

## Sequence variation and detection of a functional promoter polymorphism in the lysozyme c gene from Ragusano and Grigio Siciliano donkeys

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Complete List of Authors:	Cosenza, Gianfranco; University of Naples Federico II, Deparment of Agriculture Ciampolini, Roberta; University of Pisa, Dipartimento di Scienze Veterinarie Iannaccone, Marco; University of Naples Federico II, Department of Agriculture Gallo, Daniela; University of Naples Federico II, Department of Agriculture Auzino, Barbara; University of Pisa, Department of Veterinary Sciences Pauciullo, Alfredo; Università degli Studi di Torino, Scienze Agrarie, Forestali ed Alimentari
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Sequence variation and detection of a functional promoter polymorphism in the
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- lysozyme c gene from Ragusano and Grigio Siciliano donkeys
- G. Cosenza<sup>\*1</sup>, R. Ciampolini<sup>†1</sup>, M. Iannaccone<sup>\*</sup>, D. Gallo<sup>\*</sup>, B. Auzino<sup>†</sup>, A. Pauciullo<sup>‡</sup>
- \* Department of Agricultural Sciences, University of Naples Federico II, Portici (NA), Italy
- † Department of Veterinary Sciences, University of Pisa, Pisa, Italy.
- <sup>‡</sup> Department of Agricultural, Forest and Food Science, University of Torino, Grugliasco (TO), Italy
- <sup>1</sup> These authors contributed equally to this work.

Donkey LYZ gene variability and promoter study.

- 3 Address for correspondence:
  - M. Iannaccone, Department of Agricultural Sciences, University of Naples 'Federico II', Portici
- 15 (NA), Italy. E-mail: <u>marco.iannaccone@unina.it</u>

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Lysozyme is antimicrobial agent in milk and inhibits growth of pathogenic bacteria<sup>1, 2</sup>. The vertebrate lysozyme gene family includes three genes: lactalbumin (LALBA), calcium-binding lysozyme (LYSC1) and lysozyme c (LYZ)<sup>3</sup>. The latter is the most studied in human, camel, ruminants and equine milk. Donkey milk contains 1.0 mg/mlL lysozyme c, a higher concentration than in milk from ruminants (traces) and humans (0.12 mg/mL)<sup>5</sup>. While genetic variants have been already reported in donkey LYSC1<sup>6</sup>, so far no information is available on the molecular variation in LYZ locus in this species. In the present study we report molecular variation of the donkey LYZ gene and the detection of functional polymorphisms. Blood samples were collected from 27 donkeys of 2 breeds (15 Ragusano and 12 Grigio Siciliano) and DNA was extracted. Using primers designed on donkey genome sequence (GeneBank NW 014638180.1, complement 1781536..1786728), we sequenced for three donkeys of both breeds the complete LYZ gene (5136 bp, 4 exons), encoding a protein of 148 aa, with 342 bp promoter and 1017 bp 3'UT sequence (Figure S1). We found two variants A (GenBank MG600140) and B (MG600141), which differ at 32 positions. (Table S1). Transition g.4443G>A changes the open reading frame [p.(Ser147Phe)]. In the promoter region SNP g.203C>T alters a putative binding site of the transcription factor NF-1 (Transfact 7.0 database), which is involved in regulation of milk protein genes<sup>7</sup>. The presence of the C in position 203 and G in position 4443 represent the ancestral state of the gene, because they have also been observed in all the LYZ sequences available in database for donkey and horse species. To assess the effect of this SNP on the LYZ promoter activity, we amplified and cloned a DNA region of 1102 bp from 4 homozygous individuals (two g.203CC and two g.203TT, see Appendix S1. for more details) in the pGL3 basic vector (Promega), upstream of the reporter gene. The two different constructs g.203C and g.203T with the pGL vector as control were used to transfect transiently a HEK293. After 24 h, the reporter activity of the variants was measured using the luciferase assay system (Promega).

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The T variant of this SNP negatively affects the promoter activity of the donkey LYZ gene (Figure 1). Therefore, we suppose an effective role of this binding site in the expression of the LYZ in donkey milk, as demonstrated also in other candidate genes for the qualitative and-quantitative variations of milk in buffalo<sup>8, 9</sup>, sheep and goats<sup>10, 11, 12</sup>. A PCR-RFLP assay by AvaI (Figure S2) indicated in the limited number of samples a frequency of the C allele of 0.72. It will be interesting to link this to a phenotype, presumably a trait linked to disease resistance.

## **Conflict of interest**

The Authors have no conflict of interest to declare.

## Acknowledgements

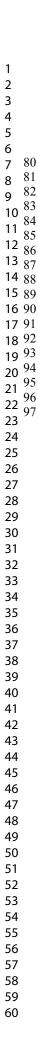
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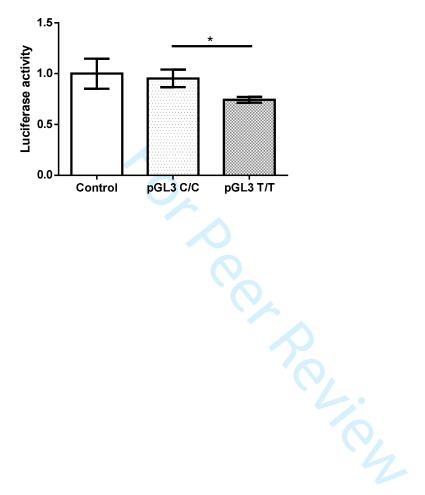
## Legends to figure

**Fig. 1.** The SNP g.203C>T influences the activity of the promoter LYZ in donkey. Luciferase activity measured in HEK293 cell transfected with pGL control. Total amount of protein was measured and used for normalizing luciferase activity. Data are shown as mean  $\pm$  SEM and are representative of three independent experiments. Data elaboration was performed using graphpad prism6 software. \*P<0.05, Student's **t**-test.





**Figure** 



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