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Karyotypes of *Pneumocystis carinii* from Korean Rats

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Abstract: Molecular karyotyping was applied to *Pneumocystis carinii* (Pc) from two strains of experimental rats, Sprague Dawley (SD) and Fisher (F), in Korea. Field inversion gel electrophoresis and contour clamped homogeneous electric field electrophoresis resolved 15 chromosomal bands from the Pc. The size of the bands was estimated 270kb to 684kb from SD rats, and 273kb to 713 kb from F rats. The bands of 283 kb from SD rats and of 273 kb from F rats stained more brightly suggesting duplicated bands. Total number of chromosomes was at least 16, and total genomic size was estimated 7×10^6 bp. All of the bands from F rats hybridized to the probe of repeated DNA sequences of Pc and the band of 448 kb size was proved to contain rDNA sequences, but Pc. chromosome bands from SD rats showed no reactions to the probes. The 2 different karyotypes of *P. carinii* from 2 strains of rats were maintained consistently for 2 years.

Key words: *Pneumocystis carinii*, albino rat strain, karyotype strains

INTRODUCTION

Pneumocystis carinii (Pc) is an important opportunistic pathogen in immune compromised humans over the world (Walzer *et al.*, 1989). Human cases also had been recorded in Korea (Cheong *et al.*, 1983), and the serologic finding by Hong (1991) revealed its saprophytous prevalence among people in Korea.

Taxonomic position of Pc is still controversial (Frenkel *et al.*, 1990). Furthermore, the basic requirement for *in vitro* cultivation is not fully understood yet (Cushion and Ebbets, 1990). The shortage of basic knowledge on the nature of this protist makes further studies difficult. Therefore, more intensive basic researches on the fastidious organism have been required.

Pulsed field gradient gel electrophoresis (PFGE) is a newly introduced method to resolve chromosome-sized DNA molecules in agarose matrix by repeatedly altered direction of electric field (Schwartz and Cantor, 1984; Carle *et al.*, 1986; Chu *et al.*, 1986; Lai *et al.*, 1989). The technique enables us to analyze the chromosomes of the microscopic organisms whose morphological karyotypes are not available. Actually PFGE has provided much information on the chromosomes of important protozoan parasites or fungi.

The karyotyping method had been applied to Pc by 3 research groups (Yoganathan *et al.*, 1989; Lundgren *et al.*, 1990; Hong *et al.*, 1990), and their results showed different karyotypes.

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Furthermore, Hong *et al.* (1990) found different patterns by the host, rats and humans, and variations even by the colony of experimental animals. These findings suggest that Pc should be a complex group of genetically various organisms though they are indistinguishable microscopically.

In this context, the karyotypic nature of Pc should be evaluated by various localities and different animals. The present study aimed to observe the karyotypes of Pc from Korean rats, and to compare with previous findings.

MATERIALS AND METHODS

1. **Induction of Pc infection in albino rats:** Adult albino rats of Sprague-Dawley(SD) or Fisher(F) strains were suppressed of their immunity by subcutaneous injection of methylprednisolone(Depomedrol®, Upjohn Co., USA), 2 mg each a week. The 2 strains were reared separately with commercial rat diet and tap water mixed with ampicillin. Each batch of the animals contained 20 rats.

2. **Purification of Pc and embedded lysis of the cells:** The rats kept over 8 weeks were sacrificed and their lungs were removed. The lungs were chopped and homogenized by a stomacher(Stomacher Lab Blender 80, Seward Medical, UK). After filtration through the cotton gauze, host blood cells and pneumocytes were lysed by successive treatment in 0.05M ammonium chloride and hypotonic solution. The homogenate was filtered through membranes of 10 μm pore for further purification. Host DNAs were lysed by DNase I(Boehringer Mannhaim,

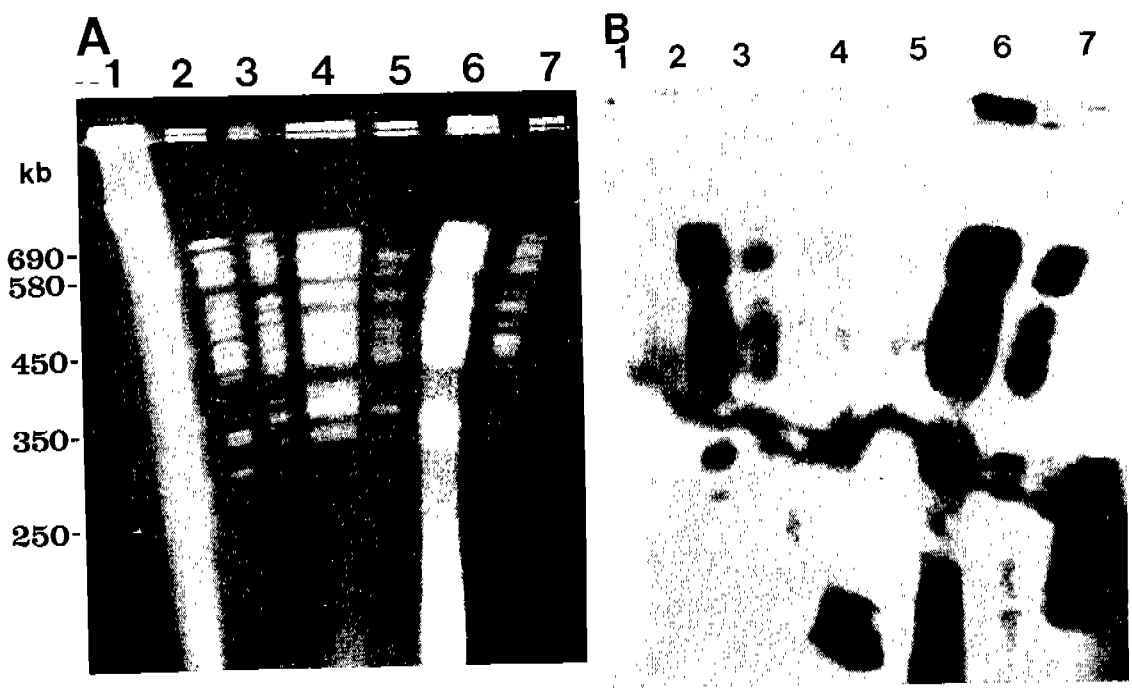

Fig. 1. **A**: CHEF gel (C15) run in 1% agarose under 95 V for 120 hrs, initial A time 25 sec, final A time 150 sec, A/B ratio 1, 1/2X TBE buffer at 15°C. Lanes loaded with size marker and *Pneumocystis carinii* (Pc) plugs. 1. *Saccharomyces cerevisiae* AB 972, a size marker; 2. USD6-2, Pc from Sprague Dawley(SD) rats in the USA; 3. UL8-5, Pc from Lewis rats in the USA; 4. SD5-1, Pc from SD rats (1/24/91); 5. SD8-2, Pc from SD rats (4/12/91); 6. F14, Pc from Fisher(F) rats(12/31/90); 7. F15, Pc from F rats (7/12/91). **B**: Autoradiograph after hybridization of P_{32} labeled Rp3-1 probe to the Southern blot of the gel Fig. 1A(C15), exposed to the film after high stringency washing. The lanes 2(USD6-2), 3(UL8-5), 6(F14), and 7(F15) showed strong signals in all bands suggesting that they share common repeat sequences of Pc.

Germany), and the cells were trapped in equal volume of 1.2% low melting point agarose (BRL Co., USA). The standard concentration of cells was $5\times10^8/125\ \mu l$. The gels were treated overnight in 0.25 mg/ml solution of proteinase K. The gel blocks were stored in refrigerated 0.5 M EDTA (pH 9.0) before use.

3. **PFGE:** PFGE was carried out by contour clamped homogeneous electric field gel electrophoresis (CHEF) and field inversion gel electrophoresis (FIGE). DNA size marker of a yeast (*Saccharomyces cerevisiae*, AB 972) was loaded on the gels with Pc samples. The standard running conditions were 1% agarose gel in 0.5× TBE buffer (45 mM Tris, 45 mM boric acid, 125 mM EDTA) at 15°C. Further details of switching time were changed by individual gels. The gels were stained with ethidium bromide and observed by UV illumination.

4. **Southern transfer and DNA-DNA hybridization:** The gels were sequentially soaked in 0.25 M HCl, 0.5 M NaOH, and 1.5 M NaCl, and the DNAs in the gel were transfered onto nylon membrane (Nylon 66 plus, Hoefer Scientific, USA) overnight in 6×SSC (0.5 M NaCl, 0.1 M NaCi). The probes were made by nick translation incorporating P_{32}-dATP to rat whole genomic DNA, repetitive sequence DNA (Stringer *et al.*, 1991), and rDNA library (Stringer *et al.*, 1989). Hybridization conditions were as usual in 50% formamide solution. The radioactivity was visualized by autoradiography. The procedures followed the manual of Sambrook *et al.* (1989).

RESULTS

1. **Karyotypes:** Table 1 listed the parameters of gel blocks of Pc used in this study. CHEF and FIGE separated 15 different chromosomal bands, one of which was stained more intensely than others (Figs. 1, 2, 3 & 4). The band seemed to be overlapped by two or more molecules of same size, therefore, the chromosomes were 16 at least. The Pc from 2 strains of rats gave different

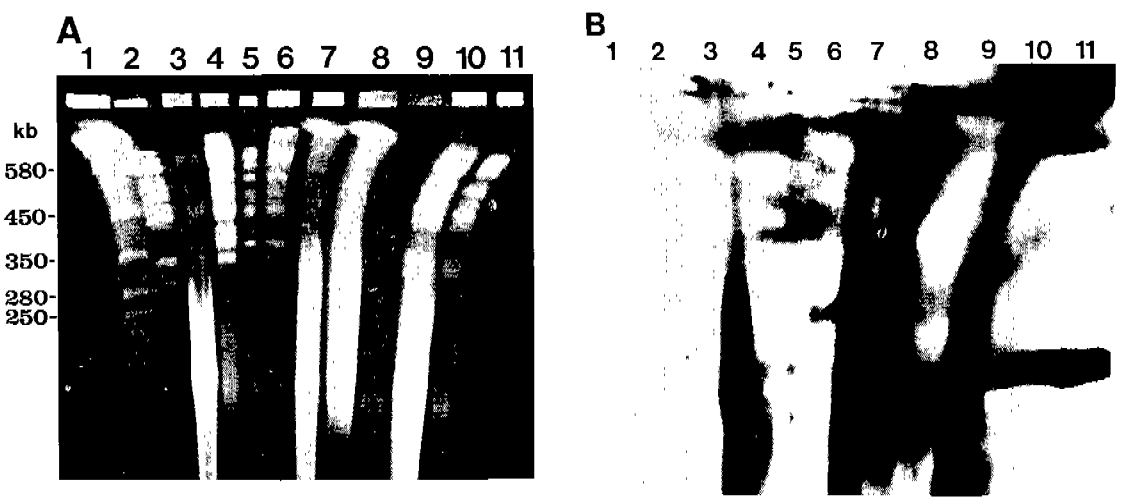

Fig. 2. **A**: CHEF gel (C13) of 0.8% agarose run under conditions of 105 V, 72 hrs, initial A time 25 sec, final A time 110 sec, and 1/2X TBE buffer at 15°C. Loaded plugs are 1. Size marker *Saccharomyces cerevisiae* AB 972; 2. USD5-2, Pc from SD rats in the USA; 3. SD3, Pc from SD rats (11/13/90); 4. SD5-2, Pc from SD rats (1/24/19); 5. SD8-1, Pc from SD rats (4/12/91); 6. SD8-2, Pc from SD rats (4/12/91); 7. SD12, Pc from SD rats (8/20/91); 8. F1-1, Pc from F rats (3/26/90); 9. F1-3, Pc from F rats (4/2/90); 10. F14, Pc from F rats (12/31/90); 11. F15, Pc from F rats (7/12/91). **B**: Autoradiograph of hybridization of P_{32} labeled rat DNA probe to the blot of Fig. 2A (C13) after washing in high stringency solution. Intense signals are observed on the plugs in the trough, megabase-sized bands, and smear backgrounds. The resolved bands between 270 kb and 700 kb show no reactions. Also the reactions differ by individual samples.

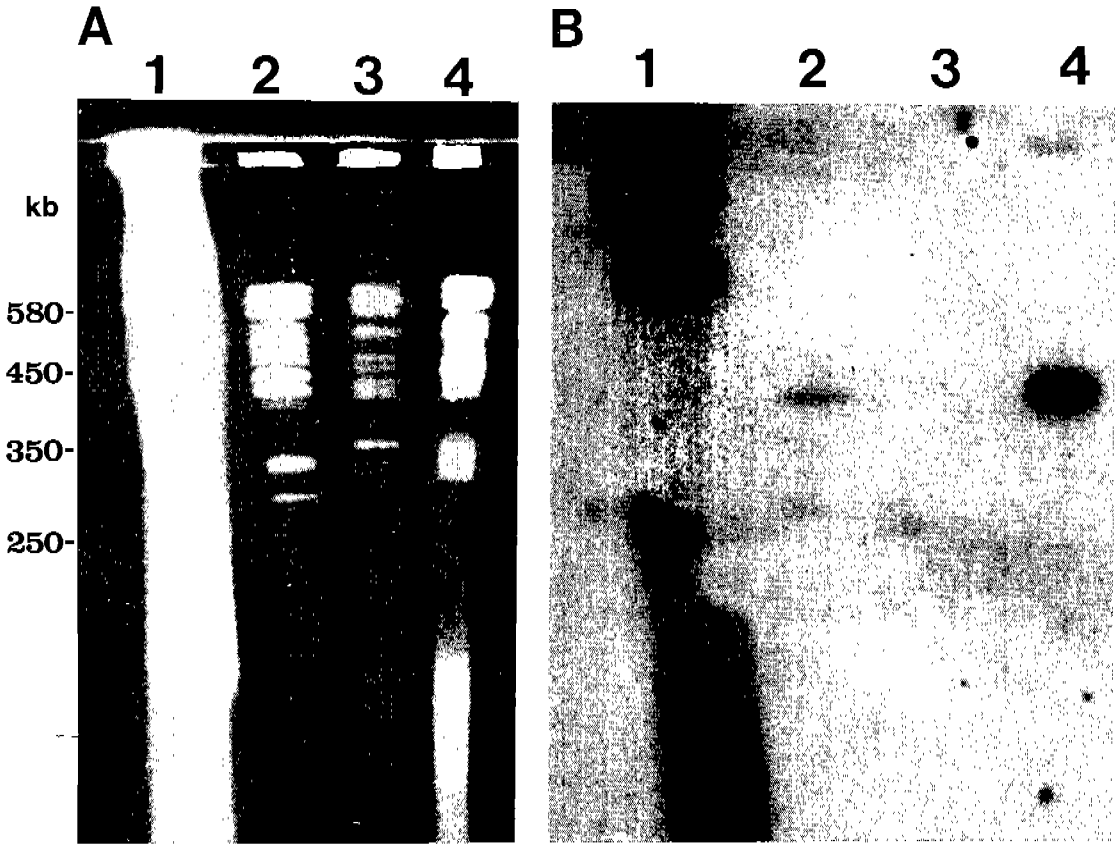

Fig. 3. **A**: CHEF gel(C14) of 1% agarose run under the parameters 90 V, 96 hrs, initial A time 25 sec, final A time 120 sec, A/B ratio 1, 1/2X TBE buffer at 15°C. The samples loaded are 1. *Saccharomyces cerevisiae* AB 972; 2. USD6-2, Pc from SD rats in the USA; 3. SD8-2, Pc from SD rats (4/12/91); 4. F15, Pc from F rats (7/12/91). **B**: Autoradiograph of the Fig. 3A(C14) gel blot, hybridized to the probe of 19c-1 rRNA genomic DNA. The 448 kb band of F15 reacted strongly. The bands of the yeast and one band of USD6-2 also showed signals.

karyotypes, from 270 to 684 kb in SD group and from 273 to 713 kb in F group as estimated by the distance of migration in the gels. The karyotype patterns were schematically shown in Fig. 5. The pattern of each rat strain was maintained same for 2 years. The karyotypes suggest the haploid genomic size of Pc from Korean rats is in the order of 7×10^6 bp.

2. **Hybridization:** The probe of repetitive sequences (Stringer *et al.*, 1991) hybridized to all of 15 bands in F samples, but not to those in SD group (Fig. 1). The chromosomal bands from SD or F samples showed little signal to the probe of rat genomic DNA. However, the large molecules of megabase size and the smears in the lower part of some samples reacted strongly (Fig. 2). The probe of rDNA hybridized to the band of 448 kb of F15 (Fig. 3), but any band from SD was not visualized.

DISCUSSION

Yoganathan *et al.* (1989) applied OFAGE and CHEF to Pc from rats, and observed 16 to 20 bands from 320 kb to 1.5 Mb. Lundgren *et al.* (1990) obtained 13 chromosomes from rat Pc by TAFE. Those are different from the present finding, i.e., the former had more bands and larger genomic size and the latter produced less bands.

The karyotypes of Pc by the present observation are very similar to those of Hong *et al.*

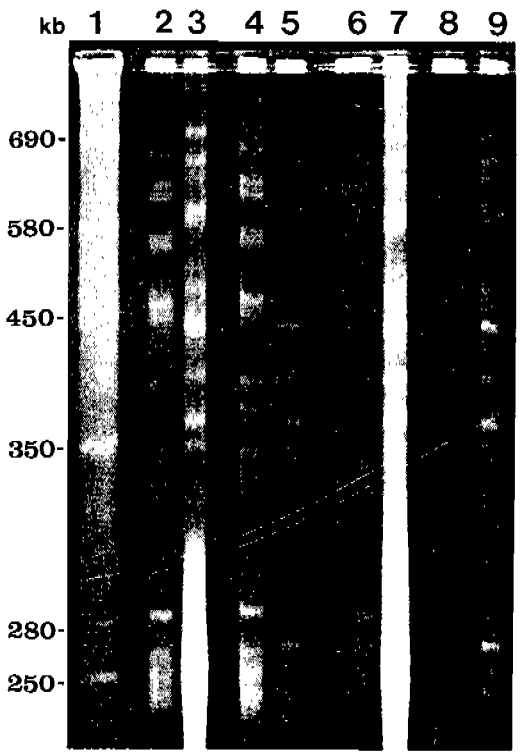

Fig. 4. The FIGE gel, F30, run under conditions of 1% agarose, 50 sec forward and 25 sec backward, 105 V, 96 hrs, and 1/2X TBE buffer 14°C. Loaded samples are 1. Size marker *Saccharomyces cerevisiae* AB 972; 2. SD8-1; 3. F14; 4. SD8-1; 5. F15; 6. SD8-2; 7. F14; 8. SD8-2; 9. F15.

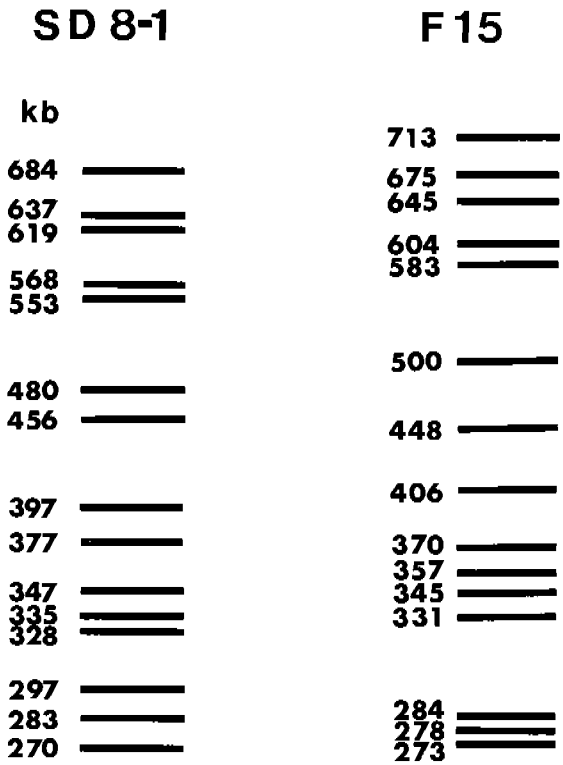

Fig. 5. Estimated size of chromosomal molecular bands resolved from SD8-1 and F15. The 283 kb band of SD8-1 and the 273 kb band of F15 are suggestive of doublet of 2 or more bands of same size.

(1990) in spite of geographical segregation between Korea and the America. Especially the Pc from F rats of the present study has a similar pattern with that of SD rats from America in the number and size range of the chromosomes, genomic size, presence of one duplicated band, location of rDNA on the 7th band from the top, and the conserved repeated sequences in all bands. However, they are not exactly same. As found in the figures, each Pc from American SD rats, American Lewis rats, Korean SD rats, Korean F rats shows different karyotypes.

Variation of the karyotype has been observed in *Leishmania* (Giannini *et al.*, (1986), *Plasmodium* (Kemp *et al.*, 1985; Janse *et al.*, 1992), and *Candida* (Soll *et al.*, 1987 & 1988; Merz *et al.*, 1988). The organsims of a species are further subdivided into strains by various characteristics. The strain may be designated by geographical distribution, host species, drug resistance, clinical manifestations, antigenicity, *etc.* Different strains can represent different karyotypes. Little information, however, is available to match the phenotypic strain and the karyotype variation of any species until now.

In *P. carinii*, no strain has been approved. Although Pc are found from various mammals, they are morphologically identical. Only major antigenic determinants are known to be varied between human Pc and rat Pc (Peglow *et al.*, 1990). The present finding and Hong *et al.* (1990)'s observation suggest a strong evidence of Pc's genetic variations. The karyotype variant is suspected to be induced by mutation (Janse *et al.*, 1992). However, the karyotype variation observed in Pc is not regarded so profound to make genetic diversity. The difference may be

Table 1. List of Pc samples from albino rats

Samples	Date of sampling	Duration	No. rats	No. of nuclei
Sprague-Dawley rats				
S D 1	4/24/90	6w	3	2×10^8
S D 2	9/24/90	7w	3	1×10^9
S D 3	11/13/90	9w	1	1.1×10^9
S D 4	12/14/90	9w	2	less than 10^6
S D 5	1/24/91	11w	3	5.4×10^8
S D 6	4/ 5/91	9w	4	4.7×10^7
S D 7	4/ 5/91	5w	2	less than 10^6
S D8-1	4/12/91	6w	6	3.8×10^8
S D8-2	4/12/91	6w	12	1.1×10^9
S D 9	6/17/91	5w	4	less than 10^6
S D10	7/ 3/91	5w	7	Not counted
S D11	7/26/91	9w	8	7.5×10^8
S D12	8/20/91	7w	14	4.2×10^7
S D13	8/28/91	6w	6	less than 10^6
Fisher rats				
F 1-1	3/26/90	8w	1	1×10^8
F 1-2	3/26/90	8w	1	1×10^8
F 1-3	4/ 2/90	9w	1	2.9×10^{10}
F 2-1	6/ 1/90	9w	3	2.2×10^8
F 2-2	6/12/90	10w	1	1×10^8
F 3-1	1/10/91	9w	2	2.6×10^8
F 3-2	1/10/91	9w	1	2.8×10^8
F12	5/ 2/91	12w	5	6.5×10^7
F13	5/ 9/91	13w	6	1.5×10^7
F14	7/ 5/91	8w	5	2.1×10^8
F15	7/12/91	3w	11	2.4×10^9

only superficial by aberrant deletion, superimposition, or translocation of the telomere between chromosomes. More data should be filed for further interpretation. Furthermore, the bands from SD Pc were hardly hybridized to the probes of repeated sequences or rDNA. Since the gels contained enough amount of DNA for the hybridization, the organisms from SD rats seemed to share no sequence homology with Pc from F rats. This should be a subject for further clarification.

Although the biological meaning of karyotype variation is still uncertain in Pc, it may be one of differential indices of strains. Since PFGE is an excellent tool to trace the same karyotype strain, it can prove the transmission modus and spreading course of Pc. Also it may supply fundamental information for its taxonomy when genetic mapping is achieved.

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=국문초록=

한국산 흰쥐 카리니주폐포자충의 핵형

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홍성태 · 김병일* · 고원규 · 유제란 · 국진아 · 채종일 · 윤종구* · 이순형

한국 내에서 사육 공급하는 실험실용 흰쥐를 실험적으로 면역억제하여 *Pneumocystis carinii* (Pc)를 발현시키고, 이를 순수하게 모아서 전기영동을 이용한 핵형을 분석하였다. Field Inversion Gel Electrophoresis와 Contour Clamped Homogeneous Electric Field Electrophoresis를 사용하여 분리한 염색체 밴드는 Sprague Dawley(SD)와 Fisher(F) 계 흰쥐 모두에서 15개 씩 이었다. 크기는 SD 흰쥐의 경우 270~684 kb, F흰쥐에서 얻은 표본에서 273~713 kb에 있었다. 이 중에서 SD 흰쥐에서 얻은 것은 283 kb의 염색체가, F흰쥐의 표본은 273 kb의 염색체가 특히 강하게 염색되어 두 개 이상의 같은 크기 분자가 중첩된 것으로 보인다. 그러므로 전체 염색체는 최소한 16개이며 각 염색체의 크기로 계산된 염색체 내 전체 유전자의 크기는 7×10^6 bp의 수준에 있다. F흰쥐에서 유래한 Pc는 전 염색체 분자가 공유하는 반복 염기서열을 가지며, 448 kb 염색체가 rRNA의 유전자를 갖고 있었다. 그러나 SD흰쥐의 Pc 표본에서는 어느 염색체 분자에서도 반복 염기서열과 rRNA 유전자를 확인하지 못하였다. 두 개의 흰쥐 계통 군은 각각 2년간 같은 핵형의 Pc를 유지하였다. (이 연구는 한국과학재단 1990년도 일반과제 연구비 지원에 의하여 수행되었음)

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