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Nucleolar Organizer Region Variants as a Risk Factor for Down Syndrome

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SUMMARY

An unusual nucleolar organizer region (NOR) heteromorphism was noted among 13 of 41 parents in whom nondisjunction leading to trisomy 21 was known to have occurred. In contrast, only one of these double NOR (dNOR) variants was found among the 41 normal spouses and none were seen among 50 control individuals. In two dNOR(+) families, a second child with trisomy 21 was conceived. In both families, the extra chromosome in each child was contributed by the parent who carried the dNOR variant and resulted from a recurrent meiosis I error. Our data suggest that the dNOR heteromorphism may play a role in meiotic nondisjunction and could be associated with as much as a 20-fold increased risk for having offspring with trisomy 21.

INTRODUCTION

Determination of the parental origin of the extra chromosome in trisomy 21 provides a logical approach to the analysis of factors that may influence nondisjunction. Previous investigators have shown that the parental origin of nondisjunction can be determined in approximately 50% of families studied using

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heritable morphological and quinacrine (QFQ)-staining heteromorphisms alone [1]. When used in conjunction with QFQ, however, the additional heteromorphism that can be detected at the nucleolar organizer region (NOR) of chromosome 21 permits the parental origin of nondisjunction to be determined in approximately 80% of the families studied [2]. Metaphase chromosomes bearing active NORs are often found to be spatially associated [3]. Since satellite associations have been considered to be a factor that may contribute to the occurrence of nondisjunction [4, 5], our study was also designed to investigate the possible role of the NOR heteromorphisms on other acrocentric chromosomes in the etiology of nondisjunction in families where the parental origin of the extra chromosome 21 could be determined.

MATERIALS AND METHODS

Fifty unselected families having a child with trisomy 21 were ascertained from cytogenetic service laboratory records of the Medical College of Virginia (1972–1982). Voluntary participation was obtained from responses to letters sent to all families. A control sample of 50 healthy normal individuals was ascertained from a population-based twin panel established at the same institution. Only one member of each of the 50 twin pairs was selected at random for inclusion in the control population. Ten milliliters of heparinized blood were obtained from all study participants. Duplicate lymphocyte cultures were established for 72 hrs, and midmetaphase spreads were prepared using standard procedures [6].

The slides were stained for NOR analysis according to a modification of the method of Bloom and Goodpasture [7] and were simultaneously counterstained with quinacrine mustard (QM) dihydrochloride for unequivocal chromosome identification. In preparation for QM and NOR staining, slides were dehydrated through a series of 10-min soaks in 100%, 90%, 70%, and 30% methanol, followed by a 10-min immersion in McIlvaine's buffer (pH 5.4; 0.1 M citric acid and 0.2 M Na_2HPO_4). Each slide was then rinsed 10 times in distilled water and blotted dry. Subsequently, three drops of SI solution (50% silver nitrate [$AgNO_3$] in Megapure water) were distributed equally over the slide, a coverslip was added, and the slide was heated (60°C) on a hot plate for 30 seconds. The coverslip was immediately removed by rinsing with Megapure water, and the slide was blotted dry. One drop each of SII (7.5 ml NH_4OH added to 4g $AgNO_3$ in 5 ml distilled H_2O) and F (3% formalin, pH 7.2) solutions were then placed on the upper left edge of each slide and a coverslip added. The staining progress was monitored in a central position on the slide by unfiltered phase microscopy. By following this methodology, a gradient of decreasing staining intensity was established from left to right on the slide. Following completion of the reaction, at which point distinct terminal black dots appeared on golden acrocentric chromosomes, the coverslip was removed, the slide rinsed in Megapure water, and stained in a 0.005% solution of QM (Sigma, #Q-2000, St. Louis, Mo.) in McIlvaine's buffer, pH 5.4, for 26 min. Following staining, each slide was rinsed 10 times in McIlvaine's solution, soaked for 10 min in this solution, and allowed to air dry at room temperature in the dark for at least 2 hrs prior to viewing. All slides were numerically coded and scored without knowledge of family relationships.

The QFQ and NOR heteromorphisms were assessed simultaneously using a Leitz Ortholux II microscope equipped with both ultraviolet and visible light sources. The QFQ staining intensities of the short arm and satellite regions of the acrocentric chromosomes were classified on a scale of 1–5 according to standards established at the Paris conference [8]. The staining intensities of the NOR heteromorphism were scored, on a scale of 0–4, according to a modification of the semiquantitative procedure of Markovic et al. [9], as adapted by Morton et al. [10]. Ten midmetaphase spreads were examined and photographed from each individual.

Following identification of the family relationships, the parental origin of nondisjunction was inferred from the distribution of the QFQ and/or NOR heteromorphisms in the parents and their offspring as described above. Additional morphological comparisons of the number 21 chromosomes were then performed from photographs to further confirm or assist with these assignments.

The Down syndrome probands and their parents were classified according to their chromosome 21 heteromorphism phenotypes, and maximum likelihood estimates of the frequency of maternal and paternal nondisjunction at the first- and second-meiotic divisions, respectively, were obtained following the method of Jacobs and Morton [11]. Statistical comparisons of the mean ages between parental groups were performed using Duncan's New Multiple Range Test [12].

RESULTS

By employing both the QFQ and NOR heteromorphisms, the parental origin of nondisjunction could be determined in 41/50 (82%) of the families. In 16/50 (32%) of the families, the assignments could have been made using only QFQ and/or morphological variants. In 25 cases, the inclusion of NOR staining permitted the parental origin to be determined (table 1). Thus, the use of NOR staining increased the number of families for whom the parental origin of the extra chromosome could be assigned by a factor of 2.6.

The QFQ and NOR heteromorphisms of all 10 acrocentric chromosomes (in addition to QFQ heteromorphisms on chromosomes 3 and 4) were also compared in parents and children in an attempt to confirm the reported paternity. No genetic inconsistencies or evidence for paternity exclusion were observed in any of the 50 families studied.

TABLE 1

SUMMARY OF HETEROMORPHISMS USED IN ASSIGNING THE PARENTAL ORIGIN OF CHROMOSOMES 21

Heteromorphism(s)	No. families
NOR heteromorphism not required:	
QFQ* only	3
QFQ or NOR†‡	3
QFQ and M§‖	6
QFQ or NOR and M	4
Subtotal	16
NOR heteromorphism required:	
NOR only	7
NOR and QFQ	10
NOR and M	5
NOR, QFQ, and M	3
Subtotal	25
Parental origin indeterminant	9
Total	50

* QFQ Heteromorphism detected by Q-bands by fluorescence with quinacrine.

† NOR = nucleolar organizer region heteromorphisms.

‡ Either QFQ or NOR could be used independently to assign parental origin.

§ M = morphological variants.

‖ Both QFQ and M were required to assign parental origin.

Following determination of the chromosome 21 phenotypes in the patients with Down syndrome and their parents, maximum likelihood estimates of the frequencies of maternal and paternal nondisjunction of the first- and second-meiotic divisions, respectively, were determined from the conditional probabilities associated with the phenotype combination of each trio (APPENDIX). Maternal errors were found to be more common (69.3%) than paternal errors (30.7%), and nondisjunction occurred more often during meiosis I (MI) than meiosis II (MII) in both males (MI—72%; MII—28%) and females (MI—93.2%; MII—6.8%).

In 15 of 50 families, an unusual NOR staining variant was observed on one of the 10 acrocentric chromosomes in at least one of the parents. This variant has been termed a "double NOR" [13], which we abbreviate as "dNOR." It appears morphologically to consist of a doubling or duplication of the NOR region and was found to occur on any one of the five pairs of the acrocentric chromosomes (fig. 1). The dNORs varied in their appearance from two completely separated areas of silver-grain deposition, as seen on the chromosome 15 at the bottom of figure 1, to two more or less confluent areas of silver staining as seen in the rest of the chromosomes. The latter form of the dNOR phenotype could be readily distinguished from a single intensely silver-stained chromosome by its dumbbell shape and the presence of elongated stalks when viewed with QFQ, a finding that was almost invariably present when a dNOR variant was observed.

Both regions of silver staining on a dNOR variant are not seen in every cell. The observed frequency of cells in which double-stained regions were present ranged from 50% to 100% (table 2). The elongation of the stalks appeared to be a more consistent, if less obvious, feature that could often be observed even in dNOR chromosomes that did not exhibit two regions of silver staining. Finally, much of the phenotypic heterogeneity in the expression of the dNOR trait did appear to be consistent from cell to cell. Thus, in the dNOR-positive individual from family 7 (fig. 1, bottom chromosome 15 at the bottom of this figure), all of the chromosomes that exhibited the dNOR phenotype showed a wide separation in the two silver-stained regions.

The parental origin of nondisjunction could be determined in 13/15 dNOR(+) families, and as shown in table 2, in each of the informative families, the parent carrying the dNOR was also determined to be the source of the nondisjunctional error (χ^2 = 10.08, 1 df, $P < .001$). Family 8 was uninformative with respect to this association, since both parents had a dNOR variant, even though the extra chromosome 21 in the child was shown to have been contributed by the mother in this family.

The segregation ratio for dNOR variants in the Down syndrome offspring of dNOR(+) couples was 0.41, which did not differ significantly from a random segregation pattern (table 2).

dNOR variants were observed in 13/41 (32%) of the parents in whom nondisjunction was known to have occurred and in 2/18 (11.1%) of the indeterminate parents (among one-half of whom nondisjunction had occurred). In contrast, only one of the 41 (2.4%) normal spouses and none of the 50 (0%) control

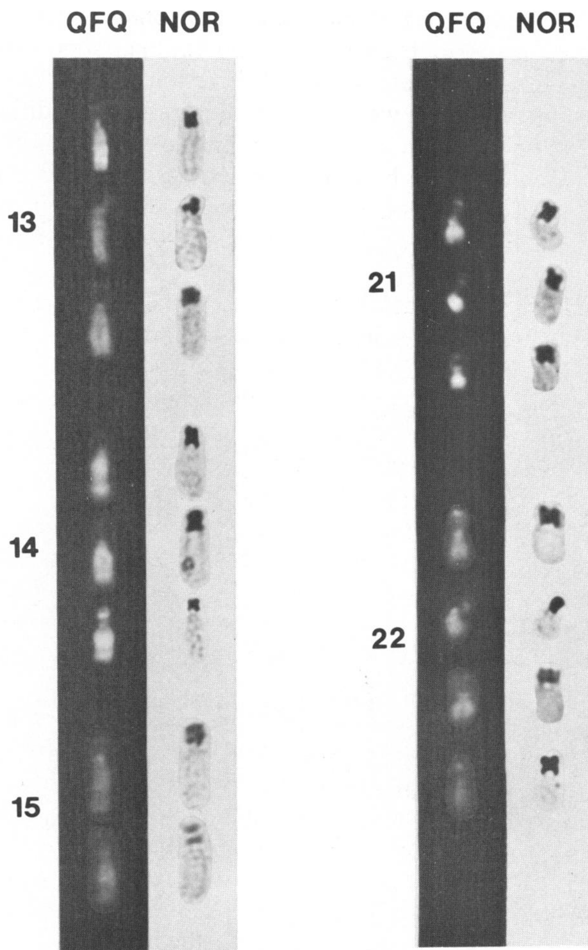

FIG. 1.—Appearance of the double NOR variant chromosomes. The 15 acrocentric chromosomes with dNORs as they appear with QFQ staining (*dark column*) and silver, or NOR, staining (*light column*). The dNORs may appear as two completely separated regions (as in the chromosome 15 at the bottom of this figure) or as two somewhat confluent regions (as seen in the other chromosomes) depending on chromosome morphology and the nature of the staining reaction. The dNOR variants are also characterized by elongated stalks as seen in the QFQ staining of these chromosomes.

subjects were noted to have a dNOR variant. The departure of the frequencies of dNORs from a random distribution among these groups was highly significant (χ^2_3 = 27.95, $P < .005$).

The meiotic stage of nondisjunction could be determined in 11 of the 13 informative dNOR(+) families, and in each case, the nondisjunction was found to have occurred during MI (table 3). In contrast, while MI errors were also observed more frequently among dNOR(−) families, MII errors were encountered in this group among both males (no. = 5) and females (no. = 2). How-

TABLE 2

DISTRIBUTION OF dNOR(+) PHENOTYPE AND PARENTAL ORIGIN OF NONDISJUNCTION

FAMILY NO.	dNOR(+) PHENOTYPE: Chromosome	dNOR(+) PHENOTYPE: Frequency	dNOR(+) PARENT	ORIGIN OF NDJ	INHERITANCE OF dNOR(+) BY PROBAND
1	13	10/10	M*	M	Yes
2	13	7/10	M	M	No
3	13	6/10	F†	F	No
4	14	7/10	F	F	No
5	14	7/10	F	F	No
6	14	6/10	M	M	No‡
7	15	6/10	M	M	No
8*	15	8/10	M	M	No
8†	21	5/10	F	M	No
9	21	5/10	M	M	Yes
10	21	5/10	M	M	Yes
11	21	6/10	M	?	Yes
12	22	8/10	M	?	No
13	22	6/10	F	F	No‡
14	22	6/10	M	M	Yes
15	22	6/10	M	M	No

* M = mother.
† F = father.
‡ The proband did *not* inherit the dNOR. However, a second child with DS *did* inherit the dNOR.

ever, this trend toward more MII errors in the dNOR(−) couples was not significant in the present sample (table 3).

There were no statistically significant differences in mean parental age, at the time of conception of the affected child, between any of the parental groups studied (table 3).

Reproductive histories were available for 48 of 50 Down syndrome families (table 4). Trisomy 21 had recurred in two families or two of 84 siblings (2.4%) of the affected probands. In both of these families, an MI error had recurred in a parent who carried a dNOR variant. In one family, nondisjunction recurred in a father who has a dNOR on a chromosome 22. This couple also reported having a first-trimester spontaneous abortion in addition to two normal children. In the second multiplex family, the mother carried a dNOR on a chromosome 14 and was shown to have been the source of the extra chromosome 21 in both affected children. From this observation of two recurrences of trisomy 21 offspring among a total of 32 viable pregnancies to dNOR(+) couples ascertained through a proband with trisomy 21, the recurrence risk was estimated to be approximately 6%. In contrast, the recurrence risk to dNOR(−) couples (0/50 viable pregnancies) may well be less than the 1%–2% figure that is usually quoted after the birth of a child with nontranslocation Down syndrome.

Although a positive trend toward an increased incidence of spontaneous abortions was observed among the dNOR(+) couples (7/41) as compared to the dNOR(−) couples (7/57), the differences did not approach significance in this sample (table 4; $\chi^2_1 = .41$, $P > .05$). Unfortunately, since the chromoso-

TABLE 3

DISTRIBUTION OF PARENTAL AND MEIOTIC ORIGIN OF NONDISJUNCTION

ORIGIN OF NONDISJUNCTION	STAGE OF MEIOSIS I	II	?	TOTAL	MEAN PARENTAL AGE Mother	Father
dNOR(+) families:						
Maternal	7	...	2	9	29.4 × 2.0	31.4 ± 2.2
Paternal	4	...	...	4	30.9 ± 4.2	33.4 ± 3.9
Indeterminate .	...	...	2	2	30.2 ± 5.6	34.7 ± 7.0
Total	11	...	4	15	29.9 ± 1.9	32.0 ± 1.2
dNOR(−) families:						
Maternal	16	2	1	19	30.3 ± 1.8	33.3 ± 1.8
Paternal	6	3	...	9	28.6 ± 1.7	29.2 ± 1.8
Indeterminate .	...	...	7	7	28.7 ± 1.4	29.7 ± 1.5
Total	22	5	8	35	29.5 ± 1.2	31.8 ± 1.2
All families:						
Maternal	23	2	3	28	30.0 ± 1.4	32.6 ± 1.4
Paternal	10	3	...	13	29.3 ± 1.8	30.6 ± 1.9
Indeterminate .	...	...	9	9	29.0 ± 1.8	31.0 ± 2.2
Total	33	5	12	50	29.6 ± 1.0	31.8 ± 1.0

* Mean ± SE of age in yrs.

mal constitution was not known in any of the spontaneous abortuses, a direct comparison of the rate of aneuploid-to-euploid conceptuses between the dNOR(+) and dNOR(−) couples could not be made.

DISCUSSION

When used in conjunction with QFQ variants, NOR heteromorphisms more than doubled the number of families in which the parental origin of the extra chromosome 21 could be inferred. In our studies, the origin of nondisjunction was shown to be approximately 70% maternal and 30% paternal, respectively. These results are in good agreement with previous studies [1] and suggest that our sample is representative of most families having children with trisomy 21.

The observation of a high frequency of Down syndrome parents with a

TABLE 4

REPRODUCTIVE OUTCOME IN DOWN SYNDROME FAMILIES STUDIED

Outcome	dNOR(+)	dNOR(−)
Normal liveborn	32	50
Trisomy 21*	2	0
Spontaneous abortions	7	7
Total	41	57

* Excluding probands.

TABLE 5

PREVIOUSLY REPORTED dNOR(+) INDIVIDUALS

Reference	dNOR chromosome(s)	Reason for referral
Henderson and Atwood [14]	15	Unknown
Archidiacono et al. [13]	15	Unknown
	21	Unknown
Miller et al. [15]	14	Normal
Lau et al. [16]	14	Normal
Martin et al. [17]	21	Normal and 47,XXY
Balicek and Zizka [18]	22	Mental retardation
	21	47,+21
	21	Multiple aborter
	22	Anomalies
	14	Mother, 47,+21*
	22	Mental retardation with anomalies
	14	Father, FA†
	14	Mental retardation with anomalies
	13	Mental retardation with anomalies
	22	Father, NTD‡
	13	Mother, 47,+21
	14	Mother, MR
	13	Mother, anomalies
	15	Mother, MR
Sofuni et al. [19]	14	Normal children of parents exposed to A-bomb
	22	Normal children of parents exposed to A-bomb
	13	Normal children of parents exposed to A-bomb
	22	Normal children of parents exposed to A-bomb
Bernstein et al. [20]	22	Ambiguous genitalia
Jotterand-Bellomo and Van Melle [21]	13	Unknown
	15	Unknown

*Mother of child with 47,+21.
† Father of child with Fanconi anemia.
‡ Father of child with neural tube defect.

dNOR on one of their acrocentric chromosomes was unexpected. However, in view of our findings, it no longer seems fortuitous that four of the 27 previously reported examples of dNORs occurred either in aneuploid patients or their parents (table 5). For example, to illustrate the value of NOR heteromorphisms for determining the parental origin of nondisjunction, Mikkelsen published a photograph (her fig. 1) of what appears to be a typical dNOR variant on a chromosome 21 in both a child with trisomy 21 and its mother [22]. As in our families, the nondisjunction in this case was noted to have occurred during MI in the parent who carried the dNOR variant. Additional support for our findings is provided by many studies of Down syndrome families in whom acrocentric variants with elongated stalks have been noted [23–28]. Although silver staining was not performed in these studies, the morphology of these variants as seen with QFQ- and Giemsa (GTG)-banding is quite consistent with that of our dNOR chromosomes. Of special interest is the report by Crandall and Ebbin [29] of a couple who had two spontaneous abortions, one offspring with trisomy

18 and one child with trisomy 21. Although, the father was not available for analysis, cytogenetic studies of the mother showed 46 chromosomes, with a variant chromosome 21 showing elongated stalks that closely resembles the morphology of the dNOR variants in our series. Recurrences of Down syndrome within families in whom Giemsa stained or GTG-banded chromosomes were noted that appear to be consistent with dNOR variants have also been reported by Shaw [30], Dhadial and Pfeiffer [31], and Werner et al. [32], the latter being a recurrence of apparent mosaicism for trisomy 21.

Two possible mechanisms for the role of the dNOR variant in nondisjunction include the promotion of nucleolar persistence and the facilitation of nonhomologous pairing and/or crossing over. The first of these suggested mechanisms was investigated in an indirect manner by an examination of the satellite associations of the dNOR variants and will be presented elsewhere [33]. Briefly, however, it is known from mitotic studies of somatic cells that the number and/or transcriptional activity of rDNA genes, the frequency of chromosomal associations, and the intensity of silver staining are generally found to be positively correlated [34–38]. It has also been shown that, on the average, chromosome 21 has the greatest staining intensity [39], a finding that we also noted in our present study [33]. Furthermore, human meiotic studies of both oocytes and spermatocytes have shown that active NORs are observed and often associated throughout most of MI, but not during MII [40, 41]. The number of bivalents involved in these associations has been noted to vary from one to three, thus resulting in the ribosomal genes on four to 12 chromatids being temporarily juxtaposed [40]. It seems possible, therefore, that in the germ cells, acrocentric chromosomes with dNORs may possess extra binding sites that promote associations between nonhomologs that may contribute to nondisjunction. Since NORs are active only during MI, this hypothesis is consistent with the exclusive occurrence of MI errors among our parents in whom a dNOR was noted and could possibly explain why MI errors occur more often among females, in whom these associations would exist for longer periods of time.

If the dNOR is a causal factor in nondisjunction, the associated risk of Down syndrome can be estimated from the frequency of the trait in the general population and among individuals in whom nondisjunction has occurred. If one assumes the incidence of live-born patients with trisomy 21 to be .001 [42], the proportion of cases arising from a dNOR parent to be .32 (13/41), and the incidence of the dNOR trait in the general population to be .02, our data suggest that possession of a dNOR variant may be associated with as much as a 20-fold increase in the risk for having a child with Down syndrome (table 6). This observation raises the possibility that the increased recurrence risk of Down syndrome for couples who have previously had an affected child with trisomy 21 may be explained largely by the subset of families, in whom nondisjunction recurs in a parent who carries a dNOR. Although we have documented only two such families to date (table 2, families nos. 6 and 13), we predict that in a majority of families the recurrence of nondisjunction involving chromosome 21 will be found to be attributable to a parent who carries a dNOR variant.

Our findings show how the analysis of NOR heteromorphisms can be of

TABLE 6

CALCULATION OF RISK ESTIMATES FOR dNOR PHENOTYPE

PARENTAL dNOR PHENOTYPE	FREQUENCY OF OFFSPRING	
	Trisomy 21	Euploid
(+)	(a) 13/41 × 0.001 = 3.2×10^{-4}	(b) 0.02 × 0.999 = 2.0×10^{-2}
(−)	(c) 28/41 × 0.001 = 6.8×10^{-4}	(d) 0.98 × 0.999 = 9.8×10^{-1}

NOTE: Absolute risk—dNOR(+): a/(a + b) = 1.57%; dNOR(−): c/(c + d) = 0.07%. Relative risk—dNOR(+)/dNOR(−) = 22.4.

value in the management of Down syndrome. When present in a parent or relative, the dNOR variant may constitute an important new indication for the antenatal monitoring of at risk pregnancies.

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APPENDIX
FAMILIAL CHROMOSOME 21 HETEROMORPHISMS AND THE CONDITIONAL PROBABILITY OF MEIOTIC ERRORS IN dNOR(+) AND dNOR(−) FAMILIES

A. Familial chromosome 21 heteromorphisms and the conditional probability of meiotic errors in dNOR(+) families

Family No.	dNOR(+) Chromosome	Chromosomes 21* Mother	Father	Child	Informative Heteromorphism(s)	Conditional Probabilities MI†	MII‡	PI§	PII‖
1	13	AA	BB	AAB	NOR# and M**	1	1	0	0
2	13	AB	CD	ABD	NOR and M	1	0	0	0
3	13	AB	CD	ACD	NOR	0	0	1	0
4	14	AB	CD	BCD	NOR and M	0	0	1	0
5	14	AB	CD	BCD	QFQ# or NOR, and M	0	0	1	0
6	14	AB	CD	ABD	QFQ or NOR	1	0	0	0
7	15	AB	CD	ABC	QFQ, NOR, and M	1	0	0	0
8a	15	AB	BC	AAB	QFQ and NOR	1	1	0	0
b ...	21	...	...	...	...	...	...	...	...
9	21	AB	CD	ABC	NOR	1	0	0	0
10	21	AB	CD	ABD	QFQ and NOR	1	0	0	0
11	21	AB	BC	BBC	None	0	¼	½	0
12	22	AB	BC	ABB	None	½	0	0	¼
13	22	AB	CD	BCD	QFQ and M	0	0	1	0
14	22	AB	CD	ABD	QFQ, NOR, and M	1	0	0	0
15	22	AB	CD	ABC	QFQ and NOR	1	0	0	0

(Appendix continues on next page)

APPENDIX *(continues)*

B. Familial chromosome 21 heteromorphisms and the conditional probability of meiotic errors in dNOR(−) families

Family no.	Chromosome 21 Mother	Chromosome 21 Father	Chromosome 21 Child	Informative Heteromorphism(s)	Conditional probabilities MI	MII	PI	PII
16	AB	BC	BCC	QFQ	0	0	0	½
17	AB	CD	ABC	QFQ and M	1	0	0	0
18	AB	CD	ABC	NOR and M	1	0	0	0
19	AB	AC	BBC	NOR	0	½	0	0
20	AB	AB	AAB	None	1	½	1	½
21	AB	CD	ADD	QFQ or NOR	0	0	0	1
22	AB	CD	ACD	QFQ, NOR, and M	0	0	1	0
23	AB	CD	ABC	NOR	1	0	0	0
24	AA	AB	AAB	None	½	½	1	0
25	AB	CD	ABD	QFQ and M	1	0	0	0
26	AB	BC	ABB	None	½	0	0	¼
27	AB	CD	ABD	QFQ or NOR, and M	1	0	0	0
28	AB	CD	ABD	NOR and M	1	0	0	0
29	AB	CD	ABC	QFQ and NOR	1	0	0	0
30	AB	CD	ABC	NOR	1	0	0	0
31	AB	CD	BCC	QFQ or NOR	0	0	0	1
32	AB	CD	ACD	QFQ and M	0	0	1	0
33	AB	CD	BCC	QFQ and NOR	0	0	0	1
34	AB	AC	ABC	None	½	0	½	0
35	AB	BC	ABB	None	½	0	0	¼
36	AB	CD	ABD	QFQ and NOR	1	0	0	0
37	AB	CD	ABD	QFQ and NOR	1	0	0	0
38	AB	CD	ABC	QFQ	1	0	0	0
39	AB	BC	ABC	None	½	0	½	0
40	AB	CD	ACD	QFQ and M	0	0	1	0
41	AB	CD	ABC	NOR	0	0	1	0
42	AA	BC	AAB	QFQ	1	1	0	0
43	AB	CD	BCD	QFQ or NOR, and M	0	0	1	0
44	AB	CD	ABC	NOR	1	0	0	0
45	AB	CD	ABC	QFQ or NOR, and M	1	0	0	0
46	AB	CD	ABD	QFQ and NOR	1	0	0	0
47	AB	CD	BCD	QFQ and NOR	0	0	1	0
48	AB	CD	ABC	QFQ and NOR	1	0	0	0
49	AB	CD	AAC	QFQ and M	0	1	0	0
50	AB	AC	AAB	None	½	0	0	¼

* The letters represent differentiable chromosomes 21 as determined with staining or structural variants. For example, in family 2, each of the four parental chromosomes 21 could be distinguished from one another and were thus assigned letters A, B, C, and D.

† MI = maternal meiosis I error.

‡ MII = maternal meiosis II error.

§ PI = paternal meiosis I error.

‖ PII = paternal meiosis II error.

NOR = nucleolar organizer region heteromorphism.

**M = morphological heteromorphism.

†† QFQ = quinacrine heteromorphism.

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