AUTHOR'S PAGE PROOFS:
NOT FOR CIRCULATION

CSIRO PUBLISHING

*Reproduction, Fertility and Development*
http://dx.doi.org/10.1071/RD14511

# Association of a region of bovine chromosome 1 (BTA1) with age at puberty in Angus bulls

*María E. Fernández*[A,C], *Alberto Prando*[B], *Andrés Rogberg-Muñoz*[A], *Pilar Peral-García*[A], *Andrés Baldo*[B], *Guillermo Giovambattista*[A] *and Juan P. Lirón*[A]

[A]Instituto de Genética Veterinaria (IGEVET), CCT La Plata – CONICET – Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, Calle 60 y 118 s/n, La Plata B1900AVW, CC 296, Argentina.

[B]Departamento de Producción Animal, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, Calle 60 y 118 s/n, La Plata B1900AVW, CC 296, Argentina.

[C]Corresponding author. Email: ggiovam@fcv.unlp.edu.ar

**Abstract.** Age at puberty is an important component of reproductive performance in cattle, so it is important to identify genes that contribute to the regulation of the onset of puberty and polymorphisms that explain differences between bulls. In a previous study, we found putative associations between age at puberty in Angus bulls and single-nucleotide polymorphisms (SNPs) in Chromosomes 1 and X. In the present work we aimed to confirm these findings in a larger sample of Angus bulls ($n = 276$). Four SNPs located in these regions were genotyped using SEQUENOM technology and the genotypes obtained were tested for association with age at puberty. The results showed that SNPs rs135953349 and rs110604205 on BTA1 were still significantly associated with age of puberty estimated at progressive sperm motility of 10% ($P < 0.05$). The association previously found on Chromosome X could not be confirmed. Analysis of the bovine genome revealed that the associated region (99.17–99.99 Mb) contained four predicted loci: myelodysplasia syndrome 1 (MDS1) and ecotropic virus integration site 1 (EVI1) complex locus (MECOM), eGF-like and EMI domain-containing 1 pseudogene-like (LOC100337483), microRNA mir-551b (MIR551B) and mCG140927-like (LOC100139843). The results obtained could contribute to the understanding of puberty regulation and could be useful for further identification and annotation of gene function in the context of reproduction.

**Additional keywords:** gene, genetics, genotyping, livestock, polymorphism.

Received 23 December 2014, accepted 10 March 2015, published online xx xxxxx xxxx

## Introduction

Puberty is an important target for genetic improvement and early prediction using genetic markers is therefore a goal for livestock breeding. The identification of genes contributing to genetic variation in puberty can assist with the selection of early-pubertal bulls, reducing the generation interval and increasing fertility (Lesmeister *et al.* 1973; Fortes *et al.* 2012*a*, 2012*b*; Hawken *et al.* 2012). However, only a few chromosome regions have been associated with age at puberty in bulls. Results from previous work supports the hypothesis that different gene pathways are involved in different processes required for arrival at puberty. For example, insulin-like growth factor 1 (IGF1) was associated with age at 28 cm of scrotal circumference (SC28) in Angus bulls (Lirón *et al.* 2012). In addition, genomic regions on Chromosomes 2, 14 and X were associated with traits measured throughout puberty in Brahman bulls, which yielded positional candidate genes on these chromosomes. In this latter case, a pleomorphic adenoma gene 1 (PLAG1) located on *Bos taurus* chromosome 14 (BTA14) was detected, which contained polymorphisms associated with IGF1 concentration and SC at 12 months of age, and a single-nucleotide polymorphism (SNP) located in inhibin α-subunit (INHA) on BTA2 was associated with inhibin levels at 4 months in Brahman bulls (Phillips 2005; Fortes *et al.* 2011, 2012*b*). Furthermore, a region on Chromosome X was associated with age at 26 cm of scrotal circumference (SC26) and it includes three positional and functional candidate genes: androgen receptor (AR), and *TAF1* and *TAF9B*, both TATA box binding protein (TBP)-associated factors (*TAF*; (Hiller *et al.* 2004; Verhoeven *et al.* 2010).

In a previous work, we performed an exploratory genome-wide association study (GWAS) that involved the genotyping of extreme animals to identify genetic variants associated with age at puberty in Angus bulls. SNPs were tested against three estimated puberty ages: age at SC28, age at progressive motility of 10% (M10) and age at sperm concentration of 50 million (C50). As a result, we detected chromosome regions associated

PROOF ONLY

with the estimated puberty ages, selected tag SNPs and confirmed these associations in a larger population of Angus males, which allowed us to find positional candidate genes that have been implicated in processes related to the development of spermatogenesis: insulin gene enhancer protein 1 (*ISL1*), pelota homolog (*PELO*), follistatin (*FST*) and spermatogenic leucine zipper 1 (*SPZ1*) (Fernández *et al.* 2014). In addition, using the same GWAS, we detected a region potentially associated with age at puberty on BTA1, located between 99.17 and 99.99 Mb and covering 14 SNPs with a maximum of significance at $P < 0.0001$ (rs135953349 and rs110604205; see Figs S1 and S2, available as Supplementary Material to this paper) and two SNPs on Chromosome X (rs135795150 and rs110798626) showing an association with age at M10. The objective of the present work was to confirm the associations previously detected between SNPs in BTA1 and BTAX and age at puberty in a larger sample of Angus bulls.

## Materials and methods

In order to achieve the objective, the associated SNPs on Chromosome 1 (rs135953349 and rs110604205) and X (rs135795150 and rs110798626) detected in the previous exploratory GWAS (M. E. Fernandez, J. P. Liron, A. Prando, A. Rogberg-Muñoz, P. Peral-Garcia, A. Baldo and G. Giovambattista, pers. comm.), were genotyped in the whole population of 276 bulls using the SEQUENOM platform by GeneSeek Inc. genotyping services (Lincoln, NE, USA). These SNPs belonged to the Illumina BovineHD Genotyping BeadChip (http://www.illumina.com/Documents//products/datasheets/datasheet_bovineHD.pdf, verified 18 March 2015). Animal samples, phenotypic measurements and estimation of puberty ages used in this work were described in detail in Lirón *et al.* (2012). Marker genotypes were tested against the three puberty ages mentioned above utilising the MIXED procedure implemented in SAS 9.0 software (SAS Institute Inc., Cary, NC, USA). The lineal mixed model used to analyse the association between puberty age and genotypes was the following:

$$Y_{ijkl} = \mu + S_i + G_j + B_k + O_l + \beta(x_{ijkl} - x) + e_{ijk},$$

where $Y_{ijkl}$ = phenotypic observation of bull I, $\mu$ = overall mean, $S_i$ = fixed effect of ith year, $G_j$ = fixed effect of jth genotype, $B_k$ = fixed effect of kth herd, $O_l$ = random effect of lth sire, $\beta(x_{ijkl}–x)$ = regression on bodyweight at 300 days and $e_{ijkl}$ = random error.

## Results and discussion

An exploratory GWAS performed using the extreme animals from the phenotypic distribution of puberty showed putative association between two SNPs (rs135795150 and rs110798626) located on Chromosome X and age at puberty in Angus bulls. This preliminary result was in agreement with Fortes *et al.* (2012*a*), who reported a region on the same chromosome associated with age at puberty estimated at 26 cm of scrotal circumference. However, the analysis of these SNPs in the whole population (276 bulls) did not allow us to confirm the association previously observed. These results could be consequence of diverse factors, such as: (1) false positives, (2) sampling effects that in the case of the preliminary study could be more important (36 animals), (3) the correspondence between the marker effect and the sample size of the whole population and (4) genetic interactions (e.g. epistasis), among others. Considering only these results we cannot discard an influence of BTX in the processes related to puberty.

**Table 1. Association study between SNPs on Chromosome 1: genotypes and estimated age at lineal progressive motility of 10%**

Bonferroni's correction was applied to adjust *P* value. *n* = number of individuals

| Haplotype (rs135953349/rs110604205) | *n* | Age at M10 (days) (mean ± s.e.m.) | Genotype effect *P* value |
|---|---|---|---|
| CA/CA | 156 | 279.61 ± 5.21 | 0.024 |
| CA/TG | 86 | 265.49 ± 5.96 | |
| TG/TG | 16 | 253.27 ± 13.70 | |

In the case of Chromosome 1, the results obtained allowed us to confirm the association of two SNPs (rs135953349 and rs110604205) located on this chromosome with age at puberty estimated at M10, but not with SC28 or C50 ($P < 0.05$, adjusted by Bonferroni's correction; Table 1). These SNPs are in complete linkage disequilibrium in the studied Angus bulls, in a linkage block between 99.17 and 99.99 Mb (Fig. S1). It is noteworthy that McClure *et al.* (2010) detected a Quantitative Trait Loci (QTL) on Chromosome 1 (87.12–99.71 cM) for scrotal circumference, supporting the hypothesis concerning the existence of genes involved in puberty in that chromosomal region.

The search for genes within and nearby the genomic regions harbouring the associated SNPs yielded the following predicted loci: myelodysplasia syndrome 1 (MDS1) and ecotropic virus integration site 1 (EVI1) complex locus (MECOM, located from 98516835 to 99144551 Mb), eGF-like and EMI domain containing 1 pseudogene-like (LOC100337483, located from 99421592 to 99467114 Mb), microRNA mir-551b (MIR551B, located from 99671696 to 99671790 Mb) and mCG140927-like (LOC100139843, located from 99478228 to 100027341 Mb).

Until now, these loci are not known to affect puberty or play a role in reproduction. Two of these loci (LOC100337483 and LOC100139843) have no known reported functions. However, microRNAs are known to play an important role in gene expression regulation, affecting nearly every biological process examined, such as developmental timing, differentiation, cell proliferation and apoptosis (Ambros 2004; Bartel 2004; Alvarez-Garcia and Miska 2005). Furthermore, it has been proven that microRNAs have an important role in hormone regulation. Ye *et al.* (2013) revealed that some microRNAs are likely to take part in the gonadotrophin-releasing hormone (GnRH) signalling pathway and consequently across the entire hypothalamic–pituitary–gonadal (HPG) axis and in reproduction control. Another study showed that the predicted gene targets of differentially expressed microRNAs are involved in processes affecting sperm morphology, sperm motility and spermatogenesis (Curry *et al.* 2011). In the particular case of the microRNA MIR551B found in this work, there is no report about its involvement in any processes related to puberty. However, Kubo *et al.* (2013), using a rat model, found that

PROOF ONLY

MIR551B could regulate the expression of several target genes, including those belonging to transforming growing factor-β (TGF-β) pathways, cell proliferation and apoptosis. It has been shown that these genes govern the pubertal processes at a higher hierarchical level of control (Loveland and Hime 2005; Itman *et al.* 2006; Ojeda *et al.* 2010). For this reason, one could think that microRNAs have important but not yet well-understood mechanistic roles in reproduction-related processes.

MECOM, also known as ecotropic virus integration site 1 (EVI1) or myelodysplasia syndrome 1 (MDS1), is an oncogenic transcription factor involved in many signalling pathways for both repression and activation of the cell cycle, haematopoiesis, apoptosis, development and cell differentiation and proliferation-related genes (Bard-Chapeau *et al.* 2013; Kustikova *et al.* 2013). The roles of MECOM are not completely understood, but it has been shown that this gene is involved in the downstream signalling pathway of the TGF-β superfamily (Sood *et al.* 1999). MECOM suppresses the transcriptional activity of Smad proteins 2 and 3, both known repressors of the TGF-β pathway (Kurokawa *et al.* 1998; Shi and Massagué 2003). Numerous pieces of evidence have shown that the TGF-β superfamily of proteins such as inhibins, activins and bone morphogenetic protein (BMP), are involved in regulating important cellular functions and, with respect to puberty, are vital for governance of testis development and spermatogenesis as we mentioned above (Alliston *et al.* 2005; Itman *et al.* 2006).

## Conclusion

Given the importance of age at puberty in the reproductive performance of cattle and the limited knowledge on genetic components regulating this characteristic, the availability of molecular marker information and technologies has turned out to be an opportunity to improve animal breeding programs by the inclusion of highly valued genetic traits. The identification of candidate genes for age at puberty has the potential to advance research related to male fertility and could assist in selection for early onset of puberty in cattle. Our results allowed us to confirm the association of two SNPs located on Chromosome 1 (99–100 Mb) with age at puberty. This region comprised two loci, MIR551B microRNA and MECOM, which have been described as regulators of TGF-β pathways. In relation to puberty, the TGF-β superfamily of transcription factor genes is vital for the regulation of testis development and spermatogenesis in mammals. In order to confirm the influence of those genes over the onset of puberty and map the causative mutations, further analysis of fine mapping and re-sequencing would be necessary. This information could be used to include causative SNPs in the chips used for genetic evaluation, increasing their information content and power.

## References


Alliston, T., Ko, T. C., Cao, Y., Liang, Y. Y., Feng, X. H., Chang, C., and Derynck, R. (2005). Repression of bone morphogenetic protein and activin-inducible transcription by Evi-1. *J. Biol. Chem.* **280**, 24227–24237. doi:10.1074/JBC.M414305200

Alvarez-Garcia, I., and Miska, E. A. (2005). MicroRNA functions in animal development and human disease. *Development* **132**, 4653–4662. doi:10.1242/DEV.02073

Ambros, V. (2004). The functions of animal microRNAs. *Nature* **431**, 350–355. doi:10.1038/NATURE02871

Bard-Chapeau, E. A., Gunaratne, J., Kumar, P., Chua, B. Q., Muller, J., Bard, F. A., Blackstock, W., Copeland, N. G., and Jenkins, N. A. (2013). EVI1 oncoprotein interacts with a large and complex network of proteins and integrates signals through protein phosphorylation. *Proc. Natl. Acad. Sci. USA* **110**, E2885–E2894. doi:10.1073/PNAS.1309310110

Bartel, D. P. (2004). MicroRNAs: genomics, biogenesis, mechanism and function. *Cell* **116**, 281–297. doi:10.1016/S0092-8674(04)00045-5

Curry, E., Safranski, T. J., and Pratt, S. J. (2011). Differential expression of porcine sperm microRNAs and their association with sperm morphology and motility. *Theriogenology* **76**, 1532–1539. doi:10.1016/J.THERIOGENOLOGY.2011.06.025

Fernández, M. E., Lirón, J. P., Prando, A., Rogberg-Muñoz, A., Peral-García, P., Baldo, A., and Giovambattista, G. (2014). Evidence of association of a BTA20 region peaked in ISL1 with puberty in Angus bulls. *Livest. Sci.* **167**, 9–18. doi:10.1016/J.LIVSCI.2014.05.009

Fortes, M. R. S., Reverter, A., Nagaraj, S. H., Zhang, Y., Jonsson, N. N., Barris, W., Lehnert, S., Boe-Hansen, G. B., and Hawken, R. J. (2011). A single nucleotide polymorphism-derived regulatory gene network underlying puberty in two tropical breeds of beef cattle. *J. Anim. Sci.* **89**, 1669–1683. doi:10.2527/JAS.2010-3681

Fortes, M. R. S., Reverter, A., Hawken, R. J., Bolormaa, S., and Lehnert, S. A. (2012*a*). Candidate genes associated with hormone levels of inhibin, luteinising hormone and insulin-like growth factor 1, testicular development and sperm quality in Brahman bulls. *Biol. Reprod.* **87**, 58. doi:10.1095/BIOLREPROD.112.101089

Fortes, M. R. S., Lehnert, S. A., Bolormaa, S., Reich, C., Fordyce, G., Corbet, N. J., Whan, V., Hawken, R. J., and Reverter, A. (2012*b*). Finding genes for economically important traits: Brahman cattle puberty. *Anim. Prod. Sci.* **52**, 143–150. doi:10.1071/AN11165

Hawken, R. J., Zhang, Y. D., Fortes, M. R., Collis, E., Barris, W. C., Corbet, N. J., Williams, P. J., Fordyce, G., Holroyd, R. G., Walkley, J. R., Barendse, W., Johnston, D. J., Prayaga, K. C., Tier, B., Reverter, A., and Lehnert, S. A. (2012). Genome-wide association studies of female reproduction in tropically adapted beef cattle. *J. Anim. Sci.* **90**, 1398–1410. doi:10.2527/JAS.2011-4410

Hiller, M., Chen, X., Pringle, M. J., Suchorolski, M., Sancak, Y., Viswanathan, S., Bolival, B., Lin, T. Y., Marino, S., and Fuller, M. T. (2004). Testis-specific TAF homologs collaborate to control a tissue-specific transcription program. *Development* **131**, 5297–5308. doi:10.1242/DEV.01314

Itman, C., Mendis, S., Barakat, B., and Loveland, K. L. (2006). All in the family: TGF-beta family action in testis development. *Reproduction* **132**, 233–246. doi:10.1530/REP.1.01075

Kubo, E., Hasanova, N., Sasaki, H., and Singh, D. P. (2013). Dynamic and differential regulation in the microRNA expression in the developing and mature cataractous rat lens. *J. Cell. Mol. Med.* **17**, 1146–1159. doi:10.1111/JCMM.12094

Kurokawa, M., Mitani, K., Irie, K., Matsuyama, T., Takahashi, T., Chiba, S., Yazaki, Y., Matsumoto, K., and Hirai, H. (1998). The oncoprotein Evi-1 represses TGF-beta signalling by inhibiting Smad3. *Nature* **394**, 92–96. doi:10.1038/27945

Kustikova, O. S., Schwarzer, A., Stahlhut, M., Brugman, M. H., Neumann, T., Yang, M., Li, Z., Schambach, A., Heinz, N., Gerdes, S., Roeder, I., Ha, T. C., Steinemann, D., Schlegelberger, B., and Baum, C. (2013). Activation of Evi1 inhibits cell-cycle progression and differentiation of haematopoietic progenitor cells. *Leukemia* **27**, 1127–1138. doi:10.1038/LEU.2012.355

Lesmeister, J. L., Burfening, P. J., and Blackwell, R. L. (1973). Date of first calving in beef cows and subsequent calf production. *J. Anim. Sci.* **36**, 1–6. doi:10.2134/JAS1973.3611

Lirón, J. P., Prando, A., Fernández, M. E., Ripoli, M. V., Rogberg-Muñoz, A., Goszczynski, D. E., Posik, D. M., Peral-García, P., Baldo, A., and


PROOF ONLY

Giovambattista, G. (2012). Association between GNRHR, LHR and IGF1 polymorphisms and timing of puberty in male Angus cattle. *BMC Genet.* **13**, 26. doi:10.1186/1471-2156-13-26

Loveland, K. L., and Hime, G. (2005). TGFβ superfamily members in spermatogenesis: setting the stage for fertility in mouse and Drosophila. *Cell Tissue Res.* **322**, 141–146. doi:10.1007/S00441-005-0008-0

McClure, M. C., Morsci, N. S., Schnabel, R. D., Kim, J. W., Yao, P., Rolf, M. M., McKay, S. D., Gregg, S. J., Chapple, R. H., Northcutt, S. L., and Taylor, J. F. (2010). A genome scan for quantitative trait loci influencing carcass, post-natal growth and reproductive traits in commercial Angus cattle. *Anim. Genet.* **41**, 597–607. doi:10.1111/J.1365-2052.2010.02063.X

Ojeda, S. R., Dubay, C., Lomniczi, A., Kaidar, G., Matagne, V., Sandau, U. S., and Dissen, G. A. (2010). Gene networks and the neuroendocrine regulation of puberty. *Mol. Cell. Endocrinol.* **324**, 3–11. doi:10.1016/J.MCE.2009.12.003

Phillips, D. J. (2005). Activins, inhibins and follistatins in the large domestic species. *Domest. Anim. Endocrinol.* **28**, 1–16. doi:10.1016/J.DOMANIEND.2004.05.006

Shi, Y., and Massagué, J. (2003). Mechanisms of TGF-beta signalling from cell membrane to the nucleus. *Cell* **113**, 685–700. doi:10.1016/S0092-8674(03)00432-X

Sood, R., Talwar-Trikha, A., Chakrabarti, S. R., and Nucifora, G. (1999). MDS1/EVI1 enhances TGF-beta1 signalling and strengthens its growth-inhibitory effect but the leukaemia-associated fusion protein AML1/MDS1/EVI1, product of the t(3;21), abrogates growth inhibition in response to TGF-beta1. *Leukemia* **13**, 348–357. doi:10.1038/SJ.LEU.2401360

Verhoeven, G., Willems, A., Denolet, E., Swinnen, J. V., and De Gendt, K. (2010). Androgens and spermatogenesis: lessons from transgenic mouse models. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **365**(1546), 1537–1556. doi:10.1098/RSTB.2009.0117

Ye, R. S., Xi, Q. Y., Qi, Q., Cheng, X., Chen, T., Li, H., Kallon, S., Shu, G., Wang, S. B., Jiang, Q. Y., and Zhang, Y. L. (2013). Differentially expressed miRNAs after GnRH treatment and their potential roles in FSH regulation in porcine anterior pituitary cell. *PLoS ONE* **8**(2), e57156. doi:10.1371/JOURNAL.PONE.0057156

PROOF ONLY