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

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

To cite this article: Stefania Dall'Olio, Luca Fontanesi, Lucia Tognazzi, Luca Buttazzoni, Maurizio Gallo & Vincenzo Russo (2011) ESR1 and ESR2 gene markers are not associated with number of piglets born alive in Italian Large White sows, Italian Journal of Animal Science, 10:3, e35, DOI: 10.4081/ijas.2011.e35

To link to this article: https://doi.org/10.4081/ijas.2011.e35
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 Russo1
1Dipartimento di Protezione e Valorizzazione Agroalimentare, Università di Bologna, Italy
2Associazione 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 PvuII 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 genomics 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 PvuII 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 PvuII 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 PvuII and ESR2 c.949G>A polymorphisms on prolificacy of Italian Large White (ITLW) sows.

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 Pietrain (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 PvuII 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 iPLEX Gold reagents. Amplification primer sequences were: forward = AGCTTGGATCTGTTTATGG; GAACCTGG and reverse = AGCTTGGATGCTTATTG; GCACTGGG (PCR product of 119 bp, Tm=59.05).

To assess 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, chi²-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 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; Goliason and Wolf, 2004; Santana et al., 2006). Other studies were not able to find any association between the ESR1-PvuII polymorphism and litter size (Depuydt et al., 1999; Drögemüller et al., 2001; Lisvile et al., 2001; Gibson et al., 2002; Iserl et al., 2002; Kniec 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 PvuII and ESR2 c.949A>G polymorphisms in two extreme and discordant groups of ITLW sows for NBA1 EBVs.

| Loci (Groups) | 1<2 | Number of sows | Allele frequencies | Genotype frequencies |
|---------------|-----|----------------|-------------------|---------------------|
|               | 1   | 2              | 1                 | 2                   |
|               | 1   | 2              | 1                 | 2                   |
| ESR1 (High)   | A>B | 220            | 0.616             | 0.384               |
| ESR1 (Low)    | A>B | 220            | 0.623             | 0.377               |
| ESR2 (High)   | A>G | 220            | 0.649             | 0.352               |
| ESR2 (Low)    | A>G | 220            | 0.655             | 0.345               |

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

[page 186] [Ital J Anim Sci vol.10:e35, 2011]
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.

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

| Pigs                                           | Number of pigs | Allele frequencies | References |
|------------------------------------------------|----------------|--------------------|------------|
| Italian Large White                            | 1772           | 0.58               | Current work |
| Italian Landrace                               | 50             | 0.65               | Current work |
| Italian Duroc                                  | 91             | 0.65               | Current work |
| Pietrain                                       | 20             | 0.53               | Current work |
| Torbiscal                                      | 150            | 0.36               | Muñoz et al., 2004 |
| Guadyerbas                                     | 46             | 0.10               | Muñoz et al., 2004 |
| F2 sows [Large White x Landrace] x Leicoma     | 123            | 0.43               | Buske et al., 2006b |
| half-sib F1 sows (40 German Landrace x 1 Duroc)| 129            | 0.34°              | Buske et al., 2006b |
| Composite Chinese-European line                | 408            | 0.25               | Muñoz et al., 2007 |
| (Meishan and Jiaxing x hyperprolific French LW)|                |                    |            |
| 4-line composite population (Landrace x Duroc x Yorkshire)| 1417      | 0.51               | Rempel et al., 2010 |

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

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-PvuII and ESR2 c.949A>G candidate SNPs for litter size in ITLW breed. These two polymorphisms segregated 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-PvuII polymorphism is not associated with NBA1 EBV variability in ITLW sows belonging to extreme and discordant EBV groups for NBA1. Therefore ESR1-PvuII 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.

References

Alfonso, L., 2005. Use of meta-analysis to combine candidate gene association studies: application to study the relationship between the ESR PvuII polymorphism and sow litter size. Genet. Sel. Evol. 37:417-435.

Buske, B., Sternstein, I., Brockmann, G., 2006a. QTL and candidate genes for fecundity in sows. Anim. Reprod. Sci. 95:167-183.

Buske, B., Sternstein, I., Reißmann, M., Brockmann, G., 2006b. Detection of novel single-nucleotide polymorphisms (SNPs) in the CYP21 gene and association analysis of two SNPs for CYP21 and ESR2 with litter size in a commercial sow population. J. Anim. Breed. Genet. 123:343-348.

Buske, B., Sternstein, I., Reißmann, M., Rein- ecke, P., Brockmann, G., 2006c. Analysis of association of GP53, FUT1 and ESR2 genotypes with litter size in a commercial pig cross population. Arch. Tierz. 49:259-268.

Chen, K.F., Huang, L.S., Li, N., Zhang, Q., Luo, M., Wu, C. X., 2000. The genetic effect of estrogen receptor (ESR) on litter size traits in pigs. Acta Genetic Sinica 27:853-857.

Dall’Olio, S., Fontanesi, L., Tognazzi, L., Russo, V., 2010. Genetic structure of candidate genes for litter size in Italian Large White pigs. Vet. Res. Commun. 34:S203-S206.

Depuydt, J., De Smet, S., Grijsppeertd, K., Herman, L., 1999. Association study of an Aval and PvuII polymorphism at the porcine estrogen receptor (ESR) gene with litter size. Arch. Anim. Breed. 42:172-174.

Distl, O., 2007. Mechanisms of regulation of litter size in pigs on the genome level. Reprod. Domest. Anim. 42:10-16.

Driegemüller, C., Hamann, H., Distl, O., 2001. Candidate gene makers for litter size in different German pigs lines. J. Anim. Sci. 79:2565-2570.

Ekine, C.C., Rowe, S.J., Bishop S.C., de Koning D.J., 2010. What is the best phenotype for genome-wide association studies in data with defined pedigrees? No. E0263 in Proc. 9th World Congr. Genet. Appl. Livest. Prod., Leipzig, Germany.

Gibson, J.P., Jiang, Z.H., Robinson, J.A.B., Archibald, A.L., Haley, C.S., 2002. No detectable association of the ESR PvuII mutation with sows productivity in Meishan x Large White F2 population.
Anim. Genet. 33:448-450.
Goliasova, E., Wolf, J., 2004. Impact of the ESR gene on litter size and production traits in Czech Large White pigs. Anim. Genet. 35:293-297.
Horogh, G., Zsolnai, A., Komlosi, I., Nyiri, A., Anton, I., Fusus, L., 2005. Oestrogen receptor genotypes and litter size in Hungarian Large White pigs. J. Anim. Breed. Genet. 122:56-61.
Hu, Z.-L., Fritz, E.R., Reecy, J.M., 2007. Animal QTLdb: a livestock QTL database tool set for positional QTL information mining and beyond. Nucleic Acids Res. 35:D604-609.
Isler, B.J., Irvin, K.M., Neal, S.M., Moeller, S.J., Davis, M.E., 2002. Examination of the relationship between the estrogen receptor gene and reproductive traits in swine. J. Anim. Sci. 80:2334-2339.
Kmiec, M., Dvorak, J., Vrtkova, I., 2002. Study on a relation between estrogen receptor (ESR) gene polymorphism and some pig reproduction performance characters in Polish Landrace breed. Czech J. Anim. Sci. 47:189-193.
Linville, R.C., Pomp, D., Johnson, R.K., Rothshild, M.F., 2001. Candidate genes analysis for loci affecting litter size and ovulation rate in pigs. J. Anim. Sci. 79:60-67.
Muñoz, G., Ovilo, C., Amills, M., Rodriguez, C., 2004. Mapping of the porcine oestrogen receptor 2 gene and association study with litter size in Iberian pigs. Anim. Genet. 35:242-244.
Muñoz, G., Ovilo, C., Estellé, J., Silió, L., Fernández, A., Rodriguez, C., 2007. Association with litter size of new polymorphisms on ESR1 and ESR2 genes in a Chinese-European pig line. Genet. Sel. Evol. 39:195-206.
Noguera, J.L., Varona, L., Gomez-Raya, L., Sanchez, A., Babot, D., Estany, J., Messer, L.A., Rothschild, M., Perez-Enciso, M., 2003. Estrogen receptor polymorphism in Landrace pigs and its associations with litter size performance. Livest. Prod. Sci. 82:53-59.
Rempel, L.A., Nonneman, D.J., Wise, T.H., Erkens, T., Peelman, L.J., Rohrer, G.A., 2010. Association analyses of candidate single nucleotide polymorphisms on reproductive traits in swine. J. Anim. Sci. 88:1-15.
Santana, B.A.A., Biase F.H., Antunes R.C., Borges M., Machain Franco M., Goulart L.R., 2006. Association of the estrogen receptor gene Pvull restriction polymorphism with expected progeny differences for reproductive and performance traits in swine herds in Brazil. Genet. Mol. Biol. 29:273-277.
Southwood, O.I., van der Steen, H., Eckardt, G.R., Tuggle, C.K., Helm, J., Vaske, D.A., Mileham, A.J., Plastow, G.S., 1997. Effect of the estrogen receptor locus on reproduction and production traits in four commercial pigs lines. J. Anim. Sci. 75:3138-3142.
Spötter, A., Distl, O., 2006. Genetic approaches to the improvement of fertility traits in the pigs. Vet. J. 172:234-247.
van Rens, B.T., de Groot, P.N., van der Lende, T., 2002. The effect of estrogen genotype on litter size and placental traits at term in F2 crossbred gilts. Theriogenol. 57:1635-1649.