Position of the \textit{Phi} and \textit{Po2} loci in the \textit{Hal} linkage group in pigs

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(received 17 May 1988, accepted 8 October 1988)

Summary — Families of Swiss Landrace (165 litters with 1348 offspring) were tested for halothane sensitivity, \textit{A-O(S)}, \textit{H}, \textit{Phi}, \textit{PGD} and \textit{Po2} phenotypes. Informative matings for the determination of the gene sequence of these linked loci were selected. Recombinations were observed between \textit{Phi-Hal}, \textit{Phi-H} and \textit{H-Po2}. On the basis of these results the most likely order of loci is \textit{Hal-Phi-H}. Confirmation for a locus for genes for \textit{Po2} separate from the locus for \textit{H} is presented. The location of \textit{Po2} is between \textit{H} and \textit{Pgd}. A gene order \textit{S-Hal-Phi-H-Po2-Pgd} is proposed.

Introduction

The linkage between the \textit{H} blood group locus and the loci for the variants of 6-phosphogluconate dehydrogenase (\textit{Pgd}) and phosphohexose isomerase (\textit{Phi}) was first described by Andresen (1971). Rasmussen & Christian (1976) reported an association between \textit{H} genotypes and susceptibility to halothane-induced stress. Jørgensen \textit{et al.} (1976) postulated that the association between \textit{H} and porcine stress and the linkage of \textit{Phi} and \textit{H} was the causal link for the association between \textit{Phi} genotypes and stress susceptibility. The inheritance of halothane-induced stress has been shown to be controlled by a recessive gene at a single locus (\textit{Hal}) with incomplete penetrance (Ollivier \textit{et al.}, 1975; Smith &
Bampton, 1977). \( N \) symbolizes \( Hal^N \) and \( n, Hal^n \). Pigs that are \( N/N \) or \( N/n \) should therefore be \( HAL^- \) non reactors and \( n/n \) signifies a \( HAL^+ \) reactor.

Linkage studies between \( Hal \) and \( Phi \) have not made it possible to place the \( Hal \) locus accurately within the linkage group. Using a method for calculation of relative linkage disequilibrium coefficients, Andresen (1979) proposed that \( Hal \) was located between \( Phi \) and \( H \). The gene order \( Phi-Hal-H-Pgd \) was also supported by Rasmusen et al. (1980). Their data, however, did not permit them to distinguish between the order \( Phi-Hal-H-Pgd \) as opposed to \( Hal-Phi-H-Pgd \). Guérin et al. (1983) described two recombinants which supported the order as \( Hal-Phi-Pgd \). The recombinants were both \( HAL^- \) offspring of matings between \( Hal^Nn \) and \( Hal^n/n \) animals. The failure of these animals to react to halothane could, however, have resulted from the incomplete penetrance of the \( Hal^n \) gene.

The \( S \) locus controls the expression of the \( A \) and \( O \) antigens of the \( A-O \) blood group system in pigs by an epistatic interaction (Rasmusen, 1964 and Hojny & Häla, 1965). Two alleles are known, \( S \) being dominant over \( s \).

The relationship between \( A-O \) blood group phenotypes determined by genes at the \( S \) locus and \( HAL^+ \) animals (Rasmusen & Christian, 1976) was in agreement with the associations found between \( A-O \) and \( H \) blood group systems (Rasmusen, 1972). Hojny (1974) suggested that this association resulted, indirectly, from the genetic linkage between the \( H \) system and the \( S \) locus. Rasmusen (1981) proposed the order \( Phi-Hal-S-H-Pgd \) on the basis of recombinants between \( S \) and \( H \) as well as between \( S \) and \( Phi-Hal \). Later, two other reports provided evidence that the \( S \) locus is not within the \( Phi-Pgd \) region, but adjacent to \( Phi \) (Hojný et al., 1985; Van Zeveren et al., 1985).

Recently it has been found that the serum postalbumin-2 (\( Po2 \) locus) also belongs to the \( S- (Phi-Hal-H)-Pgd \) linkage group and is probably located between the \( H \) and \( Pgd \) loci (Juneja et al., 1983; Gahne & Juneja, 1985; Čepica et al., 1986).

The aim of this paper is to reconsider the gene order in the linkage group, especially of the \( Phi \) and \( Po2 \) loci and to establish the haplotypes (including \( Hal \) genotypes) in a population of Swiss Landrace pigs. Estimation of recombination frequencies is given elsewhere (Vögeli et al., 1988).

**Materials and Methods**

*Description of the data*

Data for this study came from Swiss Landrace pigs kept at the experimental station of the Institute of Animal Sciences during the period 1983-1988. The total number of offspring was 1348. The animals came from 165 litters produced by 29 boars and 64 sows over 3 successive generations.

*Halothane test*

At an age of 8 to 12 weeks the animals were tested for halothane sensitivity by the method of Eikelenboom & Minkema (1974). The anesthetic was a mixture of oxygen and 4% halothane (1.5 liters/min). Negatively reacting animals were exposed for 5 min. In \( HAL^+ \) pigs the anesthesia was withdrawn as soon as the symptoms of hyperthermia (muscular rigidity, increased heart rate and elevated body temperature) became apparent.
Serological tests
The A and 0 reagents were prepared from normal serum of 2 goats and were used in the hemolytic test. The alloimmune anti-Aw was applied in the dextran agglutination test.

The blood group factors Ha and Hc were tested using two reagents each. One of each exhibited dosage effects, i.e., they hemolysed red blood cells of homozygous (Ha / Ha, Hc / Hc) pigs sooner than those derived from heterozygous (Ha / Ha-, Hc / Hc-) pigs. The validity of the reaction pattern of these reagents was verified in International Pig Comparison Tests (1984 and 1987, the latter being organized by our laboratory).

Electrophoresis
The Phi and Pgd phenotypes were determined by horizontal one-dimensional agarose or starch-gel electrophoresis of hemolysates of erythrocytes (Saison & Giblett, 1969; Gahne & Juneja, 1985). The Po2 variants were detected by two-dimensional electrophoresis by the method of Juneja et al. (1983).

Parentage control
Tests for other blood marker systems (B, G, ADA, PGM, Pl1, PO1A, PI2) were conducted on all animals for the exclusion of incorrect pedigrees.

Haplotyping
The method used in the present study to determine the haplotypes was based on deducing linkage phases involving Hal and marker loci of both the parents and their offspring. A detailed description of the procedure is given by Vögeli et al. (1988). Several instances of crossing over were observed in progeny from multiheterozygous parents mated to multihomozygous parents. These were used to determine the order of the loci.

Results
Table I provides a summary of recombinations involving the Hal and Phi loci recovered in progeny from matings in which one parent was at least triply heterozygous and the other doubly or multiply homozygous. All the recombinations are informative with respect to the location of the Phi locus. The structure of parental haplotypes was inferred from various informative matings.

Assuming that the gene order is Phi-Hal-H as suggested by Rasmusen (1981) and not Hal-Phi-H, the first 5 recombinants of the first 3 matings given in Table I would have required the occurrence of a double crossover, i.e., a crossover between Phi and Hal as well as a crossover between Hal and H which is statistically extremely unlikely.

Mating of boar 8888 with female 8849 produced a recombinant (offspring 9925) resulting in an unexpected halothane negative reaction of this offspring. This recombinant could be explained as being a result of double crossover. However, incomplete penetrance of Hal+/Hal- seems more likely. Unfortunately, the recombinant offspring 9925 was not saved for breeding to determine his actual genotype. In the offspring of animals with Hal+/Hal- genotype mated to Hal+/Hal-, about 10% are classified as HAL- (Gahne & Juneja, 1985). The failure of one offspring from a total of six to react to halothane could well be the result of incomplete penetrance of the Haln gene. From these considerations the gene order of Hal-Phi-H is suggested.
Table II shows most informative recombinants between S, Hal, Phi and H on one side and Po2 and Pgd on the other. All five recombinants are informative in determining the position of the Po2 locus. From these data the gene order is H-Po2-Pgd as proposed by Juneja et al. (1983). If the gene order were Po2-H-Pgd, all 5 recombinants could only have resulted from double crossovers (Phi Î-P02 Î-H-Pgd), which is highly improbable.

Table III shows the parents and offspring of 2 litters which include recombinants involving a crossover between loci for Phi and H types. These marker loci are also consistent with a gene order of Phi-H-Po2 as opposed to H-Phi-Po2.

### Discussion

The expected Hal genotype of offspring receiving (a) recombinant haplotype (s) can be determined if the sequence between Hal and marker loci has been established. The most likely order of the marker loci including Hal was indicated as S-(Phi-Hal) -(H-Po2)-Pgd by Hojný et al. (1985) and van Zeveren et al. (1985). As these authors did not detect crossing over between Phi and Hal, they could neither prove nor disprove the reverse
sequence for the Phi and Hal loci proposed by Guérin et al. (1983). However, they confirmed that the two loci are located very close to each other.

The most important contribution of this paper is the evidence that the Phi locus is located, most probably, between Hal and H as proposed by Guérin et al. (1983) and van Zeveren et al. (1988) and not between S and Hal as previously reported by Andresen (1981) and Rasmusen (1981). This location is more firmly established by complex S-Hal-Phi-H-Po2-Pgd haplotypes of the majority of parents and offspring, including recombinants. Probably because of incomplete penetrance of the Hal gene one animal with presumed genotype Hal^h / Hal^h failed to react to halothane. Two recombinants (Table I,
offspring 275 and 695) being informative with respect to the location of the Phi locus were classified as HAL+. These two reactors certainly are Hal !l Hal ! homozygotes because the probability of a Hah l HalN or HalN / Hain pig being falsely tested as HAL+ is very low (Vögel et al., 1988).

The data in Tables II and III are consistent with a gene order of Phi-H-Po2-Pgd. The data assembled in Tables I, II and III and those contained in earlier publications indicate a gene order S-Hal-Phi-H-Po2-Pgd. The knowledge of the halothane locus and its linkage relationships is already being used in practical animal breeding to reduce the frequency of the Hal ! gene (Gahne & Juneja, 1985; Vögel et al., 1988). Looking to the future, molecular analysis of the halothane linkage group may provide a means for identifying more reliable markers for the stress genes as well as the identity of the halothane gene itself. One step in this development is the assignment of the Hal linkage group to chromosome 6 by in situ hybridization (Davies et al., 1988).

Acknowledgements

This work was supported by grants of the ETH-Zürich, the Commission for Support of Scientific Research, Berne, the Swiss Performance Testing Station, Sempach, and other public and private organizations in Switzerland. Appreciation is expressed to my coworkers Christine Karonis, Barbara Kuhn and R. Kühne for excellent technical assistance and Drs. N.S. Fechheimer, Valerie Madison, Catherine Marguerat and G. Stranzinger for comments on the manuscript. Data collection on the experimental farm are made possible by Dr. C. Gerwig and A. Kaufmann.
References

Andresen E. (1971) Linear sequence of the autosomal loci PHI, H and 6-PGD in pigs. Anim. Blood Groups Biochem. Genet. 2, 119-120

Andresen E. (1979) Evidence indicating the sequence Phi, Hal, H of the three closely linked loci in pigs. Nord Veterinaermed. 31, 443-444

Andresen E. (1981) Evidence for a five-locus linkage group involving direct and associative interactions with the A-O blood group locus in pigs. In: (E. Brummerstedt ed.), Papers dedicated to Prof. Dr. J. Moustgaard, 208-212. The Royal Danish Agricultural Society, Copenhagen.

Čepica S., Hradecký J., Hojny J., Kuryl J. & Grzybowski G. (1986) Localization of the Po2 locus in the S, Phi, Hal, H, Po2, Pgd linkage group in pigs. Anim. Genet. 17, 283-286

Davies W., Harbitz L, Fries R., Stranzinger G. & Hauge J.G. (1988) Porcine malignant hyperthermia carrier detection and chromosomal assignment using a linked probe. Anim. Genet. 19, 203-212

Eikelenboom G. & Minkema D. (1974) Prediction of pale, soft, exudative muscle with a non-lethal test for the halothane-induced porcine malignant hyperthermia syndrome. Tijdschr. Diergeneesk. 99, 421-426

Gahne B. & Juneja R.K. (1985) Prediction of the halothane (Hal) genotypes of pigs by deducing Hal, Phi, Po2, Pgd haplotypes of parents and offspring: results from a large-scale practice in Swedish breeds. Anim. Blood Groups Biochem. Genet. 16, 265-283

Guérin G., Ollivier L. & Sellier P. (1983) Etude de groupe de liaison Hal, Phi et Pgd chez le Porc: disposition relative des trois locus et estimation des taux de recombinaison. Genet. Sel. Evol. 15, 55-64

Hojny J. (1974) H blood group genotypes and expression of A and O antigens in pigs. Anim. Blood Groups Biochem. Genet. 5, 3-10

Hojny J. & Hálá K. (1965) A contribution to the study of the blood group system A in pigs. In: Blood groups of animals (J. Matousek ed.). Publishing House of the Czechoslovak Academy of Sciences, Prague. pp. 155-161

Hojny, Čepica S. & Hradecký J. (1985) Gene order and recombination rates in the linkage group S-Phi-Hal-H-(Po2)-Pgd in pigs. Anim. Blood Groups Biochem. Genet. 16, 307-318

Juneja R.K., Gahne B., Edfors-Lilja I. & Andresen E. (1983) Genetic variation at a pig serum protein locus, Po-2 and its assignment to the Phi, Hal, S, H, Pgd linkage group. Anim. Blood Groups Biochem. Genet. 14, 27-36

Jørgensen P.F., Hylgaard-Jensen J., Moustgaard J. & Eikelenboom G. (1976) Phosphohexose isomerase (PHI) and porcine halothane sensitivity. Acta Vet. Scand. 17, 370-372

Ollivier L., Sellier P. & Monin G. (1975) Détérminisme génétique du syndrome d'hyperthermie maligne chez le porc de Piétrain. Ann. Génét. Sél. Anim. 7, 159-166

Rasmusen B.A. (1964) Gene interaction and the A-O blood group system in pigs. Genetics 50, 191-198

Rasmusen B.A. (1972) Gene interaction and the A-O and H blood group systems in pigs. Anim. Blood Groups Biochem. Genet. 3, 169-172

Rasmusen B.A. (1981) Linkage of genes for PHI, halothane sensitivity, A-O inhibition, H red blood cell antigens and 6-PGD variants in pigs. Anim. Blood Groups Biochem. Genet. 12, 207-209

Rasmusen B.A. & Christian L.L. (1976) H blood types in pigs as predictors of stress susceptibility. Science 119, 947-948

Rasmusen B.A., Beece C.K. & Christian L.L. (1980) Halothane sensitivity and linkage of genes for H red blood cell antigens, phosphohexose isomerase (PHI) and 6-phosphogluconate dehydrogenase (6-PGD) variants in pigs. Anim. Blood Groups Biochem. Genet. 11, 93-107

Saison R. & Giblett E.R. (1969) 6-phosphogluconic dehydrogenase polymorphism in the pig. Vox Sanguiinis 16, 514-516

Smith C. & Bampton P.R. (1977) Inheritance of reaction to halothane anaesthesia in pigs. Genet. Res. 29, 287-292
Van Zeveren A., Van de Weghe A., Bouquet Y. & Varewyck H. (1985) The position of the epistatic S locus in the halothane linkage group in pigs. Anim. Blood Groups Biochem. Genet. 16, 297-305

Van Zeveren A., Van de Weghe A., Bouquet Y. & Varewyck H. (1988) The porcine stress linkage group. II. The position of the Halothane locus and the accuracy of the Halothane test diagnosis in Belgian Landrace pigs. J. Anim. Breed. Genet. 105, 187-194

Vögeli P., Gerwig C. & Schneebeli H. (1983) The A-O and H blood group systems, some enzyme systems and halothane sensitivity of two divergent lines of Landrace pigs using index selection procedures. Livest. Prod. Sci. 10, 159-169

Vögeli P., Kühe R., Gerwig C., Kaufmann A., Wysshaar M. & Stranzinger G. (1988) Bestimmung des Halothangenotyps (Hal+) mit Hilfe der S, Phi, Hal, H, Po2, Pgd Haplotypen von Eltern und Nachkommen beim Schweizerischen Veredelten Landschwein. Züchtungskunde 60, 24-37