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A well-known mutation in *RYR1* alters distribution of adipose tissue in gilts

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The swine *RYR1* (*ryanodine receptor 1*) gene is a major gene for meatiness, but its effect on the fatness and location of fat deposition is less known. A known mutation in this gene is responsible for a drastic deterioration of meat quality. We provide evidence that the mutation (*c.1843^T*) alters fat distribution between back fat and abdominal fat, which are of different value in meat processing. The study included 486 gilts representing the Polish Landrace, PL (*n*=242) and synthetic line L990 (*n*=244). All gilts were classified into 3 clusters according to their predisposition to fat distribution between visceral and subcutaneous tissues. We found a relationship between this classification and RYR1. The mutation c.1843C>T changed the distribution of body fat between these tissues in PL and L990 (*P*=0.0384), and in L990 separately (*P*=0.0277). No evidence for such an effect was observed when PL was analyzed separately. Compared to the *CC* homozygotes the *T* allele was associated with a lower abdominal fat deposition and heterozygous gilts tended to allocate adipose tissue in back fat; however, the effect on fat distribution was independent of general fatness of a pig.

KEYWORDS: fat distribution / obesity / pig / SNP / *RYR1*

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The pig *RYR1* (*ryanodine receptor 1, halothane gene*) gene has been extensively studied and is now considered to be a major gene for meat quality and meatiness. The recessive variant (*c.1843*^{T}) is responsible for susceptibility to stress and the occurrence of PSE (pale, soft, exudative) meat [Fujii *et al*. 1991, Bates *et al*. 2012]. Studies on pig *RYR1* variants helped to explain the molecular background of human malignant hyperthermia [MacLennan *et al*. 1990]. A recent meta-analysis summarizing previous reports from 74 experimental groups showed that heterozygous pigs have lower meat pH, altered meat color coordinates and decreased lean meat content than *RYR1*^{T}-free pigs [Salmi *et al*. 2010]. There is, however, much less evidence concerning its effect on fatness and location of fat deposition. Adipose tissue is important for the efficiency of pig production, since intramuscular fat directly determines meat quality, whereas back fat and abdominal fat are used in meat processing.

Pig fatness may be considered as a model phenotype for complex human obesity due to the resemblance in body size, physiology and diet between humans and pigs [Lunney 2007, Switonski *et al*. 2010]. The usefulness of this species as a model in studies on obesity-related diseases, including type 2 diabetes was highlighted by Kogelmann *et al*. [2013]. Interestingly, it has been recently shown in the human that an excessive accumulation of visceral (abdominal) fat is a better marker of risk of prediabetes and type 2 diabetes mellitus than general obesity [Neeland *et al*. 2012]. Thus, the aim of the present study was to analyse the effect of the *RYR1-1843* genotypes on abdominal fat weight and visceral and subcutaneous fat deposition in modern pigs.

Material and methods

The study included 486 gilts representing the Polish Landrace PL (*n*=242) and synthetic line L990 (*n*=244). All animals were fed *ad libitum* with a commercial mixed feed, slaughtered at 100 kg, weighed and dissected at a local pig testing station. The following traits were measured: abdominal (visceral) fat weight (kg), back fat thickness (cm) over the shoulder, on the back, over the *sacrum* points I, II, III, at points C1 (on a vertical line extending from the height of the *longissimus dorsi* muscle) and K1 (on a vertical line extending from the edge of the *longissimus dorsi*); back fat of loin with skin (kg), back fat of ham with skin (kg), and lean meat content (%). DNA was isolated from blood samples collected from animals before slaughter. The genotypes of the *RYR1 locus* (*c.1843C>T*) were determined using the PCR-RFLP method [Fujii *et al*. 1991]. The association study was based on a mixed linear model, which included the fixed effects of breed, *RYR1* genotype, and breed x *RYR1* interaction, age at slaughter and right half-carcass weight as covariates, and the random effect of sire. Statistical calculations were performed with the R software v3.0.2. The package *lme4* was used to solve the mixed model.

The following procedure was used to define the predisposition of a gilt to a particular fat deposition: eight traits (abdominal fat weight and the 7 measurements of back fat thickness measured on each gilt) were standardized over the population

to the mean 0 and variance 1. Next, for each gilt separately we calculated the mean and variance of the 8 transformed measurements recorded for the gilt and we further transformed the 8 values by subtracting the mean and dividing by the variance. This double standardization (within a population and within an individual) defines a fat distribution of an individual: e.g. a negative value for a particular standardized fatness trait (abdominal fat or one of the 7 back fat thickness measurements) indicates a low fat deposition in this body part in comparison to the average fatness observed for that animal. Note, this procedure produces a fatness 'profile' for each pig. A profile consists of 8 transformed values. A profile is not connected to general fatness observed on an individual, therefore a similar profile may be assigned to a generally fat and a lean pig.

The profiles of all pigs were then analyzed with the k-means classification procedure in order to cluster all gilts into the predefined number of 3 clusters. The k-means clustering method aims to partition [Hartigan and Wong 1979] objects into *k* clusters to obtain the minimum variance inside the clusters. The number of clusters is pre-declared. In this study we set the number of clusters to 3 because we hypothesize that such a classification may be directly related with 3 genotypes observed for the polymorphic site within the *RYR1* gene. The association between the 3 clusters and the *RYR1* polymorphism was examined by the chi-square test.

Results and discussion

The frequency of the recessive *T* allele was 0.26 and altogether 36 gilts were *TT* homozygotes (Tab. 1). The low frequency of the *T* allele in both pig samples is

Table 1. Genotype and *T* allele frequency of the *RYR1* gene of 486 gilts representing Polish Landrace (PL) and synthetic line (L990)

Breed/synthetic line	*RYR1*^{T}	Genotype *RYR1*			Total
		CC	CT	TT	
PL	13%	187 (38%)	46 (9%)	9 (2%)	242
L990	40%	77 (16%)	140 (29%)	27 (6%)	244
Total	26%	264 (54%)	186 (38%)	36 (8%)	486

in agreement with the breeding strategy to reduce the incidence of PSE meat. This minor group, however, was of sufficient size for an across-breed association study. We calculated 5 significant ($P<0.05$) associations that included abdominal fat weight and two back fat thickness measurements (Tab. 2). Gilts with the *TT* genotype had a lower back fat thickness and a reduced abdominal fat weight when compared to the other genotypes. The insignificant interaction suggests that these 3 effects of the *TT* genotype are consistent across breeds. However, the breed x *RYR1* interaction for the correlated trait of lean meat content was significant and may indicate that the *RYR1* effect is breed-dependent, although this dependency was not always detected. Moreover, the *T* allele determined the lower weight of back fat with skin and a favorable lean

Table 2. Association between SNP (c.1843C>T) in the pig *RYR1* gene and fatness traits in Polish Landrace and the synthetic line L990 – the marginal means for genotype groups, the degree of dominance (*d/a*, the ratio of genotypic values for CT and CC genotype), the significance of breed x *RYR1* interaction and *P*-value for the *RYR1* effect.

Trait	CC (n=264)	CT (n=186)	TT (n=36)	d/a (%)	$P_{\text{interaction}}$	P
Abdominal fat (kg)	0.599±0.016	0.563±0.017	0.484±0.029	37	0.182	0.001
BFT[1] over shoulder (cm)	2.413±0.039	2.484±0.043	2.362±0.082	378	0.259	0.180
BFT over back	1.447±0.032	1.429±0.035	1.282±0.069	78	0.523	0.079
BFT at *sacrum* I	1.712±0.040	1.707±0.043	1.497±0.078	95	0.371	0.019
BFT at *sacrum* II	1.025±0.029	1.012±0.032	0.839±0.058	86	0.052	0.007
BFT at *sacrum* III	1.579±0.039	1.569±0.042	1.416±0.078	88	0.136	0.117
BFT at point K1	0.997±0.028	0.968±0.031	0.858±0.062	58	0.515	0.110
BFT at point C1	1.205±0.033	1.204±0.036	1.142±0.071	97	0.064	0.677
Back fat of loin with skin (kg)	1.789±0.030	1.763±0.032	1.650±0.059	63	0.061	0.083
Back fat of ham with skin (kg)	1.710±0.022	1.658±0.024	1.513±0.043	47	0.001	$1x10^{-4}$
Lean meat content (%)	58.39±0.285	58.82±0.302	60.86±0.524	65	0.011	$3x10^{-5}$

[1]BFT – back fat thickness measured after slaughter, points K1 and C1 were on a vertical line extending from the edge and the height of the *longissimus dorsi* muscle, respectively.

meat content. The heterozygous gilts are more shifted towards the *CC* homozygotes, as evidenced by the positive dominance coefficients. The decreased adipose tissue accumulation in the *TT* gilts may be explained by a higher energy consumption due to the altered function of Ca^{++} channels. It was shown that an increased energy depletion takes place in *post mortem* muscle of *TT* pigs [Shen *et al*. 2007].

The average profiles within each of the three clusters are presented on Fig. 1. We found no statistical evidence that the clusters were connected with breed, average

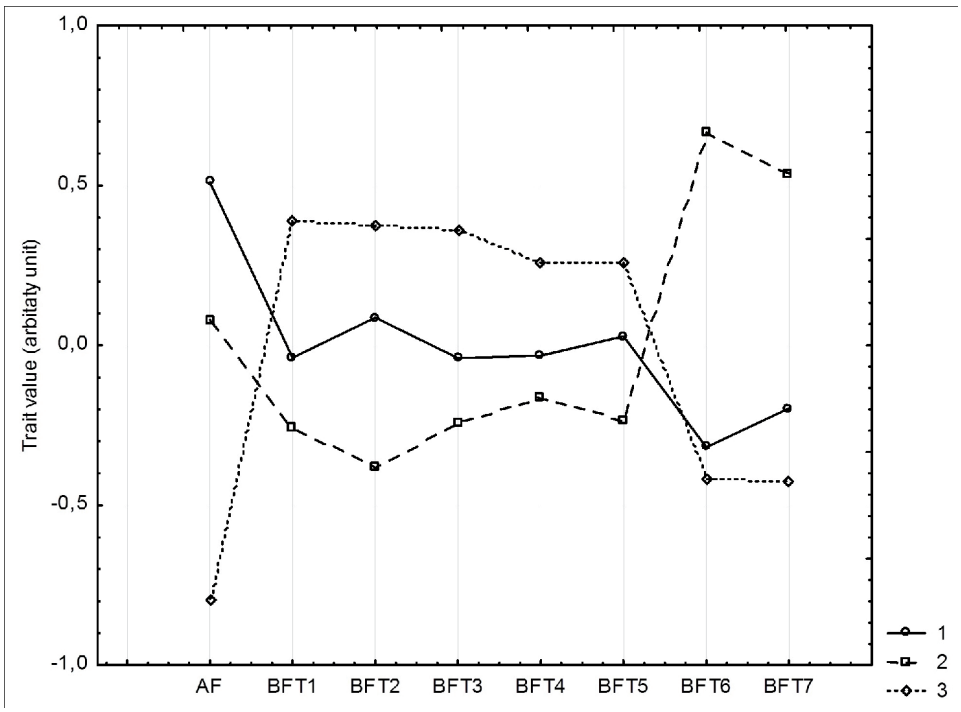

Fig. 1. Average fatness profiles in three clusters of gilts (denoted by 1, 2 and 3). Each point represents a mean value of standardized trait observations within a cluster of gilts. Traits are expressed in arbitrary units. Gilts were grouped into an arbitrarily assumed number of 3 clusters based on 8 fatness traits: abdominal fat weight (AF) and back fat thickness (BFT) measured at 7 different body points. The clustering was based on the k-means method.

body weight, age at slaughter or any particular fatness traits. We observed, however, that the *RYR1* c.1843C>T mutation was associated with fatness cluster in the group of both populations (P=0.0384, Tab. 3) and in L990 separately (P=0.0277). No evidence for such an effect was observed when PL was analyzed separately. Compared to the *CC* homozygotes the *T* allele was associated with a lower abdominal fat deposition and heterozygous gilts tended to deposit adipose tissue in back fat at points K1 and C1 (Tab. 3). To check whether other polymorphisms known to be associated with pig fatness (in the same populations) also change fat distribution we used additional data on *FTO (SNP g-167T>G)*, *CART (short tandem repeat polymorphism* $(CA)_2(CG)_n(CA)_n$*), IL6 (SNP g.61T>C) , TNF (SNP g.6464C>T)* and *UCP3 (SNP g.946C>T) genes*, previously described by Cieslak *et al*. 2009, Stachowiak *et al*. 2009, Szydlowski *et al*. 2011, and Szydlowski *et al*. 2012. We performed additional association analysis between the classification of gilts based on the fatness profile and each of the five polymorphisms separately. We observed that none of the five polymorphisms showed association with fatness profiles. These negative results clearly indicate that not all polymorphisms that modify fatness also change fat distribution.

Table 3. Association between SNP (*c.1843C>T*) polymorphism in the swine *RYR1* gene and fatness class: frequency of genotypes in gilts representing Polish Landrace and the synthetic line L990

Fatness class	CC	CT	TT
1 (increased abdominal fat)	108 (40.9%)	55 (29.5%)	14 (38.9%)
2 (medium abdominal fat)	91 (34.5%)	77 (41.4%)	8 (22.2%)
3 (lower abdominal fat)	65 (24.6%)	54 (29.1%)	14 (38.9%)
Total	264 (100%)	186 (100%)	36 (100%)

Moreover, the results confirm that our definition of fat distribution in the body is independent of individual fatness measurements.

It has been well established that most genes expressed in peripheral tissues may regulate energy homeostasis and body weight. Expression of many of them is controlled by intracellular free calcium ions (Ca^{2+}) in different types of cells, e.g. adipocytes and myocytes [Dolmetsch *et al*. 1998, Aizman *et al*. 2001]. We showed that the mutation disrupting the function of ryanodine receptor 1 leads to reduced visceral fat weight in gilts and a change in fat distribution between visceral and subcutaneous fat tissues. The effect on fat distribution is visible even in lean gilts. Our results are also important for the human, because numerous mutations were identified in the human *RYR1* gene [Hwang *et al*. 2012] and it was suggested that excess of abdominal fat increases the risk of diabetes, particularly in the Chinese [He *et al.* 2013]. The gilts were in the 6th month of life, which is equivalent to adolescence in the human.

We can further hypothesize that other polymorphisms regulating *RYR1* activity or its metabolic pathways may influence abdominal fat weight. Recently it was found that also ryanodine receptor 3 (Ryr3) plays a role in the regulation of adiponectin expression in mouse preadipocytes (3T3-L1 cell line) [Tsai *et al*. 2013]. These authors showed that knock-down of *Ryr3* increases serum adiponectin concentration, enhances insulin sensitivity and influences glucose level. In another study the expression of several genes related to the circadian rhythm, inflammation and oxidative stress correlated significantly with visceral fat accumulation [Yamaoka *et al*. 2012].

In conclusion, in this study we developed a statistical procedure that, based on multiple observations of different fatness traits, describes an individual predisposition to fat deposition between two different fat tissues. We further showed that the known mutation in *RYR1* (c.1843C>T) is associated with pig predisposition to different fat deposition patterns and partly alters the distribution of body fat between visceral and subcutaneous tissues in young gilts. Our study also indicates that the use of the pig as a model for human obesity-related diseases should consider genotype at the *RYR1 locus*, since its polymorphism influences adipose tissue deposition.

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