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Meiotic Crossing-Over in Nondisjoined Chromosomes of Children with Trisomy 21 and a Congenital Heart Defect

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Summary

We have used DNA polymorphisms to study meiotic crossovers of chromosome 21q in 27 nuclear families. Each family had a child with Down syndrome and a congenital heart defect. Twenty DNA polymorphisms on chromosome 21 were used to determine parental and meiotic origin of nondisjunction and to identify crossovers. Twenty-four cases were of maternal origin, and three were of paternal origin. Twenty-two unequivocal crossover events were identified. Sixteen crossovers were observed in 22 chromosome pairs nondisjoining at the first meiotic division (MI), and six crossovers were observed in five chromosome pairs nondisjoining at the second meiotic division. Fifty percent of crossover events in MI nondisjunction are detectable by molecular genetic means. Thus, the results suggest that, in this sample, each nondisjoined chromosome 21 pair has been involved in at least one crossover event.

Introduction

The frequency of crossovers in nondisjoining chromosomes in trisomy 21 is controversial. The occurrence of crossovers at reduced frequency has been suggested by studies of somatic cells, which report direct (cytogenetic) or indirect (DNA) evidence of crossover events (Hassold and Jacobs 1984; Warren et al. 1987; Galt et al. 1989; Meijer et al. 1989; Sherman et al. 1991, 1992). Direct meiotic observation has led to the hypothesis that, alternatively, there may be increased recombination in nondisjoining chromosomes (Hultén 1990). Tanzi and coworkers (1992) observed a relative reduction, with increasing maternal age, in crossing-over in the telomeric region and suggested a possible relationship with the maternal age effect in Down syndrome. Petersen et al. (1992) presented evidence suggesting the occurrence of recombination between cytogenetic heteromorphisms of the short arm and pericentromeric short sequence repeat (SSR) markers.

In studies of recombination in nondisjoining chromosomes 21, the increasing availability of highly informative DNA markers increases resolution. In particular, the highly informative SSR polymorphisms, in the centromeric region and elsewhere, facilitate determination of parental and meiotic origin of nondisjoined chromosomes (Antonarakis et al. 1992; Petersen et al. 1992).

A crossover event centromeric (proximal) to a given marker is detectable only if the nondisjoining parent is heterozygous at the marker locus. Crossing-over at the first meiotic division (MI) is reflected as reduction to homozygosity, shown diagrammatically in figure 1. It is well recognized, as implied by the figure and legend, that even in informative matings, only 50% of crossover events in chromosomes nondisjoining at MI will be detectable. When nondisjunction occurs at the second meiotic division (MII), a crossover event centromeric to a marker is reflected as retention of heterozygosity for the marker. All crossovers involving chromosomes nondisjoining at MII will be detectable.

Congenital heart defects affect 40% of children with Down syndrome (Smith 1976, p. 7). It has been sug-

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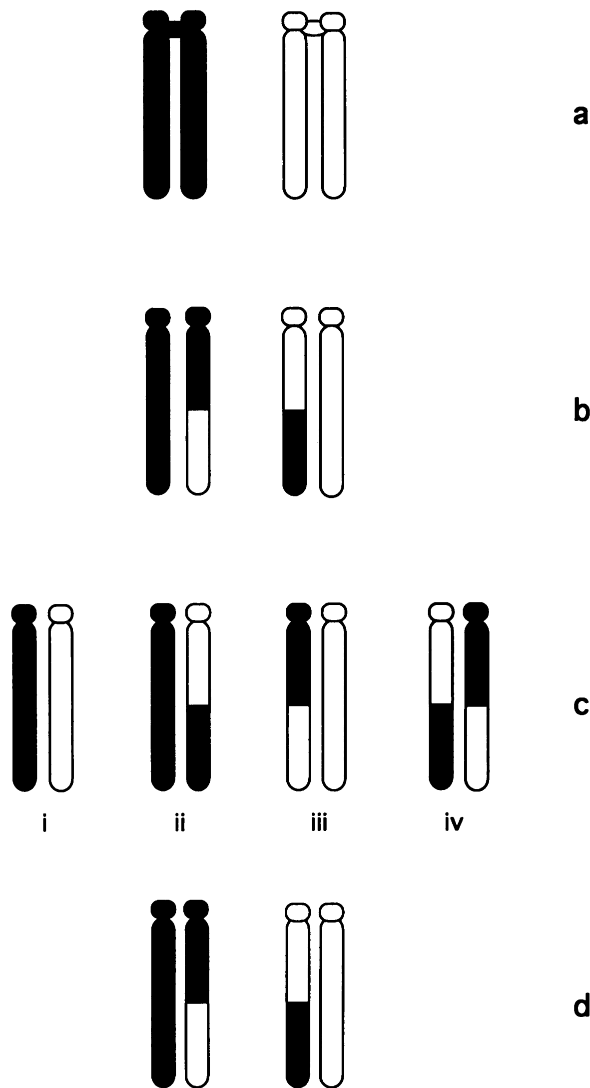

Figure 1 Detection of crossover events in nondisjoining chromosomes 21. Diagrams show chromosomes contributed by the nondisjoining parent only. *a*, Chromosomes at MI, prior to crossover. *b*, Result of one chiasma between these two chromosomes. *c* and *d*, Pairs of chromosomes contributed to the gamete by the nondisjoining parent. *c*, Nondisjunction at MI. There are four possible chromosome pairs which can pass to the gamete, assuming independent assortment. *i*, Neither chromosome took part in the crossover event; offspring will receive parental genotype. *ii* and *iii*, One chromosome was involved in the crossover event; offspring genotype will correspond to parental genotypes *centromeric* to the crossover site. Offspring genotype will show reduction to homozygosity *telomeric* to the crossover site. *iv*, Both chromosomes were involved in the crossover event; offspring will receive parental genotype. Thus, the crossover event is not detectable. *d*, Nondisjunction at MII. There are two possible chromosome pairs which can pass to the gamete. One chromosome of each pair took part in the crossover event; offspring will receive parental genotype *telomeric* to the crossover site and will show reduction to homozygosity *centromeric* to the crossover site. The diagrams show a single crossover event, but the principles extend to any number. Thus, from (*c*), given informative markers to either side of a crossover site, crossover events preceding MI nondisjunction can be detected in only 50% of the gametes (and trisomic offspring) produced (*c* [*ii*] and [*iii*]). By molecular genetic means, 50% of the chromosomes which have, in fact, been involved in crossover events cannot be identified (*c* [*iv*]).

gested (Kurnit et al. 1987) that this may result from a combination of genetic and stochastic effects. While heart defects also occur in other autosomal trisomies, the types and frequencies of defects observed vary; in Down syndrome, there is an excess of atrioventricular septal defect (AVSD) and an increased prevalence of tetralogy of Fallot (Smith 1976, p. 7).

Embryonic cardiac development and septal fusion are complex processes, likely to require the accurate coordination of expression of many genes which may be on different chromosomes. In view of the high incidence of congenital heart defects in Down syndrome, it seems probable that one or more of the genes involved in heart development lies on chromosome 21. As an initial step in an extensive study of the molecular genetic basis of congenital heart defects in Down syndrome, we studied the parental and meiotic origin of nondisjunction in families having a trisomic child with a congenital heart defect. We present findings suggesting that, in parents of this subset of children, each nondisjoining chromosome is involved in at least one crossover event.

Subjects, Material, and Methods

Subjects

DNA samples from 27 families were used for polymorphism studies, with their consent. Most (22) families were ascertained through medical records of an affected child with Down syndrome and a congenital heart defect, seen and treated at the Department of Cardiology, Institute of Child Health, or the Hospital for Sick Children, London. Most probands were referred to this major center soon after birth. Five additional families were ascertained through the Down's Heart Group (a family support association). Fifteen of the children were born with an AVSD (families 12, 66, 67, 71, 76, 90, 101, 123, 130, 131, 133, 900, 903, 904, and 906; families 12 and 76 also had tetralogy of Fallot); three were born with tetralogy of Fallot alone (families 52, 100, and 907); seven were born with ventricular septal defect (families 26, 68, 81, 85, 125, 137, and 901; 26 also had atrial septal defect); one was born with atrial septal defect alone (family 64), and one with a mitral valve defect (family 20). None of the parents was related. All the parents were European Caucasians, ex-

Table 1

Conditions for Detection of Polymorphisms Using PCR

Locus	Denaturation Temperature (°C)	Denaturation Time (s)	Annealing Temperature (°C)	Annealing Time (s)	Extension Temperature (°C)	Extension Time (s)	No. of Cycles
D21S215[a]	94	30	58	30	72	30	30
D21S120[b]	93	60	65	60	72	60	35
IFNAR[c]	94	30	58	30	72	30	30
HMG14[d]	94	30	58	30	72	60	30
PFKL[e]	94	30	56	30	72	30	30

[a] Conditions modified from those reported by Warren et al. (1992).
[b] Conditions modified from those reported by Burmeister et al. (1990).
[c] Conditions modified from those reported by McInnis et al. (1991).
[d] Conditions modified from those reported by Petersen et al. (1991*a*).
[e] Conditions modified from those reported by Polymeropoulos et al. (1991).

cept for two (in different families) of Chinese origin. In the cases of maternal origin (below), the mean maternal age at the birth of the affected child was 31.26 ± SEM (standard error of the mean) 1.07 years (range 18–46 years); no parental age information was available for one family. All subjects with Down syndrome had free trisomy 21, confirmed cytogenetically either shortly after birth or for this study. None showed mosaicism. Fourteen probands were female, 13 male; their ages were between 2 and 17 years (mean 9.2 years) at venepuncture. Blood for polymorphism studies was collected by peripheral venepuncture and was stored at −20°C.

DNA Polymorphisms

RFLPs.—The following cloned DNA probes (reviewed by Cox and Shimizu 1991), in order (21cen→21qter) were used to detect RFLPs (listed in Cox and Shimizu [1991], except as specified) by hybridization to Southern blots of agarose gels of electrophoresed genomic DNA digested with the following restriction enzymes: *D21S16, Xba*I; *D21S13, Taq*I; *D21S46, Pvu*II (M. J. Farrer, unpublished results); *D21S1/D21S11, Eco*RI; *D21S8, Hin*dIII; *D21S111, Sac*I; *D21S82, Eco*RI; *D21S53, Sac*I; *D21S42, Taq*I; *COL6A1, Bam*HI C (Davies et al. 1993); *COL6A1, Taq*I and *Bam*HI B; and *COL6A2, Kpn*I (Francomano et al. 1991).

VNTRs.—The *COL6A1* probe was used to detect two VNTRs on Southern blots of restriction enzyme digests of genomic DNA with *Taq*I (Francomano et al. 1991) and *Bam*HI (Davies et al. 1993).

SSRs.—SSR polymorphisms at loci *D21S215, D21S120, IFNAR, HMG14,* and *PFKL* were also studied. Conditions are listed in table 1 (after Warren et al. 1992; Burmeister et al. 1990; McInnis et al. 1991; Petersen et al. 1991*a;* Polymeropoulos et al. 1991, respectively).

Table 2

Observed Crossover Events, by Nondisjoining Parent

No. of Crossovers	Mother MI	Mother MII	Father MI	Father MII
0	11	0	1	0
1	5	3	0	1
2	3	1	1	0
3	1	0	0	0

Note.—All but one of these families were informative for meiotic division of nondisjunction at one or more of the four most centromeric markers *D21S215, D21S120, D21S16,* and *D21S13* (see text).

Methods

DNA was prepared from frozen whole blood samples by a modification of the method of Kunkel and colleagues (1977), as described elsewhere (Kessling et al. 1992). Restriction enzyme digests were carried out using the enzymes specified under the manufacturer's (Bethesda Research Laboratories) recommended conditions. To detect RFLPs and VNTRs, electrophoresis was carried out on 0.8% or 1% agarose gels. Southern blotting, hybridization, and autoradiography were carried out as described by Kessling et al. (1992).

PCR (Saiki et al. 1985) and PAGE were used to detect SSR polymorphisms. Amplification was carried out using 20 ng of DNA in 12.5 μl of reaction mixture (10

mM Tris-HCl, pH 8; 50 mM KCl; 1.5 mM $MgCl_2$; 0.01% gelatin; 200 μM each of dCTP, dGTP, and dTTP; 40 μM dATP; 400 μM [^{35}S]dATPαS [Amersham], 1 μM with respect to both oligonucleotide primers; and 1 unit of *Taq* polymerase [Cambridge Biotechnology]), under the conditions detailed in table 1. Electrophoresis was carried out in 6% polyacrylamide DNA sequencing gels (National Diagnostics) at 60–70 W for 3–5 h depending on the size of the PCR product. Gels were dried and exposed to X-ray film (Kodak X-OMAT AR) at room temperature for 16–48 h.

Observed Genotypes

Autoradiographs were scored for SSR polymorphism typing, and autoradiographs of Southern blots were scored independently by two observers for allelic dosage. Discrepant typings were repeated or rejected. In our hands, visual assessment of the results of SSR polymorphisms did not allow consistent scoring of allelic dosage in heterozygotes with trisomy 21; therefore, no dosage assessments have been included for these markers. This may be because the radioactive labeling was by incorporation into the PCR product, rather than by end-labeling of an oligonucleotide primer.

Results

The parent of origin of the third chromosome 21 was the mother in 24 cases, the father in 3. Twenty-six families were informative for the meiotic origin of nondisjunction at one of the four most centromeric markers (*D21S215, D21S120, D21S16,* or *D21S13;* there was no family for which *D21S16* was the most centromeric informative marker). No recombination has been shown between *D21S215* and the alphoid centromeric repeat (Jabs et al. 1991). In 21 families, these markers showed nondisjunction to have occurred at MI; in five families, nondisjunction occurred at MII. MI nondisjunction was inferred from retention of parental heterozygosity, and MII nondisjunction from reduction to homozygosity, on the assumption of no crossing-over between the most proximal informative marker and the centromere. These findings agree well with previously published observations (Sherman et al. 1991; Antonarakis et al. 1992). In the eight cases in which *D21S120* or *D21S13* was the most centromeric informative marker, there is a small possibility that meiotic origin was misassigned, as these loci have been shown to be 6 cM from the alphoid repeats (Jabs et al. 1991).

The meiotic origin of nondisjunction was inferred from the most centromeric informative marker, as described above. More distal loci were used to define crossover events as follows: A crossover event in MI nondisjunction was assigned proximal to a locus showing reduction to homozygosity. In MII nondisjunction, a crossover event was assigned proximal to a locus showing retention of heterozygosity. Additional crossovers were assigned to the same chromosome if more telomeric (distal) loci showed nonreduction or loss of heterozygosity. In one family (family 81) we have assumed nondisjunction to be of maternal origin. This is the more conservative assumption, as paternal origin presupposes five crossover events. Table 2 shows the numbers of unequivocal crossover events observed, classified by parental and meiotic origin of nondisjunction. Neither family with a parent of Chinese origin showed any unequivocal crossover events. The mean number of observed crossover events per nondisjunction in all these families was 0.72 (±SEM 0.20) for MI nondisjunction and 1.20 (±SEM 0.20) for MII nondisjunction. One family, included in the table, was uninformative at the three most centromeric loci and had no detectable crossover events. In this family, results from the most centromeric informative locus (*D21S8*) implied MI nondisjunction. Without this family, the mean frequency of observed crossover events per MI nondisjunction is increased to 0.76 (±SEM 0.20).

Table 3 shows the genotypes of all individuals typed, the informative markers flanking assigned crossovers, and the regional distribution of these crossover events along the chromosome. In all but one of the families informative for recombination events, the most distal informative marker lay within the *COL6A1–COL6A2* gene cluster. The data are compatible with an area of increased recombination in the subtelomeric region.

There was no apparent relationship between the severity of the heart defect and the number of crossovers observed (data not shown), although the numbers of children with each individual cardiac defect are small. There was no evidence for any region of uniparental dizygosity (identical alleles from the same parent) shared among all children with a heart defect (table 3), or among children sharing the same heart defect (data not shown).

Discussion

On average, 0.72 crossover events were detected per MI nondisjunction, and 1.20 crossover events per MII nondisjunction. As shown in figure 1, in MI nondisjunction, 50% of crossover events are not detectable using DNA polymorphisms. A corollary is that for every crossover event detected in MI, another such event can be inferred. Hence our findings suggest that,

Table 3

Individual Genotypes and Regional Distribution of Crossovers

Locus and Individual	Family Number												
	12 (F)	20 (M)	26 (F)	52 (M)	64 (F)	66 (M)	67 (F)	68 (M)	71 (F)	76 (F)	81 (M)	85 (M)	90 (F)
D21S215:													
Mother	13	24	12	12	13	13	13	12	12	12	12	34	23
Father	24	13	23	34	23	22	23	23	23	34	22	12	14
Affected child	14	234	123	124	123	123	123	123	12	123	12	234	124
Inference	R	mNR	NR	mNR	NR	mNR	NR	NR	(pR)	mNR	U	mNR	pNR
D21S120:													
Mother	12	23	12	23	23	**11**	12	12	24	13	12	12	12
Father	12	12	23	13	13	**12**	23	23	13	23	12	13	13
Affected child	12	123	123	123	123	**12**	12	12	124	12	12	12	12
Inference	U	NR	NR	NR	NR	**U**	(pR)	(pR)	mNR	R	U	(pR)	(pR)
D21S16:													
Mother	11	11	11	11	12	**11**	11	11	12	11	11	11	11
Father	11	11	11	11	11	**11**	11	11	11	11	12	12	11
Affected child	111	111	111	111	112	**111**	111	111	112	111	112	111	111
Inference	U	U	U	U	(mNR)	**U**	U	U	(mNR)	U	(pNR)	(pR)	U
D21S13:													
Mother	12	22	11	12	**22**	12	11	11	11	12	22	22	12
Father	12	11	11	12	**12**	11	22	11	11	12	12	12	12
Affected child	122	122	111	112	**NT**	122	112	111	111	122	122	122	111
Inference	U	m	U	U	**NT**	mR	m	U	U	U	(pNR)	(pNR)	(R)
D21S46:													
Mother	11	11	11	11	**11**	**11**	11	11	11	11	11	12	**11**
Father	11	11	11	11	**11**	**11**	11	11	11	11	11	11	**11**
Affected child	111	111	111	111	**111**	**111**	111	111	111	111	111	112	**111**
Inference	U	U	U	U	**U**	**U**	U	U	U	U	U	(mNR)	**U**
D21S1/D21S11:													
Mother	12	11	12	12	**11**	**11**	11	12	12	11	11	12	**11**
Father	11	11	22	12	**11**	**12**	11	11	12	11	12	11	**11**
Affected child	122	111	122	12	**111**	**112**	111	112	122	111	111	112	**111**
Inference	mR	U	(mNR)	U	**U**	**(pNR)**	U	(mNR)	U	U	(pR)	(mNR)	**U**
D21S8:													
Mother	22	22	12	22	12	**22**	12	11	22	22	12	11	**12**
Father	22	12	11	12	22	**12**	11	12	22	12	11	12	**22**
Affected child	222	122	112	222	222	**122**	112	111	222	222	112	111	**122**
Inference	U	(pNR)	(mNR)	(pR)	(mR)	**(pNR)**	(mNR)	(pR)	U	(pR)	(mNR)	(pR)	**(mNR)**
D21S111:													
Mother	12	11	12	12	**11**	**11**	11	11	22	12	11	22	22
Father	11	11	12	12	**22**	**12**	12	12	11	12	12	22	12
Affected child	122	111	122	122	**12**	**111**	111	111	122	122	111	222	122
Inference	mR	U	U	U	**U**	**(pR)**	(pR)	(pR)	m	U	(pR)	U	(pNR)
D21S82:													
Mother	23	23	22	12	**22**	23	33	33	22	33	23	22	22
Father	12	22	11	22	**23**	12	12	23	23	22	12	11	12
Affected child	222	223	122	122	**222**	223	133	333	223	233	123	122	122
Inference	R	(mNR)	m	(mNR)	**(pR)**	(mNR; pR)	m	(pR)	(pNR)	m	NR	m	(pNR)
IFNAR:													
Mother	**12**	13	12	13	**12**	**NT**	34	33	23	12	11	13	11
Father	**23**	24	23	24	**13**	**NT**	12	12	12	34	12	12	23
Affected child	**12**	123	12	134	**12**	**NT**	134	13	123	23	111	13	123
Inference	**(pR)**	mNR	(mNR)	mNR	**(pR)**	**NT**	mNR	(pR)	NR	R	(pR)	(pR)	pNR
HMG14:													
Mother	**13**	13	13	13	12	**33**	11	12	13	12	12	22	12
Father	**23**	24	NT	23	34	**12**	23	34	24	13	22	12	13
Affected child	**13**	123	13	13	123	**23**	12	123	134	13	12	12	123
Inference	**(pR)**	mNR	U	(pR)	mNR	**(pR)**	(pR)	mNR	mNR	(mR)	U	U	NR

(Gender of Proband)

100 (M)	101 (F)	123 (M)	125 (M)	130 (M)	131 (M)	133 (M)	137 (M)	900 (F)	901 (F)	903 (F)	904 (F)	906 (F)	907 (F)
14 23 124 mNR	23 11 13 (mR)	24 13 12 R	23 14 234 mNR	14 33 134 mNR	12 22 12 U	12 12 12 U	12 13 123 NR	12 23 123 NR	NT NT NT NT	23 12 123 NR	23 14 134 pNR	12 13 12 (pR)	33 12 23 (pR)
23 12 123 NR	12 12 222 R	22 11 12 U	12 22 12 U	**12** **23** **12** **(pR)**	12 12 12 U	13 12 12 (mR)	23 14 234 mNR	13 12 13 (pR)	12 23 23 (mR)	12 23 122 (mNR; pR)	12 12 12 U	23 12 23 (pR)	34 12 134 mNR
12 11 112 (mNR)	12 12 222 R	11 11 111 U	11 11 111 U	**11** **12** **111** **(pR)**	11 11 111 U	**11** **11** **111** **U**	11 11 111 U	11 11 111 U	**11** **11** **111** **U**	11 11 111 U	11 11 111 U	11 11 111 U	11 11 111 U
22 11 122 m	12 12 122 U	11 11 111 U	12 11 NT NT	**12** **NT** **112** **NT**	12 22 122 (mNR)	**11** **22** **112** **m**	11 11 111 U	11 12 111 (pR)	**12** **12** **112** **U**	12 12 122 U	12 22 222 (mR)	12 11 112 (mNR)	11 11 111 U
12 11 112 (mNR)	11 11 111 U	12 12 222 R	11 11 111 U	**11** **11** **111** **U**	12 11 111 (mR)	**11** **11** **111** **U**	12 NT NT NT	11 12 NT NT	**11** **11** **111** **U**	11 11 111 U	12 12 112 U	11 11 111 U	11 11 111 U
11 12 112 (pNR)	12 11 112 (mNR)	11 11 111 U	11 11 111 U	**11** **12** **112** **(pNR)**	**12** **12** **122** **U**	**11** **11** **111** **U**	12 12 122 U	11 11 111 U	**11** **22** **112** **m**	11 12 112 (pNR)	22 22 222 U	12 12 122 U	12 12 112 U
11 22 112 m	12 22 122 (mNR)	12 22 222 (mR)	12 12 112 U	**22** **22** **222** **U**	**12** **12** **122** **U**	**12** **12** **122** **U**	22 22 222 U	22 22 222 U	12 22 122 (mNR)	22 12 122 (pR)	22 22 222 U	22 22 222 U	12 22 12 U
11 11 111 U	11 12 111 (pR)	12 22 112 mR	11 12 112 (pNR)	**11** **22** **112** **m**	12 11 112 mNR	**12** **12** **122** **U**	22 12 222 (pR)	12 22 122 (mNR)	22 12 222 (pR)	11 22 112 m	22 22 222 U	12 11 112 (mNR)	11 12 122 (pNR)
13 23 133 (mNR; pR)	**22** **22** **222** **U**	22 22 222 U	22 23 222 (pR)	**12** **12** **112** **U**	12 23 122 (mNR; pR)	12 22 122 (mNR)	23 22 223 (mNR)	12 22 122 (mNR)	22 22 222 U	22 22 222 U	22 23 223 (pNR)	23 33 233 (mNR)	22 22 222 U
12 **22** **12** **U**	**13** **22** **23** **(mR)**	22 12 222 (pR)	13 24 134 mNR	**12** **12** **12** **U**	23 12 123 NR	**11** **23** **12** **(pR)**	12 23 12 (pR)	12 12 12 U	23 13 123 NR	12 11 12 U	12 13 13 (mR)	**23** **13** **23** **(pR)**	12 34 123 mNR
13 **23** **13** **(pR)**	**12** **33** **13** **(mR)**	13 24 34 R	23 12 123 NR	13 24 12 R	13 12 13 (pR)	**12** **22** **12** **U**	24 13 124 mNR	NT 13 123 NR	23 14 234 mNR	13 23 13 (pR)	13 33 13 U	**22** **12** **222** **(pR)**	13 23 123 NR

(continued)

Table 3 (continued)

Locus and Individual	Family Number												
	12 (F)	20 (M)	26 (F)	52 (M)	64 (F)	66 (M)	67 (F)	68 (M)	71 (F)	76 (F)	81 (M)	85 (M)	90 (F)
D21S53:													
Mother	**11**	12	12	11	11	**11**	12	12	12	12	11	12	12
Father	**12**	11	22	11	12	**12**	11	12	11	11	11	12	12
Affected child	**111**	112	122	111	112	**112**	112	122	112	111	111	122	122
Inference	**(pR)**	(mNR)	(mNR)	U	(pNR)	**(pNR)**	(mNR)	U	(mNR)	(mR)	U	U	U
D21S42:													
Mother	**11**	11	12	12	11	**11**	11	12	12	11	11	11	12
Father	**11**	11	22	11	12	**11**	11	11	11	11	11	12	11
Affected child	**111**	111	122	112	112	**111**	111	112	112	111	111	111	12
Inference	**U**	U	(mNR)	(mNR)	(pNR)	**U**	U	(mNR)	(mNR)	U	U	(pR)	U
PFKL:													
Mother	13	23	23	12	13	NT	12	13	12	23	13	11	23
Father	23	12	NT	12	24	NT	11	22	22	12	23	11	12
Affected child	123	123	123	12	134	NT	12	123	12	222	123	111	12
Inference	NR	NR	NR	U	mNR	NT	U	mNR	U	R	NR	U	(mR)
COL6AI BamHI B:													
Mother	11	12	12	11	11	**11**	11	12	11	11	12	12	11
Father	12	11	11	11	11	**11**	11	11	11	11	11	11	11
Affected child	112	112	112	111	111	**111**	111	112	111	111	112	112	111
Inference	(pNR)	(mNR)	(mNR)	U	U	**U**	U	(mNR)	U	U	(mNR)	(mNR)	U
TaqI RFLP:													
Mother	22	12	12	11	11	**11**	11	12	11	11	12	12	11
Father	12	22	11	11	11	**11**	11	11	11	11	11	22	11
Affected child	122	122	112	111	111	**111**	111	112	111	111	112	12	111
Inference	(pNR)	(mNR)	(mNR)	U	U	**U**	U	(mNR)	U	U	(mNR)	U	U
TaqI VNTR:													
Mother	12	**22**	24	14	12	12	23	11	23	22	12	13	12
Father	12	**11**	13	23	22	23	12	22	14	12	11	22	23
Affected child	122	**12**	234	134	NT	223	123	12	123	122	112	123	123
Inference	U	**U**	mNR	mNR	U	(mR; pNR)	NR	U	mNR	(pNR)	(mNR)	mNR	NR
BamHI VNTR:													
Mother	13	13	12	NT	11	11	22	12	13	23	23	NT	13
Father	23	22	22	NT	22	12	13	13	12	13	13	NT	22
Affected child	133	233	12	NT	12	112	12	112	113	133	123	NT	122
Inference	(mNR; pR)	mR	U	NT	U	(pNR)	U	(mNR; pR)	(mNR; pR)	(mR; pNR)	NR	NT	p
BamHI C:													
Mother	12	11	12	11	12	11	11	13	11	11	11	11	12
Father	12	12	11	11	11	11	12	12	12	12	12	22	12
Affected child	112	112	112	111	112	111	111	123	112	112	111	112	122
Inference	U	(pNR)	(mNR)	U	(mNR)	U	(pR)	NR	(pNR)	(pNR)	(pR)	m	U
COL6A2 KpnI:													
Mother	12	11	12	11	11	11	12	11	22	12	12	11	12
Father	11	22	22	12	22	12	12	11	12	12	12	11	12
Affected child	12	112	12	112	12	111	112	111	122	112	112	111	122
Inference	U	m	U	(pNR)	U	(pR)	U	U	(pNR)	U	U	U	U
Observed crossovers	1	1	0	0	2	3	0	0	0	1	(m0; p5)	0	2
Origin of nondisjunction	mMII	mMI	mMI	mMI	mMI	mMI	mMI	mMI	mMI	mMI	m(I)	mMI	pMI

NOTE.—Order of loci is from centromere (top) to telomere (end). Affected child = child affected with trisomy 21 and a congenital heart defect. We designate the more common allele "1" and the more rare allele "2" for RFLPs; this has been done in all cases except for *D21S82* and *D21S8*, for which nomenclature is in accordance with published reports. For the *COL6A1 BamHI* C RFLP, families 68 and 133 showed a rare third allele, designated "3". For SSRs and VNTRs, again in accordance with custom, the numbering is specific only to individual alleles segregating within a family. Typings for informative markers flanking putative crossover sites are underlined; **typings for loci within regions in which a crossover event must have occurred are indicated in different, boldface type.** Abbreviations for inferences are: m = maternal; p = paternal; NR = nonreduction; R = reduction; U = uninformative; and NT = no typing available. Parentheses enclose inferences conditional on parent of origin, e.g., "(pNR)" indicates that, from the results at this locus, if the nondisjunction event were of paternal origin, there would be nonreduction at this locus. Locus order within *COL6A1* is the most parsimonious order compatible with current data and preliminary mapping (G. E. Davies, unpublished results). Where only a two-allele genotype is given for a heterozygous individual with Down syndrome, this indicates that we were not able confidently to assign allelic dosage for that individual by visual means.

(Gender of Proband)													
100 (M)	101 (F)	123 (M)	125 (M)	130 (M)	131 (M)	133 (M)	137 (M)	900 (F)	901 (F)	903 (F)	904 (F)	906 (F)	907 (F)
12 **12** **112** **U**	**12** **22** **122** **(mNR)**	11 11 111 U	12 11 112 (mNR)	12 11 122 mR	12 11 112 (mNR)	12 11 122 mR	11 11 111 U	**11** **12** **112** **(pNR)**	11 12 112 (pNR)	12 12 112 U	11 11 111 U	**22** **12** **222** **(pR)**	12 12 112 U
11 **11** **111** **U**	11 12 112 (pNR)	12 12 122 U	11 11 111 U	11 11 111 U	11 11 111 U	11 12 112 (pNR)	12 11 112 (mNR)	**12** **12** **112** **U**	11 22 112 m	11 12 112 (pNR)	12 12 122 U	12 11 111 (mR)	11 11 111 U
11 **11** **111** **U**	13 24 234 pNR	NT NT NT NT	12 22 12 U	13 12 13 (pR)	14 23 124 mNR	12 12 12 U	23 13 23 (pR)	23 NT 12 (mR)	23 13 123 NR	NT NT NT NT	12 12 12 U	13 24 34 R	24 13 234 mNR
12 11 111 (mR)	11 11 111 U	11 11 111 U	12 11 112 (mNR)	11 11 111 U	11 11 111 U	11 11 111 U	12 11 112 (mNR)	**11** **11** **111** **U**	11 11 111 U	11 11 111 U	11 12 112 (pNR)	11 11 111 U	11 11 111 U
12 11 111 (mR)	11 11 111 U	11 11 111 U	12 11 112 (mNR)	11 11 111 U	11 11 111 U	11 11 111 U	12 11 112 (mNR)	**11** **11** **111** **U**	11 11 111 U	11 11 111 U	11 12 112 (pNR)	11 11 111 U	11 11 111 U
12 33 113 mR	22 12 122 (pNR)	23 13 122 mR	12 22 122 (mNR)	12 23 222 R	22 13 223 m	22 13 23 (pR)	13 12 123 NR	13 23 133 (mNR; pR)	13 23 133 (mNR; pR)	34 12 234 mNR	12 12 12 U	13 23 113 mR	13 22 123 mNR
NT NT NT NT	23 13 133 (pNR; mR)	13 22 123 mNR	12 23 122 (mNR; pR)	11 NT 12 U	22 13 223 M	22 12 222 (pR)	NT NT NT NT	11 22 112 m	NT NT NT NT	23 12 23 (pR)	23 13 123 NR	12 12 112 U	12 33 123 mNR
11 11 111 U	11 12 112 (pNR)	11 12 111 (pR)	11 11 111 U	12 11 111 (mR)	12 11 112 (mNR)	13 12 133 mR	11 11 111 U	12 12 112 U	12 11 112 (mNR)	12 11 112 mNR	11 11 111 U	12 12 222 R	11 11 111 U
12 12 112 U 1	12 11 112 (mNR) 1	11 12 112 (pNR) 1	22 11 122 m 0	11 11 111 U 1	11 11 111 U 2	11 11 111 U 2	11 12 111 (pR) 0	12 11 12 U 2	11 12 112 (pNR) 1	12 11 12 U 0	12 11 111 (mR) 0	11 11 111 U 1	12 11 112 (mNR) 0
mMI	pMII	mMII	mMI	mMI	mMI	mMII	mMI	mMI	mMII	mMI	pMI	mMI	mMI

in this sample, there has been at least one chiasma in each meiosis leading to nondisjunction.

Where both chromosomes inherited from the nondisjoining parent have been involved in the same crossover event (fig. 1, *c*[*iv*]), the reciprocal recombination of genetic material does not result in any recombination of the parental genotype transmitted to the child. The crucial point in the controversy concerning nondisjoining chromosomes is not the recombination of the parental genotype but the frequency of chiasma formation. Direct meiotic observation has shown an average of 1.05 ± SD 0.23 chiasmata per normal male chromosome 21 bivalent (Hultén 1976; Laurie and Hultén 1985). An estimate of the relative frequency of chiasmata per female bivalent can be obtained by comparing the relative lengths of the male and female genetic maps. Estimates of female:male map length vary from 161:132 cM (Kosambi map function, CEPH data of Petersen et al. 1991*b*) to 118:86 cM (using the Venezuelan pedigree data of Tanzi et al. [1992]). Using these female:male ratios with the male chiasma frequency of Laurie and Hultén (1985), we can estimate that in females, on average 1.28–1.44 chiasmata occur per chromosome 21 bivalent per meiosis. From this approximation and our results, we cannot exclude the possibility that chiasmata have occurred at normal frequency in nondisjoining chromosomes in the sample we have studied. Our findings support the view that nondisjunction may not be a consequence of lack of chiasma formation.

Our sample consists of families with a child having Down syndrome and a congenital heart defect. Studies of trisomic individuals without heart defects are in progress. While one would not expect the mechanisms of nondisjunction to differ in children with and without cardiac defects, we cannot exclude the possibility that the crossover frequency we observe is a feature of trisomy 21 with an accompanying congenital heart defect.

A major factor limiting detection of crossover events is the availability of informative markers flanking the crossover sites. Nine of the observed crossover events (table 3) were detected using informative markers at or distal to *HMG14*. For 26 of the 27 families studied, the *COL6A1*-*COL6A2* gene cluster provided the most telomeric informative marker. Five of the crossover events would not have been detected if the *COL6A1*-*COL6A2* markers had not been included. Detection depends on the degree to which a nondisjoining parent is heterozygous at loci studied: no crossover events will be detected if the nondisjoining parent is homozygous at all loci, resulting in further underestimates of crossover frequency. As demonstrated in recent studies (Antonarakis et al. 1992; Petersen et al. 1992), the use of SSR polymorphisms has facilitated both identification of the nondisjoining parent and the detection of crossover events. The use of the SSR markers and of the additional *COL6A1*-*COL6A2* region informative markers may account for the difference between our results and those previously published (Warren et al. 1987; Meijer et al. 1989; Sherman et al. 1991; Sherman 1992). The importance of subtelomeric markers is supported by the observation, in normal meiosis, that when a chromosome 21 is involved in two chiasmata, the distal chiasma tends to be subtelomeric (Hultén et al. 1990); thus a lack of sufficient informative markers in molecular genetic studies would tend to result in the underestimation of recombination and thus chiasma frequency (M. Hultén, personal communication).

Our findings from this sample suggest that in nondisjunction leading to Down syndrome, each pair of chromosomes 21 is involved in at least one crossover event. Our data are compatible with the hypothesis that crossing-over occurs at normal frequency in nondisjoining chromosomes.

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