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A note on associations between polymorphism within the 2,4-dienoyl-CoA reductase gene (*DECR1*) and growth rate of Polish Landrace boars*

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ABSTRACT

The V54L missense mutation within the *DECR1* gene, which encodes a mitochondrial 2,4- dienoyl-CoA reductase, was investigated to determine whether this polymorphism is associated with growth rate (daily gains), meat content and selection index in Polish Landrace boars kept under uniform feeding and environmental conditions (one herd). The genotype of 334 boars was determined by PCR-RFLP, identifying 112, 162, and 60 boars bearing genotypes CC, CG and GG, respectively. Statistical analysis was carried out by the General Linear Model (GLM) procedure, including fixed effects of *DECR1* genotype, sire, and birth season. Significant differences ($P<0.01$) between boars with CC and GG genotypes were found. Boars with genotype CC showed the highest daily gains (860.7 g ± 46.3), in comparison with boars bearing the GG genotype (841.7 g ± 53.6). The current findings support the hypothesis that *DECR1* V54L polymorphism is a promising marker of growth rate in pigs.

KEY WORDS: pig, *DECR1*, polymorphism, growth rate, PCR-RFLP

INTRODUCTION

Improving meat performance is one of the most challenging tasks in commercial pig breeding. Although carcass trait parameters are routinely measured and used in practical selection, their molecular background remains mostly unknown. Using a molecular approach, only several SNPs, namely, the missense mutation in *RYR1* (Fuji et al., 1992), and in *PRKAG3* (Milan et al., 2000) have major effects

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on lean meat content and meat quality, as well as the point mutation in intron 3 of *IGF2* (Van Laere et al., 2003) underlying a major QTL for muscle growth and lean meat content. Searching for new functional DNA polymorphisms and testing their associations with meat performance traits in pigs has been the purpose of many studies during last two decades (reviewed by Brym and Kaminski, 2006). Among 88 SNPs analysed by our group, the missense mutation V54L within the *DECR1* gene encoding a mitochondrial 2,4 dienoyl CoA reductase (Clop et al., 2002) showed balanced genotype and allele distribution in major commercial porcine breeds (Kaminski et al., 2008), giving a chance for reliable association studies. The key role of *DECR1* in fat and protein metabolism encouraged us to investigate whether this polymorphism is associated with growth rate (daily gains), meat content and selection index in Polish Landrace boars.

MATERIAL AND METHODS

Animals, management and sampling

Three hundred and thirty-four Polish Landrace boars were included in the analysis. Boars were kept under the same feeding and environmental conditions (one herd). All boars were genotyped using PCR-RFLP (Clop et al., 2002) with small modifications. Briefly, 0.5 ml of blood was taken from animals and genomic DNA was isolated from leukocytes by the MasterPure™ Genomic DNA Purification Kit (Epicentre Biotechnologies). The primers had the following sequence: forward 5'- AGTTTTTCAGTTATGGGACAAAAA -3', reverse 5'- CACTGAGCACCTAGGCTGGA -3'. To produce a 190 bp fragment of the *DECR1* gene the PCR mix composition was: 0.4 µl of the forward and reverse primers (40 pmol each), 0.5 U of Tth polymerase (Biotools), 2.5 µl reaction buffer: 75 mM Tris-HCl (pH 9.0), 2 mM $MgCl_2$, 50 mM KCl, 20 mM $(NH_4)_2SO_4$, 1.0 µl dNTP (2.5 mM each, Epicentre), about 150 ng of genomic DNA and H_2O *ad* 25 µl. Samples were amplified in an MJ Research thermocycler under the following conditions: 3 min/94°C and 35 cycles of 95°C/25 s, 51°C/25 s, 72°C/25 s. The PCR products were then digested by Bfa I (37°C/3h) to generate restriction fragments and electrophoresed in 3% agarose gel with ethidium bromide (AmpliSize, Bio-Rad). The results were visualized, analysed and documented by the use of a Fluor-S MultiImager (Bio-Rad).

All boars were tested for the *RYR1* genotype by the method described earlier (Kaminski et al., 2002) and were found to be free of allele T.

Traits were measured and calculated following obligatory and standardized instructions from the Regional Animal Breeding Center in Łódz.

GR (growth rate) is standardized at the age of 180 days and is estimated by the formula:

$$GR = \frac{616974\frac{Z}{W}}{-0.0127W^2 + 6.2843W - 102.72}$$

where: Z - body weight (kg) on the day of measurement; W - age (in days) on the day of measurement.

MC is measured between the 170-210th day of a boar's life and is calculated as:

$$MC = -0.4776\ P_{2ST} - 0.4593\ P_{4ST} + 0.3486\ P_4M_{ST} + 48.9829$$

where: P_{2ST} - standardized backfat thickness at point P2 (behind the last rib, 3 cm from the middle line of the back), P_{4ST} - standardized backfat thickness at point P4 (behind the last rib, 8 cm from the middle line of the back); P_4M_{ST} - the height of loin at point P4.

GR and backfat thickness were transformed and adjusted to 180 days of life and 110 kg body weight.

SI is calculated using the formula:

$$SI = 0.1556\ GR + 3.1023\ MC - 179.4935$$

where: GR - standardized growth rate at the age of 180 day, MC - percentage of meat estimated as above.

Statistical analysis

Statistical analysis was carried out by the GLM procedure (STATISTICA 6.0). The following model was used:

$$Y_{ijkl} = \mu + G_i + B_j + S_k + e_{ijkl},$$

where: Y_{ijkl} - analysed trait, μ - overall mean, G_i - fixed effect of genotype ($i = 1,...3$), B_j - fixed effect of the birth season ($j = 1,...4$), S_k - fixed effect of the sire ($k = 1,...35$), e_{ijkl} - random error.

Significance of differences between the means for each group was tested by the use of Duncan's test.

RESULTS

PCR-RFLP yielded three distinct genotypes (Figure 1). Among the analysed Landrace boars, 112 CC, 162 CG and, 60 GG genotypes were identified. Allele

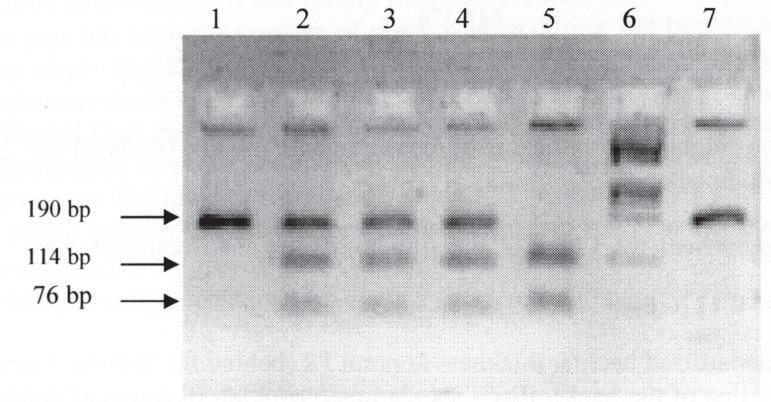

Figure 1. Example of DECR1 genotyping by PCR-RFLP technique
Path 1 - GG genotype (190 bp); paths 2-4 - CG genotype (190, 114, 76 bp); path 5 - CC genotype (114, 76 bp); path 6 - DNA size marker ΦX 174 HaeIII; path 7 - PCR product (190 bp) uncut by Bfa I

frequency was 0.577 and 0.423 for C and G, respectively. Statistical analysis revealed significant associations between *DECR1* genotypes and growth rate (Table 1). Highly significant differences (P<0.01) between boars having

Table 1. Statistical analysis of DECR1 polymorphism effect on growth rate, meat content and selection index in Landrace boars

DECR1 genotype	Number of animals	Trait $\bar{x}$ ± SD		
		growth rate, g	meat content, %	index
CC	112	860.7 ± 46.3^{A}	59.6 ± 1.52	139.9 ± 8.69
CG	162	850.5 ± 50.3	59.7 ± 1.67	138.8 ± 10.47
GG	60	841.7 ± 53.6^{A}	59.9 ± 1.75	139.4 ± 11.13

capital letters indicate differences at P<0.01

genotypes CC and GG were found. Boars with genotype CC showed the highest daily gains (860.7 g ± 46.3) in comparison with boars bearing genotype GG (841.7g ±53.6). No statistically significant effect of *DECR1* genotype on meat content and index value was observed.

DISCUSSION

Growth rate, as a quantitative polygenic trait, is affected by the interplay of many genes and environmental factors. Searching for single genes and causative mutations is a challenging task in many groups involved in QTL mapping projects

(PigQTLdb, www.animalgenome.org). QTLs responsible for both carcass yield and meat quality are of particular value, because they give a chance for simultaneous selection of meat yield and quality traits. When validating a set of pre-selected candidate SNPs addressed for diverse application in pig breeding (SNiPORK chip; Kaminski et al., 2008) we noted that *DECR1* had balanced genotype and/allele frequency, predisposing this SNP for wider association studies. To verify its possible effect on growth rate and meat content, two basic traits measured on farms for selection purposes, 334 highly selected Landrace boars were genotyped by PCR-RFLP. All boars were tested as free of the *RYR1 T* allele, commonly known to influence meat quality. Collecting pigs without the *RYR1 T* allele was possible as an effect of a long-term program eliminating this allele from reproductive schemes of the herd involved in this study. Polish Landrace has a relatively low frequency of allele *T* that tends to decrease every year (Kaminski et al., 2002). Statistical analysis revealed a significant effect of *DECR1* polymorphism on growth rate. The reported effect of *DECR1* polymorphism on growth rate is supported by the physical location of the gene on chromosome 4 harbouring at least 3 QTL for average daily gain (PigQTLdb, www.animalgenome.org). The porcine DECR1 gene maps close to the region of chromosome 4 in which several authors have localized quantitative trait loci (QTLs) affecting carcass and growth traits as well as fatty acid metabolism (Perez-Enciso etal., 2000; Walling et al., 2000; Cepica et al., 2003). Linkage and physical mapping data indicate region 4q15-q16 as a locus for *DECR1* (Davoli et al., 2002a, b). According to Stefanon et al. (2004), *DECR1* is involved in the control of fat and protein deposition.

Our results are partially in contrast with report of Amills et al. (2005). Association studies on 470 highly selected Landrace revealed differences among genotypes (of two SNPs being in LD) for isocitrate dehydrogenase activity and selected meat quality traits: *Longissimus thoracis* pH, lightness and redness (Amills et al., 2005). This group did not report significant associations with growth and carcass traits. The inconsistency may be due to differences in population origin and size as well as the statistical model applied (GLM vs haplotype contrasts). Among other possible candidate genes located within QTL interval 71-86cM on the long arm of SSC4, a polymorphism within the FABP4 gene (adipocyte fatty acid binding protein) mapped physically at position 4q12 (Szczerbal et al., 2007) revealed no association with fatness traits in Polish Landrace pigs (Chmurzyńska et al., 2008). Our current findings support the hypothesis of the more general role of *DECR1* V54L polymorphism, namely its participation in biosynthetic processes also expressed in growth regulation. The increase of body weight did not affect meat content, suggesting that the *DECR1* gene is expressed in many tissues and influences meat quality parameters (Amills et al., 2005) rather than meat yield. Because polymorphism relies on changing the amino acid sequence (V54L), it

could be hypothesized that it affects enzyme activity and efficiency of beta-oxidation of fatty acids. Biochemical studies are necessary to verify this assumption.

The results presented in this paper indicate that *DECR1* polymorphism is one of the best validated SNP in the SNiPORK chip being steadily developed and enriched with new polymorphisms applicable in MAS.

CONCLUSIONS

Current findings support the hypothesis that *DECR1* V54L polymorphism is an effective genetic marker of growth rate in Landrace pigs. Also taking into account its influence on meat quality traits (reported by other authors), *DECR1* polymorphism should be considered a promising marker for use in MAS (Marker Assisted Selection).

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