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ASSOCIATIONS BETWEEN POLYMORPHISMS IN THE *DIO3* GENE AND REPRODUCTIVE TRAITS AND CARCASS PERFORMANCE IN PIGS*

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Abstract

Recently, *DIO3* gene has been proposed as a candidate gene for litter size in pigs. Moreover, it was shown that polymorphism in this gene is associated with carcass traits. In this study we identified several SNPs within coding sequence of *DIO3* by HRM method and performed association study between two polymorphisms and reproductive and carcass traits in pigs bred in Poland. Analysis of 350 pigs of Landrace and Large White breed revealed several significant associations for *rs80999359*, like period between the second and third parities (2IP)(P<0.0008) in the whole population, period between the third and fourth parities (3IP) (P<0.022), number of piglets born alive (L3NBA) (P<0.0084) and number of piglets at 21 days (L3NB21d) (P<0.0176) at the third parity in Large White as well as period between the second and third parities (2IP) (P<0.0012) in Landrace breed. The second polymorphism (*rs80983654*) was associated with 1IP (P<0.0218), number of piglets born alive at the fourth parity (L4NBA, P<0.027), number of piglets at 21 day at the fourth litter (L4NB21d, P<0.01), in the whole population, average number of piglets born alive (ANBA, P<0.01250), average number of piglets at 21 day (ANB21d, P<0.009), average interparity period (AIP, P<0.016), age at the first parity (1AP, P<0.003), (1IP, P<0.001, L4NBA, P<0.017, L4NB21d, P<0.005) in Large White breed. In contrast, we have found only few associations between *DIO3* polymorphisms and carcass traits. *rs80999359* was associated with backfat thickness (p<0.01) while *rs80983654* with the weight of ham. Our results suggest that polymorphisms within *DIO3* gene may be associated with reproductive traits.

Key words: *DIO3* gene, reproductive traits, carcass, pigs, SNP

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Reproductive traits influence significantly on profitability of pig breeding, yet they are low heritable and it is difficult to obtain substantial progress in these traits by classical selection. Therefore, the identification of new genes engaged in modulation of reproductive performance is very important. Currently, there are only few genes for which associations with fertility have been well proved. One of the best studied genes regarding fertility of pigs is *ESR1* gene. Nevertheless, meta-analysis performed by Alfonso (2005) revealed the high and significant heterogeneity among different *ESR1* studies.

On the other hand, genetic background of carcass performance in pigs is much better explored. However, data on new major genes engaged in modulation of carcass traits are strongly demanded.

DIO3 gene is located within *DLK1-DIO3* domain which is known to contain a few imprinted protein coding genes and noncoding RNA. In the sheep this domain is called "callipyge" since mutation within intragenic region of domain results in muscle hypertrophy (Cockett et al., 1996). In the pig, *DIO3* gene consists of single exon and produces Iodothyronine Deiodinase type III (D3) – enzyme which catalyzes the inactivation of thyroid hormone (TH) by inner ring deiodination of the prohormone thyroxine (T4) and the bioactive hormone 3,3',5-triiodothyronine (T3) to inactive metabolites, 3,3',5'-triiodothyronine (RT3) and 3,3'-diiodothyronine (T2), respectively (Tsai et al., 2002; Hernandez et al., 2006). D3 protects tissues and organs from excess of TH during prenatal development. It is highly and ubiquitously expressed in fetus while in the adult animals its expression is much lower and restricted to the Central Nervous System (CNS), skin, uterus, ovary and adrenal (Kester et al., 2008; Charalambous and Hernandez, 2013). It was shown that D3-deficient mice exhibit growth retardation and reduced fertility and viability, which suggests that *DIO3* plays a critical role in growth and development (Hernandez, 2005).

Recently, several Single Nucleotide Polymorphisms (SNPs) have been identified in porcine *DIO3* gene (Yang et al., 2009; Qiao et al., 2012). Yang et al. (2009) identified *DIO3* A744C polymorphism (AY533208) but did not find any associations between it and carcass traits. Conversely, Qiao et al. (2012) showed that (A/C 687) SNP located in exon is significantly associated with fat deposition and carcass traits, including lean meat percentage (LMP), fat meat percentage (FMP), ratio of lean to fat (RLF), shoulder fat thickness (SFT), sixth-seventh rib fat thickness (RFT), buttock fat thickness (BFT), loin eye area (LEA), and intramuscular fat content (IMF). What is more, it has been suggested that *DIO3* may be a candidate gene for litter size in pigs, after the association study performed on two large populations. Authors identified SNP with a significant imprinting effect, closely linked to *DIO3* gene (Coster et al., 2012). *DLK1-DIO3* domain has been also investigated in cattle and several SNPs associated with milk yield, subcutaneous fat levels, and progeny carcass conformation as well as perinatal mortality, calving interval and gestation length has been found within this domain (Magee et al., 2011)

Imprinting status of *DIO3* gene has been investigated in pigs, sheep, mice, human and wallabies (http://www.geneimprint.com/site/genes-by-name). Generally, it is known as a paternally expressed gene, however in some regions of brain, biallelic or maternal expression was observed in mice (Hernandez et al., 2006; Charalambous

and Hernandez, 2013). Moreover, it was shown that only paternal allele of the *DIO3* gene was expressed in the 30 day porcine embryo and 65 day fetus tissues (Qiao et al., 2012).

DIO3 gene seems to play an important role in mammalian growth, development and reproduction, thus we hypothesized that the polymorphisms within this gene might affect economically important traits in pigs. The aim of our study was to evaluate the associations between two polymorphisms in the *DIO3* gene*:* (accession numbers: *rs80999359* and *rs80983654*) with reproductive traits and carcass performance in pigs. One of them (*rs80983654*) has been previously examined by Qiao et al. (2012), while the other is newly described (*rs80999359*).

Material and methods

Animals and data collection

In the experiment 782 gilts of five different breeds (Duroc, Pietrain, Puławska, Polish Landrace and Polish Large White) were included in the analysis of carcass traits and 350 gilts in the analysis of reproductive traits. The animals came from four Testing Stations of the National Research Institute of Animal Production: Pawłowice, Rossocha, Mełno and Chorzelów and were kept in uniform housing and feeding conditions.

The carcass traits were evaluated during the slaughter when the animals reached the weight of approximately 100 kg. The following parameters were measured: carcass yield (CY) %, weight of loin (WL) kg, weight of tenderloin (WT) %, weight of ham (WH) kg, backfat thickness (mean of the measures in five points) (BT) cm, loin eye area (LEA) cm^2, percent of the meat in the carcass (MP) %, weight of the main cuts (MC) kg. All parameters were assessed according to the uniform procedures of Testing Stations.

The reproductive data was collected during four subsequent parities and included: number of piglets born alive in the first and subsequent parities, respectively (L(1–4)NBA), number of piglets at the 21st day in the first and subsequent parities, respectively (L(1–4) NB21d), average number of piglets born alive from parities 1–4 (ANBA), average number of piglets at the 21st day from parities 1–4 (ANB21d), age of sow at the first parity (1AP, days), first interparity period (1IP, days), second interparity period (2IP, days), third interparity period (3IP, days), average interparity period (AIP, days). The number of the animals in each association analysis varied because not all sows had four litters (Tables 2–3).

DNA isolation and High Resolution Melting (HRM) procedure and sequencing

DNA was isolated from the whole blood using Wizard Genomic Purification kit (Promega, Madison, Wi, USA), according to the manufacturer`s protocol with minor modifications. In order to identify new SNPs in the *DIO3* gene five pairs of primers have been designed using Primer 3 software on the basis of the *DIO3* reference se-

quence (NCBI accession number: NM_001001625.2) (Table 1). The obtained products (250–350 bp length) covered almost the whole *DIO3* gene. Next, we conducted High Resolution Melting (HRM) on Eco Real Time PCR System (Illumina, San Diego, CA, USA). The HRM reaction was performed on 16 DNA samples obtained from three different pig breeds (Large White, Landrace, Pietrain). The reaction was carried out using KAPA HRM FAST PCR Kits (KAPA Biosystem, Wilmington, Massachusetts, USA) in 10 µl volume according to attached procedure. The samples with distinct melting curves were sequenced using Dye Terminator Cycle Sequencing (DTCS) Quick Start Kit (Beckman Coulter, Brea, CA, USA) on capillary sequencer CEQ8000 Genetic Analysis System (Beckman Coulter).

Table 1. Primers designed for fragments of *DIO3* gene amplification

Name	Sequence	Annealing temperature	Positions of amplicon according to *NM_001001625.2* sequence
dio3_1_f	GAGTCTCCCGCCAATTGAA	55°C	87–403
dio3_1_r	CCACTTCAGTTTCAGGCTCA		
dio3_2_f	GGGTGAGCCTGAAACTGAAG	55°C	381–723
dio3_2_r	GACGTCGCGCTGGTACTT		
dio3_3_f	GACGTCGACTTCCTCATCATC	55°C	718–1029
dio3_3_r	GTAGCGCTCCAGCCAGGTA		
dio3_4_f	CCGATGGCTACCAGGTCTC	55°C	980–1303
dio3_4_r	TTAGAGTGAGCCAGGCAACA		
dio3_5_f	TGTTGCCTGGCTCACTCTAA	55°C	1283–1648
dio3_5_r	CTCGTGAGCATCCCAAATG		

Table 2. Frequency of alleles and genotypes of analysed polymorphisms

	n	*DIO3 rs80999359*					*DIO3 rs80983654*				
		genotypes			allele		genotypes			allele	
		CC	CT	TT	C	T	GG	GT	TT	G	T
Duroc	89	1.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00
Landrace	419	0.77	0.21	0.02	0.88	0.12	0.92	0.08	0.01	0.96	0.05
Pietrain	95	0.99	0.01	0.00	0.99	0.01	0.92	0.08	0.00	0.96	0.04
Puławska	105	1.00	0.00	0.00	1.00	0.00	0.97	0.03	0.00	0.99	0.01
Large White	418	0.94	0.05	0.00	0.97	0.03	0.61	0.34	0.05	0.78	0.22

PCR-RFLP

In the whole population, we have genotyped two polymorphisms: *rs80999359* and *rs80983654* in the *DIO3* gene by PCR-RFLP. The PCR for fragment of *DIO3*

gene containing *rs80999359* was performed with primers *dio3_2_f, dio3_2_r* (Table 1), while for the fragment containing *rs80983654* we used primers designed previously by Qiao et al. (2012). Both PCRs were performed using RedTaq Ready-Mix PCR Reaction Mix with $MgCl_2$ (Sigma, Saint Louis, MO, USA) in a final volume of 10 µl. We have used NEBcutter V2.0 software (http://tools.neb.com/NEBcutter2/index.php) to find the restriction enzyme (*Bae*GI) (Biolabs, New England, USA) for discriminating genotypes in *rs80999359*. For *rs80983654* genotyping we used *Bsa*HI enzyme (Biolabs). 10 µl of both PCR products were mixed with appropriate enzyme, buffer, BSA and water according to manufacturer's protocol. The mixture was incubated overnight at 37°C. Next the mixtures were subjected to electrophoresis in a 2% agarose gel *(rs80983654)* and 4% agarose gel (*rs80999359)*.

Table 3. Means and standard deviations (Std Dev) of analysed reproductive traits

Variable	Mean	Std Dev	Mean	Std Dev
	Large White		Landrace	
1AP	364.67	36.25	358.31	35.68
1IP	164.60	30.25	171.83	33.30
2IP	158.68	18.72	163.17	24.15
3IP	156.97	15.96	160.66	22.91
AIP	158.11	12.35	162.09	16.07
ANB21d	11.86	0.94	11.81	0.84
ANBA	12.45	1.02	12.31	0.93
L1NB21d	11.57	1.53	11.15	1.63
L1NBA	12.07	1.62	11.54	1.71
L2NB21d	12.09	1.63	11.83	1.42
L2NBA	12.71	1.77	12.29	1.53
L3NB21d	12.18	1.75	12.21	1.51
L3NBA	12.73	1.80	12.68	1.69
L4NB21d	11.95	1.74	12.15	1.67
L4NBA	12.60	1.76	12.84	1.81

(L(1–4) NBA) – number of piglets born alive in the first and subsequent parities, respectively, (L(1–4) NB21d) – number of piglets at the 21st day in the first and subsequent parities, respectively; (ANBA) – average number of piglets born alive from 4 parities; (ANB21d) – average number of piglets at 21st day from 4 parities; (1AP) (days) – age at the first parity; (1IP) (days) – first interparity period; (2IP) (days) – second interparity period; (3IP) (days) – third interparity period; (AIP) days – average interparity period.

Statistical analysis

Statistical analysis was performed with SAS software (SAS Institute, Cary, NC, v.8.02,2001), MIXED procedure, using the following model for the carcass traits:

$$Y_{ijklm} = \mu + d_i + g_j + h_k + s_l + (d*g)_{ij} + \alpha(xijk) + e_{ijklm}$$

where:

Y_{ijklm} is ijklm phenotypic value,
μ is the mean,
d_i, fixed effect of the breed,
g_j, fixed effect of the genotype,
h_k, fixed effect of the testing station,
sl, random effect of the sire,
α(xijk), correction for half-carcass weight,
$(d*g)_{ij}$, interaction between genotype and the breed,
e_{ijklm}, random error.

The interaction was only included if significant. Significance of the differences between genotypes was tested with Least Squares Means procedure.

For the reproductive traits the following model was used:

$$Y_{ijklm} = \mu + d_i + g_j + h_k + i_l + j_m + (d*g)_{ij} + cov(f) + e_{ijklm}$$

where:

y_{ijklm} – ijklm, phenotypic value,
μ – mean,
d_i – fixed effect of the breed,
g_j – fixed effect of the genotype,
h_k – fixed effect of the herd,
i_l – random effect of the sire,
j_m – random effect of the year,
cov (f) – correction for farrowing age,
$(d*g)_{ij}$ – interaction between genotype and the breed,
e_{ijklm} – random error.

The interaction was only included if significant. Significance of the differences between genotypes was tested with multiple comparison Tukey–Kramer test at the 1% and 5% level.

Results

Identification of new SNPs in *DIO3* gene

HRM analysis and sequencing of samples with different melting curves revealed the presence of three SNPs in the analysed samples. All of them have been deposited to the dbSNP NCBI database (accession numbers: *rs80807884, rs80999359, rs323757288*) and are located on chromosome 7, at: 130204060 bp, 130204023 bp and 130204258 bp, respectively. According to the Ensemble database all of them are an upstream gene variants when ENSSSCT00000002823 transcript is affected. Nevertheless, when ENSSSCT000000028234 transcript is affected, variants displays different effects: *rs80807884* is a synonymous variant *(A/G)*, *rs80999359* is a missense variant (*C/T)* changing valine into methionine, while *rs323757288* is a stop gained variant *(A/T)*.

Frequency of alleles and genotypes at *rs80999359* and *rs80983654*

For further investigation we have chosen the mutation *rs80999359* because preliminary studies suggested that it was polymorphic in our populations and previously described *rs80983654* synonymous SNP (Qiao et al., 2012), for which some associations with economically important traits have been described. We have genotyped in total 778 animals by PCR-RFLP (Figure 1 and 2). Unfortunately, both polymorphisms were relatively low polymorphic in the analysed breeds of pigs (Table 2). Allele T of *rs80999359* was absent in Puławska breed and Duroc, while in the other breeds its frequency was low (Pietrain – 0.01, Polish Large White – 0.03, Polish Landrace – 0.12). For the second SNP – *rs80983654* the most frequent was allele G. Allele T was only present in the Large White breed (0.22), while in the other breeds its frequency was below 0.05.

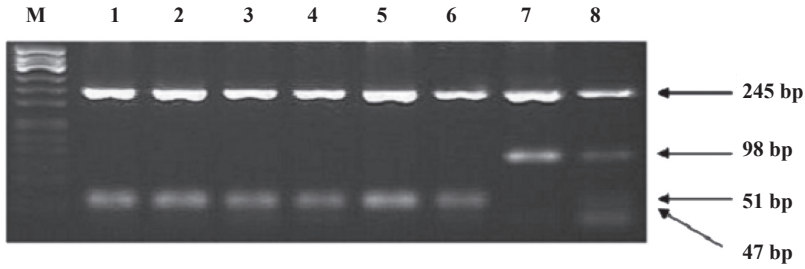

Figure 1. Results of the PCR-RFLP (*BaeG*I) analysis of *DIO3 rs80999359* polymorphism. Allele C: 245 + 51 + 47 bp, allele T 245 + 98 bp. M – marker; line 1–6 – CC homozygotes; line 7 – TT homozygotes; line 8 – heterozygote CT

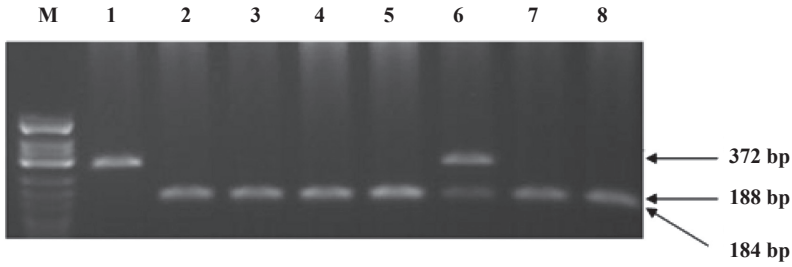

Figure 2. Results of the PCR-RFLP (*BsaH*I) analysis of *DIO3 rs80983654* polymorphism. Allele G: 188 + 184 bp, allele T: 372 bp. M – marker; line 1 – homozygote TT; lines 2, 3, 4, 5, 7, 8 – homozygotes GG; line 6 – heterozygote GT

Association analysis between *rs80999359* and *rs80983654* and reproductive traits

We have analysed the association between both polymorphisms and reproductive traits only for LW and Landrace breeds because they are maintained in Poland as a maternal component and are the most numerous. Means and standard deviations of analysed reproductive traits and carcass traits are presented in Tables 3

and 4, respectively. Moreover we have excluded from the analysis animals with minor homozygous genotypes since their frequency was very low for both SNPs. Association analysis between *rs80999359* and reproductive traits revealed that in the whole analysed population, the animals with CC genotype were characterized by shorter interparity period between the second and third litters (2IP) (P<0.01), which makes them more efficient for breeding than CT animals (Table 3). When breeds were analysed separately 2IP was significantly shorter in CC animals of Landrace breed (Table 5) (P<0.01) but not in Large White breed (Table 5). Nevertheless, period between 3 and 4 parities (3IP) was significantly shorter in CC animals of LW breed (P<0.05). On the other hand, number of piglets born alive (3NBA) and number of piglets at 21 days at the third parity (3NB21d) were higher in CT animals of LW breed (Table 5).

Table 4. Means and standard deviations (Std Dev) of analysed carcass traits

Variable	Mean	Std Dev	Mean	Std Dev
	Large White		Landrace	
CY (%)	77.23	2.61	76.85	2.14
WT (kg)	0.38	0.06	0.40	0.05
WL (kg)	7.86	0.76	7.82	0.73
WH (kg)	9.13	0.64	9.27	0.56
BT (cm)	1.34	0.33	1.32	0.34
LEA (cm^2)	51.07	5.66	54.03	6.05
MP (%)	60.64	3.12	61.61	3.47
MC (kg)	23.85	1.49	24.29	1.38

(CY) % – carcass yield, (WL) kg – weight of loin, (WT) kg – weight of tenderloin, (WH) kg – weight of ham, (BT) cm – backfat thickness (mean of the measures in five points), (LEA) cm^2 – loin eye area, (MP) % – percent of the meat in the carcass, (MC) kg – weight of the main cuts.

When the second polymorphism was analysed (*rs80983654)* in the whole population we identified significant associations with 1IP, 4NBA and 4NB21d (P<0.05) (Table 6). Heterozygous animals (GT) presented higher values of these traits. Heterozygous animals (GT) had also higher values of 3NBA when Landrace breed was analysed separately. On the other hand, we observed that GG animals are characterized by higher values of 2NBA (P<0.01) and 2NB21 (P<0.05) in this breed (Table 6). In the Large White breed, animals with GT genotype presented higher values of ANBA (P<0.05), ANB21d (P<0.01), 4NBA (P<0.05), 4NB21d (P<0.05), AIP (P<0.05) and 1IP (P<0.01). On the contrary, homozygous animals (GG) were characterized by lower age of first parity (1AP) P<0.01).

Table 5. Results of the association analysis between *DIO3 rs80999359* polymorphism and reproductive traits

Trait	Whole population						Large White						Landrace					
	No.	CC		CT			No.	CC		CT			No.	CC		CT		
		mean	SE	mean	SE	P-value		mean	SE	mean	SE	P-value		mean	SE	mean	SE	P-value
ANBA	197	12.35	0.08	12.60	0.18	0.1730	113	12.38	0.10	13.25	0.45	0.1319	84	12.30	0.12	12.40	0.17	0.9307
ANB21d	197	11.81	0.07	12.00	0.15	0.1583	113	11.80	0.09	12.50	0.35	0.1120	84	11.84	0.11	11.84	0.16	0.8672
AIP	336	157.57	0.98	164.58	3.20	0.182	185	152.56	12.75	163.19	5.19	0.4358	151	157.57	1.63	165.03	3.93	0.1250
1AP	348	364.30	2.20	354.08	4.17	0.5275	190	367.41	2.89	355.46	8.03	0.7123	158	359.74	3.36	353.59	4.93	0.5126
L1NBA	348	11.81	0.10	11.72	0.27	0.5345	190	12.02	0.12	12.23	0.47	0.8130	158	11.48	0.17	11.54	0.33	0.4876
L1NB21d	348	11.43	0.10	11.06	0.25	0.0861	190	11.60	0.11	11.54	0.42	0.5013	158	11.18	0.16	10.89	0.30	0.2620
L2NBA	336	12.52	0.10	12.51	0.26	0.8695	185	12.72	0.14	13.00	0.62	0.8195	151	12.23	0.14	12.33	0.27	0.8116
L2NB21d	336	11.95	0.10	11.84	0.21	0.9215	185	12.06	0.13	12.08	0.51	0.7798	151	11.79	0.14	11.75	0.23	0.9789
1IP	336	166.81	1.97	167.76	3.64	0.9947	185	164.53	2.37	161.31	4.13	0.8846	151	170.22	3.39	170.08	4.69	0.5315
L3NBA	280	12.67	0.11	13.15	0.29	0.1319	153	12.70	0.14	14.09	0.79	**0.0084**	127	12.62	0.17	12.80	0.25	0.9720
L3NB21d	280	12.18	0.11	12.66	0.25	0.1212	153	12.14	0.14	13.36	0.68	**0.0176**	127	12.24	0.16	12.40	0.22	0.8931
2IP	280	157.69	1.18	170.00	4.57	0.0008	153	158.51	1.68	163.55	5.37	0.2710	127	156.49	1.54	172.37	5.91	**0.0012**
L4NBA	197	12.63	0.12	13.07	0.37	0.3964	113	12.53	0.15	12.00	0.87	0.4732	84	12.81	0.21	13.41	0.38	0.2451
L4NB21d	197	11.96	0.13	12.31	0.32	0.0825	113	11.88	0.16	11.29	0.64	0.4671	84	12.10	0.21	12.64	0.35	0.3905
3IP	197	155.45	1.16	166.24	5.39	0.0755	113	154.44	1.31	171.71	8.84	**0.0219**	84	157.16	2.19	164.50	6.59	0.8600

(L(1–4) NBA) – number of piglets born alive in the first and subsequent parities, respectively; (L(1–4) NB21d) – number of piglets at the 21st day in the first and subsequent parities, respectively; (ANBA) – average number of piglets born alive from 4 parities; (ANB21d) – average number of piglets at 21st day from 4 parities; (1AP) (days) – age at the first parity; (1IP) (days) – first interparity period; (2IP) (days) – second interparity period; (3IP) (days) – third interparity period; (AIP) days – average interparity period; No. – number of animals, SE – standard error, () – number of animals in each genotype group; bolded – P-values≤0.05.

Table 6. Results of the association analysis between *DIO3 rs80983654* polymorphism and reproductive traits

Trait	No.	Whole population					No.	Large White					No.	Landrace				
		GG		GT				GG		GT				GG		GT		
		mean	SE	mean	SE	P-value		mean	SE	mean	SE	P-value		mean	SE	mean	SE	P-value
ANBA	196	12.28	0.0	12.75	0.1	0.10	112	12.28	0.1	12.76	0.1	**0.01**	84	12.27	0.1	12.71	0.42	0.64
ANB21d	196	11.76	0.0	12.11	0.1	0.33	112	11.69	0.1	12.16	0.1	**0.00**	84	11.82	0.1	11.82	0.39	0.84
AIP	334	151.79	1.18	155.2	1.53	0.22	185	160.22	1.69	154.03	1.33	**0.01**	149	159.39	1.67	161.76	6.7	0.51
1AP	344	361.48	2.1	371.82	5.0	0.57	188	361.16	2.8	379.11	5.7	**0.00**	156	361.78	3.1	345.65	7.10	0.23
L1NBA	344	11.70	0.1	12.15	0.1	0.40	188	12.04	0.1	12.16	0.2	0.75	156	11.40	0.1	12.12	0.32	0.92
L1NB21d	344	11.26	0.1	11.81	0.1	0.10	188	11.53	0.1	11.82	0.2	0.29	156	11.01	0.1	11.76	0.33	0.55
L2NBA	334	12.46	0.1	12.65	0.2	0.85	185	12.66	0.1	12.95	0.2	0.24	149	12.27	0.1	11.47	0.40	**0.00**
L2NB21d	334	11.91	0.0	11.93	0.2	0.57	185	12.01	0.1	12.20	0.2	0.42	149	11.81	0.1	10.87	0.36	**0.01**
1IP	334	167.88	2.0	160.76	2.6	**0.02**	185	167.14	2.3	158.15	2.6	**0.00**	149	168.57	2.8	171.00	7.43	0.29
L3NBA	278	12.64	0.1	13.00	0.2	0.18	152	12.76	0.1	12.81	0.2	0.90	126	12.53	0.1	13.82	0.63	**0.00**
L3NB21d	278	12.20	0.1	12.27	0.2	0.71	152	12.17	0.1	12.19	0.2	0.97	126	12.23	0.1	12.64	0.59	0.31
2IP	278	159.58	1.4	157.27	2.5	0.43	152	158.98	1.9	157.23	2.9	0.31	126	161.12	2.0	157.45	4.61	0.73
L4NBA	196	12.61	0.1	13.07	0.2	**0.02**	112	12.30	0.2	13.08	0.2	**0.01**	84	12.91	0.1	13.00	0.87	0.81
L4NB21d	196	11.91	0.1	12.48	0.2	**0.01**	112	11.59	0.2	12.54	0.2	**0.00**	84	12.22	0.1	12.14	0.67	0.99
3IP	196	158.91	1.5	155.52	2.6	0.49	112	158.18	1.9	153.21	2.0	0.08	84	159.60	2.4	168.43	12.94	0.45

(L(1–4) NBA) – number of piglets born alive in the first and subsequent parities, respectively; (L(1–4) NB21d) – number of piglets at the 21st day in the first and subsequent parities, respectively; (ANBA) – average number of piglets born alive from 4 parities; (ANB21d) – average number of piglets at 21st day from 4 parities; (1AP) (days) – age at the first parity; (1IP) (days) – first interparity period; (2IP) (days) – second interparity period; (3IP) (days) – third interparity period; (AIP) days – average interparity period; SE – standard error *, () – number of animals in each genotype group; bolded – P-values≤0.05.

Table 7. Results of the association analysis between *DIO3 rs80999359* polymorphism and carcass traits

Trait	No.	Whole population					No.	Large White					No.	Landrace				
		CC		CT				CC		CT				CC		CT		
		mean	SE	mean	SE	P-value		mean	SE	mean	SE	P-value		mean	SE	mean	SE	P-value
CY (%)	778	76.58	0.1	77.04	0.3	0.47	26	77.24	0.17	77.02	0.8	0.20	22	76.71	0.16	77.04	0.33	0.78
WT (kg)	778	0.38	0.0	0.40	0.0	0.70	26	0.38	0.00	0.38	0.0	0.72	22	0.40	0.00	0.40	0.01	0.36
WL (kg)	778	7.65	0.0	7.84	0.1	0.85	26	7.84	0.05	8.10	0.1	0.40	22	7.79	0.05	7.78	0.11	0.60
WH (kg)	778	9.25	0.0	9.31	0.0	0.89	26	9.18	0.04	8.88	0.1	0.40	22	9.31	0.04	9.42	0.07	0.49
BT (cm)	778	1.32	0.0	1.22	0.0	**0.01**	26	1.35	0.02	1.20	0.0	0.12	22	1.32	0.03	1.22	0.04	**0.01**
LEA (cm^2)	778	52.74	0.2	54.33	0.6	0.11	26	51.06	0.36	51.86	1.8	0.59	22	53.16	0.44	54.84	0.74	**0.04**
MP (%)	778	61.79	0.1	62.29	0.4	0.10	26	60.56	0.20	61.63	1.0	0.50	22	61.40	0.27	62.40	0.44	0.10
MC (kg)	778	24.04	0.0	24.48	0.1	0.11	26	23.81	0.09	23.90	0.4	0.55	22	24.14	0.11	24.60	0.16	0.09

(CY) % – carcass yield, (WL) kg – weight of loin, (WT) kg – weight of tenderloin, (WH) kg – weight of ham, (BT) cm – backfat thickness (mean of the measures in five points), (LEA) cm^2 – loin eye area, (MP) % – percent of the meat in the carcass, (MC) kg – weight of the main cuts; SE – standard error *, () – number of animals in each genotype group; bolded – P-values<0.05.

Table 8. Results of the association analysis between *DIO3 rs80993654* polymorphism and carcass traits

Trait	Whole population						Large White						Landrace					
	No.	GG		GT			No.	GG		GT			No.	GG		GT		
		mean	SE	mean	SE	P-value		mean	SE	mean	SE	P-value		mean	SE	mean	SE	P-value
CY (%)	759	76.40	0.10	77.53	0.25	0.3705	242	76.60	0.19	77.84	0.28	0.1190	226	76.81	0.15	77.25	0.61	0.9213
WT (kg)	759	0.38	0.00	0.39	0.01	0.5662	242	0.38	0.00	0.38	0.01	0.3766	226	0.40	0.00	0.39	0.01	0.2100
WL (kg)	759	7.59	0.03	8.00	0.07	0.1095	242	7.67	0.06	8.08	0.07	0.0637	226	7.80	0.05	7.93	0.22	0.9811
WH (kg)	759	9.27	0.03	9.19	0.06	**0.0432**	242	9.14	0.05	9.17	0.07	0.1216	226	9.35	0.04	9.27	0.11	0.1639
BT (cm)	759	1.29	0.01	1.37	0.03	0.2781	242	1.29	0.03	1.42	0.03	0.1475	226	1.32	0.02	1.19	0.06	0.2083
LEA (cm^2)	759	53.09	0.28	52.38	0.64	0.8602	242	50.16	0.42	51.92	0.69	0.2344	226	53.75	0.40	54.15	1.65	0.4863
MP (%)	759	62.09	0.15	60.78	0.33	0.2893	242	60.76	0.23	60.26	0.36	0.5346	226	61.57	0.24	61.70	0.76	0.4431
MC (kg)	759	24.10	0.07	24.06	0.15	0.2830	242	23.67	0.12	23.97	0.17	0.5624	226	24.25	0.10	24.53	0.32	0.4789

(CY) % – carcass yield, (WL) kg – weight of loin, (WT) kg – weight of tenderloin, (WH) kg – weight of ham, (BT) cm – backfat thickness (mean of the measures in five points), (LEA) cm^2 – loin eye area, (MP) % – percent of the meat in the carcass, (MC) kg – weight of the main cuts; SE – standard error *, () – number of animals in each genotype group; bolded – P-values<0.05.

Association analysis between *rs80999359* and *rs80983654* and carcass traits

We have found significant associations between polymorphisms in *DIO3* gene and only a few carcass traits. We observed that animals with CT genotype in *rs80999359* were characterized by lower backfat thickness than animals with CC genotype ($P<0.01$) in the whole population (Table 7). The same association was observed in Landrace breed, but not in Large White when breeds were analysed separately ($P<0.01$). Additionally, higher values for LEA were observed in CT animals of Landrace breed ($P<0.04$) (Table 7). The second polymorphism *rs80983654* was associated only with WH ($P<0.04$). Animals with GG genotype had heavier ham than GT animals when the whole population was analysed together but not in separate breeds (Table 8).

Discussion

The frequency of alleles at *rs80983654* observed in our study was contrary to that presented by Qiao et al. (2012) who noticed high frequency of T allele (0.93) in Landrace and Yorkshire pigs. Nevertheless in the indigenous Chinese breeds the most frequent was allele G (Qiao et al., 2012). There is no information about frequency of alleles at the second polymorphism (*rs80999359)* in other populations of pigs.

Our results suggest that polymorphisms within *DIO3* gene may be associated with reproductive traits in pigs. Nevertheless, we observed some conflicting results between breeds or between similar traits. This may be connected with parental imprinting of *DIO3* gene. Heterozygous animals may represent different phenotypes depending on parental origin of alleles. In our study we did not have information about parental genotypes of analysed animals. Moreover, minor allele frequency in both polymorphisms was too low to include them into statistical analysis. These aspects might have decreased the statistical power of the association detection (Distl, 2007). Identification of genes or markers influencing prolificacy of pigs is challenging because the heritability of these traits is low (Coster et al., 2012). Recently we have performed an association study on larger number of animals from the same populations, using the same statistical model and failed to find significant associations between polymorphisms in *GNAS, IGF2* and *MC4R* genes and reproductive traits (Oczkowicz et al., 2013). Interestingly, Coster et al. (2012) investigated associations of SNPs in the fifteen imprinted regions in porcine genome with prolificacy and found significant effects in a region proximal to *DIO3* gene but not in *IGF2* gene. In cattle, associations between SNPs in *DLK1-DIO3* domain and economically important traits have been investigated (Magee et al., 2011). Significant effects of polymorphisms in the neighbourhood of *MEG3* and *MEG8* genes were found on gestation length, perinatal mortality and calving interval in that study. All this data together with our results suggests that *DLK1-DIO3* domain is a promising candidate region for identification of causative mutation affecting prolificacy in pigs.

Qiao et al. (2012) identified several associations between *rs80983654* and carcass and meat quality traits in the 312 pigs of Large White × Meishan F2 resource

family. Significant additive effects were observed on lean meat percentage, fat meat percentage, ratio of lean to fat, shoulder fat thickness, sixth-seventh rib fat thickness, buttock fat thickness, loin eye area, meat colour value and significant dominant effects on *longissimus dorsi* intramuscular fat, lean meat percentage, *biceps femoris* meat colour value, *longissimus dorsi* intramuscular fat and *longissimus dorsi* water holding capacity. They concluded that allele G is associated with the increase of fat deposition traits, but decrease of traits related to muscle traits. Our observation is partially conflicting to that observed by Qiao et al. (2012) since in our population allele G was associated with increased weight of ham. These discrepancies suggest that *rs80983654* is not a causative mutation, but rather is in linkage disequilibrium with another polymorphism – causative for muscle traits. It is possible that linkage phase was different in Polish and Chinese populations and contrasting frequency of G allele in these populations is a result of selection pressure for lean meat percentage. On the other hand, our analysis was performed on larger population but did not include TT homozygous animals because this genotype was almost absent. This factor might have influenced the statistical power of the analysis, especially when we take into account the complex manner of expression of genes in *DLK1-DIO3* domain.

Conclusions

Our experiment showed that both analysed mutations are little polymorphic in pigs bred in Poland. The low power of the analysis and the lack of the information on parental origin of the alleles might have affected association analysis with reproductive and carcass traits. We observed some associations for reproductive traits, most pronounced in Large White breed, while for carcass traits the associations seemed to be weaker. We concluded that both polymorphisms are not causative, but it is very probable that the analysed region comprises mutation which is directly involved in modulation of fertility in pigs.

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