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BRIEF NOTE

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Processed pseudogene confounding the identification of a putative lethal recessive deletion in the bovine 60S ribosomal protein L11 gene (*uL5*)

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Background: In higher eukaryotes, impaired ribosome biogenesis and function can result in specific phenotypes, the so-called ribosomopathies.[1] In humans at least six ribosomopathies have been described, i.e. Diamond–Blackfan anemia (DBA), 5q-syndrome, Shwachman–Diamond syndrome, X-linked dyskeratosis congenita, Treacher Collins syndrome and cartilage hair hypoplasia.[1,2] DBA belongs to a rare group of disorders known as inherited bone marrow failure syndromes.[3] DBA7 is caused by variants of the 60S ribosomal protein L11 gene (*uL5*, formerly *RPL11*).[4–6] So far naturally occurring *uL5* defects have only been described in humans. Owing to the role of *uL5* in ribosome biogenesis and its association with fatal ribosomopathies, we addressed the question whether lethal *uL5* variants exist in cattle. Several deleterious variants have been identified, including a 2 bp deletion resulting in a frameshift and premature stop (ENSBTAG00000020905: g.129,195,922_129,195,923del; ARS-UCD1.2; rs381576999). To monitor this variant a probe located on BTA2 between positions 129 195 924 and 129 195 973 (ARS-

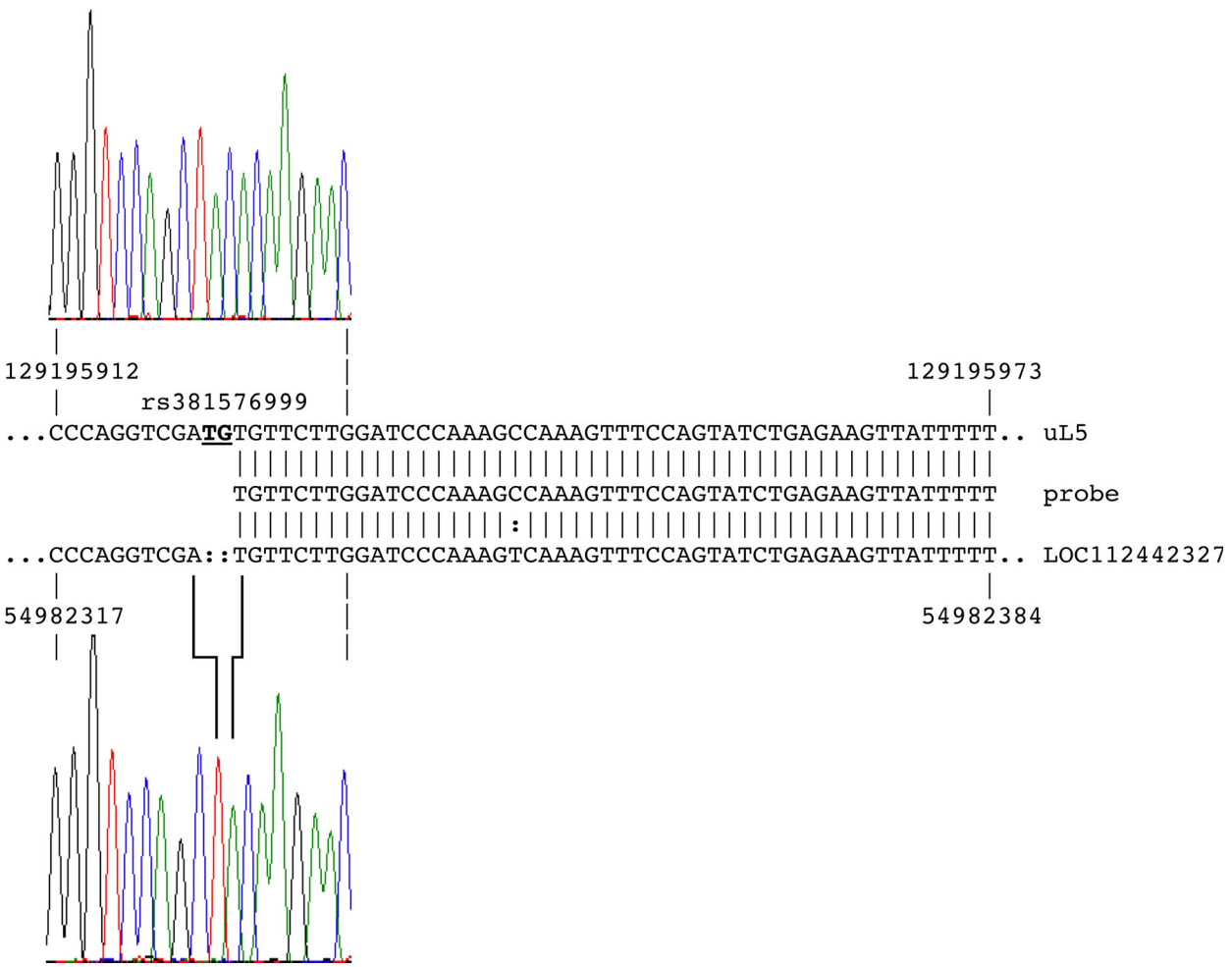

Figure 1 Alignment of *uL5* (BTA2) with the BovineLD BeadChip probe and the processed *uL5* pseudogene (LOC112442327, BTA18) between the indicated positions (ARS-UCD1.2). The putative 2 bp deletion (rs381576999) is underlined and bold. The upper and lower chromatograms show *uL5* and the *uL5* pseudogene from the same individual. At both locations the individual has the homozygous genotype of the bovine reference genome (ARS-UCD1.2), which may be mistaken for an heterozygous genotype at *uL5*.

UCD1.2) has been included as an expert-selected marker in the custom add-on part of the BovineLD BeadChip (Fig. 1).

Variant detection: The presence of the deletion was tested in 370 527 cattle, including British Angus, Charolais, Braunvieh, Belted Galloway, Simmental, Dexter, German Black Pied cattle, Gelbvieh, Hereford, Limousin, Red Holstein, German Red, Holstein, Shorthorn, Uckermärker, Wagyu, Welsh Black and Belgian Blue. We found 299 218 homozygous wt and 71 249 apparently heterozygous cattle but no homozygous carriers.[7] A complete cluster separation and high GC scores of the *uL5* SNP excluded any technical bias by the chip-based genotyping method. However, we did not detect the putative deletion by Sanger sequencing of PCR-amplified *uL5* gene segments in 10 randomly chosen heterozygous cattle (primer sequences are listed in Table S1). In addition, the BeadChip probe matches almost perfectly (49/50 nucleotides) to a processed *uL5* pseudogene on BTA18 (LOC112442347; position 54 982 088–54 982 687). Sequencing of a PCR fragment of this pseudogene (primer sequences are listed in Table S1) from an individual scored as heterozygote revealed the 2 bp deletion of rs381576999 (Fig. 1). To verify the indel within *uL5* and its processed pseudogene, 1323 (*uL5*) and 346 (pseudogene) random cattle samples were genotyped using FRET[8] (primer sequences are listed in Table S1). None of the 1323 individuals analyzed carried the deletion in *uL5*, whereas all 346 cattle were homozygous carriers of the deletion in the pseudogene. In order to further prove the suspected genotyping error by the pseudogene, we interrogated the genomic region for the presence of a haplotype with correlation to the chip data. A total of 82 014 samples were used, where 54 SNPs around *uL5* (±1.5 Mb up- and downstream) were phased using BEAGLE (version 3.32)[9] omitting position rs381576999. No haplotype with a significant correlation to the rs381576999 chip-genotype nor any suspected lethal haplotype could be established (Fig. S1).

Comments: We conclude that there is currently no evidence for the existence of the rs381576999 indel in the functional *uL5* gene or in its pseudogene on BTA18. Instead, our results suggest that the deletion has been fixed in the pseudogene, but is not scored reliably in the BovineLD BeadChip assay. These data demonstrate how pseudogenes interfere with the scoring of high-throughput genotyping platforms and that bead-arrays are not suitable for assaying polymorphisms in sequences that are not single-copy.

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References

1 Narla A. & Ebert B.L. (2010) *Blood* **115**, 3196–205.
2 Turi Z. *et al.* (2019) *Aging (Albany NY)* **11**, 2512–40.
3 Lipton J.M. & Ellis S.R. (2009) *Hematol Oncol Clin North Am* **23**, 261–82.
4 Carlston C.M. *et al.* (2017) *Am J Med Genet A* **173**, 2622–7.
5 Ban N. *et al.* (2014) *Curr Opin Struct Biol* **24**, 165–9.
6 Nicolas E. *et al.* (2016) *Nat Commun* **7**, 11390.
7 Rodriguez S. *et al.* (2009) *Am J Epidemiol* **169**, 505–14.
8 Bunt G. & Wouters F.S. (2017) *Biophys Rev* **9**, 119–29.
9 Herzig A.F. *et al.* (2018) *Genet Epidemiol* **42**, 201–13.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 Comparison of heterozygous haplotypes.
Table S1 Primer and FRET probe sequences used for PCR, sequencing and FRET genotyping.