Genet Sel Evol (1991) 23, suppl 1, 226s-230s

Localization on pig chromosome 6 of five markers: GPI, APOE, TGFβ1, ENO1 and PGD, carried by human chromosomes 1 and 19, using *in situ* hybridization

M Yerle, M Dalens, O Galman, J Gellin

Institut National de la Recherche Agronomique, Laboratoire de Génétique Cellulaire, BP 27, 31326 Castanet-Tolosan, France

(Proceedings of the 9th European Colloquium on Cytogenetics of Domestic Animals; Toulouse-Auzeville, 10-13 July 1990)

pig / molecular *in situ* hybridization / comparative mapping / halothane gene

INTRODUCTION

In the pig, the linkage group S-GPI-HAL-A1BG-PGD bearing the halothane gene (HAL: responsible for malignant hyperthermia (MH)) has been assigned to the p12 → q21 region of chromosome 6 by Davies *et al* (1988) and Chowdhary *et al* (1989). This region includes part of the short arm, the centromere, and part of the long arm. Using *in situ* hybridization combined with high-resolution chromosome banding analysis, we increased the precision of the localization of glucose phosphate isomerase (GPI) on pig chromosome 6. Then, with the objective of finding new markers around the HAL gene, we selected information from comparative mapping. GPI and 6-phosphogluconate dehydrogenase (PGD) are linked in the pig, but are located on different chromosomes in many other species, including man and mouse. GPI is situated on human chromosome 19 (q13.1 region) and PGD on human chromosome 1 (pter → p36.13 region). Thus, parts of pig chromosome 6 correspond to parts of human chromosomes 1 and 19. We chose markers near the human GPI and PGD loci: APOE (apolipoprotein E), TGFβ1 (transforming growth factor β1), ENO1 (enolase 1), in addition to GPI and PGD, to study their localization in the pig by molecular *in situ* hybridization.

MATERIALS AND METHODS

All the techniques used have been described elsewhere (Yerle *et al*, 1990a). The chromosomes were G-banded before hybridization using the GTG technique. The technique of Rønne *et al* (1987) was slightly modified and used to obtain

prometaphase chromosomes with high-resolution G-banding (Yerle *et al*, 1991). The best metaphases were selected and photographed before hybridization. Metaphase and prometaphase chromosomes were classified according to the recommendations given by the Committee for the Standardized Karyotype of the Domestic Pig (1988).

The characteristics of the DNA probes used have been described elsewhere (Yerle *et al*, 1990a, b).

For each marker, a histogram showing the grain distribution on the chromosomes was produced. One of these histograms is presented in figure 1. A statistical evaluation of the number of silver grains per unit chromosome length was made using a Poisson distribution.

RESULTS AND DISCUSSION

The results, which are summarized in table I, demonstrate that the markers are situated on the long arm of pig chromosome 6 and, more precisely, GPI on band q12, APOE on band q21.2, TGFβ1 in the cen → q21 region, ENO1 in the q22 → q24 region, and PGD in the q22 → q25 region.

Table I. Localization of GPI, APOE, ENO1, PGD and TGFβ1 on pig chromosome 6 by molecular *in situ* hybridization.

Locus	*No of cells analyzed*	*Total no of grains on the chromosomes*	*No of grains on chromosome 6*	*No of grains in the precise region of localization*
GPI	metaphases: 74		219	106 (48%) in p1.1 → q12
	prometaphases: 67		80	52 (65%) in cen → q12 42 (53%) on band q12
APOE	metaphases: 158	914	112 (12%)	48 (43%) in cen → q21
	prometaphases: 28		42	22 (52%) in q12 → q21.2 19 (45%) on band q21.2
ENO1	metaphases: 201	1413	216 (15%)	102 (47%) in q22 → q24
PGD	metaphases: 75	714	181 (25%)	96 (53%) in q22 → q25
TGFβ1	metaphases: 79	559	96 (17%)	61 (64%) in cen → q21

From these data, it can be concluded that pig chromosome 6q contains regions homologous to human chromosome 19 (region q13.1 → q13.2) and human chromosome 1 region (pter → p36.13), which in turn are homologous to regions of murine

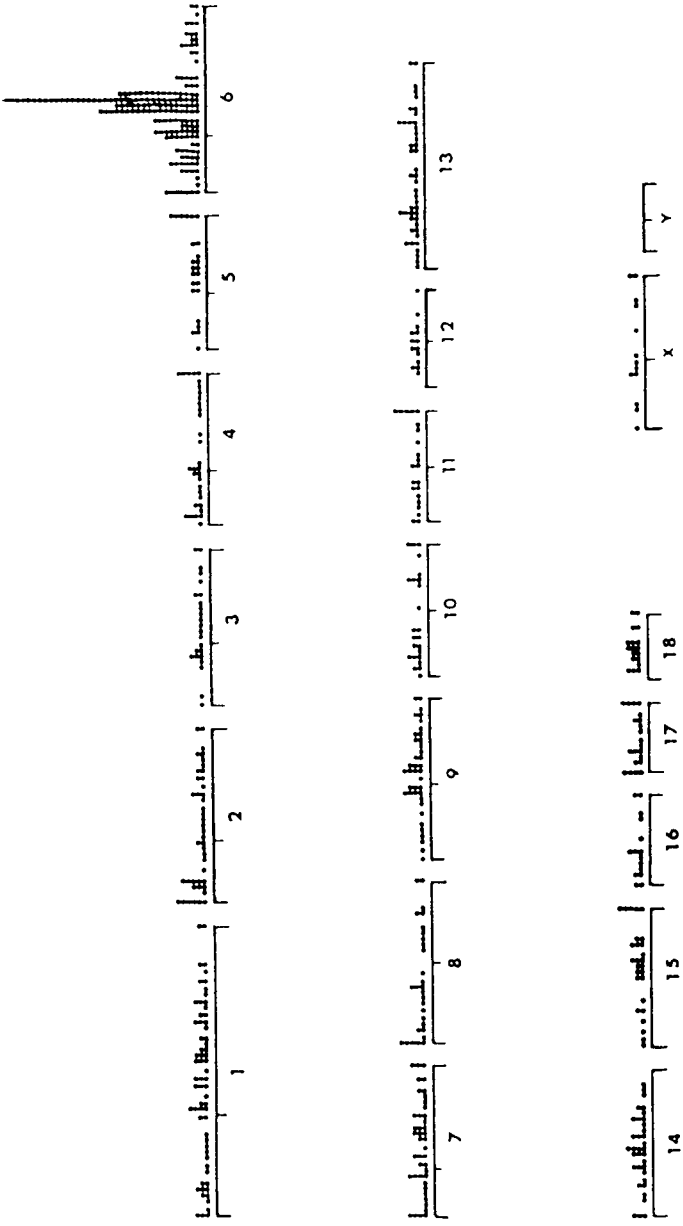

Fig 1. Chromosomal assignment of PGD by *in situ* hybridization. Histogram showing the grain distribution in 75 pig metaphases. A highly significant number of grains was found on chromosome 6.

chromosomes 7 and 4, respectively (fig 2). It is of further interest that the locus responsible for malignant hyperthermia (MH) in man has recently been mapped to the q13.1 region of HSA 19 (McCarthy *et al*, 1990). The assignment of the ryanodine receptor gene (RYR) to HSA 19 cen → q13.2 in man (MacLennan *et al*, 1990), to chromosome 6 region p11 → q21 in the pig (Harbitz *et al*, 1990) and to chromosome 7 in the mouse (Cavanna *et al*, 1990) further enhances the likelihood that the same gene is responsible for MH in man and pig. However, another gene, the hormone-sensitive lipase (LIPE) gene, which has been localized in the same region of human chromosome 19, could also be a good candidate for the gene responsible for malignant hyperthermia as suggested by Levitt *et al* (1990).

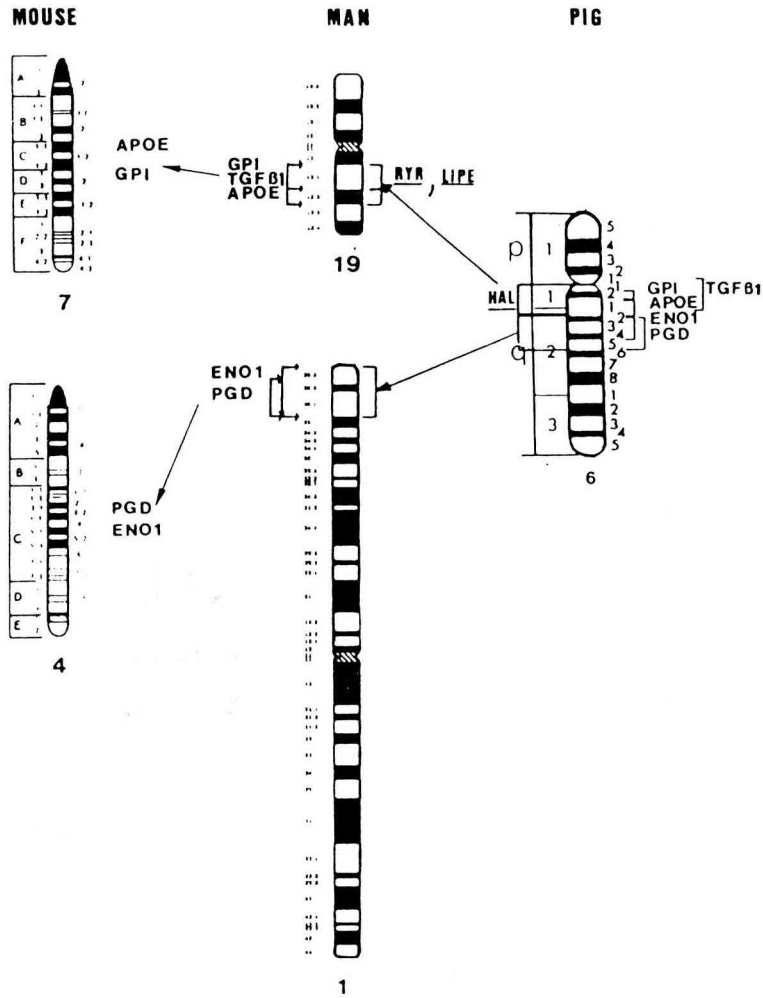

Fig 2. Comparative mapping in man, pig and mouse. Determination of homologous regions.

The results presented here confirm that the conservation of the syntenic groups around GPI and PGD is also maintained in pigs and that malignant hyperthermia in man and pigs is likely to be the result of mutations in homologous genes.

REFERENCES

Cavanna JS, Greenfield AJ, Jonhson KJ, Marks AR, Nadal-Ginard B, Brown SDM (1990) Establishment of the mouse chromosome 7 region with homology to the myotonic dystrophy region of human chromosome 19q. *Genomics* 7, 12-18

Chowdhary BP, Harbitz I, Mäkinen A, Davies W, Gustavsson I (1989) Localization of the glucose phosphate isomerase gene to the p1.2 → q2.1 segment of chromosome 6 in pig by *in situ* hybridization. *Hereditas* 111, 73-78

Committee for the Standardized Karyotype of the Domestic Pig (1988) Standard karyotype of the domestic pig. *Hereditas* 109, 151-157

Davies W, Harbitz I, Fries R, Stranzinger G, Hague JG (1988) Porcine malignant hyperthermia carrier detection and chromosomal assignment using a linked probe. *Anim Genet* 19, 203-212

Harbitz I, Chowdhary B, Thomsen P, Davies W, Kaufman U, Kran S, Gustavsson I, Christensen K, Hauge J (1990) Assignment of the porcine calcium release channel gene, a candidate for the malignant hyperthermia locus to the 6p11 → q21 segment of chromosome 6. *Genomics* 8, 243-248

Levitt RC, McKusick VA, Fletcher JE, Rosenberg H (1990) Gene candidate. Letter *Nature* 345, 297-298

McCarthy TV, Healy JMS, Heffron JJA, Lehane M, Deufel T, Lehmann-Horn F, Farrall M, Johnson K (1990) Localization of the malignant hyperthermia susceptibility locus to human chromosome 19q12 → 13.2. *Nature* 343, 562-563

McLennan DH, Duff C, Zorgato F, Fujii J, Phillips M, Korneluk RG, Frodis W, Britt BA, Worton RG (1990) Ryanodine receptor gene is a candidate for predisposition to malignant hyperthermia. *Nature* 343, 559-561

Rønne M, Stefanova V, Di Berardino D, Poulsen BS (1987) The R-banded karyotype of the domestic pig (*Sus scrofa domestica* L). *Hereditas* 106, 219-231

Yerle M, Gellin J, Dalens M, Galman O (1990a) Localization on pig chromosome 6 of markers GPI, APOE, ENO1 carried by human chromosomes 1 and 19 using *in situ* hybridization. *Cytogenet Cell Genet* 54, 86-91

Yerle M, Archibald AL, Dalens M, Gellin J (1990b) Localization of the PGD and TGFβ1 loci to pig chromosome 6q. *Anim Genet* 21, 411-417

Yerle M, Galman O, Echard G (1991) The high-resolution G-T-G banding pattern of pig chromosomes. *Cytogenet Cell Genet* 56, 45-47