Am. J. Hum. Genet. 51:1015–1027, 1992

DNA Polymorphism Analysis in Families with Recurrence of Free Trisomy 21

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Summary

We used DNA polymorphic markers on the long arm of human chromosome 21 in order to determine the parental and meiotic origin of the extra chromosome 21 in families with recurrent free trisomy 21. A total of 22 families were studied, 13 in which the individuals with trisomy 21 were siblings (category 1), four families in which the individuals with trisomy 21 were second-degree relatives (category 2), and five families in which the individuals with trisomy 21 were third-degree relatives, that is, their parents were siblings (category 3). In five category 1 families, parental mosaicism was detected, while in the remaining eight families, the origin of nondisjunction was maternal. In two of the four families of category 2 the nondisjunctions originated in individuals who were related. In only one of five category 3 families, the nondisjunctions originated in related individuals. These results suggest that parental mosaicism is an important etiologic factor in recurrent free trisomy 21 (5 of 22 families) and that chance alone can explain the recurrent trisomy 21 in many of the remaining families (14 of 22 families). However, in a small number of families (3 of 22), a familial predisposing factor or undetected mosaicism cannot be excluded.

Introduction

Trisomy 21 is the most common known genetic cause of mental retardation. Free trisomy 21 accounts for approximately 95% of cases (Giraud and Mattei 1975). Free trisomy 21 in more than one sibling occurs rarely; the recurrence risk in families with one affected child was estimated to be 1%–2%, on the basis of empiric or prenatal data (Mikkelsen and Stene 1979; Daniel et al. 1982). The frequency of trisomy 21 in second-degree (uncle-aunt/nephew-niece combinations) or third-degree (first cousins) relatives is not firmly established (Tamaren et al. 1983; Abuelo et al. 1986; Eunpu et al. 1986).

Possible explanations for the recurrence of free trisomy 21 in families include: (i) parental mosaicism, as has been reported by Harris et al. (1982), Uchida and Freeman (1985), and Nielsen et al. (1988); (ii) a genetic predisposition or factor that favors nondisjunction (for discussion of this hypothesis, see Alfi et al. 1980; Yokohama et al. 1981; DeVoto et al. 1985); (iii) environmental predisposing factors, including hy-

Received April 10, 1992; final revision received June 24, 1992.

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0002-9297/92/5105-0010$02.00

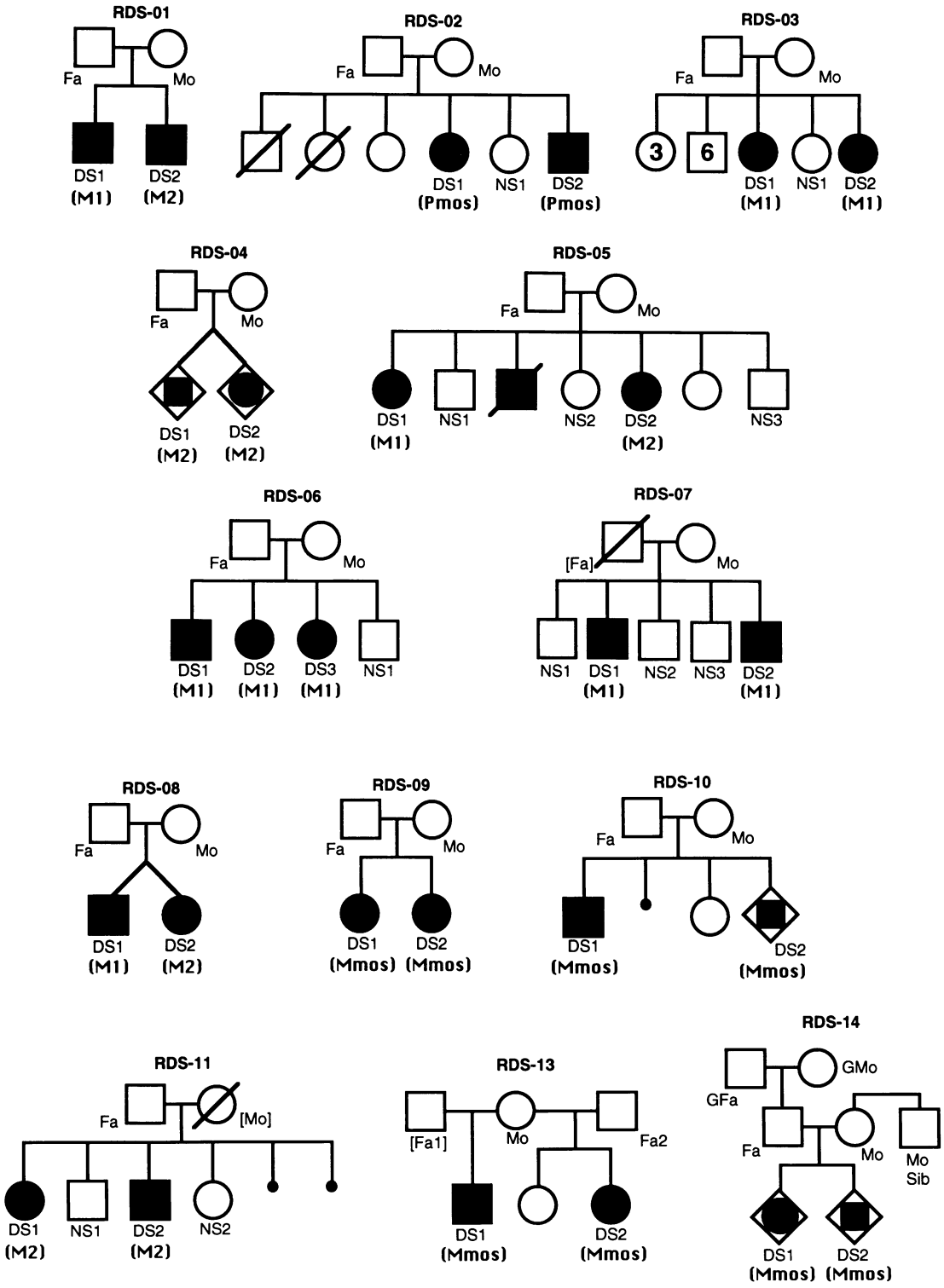
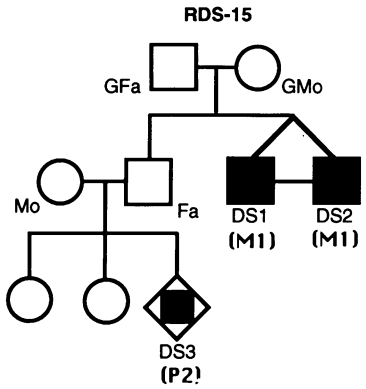
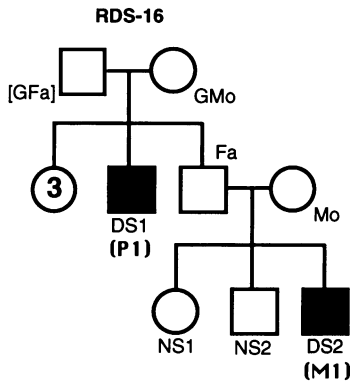
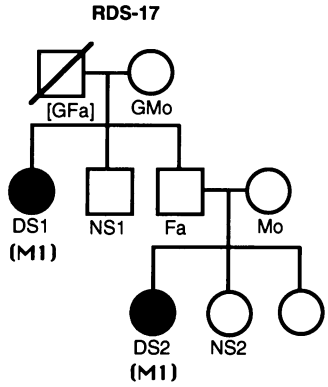
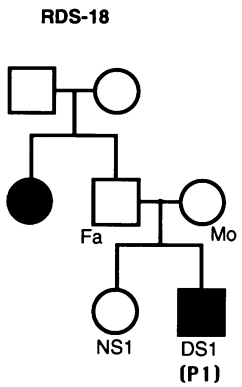


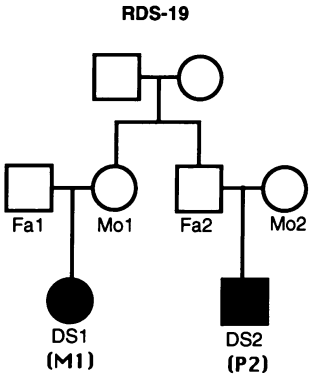
Figure 1 Pedigrees of families with two or more individuals with free trisomy 21. Blackened squares indicate males affected with Down syndrome. Blackened circles indicate affected females. Unblackened squares and circles indicate unaffected males and females, respectively. Numbers inside unblackened symbols indicate total number of unaffected siblings. The diamond enclosing a square indicates a male fetus, and the diamond enclosing a circle indicates a female fetus. Small dots indicate abortuses or miscarriages, for which no other information is known. Fa = father; Mo = mother; DS = Down syndrome; NS = normal sibling. The abbreviations for the origin of the supernumerary chromosome 21 are as in the legend to table 1.

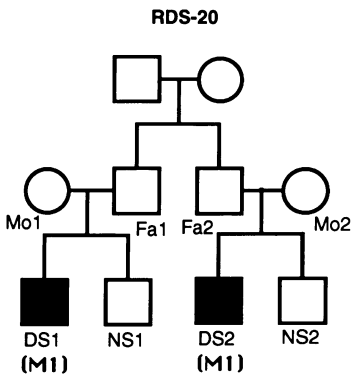

RDS-15
GFa
GMo
Mo
Fa
DS1
(M1)
DS2
(M1)
DS3
(P2)

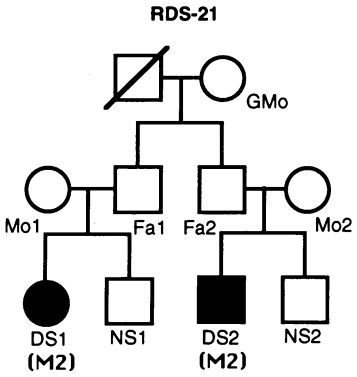

RDS-16
[GFa]
GMo
3
Fa
DS1
(P1)
Mo
NS1
NS2
DS2
(M1)

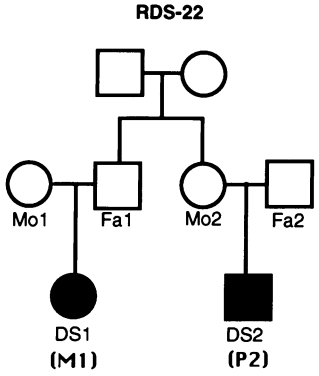

RDS-17
[GFa]
GMo
DS1
(M1)
NS1
Fa
Mo
DS2
(M1)
NS2

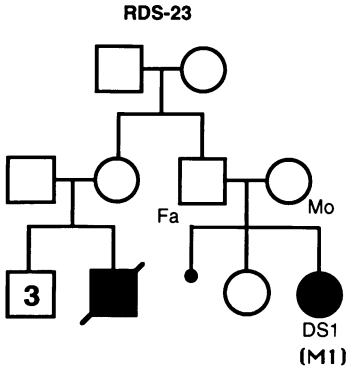

RDS-18
Fa
Mo
NS1
DS1
(P1)


RDS-19
Fa1
Mo1
Fa2
Mo2
DS1
(M1)
DS2
(P2)


RDS-20
Mo1
Fa1
Fa2
Mo2
DS1
(M1)
NS1
DS2
(M1)
NS2


RDS-21
GMo
Mo1
Fa1
Fa2
Mo2
DS1
(M2)
NS1
DS2
(M2)
NS2


RDS-22
Mo1
Fa1
Mo2
Fa2
DS1
(M1)
DS2
(P2)


RDS-23
Fa
Mo
3
DS1
(M1)

pothyroidism, toxins, etc. (Epstein 1989); (iv) the presence of double nucleolus organizing regions in acrocentric chromosomes (Jackson-Cook et al. 1985), however, this hypothesis has not been confirmed by other laboratories (Schwartz et al. 1989); and (v) chance alone.

Analysis of DNA polymorphisms on the long arm and the pericentromeric region of chromosome 21 in families with trisomy 21 can be used to establish the parental origin and meiotic stage of nondisjunction (Antonarakis et al. 1991, 1992; Sherman et al. 1991) in almost all families. The analysis of DNA polymorphisms in the rare families with more than one individual affected with free trisomy 21 (47, + 21) can provide valuable information for any possible genetic predisposing factor. In this paper we have collected and studied 22 families with more than one individual affected with free trisomy 21. The parental and meiotic origin of nondisjunction has been determined in all cases. The interpretation of the results is discussed below.

Patients, Materials, and Methods

Patients

A total of 22 families, each with more than one individual affected with free trisomy 21, were included in the study. Figure 1 shows the pedigrees of the families studied. The families were divided into the following three categories: (i) There were 12 nuclear families, each with two affected siblings (families RDS-01–RDS-05, RDS-07–RDS-11, RDS-13, and RDS-14) and one family (RDS-06) with three affected siblings. (ii) In four additional families (families RDS-15–RDS-18), the individuals with trisomy 21 were second-degree relatives. They were all related through a male individual who was the father and sibling of the patients. (iii) In five families (families RDS-19–RDS-23), the individuals with trisomy 21 were third-degree relatives, that is, they were offspring of siblings. The families of all three categories were collected from cytogenetic laboratories in France and Switzerland. The ages of mothers and fathers at the time of birth of each individual with Down syndrome are included in table 1.

Cytogenetic Analysis

Chromosomal analysis of blood lymphocytes was performed on all individuals with trisomy 21 and on their parents. Chromosome banding was performed by the RHG or GTG technique (Dutrillaux and Lejeune 1971; Seabright 1971). In order to detect mosaicism, a total of 30 metaphases per individual with trisomy 21 were analyzed, as well as 200 metaphases in each of their parents. Mosaicism in our sample is defined as the presence of at least two trisomic cells in 200 metaphases examined. No cytogenetic heteromorphisms were studied since a considerable number of DNA polymorphisms were analyzed, including several pericentromeric markers (see below).

DNA Polymorphism Analysis

The parental origin of the supernumerary chromosome 21 and the meiotic stage of the nondisjunction were detected by using DNA polymorphic markers. The following DNA polymorphisms were used after Southern blot hybridization (Southern 1975): D21S13, D21S110, D21S11, D21S8, D21S111, D21S82, D21S3, D21S112, D21S113, MX1, and COL6A1. Description of the probe-enzyme combinations, the detection method of these polymorphic markers and their mapping position on the long arm of human chromosome 21 can be found in Warren et al. (1989) and Petersen et al. (1991*b*). In addition, the following DNA polymorphisms were used after PCR amplification (Saiki et al. 1985) and detection of the alleles due to short sequence repeats (SSR) by PAGE: D21S215 (21-GT14) Warren et al. (1992), D21S120 (Burmeister et al. 1990), D21S192 (Van Camp et al. 1991), D21S213 (21-GT05) and D21S212 (21-GT10) (A. C. Warren and S. E. Antonarakis, unpublished data), D21S210 (21-GT12) Warren et al. (1992), IFNAR (McInnis et al. 1991), D21S156 (Lewis et al. 1990), and HMG14 (Petersen et al. 1990). All of these polymorphisms are due to $(GT)_n$ dinucleotide repeats, except IFNAR, which is due to a $(TAAA)_n$ repeat in the poly(A) tail of an *Alu* sequence. Description of the detection method and the scoring of polymorphic alleles per family can be found elsewhere (Petersen et al. 1991*a*). Several of the markers used are considered pericentromeric in the long arm of the chromosome (Antonarakis et al. 1992). These markers are D21S215, D21S120, D21S13, D21S192, and D21S172. The last four markers show approximately 6% recombination with a rare chromosome 21-specific polymorphism of alphoid sequences (Jabs et al. 1991), while marker D21S215 shows no recombination with the alphoid polymorphism (Warren et al. 1992). All pericentromeric markers can be used to determine the meiotic origin of nondisjunction. Not all the DNA markers were determined in all families.

Results and Discussion

Table 1 presents the genotypes of the DNA polymorphisms examined in the members of all families. This table also presents the parental and meiotic origin of each trisomy 21 and the presence of crossovers in chromosomes 21 that participated in nondisjunction.

Category I Families

There are 13 families that belong to the first category, in which the individuals with trisomy are siblings (preliminary analysis of families RDS-01 and RDS-02 has been described by Pangalos et al. [1988]). Cytogenetic analysis showed that, in 3 of these 13 families, there was parental mosaicism in blood leukocytes. In families RDS-09 and RDS-10 there was a 2% maternal mosaicism for trisomy 21 (46,XX/47,XX + 21), while in family RDS-02 there was a 2% paternal mosaicism for trisomy 21 (46,XY/47,XY + 21). Analysis of DNA polymorphisms confirmed that the parental origin of the trisomy 21 in individuals DS1 and DS2 of family RDS-02 and individual DS2 of family RDS-10 was from the parent with the mosaicism. Moreover, in family RDS-09, the DNA analysis revealed a chromosome 21 present in the individual with trisomy 21 that was not detected in the DNA of blood from either parent (see markers D21S82 and D21S112 in table 1 and D21S212 in table 1 and fig. 2), confirming the results from the cytogenetic analysis. Furthermore, analysis of DNA polymorphisms revealed potential mosaicism in two other families (RDS-13 and RDS-14) of this category, since polymorphic alleles for chromosome 21 markers found in the offspring with trisomy 21 were not present in the parents (see markers D21S156 for family RDS-13 and D21S112 for family RDS-14 in table 1; the mosaicism in family RDS-14 is probably maternal, since the "new" polymorphic allele for DNA marker D21S112 was not found in the paternal grandparents). Mosaicism that remains undetected by cytogenetic analysis can therefore be recognized after DNA analysis; however, there are cases in which mosaicism was only detected by cytogenetic analysis and was not confirmed by DNA analysis. Theoretically, mosaicism can never be detected by DNA analysis if the polymorphic alleles in the parental trisomic cells are identical.

It is of interest to note that the mosaic individuals in families RDS-09, RDS-13, and RDS-14 are probably themselves the products of meiotic nondisjunction, since they each have, in some of their cells, three different alleles at certain chromosome 21 loci tested. For example, the data suggest that the mother in family RDS-09 contains, in some of her cells, alleles 2, 3, and 5, for polymorphic marker D21S212 (see table 1).

In summary, in the set of 13 nuclear families with two siblings with trisomy 21, we detected five cases (38%) of parental mosaicism as the cause of the recurrence of trisomy 21. The mean maternal age for the first offspring with Down syndrome in these five families was 28.6 years, and the mean paternal age was 29.3 years. The presence of parental mosaicism for trisomy 21 has been previously shown to occur in families with more than one sibling with free trisomy 21 (Harris et al. 1982; Uchida and Freeman 1985; Nielsen et al. 1988). In those studies, the mean maternal age for the first offspring with Down syndrome in 11 families with more than one offspring with Down syndrome and parental mosaicism was 24.6 years.

In eight category 1 families, parental mosaicism was not detected. In these eight families there were 17 individuals with trisomy 21. The parental origin of the extra chromosome 21 in all 17 cases with Down syndrome was maternal. In all families (RDS-01, RDS-03–RDS-08, and RDS-11), there was concordance of the parental origin of the trisomy 21 for the affected siblings. The mean maternal age for the first affected offspring in these eight families was 33.6 years, and the mean paternal age was 34.5 years. These ages are not different from the mean maternal or paternal ages in large series of families with trisomy 21. Analysis of pericentromeric DNA polymorphisms revealed that the nondisjunction had occurred in maternal meiosis I in 10 (58.8%) of the 17 cases, while in 7 (41.2%) of the 17 cases the error had occurred in maternal meiosis II. There is an excess of meiosis-II errors in this small sample compared with the observed 23% among maternal meiosis errors in the study of 200 families with one child with free trisomy 21 (Antonarakis et al. 1992); however, the difference is not statistically significant ($\chi^2 = 2.48$). In three families (RDS-03, RDS-06, and RDS-07), all offspring with Down syndrome were the result of meiosis I error, while in two families (RDS-04 and RDS-11) both offspring with Down syndrome were the result of meiosis II error. However, in the remaining three families (RDS-01, RDS-05, and RDS-08), the trisomy 21 in the first affected individual was due to meiosis I error, and the trisomy in the second affected individual was the result of meiosis II error. There are two families with affected DZ twins (or, in theory, polar-body twins). In one family (RDS-04) the trisomy 21 in both affected twins was due to maternal meiosis II errors. In the other family (RDS-08) the trisomy 21 in one affected twin was the

Table 1

Families with Recurrent Trisomy 21

Families	ID No.	Age of Father/ Mother	Parental Origin[a]	Meiotic Origin[b]	Cross-over	No. of Crossovers	DS Relation	Karyotype	D21S215[b,c] (21-gt14)	D21S120[b,c]	D21S13[b,c] (*Taq*I)	D21S13 (PCR)	D21S192[b,c]	D21S110[c,d]
RDS-01:														
Fa	1598								12	22	12		11	12
Mo	1599								12	13	12		11	12
DS1	1600	36/43	Mat	M1	No	0		Trisomy 21	112	123 M1	122		111	112
DS2	1601	38/45	Mat	M2	Yes	1		Trisomy 21	222 M2	112 M2	111 M2		111	
RDS-02:														
Fa	1602							Pat Mos 2%	23		22			12
Mo	1603								13		12			11
DS1	1604	34/28	Pat Mos					Trisomy 21	333 P		122			111
DS2	1605	40/34	Pat Mos					Trisomy 21	122 P		122			122 P
NS1	1606										12			11
RDS-03:														
Fa	1607								23		12			12
Mo	1608								12		12			11
DS1	1609	48/38	Mat	M1	No	0		Trisomy 21	123 M1		122			111
DS2	1610	54/44	Mat	M1	Yes	1		Trisomy 21	122 M1		112			112
NS1	1611								13		22			11
RDS-04:														
Fa	1612										12			11
Mo	1613										12			12
DS1	1614	32/31	Mat	M2	Yes	1		Trisomy 21			222 M2			112 nr
DS2	1615	32/31	Mat	M2	Yes	1		Trisomy 21			222 M2			122 M r
RDS-05:														
Fa	1616								12		22			12
Mo	1617								23		12			11
DS1	1618	28/22	Mat	M1	No	0		Trisomy 21	123 M1		122 M1			112
DS2	1619	35/29	Mat	M2	Yes	1		Trisomy 21	122 M2		112 M2			112
NS1	1621								22		12			11
NS2	1620								12		12			12
NS3	1622								22		12			11
RDS-06:														
Fa	1623										22			22
Mo	1624										12			11
DS1	1625	25/24	Mat	M1	No	0		Trisomy 21			122 M1			112 M
DS2	1626	30/29	Mat	M1	Yes	1		Trisomy 21			122 M1			112 M
DS3	1627	36/35	Mat	M1	Yes	1		Trisomy 21			122 M1			112 M
NS1	1628										12			12
RDS-07:														
[Fa]									[?2]		[12]			[?1]
Mo	1629								13		22			11
DS1	1630	36/36	Mat	M1	No	0		Trisomy 21	123 M1		122			111
DS2	1631	44/44	Mat	M1	Yes	1		Trisomy 21	123 M1		122			111
NS1	1632								12		22			11
NS2	1633								23		22			11
NS3	1634								23		22			11
RDS-08:														
Fa	1612								23		12			11
Mo	1613								12		22			11
DS1	1614	30/36	Mat	M1	No	0		Trisomy 21	123 M1		222			111
DS2	1615	30/36	Mat	M2	Yes	1		Trisomy 21	222 M2		222			111
RDS-09:														
Fa	2218								12		22			12
Mo	2219							Mat Mos 2%	23		12			11
DS1	2220	23/26	Mat Mos					Trisomy 21	123		122			111
DS2	2221	26/29	Mat Mos					Trisomy 21	123		122			111
RDS-10:														
Fa	3254								12	12	22			12
Mo	3255							Mat Mos 2%	13	22	12			11
DS1	3256	30/28	Mat Mos					Trisomy 21	123	122	122			111
DS2	3257	34/32	Mat Mos					Trisomy 21	133 M	122	122			
RDS-11:														
Fa	3258								12		22			11
[Mo]									[?2]		[12]			[?1]
DS1	3259	41/39	Mat	M2	Yes	2		Trisomy 21	122		222 M2			111
DS2	3260	44/42	Mat	M2	Yes	1		Trisomy 21	222		222 M2			111
NS1	3261								22		12			11
NS2	3262								12		12			11

Alleles Per Marker at Loci													
D21S11[c,d]	D21S8[c,d]	D21S210[c,d] (21-gt12)	D21S111[c,d]	D21S213[d] (21-gt05)	D21S82[a,c,d]	IFNAR[c,d]	D21S3[c,d]	D21S156[a,c,d]	HMG14[c,d]	MX1[c,d]	D21S212[a,c,d] (21-gt10)	D21S113[d]	D21S112[a,c,d]	COL6A1[c,d]
11	11				23		11				12	11	12	11
22	12				13		22				12	12	33	11
122 M	**112 nr**				**123 nr**		**122 M**				112	**112 nr**	**133 M**	111
122 M	**112 nr**				**133 nr**		**122 M**					**112 nr**	**133 M**	111
12	12		11		22		12			11	12		12	11
22	11		22		33		12			12	13		23	11
222	**122 P**		**112 P**		**223 P**		122			111	111		222	111
112 P	**122 P**		**112 P**		**223 P**		222			112	113		223	111
22	11		22		33 !		11				13		23	11
22	11		12		22		11			12	12		12	11
22	11		12		13		12			22	23		34	11
222	111		122		**123 M nr**		**112 nr**			122	**223 nr**		**234 M nr**	111
222	111		112		**123 M nr**		**112 nr**			122	**223 nr**		**233 M r**	111
22	11		22		12					22	12		13	11
12	12				23		12	11	12	22	24	11	12	11
12	12				23		12	12	34	22	13	11	13	11
122	122				223		122	**112 nr**	**134 M nr**	222	**134 M nr**	111	**113 nr**	111
122	**111 r**				233		**111 r**	**122 M r**	**133 M r**	222	**134 M nr**	111	**113 nr**	111
12	11				11		12			12	13		12	
11	11				13		12			12	12		34	
111	111				**113 nr**		122			122	**112 nr**		**134 M nr**	
111	111				**133 M r**		112			122	**123 nr**		**134 M nr**	
12	11				11		22			12	11		23	
11	11				13		11			12	23		14	
12	11				13		11			12	11		14	
12	12		12		22		22			12	11		12	12
11	22		12		22		22			22	22		34	23
111	122		112		222		222			222	**122 M**		**234 M nr**	**223 nr**
112	222		112		222		222			222	**122 M**		**233 M r**	**222 r**
112	222		112		222		222			222	**122 M**		**244 M r**	**233 M r**
11			12		22		22			12	12		14	13
[?1]	[?1]				[?2]		[12]			[?2]	[14]		[12]	[12]
11	11				22		12			12	23		34	12
111	111				222					122	**123 M nr**		**234 M nr**	112
111	111				222		**222 r**			**222 r**	**122 M r**		**133 M r**	112
11	11				22		11			12	34		14	22
11	11				22		22			22	12		23	11
11	11				22		11			12	34		14	22
12	11		12		23		22			12	12		12	11
12	11		22		22		12			11	11		34	13
112	111		222		222		**122 nr**			112	111		**234 M nr**	**113 nr**
122	111		122		223		**122 nr**				111		**234 M nr**	**113 nr**
11			12		12					12	12		12	11
11			11		12					12	34		34	12
111			112		**123 Mos**					122	**235 Mos**		**135 Mos**	111
111			112		112					112	**135 Mos**		**235 Mos**	111
12								12	23	22		12	23	11
12								13	12	22		12	13	11
122						122		123	123	222		112	123	111
122						122		123	123	222			333	111
12					13			12	12	11	11	11	12	24
[?1]					[?2]			[23]	[13]	[?1]	[?2]	[?1]	[34]	[13]
122					**122 M**			**123 nr**	**123 nr**	111	**122 M**	111	**133 M r**	**112 M r**
111					**223 M**			**223 nr**	**113 nr**	111	112	111	**234 M nr**	**134 M nr**
12					23			13	12	11	12	11	13	12
12					12			13	12	11	12	11	13	12

(continued)

Table 1 (continued)

Families	ID No.	Age of Father/ Mother	Parental Origin[a]	Meiotic Origin[b]	Cross-over	No. of Crossovers	DS Relation	Karyotype	D21S215[b,c] (21-gt14)	D21S120[b,c]	D21S13[b,c] (*TaqI*)	D21S13 (PCR)	D21S192[b,c]	D21S110[c,d]
RDS-13:														
DS1	3266	25/25	Mat Mos					Trisomy 21			112			122
[Fa1]														
Mo	3264										12			12
Fa2	3263										12			11
DS2	3265	29/29	Mat Mos					Trisomy 21			122			122
RDS-14:														
Fa	3301								12	12	11			
Mo	3300								22	23	11			
DS1	3299	30/36	Mat Mos					Trisomy 21	122	123	111			
DS2	3584	32/38	Mat Mos					Trisomy 21	122		111			
PGFa ...	3916													
PGMo...	3915													
MoSib...	3917													
RDS-15:														
GFa	1639								22	13	12		11	11
GMo	1640								12	23	22		12	12
DS1	1641	33/27	Mat	M1	No	0		Trisomy 21	**122 M1**	**123 M1**	222		**112 M1**	**112 nr**
DS2	1642	33/27	Mat	M1	No	0		Trisomy 21	**122 M1**	**123 M1**	222		**112 M1**	**112 nr**
Fa	1643								22	33	22		[12]	
Mo	1644								12	12	12		12	12
DS3	1645	33/33	Pat	P2	No	0	Related	Trisomy 21	122	**233 P**	122		**222 P2**	122
RDS-16:														
[GFa] ...									[?1]	[13]				
GMo	1646								11	11	12			12
DS1	1647	42/39	Pat	P1	No	0		Trisomy 21	111	**113 P1**	122		112	122
Fa	1648								11	13	22		12	11
Mo	1649								12	12	12		11	11
DS2	1650	39/33	Mat	M1	Yes	2	Unrelated	Trisomy 21	**112 M1**	**112 M1**	**122 M1**		112	111
NS1	1651								11	13	22		11	11
NS2	1652								11	13	22		11	11
RDS-17:														
[GFa] ...									[12]	[1?]	[2]	[2]	[12]	[12]
GMo	1662								22	12	11	12	11	22
DS1	1663	??/38	Mat	M1[e]				Trisomy 21	122	112	112	122	112	222
NS1	1664								22	11	12	22	11	12
Fa	1665								22	12	12	12	11	12
Mo	1666								22	13	12	22	22	11
DS2	1667	33/32	Mat	M1	No	0	Unrelated	Trisomy 21	222	**123 M1**	112	122	**122 M**	112
NS2	1668								22	11	12	22	12	11
RDS-18:														
Fa	3273								12		12			
Mo	3272								11		12			
DS1	3274	41/39	Pat	P1	Yes	1	Related	Trisomy 21	**112 P1**		112			
NS1	3271								12		12			
RDS-19:														
Fa1	3275								23		22			12
Mo1	3276								14		22			11
DS1	3277	45/37	Mat	M1	No	0		Trisomy 21	**134 M1**		222			112
Fa2	3278								14		22			11
Mo2	3279								24		22			12
DS2	3280	42/39	Pat	P2	Yes	2	Related	Trisomy 21	**112 P2**		222			111
RDS-20:														
Fa1	3799								24	11			12	
Mo1	3800								22	13			33	
DS1	3801	33/23	Mat	M1	No	0		Trisomy 21	224	**113 M1**			**133 M**	
NS1	3805								22	11			23	
Fa2	3802								13	22			11	
Mo2	3803								22	11			13	
DS2	3804	35/36	Mat	M1	Yes	1	Unrelated	Trisomy 21	**122 M**	**112 M**			**113 M1**	
NS2	3806								23	12			13	

Table 1 (continued)

Alleles Per Marker at Loci													
D21S11[c,d]	D21S8[c,d]	D21S210[c,d] (21-gt12)	D21S111[c,d]	D21S213[d] (21-gt05)	D21S82[a,c,d]	IFNAR[c,d]	D21S3[c,d]	D21S156[a,c,d]	HMG14[c,d]	MX1[c,d]	D21S212[a,c,d] (21-gt10)	D21S113[d]	D21S112[a,c,d]	COL6A1[c,d]
112					222			145 M Mos	113	112		112	111	244
					[?2]				[?3]				[?1]	[?2]
12					22			14	11	22		12	12	14
11					23			23	12	12		12	34	12
112					222			125 M Mos	112	122		122	124 M	114
		12		12	22	12		13	12		22		15	
		23		12	13	22		12	23		13		24	
		133 M		122	112 M	122		122 M	133 M		123 M		134 Mos	
		223		112	123 M	222					123 M		124 M	
													15	
													16	
													26	
11	11		12		22	33	11						12	
11	12		12		12	12	12						33	
111	112 nr		122		122 nr	123 M nr	112 nr				234 nr		133 M	123 nr
111	112 nr		122		122 nr	123 M nr	112 nr				234 nr		133 M	123 nr
			11		12	[23]	[?1]				23		13	
11	11		11		33	14	11				15		45	12
111	111		111		113 P r	122 P r	111				335 P r		334 P r	111
											[1]			[23]
11	11		11		23		12				23		23	11
111	111		111		223		122				123 nr		123 nr	123 P nr
11	11		11		13		12				12		13	23
11	11		12		13		12				[34]		45	
111	111		112 nr		333 r		112				134 M nr		145 M nr	233
11	11		11		13		12				13		14	33
11	11		11		13		12				13		14	33
[2]	[12]				[3]					[2]	[1]		[14]	[1]
12	11				12		12			11	23		23	12
122	112				122		112			112	123 nr		123 M nr	122
22	11				23		12			12	13		34	11
12	11				13		12			12	13		13	22
12	12				13		12			22	11		56	34
112	112 nr				113					122	113		356 M nr	234 M nr
22	12				33		22			12	13		35	24
12					23	12			12	12	23	22	12	23
22					23	13			34	12	11	12	23	13
122 nr					223	123 nr			124 P nr	112	123 P nr	222	123 nr	223 P r
12					22	12			23	12	12	22	13	12
11					22			13	13	22	14	12	34	12
12					22			24	12	22	34	11	12	11
112 nr					222			124 M nr	112 nr	222	344 nr	112	124 M nr	111
12					22			44	14	22	34	12	23	12
12					12			11	45	12	12	23	14	23
122					222			144 P	115 P r	122	234 P nr	123 nr	123 P nr	112 P r
		12			12	11			23		22		11	22
		13			11	12			11		13		12	13
		113 nr			111	112 nr			112 M		123 M nr		112 nr	123 M nr
		12			11	12			13		12		11	12
		11			12	11			23		24		12	12
		14			22	11			14		22		34	23
		114 nr			222	111			112 M r		222		144 M r	222 r
		14			12	11			13		24		13	22

(continued)

Table 1 (continued)

Families	ID No.	Age of Father/ Mother	Parental Origin[a]	Meiotic Origin[b]	Cross-over	No. of Crossovers	DS Relation	Karyotype	D21S215[b,c] (21-gt14)	D21S120[b,c]	D21S13[b,c] (*TaqI*)	D21S13 (PCR)	D21S192[b,c]	D21S110[c,d]
RDS-21:														
GMo	1653								23		12			11
Fa1	1654								23	23	22			12
Mo1	1655								22	12	11			11
DS1	1656	27/25	Mat	M2	Yes	1		Trisomy 21	222	**112 M2**	112			111
NS1	1657								23		12			12
Fa2	1658								13	12	11			11
Mo2	1659								23	12	22			11
DS2	1660	26/22	Mat	M2	Yes	2	Unrelated	Trisomy 21	**223 M2**	112	**122 M**			111
NS2	1661										12			11
RDS-22:														
Fa1	3608								12	34	11		22	
Mo1	3607								22	14	12		24	
DS1	3606	24/24	Mat	M1	Yes	3		Trisomy 21	222	**144 M1**	**112 M1**		**224 M1**	
Fa2	3611								33	23	11		13	
Mo2	3610								12	34	11		22	
DS2	3609	23/27	Pat	P2	Yes	1	Unrelated	Trisomy 21	**133 P**	**223 P2**	111		**233 P2**	
RDS-23:														
Fa	3284										22			11
Mo	3285										12			11
DS1	3286	37/30	Mat	M1	Yes	1	Unrelated	Trisomy 21			**122 M1**			111

NOTE.—DNA polymorphism analysis of members of families with recurrent free trisomy 21. The individuals studied correspond to members of the pedigrees shown in figure 1. The DNA polymorphic markers studied have been arranged from left to right, from the more centromeric to the most telomeric (the order of the polymorphic loci has been determined in Petersen et al. [1991] and by S. E. Antonarakis and A. Chakravarti, unpublished linkage map). Informative data are printed in boldface type. Alleles in brackets are those inferred from the other data in the family. The meiotic origin of the extra chromosome 21 using pericentromeric DNA markers was often established, given the parental origin determined, by using the results from other markers (e.g., in family RDS-03 the meiotic origin of the extra chromosome 21 in individual DS1 was assigned as maternal meiosis I error since the parental origin of nondisjunction was maternal, as determined by markers D21S82 and D21S112).

[a] Mat = Maternal; Pat = paternal; Mos = mosaicism.

[b] M1 = Maternal meiosis I error; M2 = maternal meiosis II error; P1 = paternal meiosis I error; P2 = paternal meiosis II error.

[c] M = Maternal origin of the extra chromosome 21; P = paternal origin of the extra chromosome 21.

[d] nr = Nonreduction to homozygosity; r = reduction to homozygosity.

[e] The meiotic origin of nondisjunction was determined by haplotyping pericentromeric polymorphisms.

result of maternal meiosis I error, while the trisomy 21 in the second affected twin originated from a maternal meiosis II error (see DNA marker D21S215 of table 1). In all seven cases with maternal meiosis II errors, crossovers have been observed in the chromosomes 21 that participated in nondisjunction. These results exclude the possibility of postzygotic (mitotic) error as the cause of these trisomies. In 9 of the 10 cases of maternal meiosis I errors in which enough DNA polymorphic markers on the long arm of chromosome 21 have been studied, crossovers have been observed in four cases, while in the remaining five cases no crossovers have been detected. This is in agreement with the proposed hypothesis of reduced recombination in meiosis I in trisomy 21 (Warren et al. 1987), which has been subsequently confirmed by Sherman et al. (1991) and Antonarakis et al. (1992).

In summary, in these eight families with two affected siblings and no paternal mosaicism, there is no apparent difference from the usual families with one affected child. We therefore presume that the recurrence of individuals with trisomy 21 in the same nuclear family is the result of chance alone. Assuming that the frequency of trisomy 21 in the population is 1/700 liveborn, we expect that 1/490,000 families with two children will have two affected individuals with trisomy 21 by chance. In conclusion, this study suggests that parental mosaicism is an important and frequent cause of recurrent trisomy 21 in nuclear families, since it has been found in about 40% of the families; however chance alone accounts for the remaining 60% of the families.

Category 2 Families

In this category of four families, the individuals with Down syndrome are second-degree relatives. In all of these families the individuals with trisomy are related through a male individual (families RDS-15–RDS-18 of fig. 1). In families RDS-16 and RDS-17, the parental origin of nondisjunction in the Down syndrome of the

Table 1 (continued)

Alleles Per Marker at Loci													
D21S11[c,d]	D21S8[c,d]	D21S210[c,d] (21-gt12)	D21S111[c,d]	D21S213[d] (21-gt05)	D21S82[a,c,d]	IFNAR[c,d]	D21S3[c,d]	D21S156[a,c,d]	HMG14[c,d]	MX1[c,d]	D21S212[a,c,d] (21-gt10)	D21S113[d]	D21S112[a,c,d]	COL6A1[c,d]
12	12		12		23		22			11	44		13	13
11	11		22		23		22			12	22		12	13
12	11		12		12		11			22	35		24	23
122 M r	111		122 nr		123 nr		112 M			222	235 M nr		224 nr	123 nr
11	12		22		12		12			22	23		24	12
12	12		12		23		22			11	44		23	11
11	11		12		11		12			11	16		15	12
112	112		111 r		112 M		122 nr			111	146 M nr		125 M nr	122 M r
11	11		12		13		22			11	46		12	12
		11		12	11	23					12		11	
		23		12	23	13					13		23	
		123 M nr		112	133 M r	133 nr					123 nr		133 M r	
		22		12	12	12					14		22	
		12		13	22	34					22		12	
		122		112 nr	122 nr	124 P nr					124 P nr		122	
11					13			12	11	22		12	14	11
12					22			34	12	11		13	23	12
112 nr					223 M			234 M nr	112 nr	112 M		112 r	122 M r	122 M r

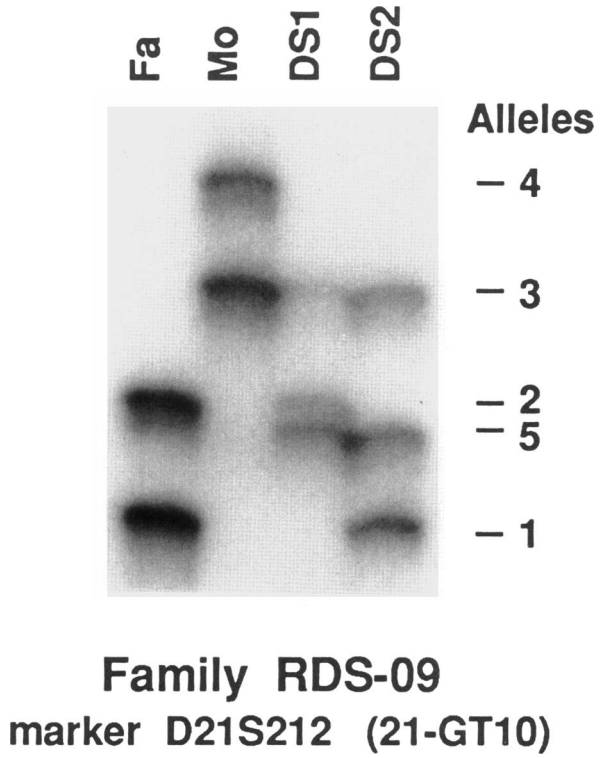


Figure 2 Representative autoradiogram of the study of the origin of the extra chromosome 21 in individuals with Down syndrome. The alleles for DNA dinucleotide repeat marker D21S212 (21-GT10) are shown. The father (Fa) has alleles 1 and 2; the mother (Mo) shows alleles 3 and 4. The first offspring with Down syndrome (DS1) has alleles, 2, 3, and 5, while the second offspring with Down syndrome (DS2) has alleles 1, 3, and 5. Allele 5 comes from the mother, who cytogenetically shows mosaicism for trisomy 21.

third generation (designated "DS2" in the appropriate pedigrees in fig. 1) was maternal, and, therefore, the nondisjunction originated in unrelated individuals in those pedigrees. Data on the meiotic origin of nondisjunction in these families are included in table 1.

In families RDS-15 and RDS-18 the parental origin of nondisjunction in the Down syndrome of the third generation (designated DS3 for pedigree RDS-15 and DS1 in pedigree RDS-18 in fig. 1) was paternal, and, therefore, the nondisjunction apparently originated in individuals in those pedigrees who were related. In these fathers (individuals "Fa" of pedigree RDS-15 and "Fa" of pedigree RDS-18 in fig. 1), no mosaicism has been observed either by cytogenetic or DNA analysis. The relationship of the origin of nondisjunction in families RDS-15 and RDS-18 can be attributed to chance alone; however, the fact that paternal nondisjunction for trisomy 21 is rare (about 5%; Antonarakis et al. 1991; Sherman et al. 1991) in the general population suggests that these two families may be different from the ordinary families with trisomy 21. In family RDS-15, DNA polymorphism analysis of

pericentromeric markers showed that the paternal nondisjunction of individual DS3 occurred in the second meiotic division. Further analysis of DNA polymorphisms in 21q suggested that there was no recombination in the chromosomes that participated in nondisjunction. The presence of two chromosomes identical at all polymorphic loci analyzed that originate from one parent can be explained by (i) meiosis II error without a crossover event in the preceding meiosis I; (ii) paternal mosaicism that has not been discovered by the cytogenetic and DNA analysis, or (iii) mitotic error. In the last case the origin of trisomy 21 is somatic, involving the paternal chromosome. The paternal chromosome 21, which is present in two copies in individual DS3 of family RDS-15, is identical at the pericentromeric region to one of the grandmaternal chromosomes that participated in the maternal nondisjunction that causes trisomy 21 in the monozygotic twins DS1 and DS2. In family RDS-18 the trisomy 21 in individual DS1 was due to an error in meiosis I in the paternal germ cells. DNA was not available from all members of this pedigree in order to study the nature of the chromosomes 21 that participated in the two nondisjunction events.

Category 3 Families

In this category of five families, the individuals with Down syndrome are third-degree relatives, that is, their parents are siblings. In pedigrees RDS-19, RDS-22, and RDS-23 the parents of the individuals with Down syndrome are brothers and sisters, while in pedigrees RDS-20 and RDS-21, the parents of the individuals with Down syndrome are brothers (see fig. 1). In four pedigrees, namely RDS-20–RDS-23, the parents in which nondisjunction had occurred were not blood relatives, and, therefore, the occurrence of two individuals with Down syndrome in these extended pedigrees can be attributed to chance. In family RDS-19 the parents in whom nondisjunction had occurred were a brother and sister. The analysis of pericentromeric DNA markers in this pedigree showed that the error for individual DS1 was in maternal meiosis I, while for individual DS2 the error was in paternal meiosis II. A mitotic error in the latter case has been excluded, since crossover events have been detected in chromosomes 21 that participated in the paternal nondisjunction. Although a predisposing factor to nondisjunction cannot be excluded in this family, chance alone also can be the explanation of the recurrent Down syndrome. It is of interest that, among the nine individuals with Down syndrome studied in this category, there was an excess of paternally derived trisomy 21 (two of nine cases), but the sample is too small to derive any conclusions.

Concluding Remarks

The aim of the study was to detect a possible genetic predisposing factor in trisomy 21. We therefore chose and collected 22 families with two affected individuals, in order to maximize the possibility of detecting such a genetic predisposition by using the powerful and unequivocal analysis of DNA markers on chromosome 21. With the exception of parental mosaicism in the relatively small sample studied, no other major genetic predisposing factor has been identified, and chance alone seems to be the main reason for the recurrence of free trisomy 21 within families.

Acknowledgments

This study was supported by NIH grant HD24605 to S.E.A. We thank Drs. A. Holler and E. Buehler for cytogenetic analyses. This work was presented in a preliminary form at the annual meeting of the American Society of Human Genetics, in New Orleans, October 12–15, 1988 (Am J Hum Genet 43:92, 1988).

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