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Cytogenetic Studies in Dupuytren Contracture

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Summary

Dupuytren contracture is a connective tissue disease mainly confined to Caucasians. It is characterized by nodular growth and proliferation of collagen in the palmar and plantar fascias. Autosomal dominance with variable penetrance is considered the most likely mode of inheritance. The goal of the present study was to examine the cytogenetics of this common benign neoplasia. Chromosome studies were performed on the nodular growth of eight patients with Dupuytren contracture, all of whom showed chromosome abnormalities that included numerical and structural clones, random numerical and structural aberrations, prophasing, and premature centromere separation. Numerical clones of trisomies 7 and/or 8, as well as some random structural aberrations, were considered to represent in vivo abnormalities, whereas most structural clones appeared likely to be the results of rapid and selective in vitro growth of particular cells. The disease process occurring in Dupuytren contracture was found to involve marked chromosome instability, as well as some in vivo clonal formation. Transverse fascial tissue, usually considered to be uninvolved in the disease process, unexpectedly showed all the same types of abnormalities as the nodular tissue. This indicates a more widespread distribution of disease in the tissues than previously suspected. The findings in the present study are similar to those in various malignant and benign types of tumorous growth and suggest the importance of further cytogenetic investigation into other conditions of benign growth.

Introduction

Dupuytren contracture is a connective tissue disease, mainly confined to Caucasians and most prevalent in individuals of northern European extraction (Hueston 1982). It is sometimes familial and is considered by Hunter et al. (1975) as perhaps the most common heritable disorder of connective tissue. More than 25% of the male population over 60 years old in Celtic races are said to have Dupuytren contracture (Hueston 1982). This disorder is classified by the World Health Organization as fibromatosis and is histologically similar to fibrosarcoma. Autosomal dominance with variable penetrance is considered the most likely mode of inheritance (McKusick 1986, p. 205). Women develop Dupuytren contracture at least half as often as men, but it is poorly expressed in females. Recurrence and/or extension is common in young patients with a strong diathesis. There is anecdoctal association with alcoholism, chronic pulmonary disease, diabetes, epilepsy, and mechanical injury or stress, although in one study no correlation was found between these conditions and bilateral incidence of the disease (Gelberman et al. 1980).

The disease typically involves the palmar and, less commonly, the plantar fascias. It evolves from an undifferentiated fibroplasia to well-organized fibroplasia and collagen bands and eventually to sizable nodules and thick wavy bundles of collagen fibers with contracture of the digits. Biochemical changes in the palmar fascia include increased collagen and hexosamine contents and the presence of galactosamine in the most severely involved tissue. Type III collagen, which is absent from normal adult palmar fascia, is abundant in this tissue of patients with Dupuytren contracture (Brickley-Parsons et al. 1981). Although these changes are greatest in the nodule, they are also present in the

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cord tissue and in the adjacent, apparently uninvolved grossly and histologically normal fascias. This indicates widespread distribution of disease in the palmar aponeurosis (Brickley-Parsons et al. 1981). Histologic and biochemical changes suggest an uncontrolled cellular proliferation, similar to fibrosarcoma.

Previous cytogenetic studies showed acquired abnormalities in the palmar fascia in four of six patients (Bowser-Riley et al. 1975) and in eight of 26 patients (Sergovich et al. 1983). In the earlier study three of the four had structural abnormalities, whereas in the later study six of the eight had numerical abnormalities. The latter study included one patient with trisomy 7 (+7) and six patients with trisomy 8 (+8). Our goal in this preliminary study was to examine the cytogenetics of this common benign neoplasia.

Material and Methods

Eight patients referred for surgery were studied without preselection. There were two women and six men aged 23 to 81 years. The clinical and familial characteristics are summarized in table 1. Of interest, the 23-year-old male had fascial thickening of a year's duration associated with previous injury. The family history was positive for alcoholism in three patients, cancer in four, and Dupuytren contracture in three. One patient (B5507) reported excessive alcohol intake. Nodular tissue was cultured from each patient. Self-controls were represented by normal skin biopsies from two patients, transverse palmar fascia (not usually considered to be involved in this disease process) taken 1 cm distant from the nodule in two patients, and transverse palmar fascia taken 5 cm distant from the nodule in five patients. The skin biopsy from patient D5390 failed to grow, and control was provided by a parallel fibroblast culture grown simultaneously and in the same batch of medium. A similar parallel control culture was analyzed as a secondary control for patient B5507 (table 2). All tissues were cultured in McCoy's 5A modified medium supplemented with 20% fetal bovine serum and antibiotics. Chromosome preparations were made as soon as possible using 0.4% KCl hypotonic for 15 min followed by three washes in 3:1 methanol:acetic acid fixative. G-banding was accomplished with the trypsin technique of Seabright (1971), using Giemsa instead of Leishmann's stain.

Results

The results are summarized in table 2 and the ideogram (fig. 1). For purposes of the present study, a hyperdiploid clone was defined as two or more cells having the same additional chromosome whereas clonal hypodiploidy required loss of the same chromosome from three or more cells. The incidence of random chromosome abnormalities in long-term cultures in this laboratory is 3.5%. Control cultures from skin biopsies grew more slowly than the fascial tissue, and nodular tissue generally grew the most rapidly. All tissues, except the normal skin biopsies, had chromosome abnormalities. One exception was the skin culture from patient B5507, which grew so slowly that it was in culture longer than any other tissue. In this specimen, a clone of t(5;15) accounted for the 23 cells with a structural abnormality (table 2), and the two numerically abnormal cells had trisomy 2. The secondary control fibroblast culture for B5507 showed no abnormalities. Three patients had clones of +7, and another had one cell with +7. Four patients had clones of +8, and an-

Table 1

Clinical and Familial Characteristics

Patient (Sex)	Age (years)	Age at Onset (years)	Duration before Surgery (years)	Familial History			Manual Labor
				Dupuytren	Alcoholism	Cancer	
1426-86 (F)	53	50	3	+	+	+	+
M4719 (F)	70	59	11	–	–	–	–
C4741 (M)	81	66	15	–	–	+	–
V5195 (M)	56	46	10	–	+	+	+
D5239 (M)	23	22	1	–	–	–	+
B5269 (M)	52	43	8	+	–	–	+
D5390 (M)	39	30	9	+	+	+	–
B5507 (M)	65	60	5	–	–	–	+

Table 2

Summary of Findings

Patient	Days in Vitro	No. of Cells Counted/Analyzed	No. of Cells Abnormal	Type Abnormality[a]		
				Numerical	Structural	Other
1426-86:						
Nodule	14(1)[b]	42/27	11		6	5
Nodule	25(1)	27/20	3			
Control[c]	ND					
Skin	41(2)	65/42	0			
M4719:						
Nodule	46(3)	65/48	9	5	4	
Control	43(2)	65/48	6	3	3	
C4741:						
Nodule	25(1)	65/48	16	9	7	
Control	34(1)	65/47	13	5	7	1
V5195:						
Nodule	19(1)	67/52	10	2		8
Nodule	49(3)	60/44	5		5	
Control	49(2)	60/43	2	2		
D5239:						
Nodule	32(2)	64/49	5		1	4
Control	43(4)	61/40	3	1		2
B5269:						
Nodule	29(3)	51/41	9	2	9	8
Nodule	41(2)	96/60	7		4	3
Control	41(4)	64/41	5		4	1
D5390:						
Nodule	46(4)	54/54	5		5	
Nodule	48(3)	49/48	2		2	
Nodule	49(4)	52/52	3	2	1	
Control	ND					
Skin[d]	39(2)	50/50	0			
B5507:						
Nodule	52(4)	52/50	15	2	12	1
Nodule	63(5)	51/51	2		2	
Control	52(4)	51/51	6	3	3	
Skin	68(4)	47/47	25	2	23	
Skin[d]	33(3)	50/50	0			

NOTE.—ND = not done.

[a] Other types of abnormalities include prophasing and PCS.

[b] Numbers in parentheses are number of subcultures before harvest.

[c] Control tissue is grossly normal transverse palmar fascia.

[d] Skin from non-Dupuytren patient cultured in parallel in same medium.

other had one cell with +8. One patient had three separate numerical clones: +7; +8; and +7,+13,–15. The only other numerical clone was +10 in one patient. Structural aberrations were varied and mostly random (fig. 2), but some were clonal. With one exception, structural clones were peculiar to each patient. A translocation between the long arms of chromosomes 7 and 20 [t(7;20)(q22;q13.3)] was observed as a clone in one patient (fig. 3), and a clone interpreted as the same translocation was seen in another patient. Prophasing, al-

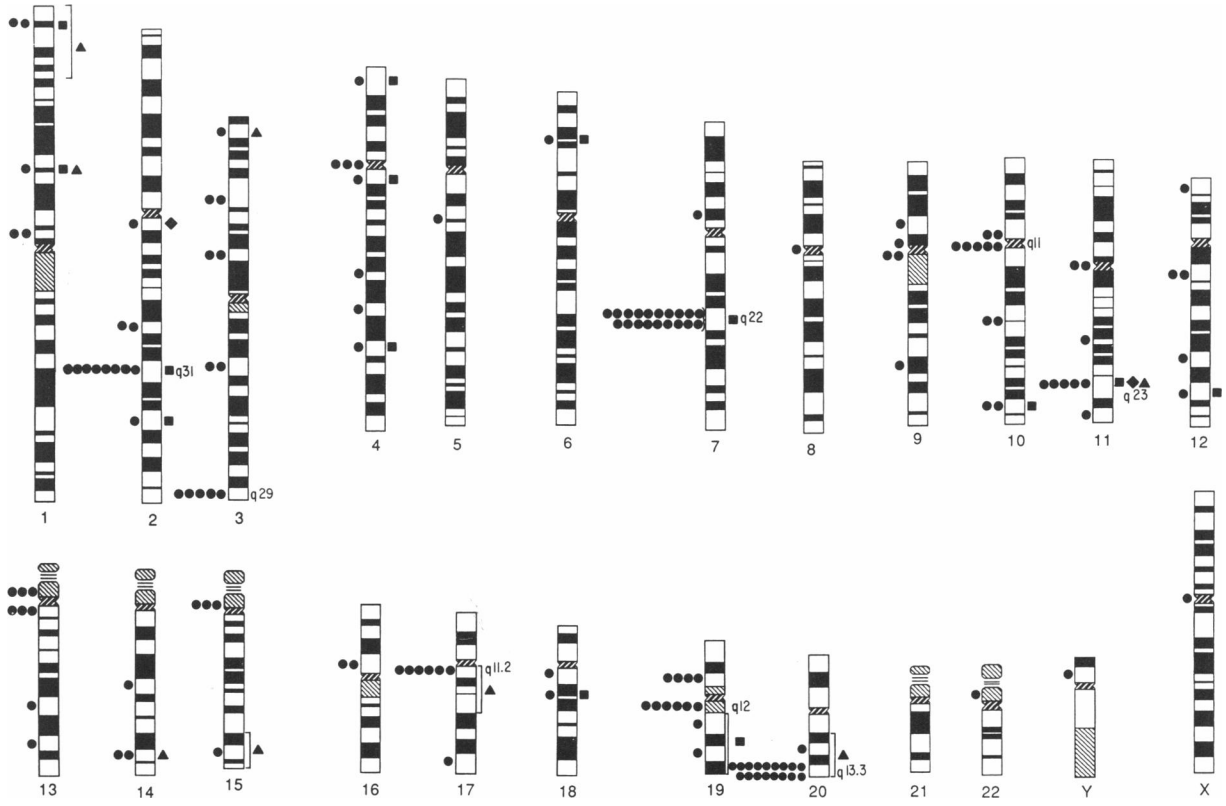

Figure 1 Total breakpoints (●), to the left of each chromosome, observed in 897 cells analyzed from nodular and transverse fascial tissues of eight patients. Locations of common (■) and rare (◆) fragile sites and oncogenes (▲) are shown to the right of each chromosome. Ideogram figures are from Adler and Willis (1987).

ternatively known as premature chromosome condensation (PCC) (fig. 4) was seen in only two patients, but premature centromere separation (PCS) was fairly common. Breakpoints were observed at locations of 14 common and two rare fragile sites and in the vicinity of eight oncogenes (fig. 1) as listed by the Eighth International Human Gene Mapping Workshop, 1985 (Berger et al. 1985). Patients with familial Dupuytren contracture displayed types of cytogenetic abnormalities similar to those of the rest of the group.

Discussion

The cytogenetics of hematological disorders have been widely studied, and an abundance of data on solid tumors is now being amassed. There has been, in contrast, very little cytogenetic investigation of benign neoplasias. While Dupuytren nodules are not thought of as tumors, they do represent a benign neoplastic growth. Although benign tumors are generally believed to be cytogenetically normal, nonrandom abnormalities have been described in meningiomas, mixed salivary-gland tumors, large-bowel adenomas, and lipomas (Sandberg 1983, pp. 459–462; Mark and Dahlenfors 1986).

Structurally abnormal clones in Dupuytren tissues were unique to each culture, with the possible exception of t(7;20), believed to have occurred in two patients, indicating probable in vitro clonal development of random abnormalities. Cells with an extra chromosome 7 or 8 appeared in more than one culture from some patients, indicating probable in vivo origin. The observation of +8 cells in five of our eight patients confirms the finding of Sergovich et al. (1983) that this is a frequent aberration associated with Dupuytren contracture, as well as with other neoplasias. Thus it appears that, analogous to the situation described in benign pleomorphic adenomas (Mark and Dahlenfors 1986), the fascial tissue in Dupuytren contracture suffers DNA damage and misrepair in the growth process manifested as pronounced chromosome instability lead-

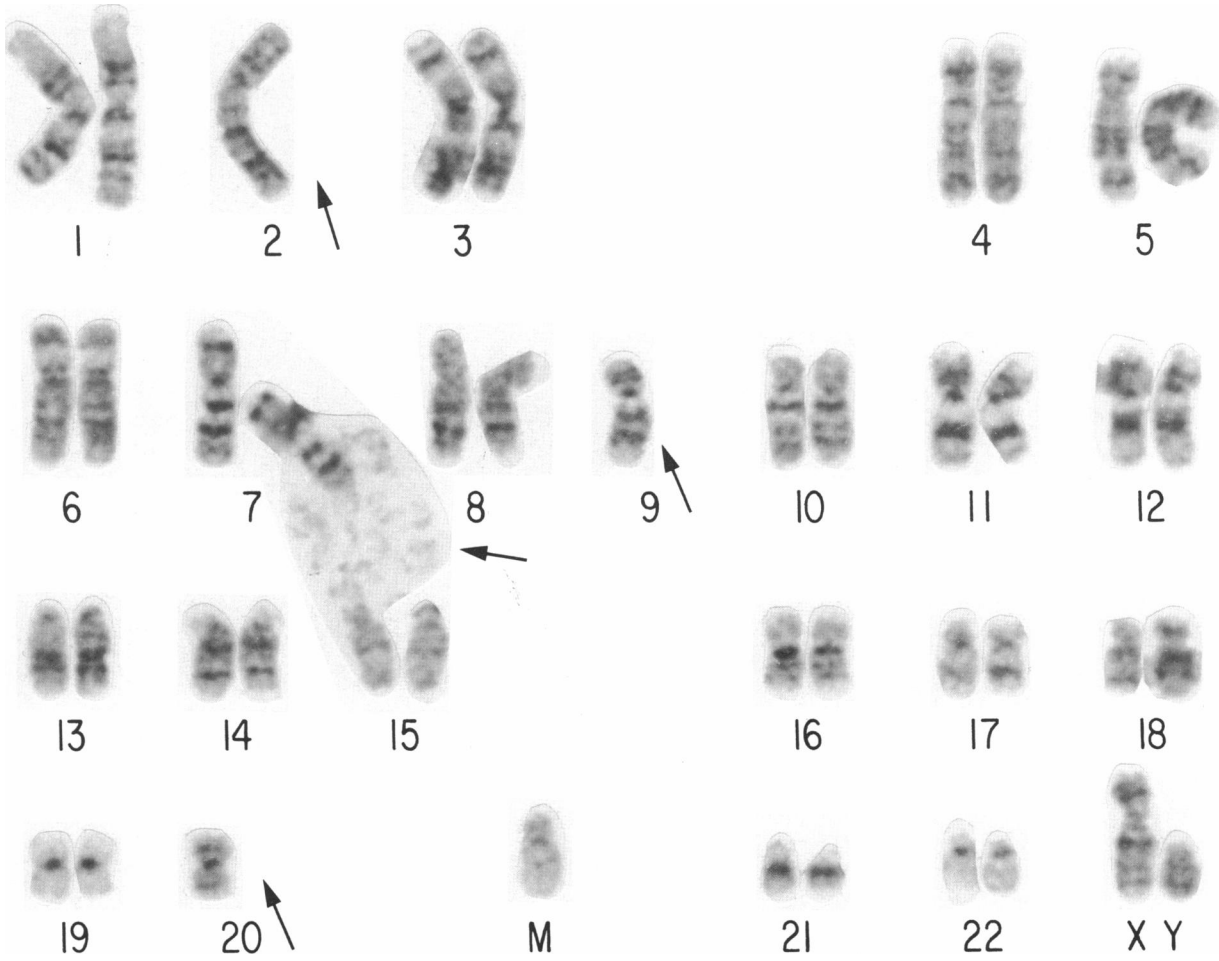

Figure 2 Karyotype of patient V5195 representative of random aberrations: 44,XY,-2,-9,-20,+mar,pulver.

ing to many variant cells. An additional similarity was the prevalence of +8 and +7 as numerical abnormalities.

Other observations of interest and possible significance were breakpoints in the region of the procollagen ∂1 (III) gene (2q24.3–2q31) (Emanuel 1985) in four patients; breakpoints at 17q11, a candidate for the location of the neurofibromatosis gene (Barker et al. 1987; Schmidt et al. 1987), in two patients; and deletion 3p, with breakpoints 3p14 to 3p22, which has been reported in various neoplasias and was seen as a random occurrence in three of our patients (fig. 3).

It is important to note that abnormalities were found in all specimens taken from the grossly normal transverse palmar fascia that is generally considered not to be involved in the Dupuytren disease process. It was originally thought that this tissue would serve as an appropriate self-control, and it is listed in table 2 as "Control." Our findings make it clear, however, that the transverse fascia is also affected by the disease process, whereas the skin fibroblasts are not.

The abnormalities seen in the skin culture of patient B5507 are considered most likely to represent in vitro clone formation fostered by the long in vitro time and to reflect an inherent tendency toward chromosome instability. Interpretation in this patient is confounded by his history of excessive alcohol consumption. Active alcoholics have been reported to have increased chromatid-type aberrations in peripheral lymphocytes, which disappear during abstinence. Recovering (dry) alcoholics, however, have an increased frequency of stable, chromosome-type aberrations (Obe et al. 1980). The effects of alcohol consumption on fibroblasts in humans have not been investigated.

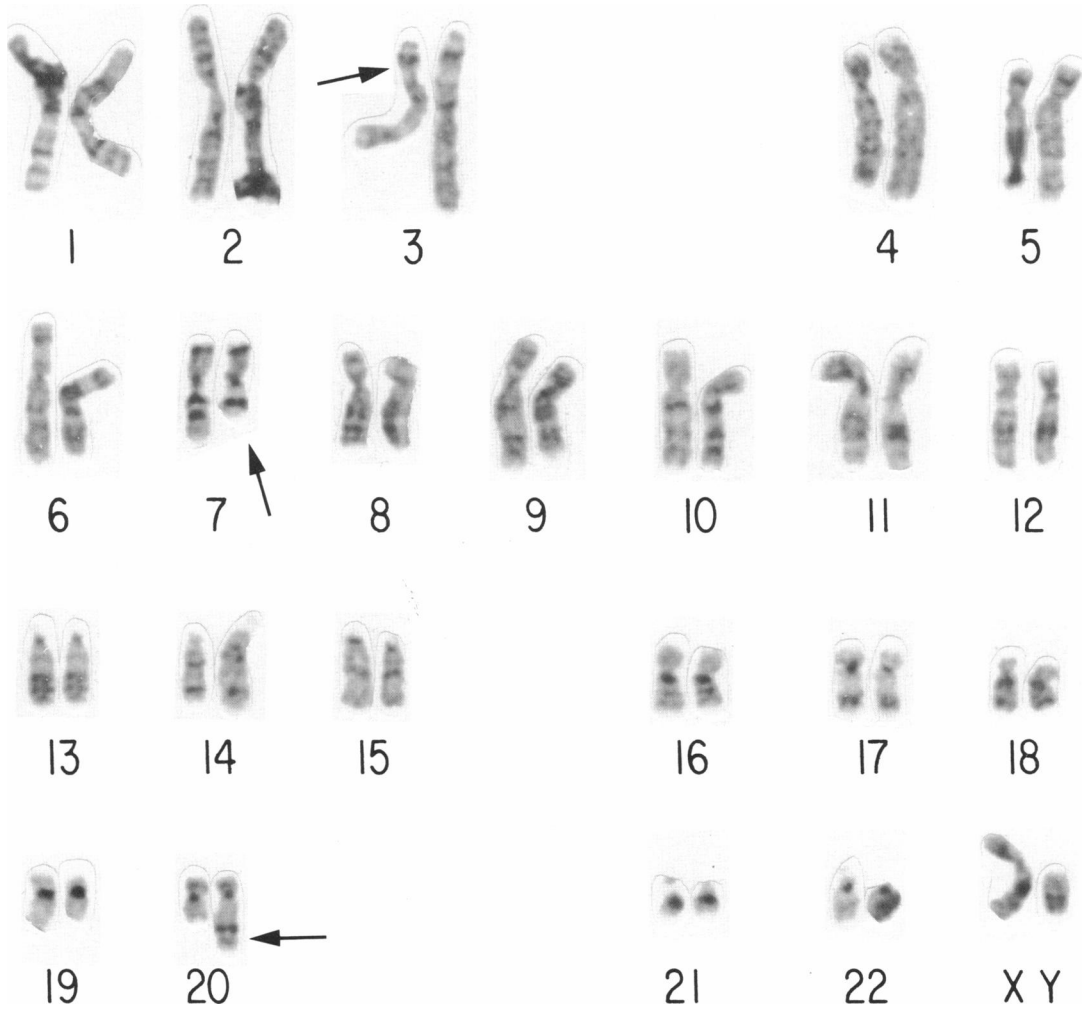

Figure 3 Karyotype of patient B5507 showing interstitial deletion of the short arm of chromosome 3 and a clonal translocation between chromosomes 7 and 20: 46,XY,del(3)(p21p23),t(7;20)(q22;q13.3).

In conclusion, we found that nodular tissue from all Dupuytren patients showed chromosome abnormalities that included numerical and structural clones, random numerical and structural aberrations, prophasing, and PCS. Numerical clones of +7 and +8, as well as some random structural aberrations, were considered to represent in vivo abnormalities, whereas most structural clones appeared likely to have resulted from proliferation of particular cells with a selective growth advantage in the in vitro environment. The Dupuytren disease process was found to involve marked chromosome instability, as well as some in vivo clonal formation. Transverse fascial tissue unexpectedly showed all the same types of aberrations as the nodular tissue. This indicates a more widespread distribution of disease in the tissues than previously suspected and supports the findings of Brickley-Parsons et al. (1981) of biochemical changes in grossly and histologically normal fascia. The findings in the present study are similar to those in various malignant and benign types of tumors and suggest the importance of further cytogenetic investigation into other conditions of benign growth.

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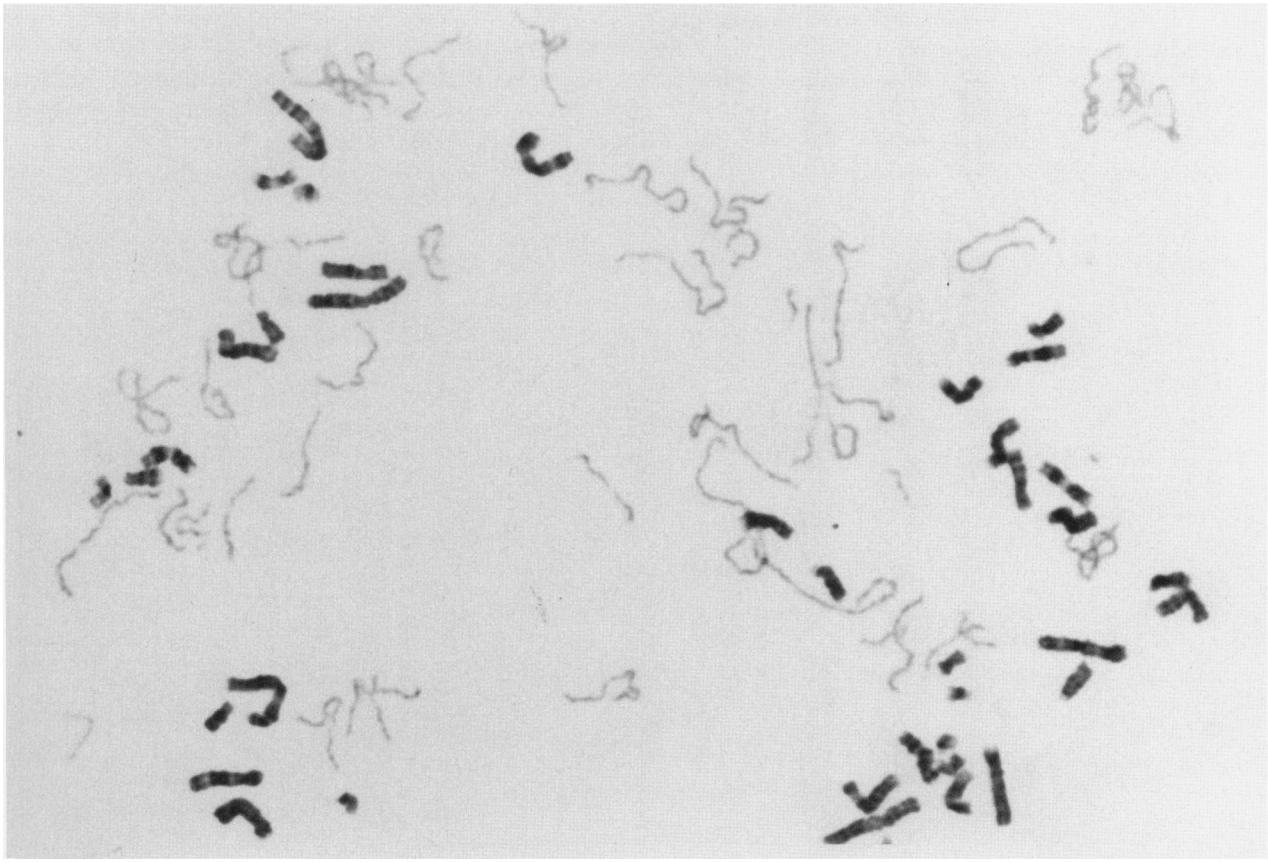

Figure 4 One cell showing prophasing (i.e., PCC) from patient 1426–86.

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