A deletion in the bone morphogenetic protein 15 gene causes sterility and increased prolificacy in Rasa Aragonesa sheep

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doi:10.1111/j.1365-2052.2008.01707.x

A deletion in the *bone morphogenetic protein 15* gene causes sterility and increased prolificacy in Rasa Aragonesa sheep

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Summary

Bone morphogenetic protein 15 (BMP15) is a member of the transforming growth factor beta superfamily, is specifically expressed in oocytes and is essential for sheep prolificacy. Reported mutations in this gene cause increased ovulation rate and infertility in a dosage-sensitive manner. In this work, a new naturally occurring mutation in the *BMP15* gene from the ovine Rasa Aragonesa breed is described. This mutation is a deletion of 17 bp that leads to an altered amino acid sequence and introduces a premature stop codon in the protein. Highly significant associations ($P < 0.0001$) were found between the estimated breeding value for prolificacy and the genotype of *BMP15* in Rasa Aragonesa animals with high and low breeding values for this trait. As for other mutations in *BMP15*, this new mutation is associated with increased prolificacy and sterility in heterozygous and homozygous ewes respectively.

Keywords *BMP15*, *FecX*, prolificacy, Rasa Aragonesa, sheep, sterility.

Genetic studies on sheep prolificacy have indicated that litter size and ovulation rate can be determined by the action of single genes, named fecundity (*Fec*) genes, with major effects (Davis 2004, 2005). One of them located on the X chromosome and known as the *FecX* locus is the *bone morphogenetic protein 15* (*BMP15*) gene (Dube *et al.* 1998). Five different *FecX*-mutated alleles led to increased ovulation rates in heterozygous ewes and sterility in homozygous ewes (Galloway *et al.* 2000; Hanrahan *et al.* 2004; Bodin *et al.* 2007). In particular, heterozygous ewes with the $FecX^I$ (Inverdale), $FecX^H$ (Hanna), $FecX^B$ (Belclare), $FecX^G$ (Galway) or $FecX^L$ (Lacaune) alleles exhibited one to two additional ovulations compared with non-carriers, whereas homozygous ewes were sterile. The $FecX^I$, $FecX^L$ and $FecX^B$ mutations cause non-conservative amino acid substitutions at positions 31, 53 and 99, respectively, within the BMP15 mature protein. Interestingly, $FecX^I$ and $FecX^B$ mutant ewes exhibit the same phenotype as the carriers of the $FecX^G$ and $FecX^H$ mutations, which introduce a premature stop codon at positions 239 and 291 of the BMP15 pro-protein and obviously impair the production of the biologically active mature form. Similarly the $FecX^L$ (Lacaune) allele is unable to produce a biologically active BMP15 product (Bodin *et al.* 2007). From these data it appears that these mutations induce loss of function in BMP15 activity, leading to increased ovulation rate or sterility in a dosage-sensitive manner.

The Rasa Aragonesa sheep breed (about 2 million animals) belongs to the so-called entrefino type, with short wool and wool fibres of medium thickness. They are polled, have wool-less heads and are used mainly for meat. The area of distribution of the Spanish Rasa Aragonesa sheep is in northeast Spain (Arranz *et al.* 1998). During the early 1990s, the cooperative 'Carnes Oviaragón S.C.L.' implemented a selection scheme designed to improve prolificacy on associated farms. Estimated breeding values (EBVs) were estimated on the whole population by an animal model, based on a progeny test. This breeding programme was performed in a nucleus of 130 000 Rasa Aragonesa adult ewes (196 flocks), mainly produced during three lambings in 2 years, with low prolificacy (1.3). This design was enhanced by the dissemination of elite sire semen, which spread among a large number of flocks into and out of the nucleus. The Rasa Aragonesa breed exhibited a large increase in prolificacy during the 'Carnes Oviaragón S.C.L.' selection programme, which led to the suspicion that a gene with a major effect on prolificacy existed.

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Accepted for publication 13 January 2008

In recent years, the presence of a major gene was suspected after analysing the distribution of EBVs for daughters of three sires that had high EBVs and that participated in the 'Carnes Oviaragón S.C.L.' selection programme for prolificacy. All daughters of these sires repeatedly presented very high litter sizes and high EBVs, while daughters of sons of these sires repeatedly presented one lamb and low EBVs. All sons of the sires show low EBVs, too. This type of segregation is typical of genes linked to the X chromosome. EBVs (in standard deviations) of the three sires, estimated based on the litter sizes of their daughters, were 2.24, 1.68 and 1.51 for sires 4455 (N = 598 daughters), 619 (N = 180 daughters) and 382 (N = 106 daughters) respectively.

The *BMP15* sequences of the three sires with higher EBVs in the selection programme were determined in order to evaluate whether this gene was involved in increased prolificacy. Furthermore, the *BMP15* sequences from three ewes with low EBVs were also determined. PCR was performed with primers designed from published sheep genomic sequences of *BMP15* exons 1 and 2 (AF236078 and AF236079 respectively). PCR was carried out under standard conditions with primers for exon 1 (forward, 5′-TTGCTGAACACCAAGCTTTT-3′; reverse, 5′-CCCCTCCCA CCAGAACAATA-3′) and exon 2 (forward, 5′-CATCTCAAG GCTGCTTGTCA-3′; reverse, 5′-CTGGGCAATCATACCCT CAT-3′). The PCR products were sequenced on an ABI 310 sequencer (Applied Biosystems). Sequences were aligned using CLUSTALW (http://www.ebi.ac.uk/clustalw/) software, and compared against the databases GenBank and EMBL.

The three sires with high EBVs revealed a novel mutation in *BMP15*: a deletion of 17 nucleotides (c.525_541delTG GGTCCAGAAAAGCCC) in the reference ovine sequence AF236079. This deletion, located at the beginning sequence of exon 2, changes codon 154, shifts the reading frame with an altered amino acid sequence and introduces a premature stop codon at position 208 (Fig. 1). The protein truncation is located before the coding region of the mature protein, producing a non-functional peptide designated as p.W154NfsX55 (for the BMP15 reference sequence, see AAF81688). The new allele of the *BMP15*/*FecX* gene in the Rasa Aragonesa population was named $FecX^R$, following the nomenclature for previous fecundity genes.

The allele $FecX^R$ was later found in the hemizygous state in five other Rasa Aragonesa sires, which had the highest EBVs of the 'Carnes Oviaragón S.C.L.' breeding programme. Thirty-five sires with negative or low positive values did not possess the $FecX^R$ allele.

In order to identify ewes that were homozygous for $FecX^R$, 81 blood samples of animals (39 rams; 42 ewes) produced from a multiple ovulation and embryo transfer (MOET) programme, which considers matings between animals with high EBVs, were analysed. *BMP15* genotypes were determined by PCR using primers flanking the $FecX^R$ polymorphism. Amplification was carried out under standard conditions with the following primers: 5′-CTCTGAGACCAA ACCGGGTA-3′ (forward) and 5′-TTTGAGGAGCCTCTT CCTGA-3′ (reverse). PCR products were separated by standard electrophoresis in a 4.5% agarose gel. PCR amplification of the $FecX^R$ and $FecX^+$ alleles produced fragments of 143 and 160 bp respectively. In total, four homozygous ewes for $FecX^R$ were found but only two were alive. Laparoscopic observations displayed an infantile genital tract. The ovaries did not carry any obvious follicular structures, nor did they resemble the streak phenotype observed in Inverdale ($FecX^I$) and Lacaune ($FecX^L$) homozygous ewes. The frequency of the $FecX^R$ allele was 0.64 and 0.21 for ewes and rams respectively (three hemizygous rams were found). These differences were due to the composition of parents in the MOET programme, in which the most-used sires were hemizygous for the $FecX^R$ allele. Furthermore, 63 ewes from a commercial flock of the 'Carnes Oviaragón S.C.L.' breeding scheme were tested, and two heterozygous $FecX^R$ ewes were found. In other populations analysed (not connected to the scheme), the $FecX^R$ allele was not found.

To confirm the association between the Rasa Aragonesa allele and prolificacy EBVs, two groups of sheep were established with extreme values for prolificacy. Animals selected for these groups were in the highest (H) 1.17% and lowest (L) 1.09% tails of the distribution of the nucleus of the 'Carnes Oviaragón S.C.L.' breeding scheme. Samples included 207 ewes with EBVs equal or higher than +0.98 (in standard deviations) (tail H: N = 97, EBV = 1.5) and with EBVs equal or lower than −0.76 (in standard deviations) (tail L: N = 110, EBV = −1.11) prolificacy. These animals represented the 9.1% and 4.6% of animals with EBVs equal or greater than +0.98 and equal or lower than −0.76 respectively. All animals belonged to the selection nucleus of the 'Carnes Oviaragón S.C.L.' breeding scheme, and flocks were connected by artificial insemination. EBVs for prolificacy came from the 2006 (BLUP) evaluation. Furthermore, ewes had at least five lambings. ANOVA test were performed using the GLM Procedure of SAS version 6.12 (SAS Institute Inc.).

Results showed that all animals of the low prolificacy tail were homozygous for the $FecX^+$ allele. Thirty-two ewes from the high tail presented heterozygous genotypes for the $FecX^R$ allele, including the 16 ewes with the highest EBVs (ranging from 2.05 to 3.4 in standard deviations). Highly significant differences in allelic frequencies between the two tails were found ($P < 0.0001$). Also, highly significant differences ($P < 0.0001$) in prolificacy EBVs and genotypes of the animals in the high tail were found, validating the association between highest genetic values and heterozygous ewes. Moreover, 145 different ewes with EBVs below −0.76 (in standard deviations), which were not tested in the association studies, have been analysed, and $FecX^R$ allele was not detected.

Figure 1 Amino acid sequence alignment between the protein products of the *FecX*^{R} and *FecX*$^{+}$ alleles of *BMP15*. The Rasa Aragonesa allele (*FecX*^{R}) results in a deletion of six amino acids compared with the *FecX*$^{+}$ sequence from a wild-type ewe (AAF81688). The underlined sequence corresponds to the mature protein with physiological activity. Boxes show amino acid alterations that result from other mutations in *BMP15*. Asterisks indicate identity; colons and full stops indicate full conservation of strong and weak groups respectively.

The present work reports a new *FecX* allele in the ovine *BMP15* gene, which consists in a deletion of 17 bp in exon 2. This deletion leads to an altered amino-acid sequence and introduces a premature stop codon. It brings to six the number of naturally occurring mutations in the *BMP15* gene that have been associated with high prolificacy or sterility, depending on its presence in the heterozygous or homozygous state respectively. This new finding lends support to the argument of heterozygote advantage in relation to mutations in *BMP15* and *GDF9* (Gemmell & Slate 2006).

Acknowledgements

This work has been supported by the INIA (RTA 2006-140) and CDTI projects. A. Martinez-Royo is supported by an INIA grant.

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