

Supplemental Material S2: Cis-eQTL effects for the three LoF variants predicted to cause nonsense mediated RNA decay in the NZDC population.

Chr	Position	Gene - Mut.	Ref. allele	BETA	STAT	P value
8	92930920	<i>RNF20</i> - SG	T	-0.4591	-15.71	3.05E-42
11	102498942	<i>TTF1</i> - SG	A	-0.2396	-8.549	4.19E-16
26	24720154	<i>OBFC1</i> - FS	CT	-0.2734	-7.152	5.22E-12

Expression QTL analysis of candidate LoF variants from the New Zealand population was conducted using mammary RNA sequence data and genotypes called directly from the RNAseq alignments. These data represented 406 mostly Holstein-Friesian dairy cows in their second or third lactation, comprising an expanded dataset to that described previously¹. Briefly, total RNA libraries were prepared and sequenced by NZ Genomics Limited (NZGL; Auckland, New Zealand) or the Australian Genome Research Facility (AGRF; Melbourne, Australia), using 100bp paired end sequencing on the Illumina HiSeq 2000 instrument. Read data were mapped to the UMD3.1 genome using Tophat2² (version 2.0.12), yielding a mean mapped depth of 88.9 million read-pairs per individual. Gene expression for the *OBFC1*, *TTF1* and *RNF20* genes was quantified using DESeq³ (v1.14.0), outputting variance stabilisation-transformed read counts in conjunction with transcript structures defined by the Ensembl genebuild v81. Genome-wide expression outlier individuals were identified using principle component analysis in accordance with published guidelines⁴, with 374 quality-filtered animals retained for association analysis. Genotypes were called using Samtools⁵ (v1.2), and association testing was performed using PLINK⁶ (v1.90). Association models incorporated fixed effects for animal cohort, and covariates to account for population structure using Illumina BovineHD BeadChip genotypes in conjunction with the identity by state and multidimensional scaling procedure implemented in PLINK.

1. Littlejohn, M. D. et al. Expression variants of the lipogenic AGPAT6 gene affect diverse milk composition phenotypes in *Bos taurus*. *PLoS One* 9, e85757 (2014).
2. Kim, D. et al. TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. *Genome Biol.* 14, R36 (2013).
3. Anders, S. & Huber, W. Differential expression analysis for sequence count data. *Genome Biol.* 11, R106 (2010).
4. Ellis, S. E. et al. RNA-Seq optimization with eQTL gold standards. *BMC Genomics* 14, 892 (2013).
5. Li, H. et al. The Sequence Alignment/Map format and SAMtools. *Bioinformatics* 25, 2078–9 (2009).
6. Purcell, S. et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* 81, 559–75 (2007).