Clonal Origin of the Philadelphia Chromosome
From Either the Paternal or the Maternal Chromosome Number 22

By G. Gahrton, J. Lindsten, and L. Zech

The heteromorphic regions of chromosome 22 were studied with the quinacrine mustard fluorescence technique in eight patients with Philadelphia chromosome-positive chronic myelocytic leukemia and in their parents. The fluorescence pattern showed that the Philadelphia chromosome had originated from the paternal chromosome 22 in one case and the maternal in another. The other six cases were non-informative in this respect. The fluorescence pattern was consistent between cells in both the informative cases. These results speak in favor of a clonal origin of the Philadelphia chromosome from either the paternal or the maternal chromosome 22.

In a recent report it was shown that the maternal chromosome 22 had been transformed into the Philadelphia chromosome in a woman with chronic myelocytic leukemia. The tracing was achieved by analyzing the heteromorphic regions of chromosome 22 with the quinacrine mustard fluorescence technique. The Philadelphia chromosome, previously identified as a number 22 chromosome [most probably a translocation between the long arm of this chromosome and the long arm of number 9/t9q+, 22q-/(5)], sometimes demonstrates a heteromorphic fluorescence pattern of the satellite region. Fluorescent satellites may therefore appear in only one, both, or neither of the two homologs, but within the same individual the pattern seems to be relatively constant. The satellites can therefore be used as markers to trace the origin of the chromosome. The present work demonstrates that either the paternal or the maternal chromosome 22 can be transformed into the Philadelphia chromosome.

MATERIAL AND METHODS

Eight patients with Philadelphia-positive chronic myelocytic leukemia and their parents were investigated. Seven patients were untreated at the time of the chromosome investigation; the eighth had received busulfan for 2 wk prior to the marrow sampling but was not in remission. All the parents were hematologically normal. Bone marrow and blood specimens were used in all the

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patients as well as in two pairs of parents, while only blood specimens were used in the other parents. Chromosome preparations from bone marrow specimens were made directly on fresh aspirates as described previously and from conventional peripheral blood cell cultures, using phytohemagglutinin and 72 hr of incubation.

The quinacrine mustard (QM) staining was performed as follows. The slides were transferred from absolute ethanol through alcohol steps and buffer (Maellvin's disodium phosphate/citric acid buffer, pH 7.0) into the staining solution. QM dihydrochloride in aqueous solution was added to the buffer to give a final concentration of 50 μg/ml. After staining for 20 min at 20°C, the slides were washed three times in buffer and sealed with a cover slip in buffer. Metaphases were selected and photographed in a fluorescence microscope, and the negatives were analyzed, using a TV set for contrast enhancement.

RESULTS

All eight patients demonstrated a presumptive 9/22 translocation (9q+, 22q-) in their bone marrow cells (Fig. 1B). Two of the patients had weakly fluorescent satellites on the Philadelphia chromosome and no fluorescent satellites on the normal chromosome 22. No fluorescent satellites were detected on either of these chromosomes in the remaining six patients.

Family One (Fig. 2A)

(Previously reported.) All ten metaphases analyzed had a weakly fluorescent satellite on the Philadelphia chromosome and no satellite on the normal number 22 chromosome. The mother had a similarly weakly fluorescent satellite of identical morphology on one of her number 22 chromosomes and no satellite on the homologous chromosome. The father had no fluorescent satellites on any of his chromosomes 22.

Fig. 1. Chromosomes 9, 11, 12, and 22 from (A) a PHA-stimulated blood cell, and (B) a bone marrow cell from a patient (PB) with chronic myelocytic leukemia. The Ph1 chromosome (22) as well as the weakly fluorescent extra chromosomal material on the long arm of one chromosome 9 (arrow) are demonstrated in the bone marrow cell. No extra fluorescent chromosomal material from chromosome 9 is found in the Ph1-negative blood cell. Chromosomes 11 and 12, with normal weakly fluorescent tips of the long arms, are shown for comparison.
Fig. 2. Chromosomes 22 from two CML patients and their parents. The Ph$^+$ chromosome of patient A has a fluorescent satellite on the short arm similar to the satellites on one of the mother’s chromosomes 22. The Ph$^+$ chromosome of patient B has a satellite on its short arm, which has a similar fluorescence pattern to the satellites of one of the father’s chromosomes 22. Quinacrine mustard stain. (A) MA, Mother of patient A; FA, Father of patient A; PA, Patient A. (B) MB, Mother of patient B; FB, Father of patient B; PB, Patient B.

**Family Two (Fig. 2B)**

The patient had a weakly fluorescent satellite on the Philadelphia chromosome. The satellite was elongated and of a characteristic morphology. The normal number 22 chromosome had a somewhat more intensively fluorescent distal part of the short arm and a very weakly fluorescent short satellite, clearly different from the satellite on the Philadelphia chromosome (Fig. 1B). This fluorescence pattern was the same in all 15 metaphases analyzed. The father had weakly fluorescent satellites on both chromosomes 22. However, the satellite on one of these chromosomes was more elongated than on the other and had the same morphologic characteristics as the one on the Philadelphia chromosome. The mother had no fluorescent satellites on one of her number 22 chromosomes and a very weakly fluorescent short satellite on a heavily fluorescent short arm in the other. The morphology of this chromosome was the same as that of the normal chromosome 22 in the patient.

**DISCUSSION**

The data presented in the present report show that the Philadelphia chromosome can be acquired either from the maternal or the paternal chromosome 22 in patients with chronic myelocytic leukemia. So far, it appears that only one of the chromosomes 22 acquires the Philadelphia chromosome abnormality, i.e., either the one received from the mother, as in family one, or the one received from the father, as in family two. The finding that the Philadelphia chromosome abnormality was always in the same chromosome in several metaphases, is in accordance with the view that the Ph$^+$ positive cell population in chronic myelocytic leukemia is of clonal origin. However, there are other possibilities. If there is a predisposing factor in the parental chromosome, an exogenous stimulus or agent may give rise to a Ph$^+$ positive cell population by affecting either only one (unicellular or clonal) or several (multicellular) bone marrow stem cells. The present data suggest that if such a predisposing factor exists, it can be transferred via either sex.
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