



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

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7 1 **Sequence variation and detection of a functional promoter polymorphism in the**
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9 2 **lysozyme *c* gene from Ragusano and Grigio Siciliano donkeys**

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24 11 Donkey *LYZ* gene variability and promoter study.

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7 17 Lysozyme is antimicrobial agent in milk and inhibits growth of pathogenic bacteria^{1, 2}. The
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9 18 vertebrate lysozyme gene family includes three genes: lactalbumin (*LALBA*), calcium-binding
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11 19 lysozyme (*LYSCI*) and lysozyme *c* (*LYZ*)³. The latter is the most studied in human, camel,
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13 20 ruminants and equine milk. Donkey milk contains 1.0 mg/mL lysozyme *c*, a higher concentration
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15 21 than in milk from ruminants (traces) and humans (0.12 mg/mL)⁵. While genetic variants have been
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17 22 already reported in donkey *LYSCI*⁶, so far no information is available on the molecular variation in
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19 23 *LYZ* locus in this species.

20 24 In the present study we report molecular variation of the donkey *LYZ* gene and the detection of
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22 25 functional polymorphisms. Blood samples were collected from 27 donkeys of 2 breeds (15
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24 26 Ragusano and 12 Grigio Siciliano) and DNA was extracted. Using primers designed on donkey
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26 27 genome sequence (GeneBank NW_014638180.1, complement 1781536..1786728), we sequenced
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28 28 for three donkeys of both breeds the complete *LYZ* gene (5136 bp, 4 exons), encoding a protein of
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30 29 148 aa, with 342 bp promoter and 1017 bp 3'UT sequence (Figure S1). We found two variants A
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32 30 (GenBank MG600140) and B (MG600141), which differ at 32 positions. (Table S1). Transition
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34 31 g.4443G>A changes the open reading frame [p.(Ser147Phe)]. In the promoter region SNP
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36 32 g.203C>T alters a putative binding site of the transcription factor NF-1 (Transfact 7.0 database),
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38 33 which is involved in regulation of milk protein genes⁷. The presence of the C in position 203 and G
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40 34 in position 4443 represent the ancestral state of the gene, because they have also been observed in
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42 35 all the *LYZ* sequences available in database for donkey and horse species.

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44 36 To assess the effect of this SNP on the *LYZ* promoter activity, we amplified and cloned a DNA
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46 37 region of 1102 bp from 4 homozygous individuals (two g.203CC and two g.203TT, see Appendix
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48 38 S1. for more details) in the pGL3 basic vector (Promega), upstream of the reporter gene. The two
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50 39 different constructs g.203C and g.203T with the pGL vector as control were used to transfect
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52 40 transiently a HEK293. After 24 h, the reporter activity of the variants was measured using the
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54 41 luciferase assay system (Promega).

Comment [HL1]: What is the ancestral allele?

Comment [MI2]: According to your request, we have predicted the ancestral state

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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^{8, 9}, sheep and goats^{10, 11, 12}. 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.

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72 **Legends to figure**

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

Figure

