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Non-disjunction of chromosome 18

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A sample of 100 trisomy 18 conceptuses analysed separately and together with a published sample of 61 conceptuses confirms that an error in maternal meiosis II (MII) is the most frequent cause of non-disjunction for chromosome 18. This is unlike all other human trisomies that have been studied, which show a higher frequency in maternal meiosis I (MI). Maternal MI trisomy 18 shows a low frequency of recombination in proximal p and medial q, but not the reduction in proximal q observed in chromosome 21 MI non-disjunction. Maternal MII non-disjunction does not fit the entanglement model that predicts increased recombination, especially near the centromere. Whereas recent data on MII trisomy 21 show the predicted increase in recombination proximally, maternal MII trisomy 18 has non-significantly reduced recombination. Therefore, chromosome-specific factors must complicate the simple model of susceptible chiasma distributions interacting with age-dependent deterioration of the meiotic mechanism. For chromosome 18, 30% of tetrads are nullichiasmate in maternal MI non-disjunction, but nullichiasmates are not observed in maternal MII non-disjunction. Chiasma distributions from normal chromosome 18 meioses provide no evidence for normal disjunction from nullichiasmate tetrads. We extend this study to examine the remaining autosomes and find no evidence for normal disjunction from nullichiasmate tetrads generally.

INTRODUCTION

Trisomy 18 or Edwards syndrome is the second most common autosomal trisomy among liveborn children. The prevalence at birth is ~1 in 8000. The syndrome includes severe mental and growth retardation with frequent microcephaly, myelomeningocele, omphalocele, cardiac and renal malformations, leading to a 95% mortality rate within the first year of life (1–3).

Trisomy can arise as an error of meiosis or post-zygotic mitosis. Maternal meiotic error is the most frequent cause of autosomal trisomy. It increases with maternal age and has been hypothesized to involve two steps: an unfavourable chiasma distribution at diplotene in fetal life followed by age-dependent deterioration of the mechanism for meiotic disjunction, which is most severe for tetrads with susceptible distributions of chiasmata (4). For at least one sample of *Drosophila* X chromosomes (5) and for human chromosome 21 (6) reduced exchange near the centromere predisposes to non-disjunction in the first meiotic division (mat MI) and increased exchange near the centromere promotes non-disjunction in the second meiotic division (mat MII). However, it is not clear that both of these exchange patterns hold for other chromosomes, since the diversity of published results on the relation between crossing over and non-disjunction is bewildering (7). We describe here an analysis of a large sample of trisomy 18 cases, construct non-disjunction maps for both the mat MI and mat MII cases and compare the degree and distribution of recombination with a standard chromosome 18 map.

For clarity we briefly describe a number of key terms. Relevant observations deal with three phenomena: *chiasmata* between pairs of chromatids in a tetrad; *transitions* from reduced (R) to non-reduced (N) or vice versa in two non-disjoined strands; *crossovers* in a single strand. A *chiasma* is sometimes called an *exchange*, although this term has been used more loosely. Chiasmata are distinguished by number and location. Two terms have been used for absence of chiasmata. The expression *achiasmate* (8) means that there is no chiasma in the whole genome. This is characteristic of one sex in many organisms (e.g. male *Drosophila*). It leads to normal disjunction and is the rule in the species where it occurs, demonstrating a mechanism for regular disjunction that does not depend on crossing over. Achiasmate meiosis is rare in higher plants and has not been observed in vertebrates (8,9). In contradistinction, *nullichiasmate* (10) means

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that there are no chiasmata for a particular tetrad in a particular cell, other tetrads being chiasmate. The distinction between a nullichiasmate meiosis and a meiosis with chiasmata distal to the tested markers is seldom feasible. We therefore use *nullichiasmate* in the operational sense, to signify absence of chiasmata between tested markers.

It has been suggested (11) that at least one chiasma is necessary for normal disjunction. If true, the frequency of nullichiasmate tetrads should be zero in normal disjunction. Recent analysis (12) predicts zero or near zero frequencies for nullichiasmate tetrads in chromosome 21 disjunction. For chromosome 18 we have examined the frequency of normal disjunction from nullichiasmate tetrads by reconstruction of the chiasma distribution from crossovers in normal meiosis and compare this with the distribution from transitions in non-disjunction. We have extended this study to determine frequency of nullichiasmate tetrads in normal meiosis in the remainder of the autosomal genome and present a comparison with *Drosophila*.

It requires great effort to collect a critical number of trisomies for any chromosome. We have therefore combined the sample of 100 cases we describe here (Bugge sample) with 61 published cases, the Fisher sample (13), and have analysed them separately and together.

RESULTS

Study population

The Bugge sample consists of 100 trisomy 18 conceptuses and their parents. Although 14 of these families came from Great Britain, six from Norway, three from Switzerland and four from Greece, the majority of 73 came from Denmark. The 100 cases comprise 57 pre-natal, 33 live births, eight stillborn children and two spontaneous abortions. The karyotypes in 57 cases are 47, XX, +18; in 38 cases 47, XY, +18; one 47, XY, + 18 [29]/46, XY [12]; one 48, XYY +18 [30%]/47, XY, + 18 [70%]. Three cases have free trisomy 18, but the sex is unknown (Table 1).

Table 1. Study population

Ascertainment	Number of cases				Maternal age
	Total	Female	Male	Unknown sex	(mean ± SD)
Pre-natal, advanced maternal age	42	22	19[a]	1	39.6 ± 3.0
Pre-natal, other than maternal age	15	7	7[b]	1	29.7 ± 4.8
Spontaneous abortion	2	1	1	0	39.0 ± 9.9
Live birth	33	25	8	0	29.8 ± 4.2
Stillbirth	8	2	5	1	28.1 ± 15.0
Total	100	57	40	3	

[a]Including one case with 47, XY, + 18/46, XY.
[b]Including one case with 48, XYY, + 18/47, XY, + 18.

Parental origin and cell division error

The parental origin in the Bugge sample was determined in all 100 cases (Table 2). In four cases the origin of the extra chromosome 18 was paternal and all four were consistent with a post-zygotic mitotic (PZM) error or non-crossover MII. It is not possible to distinguish between the two classes. However, it must be highly unlikely that these paternal cases arose as a result of a *meiotic* event that generated only non-crossovers. Therefore, the parsimonious assumption is that these were all due to post-zygotic errors. In the remaining 96 cases the additional chromosome was of maternal origin. In 34 cases the error was mat MI, of which 15 were without evidence of crossing over. There were 49 cases of mat MII with evident crossing over and seven non-crossover cases that were either maternal PZM or MII. Again, definitive classification is not possible, however, given that there are four apparent paternal cases, it is most likely that this group includes some post-zygotic errors. Therefore, there is no justification for treating all seven as non-crossover MII. However, given such a large sample of mat MII crossovers we assume that there are a small number of mat MII non-crossovers, so there is no justification for omitting all seven from the analysis. We assumed, therefore, that there were equal numbers of paternal and maternal PZM (four cases), leaving three which we classified as mat MII non-crossover. Amongst the seven cases there were small differences in the informativeness of markers. We therefore examined the effect of including different subsets of three cases in the mat MII analysis. Differences were extremely small (data not shown) and a single subset of three cases was randomly selected for the analysis. In the Fisher sample there were two paternal cases, again assumed to be PZM (Table 2). There were three maternal cases that were either PZM or non-crossover MII. We classified one of these cases as non-crossover mat MII following the same argument.

Table 2. Classification of cases by meiotic error

	Bugge sample		Fisher sample		Total	
	No.	%	No.	%	No.	%
All	100		61		161	
Mat MI	34	34	16	26	50	31
Mat MII	52	52	36	59	88	55
Mat PZM	4[a]	4	2[a]	3	6	4
Pat PZM	4	4	2	3	6	4
Mat unknown	6	6	5	8	11	7

[a]MII non-crossovers assuming equal numbers of maternal and paternal PZM.

The two samples have similar frequencies of the type of meiotic error (Table 2). Among maternal meiotic errors MII is significantly more frequent than MI ($\chi^2 = 10.45$), whereas the opposite is true for other autosomal trisomies that have been studied in our species.

Parental age

The maternal age in the study population for the Bugge sample and the maternal age by cell division error is given in Tables 1 and 3. The parents of the four paternally derived cases were all young, with a mean maternal age of 26.8 years and a mean paternal age of 28.3. Nearly half the probands were ascertained by pre-natal diagnosis because of advanced maternal age (35 years or more). The mean maternal age for mothers in Denmark is 29.1 years, or 28.1 years excluding those who are 35 years and over at the time of childbirth (14). The mean maternal age is not significantly

elevated in any group when the cases ascertained because of advanced maternal age are excluded.

In the Fisher sample mat MII errors have significantly elevated maternal age after exclusion of probands ascertained because of the mother's advanced age (13). For mat MI errors the mother's age was elevated, but not significantly. The authors suggested that failure to reach significance was due to the small sample size. On first consideration it is surprising that the Bugge sample shows no significant elevation of maternal age for either group after exclusion of probands ascertained because of advanced maternal age (Table 3). However, the two samples differ markedly in the uptake of women at risk. In the area of Britain from which the trisomy sample was drawn only 36% of pregnant women aged 35 or more had pre-natal diagnosis (15), compared with 65–70% in Denmark (16), where the high uptake greatly reduces the power to detect an effect of increased maternal age. The observed difference of 2.2 years is in the expected direction.

Table 3. Maternal age and cell division error in cases with an additional maternally derived chromosome 18

Cell division	All cases		Excluding cases ascertained because of advanced maternal age	
	No.	Maternal age (mean ± SD)	No.	Maternal age (mean ± SD)
All	90	34.8 ± 6.1	49	30.9 ± 4.7
MI	34	35.6 ± 5.5	15	31.1 ± 4.5
MII	49	34.7 ± 7.9	30	31.2 ± 5.0
Maternal PZM or MII non-crossover	7	32.4 ± 5.9	4	28.5 ± 2.4

The mean maternal age amongst the seven cases which were either maternal MII non-crossover or PZM is 28.5 ± 2.4 years. This is not significantly different from the mean maternal age in all cases (30.9 ± 4.7 years). Fisher (13) had three cases of PZM or maternal MII non-crossover and found a higher mean maternal age of 34.0 ± 0.5 years, compared with the mean maternal age in the whole sample of 32.8 ± 1.2 years. The small numbers involved, however, precluded further examination of these differences.

Standard map

All mapping was performed with the map+ program, which constructs maps with an estimable typing error frequency (ε) and mapping parameter (*p*) (17). Fisher (13) published non-disjunction and standard maps of chromosome 18. The standard female map length was estimated at 163 cM. This is longer than the standard map presented in Table 4 (147 cM). The difference in length is a consequence of typing error filtration in the present sample (error per locus estimated at $\varepsilon = 0.0028$) and map construction under interference with a mapping parameter in the Rao function (18) estimated at $p = 0.189$.

Non-disjunction map—meiosis I

The mat MI non-disjunction map of chromosome 18 constructed from the combined Bugge and Fisher samples has a length of 91 cM, significantly shorter than the standard female map length of 147 cM ($P < 0.0001$, Table 4). When the combined sample is plotted against the standard map (Fig. 1) there is evidence of strikingly reduced recombination specifically on chromosome 18p, with a complete lack of recombination in the pericentromeric region between D18S53 and D18S45 (12 cM on the standard map). This pattern is mirrored in both Fisher and Bugge samples individually, although the results are rather different with a map length of 83 ± 15 cM in the Bugge sample and 132 ± 24 cM in the Fisher sample. The Fisher sample is small (16 cases, of which six are non-crossover) and the combined sample closely follows the curve for the Bugge sample. Although the map lengths are different, the pattern of recombination in the centromeric region is similar. It may be argued that these samples should not be combined and for this reason we present results for separate samples in Figure 1.

Table 4. Standard and non-disjunction maps (combined Bugge and Fisher samples)

	Standard	MI	MII
pter			
D18S170	0.00	0.00	0.00
D18S59	0.00	0.00	3.48
D18S63	1.91	0.00	6.94
D18S54	2.04	2.91	11.19
D18S62	7.34	5.58	20.84
D18S53	35.65	13.56	45.26
D18S71	37.27	13.56	47.08
D18S40	43.17	13.56	49.49
cen			
D18S45[a]	47.71	13.56	50.26
D18S44	47.73	15.93	53.20
D18S66	54.40	23.85	62.84
D18S36	62.98	30.49	68.24
D18S67	68.64	34.93	73.78
D18S34	68.89	38.05	75.47
D18S65	74.86	38.06	75.69
D18S46	84.55	42.37	88.72
D18S35	91.87	48.62	93.77
D18S41	96.26	51.08	93.78
D18S38	108.98	54.41	106.96
D18S51[a]	119.81	54.41	111.80
D18S42	121.62	65.19	111.80
D18S43	135.31	73.15	125.32
MBP	143.57	87.37	137.21
D18S50	143.58	87.37	137.21
D18S70	146.07	87.43	138.22
D18S11	146.61	90.98	138.22
qter			
SE (length)		10.67	7.54
No. of cases		47	84

mat MI versus standard: $t = 5.2$.
mat MII versus standard: $t = 1.1$.
mat MI versus mat MII: $t = 3.6$.
[a]Markers used only in the Fisher study.

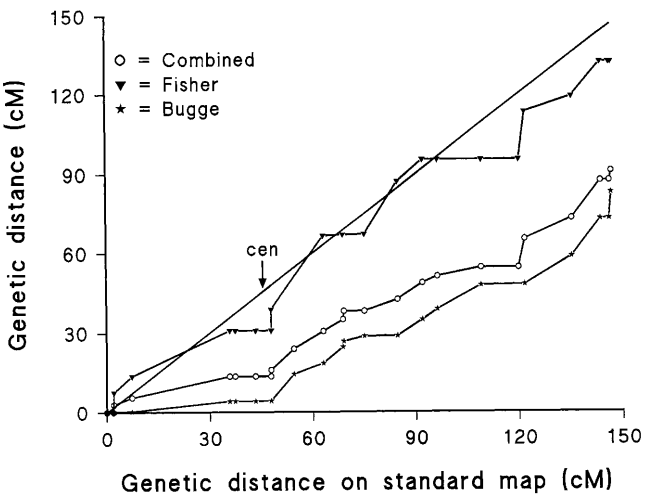

Figure 1. The figure illustrates genetic maps constructed from maternal MI cases in both separate and combined samples plotted with the p telomere at the origin. The mat MI maps are plotted against the standard map on the *x*-axis. Therefore, the standard map itself appears as a straight line through the origin. The mat MI maps are all shorter overall than the standard map, with a marked difference in the distribution of chiasmata, particularly in the centromeric region (where the slope relative to the standard map is most reduced) and also on the q arm ~110 cM from the p telomere on the standard map.

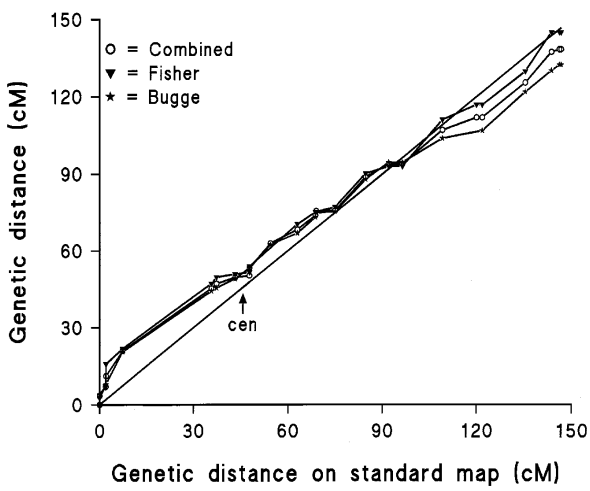

Figure 2. The figure shows maternal meiosis MII maps plotted against the standard map. Overall the combined and separate maps are slightly shorter than the standard map and show small differences in the pattern of recombination. There is no indication of a localized increase in recombination in the centromeric region.

Non-disjunction map—meiosis II

The mat MII map from the combined sample is non-significantly shorter than the standard map (138 against 147 cM, Table 4). When plotted against the standard map (Fig. 2) there is no evidence of a large alteration in the pattern of recombination for either the separate or combined samples. In particular, there is no indication that chiasmata close to the centromere are in excess, as for chromosome 21 (6). Fisher (13) found the mat MII map to be 191 ± 30 cM, compared with a standard map of 163 ± 4 cM. The present re-analysis of these data gives a map length of 145 ± 12 cM. The difference is a consequence of lack of typing error filtration and chiasma interference in the earlier analysis. The present analysis by map+ includes interference and error filtration (17).

Reconstruction of the chiasma distribution

Lamb *et al.* (12) have developed a method for reconstructing chiasma frequencies in arbitrary chromosome intervals. Their approach, however, generates negative frequencies for certain chiasma classes. We here present a novel method for reconstruction of the chiasma distribution from crossovers in normal disjunction and transitions in non-disjunction. The limitations on this theory are that crossing over in the telomeric regions is not detected, typing errors simulate double crossover (19) and post-zygotic mitotic non-disjunction (PZM) is confounded with non-crossover MII non-disjunction. We represent the probability of *j* chiasmata as q_j. By constraining the frequency of zero chiasmata (q_0) to zero we can test the null hypothesis that all tetrads are chiasmate by taking the difference in –2 log likelihood between this constrained model and a model in which q_0 is estimated as a χ^2 value with 1 degree of freedom. In this way non-crossover events are discriminated in probability from nullichiasmate meiosis.

Table 5. Number of crossovers and transitions in chromosome 18 meiosis

Data	q_0	χ^2_1 for $q_0 = 0^a$	Counts of crossovers/transitions								Length (cM)	Source
			n_0	n_1	n_2	n_3	n_4	n_5	n_6	Total		
Paternal chiasmata	0		0	25	116	4	0	0	0	145	92.8	Laurie and Hultén (20)
Paternal crossovers	0.07	0.74	24	35	19	0	0	0	0	78	92.3	CEPH v.8.1
Maternal crossovers	0		11	29	29	6	3	0	0	78	146.4	CEPH v.8.1
Maternal MI	0.25	2.10	6	2	4	1	3	0	0	16	118.7	Fisher
	0.32	5.24	15	5	7	4	0	0	0	31	80.9	Bugge
	0.30	7.33	21	7	11	5	3	0	0	47	95.6	Total
Maternal MII	0		1	1	19	9	6	0	0	36	152.7	Fisher
	0		3	4	21	13	6	1	0	48	151.0	Bugge
	0		4	5	40	22	12	1	0	84	152.2	Total

q_0, frequency of nullichiasmate tetrads; $n_0 – n_6$, counts of crossovers/transitions in each class from 0 to 6.

Table 6. CEPH v.8.1, crossover counts from breakpoint maps

Chromosome	χ^2_1 $q_0 = 0$	Sex	n_0	n_1	n_2	n_3	n_4	n_5	n_6	n_7
1		m	5	19	29	15	7	3	0	0
1		f	1	6	9	18	21	12	10	1
2		m	5	24	26	17	5	1	0	0
2		f	0	6	17	22	18	11	3	1
3		m	4	30	26	13	4	1	0	0
3		f	1	12	22	25	13	4	1	0
4		m	9	31	26	11	1	0	0	0
4		f	1	15	28	13	13	8	0	0
5		m	8	39	19	12	0	0	0	0
5	0.11	f	5	14	24	18	11	6	0	0
6	4.03	m	21	23	25	9	0	0	0	0
6		f	6	16	20	15	14	6	0	1
7		m	16	34	16	11	1	0	0	0
7	0.83	f	6	12	27	21	8	4	0	0
8		m	20	37	18	3	0	0	0	0
8		f	5	20	27	18	4	2	1	1
9	1.16	m	18	28	27	6	0	0	0	0
9		f	7	23	26	18	2	2	0	0
10		m	16	32	21	8	1	0	0	0
10		f	9	20	21	19	7	2	0	0
11		m	15	40	16	6	1	0	0	0
11		f	8	31	19	15	4	1	0	0
12		m	13	35	24	5	1	0	0	0
12		f	3	23	26	19	6	1	0	0
13	0.32	m	29	36	13	0	0	0	0	0
13	1.43	f	21	27	21	9	0	0	0	0
14		m	19	36	23	0	0	0	0	0
14	0.32	f	13	25	25	14	1	0	0	0
15	0.04	m	25	38	15	0	0	0	0	0
15	0.86	f	16	27	30	5	0	0	0	0
16		m	23	41	13	1	0	0	0	0
16		f	7	32	24	11	3	1	0	0
17		m	19	39	20	0	0	0	0	0
17		f	8	33	28	8	1	0	0	0
18	0.70	m	24	35	19	0	0	0	0	0
18		f	11	29	29	6	3	0	0	0
19	0.76	m	25	35	18	0	0	0	0	0
19		f	18	36	21	3	0	0	0	0
20	1.51	m	36	33	8	0	1	0	0	0
20		f	16	36	20	6	0	0	0	0
21	4.58	m	47	29	1	1	0	0	0	0
21	0.20	f	33	37	8	0	0	0	0	0
22	4.19	m	46	30	2	0	0	0	0	0
22		f	32	39	7	0	0	0	0	0

Table 7. Counts for *Drosophila* female X chromosome

Type	χ^2_1 for $q_0 = 0$	Counts of crossovers/transitions						Map length	Reference
		n_0	n_1	n_2	n_3	n_4	Total		
MI	11.52	49	21	24	0	0	94	–	Merriam and Frost (21)
MI crossover	15.73	99	67	22	0	0	188	59.0	Merriam and Frost (21)
MI crossover	135.92	173	21	0	0	0	194	10.8	Koehler *et al.* (5)
MII crossover	–		5	5	0	0	12	125.0	Koehler *et al.* (5)
Crossover	103.50	12776	13202	2196	63	2	28239	63.0	Weinstein (22), Table 4
Crossover	50.68	6607	7555	1913	61	0	16136	71.7	Morgan *et al.* (23)

A dash (–) indicates $q_0 = 0$.

This method (see Materials and Methods) is implemented in the computer program Exchange, available from Andrew Collins. We have used this program to examine the chiasma distributions from chromosome 18 crossovers and transitions (Table 5), from normal meiosis in the CEPH v.8.1 meiotic breakpoint maps (available on WWW at http://www.cephb.fr) (Table 6) and meiotic and non-disjunction data from *Drosophila* (Table 7). For chromosome 18 (Table 5) we find no significant evidence for nullichiasmate tetrads in normal disjunction in either paternal or maternal meiosis. In maternal MI we find significant evidence for nullichiasmate tetrads in the Bugge ($\chi^2_1 = 5.24$, $P = 0.022$) and combined samples ($\chi^2_1 = 7.33$, $P = 0.007$). There is no evidence for nullichiasmate tetrads in mat MII in either separate or combined samples.

The CEPH v.8.1 database provides meiotic breakpoint maps for chromosomes 1–22, constructed from families typed for >5000 Genethon markers. From these maps crossover distributions for both male and female meiosis can be obtained (Table 6). An examination of the reconstructed chiasma distribution and a test of the null hypothesis that $q_0 = 0$ gives only three significant χ^2 values amongst 44 tests (chromosomes 6, 21 and 22 in male meiosis). This is close to the number that would be expected under the null hypothesis at the 5% significance level. It is noticeable that these three include the two smallest chromosomes, suggesting the possibility of nullichiasmate meiosis in these cases. This is inconclusive, however, as map lengths from these crossover distributions suggest that outer markers in the breakpoint maps are not close to the telomeres in many cases. This leads to an underestimate of the number of chiasmata. The breakpoint maps from the crossover counts in males cover an autosomal length of 2536 cM, which is 89% of the length of the standard map (2853 cM; 24). In females 4128 cM is 96% of the length of the standard map, at 4298 cM. The ratio of female/male length is 1.63 in the breakpoint map, compared with 1.51 in the standard map. This indicates that the regions close to the telomere are incompletely covered in the breakpoint maps, a region where male recombination is at its highest relative to female recombination (24).

DISCUSSION

In the Bugge sample the additional chromosome 18 was a result of maternal non-disjunction in 96% of the cases. This is similar to the results found in previous non-disjunction studies of trisomy 18 (13,25–28) and of other autosomal trisomies such as trisomy 13, 15, 16, 21 and 22, which all have a low paternal error rate (29–32). The four paternally derived cases were all a result of a post-zygotic error. The probands were one stillborn and three liveborn children. Paternal meiotic non-disjunction responsible for trisomy 18 has only been described in two cases (28) and thus seems to be very rare.

The distribution of mat MI and mat MII errors for chromosome 18 is consistent in all studies (13,28), but is unique amongst autosomal trisomies, as mat MI error is responsible for the majority of non-disjunction in trisomy 13, 16, 21 and 22 (6,30,32,33). Similarities and differences between chromosomes are striking. Fisher *et al.* (13) calculated that the frequency of disomic ova due to mat MII non-disjunction is similar for chromosomes 18 and 21, but the latter has a much higher frequency of mat MI non-disjunction. Chromosomes 16 and 18 are rather similar in size, shape and female genetic map length, but they have extremely different patterns of meiotic error, with mat MI non-disjunction being very common for chromosome 16 while mat MII non-disjunction is unknown (33).

We have examined the evidence for normal disjunction from nullichiasmate tetrads in chromosome 18. Laurie and Hultén (20) did not observe a single nullichiasmate tetrad for chromosome 18 in spermatogonia (Table 5). Among 3890 autosomal tetrads there were only two apparent nullichiasmates (for chromosome 21). This was true even though they scored chiasmata conservatively and the corresponding genetic lengths for all chromosomes are consistently less than Lindsten obtained on the same material (34,35), which in turn are slightly less than indicated by linkage (24). For chromosome 18 we have tested the null hypothesis that all tetrads are chiasmate using the crossover distribution derived from CEPH v.8.1 (Table 5). The frequency of nullichiasmate tetrads (q_0) has its maximum at zero in female meiosis and is non-significantly greater than zero in males ($\chi^2_1 = 0.74$). For the combined sample there is significant evidence for nullichiasmate tetrads, at a frequency of $q_0 = 0.3$, in mat MI ($\chi^2_1 = 7.33$, $P = 0.007$). Assuming that the maternal MII non-crossovers are correctly classified, we find no evidence for nullichiasmates amongst mat MII errors.

Data from our species on mat MI non-disjunction indicate reduced recombination when compared with the standard map. For chromosome 18 the mat MI map is 62% of the length of the standard map. The mat MI map is only 46 and 75% of the standard map length for chromosomes 21 and 16 respectively (33,36). In chromosome 21 mat MI non-disjunction proximal crossing over in particular has been reported to be decreased (6,32) relative to the standard map. In chromosome 16 mat MI non-disjunction there is a dramatic reduction in recombination in the proximal region, which is 77 cM in the standard map but only 4 cM in the trisomy cases (33). Chromosome 15 shows reduced recombination

in mat MI non-disjunction, especially on proximal 15q (37). For chromosome 18 mat MI (Fig. 1) there is a marked suppression of recombination on proximal 18p and in the medial 18q region, but not on proximal 18q.

For mat MII Petersen *et al.* (30) reported recombination very close to the centromere in seven of 11 cases of trisomy 21. More recently Lamb *et al.* (6) reported a map length of chromosome 21 MII that is 1.46 ± 0.11 times the standard map, mostly the result of increased proximal recombination. However, MacDonald *et al.* (10) reported an overall decrease in recombination in maternal MII X chromosome trisomy. For chromosome 18 there is a slightly but non-significantly reduced map length in mat MII.

While there are no reliable chiasma counts for human females, the evidence is against an appreciable frequency of nullichiasmates amongst tetrads with normal disjunction (Table 6). Marker coverage of the chromosomes is incomplete, but still only three of 44 chiasma distributions give evidence against the hypothesis that $q_0 = 0$, in close agreement with the null hypothesis. While the sample size is fairly modest (78 or 79 meioses), the dogma that at least one chiasmata is required for normal disjunction appears to be true for our species, with no vestige of the achiasmate mechanism characteristic of some invertebrates and plants.

The close similarity of two recent studies on meiotic non-disjunction in *Drosophila* (5) and man (6) led to the hope that other examples would reveal a similar effect of chiasma distribution. However, the data are more complex. In *Drosophila* Merriam and Frost (21) found much the same distribution of chiasmata for mat MI non-disjunction as for the standard map, with lengths of 59.0 and 63.0 cM respectively (Table 7). There was no marker on Xp and so the possible inclusion of a few MII events cannot be excluded. In contrast, Koehler *et al.* (5) reported for mat MII non-disjunction that chiasmata were localized to distal Xq at an extremely low frequency. In a small number of mat MII events there was an excess of chiasmata, especially in proximal Xq. The estimated map lengths were 60.3 cM for the standard map, 10.8 cM for mat MI and 125.0 cM for mat MII. Assuming an absence of crossing over in the unmarked telomeric regions, the estimates of chiasma distributions in large samples analysed by Weinstein (22) and Morgan *et al.* (23) leave no doubt that nullichiasmate tetrads can disjoin normally in this species, where the male is achiasmate. The data presented here derived from the CEPH database suggest that there is no such mechanism for normal disjunction in man. Furthermore, the model proposed by Lamb *et al.* (6), in which susceptible chiasma configurations predispose to non-disjunction at both stages, is not supported by this study. The observation of increased recombination largely restricted to proximal 21q in mat MII non-disjunction suggests that bivalents with proximal chiasmata become 'entangled', non-disjoin at MI and undergo a reductional division at MII resulting in an 'MII error'. Chromosome 18 reveals no evidence for increased proximal recombination in mat MII to support this as a unifying mechanism.

Recently Angell (38) observed that among 67 MII metaphase oocytes exhibiting chromosomal abnormalities none possessed 24 *whole* chromosomes. In fact, only single chromatids (half chromosomes) were found to be present. These observations support centromere misdivision in MI and contradict the 'entanglement' hypothesis, for which the complete bivalent would be expected to non-disjoin at MI and go to one pole, followed by subsequent reduction division at MII.

MATERIALS AND METHODS

DNA analyses

DNA from the probands in the Bugge sample was extracted from blood, amniocytes, chorionic villi or fibroblasts and from the parents from EDTA anti-coagulated blood by a salting out procedure (39). A total of 28 DNA polymorphisms were studied. The following five chromosome 18 probes were used in standard Southern blot analyses (40): L2.7 (D18S6) (41); D18S21 (42); pMS1-3 (D18S19) (43); CL1-L159 (D18S17) (44); pERT-25 (D18S11) (45). A total of 23 DNA polymorphisms were detected after PCR amplification of genomic DNA (46). End-labeling of primer, PCR amplification conditions, polyacrylamide gel electrophoresis of the amplification products and autoradiography were performed according to protocols described elsewhere (29). The microsatellites used were as follows: D18S40, D18S41, D18S42, D18S43, D18S44, D18S46 and D18S50 (47), D18S53, D18S54, D18S59, D18S62, D18S63, D18S65, D18S66, D18S67, D18S70, D18S71 (48), D18S36 (49), D18S34, D18S35 (50,51), D18S170 (R.Straub, GDB), D18S38 (J.Hudson, GDB) and MBP (53). Information on the probes, primers and allele frequencies are available in the Genome Data Base and the references cited.

The parental origin of the extra chromosome 18 was determined by scoring the polymorphic alleles when three different alleles were present and by scoring the alleles combined with dosage analysis when two different alleles were present (29). There was no centromeric marker, so the meiotic stage of non-disjunction was assigned on the basis of reduction to homozygosity at the pericentromeric markers: D18S40 on the short arm and D18S44/D18S45 on the long arm of chromosome 18. It was not possible to say which was the closest to the centromere and therefore most likely to reflect zygosity at the centromere. There were only five cases in which the centromere flanking markers disagreed, thereby preventing definitive assignment to MI or MII. This must reflect recombination in this region in this small number of cases. We were anxious not to omit these from the study, thereby introducing a bias. To assign to either class we assumed that the majority of the five were MII, given the higher frequency of MII in the remainder of the sample. Using zygosity at D18S40 would have assigned the majority to mat MI, therefore it was more appropriate to use zygosity at markers D18S44/D18S45, allowing classification of four cases as mat MII and one as mat MI.

Non-disjunction mapping

Analysis was performed using the map+ program (17). Heterozygous loci in the parent contributing the additional chromosome were identified and it was determined whether these loci remained heterozygous (classified as N, not reduced to homozygosity) or were reduced to homozygosity (R) in the trisomic proband. Transitions between reduced and non-reduced states were identified for the markers tested, where informative. Only individuals from either Bugge or Fisher samples with at least 10 markers informative (R or N) were used for mapping. The theory for tetrad analysis comes from Shahar and Morton (53) and Morton *et al.* (54). The relationship between recombination (θ) and map distance (w) can be expressed as $y = 3\theta - w$, where y is the tetratype frequency (the probability of obtaining all four possible chromatids from a pair of heterozygous loci). Map+ implements this theory and incorporates

a typing error frequency ε (19) and interference parameter *p* in the Rao model (18). These parameters were estimated for a standard map constructed by map+ from the CEPH v.8 database and were assumed to be the same for the non-disjunction map.

As map length increases the value of *y* approaches 2/3 with damped oscillations, so pairs of loci at large distance are consistent with more than one recombination value. Therefore, a truncation parameter *T* is required to ignore pairs of loci separated by >*T* cM in the standard map. The appropriate value of *T* for this analysis was estimated at 55 cM, corresponding to interference in the standard maps (24).

A number of adjacent markers were combined into megaloci in both the standard and non-disjunction maps where typing was incomplete and there was no recombination between them. Combining Bugge and Fisher samples, megaloci were defined as follows: (D18S11, D18S17, D18S31); (D18S42, D18S19, D18S5); (D18S35, D18S8, DCC); (D18S67, D18S6); (D18S40, D18S32); (D18S62, D18S21).

Reconstructing the chiasma distribution

We here describe a method for reconstruction of the chiasma distribution from the observed crossovers in normal meiosis and transitions in non-disjunction. Formulae relating crossovers to underlying chiasma frequencies are known (22) and tables relating transitions in MI and MII to chiasma frequencies have been published (54).

A proportion 0.5^j of chromatids from tetrads with *j* chiasmata do not undergo crossing over and are therefore indistinguishable from products of nullichiasmate meiosis.

The probability c_i of *i* crossovers or transitions is related to the probability q_j of *j* chiasmata as

$$c_i = \sum_{j=0}^{J} k_{i|j}\, q_j \qquad i, j = 0, \ldots, J$$

where $k_{i|j}$ is the conditional probability of *i* given *j* (54). In matrix form this is $c = Kq$, where *c* and *q* are column vectors and *K* is a square matrix of order $J + 1$, and so $q = K^{-1}c$. However, this is true only in expectation and application of this theory often gives negative estimates of some values of *q* unless the sample is very large and the maximum number of crossovers is small (6,12).

Maximum likelihood by the EM algorithm gives better estimates. If n_i crossovers or transitions are observed, the likelihood is

$$L = \prod_i c_i^{\,n_i}$$

and the EM algorithm gives for the *i*th iteration

$$q_j^{(t)} = E_j^{(t-1)} / N$$

where $N = \sum_i n_i$ and

$$E_j^{(t-1)} = q_j^{(t-1)} \sum_i \left(\frac{\partial \ln L}{\partial c_i}\right)\left(\frac{\partial c_i}{\partial q_j}\right)$$
$$= q_j^{(t-1)} \sum \left(n_i\, k_{i|j} / c_i\right)$$

the derivatives being taken without constraining to $\Sigma c_i = 1$. Then all estimates of q_j are non-negative and at each iteration $\Sigma q_j = 1$.

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