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Mapping of genes belonging to the halothane linkage group in pigs using *in situ* hybridization

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INTRODUCTION

In pigs, the halothane (HAL) linkage group, which comprises the loci for glucose phosphate isomerase (GPI), HAL, the blood groups S and H, α-1-glycoprotein (A1BG) and 6-phosphogluconate dehydrogenase (PGD), has been intensively studied (see review by Archibald and Imlah, 1985). The HAL locus is important with respect to the genetic predisposition of pigs to develop porcine stress syndrome (PSS). Based on physiological evidence, the calcium release channel (CRC) of the sarcoplasmic reticulum has been proposed as a candidate for the defective protein in malignant hyperthermia (MH). Recently, the CRC gene – referred to as the ryanodine receptor (RYR) – has been found to be tightly linked to the MH gene in humans (MacLennan *et al*, 1990). The present study was undertaken to sublocalize two known member loci of the HAL linkage group, *viz* GPI and PGD, along with the localization of the CRC gene in pigs.

MATERIALS AND METHODS

The probes used were: 1) a porcine genomic GPI-specific DNA fragment, 2) a porcine PGD cDNA and 3) a porcine CRC cDNA. The probes were radioactively labeled using the random-priming method (Feinberg and Vogelstein, 1983), modified for tritium labeling (Lin *et al*, 1985). The specific activity of the probes ranged from $4–7 \times 10^8$ dpm/μg.

Chromosome preparations were obtained from pokeweed-stimulated pig lymphocytes that had been cultured for 72 h. 5-Bromodeoxyuridine was added (200 μg/ml of medium) for the final 7 h of the culture. The chromosomes were identified using the RBA-banding technique (Dutrillaux *et al*, 1973) before hybridization, and the GTG-banding technique (Popescu *et al*, 1985) after hybridization. The technique of *in situ* hybridization was essentially the same as that described by Harper and Saunders (1981) with some modifications. The concentration of the DNA probe in the hybridization mixture was 100 μg/ml (3 ng/slide). The slides were exposed for 18–25 days in light-tight boxes with dessicator at +4°C. The grains scored were plotted on the idiogram of G-banded pig chromosomes (Committee for the Standardized Karyotype of the Domestic Pig, 1988), and the results were analyzed using standard statistical procedures. Chromosome measurements according to Lin *et al* (1980) were used. The χ^2-test was used for statistical analysis.

RESULTS

For the GPI gene, a total of 124 metaphases (67 RBA-banded and 57 GTG-banded) from one hybridization experiment were analyzed and the grains plotted as shown in figure 1a. Of the total 646 grains scored, 163 (25%) were located on chromosome 6 ($P < 0.001$). Analysis of the grain distribution on this chromosome revealed that about 126 (77%) grains were clustered on the p12–q21 segment, and 106 (65%) on the cent–q21 segment. The latter area forms less than 1% of the total genome. On this basis, there is a high probability that the GPI gene is located in this segment ($P < 0.001$). There was a clear predominance of grains on the q arm near the centromere with the peak clustering on the q12 band which could be proposed as the probable site of the GPI gene.

For the PGD gene, a total of 180 GTG-banded metaphases from two hybridization experiments were analyzed, and the grains plotted as shown in figure 1b. In all, 1052 grains were scored, of which 235 (22%) were located on chromosome 6 ($P < 0.001$). Of the total number of grains on this chromosome, 21 (9%) were present on the p arm and 214 (91%) on the q arm. On the q arm, the grains mainly clustered on the 6q25–q27 segment, which had a total of 117 grains. This segment forms approximately 1% of the total genome and had 11% of the total number of grains. On this basis, the probability that the PGD gene is located in the 6q25–q27 segment is highly significant ($P < 0.001$).

For the CRC gene, a total of 208 GTG-banded metaphases from one hybridization experiment were analyzed and the grains plotted as shown in figure 1c. In all, 1456 grains were scored, of which 298 (20%) were located on chromosome 6 ($P < 0.001$). Of the total number of grains on this chromosome, 35 (12%) were present on the p arm and 263 (88%) on the q arm. On the q arm, the grains mainly clustered on the cent–q21 segment, which had a total of 192 grains. This segment forms approximately 1% of the total genome and had 13% of the total number of grains. Indications from the grain distribution are that the gene for CRC is located in the p11–q21 segment of chromosome 6, with a very high probability of it being present on the q arm in the cent–q21 segment ($P < 0.001$). The signal peaked on the q12 band, which can be proposed as the possible site of the CRC gene.

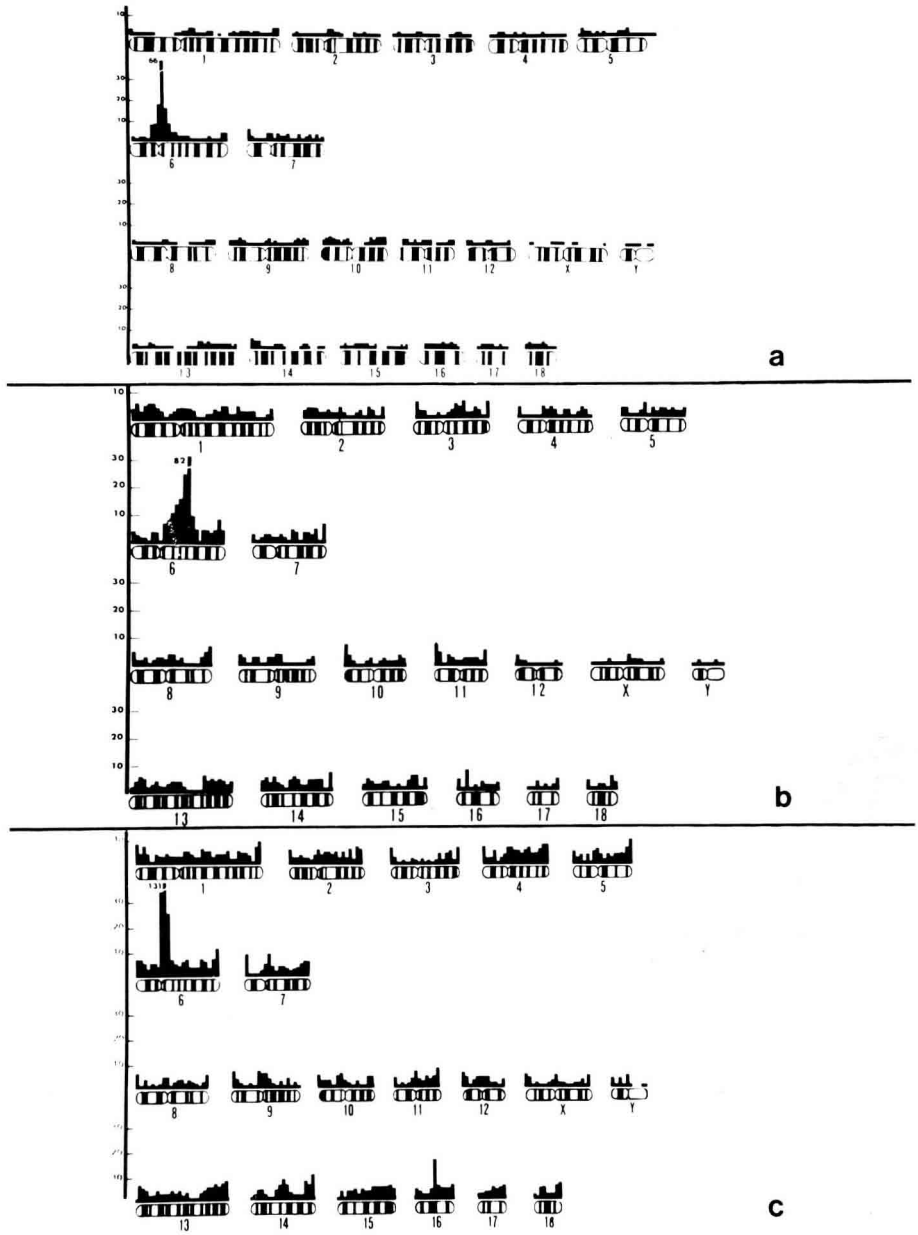

Fig 1. Grain distribution after *in situ* hybridization of (**a**) porcine genomic GPI-specific DNA, (**b**) porcine PGD cDNA (**c**) porcine CRC cDNA to pig metaphase chromosomes (courtesy of *Hereditas* and *Genomics*).

DISCUSSION

The *in situ* hybridization results thus confirm that the HAL linkage group is located on chromosome 6 in pigs, and that the CRC locus is within this complex. Interestingly, the signal of the CRC peaked on the same segment (6q12) as that of the GPI gene. Various studies have shown that, of the known loci, GPI is located closest to the HAL locus (see Archibald and Imlah, 1985). The localization of the CRC gene at almost the same site, together with the accumulated evidence of possible involvement of this gene in causing MH both in humans and pigs is a powerful argument that the basic defect in MH results from a mutation in the CRC gene. However, conclusive proof of this hypothesis will require the identification of a mutation in the CRC gene exclusively found in pigs, a mutation that directly affects the regulation of the calcium release process.

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