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Original article

Mapping the porcine *RN* gene to chromosome 15

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Summary – Microsatellite markers have been used to map the porcine *RN* gene to chromosome 15 as previously reported. This locus affects the technological quality of meat in pigs. Resource families were established by mating 16 crossbred boars (Hampshire × Piétrain) to 65 crossbred sows (Large White × Landrace). From 564 progeny meat samples were taken from the musculus longissimus dorsi 24 h post mortem and the glycogen concentration was determined. The assessment of *RN* genotypes is based on the measurement of the glycogen concentration. Boars were heterozygous (RN^-/rn^+) and sows were homozygous (rn^+/rn^+) at the *RN* locus. A subsample of 263 animals was typed with four microsatellite markers from chromosome 15. Linkage analysis confirmed that the *RN* locus maps to chromosome 15, and showed linkage to Sw936 at a distance of 4 cM.

pig / meat quality / glycogen / microsatellite marker / linkage

Résumé – La localisation du gène *RN* porcin sur le chromosome 15. *Des marqueurs microsatellites ont été utilisés pour localiser le gène* RN *du porc sur le chromosome 15 dans une étude antérieure. Ce locus influence la qualité technologique de la viande. Les familles étudiées ici ont été construites par accouplement de 16 verrats croisés (Hampshire × Piétrain) avec 65 truies croisées (Large White × Landrace). Des échantillons de viande de 564 descendants ont été extraits du muscle longissimus dorsi 24 heures post mortem pour mesurer la concentration en glycogène. La détermination du génotype* RN *est basée sur la valeur de la concentration en glycogène. Les verrats étaient hétérozygotes* (RN^-/rn^+) *et les truies homozygotes* (rn^+/rn^+) *au locus* RN. *Une sous-population de 263 animaux a été typée à l'aide de quatre marqueurs microsatellites du chromosome 15. L'analyse des liaisons génétiques confirme que le locus* RN *est localisé sur le chromosome 15 et montre qu'il est situé à une distance de 4 cM du marqueur Sw936.*

porc / qualité de la viande / glycogène / marqueur microsatellite / liaison génétique

INTRODUCTION

A new meat quality trait was defined by Naveau et al (1985), the 'rendement technologique Napole' (RTN). This parameter describes the technological yield of

'Paris ham' processing and is correlated with the glycolytic potential in muscle tissue. Complex segregation analysis (Le Roy et al, 1990) showed that this trait is influenced by a major gene, called the *RN* gene, with two alleles, the dominant RN^- allele and the recessive rn^+ allele.

Studies by Feddern (1994) revealed that the RN^- allele is present in the Hampshire line of a German crossbreding programme, but not in the Piétrain, Large White and Landrace lines. In the presence of the RN^- allele an economic loss of 1% of the carcass value was estimated when meat is cooked. Furthermore it was revealed that the glycogen concentration in muscle tissue is highly correlated with the glycolytic potential and therefore a good parameter for characterizing the *RN* locus.

Nevertheless a DNA test would simplify the determination of *RN* genotypes. The localization of the *RN* locus within the pig genome is the first step to establish such a test. During our study Milan et al (1995) mapped the *RN* locus to chromosome 15 at a distance of 18 cM from the marker S0088. To confirm this result we screened resource families with highly polymorphic microsatellite markers from chromosome 15. This paper reports the first results of this investigation.

MATERIALS AND METHODS

Animals

Resource families were created by mating 16 crossbred boars (Hampshire × Piétrain) with 65 crossbred sows (Large White × Landrace). Each boar was mated to four to seven sows and some sows were mated to two boars. The resulting 564 offspring were fattened in the experimental pig unit Hohenschulen of the Christian-Albrechts University, Kiel, and slaughtered when they reached approximately 107 kg liveweight.

Feddern (1994) demonstrated the presence of the RN^- allele in the Hampshire line, while this allele was absent in the Piétrain, Large White and Landrace lines. Thus we expected the crossbred boars to be heterozygous (RN^-/rn^+) and the crossbred sows to be homozygous (rn^+/rn^+) at the *RN*-locus. We expected an equal proportion of both possible genotypes for the offspring.

Glycogen phenotypes and RN genotypes

Meat samples were drawn from 564 offspring at 24 h post mortem out of the longissimus dorsi and stored at −20 °C for subsequent analysis of muscle glycogen concentration, because this trait has been shown to be closely related to RTN (Le Roy et al, 1996). Thawed samples were analysed with the iodine-binding method of Dreiling et al (1987). This method consists of three steps: tissue preparation, incubation with an iodine solution, and determination of the optical density.

RN genotypes for linkage analysis were determined from the muscle glycogen concentration of the animals. Sire genotypes were determined from the distribution of the glycogen concentration within full-sib families. A bimodal distribution within families gave us strong evidence that the sires were heterozygous (RN^-/rn^+),

except for two, at the *RN* locus. Previous analyses of Feddern (1994) showed that crossbred animals of the dam line had a low glycogen concentration with a unimodal distribution ($n = 71$). Thus we confirmed that the dams of our experiment had the rn^+/rn^+ genotype. The genotypes of the offspring were determined by their muscle glycogen concentrations. The heterozygous genotype (RN^-/rn^+) was attached to animals with a high glycogen concentration (> 80 μmol/g). Animals with a low glycogen concentration (< 15 μmol/g) were classified as rn^+/rn^+. These *RN*-genotypes were used for linkage analysis and animals with a glycogen concentration between 15 and 80 μmol/g were classified as uncertain genotypes and excluded from the study. The thresholds 15 and 80 μmol/g were chosen by visual inspection of the glycogen distribution. Of course these thresholds are somewhat arbitrary but analyses with different thresholds showed that results were not greatly modified.

Marker genotypes and linkage analysis

A subsample of 263 animals was genotyped with four microsatellite markers from chromosomes 15, which were selected from the USDA map (Rohrer et al, 1994 and the corresponding database) and a map reported by Ellegren et al (1994). Primers were synthesized and labelled with fluorescein. PCR amplifications were carried out on a 9600 Perkin–Elmer–Cetus thermal cycler in a microtitre format. Four different annealing temperatures and four different numbers of cycles were tried to optimize the reaction conditions for each marker. PCR products were analysed with an automated laser fluorescence detection system (ALF, Pharmacia). PCR fragments with defined lengths were used as internal standards flanking the alleles closely. One animal was analysed on each gel with the corresponding marker and used as external standard to ensure that genotypes were comparable between gels. Offspring were always analysed with their parents on the same gel in order to avoid typing errors.

The size of the amplified fragments was determined with the ALF fragment manager software. In conjunction with the ALF fragment manager, the automated linkage preprocessor (ALP) software (Mansfield et al, 1994) converts raw data into genotypic data. This system was used to size the microsatellite alleles, check Mendelian inheritance of the markers used and create an output file for linkage analysis. Alleles were defined by the size of the amplified fragments. Genotypic data were stored in a database.

Pairwise linkage analyses between the analysed marker and the *RN* locus and a multipoint linkage analysis were performed with the CRIMAP program (Green et al, 1990). Because of the assumed heterozygosity of the boars and homozygosity of the sows at the *RN* locus, only male meioses were informative for linkage analysis.

RESULTS AND DISCUSSION

Glycogen distribution

The bimodal distribution of the muscle tissue glycogen concentration (fig 1) supports the hypothesis of Le Roy et al (1994) that the *RN* locus is a major locus with two alleles and our hypothesis that the boars were heterozygous at the

RN locus. From the presence of both alleles in almost all the families it can be concluded that the RN^- alleles has a high frequency in the Hampshire population analysed.

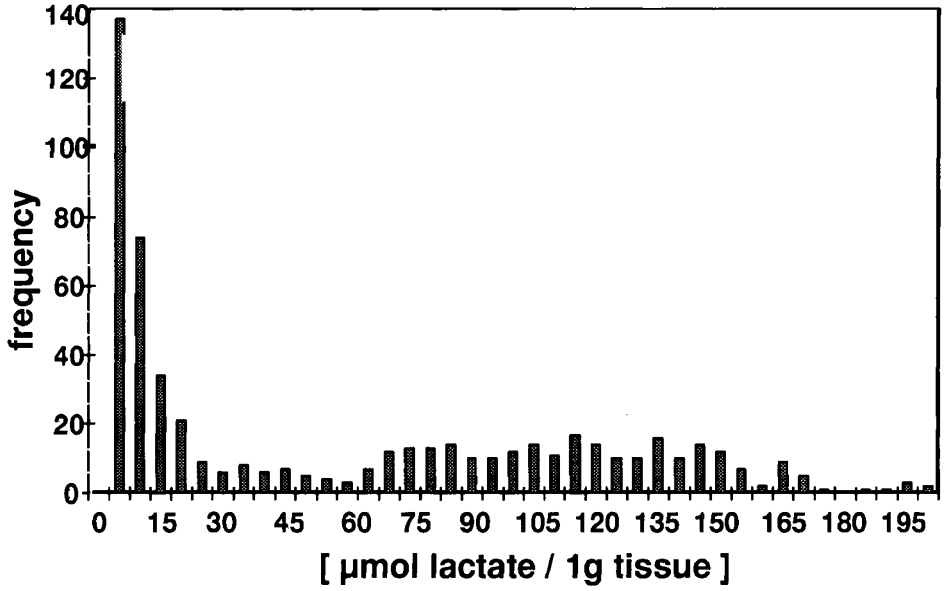

Fig 1. Glycogen concentration expressed as µmol lactate equivalents per gram wet weight in the muscle 24 h post mortem of progeny from heterozygous sires ($n = 496$).

Linkage between the markers and the RN locus

In the present study we could confirm the localization of the *RN* locus on chromosome 15. All chromosome 15 markers were linked to the *RN* locus (table I). The number of informative meioses varied between 167 and 336. Lod scores exceed the value of 3.0 for all markers. Genetic linkage between the porcine microsatellite markers Sw936 and the *RN* locus was demonstrated at a distance of 4 cM with a maximum lod score of 37.51 (table I). The *RN* locus is also linked to the marker S0088 at a distance of 20 cM, Sw964 at a distance of 21.2 cM and Swr312 at a distance of 31.6 cM.

The calculations are based only on male meioses because dams were obviously homozygous rn^+/rn^+ at the *RN* locus and consequently not informative for linkage analysis. Thus a larger sex-averaged distance between the marker Sw936 and the *RN* locus can be assumed, because of a higher recombination rate in females than in males (Ellegren et al, 1994). The distance of 20 cM between the marker S0088 and the *RN* locus is nearly the same as calculated by Milan et al (1995).

The order of the markers presented in the multipoint linkage map (fig 2) is in agreement with the map published by Rohrer et al (1994), noting however that

Table I. Pairwise linkage data to the *RN* locus.

Locus	*Number of informative meioses*	*Maximum lod score*	*Distance (Kosambi cM)*
Sw964	336	12.11	21.2
S0088	167	5.58	20.0
Sw936	323	37.51	4.0
Swr312	328	4.21	31.6

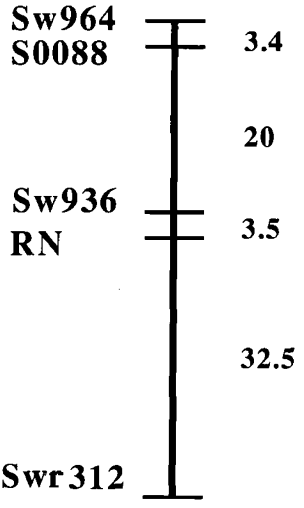

Fig 2. Multipoint map of porcine chromosome 15 around the *RN* locus. Map distances are expressed in Kosambi cM.

Swr312 has been mapped to chromosome 15 later. An important difference between the two maps exists only for the interval between markers S0088 and Sw964.

The determination of *RN* genotypes from glycogen measurements seems to be correct because misclassifications would have led to increased recombination estimates. Thus the measurement of glycogen in the muscle seems to be a good parameter to describe the *RN* locus. Nevertheless this method is relatively labour-intensive and expensive.

A marker flanking the *RN* locus at a recombination fraction of 0.04 offers the possibility of applying a positional cloning and a comparative mapping strategy. Although the gene product of the *RN* locus is still unknown, it is known from the glycogen measurements that the gene product influences the glycogen metabolism. Furthermore, comparative gene mapping studies (Johansson et al, 1995; Rettenberger et al, 1995) showed that the porcine chromosome 15 is homologous to the q-arm of the human chromosome 2. This knowledge could help to choose attractive genes from the human gene map. Subsequently the porcine sequence has to be identified and analysed for the causal mutation. The identification of the *RN* locus

and the development of a PCR-based test would offer the possibility of selecting for the *RN* locus in breeding programmes. Additionally to the measurement of the glycogen concentration, growth and carcass traits were recorded. Further analysis will reveal if the *RN* locus influences other economically important traits in pigs.

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REFERENCES

Dreiling CE, Brown DE, Casale L, Kelly L (1987) Muscle glycogen: comparison of iodine binding and enzyme digestion assays and application to meat samples. *Meat Sci* 20, 167-177

Ellegren H, Chowdhary BP, Johansson M, Marklund L, Fredholm M, Gustavsson I, Anderson L (1994) A primary linkage map of the porcine genome reveals a low rate of genetic recombination. *Genetics* 137, 1089-1100

Feddern E (1994) Verlauf der postmortalen Glykogenolyse und Muskelstrukturmerkmale bei Hampshire-Reinzuchttieren und verschiedenen Kreuzungskombinationen. PhD thesis, Kiel

Green P, Falls K, Crooks S (1990) Documentation of CRI-MAP, version 2.4, Washington, University School of Medicine, Saint Louis, MO

Johansson M, Ellegren H, Anderson L (1995) Comparative mapping reveals extensive linkage conservation – but with gene order rearrangements – between the pig and the human genomes. *Genomics* 25, 682-690

Le Roy P, Naveau J, Elsen JM, Sellier P (1990) Evidence for a new major gene influencing meat quality in pigs. *Gen Res Camb* 55, 33-40

Le Roy P, Caritez JC, Elsen JM, Sellier P (1994) Pigmeat quality: experimental study on the *RN* major locus. *Proc 5th World Congress Genet App Livest Prod* 19, 473-476

Mansfield DC, Brown AF, Green DK, Carothers AD, Morris SW, Evans HJ, Wright AF (1994) Automation of genetic linkage analysis using fluorescent microsatellite markers. *Genomics* 24, 225-233

Milan D, Le Roy P, Woloszyn N, Caritez JC, Elsen JM, Sellier P, Gellin J (1995) The *RN* locus for meat quality maps to pig chromosome 15. *Genet Sel Evol* 27, 195-199

Naveau J, Pommeret P, Lechaux P (1985) Proposition d'une méthode de mesure du rendement technologique: « la méthode Napole ». *Techni-porc* 8, 7-13

Rettenberger G, Klett C, Zechner U, Kunz J, Vogel W, Hameister H (1995) Visualization of the conservation of synteny between pigs and humans by heterologous chromosomal painting. *Genomics* 26, 372-378

Rohrer GA, Alexander LJ, Keele JW, Smith TP, Beattie CW (1994) A microsatellite linkage map of the porcine genome. *Genetics* 136, 231-245