Identification and characterization of AMPK \( \gamma \) 3 mutations in the pig

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Abstract

The RN\(^-\) (rendement Napole, French for Napole yield) phenotype is common in Hampshire pigs and is characterized by a 70% increase in glycogen content in skeletal muscle and large effects on meat characteristics (pH, water content, technological yield and lean meat content). The phenotype is controlled by an autosomal dominant allele designated RN\(^+\). The protein kinase AMP-activated \( \gamma \) 3 subunit gene, PRKAG3, which encodes the \( \gamma \) 3 isoform of AMP-activated protein kinase (AMPK), was identified as the causative gene for this phenotype by a pure positional cloning approach. There are now several lines of evidence supporting our interpretation that the RN\(^-\) phenotype is caused by a missense mutation (Arg\(^{200} \rightarrow\) Gln) in PRKAG3. Recent data from another group have revealed the presence of a third functional allele at the PRKAG3 locus, probably caused by a Val\(^{199} \rightarrow\) Ile missense mutation. This allele has opposite effects compared with RN, as it is associated with a low glycogen content. We have confirmed the phenotypic effect of this third allele in a meat-quality study of a Hampshire/Landrace intercross. A physiological characterization of RN\(^-\) carriers and normal pigs showed that the RN\(^-\) pigs utilized glycogen during exercise to the same extent as normal pigs and they showed a significantly faster resynthesis of glycogen after exercise. The results strongly suggest that the Arg\(^{200} \rightarrow\) Gln substitution is not associated with a defect in glycogen degradation, but rather with an increased glucose uptake in skeletal muscle.

Background

Strong selection on meat quality and quantity has increased the frequency of several mutations with major effects on these traits in pigs [1–3]. Studies in France on meat quality and, in particular, processing yield of ham have revealed the segregation of a major gene that affects these traits in the Hampshire breed [4]. The gene was named RN, which is an abbreviation for the French ‘rendement Napole’, meaning ‘Napole yield’, as Napole is a method for estimating the yield of cured cooked ham. Two alleles at the RN locus were defined: the recessive wild-type allele RN\(^+\) and the dominant mutant allele RN\(^-\). Subsequent phenotypic characterization revealed that the major effect of this mutation was a marked increase (approx. 70%) of the glycogen content primarily in white skeletal muscle [5,6]. Interestingly, glycogen content in liver is normal in RN\(^-\) pigs. The glycogen content in skeletal muscle of Hampshire pigs shows a bimodal distribution with almost no overlap. The two groups correspond to homozygous normal (RN\(^+\)) and RN\(^-\) carriers (RN\(^+\)/RN\(^+\) and RN\(^-\)/RN\(^-\)); RN\(^-\) appears to be fully dominant since no difference in glycogen content has been revealed between carriers and homozygous mutants. This means that pigs can be classified into these two genotypic classes simply by measuring the glycogen content from muscle biopsies or in muscle samples collected after slaughter. RN\(^-\) carriers also produce meat with a lower ultimate pH (often called acid meat) because of post-mortem degradation of the excess glycogen. Biochemical characterization has indicated that RN\(^-\) carriers have a higher oxidative capacity, measured as an increase in the activity of citrate synthase and \( \beta \)-hydroxy-acyl-coenzyme A dehydrogenase, in white muscle like longissimus but not in red muscle like semispinalis capitis [7,8].

The muscle of RN\(^-\) carriers has a higher water content and this is probably the explanation for why RN\(^-\) pigs are classified as having a higher lean meat content [9]. Lean meat content is the proportion of muscle of the total carcass weight of a pig. A major selection goal in pig breeding over the last 50 years has been to increase the lean meat content in slaughter pigs. This is probably the explanation for the very high frequency of the RN\(^-\) mutation in Hampshire pigs, which was estimated at approx. 70% before any programmes were initiated to reduce its frequency. The RN\(^-\) phenotype has not been found in other breeds, suggesting that the mutation arose in this breed and has experienced a selective sweep. Owing to the strong negative effect on processing yield, there has been a considerable interest to develop a diagnostic DNA test that can be used to eliminate the mutation from the breeding stock.

Positional cloning of the RN gene

Three European groups led by Denis Milan in Toulouse (France), Christian Looft in Kiel (Germany) and Leif Andersson in Uppsala (Sweden) collected pedigree material

Key words: glycogen, positional cloning, protein kinase AMP-activated \( \gamma \) 3 subunit (PRKAG3).

Abbreviations used: AMPK, AMP-activated protein kinase; BAC, bacterial artificial chromosome; PRKAG3, protein kinase AMP-activated \( \gamma \) 3 subunit; QTL, Quantitative Trait Locus; RN, rendement Napole (Napole yield).

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that segregated for the RN\(^{-}\) mutation and they were all able to assign the RN locus to pig chromosome 15 [10–12]. Part of pig chromosome 15 is homologous with a part of the long arm of human chromosome 2. The genetic map of human chromosome 2 around this time did not contain any obvious candidate genes for the RN locus, indicating that a positional cloning approach was required to identify the causative gene. Therefore, the three groups decided to join forces to successfully accomplish a very laborious positional cloning project in a species where the tools required for positional cloning were poorly developed at the time. The group of Patrick Chardon in Paris (France), which was in the process of establishing a porcine bacterial artificial chromosome (BAC) library [13], also joined the collaborative project. A variety of resources was developed and utilized to successfully identify the causative gene. These included (i) high resolution linkage mapping based on more than 1000 informative offspring, (ii) radiation hybrid (RH) mapping to establish a physical map of the RN region [14], and (iii) a BAC contig of the region [15]. Linkage disequilibrium mapping allowed us to map the RN gene to a region of about 200 kb [16]. Shotgun sequencing of a BAC clone from the region and subsequent bioinformatic analysis revealed four genes; one of them turned out to encode the PRKAG3 (protein kinase AMP-activated \(\gamma\) 3 subunit) gene. Sequence analysis of the Berkshire and Yorkshire founder animals revealed three missense mutations, Thr\(^{30}\) \(\rightarrow\) Ser, Gly\(^{52}\) \(\rightarrow\) Ser and Val\(^{199}\) \(\rightarrow\) Ile, that were potentially associated with the RN\(^{-}\) allele. These mutations were supported further by an extensive association study based on meat-quality traits in more than 1500 animals from five commercial lines of pigs. The most likely causative mutation is the Val\(^{199}\) \(\rightarrow\) Ile substitution that occurs at the neighbouring codon of the Arg\(^{200}\) \(\rightarrow\) Gln mutation (Figure 1); the allele associated with the Val\(^{199}\) \(\rightarrow\) Ile substitution is denoted \(m\)\(^{-}\) in Figure 1. This is a conservative substitution but valine at this position is, in fact, highly conserved among AMPK \(\gamma\) chain isoforms. The phenotypic effect of the Val\(^{199}\) \(\rightarrow\) Ile mutation is opposite to the effect of Arg\(^{200}\) \(\rightarrow\) Gln, as it reduces glycogen content and increases 

\[ pH \] in meat after slaughter. The presence of two mutations with phenotypic effects in this region implies that this part of the molecule has an important functional role.

We have recently investigated the effect of PRKAG3 alleles on meat quality and carcass composition in a cross between Landrace and Hampshire pigs [19]. All three alleles, RN\(^{-}\) (Val\(^{199}\)-Gln\(^{200}\)), \(m\)\(^{+}\) (Val\(^{199}\)-Arg\(^{200}\)) and \(m\)\(^{-}\) (Ile\(^{199}\)-Arg\(^{200}\)),
were segregating in this material. The results were in good agreement with the previous study [18] and showed that the two mutant alleles $RN^−$ and $rn^+$ are associated with opposite effects on meat quality and carcass composition traits, but the deviation from the wild-type allele $rn^+$ is more pronounced for $RN^−$. The $RN^−$ allele was associated with high glycogen content, high lean meat percentage and low ultimate pH whereas the $rn^+$ allele was associated with low glycogen content, low lean meat percentage and high ultimate pH.

**Physiological characterization of $RN^−$ pigs**

We have carried out a physiological characterization of a limited number of $RN^−$ carriers and wild-type normal ($rn^+/rn^+$) pigs (B. Essén-Gustavsson, M. Jensen-Waern, R. Jonasson and L. Andersson, unpublished work). We observed that glycogen content in heart and liver was normal in $RN^−$ carriers and the result is consistent with Northern blot data showing that $PRKAG3$ is predominantly expressed in skeletal muscle [16]. $RN^−$ carriers showed normal blood glucose and insulin levels at rest and normal glucose tolerance. Glycogen contents were also measured before and after exercise on a treadmill. The results showed that the $RN^−$ carriers utilized glycogen to the same extent as wild-type pigs and, interestingly, that they showed a faster resynthesis of glycogen after exercise. This finding demonstrates that the high glycogen content in $RN^−$ pigs is not due to a defect in glycogen degradation, but rather suggests that it is caused by a higher glucose uptake.

**Is Arg200 → Gln causative and, if so, what is the mechanism of this mutation?**

The $PRKAG3$ Arg200 → Gln mutation in pig was identified by a positional cloning approach and the significance of this mutation was supported by very strong genetic evidence. However, it is difficult to formally exclude the possibility that the $RN^−$ phenotype could have been caused by another closely linked mutation. The diagnostic DNA test for the Arg200 → Gln mutation has already been widely used by the pig-breeding industry and many thousands of breeding animals have been tested with the aim of eliminating this mutation from the breeding stock. This practical application has not revealed any conflicting data that would put in doubt that Arg200 → Gln is causing the $RN^−$ phenotype.

Strong support for the causative nature of the Arg200 → Gln mutation also comes from other studies involving the $γ1$ and $γ2$ isoforms of AMPK. The same missense mutation at the corresponding position in the CBS1 (cystathionine β-synthase domain 1) domain encoded by the human $PRKAG2$ gene (Arg202 → Gln) causes the Wolff–Parkinson–White cardiomyopathy syndrome [20] and functional studies using the Snf1–Snf4 kinase as a model suggested that this mutation leads to a constitutively active kinase [21]. The same mutation has also been introduced into the human $γ1$ isoform (Arg22 → Gln) and the functional consequences were investigated by transient expression in chimpanzee ovarian sarcoma cells [22]. The introduction of the mutation caused a marked increase in AMPK activity and the enzyme was largely AMP-independent.

We have recently generated two transgenic mice lines overexpressing (primarily in white skeletal muscle) either the wild-type form of mouse $Prkag3$ or the Arg200 → Gln mutant form (S. Marklund and L. Andersson, unpublished work). The fact that the transgenic mice expressing the Arg200 → Gln mutation show a marked increase in glycogen content in skeletal muscle confirms that this mutation must be causative and that it is not a loss-of-function mutation.

In conclusion, the data on the pig $PRKAG3$ mutation, on human $PRKAG2$ mutations, from transfection experiments with human $PRKAG1$ variants and our transgenic mice strongly suggest that Arg200 → Gln in $RN^−$ pigs is an activating mutation that increases glucose uptake. It may have other effects as well, for instance on fatty acid oxidation or synthesis. There are also a number of other important questions to address as regards the phenotypic effect of the Arg200 → Gln mutation. For instance, does this mutation provide any protection against the development of insulin resistance and Type II diabetes and does the mutation influence muscle strength and/or endurance? Also, what is the specific role of AMPK heterotrimers containing the $γ3$ chain? These questions are currently being investigated using our transgenic and knockout models.

References


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