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Short Communication

Karyotype of Hairless Guinea pig

F. Todehdehghan*, M. H. Motedayen, and S. Tiemorzadeh

Department of Venomous Animals and Antivenin Production, Razi vaccine and serum research institute, Karaj, Islamic Republic of Iran

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Abstract

Chromosomal patterns of experimental animals are useful tools for cytogenetics research and animal breeding. Chromosome investigations of the hairless guinea pig are rare, therefore, karyotype of hairless guinea pigs (twelve male and female) was studied using metaphase spreads of bone marrows and G banding techniques. The chromosomes diploid number was 2n= 64 and polymorphism of three type chromosomal pairs were observed in the genus Cavia of Iranian hairless guinea pigs. A karyotype of 24 biarmed pairs and seven pairs of acrocentric chromosomes, 2 of sex chromosomes were also seen. The findings describe the karyology of the hairless guinea pig that is produced by Razi vaccine and serum research institute of Iran that improves our knowledge about laboratory hairless guinea pig and provides basic data for further use of the animal.

Keywords: Chromosome; Guinea pig; Hairless.

Introduction

The extensive development of immunogenic and cytogenetics research has brought up the question of analysis of chromosomes of experimental animals used in these areas. The genus Cavia Pallas, 1766 has eight species (16), C. aperea, C. fulgida, C. nana, C. anolaimae, C. guianae, C. tschudii, C. magna and C. porcellus. Some authors consider the domesticated cavy as C. porcellus (23) and others consider it a subspecies of C. aperea (16). Three inhabit Brazil including; C. aperea, C. fulgida, and C. magna (24). Previous studies have reported a very constant diploid number in different species: C. porcellus with 2n = 64 (13, 17) and FN= 96 (1), C. aperea with 2n= 64, FN= 128 (8) and C. a. aperea from Pernambuco State, Brazil, with 2n= 64, FN= 116 (12). C. magna from Rio Grande do Sul State, C. aperea pamparum and C. fulgida, both from Paraná and Rio de Janeiro States, Brazil, and C. porcellus, all have 2n= 64 and FN= 128 (12). Study of mammalian chromosomes by banding methods has permitted identification of individual chromosomes. The karyotype of the guinea pig, *C. porcellus* L. has been investigated, using chromosomal preparations from various tissues of female and male animals. The diploid number was reported 2n= 64 and only four large autosomal pairs and the X chromosome were individually identifiable. One entire X chromosome and possibly the short arms of the second X in females showed late DNA replication. A polymorphism involving the short arms of the longest pair of autosomes was present in the population and this

* Corresponding author: Tel: +989125604072; Fax: +982634552194; Email: F.Todehdehghan@rvsri.ac.ir

polymorphism was shown to segregate in families (4). The existence of a considerable amount of repetitious DNA, representing about 7% of the guinea pig genome that has been demonstrated by Yunis and Yasmineh (25). Bianchi .and Ayres (2) reported the chromosome complement and patterns of heterochromatin distribution (demonstrated by the DNA d-r method) in the three different guinea pigs. Karyotype analyses showed that one of the females had a heteromorphic sex pair formed by a submetacentric X chromosome and a subterminal X chromosome originated by a shortening of the short arm (x-chromosome). The heterochromatin was mainly found in the pericentromeric areas of the autosomes and X chromosomes and in the short arm of pair 7. The Y chromosome exhibited a degree of heterochromatinization different from that of pericentromeric areas. The analysis of the heterochromatin distribution in the X chromosomes showed that the smaller size of the heteromorphic x-chromosome was probably due to a lack of heterochromatin in its short arm. Moreover, two out of the three animals studied had a heteromorphic pattern of heterochromatinization in the pair 21 characterized by heterochromatinization of the pericentromeric area in one chromosome and almost complete heterochromatinization of the other homologue. It was suggested that most of the heterochromatin disclosed by the DNA d-r method is formed by repetitious DNA; and the Y chromosome and perhaps some autosome regions in guinea pigs are formed by a type of heterochromatin with properties different from the intermediate heterochromatin. Comparative chromosome painting demonstrated that the karyotypes of two species of guinea pigs, C. porcellus and C. tschudii are identical (19). In this work the Iranian hairless guinea pig chromosomes

(chromosomal spreads) are examined microscopically to establish diploid number and chromosome morphology of this laboratory animal (3, 18) for further use in research.

Materials and Methods

Twelve hairless guinea pigs, six males and six females were selected for chromosome preparations, following bone marrow extraction method according to Deanna and Robbins procedure (6) and G-banding by Seabright method (21). Tow hours prior to the test, each guinea pig was injected intraperitoneally with 0.1 ml of 0.01% colchicine, a mitotic inhibitor. The animals were sacrificed in a humane manner (cervical dislocation), and the hind leg bones (femur and tibia) were removed. The bone marrow of each leg was flushed with 3 mL of 0.075M KCl into a centrifuge tube. The solution was gently aspirated with a Pasteur pipette until a more or less homogenous cellular suspension was produced. The cell suspension incubated for 15 minutes at about 37°C, or (can hold the tube in your hand for 15 minutes) and then centrifuged for 2 minutes at 1500 rpm. The supernatant (about 0.5 ml) was collected, and fresh, cold fixative (3:1, methanol: glacial acetic acid), was added and centrifuged as before. The supernatant was removed without disturbing the cell button. The cells were resuspended in about 1.0 ml of fixative, and then aspirated. Two to four drops of the suspension were placed on the frosted microscope slides, let the excess liquid run off and slides were air dried. The slides were stained in a Coplin jar with 2% Giemsa stain for 10 minutes and gently rinsed with distilled water, and air dried. The chromosomal spreads were examined under Olympus microscope (model, BX51TRF made in Japan) with high power and oil immersion and photos were taken by camera (Olympus, Model, DP72-22A, made in Japan). We have found the bone marrow extraction procedure to be easily adaptable to 2- 2.5 hour laboratory period. This procedure could replace that of tissue culture, which is time-consuming, expensive, and requires that the procedure be performed wholly in the laboratory (6, 14).

This descriptive study was part of a project and approved by the Razi vaccine and serum research Institutes scientific committee and the housing and use of the animals complies with ISIRI 7216-2 animal ethics guidelines (10).

Results

The chromosome's diploids number in hairless guinea pig was 64, of which twenty four pairs were biarmed and seven were acrocentric. The sexual pair consisted of a large metacentric X-chromosome which was second in size. Y chromosome was one of the largest acrocentric chromosomes. Three autosomal pairs, pairs 1, 2, and 3, identify differently. Pair 1 was the largest acrocentric chromosome in the set; pairs 2 and 3 were medium size and had a submetacentrie kinetochore location. The remaining autosomes had a subterminal or terminal kinetochore position and formed a continuous series which decreased in size without noticeable gaps; therefore, their identification was only tentative. In the female (XX-female) and in the male, the X chromosomes could be easily identified on the basis of their large size and submetacentric kinetochore location. The Y chromosome of the guinea pig has no morphological features permitting its correct identification. Accordingly the Y chromosome is

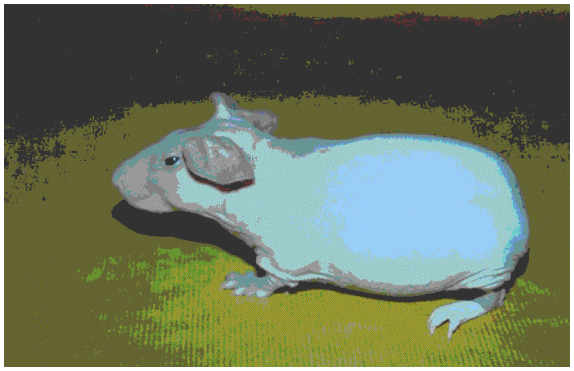

Figure 1. Hairless Guinea pig

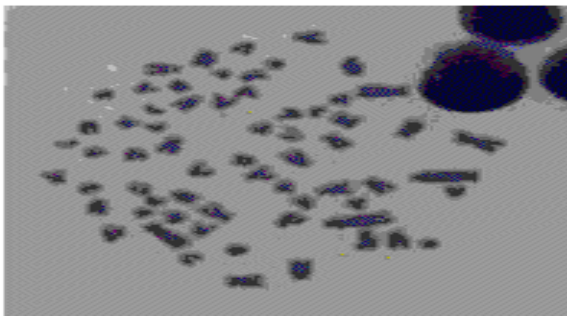

Figure 2. Karyotype of female hairless Guinea pig

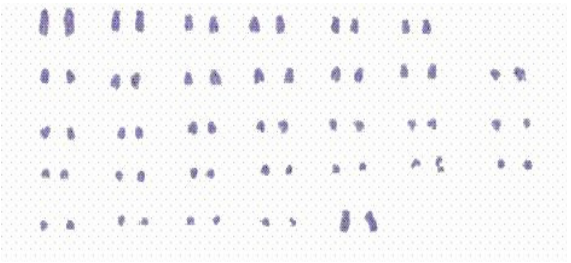

Figure 2.1. Homologous chromosomes of female hairless Guinea pig

identified as one of the smallest subterminal chromosomes of the set. The phenotype (Fig.1), karyotype and homologous chromosomes of female and male hairless guinea pig are illustrated in Figures 2–3.

Discussion

This is first chromosomal study reported for the Iranian hairless guinea pigs. Origin of the guinea pig is from lab animals breeding colonies (22) in the Razi vaccine and serum research institute, Karaj, Iran. So far, it seems that the cytological investigation on the hairless guinea pig, *Cavia porcellus*, has not been carried out. The present study has investigated the chromosomal morphology and numbers of Iranian hairless guinea

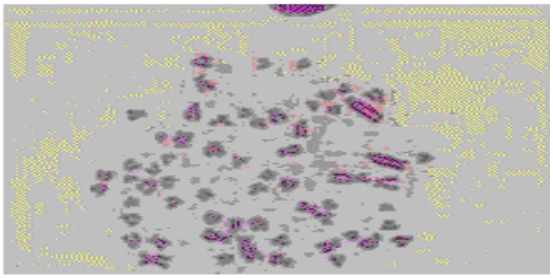

Figure 3. Karyotype of male hairless Guinea pig

Figure 3.1. Homologous chromosomes of male hairless Guinea pig

pigs, employing bone marrow metaphase and G banding method. Although the technique have resolution limitations, that make it impossible to detect small aberrations and analyze whole genome. Yet, they are rapid (14) and economical techniques to study the lab. animal karyotypes and are valuable tool in both research and diagnosis (6). With Giemsa staining centromere stains very dense, that was revealed in our results (15). G-bandings show many of major bands that contains minor bands (5, 15) according this banding pattern, homologous chromosomes were paired. X chromosome is one of longest chromosomes could be almost easily paired (5, 11, 14) and Y chromosome was constantly dark and centromeric chromatin was not obvious (5, 11, 14, 20). Five species of the genus Cavia have been cytogenetically studied. They all have a diploid number of 2n= 64 (7). The diploid chromosome number in hairless guinea pig, like haired one is 64 (Fig 2-3) in both sexes (4, 7, 13). The chromosomes can be arranged in 31 homologous autosomal pairs and a pair of sex chromosome which is homomorphism XX in the female and heteromorphy XY in the male, the X chromosome being appreciably larger than Y (Fig.3). Three pairs stand distinct, a pair of acrocentric autosomes, which are largest of all. Two other pairs have no much difference in size, but can be easily distinguished from position of their centromeres (2). The remaining homologous pairs are submetacentric autosomes (13). In some species such as the guinea pig,

the amount of repetitious DNA is remarkably large (up to about 20% of total DNA) and represents most of the constitutive heterochromatin (9, 25)**.** Results from some investigators show that the Y chromosome from various species and the Y chromosome of guinea pig exhibit distinctive properties different from that of pericentromeric heterochromatin. It is known that the heterochromatine has different properties such as, genetic inertness, late replication, high concentration of redundant DNA, differential fluorescence with fluorochromes, uncoiling in interphase and increased breakage with some drugs and virus. The polymorphic pattern of heterochromatin distribution observed in some pairs of the X chromosomes of guinea pig can be explained by assuming either a decreased or increased rate of redundancy for DNA of certain chromosome regions. All mammalian species so far studied showed a predominant kinetochore site of the constitutive heterochromatin. Consequently, it seems reasonable to assume that the presence of an almost completely heterochromatic autosome in the guinea pig complement may be caused by an excessive production of repetitive heterochromatin (9, 25)**.** However the results of present cytogenetical study show the hairless guinea pig, like five species of the genus Cavia pallas of Brazil have a diploid number of 2n= 64. The present report describes the karyology of hairless guinea pig that was produced and housed in conventional colony in Razi vaccine and serum research institute of Iran (22) and results could be used as a reference value for breeders and biomedical researchers.

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