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INTRODUCTION

Fragile sites are specific chromosomal loci prone to breakage which are observed as gaps, breaks or rearrangements under appropriate *in vitro* culture conditions. More than 100 fragile sites have been identified in human chromosomes and classified according to their mode of induction and population frequency (Sutherland and Ledbetter, 1989). All fragile sites, by definition, are inducible in culture, and induction results in their expression or elevates the proportion of cells in which fragile sites are observed if they are 'spontaneously' expressed (Sutherland and Hecht, 1985). Fragile sites are also identified as rare (occurring in less than 1% of the population) or common.

One useful fragile site-inducing agent is aphidicolin (APC). This chemical is a selective inhibitor of DNA polymerase-α, the major eukaryotic polymerase which adds deoxynucleotide monophosphates to the 3′-end of a DNA primer, during both replicative and excision repair DNA synthesis (Fry and Loeb, 1986). At low concentrations (0.2 μM), APC has been used to induce fragile sites in human lymphocyte cultures without significantly decreasing mitotic yield (Glover *et al*, 1984).

The significance of fragile sites remains unclear, although numerous hypotheses have been suggested linking fragile sites to cancer chromosome breakpoints and protooncogene locations (LeBeau, 1988), reciprocal translocation breakpoints (Riggs and Chrisman, 1989), chromosome rearrangements resulting in spontaneous abortion and infertility (Schlegelberger *et al*, 1989) and evolutionary preservation of syntenic groups (Threadgill and Womack, 1989). Chromosome aberrations, especially reciprocal translocations, are of economic importance in the domestic pig

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because they usually result in significantly reduced fertility (reviewed by Popescu *et al*, 1984).

Although extensively documented in human chromosomes, the study of fragile sites has been neglected in domestic animals. Since fragile sites are distributed non-randomly, and it has been suggested that reciprocal translocations are distributed non-randomly across the pig genome (Fries and Stranzinger, 1982), this investigation was undertaken to study common fragile sites in pig chromosomes. The initial objectives of the project were 3-fold: 1) establish protocols for studying fragile sites in chromosomes of domestic animals, specifically the pig; 2) identify band locations of fragile sites in pig chromosomes; 3) determine whether a correlation exists between common fragile site locations and reciprocal translocation breakpoints.

MATERIALS AND METHODS

Peripheral blood was collected *via* the anterior vena cavae of 2 male and 2 female purebred *Duroc* pigs, and 7 male and 8 female 3- or 4-way crossbred pigs (*Yorkshire* × *Landrace* × *Hampshire* ; *Yorkshire* × *Chester White* × *Hamsphire* ; *Yorkshire* × *Landrace* × *Hampshire* × *Duroc* ; *Yorkshire* × *Chester White* × *Hampshire* × *Duroc*). Standard cultures were established with RPMI-1640 (Gibco), 10% fetal bovine serum (Gibco), 2% phytohemagglutinin-M (Gibco), 1% l-glutamine, 0.3% sodium heparin, 0.5% gentamicin sulfate and 5% whole blood. Cultures were grown in 5% CO_2 in air at 37°C for 64.5 h. Aphidicolin (Sigma) was dissolved in ethanol and diluted with saline. Cultures received APC or the carrier solution 24 h prior to harvest.

To establish dose–response curves and determine the optimal APC concentration for study, 50 solid-stained metaphases per culture were scored for gaps, breaks and rearrangements in both crossbred and *Duroc* pigs. Chromosome preparations from controls and cultures that received 0.2 μM APC were trypsin–Giemsa (GTG-) banded, and band locations according to the international nomenclature (Committee for the Standardized Karyotype of the Domestic Pig, 1988) were assigned to observed gaps, breaks and rearrangements.

RESULTS AND DISCUSSION

Aphidicolin induced gaps, breaks and rearrangements in pig chromosomes when added to lymphocyte cultures 24 h before harvest. Induction of chromosome aberrations was dependent upon the APC concentration, and response was similar to that demonstrated for human chromosomes by Glover *et al* (1984). Gaps, breaks and rearrangements induced by 0.2 μM APC were analyzed from 345 metaphase plates from 7 individuals. A total of 345 aberrations were observed at 94 different band locations. Only 3 breaks were observed in 350 metaphases from control cultures.

So-called common fragile sites were identified by χ^2 analysis. Based on a 287-band, standard GTG karyotype, and assuming each band had an equal probability of breakage, the expected number of breaks from the 345 aberrations induced by 0.2 μM APC was 1.20 breaks/band. χ^2 analysis indicated that any band with 4

or more breakage events was significantly damaged (χ^2, 1 $df \geq 4.41$ with Yates' correction; $P < 0.05$). Band locations of fragile sites with 4 or more breakage events are listed in table I. Of 94 different breakpoints, 21 locations were significantly damaged.

Table I. Aphidicolin-induced fragile sites in domestic pig chromosomes.

No of breaks[a]	*Band locations*
4[b]	4q15, 6p15, 13q33, Xp24
5[c]	1q21.1, 11p13, 13q41, 17q21, Xq24
6[d]	1p21, 1q17, 4q23, 6q31
7[d]	4p15, 13q21
11[d]	Xq26
14[d]	1p25, Xq22
21[d]	1p23
31[d]	10p15
47[d]	4q25

[a] Number of breaks observed at specific band locations in 345 metaphases from 7 individuals.
[b] $P < 0.05$; [c] $P < 0.005$; [d] $P < 0.001$.

Breakpoints of 29 reciprocal translocations (43 different breakpoints) reported in the literature were compared to APC-induced chomosome breakpoints. Twenty-nine locations were identified as both translocation and APC-induced breakpoints. χ^2 analysis indicated that APC-induced breakage and reciprocal translocation breakpoints were not independent ($\chi^2, 12\ df = 57.30; P < 0.001$).

The primary objective of this study was to establish protocols for studying fragile sites in pig chromosomes and identify the common APC-induced fragile sites in the pig genome. At 0.2 μM concentration, as in previous human fragile site studies, APC was useful for inducing non-random gaps, breaks and rearrangements, *ie*, fragile sites. Twenty-one fragile sites located on 8 chromosomes (1, 4, 6, 10, 11, 13, 17, X) were identified. The relationship of fragile sites to reciprocal translocation breakpoints was also examined. Breakage was induced in 29 bands which had been previously identified as translocation breakpoints. Preliminary analysis indicated that the two events were not independent. Stronger conclusions cannot be drawn at this time, however, because no data are available concerning the actual population frequency of *de novo* (or environmentally induced) translocation incidents. Also, translocations which arise at some fragile sites may result in inappropriate gene expression and be lethal. These translocations would probably never be observed.

This study was designed as a preliminary examination of chromosomal fragile sites in domestic animals. A better understanding of the nature of fragile sites should provide insight for clinical and research investigations on mechanisms of environmental mutagenesis and *in vivo* induction of chromosome aberrations associated with reproductive problems.

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