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Comparative Study of Spontaneous and FUdR Induced Chromosomal Instability in Pakistani Lohi and British Suffolk Sheep – An Indication of Environmental Mutagenesis

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Abstract.- A Study to compare the extent of geno-environmental interaction expressed as fragile sites was carried out in British Suffolk and Pakistani Lohi breeds of sheep *(Ovis aries).* Spontaneous and 5-Fluorodeoxyuridine (FUdR) induced fragile sites were scored in metaphase chromosomes in two flocks of sheep reared under different environmental conditions. Means and standard errors for aberrant cell count (AC) and number of aberrations (NoA) per animal revealed highly significant differences between the two groups ($P<0.01$) belonging to distant continents of the globe. Average Number of cells with gaps and breaks (AC) was 0.56±0.15 Vs 1.91± 0.34 whereas NoA index per cell averaged 0.59±0.16 Vs 2.36±0.48 in chromosomes grown in control cultures of Lohi and Suffolk breeds respectively. Corresponding means in FUdR synchronized chromosomes were 2.18±0.33 Vs 13.26±0.85 and 2.65±0.50 Vs 21.87±1.88 in the two breeds respectively. Distribution comparison of chromosomal fragile sites grown in control cultures with no FUdR revealed seven fragile bands in Lohi compared to 24 in Suffolk. Microscopic analysis for the number of fragile bands in FUdR treated chromosomes revealed 29 and 78 autosomal bands in Lohi and Suffolk breeds respectively. Similarly the number of significantly fragile bands was 4 and 78 in the two breeds respectively. X-chromosome in Lohi was highly stable in control as well as FUdR cultures expressing no gaps and breaks whereas the Suffolk X expressed 4 fragile bands. The study suggested that long term exposure of the two flocks to entirely variable environment might have played a significant role for higher chromosomal damage in the Suffolk breed.

Keywords: Spontaneous chromosomal instability, FUdR, chromosomal instability, Pakistani Lohi, British Suffolk, environmental mutagenesis.

INTRODUCTION

With new molecular cloning insights regarding fragile sites of clinical and common importance the interest in the subject has been renewed. Althogh fragile sites in human have been the major point of interest with only a small number of studies cited in farm animals (Riggs and Ronne, 2009). Similarly Ali *et al*. (2008) published a detailed overview fragile site phenomenon in sheep genome.

Although the exact mechanism of fragile site expression still remains unclear, however some external factors have been implicated to be instrumental for their expression.

Environmental pollution is an important factor for increased rates of mutagenesis due to higher than ever industrial and automobile emissions (Darkwa, 1996). A number of reports are available describing spontaneous and chemically induced chromosomal damage in living organisms due to their exposure to heavy pollutants in the environment. Higher chromosomal lesions, congenital abnormalities and higher rates of dynamic mutations are caused by long term exposure of animals and plants to air, water and land born pollutants (Ahmad *et al*., 1998; Carlose *et al*., 2005; Di Berardino *et al*., 1983). A unique comparative investigation was planned to explore the extent of environmental genotoxicity expressed as fragile sites, in Lohi breed of Pakistan exposed to relatively pure and rural environment in the remote area of District, Okara with minimal automobile and industrial emissions compared to Suffolk sheep exposed to a highly industrial and polluted environment of Avonmouth area in Bristol,

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England.

Fragile sites are chromosomal regions frequently showing gaps, breaks and multi-radial figures *in-vitro* spontaneously or when treated with some carcinogenic substance such as FUdR Carlose *et al*. (2005). This investigation was carried out to compare the extent of spontaneous and FUdR induced fragile sites in Pakistani Lohi and British Suffolk sheep.

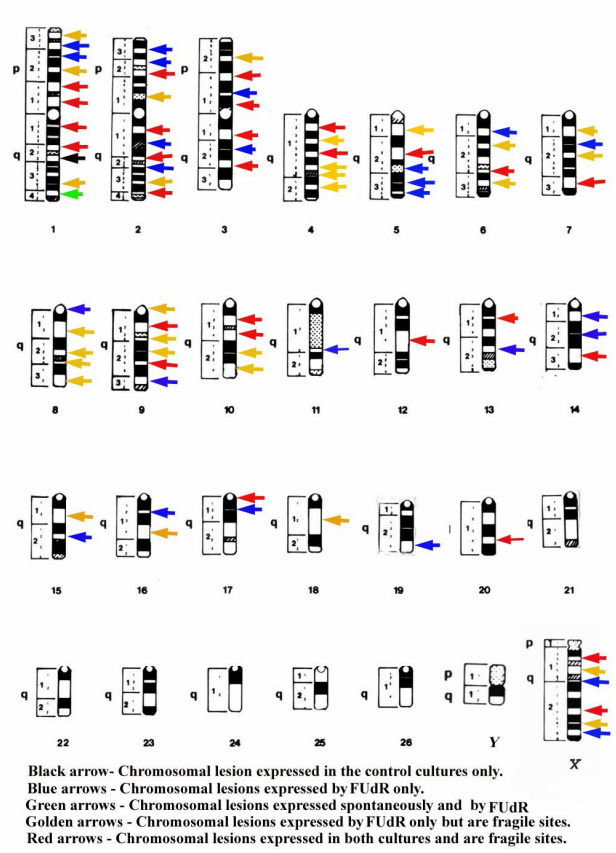

Fig. 1. Spontaneous and FUdR induced fragile site map in British Suffolk cross sheep.

MATERIALS AND METHODS

Cytogenetic data on two measures of chromosomal damage *viz*. aberrant cell count (AC), defined as the number of metaphase cells showing gaps and breaks in chromosomes or chromatids and the number of aberrations, (NoA) defined as total number of gaps and breaks in a single metaphase, was collected from 45 Suffolk and 32 Lohi sheep breeds. Blood samples of Suffolk sheep were obtained in heparinized vacutainers through jugular venepuncture and cultured in Cytogenetic Unit of the Department of Clinical Veterinary Science, Langford University of Bristol UK. Same procedure was followed to collect peripheral blood samples from 20 healthy and adult ewes and 12 breeding rams from a flock of Lohi breed maintained at Livestock Production Research Institute Bahadarnagar Okara, Pakistan and processed at the Centre of Excellence in Molecular Biology (CEMB), Lahore, Pakistan. Lohi animals were confined to pollution free environment for generations at the farm area covering about 2000 acres of land in the heart of a rural area and largely surrounded by agri-based industry.

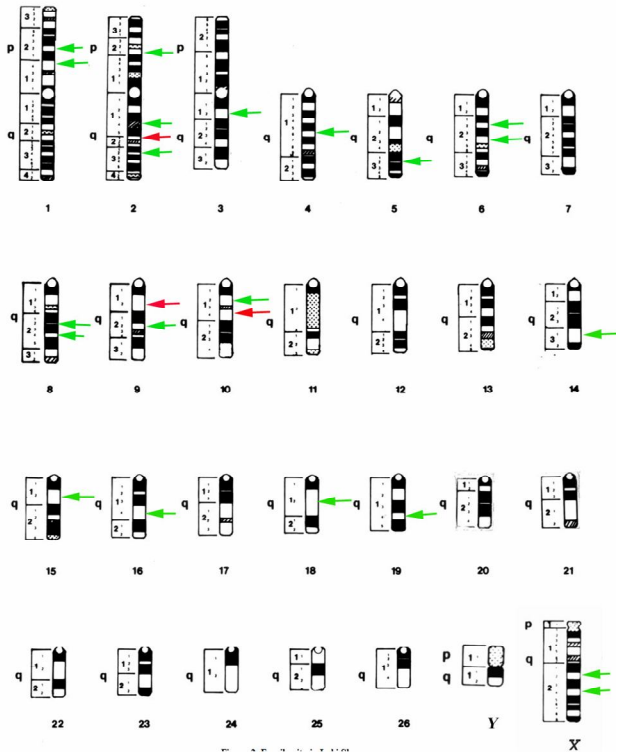

Fig. 2. Fragile site in Lohi sheep.

Short term peripheral whole blood lymphocyte cultures were established using standard protocol (Long, 1985). Control and treatment cultures from each animal were set-up in replicates with no FUdR synchronization and the experimental cultures synchronized with 5 µg/ml FUdR (Sigma-

Table I.- Comparison of the expression of folate sensitive chromosomal lesions between British and Pakistani sheep breeds at different FUdR levels.

Parameters	5µg/ml		5µg/ml		Test statistics	
	Lohi	Suffolk	Lohi	Suffolk	0µg/ml	5µg/ml
Average AC per animal	0.56± 0.15	1.91± 0.34	2.18±0.33	13.26±0.85	P < 0.05	P < 0.05
Average NoA per animal	0.59±0.16	2.36±0.48	2.65±0.50	21.87±1.88	P < 0.01	P < 0.01

F0530) 24 hour prior to harvesting. Blood cultures were harvested using standard harvesting technique. The slides were prepared and giemsa stained for microscopy.

Each slide prepared from control as well as experimental cultures, was screened to record cytogenetic data on AC and NoA. A light microscope (oil immersion x1000) was used to screen 50 good quality mitotic spreads in control and FUdR treated cultures from each animal. Aberrant cells were photographed with a manual orthomate camera using Kodak Technical Pan film. The slides were then de-stained with 3:1 methanol acetic acid fixative and stored at –20 °C until ready for GTG-banding using standard technique (Seabright, 1971). Aberrant cells with optimal GTG-bands were re-photographed and chromosomal lesions were mapped to individual chromosomal bands using G-band nomenclature (Di Berardino *et al*., 1983). The data on AC and NoA was analysed using Mann-Whitney, a non-parametric statistical test (Minitab Statistical Software Release 3.11) to determine variation in the frequency of fragile site expression between different treatments and groups. Individual chromosomal band data with gaps and breaks was analysed to identify statistically fragile bands (fragile sites) using multinomial statistical model (Bohm *et al*., 1995).

RESULTS

A comparison of AC and NoA means with standard errors between British Suffolk and Pakistani Lohi is summarised in Table I. The AC index from control cultures was 1.12% and 3.70% in Lohi and Suffolk breeds respectively. AC means per animal in metaphase cells grown in the control cultures were 0.56±0.15 and 1.91±0.34 in two breeds respectively. Statistical comparison revealed variation in AC means to be significant (P<0.05) between the two groups with Suffolk sheep expressing approximately three times more chromosomal breaks and gaps and breaks compared to Lohi animals. Chromosomal instability in Suffolk breed was manifold in 5 µg/ml FUdR treated chromosomes. Consequently AC index was 4.34% with corresponding average values per animal being 2.18±0.33 respectively in Lohi and Suffolk breeds. The scale of variation for AC means in control cultures of Suffolk was high enough to be comparable with FUdR treated cultures from Lohi breed.

NoA was taken as a measure of the extent of chromosomal instability within each cell of an individual animal to reflect the scale of chromosome damage in spontaneously or induced cells in overall metaphase cells scored and recorded. Individual NoA means in metaphase cells grown in control cultures was 1.91 ± 0.34 in Suffolk sheep. Statistical comparison revealed variation in NoA means to be significant higher (P<0.05) when compared between the two groups. Genomic instability following 5µg/ml FUdR treatment in Suffolk breed was also recoreded to be exceptionally higher for breed comparison exposed to different environmental condition. Calculated NoA index was 28.33% with corresponding average NoA values per animal being rising from 2.18 ± 0.33 in Lohi shep to 13.26 ± 0.85 respectively in Suffolk breed. The scale of variation for AC and NoA means in control cultures of Suffolk was high enough to be comparable with FUdR treated cultures from Lohi breed.

Distribution comparison between Lohi and Suffolk breeds revealed significant variation in the number of individual chromosomal bands expressing gaps and breaks (Table II). It was clearly

Table II.- Distribution comparison and identification of fragile sites between Lohi and Suffolk Sheep breeds *(Ovis aries).*

0µg/ml		5µg/ml		FSM995*	
Lohi Bands	**Suffolk Bands**	**Lohi Bands**	**Suffolk Bands**	**Lohi Bands**	**Suffolk Bands**
3q12	1p12	1p14	1p12	2q21‡	1p12
5q32	1p14	1p22	1p14	8q12‡	1p14
8q12	1q12	2p21	1p22	9q12‡	1p22
9q22	1q21	2q14	1p24	10q14‡	1p33
9q24	1q23	2q21	1p31		1q12
10q14	1q41	2q32	1p33		1q14
19q14	2p21	3q12	1q12		1q21
	2q12	4q17	1q14		1q36
	2q21	5q32	1q21		2p12
	2q41	6q22	1q36		2p21
	3p11	6q24	1q41		2q12
	3p15	8q22	2p12		2q14
	3q12	8q24	2p21		2q21
	3q24	9q12	2p23		2q34
	4q12	9q22	2p32		2q41
	4q16	10q12	2q12		3p11
	5q22	10q14	2q14		3p15
	6q26	14q31	2q21		3p22
	7q31	15q12	2q23		3q12
	9q12	16q14	2q34		3q24
	9q24	18q12	2q41		4q12
	10q12	19q14	3p11		4q14
	10q14	Xq17	3p13		4q16
	12q14	Xq22	3p15		4q18
	14q31	Xq24	3p22		4q110
	17q		3q12		4q22
	20q14		3q22		5q12
	Xq13		3q24		5q22
	Xq24		4q12		6q22
			4q14		6q26
			4q16		6q32
			4q18		7q12
			4q110		7q24
			4q22		7q31
			5q12		8q12
			5q22		8q22
			5q24		8q24
			5q32		8q32
			5q34		9q
			6q12		9q12
			6q22		9q14
			6q26		9q22
					9q24
					10q12
					10q14
					10q22
					10q24
					12q14
					13q12
					13q22
					14q31
					15q12
					16q14
					18q12
					20q14
					Xq13
					Xq15
					Xq24
					Xq26

FSM995 Software to analyse significantly fragile bands from chromosome breakage data (Bohm *et al.*, 1995).

obvious that fewer chromosomal lesions were expressed in fewer chromosomal bands in Lohi genome expressing only 7 fragile bands compared to 24 bands in the Suffolk breed in control (spontaneous chromosome damage) cultures. However, FUdR treated chromosomes in the two breeds revealed 29 and 78 autosomal bands respectively. Similarly the number of statistically fragile bands in Lohi genome was 4 compared to 58 in the Suffolk breed. Lohi X-chromosome also demonstrated high level of stability even in FUdR treated cultures with no fragile sites identified.

DISCUSSION

Number of reports describing significant variation in the expression of folate sensitive fragile sites from different subjects depending upon age, health status, breed and sex in different species are available (Carlose *et al*., 2005; Di Berardino *et al*., 1983; Kahkönen, 1988). Some evidence also exists on non significant breed variation in the expression of folate sensitive fragile sites between two breeds of dog (Stone *et al*., 1991) however, no studies have reported variation of the magnitude found in the present study.

There are a number of reports describing significantly higher chromosomal damage in mammals exposed to high level of organic environmental pollutants (Ahmad *et al*., 1998; Carlose *et al*., 2005; Stone *et al*., 1991; Hongell, 1996; Iredi *et al*., 1998; Randerath, 1999; Roderiguez *et al*., 1997). High organic energy usage by industry and automobiles, in turn, significantly increases the levels of environmental pollutants. It has been described that energy consumption in various sectors in the United Kingdom is associated with high emissions of gasses such as CO_2, SO_2, oxides of nitrogen (NOx), CO and volatile organic compounds (VOC). United Kingdom alone is reported to produce 3% of the world's total emission of CO_2 per year (Darkwa, 1996). In addition to the global climate change, the biological impact of environmental pollutants sets a foundation to accelerate environmental mutagenesis. This leads to micro and macro level mutations in chromosomal DNA resulting in elevated incidence of congenital abnormalities, developmental malformations,

abortions and fertility problems (Iannuzzi and Di-Berardino, 2008). About 25% of abortions and 2.5% of abnormal foetuses in two flocks of sheep raised in Naples were reported to be a direct result of high level of dioxins in the environment (Schoket, 1999). Similarly, significantly higher chromosomal lesions, congenital abnormalities and higher rates of dynamic mutations both in animals and humans have been reported (Sharara *et al*., 1998; Sram *et al*., 1999; Uchida *et al*., 1986; Yang and Long, 1993). It may be concluded from the chromosomal instability data presented in this study that the genomic stability exhibited by Lohi genome is attributable to its exposure to environment with relatively low level of industrial and automobile pollutants compared to Suffolk which is raised in a heavily industrial area in UK.

The analysis of spontaneously occurring and induced fragile sites also contributes to better understanding the processes shaping mammalian genome evolution. Common fragile sites are usually termed as the hotspots in the mammalian genome which tend to breakaway and relocate on different other points of the same significance to evolve new combinations of sometimes positive nature (evolution) or negative nature in the form of deleterious mutations (Sutherland and Hecht, 1985). Correlation of translocation breaktpoints with fragile sites in the present study (data not presented) and elsewhere suggests high coincidence of break sites in the two phenomena (Yang and Long, 1993; Ronne, 1997). Some scientific evidence also exists comparing the points of common fragile sites with breakpoints of evolutionary significance in different species. A high level of coincidence was found between two types of breakpoints, especially in the case of X-chromosome due to its wide spread conservation among different mammalian species Smeets and Klundert (1990); Stone *et al.* (1991); Ronne (1992).

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