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First report of GTG-banded nomenclature of Pakistani Lohi sheep (*Ovis aries*)

Ahmad ALI[1,*], Masroor Ellahi BABAR[1], Saqib MAHMOOD[2], Muhammad IMRAN[1]

[1]University of Veterinary and Animal Sciences, Lahore, 54000 - PAKISTAN

[2]Genetic Diagnostic Laboratories, Children Hospital, Lahore - PAKISTAN

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Abstract: The present study was planned to generate the G-band nomenclature of the commonly occurring Lohi sheep breed in the Punjab province of Pakistan. Whole blood samples were collected through jugular venepuncture in heparinised vacutainers. The metaphase slides were prepared using standard protocols. The results from microscopic screening of 50 good quality metaphase cells from each animal were studied and they revealed a chromosome complement of 54, XX and XY in Lohi sheep. The first 3 pairs were sub/metacentric whereas the other autosomes, including X, were classed as acro/subacrocentric. The Y chromosome was the smallest metacentric chromosome and had a star-like presentation. The trypsinisation time for optimal G-bands was highly variable, and individual chromosomes were identified by G-positive and negative band markers. The identification of chromosomes 4 & 6, 8 & 9, and 19-26 was difficult because these chromosomes were indistinct and small. The X chromosome was the largest sub/acrocentric, with a prominent G-positive band along the lower middle length. Complete G-band homology was noticed between Lohi and other reported sheep breeds from different parts of the world. The G-band ideogram of Lohi was also constructed based upon the G-band organisation observed in the Lohi genome. None of the 350 metaphase cells studied revealed chromosomal abnormalities.

Key words: Lohi sheep, karyotype, GTG bands, Pakistan, cytogenetics

Introduction

Cytogenetic diversity within different livestock species helps delineate evolutionary genetic relationships. In addition to 30 well-defined domesticated sheep breeds, 3 wild sheep breeds are found in Pakistan, including Blue sheep (*Pseudois nayanr*), Marco Polo sheep (*Ovis ammon polii*), and 3 subspecies of Urial sheep (*Ovis orientalis punjabensis, Ovis orientalis cycloceros*, and *Ovis orientalis vignei*) (1,2). No information on diploid chromosome complement is available on these species so far.

Moreover, very little information on chromosomal morphology and dys-morphology of domestic sheep breeds (*Ovis aries*) is available in Pakistan. Kari is the only sheep breed that has recently been reported to have 54, XX and XY chromosome complement (3). In addition, the standard karyotype nomenclature with precise chromosome identification is also lacking for dairy and other livestock breeds of Pakistan. The study of chromosome morphology and the precise identification of chromosomes using differential staining techniques—such as G, R, and Q-banding—constitute the first step in exploring chromosomal dys-morphology, such as translocations, autosomal, and sex chromosome aneuploidy. Such chromosomal abnormalities can cause lowered reproductive efficiency in sheep and other livestock species due

* E-mail: ahmadali65@hotmail.com

to the production of unbalanced gametes during meiosis (4). In addition, establishing a standard chromosome nomenclature for each breed, as well as breed characterisation at the molecular level, is important for the patent registration of national genetic resources. Information produced in this manner can then be incorporated to select superior stock and devise effective breeding plans. This would ultimately help to overcome the acute shortage of quality animal protein in the country.

Materials and methods

Whole blood samples from 5 Lohi ewes and 2 rams were collected in heparinised vacutainers through jugular venepuncture from an experimental flock maintained at the Livestock Production Research Institute in the Okara district of Punjab province. The blood samples were immediately transported in a flask maintained at 4 °C to the Molecular Cytogenetics and Genomics Laboratory of the University of Veterinary and Animal Sciences, Lahore. These blood samples were cultured using standard protocols (5). The culture medium of choice was RPMI-1640 (Gibco) with 20% fetal bovine serum, pokeweed mitogen, and penicillin/streptomycin.

The cultures were incubated for 72 h at 37 °C in a humidified incubator. In order to have ample choice of good quality metaphase cells, 3 replicates from each animal were set up. At the completion of a 72 h incubation period, the cultures were treated with 100 µL of Colcemid (Gibco Karyomax) and were mixed gently. The cultures were then incubated at 37 °C for 20 min and centrifuged at 1000 rpm for 10 min. The supernatant was removed, and the cells were suspended in 6-7 mL of 0.075 M KCl solution, already incubated at 28-30 °C. The cultures were incubated for another 15-20 min at the above temperature. At the conclusion of hypotonic treatment the cultures were spun down again at 1000 rpm for 10 min. The supernatant was removed just above the buffy coat, and the cultures were gently mixed with a plastic pipette before fixing the cell pellet with a 3:1 mixture of methanol and glacial acetic acid at −20 °C. A minimum of 3 fixative runs were carried out on each culture before plating out the cells using an air drying technique. Each slide was then examined by phase contrast microscopy to ascertain the quality of metaphasis and mitotic index.

To induce G-bands the slides were left overnight in the oven at 60 °C for maturation before exposing them to trypsin treatment. Each slide was exposed to a solution of 35 mg trypsin in a 70 mL PBS buffer for 10-30 s at room temperature. The trypsinised slides were then stained with 10% Giemsa Gurr buffer (Gibco) for 10 min, washed, and blot dried for microscopic analysis using an Applied Imaging (AI) Cytovision apparatus. A minimum of 50 well spread metaphase cells were scored for each animal, and the selected ones were photographed for karyotype analysis. Based on the G-banding pattern, an ideogram was also constructed using Genus software.

Results

A satisfactory G-band level was defined to allow for the precise identification of individual chromosome numbers and bands at 100 × magnification under oil immersion. The G banding experience revealed that the trypsin exposure time required to obtain optimal G-bands was highly variable. A trypsin treatment time of 10-30 s was found to be generally satisfactory to achieve optimal G-bands at different times. The optimal treatment time was determined by running couple of pilot slides. There was a general trend towards unsatisfactory banding results in humid and cold weather. The trypsin solution worked most effectively on dry and sunny afternoons.

Each metaphase cell was examined at 100 × magnification under oil immersion. A G-banded chromosome spread of Lohi sheep is illustrated in Figure 1. The diploid chromosome complement was

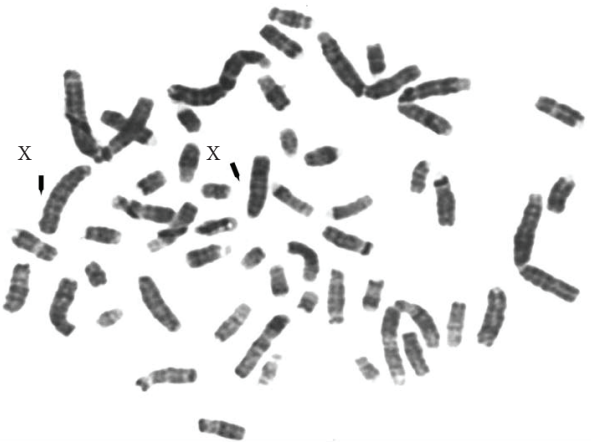

Figure 1. G-banded chromosome spread of sheep (*Ovis aries*).

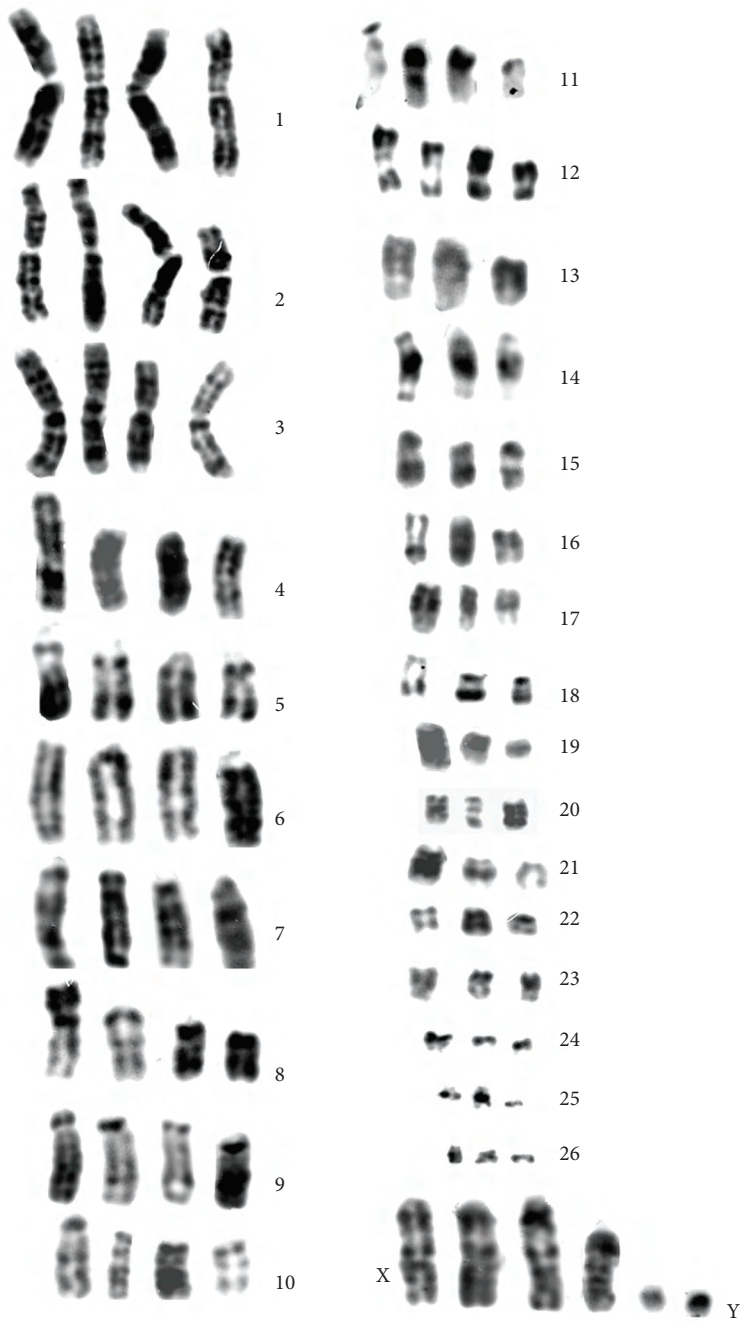

Figure 2. GTG-band nomenclature of Lohi sheep from Pakistan.

54, XX and 54, XY in females and males, respectively. The first 3 pairs were classed as sub/metacentric chromosomes having prominent *p* and *q* arms with highly distinct banding patterns. The X chromosome was the largest sub-acrocentric with minute *p* arm extensions, whereas the Y chromosome presented as an asterisk-shaped metacentric. Chromosomes 4-26 were classed as acrocentric with a descending length gradient. At a cytogenetic level the identification of chromosomes 4 & 6 was highly challenging, due to their indistinct band organisation. Therefore, great care was taken to correctly identify these 2 pairs. Similarly, chromosomes 8 & 9 were often difficult to differentiate. The precise identification

of chromosomes 19 and 20-26 also required great care due to their small size, especially when it was difficult to find optimally G-banded chromosomes in this category. All chromosomes were selected from different metaphase cells and aligned in rows of 4 each with different lengths. In addition, the G-band nomenclature (ideogram) of each chromosome was prepared alongside each row using Adobe Photoshop software, release 8. The ideogram of Lohi sheep, developed with the help of a cytovision apparatus, consisted of 252 bands. The number of G-positive bands was 115, including sex chromosomes, whereas the total number of G-negative bands was 137 in the Lohi karyotype (Figure 2).

Discussion

The G-band pattern of metaphase chromosomes corresponds to that of chromosomes observed without treatment at the pachytene stage of meiosis. The G-banding method uses either the effect of a proteolytic enzyme or mild de-naturation, which affects the interaction that stabilises the structure of different proteins and nucleic acid components of the chromatin. The G-band mechanism is based mainly upon differences in protein composition and organisation (6). It has also been suggested that trypsin treatment leads to the unfolding of protein loops and permits the protein structure associated with the alignment of AT-rich sequences, as reported by Popescu et al. (7). The results of the present study were highly inconsistent regarding standard trypsin exposure time for the satisfactory induction of G-bands, which made precise identification of individual chromosomal bands challenging. This may have been due to relative humidity, room temperature, and the variable extent of slide maturity during overnight incubation (8,9).

To overcome this difficulty, the practice of running couple of pilot slides proved extremely helpful in optimising trypsinisation time for a given set of slides. The *Ovine* chromosomes were also difficult to identify correctly due to indistinct G-band patterns between certain chromosomes, such as 4 & 6, 8 & 9, and 19, as well as the small size of chromosomes 20-26 (10,11,12). In standard nomenclature of sheep chromosomes there is, in fact, some ambiguity in assigning chromosome numbers to different chromosomes (13). The diploid chromosome complement of *Ovis aries* and the G-band pattern has not shown any significant variation across the globe, and the findings of the present study substantiated this fact. Furthermore, when compared with the published G-band nomenclature of Long (14), Matejka and Cribiu (15), and Ansari et al. (5) there was no significant variation found in the marker G-negative and G-positive bands of different sheep breeds. In the light of the homology in G-band patterns, it would be interesting to explore existing chromosomal abnormalities in Pakistani sheep breeds and compare these with previously-reported anomalies—such as translocations, aneuploidy, and sex chromosome abnormalities—in order to collect karyotype dys-morphology data on indigenous sheep breeds and investigate the impact of chromosomal aberrations on the fertility and performance of different Pakistani sheep breeds, as reported by Long (4).

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