

HIGH RESOLUTION BREAKPOINTS OF THE PHILADELPHIA TRANSLOCATION IN PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA

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Summary The breakpoints of the Philadelphia (Ph) translocation were studied precisely with high resolution chromosome banding in 19 patients with chronic myelogenous leukemia (CML). The cultures were treated with ethidium bromide to obtain elongated and finely banded chromosomes. In five cases chromosomes were examined at 850 bands/haploid set of chromosome stage and the Ph breakpoints were specified on 9q34.11 band and 22q11.21 band. The remaining 14 cases revealed the breakpoints on bands 9q34.1 and 22q11.2 at 550-650 band stage of mitosis. The data indicate that the Ph breakpoints are identical in all the 19 cases of CML.

INTRODUCTION

Recent technological advances in cytogenetics and molecular biology showed that the Philadelphia (Ph) chromosome (Nowell and Hungerford, 1960) is the result of a reciprocal translocation between one chromosome #9 and one chromosome #22 in most cases (standard translocation) (Rowley, 1973; Groffen *et al.*, 1983), but in the remaining cases the Ph translocation involves chromosomes #9, #22, and at least one other chromosome (complex translocation) (Hagemeyer *et al.*, 1984; Mitelman, 1985). Whether the Ph translocation is the standard type or the complex type, these rearrangements consistently result in a translocation of a chromosomal segment from chromosome #9 to #22 (Hagemeyer *et al.*, 1984). In association with this translocation one of proto-oncogenes, *c-abl*, is translocated from chromosome #9 to #22 (Klein *et al.*, 1982; Hagemeyer *et al.*, 1984). Interestingly, several investigators have shown the variety in the size of the Ph chromosome by banding chromosome analysis (Watt *et al.*, 1977; Verma and Dosik, 1980), which means the breakpoint

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may differ among different cases. We attempted to specify the breakpoints of the Ph translocation with elongated and finely banded chromosomes.

MATERIALS AND METHODS

Bone marrow or peripheral blood cells were obtained from 19 patients with chronic myelogenous leukemia (CML). The cells were cultured in RPMI 1640 medium supplemented with 15% fetal bovine serum for 24 hr at 37°C without mitogen. To obtain elongated and finely banded chromosomes, ethidium bromide was added to the culture for the last two hours at a final concentration of 10 µg/ml and Colcemid was added for 15 min before harvesting the cells (0.05 µg/ml) (Misawa *et al.*, 1986). Chromosome preparations were made by air drying method and G-banding was performed. Chromosome identification and karyotype designation were done according to the International System for Human Cytogenetic Nomenclature (1985).

RESULTS

All patients showed a standard or a complex Ph translocation (Table 1). The complex translocations seen in these patients were t(9;22;15) (q34.1;q11.2;q22.1) in

Table 1. High resolution breakpoints of Ph translocation in patients with chronic myelogenous leukemia.

Case No.	Name	Age	Sex	Mitotic stage, bands/haploid	Breakpoints ^a of chromosome 9 & 22
1 ^b	M. K.	47	M	550	9q34.1/22q11.2
2	K. K.	30	F	550	9q34.1/22q11.2
3	Y. H.	56	F	550	9q34.1/22q11.2
4	T. K.	62	F	550	9q34.1/22q11.2
5	T. M.	44	M	650	9q34.1/22q11.2
6 ^b	Y. T.	31	F	550	9q34.1/22q11.2
7	A. K.	62	F	850	9q34.11/22q11.21
8 ^b	M. M.	55	F	550	9q34.1/22q11.2
9	T. M.	55	M	550	9q34.1/22q11.2
10	C. T.	49	F	550	9q34.1/22q11.2
11	K. O.	58	M	850	9q34.11/22q11.21
12	M. Y.	49	F	650	9q34.1/22q11.2
13	M. H.	47	M	650	9q34.1/22q11.2
14	I. I.	74	M	550	9q34.1/22q11.21
15	S. S.	29	M	850	9q34.11/22q11.21
16	K. M.	42	M	850	9q34.11/22q11.21
17	T. O.	44	M	550	9q34.1/22q11.2
18 ^b	M. C.	27	F	550	9q34.1/22q11.2
19	M. M.	42	F	850	9q34.11/22q11.21

^a Band designation is done according to ISCN (1985). ^b Complex Ph translocation; details are described in text.

case 1, $t(4;9;22)$ (q33;q34.1;q11.2) in case 6, $t(9;22;12)$ (q34.1;q11.2;q24.1) in case 8, and $t(9;22;12)$ (q34.1;q11.2;p13.1) in case 18.

Among 19 patients examined, 14 patients were analyzed at early metaphase stage of mitosis (approximately 550–650 bands per haploid set of chromosomes) (Yunis, 1981). The breakpoints of the Ph translocation were found to be 9q34.1 and 22q11.2 on chromosome #9 and #22, respectively. In five cases the breakpoints were specified with prometaphase cells (approximately 850 bands per haploid set) (Fig. 1). The chromosome segment translocated from the terminal portion of chromosome #9 to chromosome #22 showed two dark bands: They were 9q34.12 proximally and 9q34.2 distally. Whereas, the segment translocated from the Ph chromosome to the 9q+ chromosome showed two moderately dark bands proximally (22q12.1 and 22q12.3) and two narrow bands distally (22q13.2 and 22q13.32). The band 22q11.22 which is a thin and weakly positive band and can originally be seen close to the band q12.1, could not be recognized on the Ph chromosome but seemed

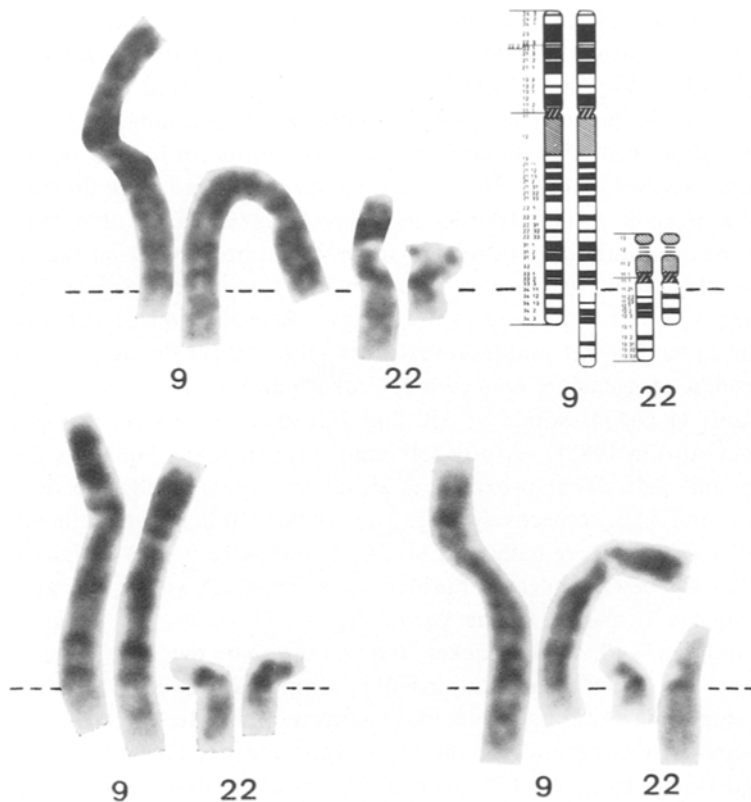


Fig. 1. Partial karyotypes of chromosomes #9 and #22 from case 11 (top), case 19 (bottom left), and case 16 (bottom right). Diagram and band designation are quoted from ISCN (1985).

to be present on the 9q+ chromosome distal to the pale region where rejoining occurred. In addition, the dark band which is seen very close to 22q11.1 band on the long arm of the Ph chromosome was well corresponded to 9q34.12 band in its staining darkness and width. Thus, the breakpoint of the Ph chromosome was assigned to 22q11.21.

DISCUSSION

The breakpoints of the Ph translocation were identical in all the 19 cases of Ph-positive CML. The breakpoints were 9q34.1 and 22q11.2 with early metaphase cells and 9q34.11 and 22q11.21 at prometaphase stage. Previous studies in which the Ph translocations were studied with high resolution banding of methotrexate synchronization culture showed that the breakpoints were the same as we identified in this study (Prakash and Yunis, 1984; Mohamed *et al.*, 1986). These results indicate that the breakage and reunion of the Ph translocation occur at identical bands in all patients regardless the Ph is derived from a standard or a complex translocation.

Several investigators reported the variety in the size of the Ph chromosome (Watt *et al.*, 1977; Verma and Dosik, 1980). They suggested that the breakpoint may not be specific but can happen anywhere on chromosome #22. Verma and Dosik (1980) described four different types of the Ph according to the size; small, average, large, and very large. However, their very large and large Ph chromosomes were derived from a variant translocation involving #22 and other chromosomes than #9. These findings are not incompatible with a breakpoint on the 22q11 band because the Ph may be involved in a further complex rearrangement and becomes actually larger in size (Sandberg *et al.*, 1985). Reevaluation of these cases using high resolution banding techniques revealed that these larger Ph are a form of masked Ph chromosome (Hagemeyer *et al.*, 1984; Prakash and Yunis, 1984).

Diagrams of chromosomes at 550 and 850 band stage were presented by the ISCN (1981) (also in 1985). At the 550 band stage the band 9q34 has three bands, pale, dark, and pale, from proximal to distal and these bands are designated as 9q34.1, 34.2, and 34.3, respectively (Fig. 2). At the 850 band stage the 9q34.1 band is further divided into three bands, 34.11, 34.12, and 34.13. This designation system indicates that the newly appeared positive band (9q34.12) will be recognized within the band 9q34.1, proximal to the 9q34.2 band. However, the proximal Giemsa positive band, 9q34.12, looks thicker and darker than the distal one, 9q34.2, as shown in Fig. 2. When a chromosome #9 is compared between 550 band stage and 850 band stage, the proximal band looks corresponding to the 9q34.2. Thus the distal thin and pale band must be the newly appeared one so that this band should be localized in the band 9q34.3. Therefore, when the designation system recommended by the ISCN is applied to, these five bands which can be recognized at 850 band stage have to be designated as 9q34.1, 34.2, 34.31, 34.32, and 34.33, respectively, as it has already been proposed by Yunis (1980).

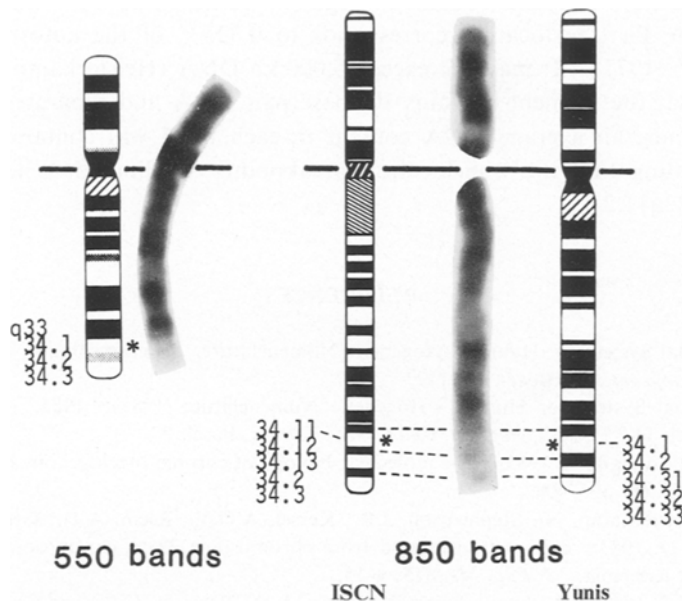


Fig. 2. Chromosome #9 at 550 band stage and at 850 band stage. Diagrams of 550 bands and 850 bands of the left side are quoted from ISCN (1985). Diagram of 850 bands on the right is proposed by Yunis (1980). Asterisks indicate the pale band where breakage and reunion occur on 9q+ chromosome.

These two different interpretations of the chromosome bands arise a problem in specifying the breakpoints of the Ph translocation because the breakpoint on chromosome #9 exists in the most proximal pale band in the band 9q34 (Fig. 2). This band is designated as 9q34.11 according to the ISCN but is 9q34.1 by a proposal of Yunis and the latter seems correct as discussed above. The other breakpoint of the Ph translocation is 22q11.21 at 850 band stage and 22q11.2 at 550 band stage.

Recent studies using molecular biology techniques demonstrated that the exact breakpoint is not point-specific and is not always an identical site. The breakpoints on chromosome #22 are clustered within an approximately 5.8-kilobase-pair region termed breakpoint cluster region or bcr (Groffen *et al.*, 1984). Moreover, the breakpoint on chromosome #9 exists in a relatively large but limited region and the break occurs over a minimum range of 50–100 kb (Heisterkamp *et al.*, 1983; Gale and Cannani, 1985).

A human nuclear DNA was estimated to contain approximately 3.3×10^9 nucleotides. Even when chromosomes are analyzed at prometaphase stage (approximately 850 bands per haploid), each chromosome band still contains an average of 2×10^6 base pairs (1–5,000 kb of DNA) (Gale and Cannani, 1985). Another esti-

mation suggested that the chromosome segment translocated from chromosome #9 to #22 in the Ph translocation corresponds to 0.325% of the autosomal genome (Hayall *et al.*, 1977). It may not exceed 5,000 kb DNA (Heisterkamp *et al.*, 1983). Assuming that the segment contains 10^7 base pair DNA and separates into 5 dark and pale bands, an average DNA content in each band will contain 2×10^6 base pairs, suggesting that the translocation breakpoints are limited to a single band 9q34.1 and 22q11.21.

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