ACTA UNIVERSITATIS AGRICULTURAE ET SILVICULTURAE MENDELIANAE BRUNENSIS

Volume LX 10 Number 5, 2012

ASSOCIATION ANALYSIS OF *INTERLEUKIN-18* GENE WITH PERFORMANCE TRAITS IN CZECH LARGE WHITE PIGS

P. Chalupová, T. Urban, A. Knoll

Received: May 3, 2012

Abstract

CHALUPOVÁ, P., URBAN, T., KNOLL, A.: *Association analysis of interleukin-18 gene with performance traits in Czech Large White pigs.* Acta univ. agric. et silvic. Mendel. Brun., 2012, LX, No. 5, pp. 97–102

This research focused on recently described *Msp*I and *Vsp*I SNPs in interleukin-18 gene and their association with selected performance traits (backfat thickness; lean meat content, average daily gain from birth, average daily gain in test, breeding value for average daily gain, for lean meat content, for reproduction and total breeding value) in a population of 344 Czech Large White sows. Both SNPs were genotyped by PCR-RFLP. In this work, these polymorphisms were associated with backfat thickness and lean meat content. Animals with genotype *AA* (*Msp*I SNP) had significantly ($P < 0.05$) lower backfat thickness and higher lean meat content (0.77 ± 0.02 and 62.16 ± 0.23, resp.) compared to *GA* (0.83 ± 0.02 and 61.40 ± 0.18, resp.). Animals with genotype *AA* (*Vsp*I SNP) had significantly ($P < 0.05$) lower lean meat content and higher backfat thickness (61.10 ± 0.36 and 0.85 ± 0.03, resp.) compared to *GA* (62.02 ± 0.16 and 0.77 ± 0.02, resp.). No association between *IL-18* polymorphisms and other performance traits was found. Our study revealed that *IL-18* could be candidate gene for backfat thickness and lean meat content in pigs.

pig, *IL-18*, polymorphism, performance traits, association

Breeding efficiency is a necessary precondition for ensuring the competitiveness of Czech pork production. The most important traits for pork production in the growing phase are lean growth, feed intake and pig survival. Consumers are more concerned about the degree of fatness or carcass merit as well as pork quality (Rothschild *et al.*, 2007). Producers have to pay attention to these traits to ensure the demand of the pork. Using marker assisted selection (MAS), the commercial pig industry is actively using this information and traditional performance information to improve pig production (Rothschild, 2003).

For association study are usually used purebred animals (Fontanesi *et al.*, 2010; Aslan *et al.*, 2012) or F2 experimental crosses (Gandolfi *et al.*, 2011). Numbers of animals of each breed range from around 100 (Aslan *et al.*, 2012) to around 300 (Fontanesi *et al.*, 2010). Results are evaluated most frequent in the statistical program SAS using mostly procedure GLM (Sironen *et al.*, 2009, Davoli *et al.*, 2012) or MIXED (Chen *et al.*, 2012).

This research focused on the two SNPs in interleukin-18 gene (*IL-18*) found previously (Chalupová *et al.*, in preparation) and their association with important performance traits in pigs.

IL-18 is a proinflammatory cytokine that belongs to the *IL-1* cytokine family and shows pleiotropic effect. *IL-18* modulates the activity of *Th1* cells, cytotoxic T lymphocytes, NK cells, macrophages, dendritic cells, and B cells (Biet *et al.*, 2002). *IL-18* was mapped to SSC9p13 (Fornout *et al.*, 2000). *IL-18* or *IL-18* receptor knockout in mouse leaded to increased food intake, obesity and insulin resistance (Netea *et al.*, 2006). Level of plasma *IL-18* was previously associated with insulin resistance independent of obesity in man (Fischer *et al.*, 2005). Plasma *IL-18* was associated with changes in insulin resistance and reduced after weight loss (Bruun *et al.*, 2007). In obese women, *IL-18* levels were associated with

body weight and abdominal fat deposition. Weight loss leaded to *IL-18* levels reduction (Esposito *et al.*, 2002). There is the increasing number of evidences that *IL-18* might be closely related to the metabolic syndrome. Moreover, cytokines are probably involved in the regulation of skeletal muscle function (Trøseid *et al.*, 2010).

MATERIALS AND METHODS

In total 344 Czech Large White purebred sows with defined relationship were selected randomly from 3 nucleus herds (N = 98, 101 and 145). The phenotypic values of backfat thickness (BFT, cm), lean meat content (LM, %) and average daily gain from birth (ADGb, g) was studied in all 3 herds and the average daily gain in test (ADGt, g), breeding value for average daily gain (EBVadg), for lean meat content (EBVlm), for reproduction (EBVr) and total breeding value (EBVt) were studied in herd 2 and 3 only. The data were collected by the Association of Pig Breeders in Bohemia and Moravia (http://www.schpcm.cz/) during the field test in compliance with appropriate methodology (Pražák and Žáková, 2005).

Polymorphisms were analyzed by PCR-RFLP method. PCR was performed in 12.5 µl reaction volume using 100 ng of porcine genomic DNA, 0.2 µM of each primer, 200 µM of each dNTP (Fermentas, EU), 1×Taq Buffer complete (containing 1.5 mM $MgCl_2$; Top-Bio, s.r.o., Prague, Czech Republic), and 0.5 U of Taq DNA Unis polymerase (Top-Bio, s.r.o.). PCR reaction was performed in GeneAmp® PCR System 9400 (Applied Biosystems, Foster City, CA) under following conditions: 95 °C/4 min, 30 × (95 °C/20 s; 55 °C/30 s; 72 °C/15 s), 72 °C/6 min.

SNP ENSSSCG00000015037:g.11237G>A in exon 2 of *IL-18* (synonymous mutation) was analyzed by *Msp*I restriction endonuclease. SNP ENSSSCG00000015037:g.19480G>A; in intron 5-6 was analyzed by *Vsp*I.

Association analysis was performed in SAS (Version 9.1.4, SAS Institute, Cary, NC, USA) by mixed linear model using REML procedure. Fixed effects of polymorphisms and herd, and random effects of sire and dam were included.

$$Y_{ijklmn} = \mu + MspI_i + VspI_j + h_k + f_l + m_m + e_{ijklmn},$$

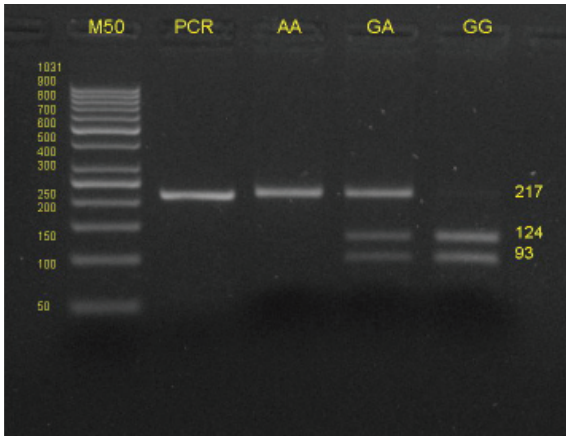
where:

Y_{ijklmn} ..phenotypic value of analysed trait
µ.....population mean
$MspI_i$..fixed effect of the ith *Msp*I genotype of *IL-18* gene (*i* =*AA*, *AG* and *GG*)
$VspI_j$...fixed effect of the jth *Vsp*I genotype of *IL-18* gene (*i* =*AA*, *AG* and *GG*)
h_k.....fixed effect of the kth herd
f_j..... random effect of the jth father
m_m..... random effect of the mth mother
e_{ijklmn}..... random error effect of each observation.

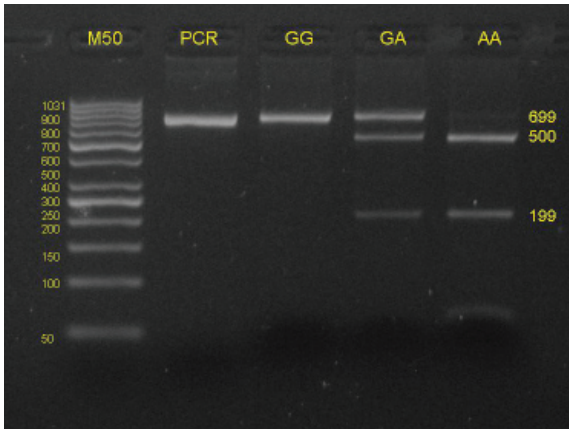
RESULTS AND DISCUSSION

PCR-RFLP

New g.11237G>A polymorphism was analyzed by *Msp*I. After digestion and visualisation, following fragments were obtained: 93 and 124 bp for allele *G*; 217 bp for allele *A* (Fig. 1). g.19480G>A polymorphism was analyzed by *Vsp*I resulting in 500 and 199 bp fragments for allele *A* and 699 bp for allele *G* (Fig. 2). Allele and genotype frequencies were calculated (Tab. I).



1: *Agarose gel (3%) showing the IL-18 g.11237G > A polymorphism after MspI digestion. M50 GeneRuler™ 50 bp DNA Ladder (Fermentas, EU)*



2: *Agarose gel (3%) showing the IL-18 g.19480G > A polymorphism after VspI digestion. M50 GeneRuler™ 50 bp DNA Ladder (Fermentas, EU)*

Association analysis

Descriptive statistics are presented in Table II. All variables had close to normal distribution.

Association analysis results are showed in Tab. III.

In *IL-18 Msp*I polymorphism, animals with genotype *AA* had significantly lower backfat thickness and higher lean meat content compared to *GA* ($P < 0.05$). In *IL-18 Vsp*I polymorphism, pigs with genotype *AA* had significantly lower lean meat

I: *Allele and genotype frequencies*

SNP	Allele frequency				Genotype frequency						N
IL-18_MspI	A	0.37	G	0.63	AA	0.15	AG	0.44	GG	0.42	344
IL-18_VspI	A	0.28	G	0.72	AA	0.08	AG	0.40	GG	0.52	344

II: *Descriptive statistics.*

	N	Mean	Variance	St. Deviation	St. Error
BFT	344	0.78	0.04	0.19	0.01
LM	342	61.93	4.69	2.17	0.12
ADGb	344	587.83	2891.47	53.77	2.90
ADGt	344	719.00	211839.90	460.26	24.82
EBVadg	344	12.84	196.55	14.02	0.76
EBVlm	344	0.69	0.36	0.60	0.03
EBVr	344	1.23	0.79	0.89	0.05
EBVt	344	932.47	419605.4	647.77	34.93

III: *Association analysis of performance traits in Czech Large White pigs*

SNP *IL-18*	genotype (N = 344)	BFT	LM	ADGb	genotype (N = 246)	ADGt	EBVadg	EBVlm	EBVr	EBVt
MspI	*AA* (51)	0.77 ± 0.02[a]	62.16 ± 0.23[a]	588 ± 6.57	*AA* (29)	995 ± 18.69	18.50 ± 2.51	1.10 ± 0.08	1.63 ± 0.10	1247.77 ± 65.29
	GA (150)	0.83 ± 0.02[a]	61.40 ± 0.18[a]	585 ± 5.11	*GA* (109)	1011 ± 15.82	18..40 ± 2.16	1.04 ± 0.07	1.64 ± 0.09	1252.45 ± 57.55
	GG (143)	0.80 ± 0.02	61.56 ± 0.26	588 ± 7.46	*GG* (108)	1005± 21.04	16.71 ± 2.70	1.00 ± 0.08	1.57 ± 0.11	1193.20 ± 69.32
VspI	*AA* (26)	0.85 ± 0.03[a]	61.10 ± 0.36[a]	591 ± 10.29	*AA* (7)	1003 ± 36.24	14.36 ± 4.50	1.08 ± 0.14	1.58 ± 0.19	1154.48 ± 111.52
	GA (139)	0.77 ± 0.02[a]	62.02 ± 0.16[a]	583 ± 4.56	*GA* (96)	1002 ± 12.37	18.26 ± 1.83	0.99 ± 0.05	1.62 ± 0.08	1245.32 ± 50.68
	GG (179)	0.78 ± 0.02	62.00 ± 0.20	586 ± 5.75	*GG* (143)	1006 ± 14.33	20.99 ± 2.07	1.08 ± 0.06	1.64 ± 0.09	1293.62 ± 55.76

Least squares means ± standard error. The same superscripts in a column show significant differences between the genotypes: [a,b] $P < 0.05$
BFT = backfat thickness; LM = lean meat content; ADGb = average daily gain from birth; ADGt = average daily gain in test; EBVadg = breeding value for average daily gain; EBVlm = breeding value for lean meat content; EBVr = breeding value for reproduction; EBVt = total breeding value

content and higher backfat thickness compared to *GA* ($P < 0.05$). Moreover, animals with genotype *AA* had lower lean meat content compared to *GG* (close to significant, $P = 0.0665$).

No association between *IL-18* polymorphisms and other performance traits (average daily gain in test, breeding values for average daily gain, for lean meat content, for reproduction and total breeding value) was found. Subsequent studies with more animals should be carried out to further support there results and to refine on association studies.

Due to the fact that these polymorphisms in *IL-18* are new, there are no association analyses in pigs done by other authors. Many studies in mouse and human indicates, that this gene is candidate for fat deposition (Esposito *et al*., 2002; Netea *et al*., 2006; Bruun *et al*., 2007; Evans *et al*., 2007; Trøseid *et al*., 2010). Based on our results, we tend to this conclusion also.

CONCLUSIONS

This pilot study revealed association between two SNPs in *IL-18* and backfat thickness and lean meat content. Because none of the polymorphisms cause structural changes in protein, the new *IL-18* polymorphisms are probably markers that are in linkage disequilibrium with unknown causative mutations. Due to the quantitative character of analysed traits and possible influencing by unknown genetic background, the results should be verified in other and/or extended populations. However, SNPs given in this research could be potentially useful in marker assisted selection and pork production efficiency.

Further studies could help to understand the physiological role of *IL-18* in lipid metabolism and muscle formation in pigs and these data could serve as a foundation for next research of these novel SNPs in *IL-18* gene.

SUMMARY

For Czech pig breeding sustainability is necessary to ensure high efficiency and competitiveness of breeds. Modern methods of molecular genetics provide a suitable tool for analysis of genetic markers. MAS (Marker Assisted Selection) can serve as a timely and efficient selection of pigs with outstanding production parameters.

This study focused on association analysis between two polymorphism in *IL-18* gene and important performance traits in pigs (backfat thickness; lean meat content, average daily gain from birth, average daily gain in test, breeding values for average daily gain, lean meat content and reproduction and total breeding value). In total, 344 Czech Large White sows selected randomly from 3 herds were tested. SNPs ENSSSCG00000015037:g.11237G>A in exon 2 of *IL-18* and ENSSSCG00000015037:g.19480G>A in intron 5-6 of *IL-18* were analysed using PCR-RFLP method and restriction endonuclease *Msp*I and *Vsp*I, respectively.

Association analysis revealed influence of *IL-18* polymorphisms to backfat thickness and lean meat content. Animals with genotype *AA* (*Msp*I SNP) had significantly ($P < 0.05$) lower backfat thickness and higher lean meat content (0.77 ± 0.02 and 62.16 ± 0.23, resp.) compared to *GA* (0.83 ± 0.02 and 61.40 ± 0.18, resp.). Animals with genotype *AA* (*Vsp*I SNP) had significantly ($P < 0.05$) lower lean meat content and higher backfat thickness (61.10 ± 0.36 and 0.85 ± 0.03 resp.) compared to *GA* (62.02 ± 0.16 and 0.77 ± 0.02 resp.). No association between *IL-18* polymorphisms and other performance traits (average daily gain in test, breeding values for average daily gain, for lean meat content, for reproduction and total breeding value) was found.

The results extend our knowledge of molecular genetic markers in pigs and could be potentially useful for genetic improvement of Czech pig populations and pork production efficiency.

Acknowledgement

This study was supported by the GAČR 523/09/0844 and realized in the European center of excellence "CEITEC – Central European Institute of Technology" supported by the project CZ.1.05/1.1.00/02.0068 from the European Regional Development Fund.

REFERENCES

ASLAN, O., HAMILL, R. M., MULLEN, A. M., DAVEY, G. C., GIL, M., GLADNEY, C. D., SWEENEY T., 2012: Association between promoter polymorphisms in a key cytoskeletal gene (Ankyrin 1) and intramuscular fat and water-holding capacity in porcine muscle. *Mol. Biol. Rep.*, 39, 4: 3903–3914.

BIET, F., LOCHT, C., KREMER, L., 2002: Immunoregulatory functions of interleukin 18 and its role in defense against bacterial pathogens. *J. Mol. Med.*, 80, 3: 147–162.

BRUUN, J. M., STALLKNECHT, B., HELGE, J. W., RICHELSEN, B., 2007: Interleukin-18 in plasma and adipose tissue: effects of obesity, insulin resistance, and weight loss. *Eur. J. Endocrinol.*, 157, 4: 465–471.

CHEN, K., HAWKEN, R., FLICKINGER, G. H., RODRIGUEZ-ZAS, S. L., RUND, L. A., WHEELER, M. B., ABRAHAMSEN, M., RUTHERFORD, M. S., BEEVER, J. E., SCHOOK, L. B., 2012: Association of the porcine transforming growth factor beta type I receptor (*TGFBR1*) gene with growth and carcass traits. *Anim. Biotechnol.*, 23, 1: 48–63.

DAVOLI, R., BRAGLIA, S., VALASTRO, V., ANNARRATONE, C., COMELLA, M., ZAMBONELLI, P., NISI, I., GALLO, M., BUTTAZZONI, L., RUSSO, V., 2012: Analysis of *MC4R* polymorphism in Italian Large White and Italian Duroc pigs: Association with carcass traits. *Meat Sci.*, 90, 4: 887–892.

ESPOSITO, K., PONTILLO, A., CIOTOLA, M., DI PALO, C., GRELLA, E., NICOLETTI, G., GIUGLIANO, D., 2002: Weight loss reduces interleukin-18 levels in obese women. *J. Clin. Endocrinol. Metab.*, 87, 8: 3864–3866.

EVANS, J., COLLINS, M., JENNINGS, C., VAN DER MERWE, L., SÖDERSTRÖM, I., OLSSON, T., LEVITT, N. S., LAMBERT, E. V., GOEDECKE, J. H., 2007: The association of interleukin-18 genotype and serum levels with metabolic risk factors for cardiovascular disease. *Eur. J. Endocrinol.,* 157, 5: 633–640.

FISCHER, C. P., PERSTRUP, L. B., BERNTSEN, A., ESKILDSEN, P., PEDERSEN, B. K., 2005: Elevated plasms interleukin-18 is a marker of insulin-resistance in type 2 diabetic and non-diabetic humans. *Clin. Immunol.*, 117, 2: 152–160.

FONTANESI, L., SPERONI, C., BUTTAZZONI, L., SCOTTI, E., COSTA, L. N., DAVOLI, R., RUSSO, V., 2010: Association between cathepsin L (CTSL) and cathepsin S (*CTSS*) polymorphisms and meat production and carcass traits in Italian Large White pigs. *Meat Sci.*, 85, 2: 331–338.

FORNOUT, S., DOZOIS, C. M., YERLE, M., PINTON, P., FAIRBROTHER, J. M., OSWALD, E., OSWALD, I. P., 2000: Cloning, chromosomal location, and tissue expression of the gene for pig interleukin-18. *Immunogenetics*, 51, 4–5: 358–365.

GANDOLFI, G., CINAR, M. U., PONSUKSILI, S., WIMMERS, K., TESFAYE, D., LOOFT, C., JÜNGST, H., THOLEN, E., PHATSARA, C., SCHELLANDER, K., DAVOLI, R. 2011: Association of *PPARGC1A* and *CAPNS1* gene polymorphisms and expression with meat quality traits in pigs. *Meat Sci.*, 89, 4: 478–485.

NETEA, M. G., JOOSTEN, L. A., LEWIS, E., JENSEN, D. R., VOSHOL, P. J., KULLBERG, B. J., TACK, C. J., VAN KRIEKEN, H., KIM, S. H., STALENHOEF, A. F., VAN DE LOO, F. A., VERSCHUEREN, I., PULAWA, L., AKIRA, S., ECKEL, R. H., DINARELLO, C. A., VAN DEN BERG, W., VAN DER MEER, J. W., 2006: Deficiency of interleukin-18 in mice leads to hyperphagia, obesity and insulin resistance. *Nat. Med.*, 12, 6: 650–656.

PRAŽÁK, Č., ŽÁKOVÁ E., 2005: Metodické pokyny hodnocení plemenných, chovných a užitkových prasat [online] Svaz chovatelů prasat v Čechách a na Moravě, 1–11. [cit. 2012-06-15]. Accessible by: http://ksz.af.czu.cz/slechteniprasat/texty/05_hodnoc.pdf.

ROTHSCHILDT, M. F., 2003: From a sow's ear to a silk purse: real progress in porcine genomics. *Cytogenet. Genome Res.*, 102, 1–4: 95–99.

ROTHSCHILD, M. F., HU, Z. L., JIANG, Z., 2007: Advances in QTL Mapping in Pigs. *Int. J. Biol. Sci.*, 3, 3: 192–197.

SIRONEN, A. I., UIMARI, P., SERENIUS, T., MOTE, B., ROTHSCHILD, M., VILKKI, J., 2009: Effect of polymorphisms in candidate genes on reproduction traits in Finnish pig populations. *J. Anim. Sci.*, 88, 3: 821–827.

TRØSEID, M., SELJEFLOT, I., ARNESEN, H., 2010: The role of interleukin-18 in the metabolic syndrome. *Cardiovasc. Diabetol.*, 9, 11: 1–8.

Address

Ing. Pavla Chalupová, Ústav morfologie, fyziologie a genetiky zvířat a CEITEC MENDELU, Mendelova univerzita v Brně, Zemědělská 1, 613 00 Brno, Česká republika, e-mail: pavla.chalupova@gmail.com