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Reciprocal Translocation and the Philadelphia Chromosome

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Summary. We examined metaphases from three patients with chronic myeloid leukaemia and a typical Philadelphia chromosome with one chromosome 9 as the recipient to determine whether the 9q+ 22q− translocation is reciprocal. Good quality G-banded photographs of the chromosomes concerned were subjected to light absorption density analysis. This provided enlarged tracings corresponding to the relevant chromosome regions and so facilitated accurate measurement. This technique has unambiguously shown that the typical Philadelphia chromosome results from a reciprocal translocation and that probably no material is gained or lost in the exchange. Furthermore, in a total of six patients for whom sequential G and C banding was performed, the chromosome 9 with the largest block of centromeric heterochromatin received the translocated material. We offer tentative explanations for this curious observation.

Introduction

There has been renewed interest regarding the question of whether human chromosome translocations are in fact always reciprocal. Hansmann et al. (1977) presented convincing evidence for reciprocity using a method of selective silver staining of acrocentric chromosomes in two carriers of balanced translocation involving acrocentric chromosomes. They concluded that G banding alone was inadequate to define the exchanged segments.

Other authors have been reluctant to acknowledge that reciprocal translocations exist (Francke, 1972; Shaw, 1972; de la Chapelle and Schröder, 1974), although they used a variety of banding methods.

The Philadelphia (Ph^1) chromosome, associated with chronic myeloid leukaemia (CML) and originally believed to be a deletion (Nowell and Hungerford, 1960), was identified by fluorescence as originating from a chromosome 22 by Caspersson et al. (1970). Rowley (1973) then showed, also by Q banding, that the missing material was actually translocated to the long arm of a chromosome 9.

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The Ph[1] chromosome itself is so small that if material is exchanged, the segment from chromosome 9 must be very short indeed, certainly not large enough to be recognised as a band. With this daunting prospect we used a combination of good quality G banding with densitometry and sequential C banding to evaluate the true nature of the Ph[1] chromosome translocation.

Materials and Methods

Patients. We selected three patients (one male and two female) with a typical Ph[1] chromosome (as described by Watt et al., 1977) and with one chromosome 9 as recipient of the translocated material. All clinical and haematological data were consistent with the diagnosis of chronic myeloid leukaemia (CML).

Cell Culture and Chromosome Preparation. RPMI 1640 (Gibco Biocult Labs Ltd.) supplemented by 20% calf serum was used for all cultures. Bone marrow cells were harvested one hour after aspiration, having been arrested at metaphase by the immediate addition of colcemid (4 µg/ml) on receipt of the sample. Processing closely followed aspiration, since we found that this yielded the best quality and quantity of metaphases. Harvesting was standard, with hypotonic pretreatment in 0.075 M KCl for 10 min followed by several fixations in three parts methanol: one part glacial acetic acid. The preparations were air dried.

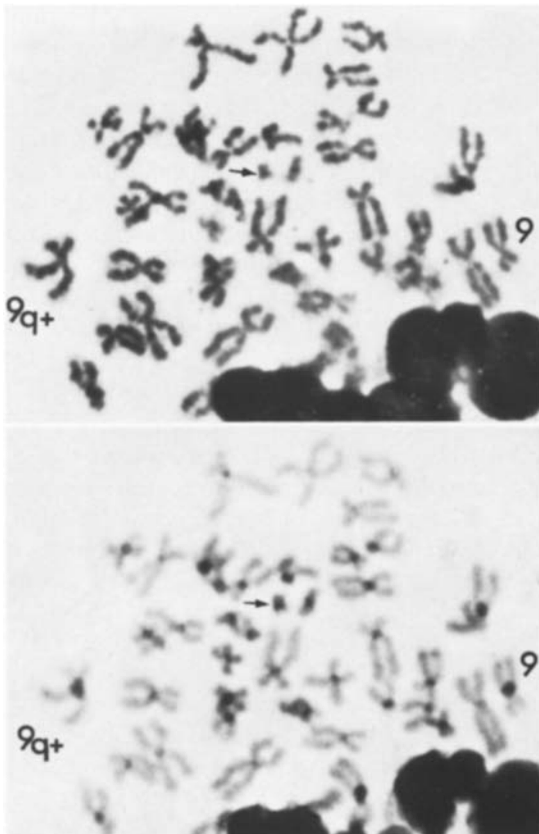

Fig. 1. Sequential G- and C-banded metaphase from J.D. (cell number 12 on Table 1)

For all preparations we used the G-banding method of Stephen (1977), which is much more suitable for bone marrow cells than any other G-banding method tried. It has the added advantage that cells can be successfully destained in alcohol after photographing and sequentially C-banded using a modification of the BSG method of Sumner (1972). This procedure demonstrated that chromosome 9 without any doubt received the material from chromosome 22.

G-banded photographs from all three patients were karyotyped and compared with their C-banded counterparts to ensure correct identification of chromosomes 9 in particular. The four chromosomes: 9, 9q+, 22, and 22q− were scanned on the densitometer (Joyce Loebl Chromoscan). Certain chromosomes were scanned down the centre of a chromatid to give the best definition of bands, because the chromatids are frequently held apart in bone marrow chromosomes (Fig. 1).

Results

Sequential G and C banding has proved invaluable in correctly assigning the translocated material to chromosome 9, especially in cells of less than perfect quality (Fig. 1). We believe this confirmatory procedure is necessary, since in our experience the marker 9q+ sometimes has an altered banding appearance and can be confused with chromosome 5, which is similar in size and banding pattern.

Figure 2 clearly shows that the distance between the midpoint of landmark 9q 31−3 to the telomere in the normal 9 (a^9) is greater than the distance from the midpoint of this landmark to the introduction of band 22q12 on the translocation chromosome 9 (b^9). In this cell, the interchange can be seen without any measurement. Figure 3 shows density scans of the four chromosomes in question in two cells from different patients. The distance a^9 is again clearly greater than b^9, suggesting that a small part of chromosome 9 was removed before the material from chromosome 22 was added. Similarly, by comparing the scan of the Ph^1 chromosome with that of the normal 22, it is clearly seen that the distance b^{22} is greater than the distance a^{22}, again favouring reciprocal translocation. Table 1 shows a series of 19 such measurements. It can be seen that a^9 is always greater than b^9, but that the increment varies between 7.4% and 56.9% of the total distance a^9+b^9, with the mean being 28.4%. Similarly b^{22} is always greater than a^{22}, with the increment varying between 7.2% and 48.4% of the total distance $a^{22}+b^{22}$ and the mean being 33.8%.

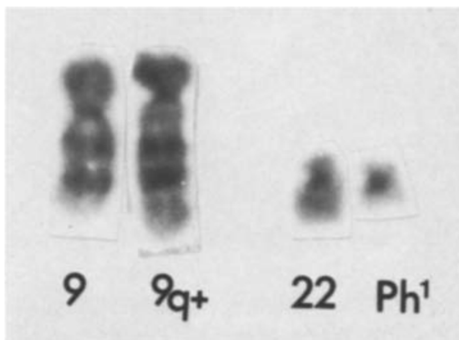

Fig. 2. Partial karyotype from A.D. (cell number 1 on Table 1)

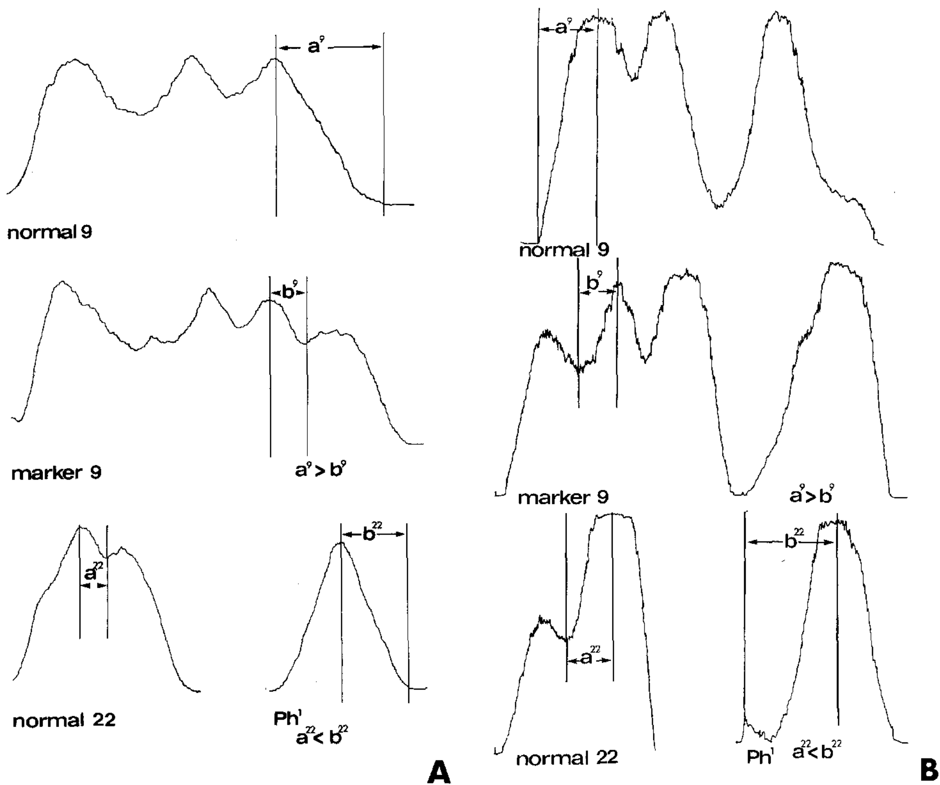

Fig. 3A and B. Densometric traces of chromosomes 9, 9q+, 22, and 22q− from **A** A.D. (cell number 1 on Table 1), scanned p→q; **B** M.G. (cell number 18 on Table 1), scanned q→p

Assuming that no DNA is lost in this translocation, then theoretically the sum of the distances measured in the two normal chromosomes ($a^9 + a^{22}$) would be equal to the sum of the distances measured on the two abnormal chromosomes ($b^9 + b^{22}$). Since there is an even distribution either side of equality, as shown in Table 1, apparently no material is lost or gained.

It is a surprising and perhaps important finding that in all six patients having the 9q+ 22q− translocation that we have examined using sequential G and C banding, the extra material has been exclusively located on the chromosome 9 with the largest block of centromeric heterochromatin.

Discussion

There would seem to be little doubt from our results that formation of the typical Ph[1] chromosome involves interchange of material between chromosomes 9 and 22. That no material is apparently lost or gained in the reciprocal exchange is an important point when an issue such as malignancy is involved.

Table 1

Patient	Cell No.	a^9	b^9	a^{22}	b^{22}	$\frac{a^9 - b^9}{a^9 + b^9}$	$\frac{b^{22} - a^{22}}{b^{22} + a^{22}}$	$(a^9 + a^{22}) - (b^9 + b^{22})$
A.D.	1	63.0	22.0	16.0	39.0	0.482	0.418	+ 18.0
	2	50.5	35.0	20.5	38.5	0.181	0.305	− 2.5
	3	30.0	18.0	—	—	0.250	—	No normal chromosome 22 available
	4	38.0	22.5	20.0	47.0	0.256	0.403	+ 11.5
	5	36.0	17.5	17.0	37.5	0.346	0.376	+ 2.0
	6	40.0	11.0	22.5	26.0	0.569	0.072	+ 25.5
	7	38.0	23.0	20.0	27.0	0.246	0.149	+ 8.0
J.D.	8	45.0	26.0	25.0	48.5	0.268	0.320	− 4.5
	9	36.5	14.0	27.0	38.0	0.445	0.169	+ 11.5
	10	37.0	19.5	20.0	47.0	0.310	0.403	− 9.5
	11	26.5	12.5	18.0	34.0	0.359	0.308	− 2.0
	12	56.0	25.0	17.0	32.0	0.383	0.306	+ 16.0
M.G.	13	32.0	22.5	19.0	43.5	0.174	0.392	− 14.5
	14	27.5	16.0	16.0	38.0	0.264	0.407	− 10.5
	15	29.0	25.0	11.0	31.5	0.074	0.482	− 16.5
	16	34.0	19.0	15.0	29.0	0.283	0.318	+ 1.0
	17	29.0	23.5	13.0	33.5	0.105	0.441	− 15.0
	18	34.5	23.0	27.0	54.0	0.200	0.334	+ 15.0
	19	26.0	17.0	12.0	34.5	0.209	0.484	− 13.5
						Mean 0.284	Mean 0.338	9 +ve 9 −ve

The considerable variation between the proportion by which a^9 and b^{22} exceed b^9 and a^{22}, respectively, likely reflects two unavoidable factors, both probably related to the relative positions of the chromosomes in the metaphase spreads: chromosomes at the periphery tend to be longer (e.g. extention of the Y chromosome described by Unnérus et al., 1967), and the intensity of the staining in the pale bands at the telomeres of the normal 9 and the Ph^1 chromosome can vary. However, the consistent unidirectional difference in the measurements supports our conclusion despite sampling error and the obvious drawbacks from spreading and staining.

The breakpoint concerned in the genesis of the Ph^1 chromosome has already been subject to much debate (Watt et al., 1977). Having in this case shown reciprocal exchange, we must review the situation and consider also the necessary breakpoint on chromosome 9. Figure 4 illustrates two possible sets of events that could result in the observed interchange. 'Possibility 1' infers that chromosome 22 has broken at the 22q11/q12 band interface, so that to fit the final picture, chromosome 9 must have broken within band 9q34. Alternatively, 'Possibility 2'

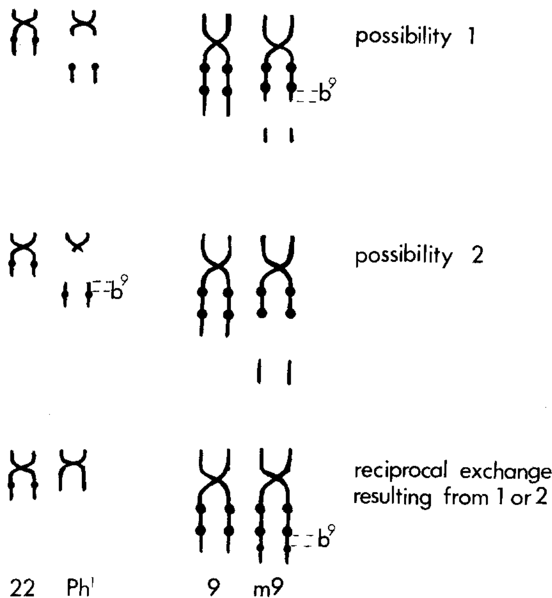

Fig. 4. Diagram illustrating two of the possible sets of breakpoints on chromosomes 9 and 22 that could result in the given reciprocal translocation

shows that chromosome 22 may equally well have broken just below the centromere, and hence chromosome 9, at the 9q33/34 band interface. Thus in the former case, the measured segment b^9 (on the observed marker 9) is derived wholly from the original chromosome 9, while in the latter case, this segment is wholly donated by the original chromosome 22. This second possibility must fulfill two criteria to be realistic: a^{22} cannot exceed b^9, and a^9 cannot exceed b^{22}. From the measurements on Table 1, it can be seen that these two conditions are satisfied, subject to the unavoidable variation. Furthermore, both possibilities illustrated represent the extreme limits where breaks could have occurred; for both chromosomes 9 and 22, one can visualise a spectrum of equally likely breakpoints, making it feasible for the segment b^9 to be composed partly of chromosome 9 material and partly of chromosome 22 material. It would appear then, that although this technique is sensitive enough to detect the reciprocal translocation, it is beyond its resolution to pinpoint the breakpoints. However, a recent paper by Buckton and O'Riordan (1976) implicates reciprocal translocation with R-banding, and their illustration favours the breakpoints illustrated in possibility 2.

Where it is not known whether any translocation is reciprocal, diagrammatic interpretations should be avoided. For example, the complex 9, 11, and 22 translocation in CML reported by Gahrton et al. (1977) may require reevaluation in the light of the information presented here.

The most conclusive evidence supporting the existence of reciprocal translocation and giving information concerning the actual breakpoints is provided by the examination of human male meiotic chromosome preparations. Ferguson-

Smith and Page (1973) examined meiosis in a man with a 10q;11q translocation, which was dubiously reciprocal by G banding. The pachytene quadrivalents, which are four times longer than most meiotic metaphase chromosomes, demonstrated reciprocity by the pattern of homologous pairing giving cross configurations. The intersection of the cross gave a good indication of the breakpoints. In addition, the presence of ring quadrivalents in some of the diakinesis cells provided evidence of crossing over in exchanged segments.

The acquired translocation resulting in the Ph^1 chromosome originates in bone marrow, and is not (as far as we know) present in germ cells, so that meiotic analysis is impossible. In any case, the putative segment from 9 would probably be too small to be resolved by this method since crossing-over is closely related to chromosome length.

More information regarding the breakpoint might be obtained if other translocations resulting in a Ph^1 chromosome were examined, but where chromosomes other than number 9 are involved. These are rarely observed, and in our series of seven patients with a typical Ph^1 chromosome, number 9 has always been implicated.

The significance of the observation that the chromosome 9 with the largest block of centromeric heterochromatin was in this series always the recipient of the translocated material from 22 remains unclear, but it may be a consequence of the fact that the paracentric region of chromosome 9 behaves as nucleolus organiser (Page, 1973). Schmid et al. (1975) confirmed that chromosome 9 associates with the acrocentric chromosomes and further showed that the degree of attraction increases proportionally with the size of the heterochromatic block. With this in mind, it would be of interest to collect data on the association frequency of the Ph^1 chromosome compared with that of the normal 22. From our small sample, we did get the impression that the Ph^1 chromosome associated more than its homologue. Perhaps the Ph^1 chromosome always stems from the chromosome 22 with the largest nucleolus organiser region. Some support for this suggestion was unknowingly given by Gahrton et al. (1974), who described a case where the Ph^1 chromosome arose clonally from the 22 of paternal origin; the 22s were distinguished by the length of the short stalks. They clearly showed that the Ph^1 chromosome originated from the 22 with the longest stalk, and it is now believed that the stalks, as distinct from the satellites, are engaged in nucleolus organization (Goodpasture et al., 1976). It would therefore seem feasible that in formation of the Ph^1 translocation, the chromosome 9 with the largest C band is likely to be engaged in interchange with the chromosome 22 with the longest stalks, since these two chromosomes are often brought close together by their strong mutual attraction, due to their common nucleolus organising role.

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