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A COMPARATIVE STUDY ON CHROMOSOME ABERRATIONS IN PERIPHERAL LYMPHOCYTES FROM BLACKFOOT DISEASE PATIENTS, THEIR RELATIVES AND CONTROLS[1]

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Sheng Wang-Wuu, Ruea-Yea Huang and Wu-Nan Wen (1985) A comparative study on chromosome aberrations in peripheral lymphocytes from Blackfoot disease patients, their relatives and controls. *Bull. Inst. Zool., Academia Sinica* **24**(2): 195-202. A total of 1535 lymphocytes from 32 Blackfoot disease patients, 700 from 14 healthy family members of these patients and 1003 from 28 healthy persons was analyzed cytogenetically. The incidences of cells with chromosomal aberrations in these three groups of people were 3.58%, 2.57%, and 2.39%, respectively. No significant difference was observed statistically among these incidences. Therefore, peripheral lymphocytes from patients with Blackfoot disease, for which chronic arsenicism has been suspected as the etiological factor, have a normal frequency of chromosome aberrations.

Analysis of chromosomal aberrations in peripheral lymphocytes of persons exposed to certain environmental mutagens has been a useful method for the detection of genetic damage. Previous reports have demonstrated an elevated frequency of chromosome aberrations in lymphocytes from psoriatic patients treated with arsenic (Nordenson *et al.*, 1979; Petres *et al.*, 1977), and from people exposed to arsenic for professional reasons—vine growers (Petres *et al.*, 1977) and workers at cooper smelter (Nordenson *et al.*, 1978). Blackfoot disease is a peripheral vascular disease prevailing in regions along the southwestern coast of Taiwan (Tseng *et al.*, 1961). Patients with Blackfoot disease (BFP) as well as inhabitants of the endemic area often show hyperpigmentation, keratosis and high risk of cancer (Tseng *et al.*, 1968; Yeh, 1973). Based on findings of clinical, epidemiological and pathological studies, this disease has been claimed to be attributed to the high content of arsenic in the drinking water of artesian wells in that area (Chen and Wu, 1962; Yeh and How, 1963). Lymphocytes from BFP

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were shown to have a significantly higher frequency of sister chromatid exchanges (SCE) than that of controls (Wen *et al.*, 1981). On the other hand, Chuang *et al.* (1981) found a normal incidence of chromosome aberration in BFP by conventional staining procedures after 3 to 4-day cultivation.

The purpose of this investigation was to compare the spontaneous frequency of chromosome aberrations of BFP with that of their healthy family members, who also reside in the endemic area. These results were further compared with the background data of healthy persons obtained in this laboratory. We applied the classical orcein staining as well as the G-banding techniques, which made possible not only the identification of individual chromosomes, but also the detection of balanced abnormalities that might accumulate after chronic exposure to certain mutagens.

MATERIALS AND METHODS

Thirty-two patients, 17 males and 15 females aged 46–82 years, and 14 healthy family members, 5 males and 9 females aged 26–74 years, were studied. Among the family members, 9 were spouses, 4 sons and 1 daughter-in-law of BFP. In the course of this study, a reference chromosome aberration incidence of this laboratory was established from 28 healthy persons, 16 males and 12 females aged 21–72. None of the subjects had been exposed to occupational or therapeutic irradiation or cytotoxic therapy by the time of this investigation.

A volume of 0.3 ml of heparinized blood was incubated at 37°C in 10 ml McCoy's 5A medium supplemented with 15% fetal calf serum, 100 units/ml Penicillin, 100 μg/ml Streptomycin, and 2% phytohemagglutinin (all materials from GIBCO) for 48 hr. Duplicate cultures were set up for each subject. In the last 1.5 hr. of incubation, cultures were exposed to colcemid (GIBCO) at a final concentration of 0.15 μg/ml. Hypotonic treatment was carried out with 0.075 M KCl for 15 min at 37°C, then the cells were fixed in freshly prepared methanol-acetic acid (3:1). After two changes of methanol-acetic acid, the cell suspension was dropped onto clean, prechilled slides. Air dried preparations were stained by aceto-orcein or G-banding procedure (Wang-Wuu and Jan, 1978) and blind-coded before analyzed.

For BFP and their relatives, 50 metaphases were analyzed from each individual, except in 4 BFP cultures where fewer cells were available for analysis. Thirty to 40 cells were scored in the healthy persons. Agreement between two observers were required for scoring any aberrations. The aberrations were recorded according to the classification system recommended by Buckton and Evans (1973). Abbreviation of chromosomal aberrations was adopted from ISCN (1978). Gaps, both chromatid and isochromatid, were recorded but not included in the calculation of aberration frequencies.

Student's *t*-test (Steel and Torrie, 1960) was used to compare the spontaneous chromosome aberration frequencies of BFP with that of their family, and the control.

RESULTS

Fig. 1 shows the distribution of chromosome aberrations in BFP, their healthy family members and the control group. The cultured lymphocytes from 28 out of 32 BFP had chromosomal aberrations. The percentage of cells with chromosome aberrations in each patient varied from 2 to 12%, with a 2–4% the most frequent. Eleven out of 14 BFP relatives had spontaneous chromosome aberrations in their cultured lymphocytes. A similar mode of variation as that of BFP was observed. However, sporadic distribution of aberration frequencies was found in 16 out of 28 healthy persons.

The frequencies and types of chromosome aberrations of these 3 groups of people are presented in Table 1. From a total of 1535 metaphase spreads examined in BFP, 55 cells

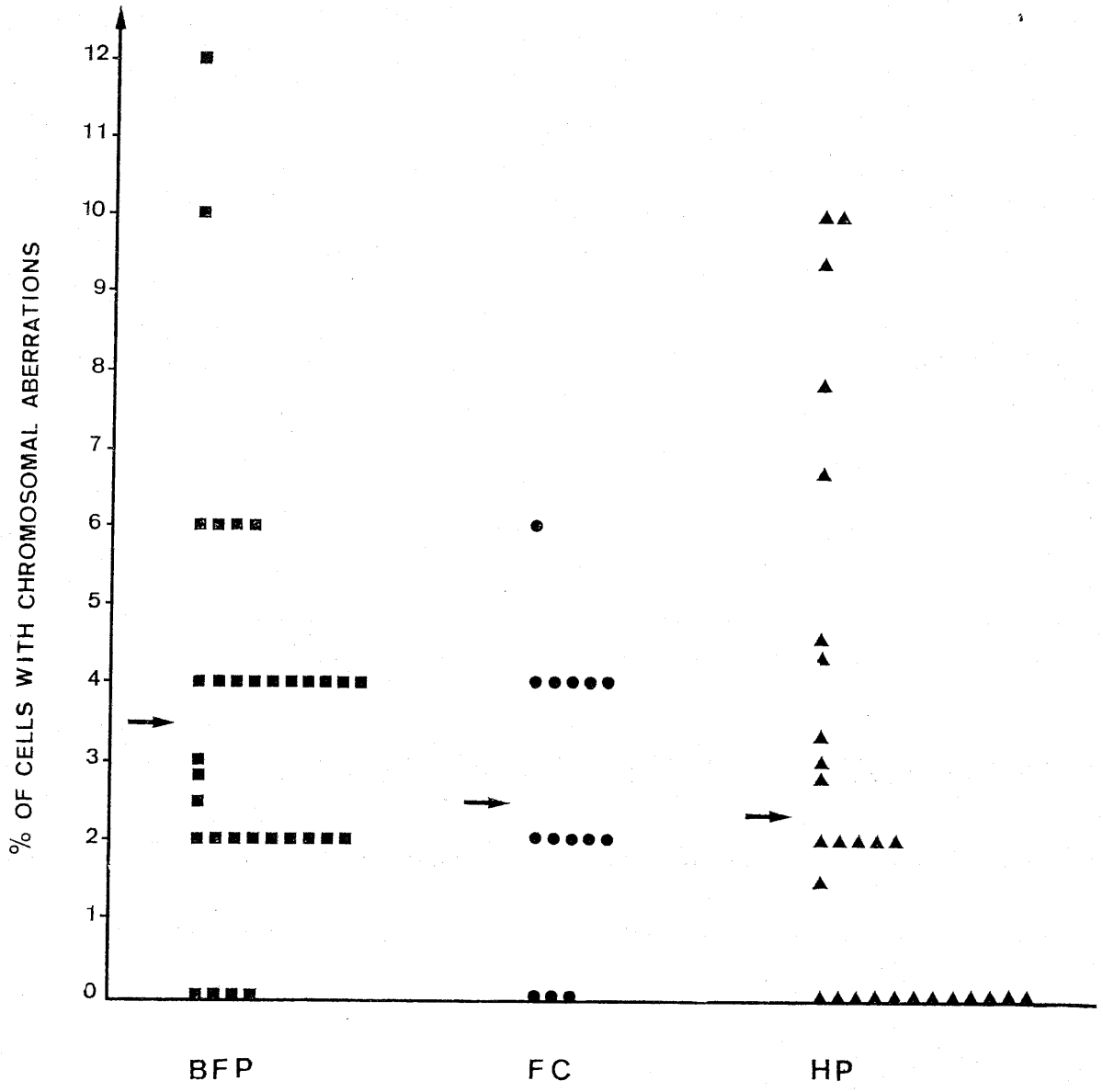

Fig. 1. Chromosomal aberrations in lymphocytes from Blackfoot disease patients (BFP=■), their healthy family (FC=●), and controls (HP=▲). The mean values are indicated by arrows.

were found to contain structural abnormalities. In the family members, 18 out of 700 analyzed cells were abnormal. Although the overall aberration frequency of BFP, 3.58%, was higher than that of the latter, 2.57%, the difference between the two groups was statistically insignificant ($t=1.142$, $p>0.05$). Taking types of aberration into consideration, chromatid break and acentric fragment were more frequent than other types of lesions observed, i. e. chromosome break, minute, dicentric chromosome, pericentric inversion, translocation, ring chromosome and chromatid exchange. Fig. 2 illustrates some of the aberrations observed in this study. The aberration frequencies of these two groups of people showed no difference from the reference background data of this laboratory, i. e., 2.39%

TABLE 1
Types and frequencies of chromosomal aberrations in lymphocytes from patients with Blackfoot disease (BFP), their healthy family members (FC) and controls (HP)

Group	No. of Subjects	No. of cells analyzed	Abn. cells		Total aberrations	No. of aberrations[a]									
			no.	%		ctb	csb	ace	min	dic	inv	t	r	cte	gap
BFP	32	1535	55	3.58	57	20	6	21	1	6	1	0	1	1	17(11)[b]
FC	14	700	18	2.57	18	4	1	10	1	1	0	1	0	0	6(5)
HP	28	1003	24	2.39	27	10	0	7	1	3	0	3	1	2	9(9)

a. Abbreviations: ctb-chromatid break; csb-chromosome break; ace-acentric fragment; min-minute; dic-dicentric chromosome; inv-pericentric inversion; t-translocation; r-ring chromosome; cte-chromatid exchange.
b. Number of cells containing gaps.

(t=2.005 and 0.650, respectively, P>0.05).

DISCUSSION

Both *in vitro* (Nakamuro and Sayato, 1961; Oppenheim and Fishbein, 1965; Paton and Allison, 1972; Wan *et al.*, 1982) and *in vivo* (Nordenson *et al.*, 1978, 1979; Petres *et al.*, 1979) studies on human lymphocytes have suggested that arsenic compounds appear to be powerful clastogens. In this study, however, we found that the frequency of chromosome aberrations in lymphocytes from BFP was higher but statistically insignificant than that of their healthy family members, who also reside in the endemic area. Furthermore, these values were comparable to that of the reference data of this laboratory, even though the latter was limitted in number. Our result is in accordance with Chuang *et al.* (1981) and Burgdorf *et al.* (1977) have found, i. e., lymphocytes from BFP consumed well water with high content of arsenic and from patients treated with Fowler's solution (1% $KAsO_2$) have a normal frequency of chromosome aberrations. The discrepancy of our observation with that of others (Nordenson *et al.*, 1978, 1979; Petres *et al.*, 1977) may be attributed to the natural history of the disease.

It is obvious from Fig. 1 that individual variation of aberration frequency existed in all three groups of people. When compared with the control group in which chromosome aberrations were observed in 16 out of 28 subjects, more individuals of BFP and the family members had chromosome aberrations and showed the clustering distribution in the range of 2-4%. We do not have a final explanation for this phenomenon at the moment. It is conceivable that genetic factors may play an important role in the yield of chromosome aberrations. Other factors such as viral infection and undefined seasonal variables (Littlefield and Goh, 1973) may have pronounced effects on clinically normal persons. Therefore, sample of a feasibly larger size is necessary to better define the spontaneous chromosomal aberration levels.

On the other hand, the production of chromosome aberration is influenced by a variety of *in vitro* factors, for instance the composition of the medium and the time of the cells are maintained in culture. Evans and O'Riordan (1975) suggested that the culture duration is the most important *in vitro* factor which influence the occurrence of chromosome aberration and therefore, in the analysis of chromosome aberrations following *in vivo* exposure to certain mutagens, blood sample should be cultured for up to 50 hr. The aberration frequencies observed in this study for all three groups agreed with previous data of 2-day cultures (Gundy and Varga, 1983; Ivanov *et al.*, 1978; Vijayalaxmi and

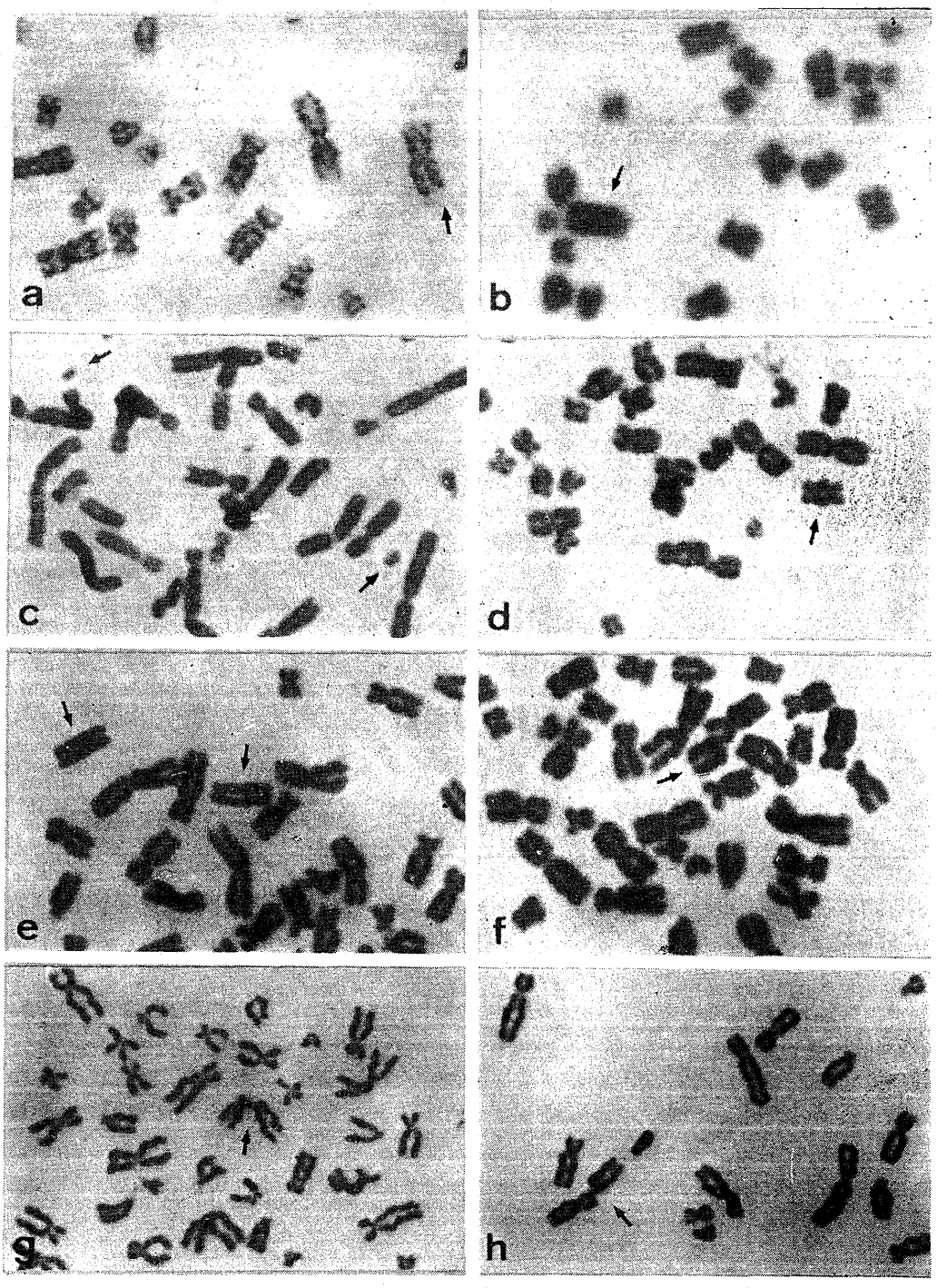

Fig. 2. Several types of structural aberrations (arrows) observed in Blackfoot disease patients (a-e), their healthy family (g), and controls (f, h). a. Terminal deletion. b. Pericentric inversion (chromosome 1). c. Minutes. d. Dicentric chromosome. e. Two acentric fragments. f. One acentric fragment. g. Chromosome break (chromosome 2). h. Chromatid break (chromosome 2).

Evans, 1982), which indicated that the spontaneous aberration frequency ranged from 1 to 4%. Difference in technique and laboratory conditions may probably lead to this variation.

When metaphase preparations are being utilized in a test system, gaps and breaks are sometimes difficult to be distinguished unambiguously. The scoring of gaps/breaks could contribute to the variation in aberration frequencies obtained by different laboratories. Since a significant increase in gaps was found in lymphocytes from workers in a nickel refinery (Waksvik and Boysen, 1982) and from workers in and around a smelter (Nordenson *et al.*, 1978), these investigators came to a conclusion that gaps may serve as a useful parameter in the monitoring of population exposed to mutagens/carcinogens. Brogger (1982) supported this conclusion and suggested that gaps and breaks are scored in the same class of aberration or are not scored at all in genotoxicology testing and monitoring. Our data in Table 1 showed that the incidence of gaps in presumably arsenic exposed BFP and their relatives were comparable to that found in the control group, i. e., 0.72, 0.71 and 0.9%, respectively. The scoring of gaps in the calculation of aberration frequency consequently lead to the same conculusion as they were left out. Therefore, the usefulness of gaps in the monitoring of exposure population is not clear until the nature of gaps is better understood and the gap-inducing substance is proved to cause segregational errors during anaphase.

In conclusion, peripheral blood lymphocytes from patients with Blackfoot disease, for which chronic arsenicism has long been suspected as the etiological factor, have a normal frequency of chromosome aberrations.

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烏脚病患者、家屬及對照組的染色體異常分析之比較

王　昇　黃瑞雅　溫五男

應用短期血淋巴球培養法分析烏脚病患者 32 人、健康家屬 14 人、及 28 位健康人的染色體異常之發生率。在 32 位患者的 1,535 個細胞中，55 個含有染色體異常結構，卽染色體異常的發生率爲 3.58%；在 14 位家屬的 700 個細胞中，18 個屬不正常者，異常的發生率爲 2.57%；而對照組的異常發生率爲 2.39%。雖然患者染色體異常之發生率較其他兩組人的爲高，但是這差異在統計上不顯著，亦卽烏脚病患者與健康人之間，不論是否曾經飲用含砷的地河井水，其染色體異常的發生率並無差別。