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Review

Radiation-induced breakpoint misrejoining in human chromosomes: random or non-random?

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Abstract

Purpose: To investigate whether radiation-induced misrejoining of chromosome breakpoints is randomly or non-randomly distributed throughout the human genome.

Materials and methods: Data were combined from as many published cytogenetic studies as possible. The percentage of radiation-induced breaks per megabase (Mb) of DNA between all human chromosomes was calculated, and the observed and expected numbers of breakpoints based on DNA content between and within chromosomes were compared.

Results: A DNA-proportional distribution of breakpoints in 14 autosomes and a statistically significant deviation from proportionality in the other eight autosomes and the sex chromosomes was found. Regression analysis showed no significant change in breakpoint frequency per Mb of DNA relative to autosome size. Analysis between chromosome arms showed a non-random distribution of induced breakpoints within certain autosomes, particularly the acrocentrics. In cases of non-random distributions, a prevalence of events was found at heterochromatic regions and/or telomeres, and a clustering of breakpoints was found near the centromeres of many chromosomes.

Conclusions: There is an approximately linear proportionality between autosomal DNA content and observed breakpoint number, suggesting that subsets of autosomes can be used to estimate accurately the overall genomic frequency of misrejoined breakpoints contingent upon a carefully selected subset. However, this conclusion may not apply to the sex chromosomes. The results also support the influence of chromatin organization and/or preferential DNA repair/misrejoining on the distribution of induced breakpoints. However, these effects are not sufficient at a global level to dismiss the value of cytogenetic analysis using a genome subset for biodosimetry.

1. Introduction

An enduring and highly significant question in the field of radiation biology has been whether the distribution of radiation-induced breakpoints is proportional to chromosome size. This has been a frequent topic of debate since early work in the field (Sax 1938, 1940). Misrejoined breakpoints are an easily detected manifestation of radiation exposure, and therefore many research groups have used these events as an endpoint in human biodosimetry studies. However, due to the time-consuming nature and potential subjectivity of chromosome banding techniques and the lack of a sufficient number of different fluorescent stains when using chromosome painting (without image processing), it has become common practice to analyse only one or a few chromosomes. These analyses of a subset of chromosomes result in conclusions that are primarily related to the chromosomes under analysis, although the data are often extrapolated to yield estimates of the overall genomic frequency of chromosome exchanges. If this extrapolation is to be feasible, it must be assumed that the chromosomes analysed are representative of the genome as a whole. This assumption requires that radiation-induced chromosome rearrangements are randomly distributed throughout the genome and depend mainly on DNA content.

Over the years numerous studies have been performed to address the assumption of a chromosome size distribution of radiation-induced chromosome breakpoints in human cells. These include both classical cytogenetic (i.e. banding) and molecular cytogenetic (i.e. chromosome painting) studies. Many research groups concluded that the distribution of radiation-induced breakpoints is not proportional to chromosome size. However, these conclusions share little agreement on which chromosomes are more or less likely to be involved in exchanges based on DNA content. For example, Fernandez *et al.* (1995) found that chromosomes 1 and 2 in lymphocytes from healthy subjects were less likely to take part in X-ray-induced exchanges compared with their DNA content. This conclusion was supported by Knehr *et al.* (1996), who found that, in general, larger chromosomes were less frequently involved in X-ray-induced symmetrical exchanges than expected according to their DNA content. In contrast, Lucas *et al.* (1992),

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in a study of γ-radiation-induced exchanges and Y-12 accident victims, and Natarajan *et al.* (1992), who studied X-ray and fast neutron-induced translocations, found that chromosome 1 was more likely to take part in exchanges when compared with its DNA content. This conclusion was supported by Boei *et al.* (1997), who performed an X-irradiation study of chromosomes 1 and 4 and found that both chromosomes were involved in exchanges more frequently than expected based on size. The contradictory results of these and other cytogenetic studies have cast doubt on the usefulness of chromosome breakpoints as an endpoint for biological dosimetry.

While some studies of human cells have shown distributions of radiation-induced chromosome breakpoints that were not proportional to chromosome size, other work has resulted in conclusions that DNA content was an important factor. For example, Natarajan *et al.* (1991), in a study of the radiation accident in Goiania, Brazil, suggested that chromosome breakage depends mainly upon the length of the chromosome. Indeed, Lucas *et al.* (1992) concluded that the significant deviation of the translocation frequency for chromosome 1 did not seem sufficiently large to invalidate the assumption that translocation frequencies are proportional to DNA content. Studies on A-bomb survivors and *in vitro* X-ray exposures have shown general agreement with chromosome size proportionality (Sachs *et al.* 1993 and Granath *et al.* 1996, respectively). Johnson *et al.* (1998a) found that γ-radiation-induced breakpoints in chromosomes 3, 5 and 6 were distributed based on relative DNA content. These results suggest that subsets of chromosomes may be useful for estimating whole genomic breakpoint frequencies.

Another consideration in the decision to use chromosome breakpoints as a study endpoint is the distribution of these events along the length of a chromosome. However, the analysis of the 'intra-chromosomal' distribution of breakpoints may not be as germane for biodosimetry purposes as the 'inter-chromosomal' distribution analysis. The important question for dosimetry is whether a particular chromosome to be analysed is representative of the genome as a whole and not whether the distribution of breakpoints is random along the length of the chromosome. However, the analysis of breakpoint location relative to centromere and telomere position may yield important information on the effects of heterochromatin, preferential DNA repair and radiation-sensitive 'hotspots' on the distribution of induced breakpoints. It may also prove useful in cancer risk assessment (i.e. radiation-induced clonal aberrations), as many malignancies have been associated with specific chromosomal rearrangements (Solomon *et al.* 1992, Rabbitts 1994, Mitelman *et al.* 1997).

Like those studies analysing the inter-chromosomal distribution of radiation-induced breakpoints, cytogenetic analyses of the breakpoint distribution within chromosomes have yielded conflicting results. For example, early work did not indicate any departure from randomness (e.g. Savage *et al.* 1973). However, more recent studies, as well as past work, have shown a reduction in the number of rearrangements near the ends of chromosomes (e.g. Tucker and Senft 1994, Cooke *et al.* 1975), while others have shown clustering of radiation-induced breakpoints at telomeres (e.g. San Ramon and Bobrow 1973, Lee and Kamra 1981, Barrios *et al.* 1989). A preferential occurrence of chromosome breakage near interbands (Dubos *et al.* 1978) and in G-negative bands (Bauchinger and Gotz 1979) has been shown, although the problem of assigning breakpoint locations when using banding techniques is well documented and has been described in detail (Savage 1977). Unfortunately, very few molecular cytogenetic studies have been performed to localize breakpoint positions.

The varied conclusions of cytogenetic studies have resulted in a very unclear picture of radiation-induced chromosome breakpoints and their distribution relative to DNA content, and which particular chromosomes, if any, are more or less likely to be involved in exchanges based on their DNA content. The contradictory nature of the results of these studies has not allowed resolution on whether the analysis of a subset of chromosomes yields accurate biodosimetry data. A thorough analysis of both the inter- and intra-chromosomal distribution of radiation-induced breakpoints may prove useful for determining whether these events are an effective endpoint for biological dosimetry and which factors may influence the observed results. The goal of this review is to combine as many cytogenetic studies as possible (both classical and molecular) to determine if any consensus exists in the long-lasting debate on the distribution of radiation-induced misrejoined breakpoints.

2. Materials and methods

2.1. *Study selection*

The human cytogenetic studies included in this review were those in which sufficient raw data were available. The minimum requirement for inclusion was the observed number of radiation-induced breakpoints in each chromosome analysed. Unfortunately, some studies could not be included

for reasons such as the absence of the number of observed breakpoints and the absence of a chromosomal categorization of the events. In other studies, individual chromosomes were analysed in different numbers of cells. A total of 17 classical cytogenetic studies (spanning 1972 through 1989) and nine molecular cytogenetic studies (spanning 1990 through the present), representing over 20 000 breakpoints, contained sufficient data for inclusion in this review. The classical studies contained analyses of all the human chromosomes while the painting studies represented only chromosomes 1–10, 12, 14 and X. Of the 26 studies included in this review, 23 represented analyses of lymphocytes and three represented analyses of fibroblasts (Lee and Kamra 1981, Dutrillaux *et al.* 1983, Kano and Little 1986).

2.2. *Breakpoint definition*

The term 'breakpoint' is used in this review for consistency, although the aberration types included for analysis varied slightly. The aberration types included in the classical cytogenetic analyses (over 14 000 breakpoints) were most often dicentrics and rings plus associated fragments (asymmetrical), and translocations and inversions (symmetrical). Other types (e.g. insertions and deletions) were included in some cases. Translocations and dicentrics were the most numerous when aberration types were categorized. The aberrations observed in the molecular cytogenetic studies (nearly 8000 breakpoints) always included translocations, and in some cases included other types (e.g. dicentrics, insertions, rings and fragments). Overall, the most common aberration types from all analyses were translocations and dicentrics. The fact that aberration type categories were frequently not included made certain analyses impossible, such as the comparisons of symmetrical and asymmetrical aberrations (i.e. translocations and dicentrics, respectively), inter- and intra-chromosomal exchanges (e.g. dicentrics and centric rings, respectively) and inter- and intra-arm aberrations (i.e. peri-and paracentric inversions, respectively), as well as the analysis of complex rearrangements.

Despite the variability in aberration types included in the reviewed studies, the number of misrejoined breakpoints represented by each aberration type are consistent throughout the studies. The number of scored events according to the classical system of quantifying induced chromosomal structural changes (Buckton and Evans 1973, Savage 1976), the chromosome painting nomenclature system of Savage and Simpson (S & S) (1994a,b), and the Protocol for Aberration Identification and Nomenclature Terminology (PAINT) (Tucker *et al.* 1995) are listed in table 1 to illustrate some of the variation among scoring systems.

According to classical terminology, aberrations are assumed to be complete exchanges. However, classical terminology does account for the observation of incomplete exchanges (e.g. a dicentric chromosome observed with two associated acentric fragments). The Savage and Simpson system quantifies events by the number of chromosomes, arms and breaks (C/A/B) involved in the aberration. This system was developed for the analysis of complex aberrations, but for comparison it is applied here to simple exchanges. PAINT quantifies the number of events by enumerating colour junctions and accounts for incomplete exchanges. Inversions and interstitial deletions are not scored when using PAINT because these events are not readily detectable by painting. Simple breaks (terminal deletions) and centric rings plus fragments are scored (0 colour junctions) using PAINT if the chromosome involved is painted, although they are usually not scored if the material involved is unpainted. Insertions are reported as two colour junctions when using PAINT. However, the difference between three breaks reported using other systems has a negligible effect on the distribution results because the frequency of insertions is very low relative to other aberration types (i.e. translocations and dicentrics).

2.3. *Inter-chromosomal breakpoint distribution*

Chi-square analysis (1 df) was used to compare the observed number of breakpoints within a particular

Table 1. The number of scored events according to three scoring systems.

Aberration	Classical	S & S	Paint	Breakpoints
Simple break (terminal deletion)	1	1/1/1	0/n.s.	1
Interstitial deletion (minute)	1	1/1/2	n.s.	2
Reciprocal translocation	1	2/2/2	2	2
Dicentric+ fragment	1	2/2/2	2	2
Paracentric inversion	1	1/1/2	n.s.	2
Pericentric inversion	1	1/2/2	n.s.	2
Centric ring +fragment	1	1/2/2	0/n.s.	2
Insertion	1	2/2/3	2	3

n.s., not scored.

chromosome with that expected based on random distribution of the breakpoints. In many studies, observed and expected numbers of breakpoints were given. If expected numbers were not provided, they were calculated based on a DNA-proportional distribution (Morton 1991) and on the basis of the chromosomes analysed in that study alone, without extrapolation to the entire genome. The observed and expected numbers of breakpoints within a chromosome were combined from all studies analysing that particular chromosome to yield a single value for each chromosome in the genome.

To analyse further the distribution of breakpoints between chromosomes, a simple equation was used to allow for direct comparison of cytogenetic studies, including those that analysed less than the entire complement of human chromosomes. Using the number of breakpoints observed in each individual chromosome analysed, the total number of observed breakpoints and the size in megabases (Mb) of the chromosomes according to Morton (1991) and references therein, the equation to determine the percentage of breaks per Mb of DNA in an individual chromosome is:

$$\frac{\%\ \text{breaks}}{\text{Mb DNA}} = \frac{\dfrac{\text{breaks in chromosome 'i'}}{\text{total breaks in study 'j'}} \times 100}{\text{Mb quantity of chromosome 'i'}} \times \frac{\text{Mb analysed in study 'j'}}{\text{Mb in human genome}}$$

where 'i' represents a particular chromosome analysed in a study and 'j' represents that study. The first part of the equation yields the percentage of breaks per Mb in each chromosome analysed, while the second part of the equation represents the fraction of the genome analysed in a particular study, and therefore is a correction factor for those studies where only a portion of the genome was analysed. If all chromosomes were analysed in a given study, then the second part of the above equation would be equal to 1.

Regression analysis was used to determine the best fit model based on minimizing the sum of squares for the relationship between the percentage of radiation-induced breaks per Mb of DNA and chromosome size. The t statistic was used to test the hypothesis that the regression line is horizontal and that chromosome size can be used to predict breakpoint frequencies.

2.4. *Intra-chromosomal, inter-arm breakpoint distribution*

To analyse the distribution of misrejoined breakpoints between chromosome arms, an arm-specific analysis of breakpoints was necessary for inclusion in this part of the review. Fifteen of the studies selected for the inter-chromosomal distribution analysis included data that could be used for the chromosome arm analysis and represented over 10 000 breakpoints.

Chi-square analysis (1 df) was used to compare the observed number of breakpoints distributed between arms of a particular chromosome with that expected based on the DNA content of each arm (Morton 1991).

3. Results

Table 2 shows the observed number of radiation-induced breakpoints and the expected number based on chromosome size in all studies combined, classical studies alone and painting studies alone. The least deviation from a DNA-proportional distribution of breakpoints occurred in groups B, C and F and the greatest deviation occurred in group G and the sex chromosomes. Two out of three chromosomes in each of groups A and D and one out of three in group E deviated significantly from a proportional distribution. Overall, breakpoints were distributed randomly in 14 autosomes (1, 4–8, 10–13, 17–20). When analysing classical studies alone, breakpoints were randomly distributed in 13 autosomes (2, 5–8, 10–13, 17–20). When analysing painting studies alone, breakpoints were randomly distributed in six autosomes (6–8, 10, 12, 14). However, only chromosomes 1–10, 12, 14 and X were examined in painting studies while the entire complement of human chromosomes was studied by classical methods (i.e. banding).

The variation in the frequency of radiation-induced breakpoints per Mb of DNA within and between human chromosomes 1, 2 and 3 is shown in figure 1. This graph allows each study included in this review to be represented by at least one data point, and shows the position of each study's data relative to others for this subset of chromosomes. The variation in the frequency of breakpoints per Mb of DNA within and among all chromosomes from every study is shown in figure 2.

The frequency of induced breakpoints, represented by the percentage of breaks per Mb of DNA, plotted against the quantity of DNA for each human chromosome is shown in figure 3. Three sets of data are represented: one set of mean values from all studies combined, one set from classical studies alone and one set from painting studies alone.

If all breakpoints were randomly distributed throughout the genome, the percentage of breaks per Mb of DNA would be 0.0304 (100%/3286 Mb).

Table 2. Inter-chromosomal distribution of radiation-induced breakpoints in the human genome.

Chromosome	Group	All studies				Classical studies				Painting studies			
		No. of breakpoints		Chi-square value	Probability	No. of breakpoints		Chi-square value	Probability	No. of breakpoints		Chi-square value	Probability
		Observed	Expected			Observed	Expected			Observed	Expected		
1	A	2806	2702	3.98	n.s.	1413	1190	41.97	<0.0001	1393	1513	9.47	0.0021
2		2289	2510	19.48	<0.0001	1104	1153	2.11	n.s.	1185	1357	21.74	<0.0001
3		1567	1755	20.16	<0.0001	883	968	7.45	0.0063	684	787	13.52	0.00024
4	B	2005	1959	1.09	n.s.	822	918	10.07	0.0015	1183	1041	19.45	<0.0001
5		1267	1248	0.28	n.s.	840	877	1.60	n.s.	427	371	8.53	0.0035
6	C	1125	1168	1.59	n.s.	805	828	0.62	n.s.	320	340	1.22	n.s.
7		1340	1262	4.84	n.s.	830	773	4.14	n.s.	510	488	0.95	n.s.
8		1024	1047	0.50	n.s.	659	701	2.52	n.s.	365	346	1.06	n.s.
9		995	857	22.28	<0.0001	727	656	7.72	0.0055	268	201	22.33	<0.0001
10		866	851	0.27	n.s.	630	651	0.70	n.s.	236	200	6.63	n.s.
11		694	651	2.80	n.s.	694	651	2.80	n.s.	–	–	–	–
12		898	845	3.32	n.s.	679	647	1.60	n.s.	219	198	2.18	n.s.
13	D	495	516	0.83	n.s.	495	516	0.83	n.s.	–	–	–	–
14		720	644	8.94	0.0028	559	493	8.83	0.0030	161	151	0.65	n.s.
15		540	479	7.65	0.0057	540	479	7.65	0.0057	–	–	–	–
16	E	520	443	13.29	0.00027	520	443	13.29	0.00027	–	–	–	–
17		460	416	4.63	n.s.	460	416	4.63	n.s.	–	–	–	–
18		350	384	3.09	n.s.	350	384	3.09	n.s.	–	–	–	–
19	F	302	303	0	n.s.	302	303	0	n.s.	–	–	–	–
20		337	326	0.40	n.s.	337	326	0.40	n.s.	–	–	–	–
21	G	275	226	10.55	0.0012	275	226	10.55	0.0012	–	–	–	–
22		345	253	33.21	<0.0001	345	253	33.21	<0.0001	–	–	–	–
X		634	833	47.73	<0.0001	365	606	95.91	<0.0001	269	227	7.63	0.0057
Y		23	197	153.55	<0.0001	23	197	153.55	<0.0001	–	–	–	–

n.s., not significant at $p=0.01$.

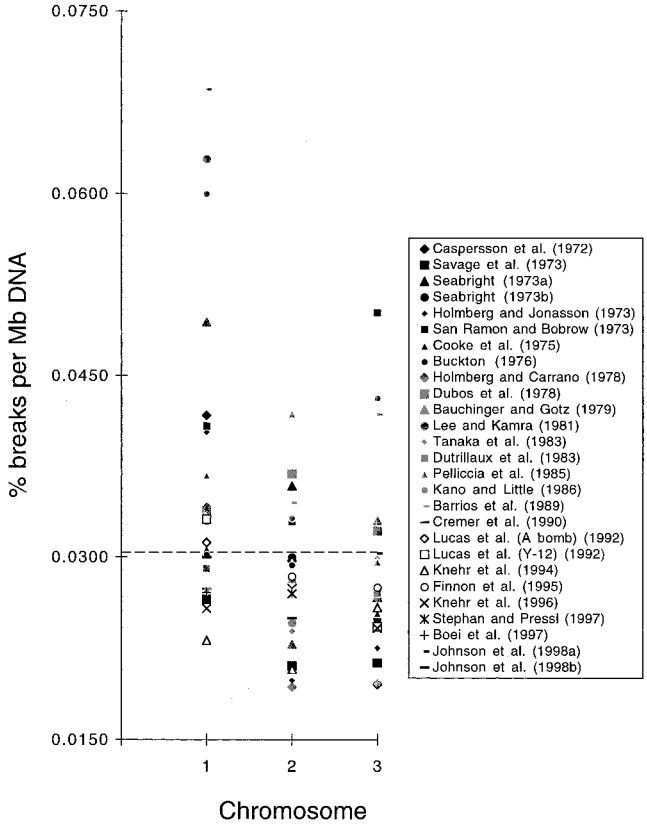

Figure 1. Percentage of radiation-induced breakpoints per megabase (Mb) of DNA by chromosome number. Values for chromosomes 1, 2 and 3 are shown, with each study represented by at least one datum. The value corresponding to a random distribution of events is represented by the dashed line (0.0304% breaks per Mb of DNA).

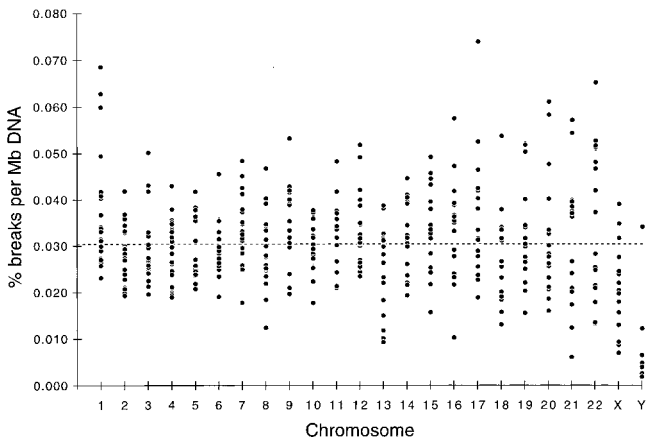

Figure 2. Percentage of radiation-induced breakpoints per megabase (Mb) of DNA by chromosome number. Every chromosome from each study is included and is represented by one datum. The value corresponding to a random distribution of events is represented by the dashed line (0.0304% breaks per Mb of DNA).

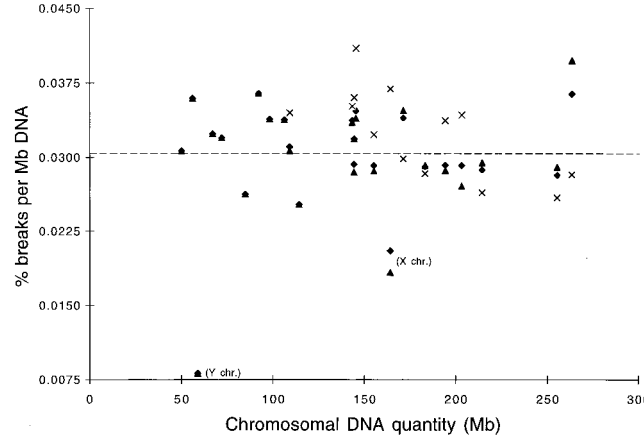

Figure 3. Percentage of radiation-induced breakpoints per megabase (Mb) of DNA by the total length of each chromosome in the human genome. Included are the mean values of all studies combined (diamonds), painting studies alone (X symbols) and classical studies alone (triangles). Values representing the sex chromosomes are indicated in parentheses. The value corresponding to a random distribution of events is represented by the dashed line (0.0304% breaks per Mb of DNA).

When regression analysis is performed on autosomal values only, there is no significant change in the frequency of radiation-induced breakpoints per Mb of DNA with increasing chromosome size in all studies combined and classical studies alone ($t = -0.711$ and -0.217, respectively, with alpha $= 0.01$, 20 df). However, there is a significant decrease in breakpoint frequency with increasing autosome size in painting studies alone ($t = -4.49$ with alpha $= 0.01$, 10 df). The addition of the sex chromosomes to the regression analysis of all studies combined and classical studies alone and of the X chromosome to the analysis of painting studies alone (the Y chromosome was not analysed by painting) does not affect the conclusions ($t = 0.466$ and 0.623, alpha $= 0.01$, 22 df for all studies combined and classical studies alone, respectively, and $t = -4.49$, alpha $= 0.01$, 11 df for painting studies alone).

Table 3 shows the observed number of radiation-induced inter-arm breakpoints and the expected number based on the size of the p and q arms. Groups B, E, F and the sex chromosomes had no inter-arm deviation from a DNA-proportional distribution of breakpoints. The number of inter-arm breaks in chromosome 1 (group A) and chromosome 9 (group C) deviated significantly from proportionality ($p = 0.0042$ and 0.00036, respectively), and the numbers in every chromosome in groups D and G deviated significantly from a DNA content-based inter-arm distribution of induced breakpoints ($p < 0.0001$).

Table 3. Intra-chromosomal, inter-arm distribution of radiation-induced breakpoints in the human genome.

Chromosome	Group	No. of breakpoints				Length in megabases[a]		Chi-square value (1 df)	Probability
		p arm		q arm					
		Observed	Expected	Observed	Expected	p arm	q arm		
1	A	564	517	499	546	128	135	8.19	0.0042
2		306	288	437	455	99	156	1.74	n.s.
3		315	300	333	348	99	115	1.44	n.s.
4	B	179	160	400	419	56	147	3.21	n.s.
5		145	159	449	435	52	142	1.73	n.s.
6	C	193	195	355	353	65	118	0.022	n.s.
7		228	228	373	373	65	106	0.0014	n.s.
8		158	154	320	324	50	105	0.14	n.s.
9		145	184	378	339	51	94	12.72	0.00036
10		143	141	318	320	44	100	0.047	n.s.
11		203	200	293	296	58	86	0.087	n.s.
12		162	137	339	364	39	104	6.47	n.s.
13	D	88	47	248	289	16	98	41.15	<0.0001
14		112	59	290	343	16	93	55.77	<0.0001
15		98	61	284	321	17	89	26.24	<0.0001
16	E	140	143	220	217	39	59	0.12	n.s.
17		101	98	220	223	28	64	0.16	n.s.
18		70	58	177	189	20	65	3.18	n.s.
19	F	83	90	118	111	30	37	0.99	n.s.
20		90	97	136	129	31	41	0.96	n.s.
21	G	107	44	94	157	11	39	114.27	<0.0001
22		82	54	150	178	13	43	19.15	<0.0001
X		125	123	200	202	62	102	0.060	n.s.
Y		6	4	10	12	13	46	2.23	n.s.
TOTAL		3843	3516	6641	6968	1102	2184	45.78	<0.0001

[a] From Morton 1991.
n.s., not significant at $p=0.01$.

4. Discussion

Analysis of the inter-chromosomal distribution of misrejoined radiation-induced breakpoints shows that 14 of the human autosomes are involved in breakpoint formation at a frequency that is proportional to their DNA content (table 2). These chromosomes (particularly groups B, C and F) are therefore most useful for estimating whole-genome breakpoint frequencies for biodosimetry. Other chromosomes (particularly group G and the sex chromosomes), which are involved in breakpoint formation significantly different from that based on DNA content, would be of doubtful utility in this capacity. Therefore, cytogenetic analysis for biodosimetry purposes appears to be acceptable and indeed useful if the appropriate subset of chromosomes is selected. By examining table 2, which shows the probability associated with the chi-square values determined from the observed numbers of breakpoints and that expected based on chromosome size, autosomes can be selected for cytogenetic analysis that would be representative of the breakpoint frequency in the genome as a whole.

While it appears that some chromosomes may not be useful for estimating genomic breakpoint frequencies, evidence suggests that these non-random distributions may be due to technical aspects. This becomes clear when the relative direction in the number of breakpoints from that expected based on DNA content is examined. For example, the number of breakpoints in chromosomes 1, 4 and X tend to be in opposite directions when comparing classical and painting studies (table 2). When the data for chromosomes 1 and 4 are pooled from classical and painting studies, the statistically significant deviations in the study types alone cancel each other out resulting in overall non-significance. In contrast, chromosomes 3 and 9 deviate significantly in the same direction when comparing classical and painting studies. The probability associated with the chi-square value in chromosome 3 decreases by greater than one order of magnitude when both study types

are combined. These observations indicate that technical effects (e.g. chromosome staining intensity and probe complexity with painting, variable staining patterns with banding and levels of chromatin condensation) are probably influencing the variable results, since there appears to be little consistency between banding and painting with respect to the number of breakpoints observed in specific chromosomes.

The variation between studies in the percentage of misrejoined breakpoints per Mb of DNA in each human chromosome is shown in figures 1 and 2. Although there is considerable variation in all chromosomes, it appears that, in general, larger chromosomes have less frequency variability (figure 2). This indicates that larger chromosomes (e.g. groups B and C) may be more useful for estimating breakpoint frequencies than smaller chromosomes (e.g. groups E and G). However, it is likely that these differences are influenced by the number of breaks per chromosome. Indeed, a 10-fold difference in breakpoint numbers between the largest and smallest chromosomes was found (2806 versus 275 breakpoints in chromosomes 1 and 21, respectively).

Some prior work has yielded results that suggest a relationship between increasing or decreasing breakpoint frequencies and increasing chromosome size (e.g. Barrios *et al.* 1989 and Knehr *et al.* 1996, respectively). However, the results of this review for all cytogenetic studies combined and classical studies alone do not show a statistically significant relationship between breakpoint frequencies and autosome size (figure 3). The results of the painting studies alone, however, do show a significant decrease in breakpoint frequency with increasing autosome size, although this could be due in part to the small number of painting studies available for analysis. It is important to note that results from the authors' painting studies alone agree with those of Knehr *et al.* (1994, 1996), and that this work contributed all of the data on chromosomes smaller than 8. More molecular cytogenetic data are needed to minimize the influence of the small sample size, although it is likely that the effect of DNA content on the misrejoined breakpoint distribution will become more pronounced as the amount of data increases.

The inclusion of the sex chromosomes in this analysis does not have a statistically significant effect on the relationship between the frequency of breakpoints per Mb of DNA and chromosome size in all studies combined, or in classical or painting studies alone. However, in studies utilizing banding techniques, the number of breakpoints observed in both sex chromosomes is far less than expected based on DNA content, especially in the Y chromosome (table 2). In fact, these two chromosomes have the highest chi-square values determined from the observed number of breakpoints and the expected number based on DNA content. The lack of recordable induced breakpoints in the sex chromosomes is a consistent observation in classical studies, although an explanation for this finding remains elusive. These results may indicate that chromatin structure or preferential DNA repair may play a role in the lack of involvement of sex chromosomes in breakpoint formation and interaction. Conversely, more breakpoints were found in the X chromosome than expected based on DNA content in painting studies alone (the Y chromosome was not analysed). This observation suggests a lack of DNA repair of the inactive X chromosome. It is also possible that cells with breaks in inactive DNA (i.e. heterochromatin) are not selected against as strongly as cells with breaks in active DNA, resulting in a preponderance of heterochromatic rearrangements. However, the contradictory results between the classical and painting studies may indicate that technical influences, not mechanistic effects, are responsible. Overall, these observations mean that the true effect of the sex chromosomes on the distribution of breakpoints must be studied further to be more fully understood.

Table 3 shows that the distribution of breakpoints in the majority of chromosomes is proportional to the size of each arm. The acrocentric chromosomes (groups D and G) have the greatest deviation from inter-arm proportionality. Since the ratio of breakpoints between p and q arms is based on DNA content in nearly all chromosomes except the acrocentrics, the use of the non-acrocentric chromosomes for biodosimetry would be further justified. However, the observation of a statistically significant increase in breakpoints on the p arms of the acrocentric chromosomes suggests an influence of heterochromatin on the distribution of induced breakpoints. It appears that heterochromatic DNA is repaired far less efficiently in some cases than other DNA sequences thus providing cytogenetic support for preferential DNA repair.

Of the non-acrocentric chromosomes represented in table 3, only chromosomes 1 and 9 showed a significant deviation from a proportional inter-arm distribution of induced breaks based on DNA content. However, the probability associated with the chi-square values in these chromosomes is at least an order of magnitude greater than that found in the acrocentrics. In chromosome 1, this deviation was due to a large number of breaks at the p arm terminus (1p3.6), which has been reported by others (Bauchinger and Gotz 1979, Kano and Little 1986, Barrios *et al.* 1989). It is not known why this region

of chromosome 1 appears to be more sensitive to radiation-induced breakpoint formation, although the presence of oncogenes at this region has been suggested as a possible explanation (Barrios *et al.* 1989). In addition to the p terminus influence, there is also a clustering of breakpoints at the subcentromeric heterochromatic site of chromosome 1 (1q1.2). While this observation does not have a significant effect on the inter-arm distribution of breaks, it does provide support for the role of heterochromatin in the breakpoint distribution.

Chromosome 9 has a clustering of events at its q arm terminus (9q3.4), thus providing more evidence for a preferential location of induced breaks at chromosome telomeres. Chromosome 9 also exhibits a clustering of breaks at its subcentromeric heterochromatin location (9q1.2). While these observations contribute to the non-random distribution of breaks on these chromosomes, other chromosomes also exhibit an increase, albeit statistically non-significant, in breaks at heterochromatic DNA regions. These include the subcentromeric heterochromatin of chromosome 16 (1q1.1) and the heterochromatic portion of the q arm of the Y chromosome (Yq1.2). These observations do not contribute significantly to a non-random distribution of induced breakpoints, although they do provide evidence for the role of chromatin organization on the breakpoint distributions.

In theory, the number of breaks present initially in the chromatin before restitution and/or misrejoining is expected to be proportional to DNA content. However, this is not directly accessible to cytogenetic observation. Therefore, the related question is whether the breakpoint number obtained from quantifying observed chromosome rearrangements is also proportional to DNA content? These are different questions because most breaks are presumably restituted and preferential restitution/misrejoining may bias the results. For example, proximity effects result in a bias for the production of rings over dicentrics (Sachs *et al.* 1997). Also, ring and pericentric inversion frequencies are expected to be proportional to a product of the DNA content of the long and short arms (Savage and Papworth 1982, Hlatky *et al.* 1992). Allocation of breakpoints for all simple, two-break interchanges (i.e. reciprocal translocations and dicentrics) is proportional to the product of the arm lengths involved (Savage and Papworth 1982, Savage 1991). The net result of these discrepancies is that the observed number of breakpoints (e.g. those contributed by the proximity-induced centric rings) may be more quadratic in chromosome content than linear (e.g. Sachs *et al.* 1993). In addition, any particular painting/staining method will reveal only some of the breakpoints (e.g. inversions are very often cryptic). However, since such discrepancies are not substantial and in some cases are unavoidable, the main questions of experimental interest concern the breakpoints observable by analysis of rearrangements. Therefore, only these breakpoints have been considered.

Many of the non-random distributions (both inter- and intra-chromosomal) are undoubtedly influenced by technical aspects. These effects have to be accounted for and minimized to allow for the accurate estimation of genomic breakpoint frequencies when using chromosome subsets. The results of this review indicate that subsets of chromosomes, particularly the non-acrocentric autosomes, can provide accurate estimates of whole genomic breakpoint frequencies. The most appropriate chromosomes for use in cytogenetic analysis for biodosimetry appear to be those that are most metacentric and possess the least amount of heterochromatin. These analyses lend further support for the role of chromatin organization in the breakpoint distribution, although this effect does not eliminate the justification for using chromosome subsets when conducting cytogenetic analyses for biodosimetry.

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