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Stefania Dall'Olio, Luca Fontanesi, Lucia Tognazzi, Luca Buttazzoni, Maurizio Gallo & Vincenzo Russo

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SHORT COMMUNICATION

ESR1 and *ESR2* gene markers are not associated with number of piglets born alive in Italian Large White sows

Stefania Dall'Olio,[1] Luca Fontanesi,[1] Lucia Tognazzi,[1] Luca Buttazzoni,[2] Maurizio Gallo,[2] Vincenzo Russo[1]

[1]Dipartimento di Protezione e Valorizzazione Agroalimentare, Università di Bologna, Italy
[2]Associazione Nazionale Allevatori Suini, Roma, Italy

Abstract

Many studies have reported that markers in the *estrogen receptor 1* (*ESR1*) and *estrogen receptor 2* (*ESR2*) genes are associated with litter size in pigs, even if inconsistent results have been obtained in different populations. We analysed the *ESR1 Pvu*II and the ESR2 AF164957:c.949G>A polymorphisms in Italian Large White (ITLW) sows to evaluate if these markers are associated with number of piglets born alive at first litter (NBA1). First, both polymorphisms were genotyped by selective genotyping in a total of 440 sows chosen according to the extreme and divergent estimated breeding value (EBV) for NBA1 (220 sows with low EBV and 220 sows with high EBV). For the *ESR1* polymorphism, no allele and genotype frequency differences were observed between the two groups (allele A=0.62 and allele B=0.38 in both two groups). For the *ESR2* polymorphism, a trend of different allele frequency between the two tails was identified (P=0.052). However, no significant association between the same *ESR2* marker and EBV NBA1 was detected analyzing 1772 ITLW sows (allele A=0.59 and allele G=0.41). As the two investigated polymorphisms were not associated with NBA1 EBVs, they seem not useful for marker assisted selection to improve this trait in the ITLW breed.

Introduction

Prolificacy is one of the most important parameters affecting reproductive efficiency of sows (pigs weaned/sow/year). To improve sow prolificacy, selection based on quantitative genetics has been less effective compared to other performance and production traits because this trait is sex-limited and measurable only after sexual maturity and has low heritability. The use of DNA-based information (marker assisted selection and gene assisted selection, MAS and GAS, respectively) in conjunction with traditional selection methods could be useful to accelerate genetic progress for litter size and female reproduction efficiency in pigs. Several candidate genes for sow prolificacy have been already evaluated in different pig breeds/lines (Buske *et al*., 2006a; Spötter and Distl, 2006; Distl, 2007). Among these genes, *estrogen receptor 1* (*ESR1*) and *estrogen receptor 2* (*ESR2*) polymorphisms have been reported to explain part of litter size variability in several pig populations. Both genes map on *Sus scrofa* chromosome 1 (SSC1) and encode proteins involved in numerous physiological mechanisms directly or indirectly affecting reproduction (Muñoz *et al*., 2004; Distl, 2007). In particular, ESR1 is involved in the development of secondary sex traits, fertility and lactation, whereas ESR2 is essential for ovulation, maturation of the ovarian follicles and growth and development of pery-implantation embryos (Muñoz *et al*., 2007). The intronic *Pvu*II recognition site polymorphism in the *ESR1* gene was the first described marker gene associated with pig litter size (Rothschild *et al*., 1994, 1996). As this polymorphism was the objective of a patent (US5550024), information on the exact nucleotide substitution and position were not reported and the *Pvu*II alleles were referred as A and B. Rothschild *et al*. (1994, 1996) indicated that *ESR1* B allele was associated with increased litter size in PIC synthetic lines made with Meishan or Large White blood. However, the effects of this polymorphism on litter size resulted contradictory (Alfonso, 2005; Buske *et al*., 2006a; Distl, 2007), so it has been largely debated whether the *ESR1 locus* could or not be introduced into breeding programs.

A few polymorphic sites have been also reported for the *ESR2* gene (Muñoz *et al*., 2004). In particular one missense mutation (c.949G>A; p.317Val>Met) in exon 5 has been shown to be associated with litter size (Buske *et al*., 2006b), but with inconsistencies among different populations (Muñoz *et al*., 2004, 2007; Buske *et al*., 2006c; Rempel *et al*., 2010).

In this study, we wanted to evaluate the effects of *ESR1 Pvu*II and *ESR2* c.949G>A polymorphisms on prolificacy of Italian Large White (ITLW) sows.

Corresponding author: Prof. Stefania Dall'Olio, Dipartimento di Protezione e Valorizzazione Agroalimentare, Sezione di Allevamenti Zootecnici, Facoltà di Agraria, Università di Bologna, via F.lli Rosselli 107, 42123 Reggio Emilia, Italy.
Tel. +39.0522.290516 - Fax: +39.0522.290523.
E-mail: stefania.dallolio@unibo.it

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Materials and methods

Animals and data

Hair root samples were collected from 1803 ITLW sows (referred as basic population) reared in six different herds in the North of Italy. All sows were registered to the Herd Book of the Italian Large White breed maintained by the National Association of Pig Breeders (Associazione Nazionale Allevatori Suini, ANAS, Italy). Number of piglets born alive at first litter (NBA1) were recorded for these sows and data were used to calculate NBA1 estimated breeding values (EBVs) by using a BLUP-single trait-animal model including effects of herd-year, month of birth, age at first farrowing, inbreeding coefficient of sow and type of mating. The NBA1 EBVs are expressed in standard deviation units around the rolling average of indexes of sows farrowing since 1990. Genealogical data were downloaded from ANAS database (*http://www.anas.it*).

In order to reduce the number of animals to be genotyped in the association analysis, we initially applied a selective genotyping approach. Within the basic population, 440 ITLW sows were selected based on their NBA1 EBVs, genealogical data (to reduce sibs and

half-sibs within tail) and balanced within farms. This sub-sample included two groups of sows (220 sows each) with extreme and divergent values for NBA1 EBVs: 1) 220 sows with highest NBA1 EBVs (tail high, H: mean and standard deviation were equal to +2.11±0.85, range from +0.10 to +4.96) derived from 66 different boars; 2) 220 sows with lowest NBA1 EBVs (tail low, L: mean and standard deviation were equal to -1.19±0.76, range from -3.23 to -0.03) derived from 51 different boars.

In addition, hair roots were collected from Italian Landrace (50 animals), Italian Duroc (91) and Piétrain (20) pigs that were used for allele frequency evaluation of the *ESR2* c.949G>A polymorphism.

Genotyping

Genomic DNA was extracted from hair roots of a total of 1964 pigs using standard procedures. The *ESR1 Pvu*II polymorphism was analysed by PCR-RFLP (Short *et al*., 1997). Digested PCR fragments (allele A = 120 bp, allele B = 65 bp + 55 bp) were electrophoresed on 10% 29:1 polyacrylamide:bisacrylamide gels and visualized by ethidium bromide staining.

The genotyping of the *ESR2* AF164957: c.949G>A SNP was performed using the Sequenom MassArray platform and i-Plex™ Gold reagents. Amplification primer sequences were: forward = ACGTTGGATGTCTGTTTTTATGGAACTGGG and reverse = ACGTTGGATGCACTTGGTCGTACAGGCTGA (PCR product of 119 bp, Tm=59.05).

To asses genotyping accuracy for the *ESR2* SNP, several samples were genotyped in duplicates, genotyping success rate (call rate percentage) was evaluated and, after computation of allele frequency, χ^2-test was done to verify Hardy-Weinberg equilibrium.

Statistical analyses

Allele and genotype frequencies of the *loci* were calculated for each NBA1 EBVs ITLW group and for each examined breed. The Chi-square test was employed to evaluate if significant differences of allele and genotype frequencies for the *ESR1* and *ESR2* markers were present between the two divergent ITLW groups made according to NBA1 EBVs.

Based on suggestive result obtained for the *ESR2* AF164957:c.949G>A SNP, all sows of the basic population were genotyped for the *ESR2* polymorphism and association analysis with NBA1 EBVs was performed by the general linear model (GLM) procedure of SAS, release 9.2 (SAS Institute Inc., Cary, NC, USA). The statistical model included fixed effect of genotype (AA, AG and GG) for *ESR2* marker as the EBVs were already corrected for the main sources of variations contributing to the variability of NBA1 (see above the method of NBA1 EBV calculation).

Results and discussion

Allele and genotype frequencies of the two analysed SNPs (*ESR1 Pvu*II and *ESR2* c.949G>A) in the two divergent groups of sows for NBA1 EBVs are given in Table 1. Both polymorphisms segregate in the ITLW breed and all three possible genotypes/locus were detected in each group. Observed genotype frequencies for the two markers corresponded to the expected ones according to Hardy-Weinberg equilibrium. At the *ESR1 locus*, allele B frequency was equal to 0.38 in both divergent ITLW groups. Many studies have investigated this polymorphic site and differences of allele frequencies among breeds/populations were reported. The B allele was the most frequent in several Chinese breeds (Alfonso, 2005), whereas this allele was not observed in German Duroc and German Landrace (Drögemüller *et al*., 2001) as well as in Italian Duroc, Pietrain, Hampshire breeds (Russo *et al*., 2004), and in lines derived from a Landrace/Large White composite population (Linville *et al*., 2001).

In ITLW no allele and genotype frequency differences were observed between the two tails for the *ESR1* polymorphism (Table 1), indicating that this marker is not associated with NBA1 EBV in the investigated breed. Inconsistencies about the effects of the two alleles of *ESR1* gene have been reported in the literature (reviewed by Alfonso, 2005; Buske *et al*., 2006a; Distl, 2007). The positive effect of allele B on total number born (TNB) or NBA was identified in synthetic lines and in European breeds (Southwood *et al*., 1995; Rothschild *et al*., 1996; Short *et al*., 1997; Chen *et al*., 2000; Horogh *et al*., 2005). However, a favorable effect of allele A on NBA was observed in Large White pigs (Van Rens *et al*., 2002; Goliasova and Wolf, 2004; Santana *et al*., 2006). Other studies were not able to find any association between the *ESR1-Pvu*II polymorphism and litter size (Depuydt *et al*., 1999; Drögemüller *et al*., 2001; Linville *et al*., 2001; Gibson *et al*., 2002; Isler *et al*., 2002; Kmiec *et al*., 2002; Noguera *et al*., 2003; Muñoz *et al*., 2007).

For the analysed *ESR2* SNP, the allele c.949A that codes p.317Met in the translated protein was the most frequent in ITLW sows (Dall'Olio *et al*., 2010). As allele frequencies for this polymorphisms were not available in pig breeds reared in Italy, we also investigated the distribution of this marker in a few breeds and compared these results with those reported from other Authors (Table 2). Allele c.949A presented higher frequency in Italian Landrace, Pietrain and in a Landrace-Duroc-Yorkshire composite population. On the other hand, allele c.949G (p.317Val) was the most frequent in Italian Duroc, Iberian populations, German commercial F1 and F2 populations, and in a composite Chinese-European pig line (Table 2).

Comparing allele frequency for *ESR2* SNP between the two extreme and discordant groups of ITLW, a notable difference (chi square difference = 3.77; P=0.052) was detected (Table 1). Based on this suggestive result, we genotyped the c.949A>G SNP in a larger sample (1772 out of 1803 samples, call rate= 0.98%) of ITLW. In this group of sows allele A and G frequencies were 0.59 and 0.41, respectively. The results of association study between NBA1 EBVs and the *ESR2* genotypes indicated that *ESR2* is not a source of variability for the investigated reproductive trait (P>0.05). The estimated means were similar for the three genotypes (estimated means ± standard error were: AA= +0.751±0.051, AG= +0.787±0.042 and GG= +0.773±0.072).

Any association between *ESR2* marker and NBA was identified in Iberian populations (Muñoz *et al*., 2004), in a Chinese-European pig line (Muñoz *et al*., 2007), and in German F2 sows belonging to two extreme performances groups for litter size (Buske *et al*., 2006c). However, in German F1 sows a significant association (P=0.034) with NBA was reported,

Table 1. Allele and genotype frequencies of the *ESR1 Pvu*II and *ESR2* c.949A>G polymorphisms in two extreme and discordant groups of ITLW sows for NBA1 EBVs.

Loci (Groups)	Alleles 1>2	Number of sows	Allele frequencies			Genotype frequencies			
			Allele 1	Allele 2	P	11	12	22	P
ESR1 (High)	A>B	220	0.616	0.384	0.945	0.395	0.449	0.155	0.677
ESR1 (Low)		220	0.623	0.377		0.378	0.493	0.129	
ESR2 (High)	A>G	220	0.648	0.352	0.052	0.390	0.514	0.095	0.108
ESR2 (Low)		220	0.584	0.416		0.323	0.523	0.154	

P, probabilities of comparison of allele and genotype frequencies between the two groups.

Table 2. Allele frequencies of the *ESR2* c.949A>G polymorphism in different pig populations.

Pigs	Number of pigs	Allele frequencies		References
		Allele c.949A (p.317Met)	Allele c.949G (p.317Val)	
Italian Large White	1772	0.59	0.41	Current work
Italian Landrace	50	0.65	0.35	Current work
Italian Duroc	91	0.04	0.96	Current work
Pietrain	20	0.53	0.47	Current work
Torbiscal	150	0.36	0.64	Muñoz *et al*., 2004
Guadyerbas	46	0.10	0.90	Muñoz *et al*., 2004
F2 sows [(Large White x Landrace) x Leicoma]	123	0.43	0.57	Buske *et al*., 2006b
half-sib F1 sows (40 German Landrace x 1 Duroc)	129	0.34°	0.66	Buske *et al*., 2006b
Composite Chinese-European line (Meishan and Jiaxing x hyperprolific French LW)	408	0.25	0.75	Muñoz *et al*., 2007
4-line composite population (Landrace x Duroc x Yorkshire)	1417	0.51	0.49	Rempel *et al*., 2010

°No sows with the c.949AA genotype were found.

with a favourable effect of the c.949AG genotype compared to the c.949GG genotype (no animals with c.949AA genotype was identified; Buske *et al*., 2006b).

Moreover, it is possible to note that no reported quantitative trait locus for any litter size trait has been mapped close to the *ESR1* and *ESR2 loci* (Buske *et al*., 2006a; Hu *et al*., 2007; *http://www.animalgenome.org/QTLdb/*, release of November 2010). Inconsistent results about the effect of these two loci could be attributed to differences of sample size, population structures, environmental factors and statistical models used, linkage phases between SNPs and causative mutations affecting litter size or epistatic interactions with population specific genetic backgrounds.

It is worth to point out that our study of association has been conducted using EBV for a prolificacy trait. According to Ekine *et al*. (2010) simulations, the use of EBVs in association studies with DNA markers, compared with uncorrected phenotypic traits, could result in a higher level of false positives (a higher level of type I error). However, as we did not find any significant effect even using EBV, we can confidently assume that the two analysed markers may not have any important effect on NBA1 in the Italian Large White breed.

Conclusions

The use of DNA-based information in conjunction with traditional selection methods could be useful to accelerate the genetic progress for litter size and female reproduction efficiency in pigs. We analysed *ESR1-Pvu*II and *ESR2* c.949A>G candidate SNPs for litter size in ITLW breed. These two polymorphisms segregate in ITLW and based on identification of the three possible genotypes/locus and on MAF values it was possible to perform association studies with NBA1 EBV.

Results of the present study indicated that the *ESR1-Pvu*II polymorphism is not associated with NBA1 EBV variability in ITLW sows belonging to extreme and discordant EBV groups for NBA1. Therefore *ESR1-Pvu*II polymorphism may not be useful in marker assisted selection programs to improve NBA1 in ITLW breed, despite the fact that in other populations and lines this SNP has been applied to this purpose.

This is the first report that analyse allele frequency distribution of the *ESR2* c.949A>G polymorphism in Italian pig breeds. The association analysis conducted in ITLW sows did not evidence any significant effect on NBA1 EBVs. Therefore, the *ESR2* c.949A>G SNP should not be used in MAS to improve NBA1 in this breed.

Additional genes should be investigated to identify SNPs affecting litter size in ITLW sows.

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