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Distribution of the polymorphic variants of genes *RYR1*, *LEP*, *GH*, *MYOG*, *MYF5*, and *GDF8* in wild boars from North-East of Poland

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The aim of this study was to characterize the polymorphism of genes *RYR1*, *LEP*, *GH*, *MYOG*, *MYF5* and *GDF8* in wild boar population inhabiting North-East of Poland. The above genes are considered candidate genes for carcass quality traits in domestic pigs. Ninety animals were tested. Genes *RYR1*, *LEP* and *MYF5* appeared to be monomorphic, whereas genes *MYOG, GH* and *GDF8* showed polymorphism similar to that observed in European pig breeds. However, no PCR product was obtained for several animals when *MYOG* and *GDF8* gene fragments were amplified, suggesting the difference between wild boars tested and domestic pig in sequence of the gene regions where primers annealed. Moreover, this suggests a possibility of identification of new variants of these genes in wild boar.

KEY WORDS: gene polymorphism / pig / wild boar

The species of wild boar, which is widespread in Europe, Asia and North Africa, includes about 27 subspecies [Herre and Rohrs 1977, Epstein 1984]. The polymorphisms of blood group systems and blood proteins were characterized in several wild boar subspecies showing marked differences within and between subspecies and populations [Hartl *et al*. 1993, Tikhonov and Bobovich 1997]. Mitochondrial genetic variation was used to investigate the relationship between two Japanese wild boar subspecies [Watanobe *et al*. 1999] as well as between wild boar and Chinese native breeds [Huang *et al*. 1999].

A clear evidence for independent domestication of European and Asian subspecies of the wild boar was established on the basis of sequencing of complete cytochrome B gene (mitochondrial) and genotyping of three nuclear genes in several breeds of European and Asian pig breeds and wild boar subspecies [Giuffra *et al*. 2000].

Wild boar appeared to be especially interesting and attractive paternal component of reference families arranged for mapping of quantitative traits *loci* (QTLs) in the pig genome, and was used in two projects on this subject realized in European laboratories [Andersson-Eklund *et al*. 1998, Geldermann *et al*. 2003]. A significant difference was expected between gene variants present in European breeds and commercial lines of pigs and those in wild boar. It was necessary for mapping QTLs determining carcass composition [Marklund 1997].

Analysis of polymorphism of blood group systems in five wild boar subspecies inhabiting Byelorussia, Lituania, Central Russia, North Caucasian and Transcaucasian regions, Kirgizian mountains and Siberia showed marked differences within and between individual subspecies and populations especially concerning the frequencies of alleles *L* and *G* [Tikhonov and Bobovich 1997].

Our earlier study on a diversity of blood proteins in wild boar inhabiting Poland showed lack of polymorphism at *locus A1BG*, analogous protein variants to those observed in domestic pig (Tf, Cp, Pi1) and of new variants not described earlier in pig (Tf, Hpx, Po1A) – Kurył and Żurkowski [1988].

The aim of the investigation presented here was to characterize the polymorphism of the selected genes in wild boar, being considered as candidate genes for meat and fat content of carcass, or known as influencing these traits in different breeds of domestic pig.

Material and methods

Hair samples were collected from 90 wild boars just after shooting in the North-East region of Poland. Three hair bulbs were used for genomic DNA isolation [Buitkamp *et al*. 1999]. PCR/RFLP polymorphism of the chosen genes was determined according to the following procedures: *RYR1* [Kamiński *et al*. 2001], *LEP* [Stratil *et al*. 1997], *GH* [Kirkpatrick 1992], *MYOG* [Soumillion *et al*. 1997], *MYF5* [Te Pas *et al*. 1999], *GDF8* [Stratil and Kopečny 1999].

Heterozygosity of population tested was estimated according to Ott [1992].

Results and discussion

The frequency of genotypes at *loci RYR1*, *LEP*, *GH*, *MYOG*, *MYF5*, and *GDF8* in the tested wild boars is shown in Table 1.

***RYR1* gene**. All animals were found to be of *CC* genotype (stress resistant) at the *RYR1 locus* that determines stress resistance/susceptibility in pigs and affects meat and

Table 1. Frequency of genotypes and alleles at the chosen *loci* and coefficient of heterozygosity (H) in the population of wild boars inhabiting North-East region of Poland

Locus	Genotype	Frequency of genotypes				Allele	Frequency of alleles	H
		observed		expected*	P			
		n	%	n				
RYR1	*CC*	90	100.0	90.0		*C*	1.00	0.00
	CT	0	0.0	0.0	ns	*T*	0.00	
	TT	0	0.0	0.0				
LEP	*CC*	90	100.0	90.0		*C*	1.00	0.00
	CT	0	0.0	0.0	ns	*T*	0.00	
	TT	0	0.0	0.0				
GH	+/+	23	25.6	28.2		+	0.56	0.51
	+/-	54	60.0	44.4	ns	-	0.44	
	-/-	13	14.4	17.4				
*MYOG***	*AA*	9	11.8	13.4		*A*	0.42	0.49
	AB	46	60.5	37.0	ns	*B*	0.58	
	BB	21	27.6	25.6				
MYF5	*AA*	90	100.0	90.0		*A*	1.00	0.00
	AB	0	0.0	0.0	ns	*B*	0.00	
	BB	0	0.0	0.0				
*GDF8***	*CC*	24	33.3	28.6		*C*	0.63	0.47
	CT	42	58.3	33.5	ns	*T*	0.37	
	TT	6	8.3	9.9				

*According to Hardy-Weinberg.
**Number of genotypes identified: 76 at the *locus MYOG* and 72 at the *locus GDF8*.

carcass traits [Kuryłł 2000 – a review]. Wild boar is known as free of $RYR1^T$ allele and this phenomenon was one of the most important criteria considered in choice of breeds for parental generation of reference family created for QTLs mapping. Müller *et al*. [2000] tested 10 European wild boars, whereas Ernst *et al*. [2003] 109 wild boars of two subspecies (*Sus scrofa scrofa* and *Sus scrofa attila*) and all these animals were of *CC* genotype. However, one out of two European wild boars used for arrangement of the Nordic reference family for QTLs mapping appeared to be heterozygote (*CT*) at the *RYR1 locus* [Marklund 1997].

***LEP* gene**. No polymorphism was observed at the *LEP locus* (*T*3469*C* point mutation) within tested population. All animals were of *TT* genotype what confirms the results of Dragos-Wendrich *et al*. [2003]. *LEP* gene – encoding leptin, a hormone secreted by adipocytes and involved in the regulation of feed intake and energy balance [Remesar *et al*. 1997] – was considered as candidate gene for carcass traits. A significant difference between *TC* and *TT* genotypes in meat and fat content of ham was shown in our earlier study [Kuryłł *et al*. 2003]. It should be mentioned that all Meishan pigs tested so far [Stratil *et al*. 1997, Dragos-Wendrich *et al*. 2003] appeared to be homozygotes *CC* at the *locus LEP*. Both alleles were found in several commercial breeds [Stratil *et al*.

1997, Kurył *et al*. 2003]. The QTLs for weight of ham relative to carcass weight (%) and fat-to-meat ratio were located on swine chromosome 18 near the *LEP* [Dragos-Wendrich *et al*. 2003] on the basis of analysis of reference family originating from crossing of Wild Boars with Meishan sows, being of *TT* and *CC* genotype at the *LEP locus*, respectively. Thus, it may suggest that *TT* genotype present in all wild boars is not associated with increasing share of fat cuts in their carcass compared to e.g. Pietrain pigs [Müller *et al*. 2000].

***GH* gene**. The frequency of genotypes at *locus GH*/*Hae*II (polymorphism in exon 2 of growth hormone gene) in the tested population of wild boars was similar to that reported by Ernst *et al*. [2003] for animals originating from Northern Germany and several regions of the Czech Republic and Slovakia. A lowest frequency (14.4%) was demonstrated by „-/-" genotype. In our earlier studies this genotype was shown to be significantly related to a highest fat thickness in different points of measurements in F_2 pigs [(Polish Large White × Zlotnicka Spotted) × [(Polish Large White × Zlotnicka Spotted)] and with lower weight of ham and ham meat in PIC pigs [Pierzchała *et al*. 1999 and Kurył *et al*. 2003, respectively]. Thus, comparing to commercial pig breeds, wild boar is not more valuable as experimental material for studyind of an effect of *GH*/*Hae*II polymorphism on carcass traits. One may suggest that this conclusion finds its confirmation in the studies by Yue *et al*. [2003] who have not found any significant QTLs in the region of *GH* gene localization (SSC12) for growth rate and carcass and meat quality traits in reference families originating from crosses Wild Boar × Meishan and Wild Boar × Pietrain. However, significant QTL for the share of lean cuts in carcass was identified in this region in Meishan × Pietrain family.

***MYOG* gene**. Genotype at the *MYOG locus* was identified in 76 out of 90 wild pigs (Tab. 1). No PCR product was obtained for the remaining 14 animals. It may suggest the presence of mutation(s) in the *MYOG* gene region where primers annealed, thus making impossible their hybridization to a template and gene fragment amplification. The frequencies of alleles *A* and *B* were almost equal to 0.5. However, only nine homozygotes *AA* (11.8%) were found within the population tested. Soumillion *et al*. [1997] tested 10 wild pigs and did not find any animal of this genotype. *MYOG* gene belongs to the *MyoD* family genes encoding proteins involved in muscle cell determination and differentiation [Te Pas and Visscher 1994]. Therefore, these genes were considered as potentially affecting meat deposition in carcass, and *BB* genotype was reported as advantageous for this trait [Te Pas *et al*. 1999, Cieślak *et al*. 2000]. No effects on muscling or growth was assigned to the *MYOG locus* by Cepica *et al*. [2003] in the Wild Boar × Pietrain and Wild Boar × Meishan families.

***MYF5* gene**. All the tested animals were homozygotes *AA* at the *locus MYF5* (Tab. 1). Similar results were reported by Te Pas *et al*. [1999] who genotyped 10 wild boars, whereas Lee *et al*. [2003] have found both variants of the *MYF5* gene – *A* and *B* – also within 10 animals tested. *MYF5* gene is a member of the *MyoD* family genes and controls processes of myogenesis. Therefore, it was also considered as candidate gene for carcass meat deposition. Dutch authors reported lack of association between

MYF5 genotype and meat production traits [Te Pas *et al*. 1999], whereas our earlier study showed that *AB* genotype was most advantageous for meat content of loin and weight of ham meat [Cieślak *et al*. 2002]. A usefulness of wild boars as founders of reference families arranged for evaluation of *MYF5* genotype on carcass traits has not been confirmed so far [Lee *et al*. 2003].

***GDF8* (*MSTN*) gene**. Genotype at the *GDF8 locus* was defined in 72 out of 90 wild boars tested (Tab. 1). No PCR product was obtained for the remaining 18 animals. The explanation of this phenomenon might be similar to that presented for the difficulties with *MYOG* gene fragment amplification. In exon 3 of the gene *GDF8* the polymorphism $C \rightarrow T$ identified with *Taq*I enzyme, was not investigated in wild boars, so far. Czech authors have tested several pig breeds and lines (Czech Meat Pig, Black Pied Prestice, Landrace, Pietrain, Hampshire, Duroc) and found the polymorphism mentioned only in Landrace pigs. Frequencies of alleles were: $T = 0.18$ and $C = 0.82$, what means that only three out of 98 animals tested were of *TT* genotype [Stratil and Kopečny 1999]. The *GDF8* (*MSTN*) gene codes for growth differentiation factor-8 (myostatin) belonging to the transforming growth factor β (TGFβ) superfamily and is essential for muscle growth and development. Mutations in the *GDF8* gene were found to cause muscular hypertrophy in cattle [Grobet *et al*. 1998]. No relationship between *GDF8* genotype and meat content of carcass was found in pigs by Cieślak *et al*. [2003] but they reported that only 13 animals of three different breeds of *TT* genotype were available for an analysis, whereas *CC* and *CT* genotypes were represented by 189 and 112 animals, respectively. *GDF8* gene was not included into QTLs mapping projects based on intercross between wild boar and domestic pigs [Andersson-Eklund *et al*. 1998, Geldermann *et al*. 2003]. A further analysis is necessary to explain a significance of *GDF8*/*Taq*I genotype for meat content of carcass. Relatively high proportion of *TT* genotypes in the population of wild boars compared to various domestic pig breeds suggests the usefulness of the former for similar studies.

Distribution of genotypes at the individual *loci* within tested population of wild boars did not differ significantly from that expected according to the Hardy-Weinberg law (Tab. 1).

Heterozygosity coefficients (H) at individual *loci* analysed in this study are shown in Table 1 and these values are similar to those reported by Ernst *et al*. [2004] for *loci*: *RYR1*, *LEP*, and *GH*. A similar information regarding *loci MYOG*, *MYF5,* and *GDF8* in wild boar was not reported, so far.

Geldermann *et al*. [2003] reported 185 markers genotyped in Wild Boar × Pietrain, Wild Boar × Meishan and Pietrain × Meishan families; more alleles appeared different between Meishan (very high fat content of carcass) and European pigs than between Pietrain (very high meat content of carcass) and wild boar. In the study presented here we identified gene variants at six *loci* and they appeared to be similar to those known in European pig breeds. This observation confirms that reported by Geldermann *et al*. [2003]. However, lack of amplification of *MYOG* and *GDF8* gene fragments in several

wild boars used suggests that point mutation(s) – SNPs – so far unknown may appear in these gene regions.

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Polimorfizm wybranych genów (*RYR1*, *LEP*, *GH*, *MYOG*, *MYF5* i *GDF8*) dzików z północno-wschodniej Polski

S t r e s z c z e n i e

Celem badań była charakterystyka polimorfizmu wybranych genów (*RYR1*, *LEP*, *GH*, *MYOG*, *MYF5*, *GDF8*) u dzików zamieszkujących północno-wschodni rejon Polski. Przebadano 90 zwierząt. Wybrane geny określane są mianem genów kandydujących (*candidate genes*) dla cech jakości tuszy świń ze względu na ich potencjalne oddziaływanie na te cechy. Trzy z nich (*RYR1, LEP* i *MYF5*) okazały się monomorficzne, podczas gdy pozostałe (*MYOG*, *GH* i *GDF8*) wykazały polimorfizm podobny do obserwowanego w europejskich rasach świń. Dla niektórych jednak zwierząt nie uzyskano produktu PCR w wyniku reakcji amplifikacji (powielania) fragmentów genów *MYOG* i *GDF8*, co sugeruje możliwość obecności mutacji w rejonach przyłączania starterów, które uniemożliwiły prawidłowy przebieg tego etapu reakcji PCR i w konsekwencji dalszych jej etapów. Można wnioskować zatem o możliwości identyfikacji u dzika nowych wariantów tych dwóch genów.