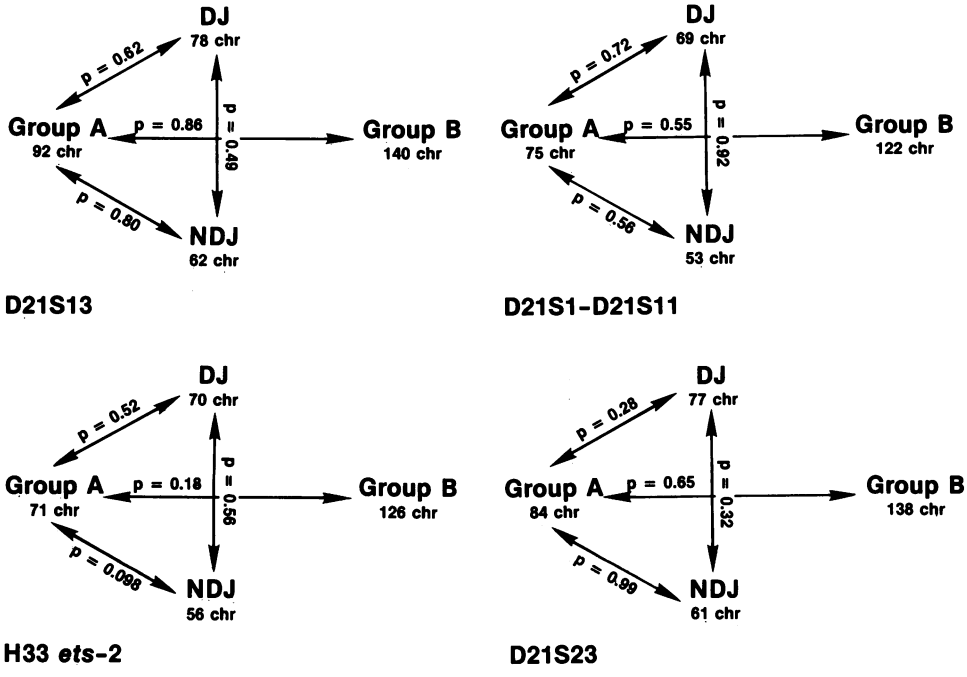

FIG. 3. The diagrams summarize how the distribution of each haplotype was compared among the various groups of chromosomes 21. Each arrow points to the two groups under comparison. The size of each group for the haplotype in question is also reported. The *P* values were obtained by the χ^2 test.

Table 1. Distribution analysis of the haplotypes identified by H33 *ETS2* probe

Group	Haplotypes, no. ++	+−	−−	Total no.		Statistical data χ^2	*P*	df
A	15	11	45	71				
B	33	30	63	126	A→B	3.47	0.18	2
DJ	17	15	38	70	A→DJ	1.32	0.52	2
NDJ	16	15	25	56	A→NDJ	4.65	0.098	2
					DJ→NDJ	1.17	0.56	2
NDJ I	14	13	22	49	A→NDJ I	4.2	0.12	2
					DJ→NDJ I	1.02	0.60	2
NDJ II	2	2	3	7	A→NDJ II	1.25	0.53	2
					DJ→NDJ II	0.35	0.84	2

Example of statistical elaboration to compare the distribution of the haplotypes in the different groups of chromosomes. The example refers to the haplotypes identified by the *ETS2* probe. Groups: A, chromosomes 21 from control parents; B, chromosomes 21 from parents with a DS child; DJ, chromosomes 21 of group B that underwent disjunction; NDJ, chromosomes 21 that did not undergo disjunction at the first meiotic division (NDJ I) or at the second (NDJ II).

reasons and for the exclusion of the most infrequent haplotypes, the total number of chromosomes considered for the statistical analysis in each group varies slightly for the different haplotypes.

Fig. 3 represents diagrammatically the summary of the overall statistical analysis. The comparison of the distributions of the haplotypes between each group and the other groups was performed by the χ^2 test for independence. The probability values (*P*) were in all cases calculated as exemplified in Table 1 that contains the data for the *ETS2* haplotypes. In the diagrams of Figs. 3, the final *P* values deduced from the overall calculations are reported. From the various comparisons it is apparent that no significant difference ($P > 0.01$) exists between groups A and B, even when the NDJ and DJ groups are considered separately. Moreover, even the distinction of NDJ groups on the basis of first (NDJI) and second (NDJII) meiotic error does not significantly affect the *P* values (Fig. 4). In the latter case, because of the small number of chromosomes of the NDJII group, the statistical test used was the Fisher exact test (FET).

A study considering only the *D21S1/D21S11* haplotypes was performed on Greek subjects by other authors (1). That study seemed to point to a correlation between the haplotype − + + + and a propensity to missegregation of the chromosome 21 marked with that haplotype. For this reason, the Greek and Northern Italian chromosome groups were compared for the haplotype in question. Table 2 shows that the Greek and Italian control groups (Group A) are indistinguishable ($P = 0.95$) as are the B ($P = 0.63$) and DJ groups ($P = 0.52$). The only significant difference ($P = 0.013$) was found for the NDJ groups.

DISCUSSION

In experimental organisms (*Drosophila*), there is evidence that genetic factors may increase the frequency of chromosome missegregation during the meiotic process. The possibility that this may also be the case in humans is intriguing. A recent study has suggested a genetic predisposition to NDJ for human chromosome 21—that is, a predisposition to Down syndrome (7). Specifically, in an investigation on a group of Greek families, chromosomes 21 marked with a particular DNA haplotype seemed to be preferentially involved in the NDJ events. The haplotype in question (− + + +) was defined by four closely linked polymorphic sites: *Bam*HI and *Msp*I in *D21S1* and *Eco*RI and *Taq* I in *D21S11*. This finding implied some association between this haplotype and a putative cis-acting genetic element influencing NDJ. If substantiated, it would represent a way to screen for couples at increased risk for trisomy 21 offspring.

To test the general applicability of that observation, we undertook an extensive investigation of a larger cohort of Northern Italian individuals. In addition to the haplotype used in the previous work, we analyzed control haplotypes for chromosome 21 sequences localized in distinct regions of

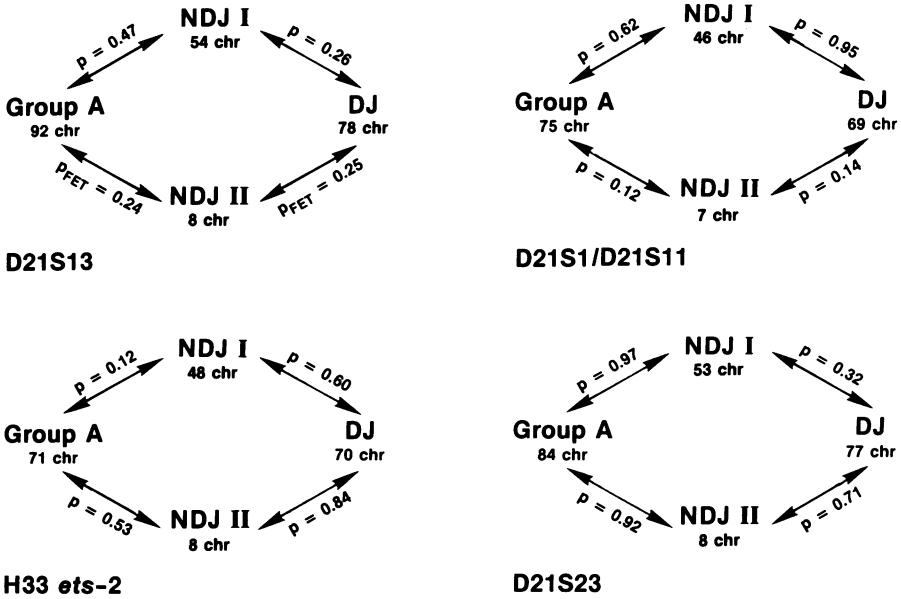

FIG. 4. Haplotype distributions of groups A and DJ are compared with those of the NDJ group, which was classified in two subgroups, NDJ I and NDJ II, according to the stage of occurrence of the meiotic error. The *P* values indicated were obtained by the χ^2 test or Fisher exact test.

Table 2. Comparison of the distribution of haplotypes identified by *D21Z1* and *D21S11* within the groups in Greek and Italian subjects

Group	Subjects	Haplotypes, no.				Statistical analysis		
		++++	−+++	+−−−	Total	χ^2	*P*	df
A	Greek	43	23	24	90	0.098	0.95	2
	Italian	34	20	21	75			
B	Greek	15	17	16	48	0.91	0.63	2
	Italian	46	35	41	122			
DJ	Greek	14	5	10	29	1.3	0.52	2
	Italian	27	19	23	69			
NDJ	Greek	1	12	6	19	8.64	0.013	2
	Italian	19	16	18	53			

Distribution of haplotypes defined by polymorphic sites identified by *D21S1* (*Bam*HI and Msp I) and *D21S11* (*Eco*RI and Taq I) within the various chromosome groups in Greek and Italian subjects. The values for χ^2, *P*, and the degree of freedom (df) are reported.

the chromosome. From the overall statistical comparisons of the distribution of the various haplotypes, we could not conclude that a particular haplotype is characteristic in the NDJ chromosomes that we considered, even when we distinguished those that were involved in the reductional segregation of meiosis I (NDJ I) from those involved in the equational segregation of meiosis II (NDJ II).

Therefore, our results do not show any genetic predisposition to NDJ linked to the arbitrary chromosome 21-specific DNA sequences analyzed, including the *D21S1* and *D21S11* sequences used in the other study (7).

The difference between our findings and the conclusion based on the Greek population is puzzling. The two ethnic groups do not seem to differ because the distribution of the haplotypes in question is identical in control subjects of group A ($P = 0.95$) and similar in the B ($P = 0.63$) and DJ ($P = 0.52$) groups (Table 2). The only significant difference is in the *P* values obtained from the comparison of groups A and NDJ, giving a *P* value of 0.56 for the Italian sample and of 0.0005 for the Greek sample (Fig. 5). The probability of 0.013 (Table 2 and Fig. 5) associated with the NDJ groups is a strong indication of a difference between the Greek and Italian subjects. While the relatively small size of the Greek sample does not invalidate the above conclusion, a larger sample of Greek subjects might have produced a less striking difference in the ++++ haplotype (Table 2), which is the largest component of the overall χ^2.

From our data, we exclude an association between specific haplotypes detected by these chromosome 21 sequences in Northern Italian individuals. As a consequence, it would not be appropriate to use these specific molecular haplotypes for identifying couples at risk for having a DS child in this population. Whether a genetic component on chromosome 21 itself may influence the tendency to chromosome 21 missegregation remains to be determined. Success in identifying this component might be achieved by focusing on probes close to the centromere. The quite consistent distance (15 centimorgans) (R. E. Tanzi, personal communication) of the *D21S1/D21S11* loci from the centromere is supportive of the findings we described. It is difficult to perceive how an aberrant cis-acting element on chromosome 21 would have remained linked over the course of the evolution to both the centromere and the distant *D21S1/D21S11* haplotype (−+++). This latter argument makes it unlikely to interpret the discrepancy between the previous study and our study on the basis of a difference in such linkage between Greeks and Northern Italians. Most likely the discrepancy might be reconciled by applying a more rigorous definition of the origin of the chromosomes in the sample of Greek subjects.

In the context of the above discussion, any application of the haplotype test (7) to clinical practice for Down syndrome prevention should in our opinion be viewed with caution.

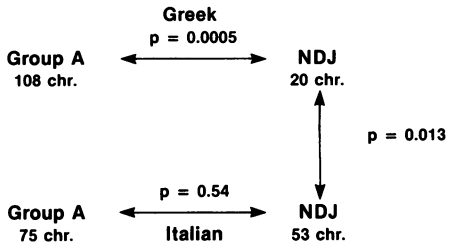

FIG. 5. The distribution of the haplotype for the *D21S1/S21S11* polymorphic sites is compared between the A and NDJ groups in the Italian and Greek subjects.

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