Genome variants associated with RNA splicing variation in bovine are extensively shared between tissues

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Abstract

Background: Mammalian phenotypes are shaped by numerous genome variants, many of which may regulate gene transcription or RNA splicing. To identify variants with regulatory functions in cattle, an important economic and model species, we used sequence variants to map a type of expression quantitative trait loci (expression QTLs) that are associated with variations in the RNA splicing, i.e., sQTLs. To further the understanding of regulatory variants, sQTLs were compare with other two types of expression QTLs, 1) variants associated with variations in gene expression, i.e., geQTLs and 2) variants associated with variations in exon expression, i.e., eeQTLs, in different tissues.

Results: Using whole genome and RNA sequence data from four tissues of over 200 cattle, sQTLs identified using exon inclusion ratios were verified by matching their effects on adjacent intron excision ratios. sQTLs contained the highest percentage of variants that are within the intronic region of genes and contained the lowest percentage of variants that are within intergenic regions, compared to eeQTLs and geQTLs. Many geQTLs and sQTLs are also detected as eeQTLs. Many expression QTLs, including sQTLs, were significant in all four tissues and had a similar effect in each tissue. To verify such expression QTL sharing between tissues, variants surrounding ($\pm$1Mb) the exon or gene were used to build local genomic relationship matrices (LGRM) and estimated genetic correlations between tissues. For many exons, the splicing and expression level was determined by the same *cis* additive genetic variance in different tissues. Thus, an effective but simple-to-implement meta-analysis combining information from three tissues is introduced to increase power to detect and validate sQTLs. sQTLs and eeQTLs together were more enriched for variants associated with cattle complex traits, compared to geQTLs. Several putative causal mutations were

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56   identified, including an sQTL at Chr6:87392580 within the 5th exon of kappa casein (*CSN3*)

57   associated with milk production traits.

58   **Conclusions:** Using novel analytical approaches, we report the first identification of

59   numerous bovine sQTLs which are extensively shared between multiple tissue types. The

60   significant overlaps between bovine sQTLs and complex traits QTL highlight the

61   contribution of regulatory mutations to phenotypic variations.

62   **Keywords:** RNA splicing, gene expression, expression QTL, sQTL, genetic correlations,

63   local genomic relationship matrices (LGRM), transcriptome meta-analysis, bovine

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Background

65 Cattle are an important source of meat and dairy products for humans worldwide. Also, cattle

66 can be used as clinical models to study genetic causes of human diseases [1]. To improve

67 productivity, health performance and efficiency of cattle, traditional selective breeding has

68 been widely used. In the last decade, genomic selection, originally developed in cattle

69 breeding, has further increased the rate of genetic improvement of complex traits in all

70 livestock species [2, 3]. However, genomic selection commonly uses genotyping arrays that

71 are based on single nucleotide polymorphisms (SNPs) of which very few have known

72 biological functions or directly impact genetic variation in production traits. Knowledge of

73 the genes involved and polymorphic sites would increase our understanding of the biology

74 and may further increase the rate of genetic improvement [4].

75 Many of the sequence variants that are associated with complex traits (quantitative trait loci

76 or QTL) are not coding variants and are presumed to influence the regulation of gene

77 expression, that is to be expression QTLs [5]. An expression QTL might be associated with

78 the variation in overall transcript abundance from the gene, which we will refer to as a gene

79 expression QTL or geQTL. In cattle and humans, geQTLs show significant enrichments for

80 mutations associated with diseases and complex traits [5-7].

81 After transcription, RNA is spliced by intron removal and exon ligation to create various

82 mature transcripts. An expression QTL associated with the changes in the expression ratio of

83 an exon to the gene implies that it alters RNA splicing. This type of expression QTL is then

84 defined as a splicing QTL or sQTL, which have been studied in humans by inferring the

85 individual splicing ratio from RNA sequence data [8]. More recently, sQTLs, identified using

86 intron information extracted from RNA sequence data, were demonstrated to have

87 fundamental links with human diseases [9, 10]. RNA splicing also results in different

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88   expression levels of exons within a gene. Thus, in theory, the type of expression QTL that

89   change the level of expression of one or several exons, i.e., exon expression QTL or eeQTLs,

90   may represent some sQTLs. However, the extent to which eeQTLs overlap with sQTLs

91   and/or geQTLs remains unclear, at least in cattle.

92   Knowledge of large mammal regulatory mutations is limited mainly to humans, where there

93   have been multiple studies reporting on expression QTLs [9, 11, 12]. In this study, we aim to

94   identify bovine cis splicing QTLs using the abundances of genes, exons and introns estimated

95   from RNA sequence data from hundreds of animals and multiple tissues along with imputed

96   whole genome sequences. We examined the extent to which sQTLs can be detected in white

97   blood cells, milk cells, liver and muscle transcriptomes and the extent to which sQTLs

98   overlap with conventional QTL associated with complex traits overlapped. To further

99   characterise the features of sQTLs, we used the counts of genes and exons to map another

100   two types of cis expression QTLs: eeQTLs and geQTLs, and then analysed their relationships

101   with sQTLs in different tissues.

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Results

104   *Data quality*

105   In total, we analysed 378 transcriptomes of 19 tissue types from 214 cattle generated from

106   four experiments covering major dairy and beef cattle breeds (Table 1, Figure 1a and

107   Additional file 1: Supplementary Methods). Following recommendations from Mazzoni and

108   Kadarmideen 2016 [13] RNA sequence quality was assessed and was detailed in Additional

109   file 1: Supplementary Methods. Based on the results produced by Qualimap 2 [14], no

110   significant events of RNA degradation were observed in all studied tissues (Additional file 2:

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111   Supplementary Figure S1). Also, according to Qualimap 2 [14], on average, 60.3% of reads

112   were mapped to exonic regions of the bovine reference genome (UMD3.1), 15.4% of reads

113   were mapped to intronic regions and 24.4% were mapped to integenic regions (Additional

114   file 3: Supplementary Table S1). Splicing junction annotation and saturation were estimated

115   using RSeQC [15]. As a small demonstration, no significant difference was observed in the

116   annotated splicing junction events in the bovine reference genome (UMD3.1), using different

117   RNA-seq alignment software including HISAT2 [16], STAR [17] and TopHat2 [18]

118   (Additional file 4: Supplementary Table S2). Although HISAT2 and STAR outperformed

119   TopHat2 for novel splicing junction events (do not exist in the current bovine UMD3.1

120   genome). Also, using a splicing junction saturation analysis for all tissues we observed

121   saturated coverage for the known splicing junctions (Additional file 2: Supplementary Figure

122   S2), though there appeared to be more potential for splicing junction discovery for the novel

123   category.

124   Animals with white blood cell RNA-seq data were evaluated for the consistency between

125   imputed genotypes from the 1000 bull genomes project [19] and RNA sequence genotypes as

126   predicted from the RNA sequence data using samtools [20]. On average, the concordance

127   between imputed sequence genotypes and RNA sequence genotypes was 0.943 (Additional

128   file 2: Supplementary Figure S3), which was consistent with the average imputation accuracy

129   (0.926) of the 1000 bull genomes project [21]. The comparison of the genotypes was detailed

130   in Additional file 1: Supplementary Methods.

131   Overall, samples from the same or similar tissues clustered together rather than clustering by

132   experiments, based on exon expression levels (Figure 1a). This was supported by further

133   analyses of the clusters where ellipses, drawn based on tissue types, were clearly separated

134   (Additional file 2: Supplementary Figure S4a,c,e), whereas ellipses drawn based on different

135   experiments overlapped (Additional file 2: Supplementary Figure S4b,d,f), at the confidence

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136   interval = 0.95 [22]. Consistent with previous reports [23], milk cells and mammary gland

137   transcriptomes were closely related.

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Table 1. Summary of experiments, data and analyses. Tissue splicing: variation in differential splicing associated with tissue types estimated using RNA sequence data from all experiments. Breed splicing: variation in differential splicing associated with Holstein and Jersey breeds estimated using RNA sequence data of milk cells from experiment III. sQTLs: cis splicing quantitative trait loci, sQTLs estimated using RNA sequence data and imputed whole genome and from experiment III and IV. Data from experiment III and IV were also used to estimate exon expression eeQTLs and gene expression geQTLs.

Tissue splicing	Breed splicing	sQTLs	Experiment	Tissue type	Sample No.	Breed	Individual No.
✓			I	18 various[a]	54	Holstein	1
✓			II	milk cells & mammary	12	Holstein	6
✓		✓	III	white blood cells	105	Holstein	105[b]
✓	✓	✓	III	milk cells	131	Holstein & Jersey	105[b] & 26
✓		✓	IV	liver	35	Angus	35
✓		✓	IV	muscle	41	Angus	41

[a]: 18 tissues from [24]. [b]: The same 105 Holstein cattle, each of which had both white blood and milk cell transcriptomic data.

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140   *Differential splicing between tissues and breeds*

141   We primarily defined a differentially spliced gene as a gene which contained exons whose

142   inclusion ratios (exon expression divided by gene expression) were significantly associated

143   with tissue or breed (FDR<0.1). To verify the significantly spliced exons, we imposed a

144   requirement that at least one adjacent intron had an excision ratio [9, 25] that was also

145   significantly (FDR<0.1) associated with tissue or breed (See methods). The FDR threshold of

146   such exon splicing events was considered as approximately 0.01 by combining the FDR

147   thresholds from exon and intron analyses (0.1 × 0.1). The overlaps of genes displaying

148   differential splicing from exon and intron analyses were shown and examined in 2 × 2 tables

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149 by Chi-square tests (Additional file 5: Supplementary Table S3). Overall, the overlap of the

150 results from exon and intron analyses was significantly more than expected by random

151 chance.

152 Using data from all experiments (Table 1), there were 8,657 genes in which at least one exon

153 had the variation in splicing associated with differences between tissue types. A list of these

154 genes with the significances of differential splicing for the exons and introns was shown in

155 Additional file 6: Supplementary Table S4. The top 10% of these significantly differentially

156 spliced genes had a GO term enrichment (FDR<0.01) of 'regulation of cellular process',

157 suggesting very general roles of these genes in cell function. There were148 genes with

158 significant differential splicing events in the milk cell transcriptome between breeds (Table 1,

159 Additional file 7: Supplementary Table S5). While these genes did not show any significant

160 GO term enrichments, they included the milk protein gene *CSN3* [26, 27], where the 5th exon

161 (6:87,392,578-87,392,750) was more commonly included in the transcript in Holstein cattle

162 than in Jersey cattle (Figure 1b).

163 *cis splicing quantitative trait loci (sQTLs)*

164 The mapping of sQTLs was based on data from 312 transcriptomes generated from

165 experiments III and IV, including white blood cells, milk cells, liver and muscle tissues

166 (Table 1). In total 207 individuals had imputed whole genome sequence data and in

167 experiment III, 105 genotyped cattle had both white blood and milk cell transcriptome data

168 (Table 1). Similar to differential splicing analyses described above, a cis-acting sQTL was

169 defined as a SNP significantly (FDR<0.1) associated with the variation in the inclusion ratio

170 of the exon (up to 1Mb away) and significantly (FDR<0.1) associated with the variation in at

171 least one adjacent introns' excision ratio [9, 25]. When analysed separately, the overlap

172 between sQTLs found by exon analyses and sQTLs found by intron analyses were

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173   significantly more than expected by random chance (Additional file 5: Supplementary Table

174   S3). After requiring that the variation in the inclusion and excision ratios for adjacent exons

175   and introns both be associated with the same SNP, 138,796 sQTLs were called in the white

176   blood cells, 28,907 sQTLs were called in the milk cells, 11,544 sQTLs were called in the

177   liver tissue and 5,783 sQTLs were called the muscle tissue (Figure 2, Additional file 5, 8:

178   Supplementary Table S3 and S6).

179   The significant sQTLs in white blood and milk cells were mapped to 929 and 283 genes,

180   respectively (Table 2). Many SNPs were significant for sQTLs due to linkage disequilibrium

181   between SNPs close to the same gene. The results do not imply many sQTLs per gene.

182   In the milk cell transcriptome, the fifth exon of *CSN3* (6:87392578-87392750), which as

183   described above was differentially spliced in Holstein and Jersey cattle (Figure 1b), and was

184   strongly associated with an sQTL (Chr6:87392580, p = 5.0e-07, Additional file 8:

185   Supplementary Table S6). This sQTL is physically located within the 5th exon of *CSN3*. Also,

186   the B allele of this sQTL increased the expression and inclusion ratio of the 5th exon and had

187   a higher allele frequency among Holstein cattle than Jersey cattle (0.79 vs 0.02). This

188   predicted that the expression and inclusion ratio of the 5th exon would be significantly higher

189   in the Holstein cattle than Jersey cattle, which was in line with the observations in Figure 1b.

190   In addition, this sQTL was also predicted to be a splice site ('splice_region_variant') by

191   Ensemble [28] and NGS-SNP software [29]. Much smaller numbers of significant sQTLs

192   were detected in liver (11,544 SNPs) and muscle (5,783 SNPs) (Figure 2c,d). This was

193   probably due to the smaller sample size (Table 1) and lower sequence depth of liver and

194   muscle from experiment IV (Additional file 1: Supplementary Methods) than that of white

195   blood and milk cells from the experiment III (Figure 2a,b, Table 2).

Table 2. Summary of expression QTLs detected. cis sQTLs: significant SNPs within ± 1Mb of the exon, associated with the variation in its inclusion ratio and also associated with the variations in the excision of an adjacent intron at the same significance level. Where the FDR threshold was approximated to 0.01 by combining FDR thresholds used in exon (FDR < 0.1) and intron (FDR < 0.1) analyses. cis eeQTLs: significant (FDR<0.01) SNPs within ± 1Mb of the exon, associated with the variation in its abundance. cis geQTLs: significant (FDR<0.01) SNPs within ± 1Mb of the gene associated with the variation in its abundance.

cis QTL type	Tissue	Expression QTL number	Gene number
sQTLs	white blood cells	138,796	929
	milk cells	28,907	283
	liver	11,544	49
	muscle	5,783	76
eeQTLs	white blood cells	802,685	6,446
	milk cells	100,844	2,102
	liver	37,322	346
	muscle	64,675	1,267
geQTLs	white blood cells	96,530	842
	milk cells	4,099	99
	liver	39,306	419
	muscle	57,054	1,150

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197   *Comparing sQTLs with exon expression eeQTLs and gene expression geQTLs*

198   Many more significant eeQTLs than sQTLs were detected in all tissues studied (Table 2). In

199   white blood and milk cells, the number of geQTL was smaller than the number of significant

200   sQTLs in white blood and milk cells (Table 2).

201   Figure 3a showed that sQTLs were a median distance of about 200 kb from the transcription

202   start site (TSS) and were slightly closer to the TSS than eeQTLs and geQTLs. All 3 classes of

203   expression QTLs had a lower percentage of intergenic SNPs and a higher percentage of

204   intronic and coding SNPs, including splice sites than the same categories across all SNPs

205   analysed (Figure 3b). Specifically, sQTLs had the highest percentage of intronic SNPs,

206   compared to eeQTLs and geQTLs. However, no consistent ranking of concentrations of

207   'Splice' SNP category for sQTLs, eeQTLs and geQTLs were observed in different tissues

208   (Figure 3b).

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209   *Shared genetic influences between cis QTL types*

210   Within each tissue, the sharing of SNPs between all three expression QTL types was

211   significantly more than expected by chance (Figure 4). However, in the white blood and milk

212   cells, which had relatively large sample size (n>=105, Table 1), the largest absolute amount

213   of SNP sharing appeared to be between sQTLs and eeQTLs (Figure 4). This was followed by

214   the amount of SNP sharing between eeQTLs and geQTLs (Figure 4a,b). In liver and muscle

215   tissue which had relatively small sample size (n<=41) and low sequencing depth, the largest

216   absolute amount of SNP sharing was between eeQTLs and geQTLs, followed by the amount

217   of SNP sharing between sQTLs and eeQTLs (Figure 4c,d).

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219   To further examine the relationship between sQTLs and eeQTLs, a two by two table of sQTL

220   and eeQTL counts in white blood and milk cells, which had comparable sample sizes, was

221   created (Additional file 9: Supplementary Table S7). This suggested that when an sQTL was

222   found, it was highly likely to be also identified as an eeQTL. For example, of 138,796 sQTLs

223   found in the white blood cells, 109,155 of them were also blood eeQTLs, but only 21,766 of

224   them were identified as blood geQTLs. Again, for these 138,796 blood sQTLs, although only

225   18,005 and 25,932 of them were milk sQTLs and eeQTLs, respectively, an even smaller

226   number, 720, of them were identified as milk cell geQTLs.

227   *Shared genetic influences between tissues*

228   Within each type of expression QTL between different tissues, the majority of the significant

229   expression QTL sharing was observed between white blood and milk cells and between liver

230   and muscle (Figure 5a). This is not unexpected since most of the white blood and milk cells

231   came from the same lactating cows and the muscle and liver from different growing Angus

232   bulls. Nevertheless, there was significant sharing of eeQTLs between milk cells and liver and

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233   between milk cells and muscle (Figure 5a). The largest amount of across-tissue expression

234   QTL sharing was observed in eeQTLs, followed by sQTLs and geQTLs (Figure 5). Where a

235   SNP was significantly associated with variation in expression in two tissues, the direction of

236   effect was usually the same in both tissues (Additional file 2: Supplementary Figure S5). The

237   correlation between effects of expression QTLs for white blood cells and milk cells

238   (Additional file 2: Supplementary Figure S5a,c,e) was stronger than that between liver and

239   muscle (Additional file 2: Supplementary Figure S5b,d,f). The sharing at the SNP level

240   between white blood cells and milk cells and between liver and muscle were also evident at

241   the exon and gene level (Figure 5b).

242   The expression QTL sharing between tissues was further examined for all types of expression

243   QTL by using a less stringent p-value (p<0.05) to test their effect (Additional file 2:

244   Supplementary Figure S6). This showed that the expression QTL sharing between tissues was

245   stronger for sQTLs and eeQTLs (Additional file 10: Supplementary Figure S6a-d), than the

246   sharing for geQTLs (Additional file 10: Supplementary Figure S6e-f). Again, more

247   expression QTL sharing was found between white blood cells and milk cells than between

248   liver and muscle. For instance, 75% of eeQTLs significant in the white blood cells at p<0.002

249   were significant in milk cells at p<0.05 (Additional file 2: Supplementary Figure 6c).

250   The correlation between estimated SNP effects on gene splicing and expression in different

251   tissues are lower in magnitude than the true correlation between SNP effects, because the

252   effects are estimated with error and these errors are independent between tissues. To estimate

253   the true correlation, we computed the genetic correlation between SNP effects in two

254   different tissues by GREML[16] using a local genomic relationship matrix or LGRM built from

255   SNPs from 1 Mb surrounding the exon or gene (Figure 5c). LGRM differs from a

256   conventional GRM by focusing on the local SNPs (in this case within 1 Mb distance) with

257   potential *cis* genetic associations with the variation in the splicing or expression level of the

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258   exon or gene. This was in agreement with the definition of the *cis* expression QTLs which

259   were also within 1Mb distance to the exon or gene in the current study. Out of 1,145 analysed

260   sQTLs (inclusion ratio of the exon), eeQTLs (expression level of the exon) and geQTLs

261   (expression level of the gene) between tissues, 598 had genetic correlations significantly

262   ($p<0.05$) different from 0, out of which 561 had genetic correlations insignificantly ($p >= $

263   0.05) different from 1. That is, in many cases, the variation in exon expression in white blood

264   cells and milk cells was associated with the same *cis* polymorphism (s).

265   Often both splicing events and exon expression within a gene were highly correlated between

266   white blood and milk cells, for instance *DDX19B*, *CTSD* and *EFF1A1* (Figure 5c). In liver

267   and muscle, exons from *HLA-DQA1* encoding major histocompatibility complexes [30] also

268   showed significant genetic correlations between tissues based on both exon expression and

269   splicing. There were more cases of eeQTLs than sQTLs and geQTLs and so there were more

270   estimates of genetic correlations between white blood and milk cells in Figure 5c. The

271   genetic correlations between eeQTLs in white blood and milk cells show a range from +1 to -

272   1 although most are close to +1. Exons with negative genetic correlations of expression

273   between white blood cells and milk cells were mapped to *SART1* [30], a post-transcriptional

274   regulator in epithelial tissues and *TTC4* with potential to mediate protein-protein interactions

275   [30]. These negative genetic correlations imply that there are mutations that increase the

276   expression of the exon in milk cells but decrease it in white blood cells. An exon within

277   *S100A10*, a cell cycle progress regulator, showed negative genetic correlation of expression

278   between liver and muscle.

279   Genetic correlations between exon expression levels in two tissues can be different between

280   exons within the same gene. For example, only the 2nd exon (5:93,942,055 -93,942,195) of

281   *MGST1* (which is associated with the variation in dairy cattle milk fat yield [27, 31]) had a

282   significant genetic correlation of expression between white blood cells and milk cells (Figure

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283   5c). This was largely due to a few eeQTLs with relatively highly significant effects (p<1e-10)

284   on the expression levels of the 2nd exon in the milk cells and a similar but less significant

285   effect (p<1e-4) on the 2nd exon expression in white blood cells (Additional file 2:

286   Supplementary Figure S7). For exons 1 and 3, the significances of the eeQTLs in both milk

287   and white blood cells were > 1e-3. For exon 4, the significance of the majority of eeQTLs in

288   both milk and white blood cells were > 1e-5 (Additional file 2: Supplementary Figure S7).

289   *Multi-transcriptome meta-analysis to increase power of expression QTLs detection*

290   Based on shared genetic effects of all types of expression QTLs across tissues, a multi-

291   transcriptome meta-analysis was introduced to increase the power to detect sQTLs, eeQTLs

292   and geQTLs (Figure 6, Table 3). For sQTLs, eeQTLs and geQTLs that had significant effects

293   (p < 0.05) in all of white blood cells, milk cells and muscle transcriptomes, their standardised

294   effects (signed t values) in each transcriptome were simply combined and tested for

295   significance against a χ^2 distribution with 1 degree of freedom. Overall, the multi-

296   transcriptome meta-analysis based on summary statistics substantially increased the power of

297   expression QTLs detection (Figure 6). The significance of multi-transcriptome expression

298   QTLs was compared with their significance in the liver transcriptome (Figure 6, Table 3). For

299   a criteria where the expression QTLs had both multi-transcriptome meta-analysis $p < 1 \times 10^{-5}$

300   and liver transcriptome analysis p < 0.05, all types of expression QTLs had significant

301   overlap of the SNPs between the meta-analysis and the single transcriptome analysis in liver.

302   In fact most of the significant sQTLs, eeQTLs and geQTLs detected by the meta-analysis

303   were also detected by the liver analysis but at a much higher p-value (Table 3).

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Table 3. The overlap between the multi-transcriptome meta-analysis of three tissues and the single transcriptome results of liver. sQTLs: splicing quantitative trait loci; eeQTLs: exon expression QTLs; geQTLs: gene expression QTLs. Liver: QTLs with single-transcriptome effects p < 0.05 in the liver. Meta-analysis: QTLs with χ^2 p < 1e-05 for the meta-analysis of white blood cells, milk cells and muscle. '+' indicates the number of SNPs met the significance criteria while '−' indicates number of SNPs failed to meet the significance criteria.

sQTLs		Meta-analysis		Overlap p
		+	-	
Liver	+	768,650	2,842,552	p < 2e-16
	-	139,551	4,301,536	

eeQTLs		Meta-analysis		Overlap p
		+	-	
Liver	+	1,052,041	3,186,839	p < 2e-16
	-	140,033	3,575,617	

geQTLs		Meta-analysis		Overlap p
		+	-	
Liver	+	178,093	473,232	p < 2e-16
	-	43,578	6,663,855	

305

306 *Overlap between expression QTLs and QTL for dairy and beef traits*

307 We examined whether cis sQTLs, eeQTLs and geQTLs were significantly enriched amongst

308 SNPs associated with economically important cattle traits. Pleiotropic SNPs significantly

309 (FDR<0.01) associated with more than one of 24 dairy traits [32] and with more than one of

310 16 beef traits [33] were tested for overlap with detected sQTLs, eeQTLs and geQTLs (Figure

311 7, Additional file 10,11: Supplementary Table S8,9). Overall, sQTLs, geQTLs and eeQTLs

312 identified in white blood and milk cells had greater overlap with SNPs associated with dairy

313 and beef traits than sQTLs, geQTLs and eeQTLs identified in liver and muscle (Figure 7).

314 sQTLs in white blood and milk cells were significantly enriched for dairy cattle pleiotropic

315 SNPs, including SNPs from the *CSN3* loci on chromosome 6 (Figure 7a,b). eeQTLs in the

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316   white blood cells had the largest absolute amount of SNPs overlapping with dairy cattle

317   pleiotropic SNPs (Figure 7b) and was the only expression QTL type with significant

318   enrichment with beef cattle pleiotropic SNPs (Figure 7b). eeQTLs in milk cells and liver also

319   had significant enrichment for dairy cattle pleiotropic SNPs (Figure 7a). Of the geQTLs, only

320   those from white blood cells had a significant enrichment with dairy cattle pleiotropic SNPs

321   (Figure 7a).

322   An example of an eeQTLs that overlaps a milk production QTL is for *MGST1*, where effects

323   of milk cell eeQTLs were highly significantly associated with their effects on milk fat yield

324   [32] (Figure 7c). Specifically, some expression QTLs with strong associations with the

325   variation in milk cell expression levels of exon 2 (Chr5:93,942,195-93,942,055) and exon 3

326   (Chr5: 93,939,244-93,939,150) of *MGST1* (Additional file 2: Supplementary Figure S7) also

327   had strong associations with the variation in milk fat yield (Figure 7c). Littlejohn et al.[31]

328   identified SNPs associated with milk yield percentage and *MGST1* expression in the

329   mammary gland, including 17 putative causal variants. Most SNPs identified by Littlejohn et

330   al. originated from whole genome sequence and so were not present on the high density SNP

331   chip data we analysed for dairy cattle pleiotropy [32] (Additional file 12: Supplementary

332   Table S10). However, 53 significant milk cell eeQTLs identified by the current study

333   overlapped with the top 200 SNPs from Littlejohn et al [31] (Additional file 12:

334   Supplementary Table S10), which was significantly more than expected by random chance.

335   The 53 eeQTLs included the SNP suggested as a putative causal candidate (Chr5:93945738)

336   [31], which was significantly associated with the variation in expression level of the third

337   exon (5:93939150-93939244) of *MGST1* in milk cells (Additional file 12: Supplementary

338   Table S10). No milk cell geQTLs was called for *MGST1*, as all of them had weak effects on

339   the whole *MGST1* gene expression in milk cells, resulting in a large FDR (Additional file 12:

340   Supplementary Table S10).

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Discussion

343   We performed a systematic analysis of cis expression QTLs (<=1Mb) in multiple tissues

344   centred around RNA splicing events, using a large number of RNA and whole genome

345   sequence data from an important domestic animal species. Overall, differential splicing

346   between tissues is ubiquitous and between breeds is common. Differential splicing between

347   individuals due to SNPs (sQTLs) occurs for many genes and is enriched with cattle complex

348   trait QTL. Within each tissue, all cis expression QTLs types showed significant overlap. Most

349   geQTLs and sQTLs were detected as eeQTLs indicating that the exon expression can be

350   altered by changing the expression of the whole gene or by changing the splicing. However,

351   an sQTL was likely to be an eeQTLs and, to a lesser extent, geQTLs. Between tissues, while

352   all QTLs types showed significant overlap between white blood cells and milk cells and

353   between liver and muscle, the strongest cross-tissue sharing appeared to be at the exon level

354   (sQTLs and eeQTLs). This is supported by many significant tissue pair genetic correlations.

355   Such cross-tissue expression QTL sharing allowed the multi-transcriptome meta-analysis of

356   expression QTL effects which substantially increases power to detect significant expression

357   QTLs.

358   The majority of significant sQTLs were detected from white blood and milk cells (Figure

359   2a,b) which also overlapped with SNP chip based complex trait QTL (Figure 7), compared to

360   sQTLs detected from liver and muscle. This is probably due to the larger sample size for

361   white blood and milk cells than for liver and muscle (Table 1) and the higher sequencing

362   depth (Additional file 1: Supplementary Methods). One of the significant white blood cell

363   sQTLs (29:44585782) for *CAPN1* is also a SNP chip based significant pleiotropic SNP for 16

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364   beef cattle traits (Additional file 11: Supplementary Table S9). This SNP is associated with

365   shear force in multiple taurine breeds [34].

366   In the milk cell transcriptome, a significant sQTL (Chr6:87392580, Figure 2a) with predicted

367   splicing function [28] within the fifth exon (6:87,392,578-87,392,750) of *CSN3* is strongly

368   associated with differential splicing between Holstein and Jerseys (Figure 1b). Variants

369   within *CSN3* have long been found to be associated with milk traits [35, 36] but only recently

370   have putative causal variants been prioritised [26]. The milk cell sQTL 6:87392580 had

371   perfect linkage disequilibrium (r =1) with the variant 6:87390576 which has been suggested

372   as a putative causal variant for effects on milk protein yield and percentage [26, 27]. Given it

373   is at a splicing site, 6:87392580 could be a putative causal variant contributing to milk

374   production in dairy cattle by altering exon splicing.

375   Compared to identified bovine cis geQTLs, cis sQTLs tended to be closer to the transcription

376   starting site (TSS) and had highest concentrations of intronic SNPs (Figure 3). In humans, cis

377   sQTLs [9, 37] were more enriched for intron SNPs than other types of QTLs. However,

378   reports of the distance between human QTLs and TSS appear to be inconsistent. While no

379   difference in enrichment of SNPs near TSS between sQTLs and geQTLs were found by the

380   human GTEx project [8], a more recent study[9] found that human geQTLs were more

381   enriched near TSS than sQTLs. Our results appear to stand in between the results of GTEx

382   project and the later findings from Li et. al. [9], where cattle sQTLs were slightly closer to

383   TSS than geQTLs. However, this difference is not significant in all tissues (Figure 3a). On

384   the other hand, significant overlap between sQTLs and geQTLs was found in this study

385   (Figure 4) and by the human GTEx project [8]. However, Li et. al. [9] found that human cis

386   sQTLs were independent of geQTLs. These inconsistent observations are likely to be due to a

387   number of differences between these studies, including definition of sQTLs, choice of tissues

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388   and populations and computational procedures. Also, these inconsistent observations also

389   suggest that we are still at the very early stage of understanding of sQTLs features.

390   Within each studied bovine tissue, the largest amount of overlap between expression QTL

391   types was found either between exon expression eeQTLs and sQTLs or between eeQTLs and

392   geQTLs (Figure 4). Further, the largest amount of enrichments of cattle pleiotropic SNPs was

393   found for eeQTLs, followed by sQTLs and geQTLs. The white blood cell eeQTLs showed

394   particularly strong enrichments of pleiotropic SNPs for dairy and beef cattle. In a large scale

395   human blood cell expression QTLs study [12], eeQTLs also showed the strongest

396   enrichments of GWAS variants, followed by sQTLs and geQTLs. Thus, focusing on exon-

397   level QTLs, including eeQTLs and sQTLs, could increase the chance of finding regulatory

398   variants for complex traits, as proposed by Guan et. al.[38].

399   A hypothesis to explain these results is that mutations in regulatory DNA may increase the

400   expression of one or more transcripts from a gene. If they increase expression of one

401   transcript then they may be detected as an eeQTL for the exons in that transcript, as a sQTL

402   for exons spliced out of that transcript or as a geQTL if this transcript forms a large part of

403   the total transcription from the gene. Thus, there is expected to be overlap between eeQTLs,

404   geQTLs and sQTLs, but at least sQTLs and eeQTLs should overlap and this is what we found

405   (Figure 4, Additional file 9: Supplementary Table S7). It appears that eeQTLs detect the

406   largest proportion of these regulatory polymorphisms provided sequencing depth is high.

407   In humans, significant cross-tissue sharing of sQTLs and geQTLs was reported [8, 39]. In our

408   study of cattle, the strongest evidence of expression QTL sharing appeared to be at the exon

409   level. This included sQTLs and eeQTLs sharing between white blood and milk cells and

410   between liver and muscle (Figure 5). When extending the examination of expression QTLs to

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411   include those with p < 0.05 (Additional file 2: Supplementary Figure S6), the exon-level

412   expression QTLs cross-tissue sharing is also the greatest.

413   We highlighted a few examples of cross-tissue shared eeQTLs along with the related exons,

414   of which the genetic correlations of the expression and splicing in different tissues were

415   significant (Figure 5c). One of these eeQTLs is located within the milk fat yield [27, 31] QTL

416   *MGST1* (Figure 5c, Additional file 2: Supplementary Figure S7). For eeQTLs associated with

417   *MGST1*, a strong positive relationship of SNP effects was observed between milk cell

418   eeQTLs and dairy milk fat yield SNPs (Figure 7c). Furthermore, the identified milk cell

419   eeQTL overlaps with previously identified putative causal variants [31] within *MGST1* for

420   milk fat percentage, thus supporting their candidacy. This overlap further supports the top

421   candidate SNP 5:93945738 with significant effects on the abundance of the third exon of

422   *MGST1* (Additional file 12: Supplementary Table S10) for milk fat traits. Overall, our

423   analysis demonstrates the significant potential of using detailed exon analysis to aid in

424   identification of putative causative mutations.

425   Based on the sharing of expression QTLs between tissues, a multi-transcriptome meta-

426   analysis which simply combined expression QTL effects to substantially increase the power

427   (Figure 6) was introduced. Using this approach, combined expression QTL effects of white

428   blood cells, milk cells and muscle were validated in the liver (Table 3). This also

429   demonstrated the significant extent of QTL sharing across tissues. Previously, Flutre et. al.

430   [39] combined data from human fibroblasts, lymphoblastoid cell lines and T-cells and found

431   that up to 88% of geQTLs were shared across tissues at FDR<0.05 level. We checked the

432   existing results of the meta-analysis combining SNP effects from tissues of white blood cells,

433   milk cells and muscle at the FDR threshold < 0.05. We found that the meta-analysis

434   identified 585,406 geQTLs with FDR < 0.05 in more than one tissue. This accounted for 69.2%

435   of total geQTLs (845,431) that were called and common in the individual geQTL analysis of

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436   white blood cells, milk cells and muscle. While there were differences in the selection of

437   tissue/cell type between our experiment and Flutre et. al, it is possible that the analysis

438   proposed by Flutre et. al with more complex procedures would be more powerful than the

439   meta-analysis introduced by us. Flutre et. al applied principal components analysis to

440   normalise their gene expression data while we used quantile normalisation which appeared to

441   show good performances in combining different transcriptome datasets [40]. However, our

442   meta-analysis is powerful for detecting and validating many expression QTLs that have an

443   effect in the same direction in multiple tissues, and is simpler to implement than that of Flutre

444   et al. A future systematic comparison of different approaches of analysing expression QTL in

445   multiple tissues would be very useful.

446   As one of earliest investigations of large animal expression QTLs, our study has its potential

447   limitations. While the overlaps between sQTLs detected with exon and intron analyses were

448   significantly more than expected by random chance, the absolute amount of overlap was still

449   small. Through all analyses, there were always many more splicing events detected by intron

450   analyses implemented by leafcutter [10] than the exon analysis (Additional file 5:

451   Supplementary Table S3). This appears to be consistent with Li et al [10], the authors of

452   leafcutter. They suggested that intron-centred analyses can be both more sensitive (lower

453   proportion of false negatives) and more accurate (lower proportion of false positives) than the

454   exon based splicing mapping methods, such as Altrans [41].

455   We found that the strongest sharing of expression QTLs was either between white blood and

456   milk cells or between liver and muscle tissues, at the threshold of FDR <0.01 (Figure 5). The

457   white blood and milk cells sampled from the same Holstein and/or Jersey cattle of experiment

458   III had a larger sample size and higher read coverage, compared to the liver and muscle

459   tissues sampled from different Angus bulls of experiment IV. The reduced expression QTL

460   sharing detected between, e.g., muscle and milk cells, could be due to differences in the tissue,

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461   the physiological state of the cattle or the breed. However, it can be also due to different

462   power in the milk cells, liver and muscle datasets compared to the white blood cell data.

463   Nevertheless, in the multi-transcriptome meta-analysis where expression QTLs with low

464   threshold were examined (p<0.05), the combined effects of all types of expression QTLs of

465   the three tissues from different experiments were highly significant (Figure 6). Many of these

466   expression QTLs were also found in liver with p<0.05 (Table 3). This evidence supports the

467   proposal that the sharing of cis expression QTL is extensive across tissues, but these shared

468   expression QTLs may not necessarily have strong effects in each studied tissue. In the latest

469   human expression QTLs mapping study (GTEx consortium) where RNA seq data of 44

470   tissues from up to 450 individuals were analysed, cis expression QTL tended to be either

471   shared across most tissues or specific to a small subset of tissues [11]. As sample numbers for

472   each tissue increased, GTEx consortium identified more tissue specific expression QTLs [11].

473   Future studies with significantly increased power and selection of cattle tissues and breeds

474   may update our current results.

475   Another potential limitation of our study is the use of imputed sequence data, which may

476   introduce imputation errors that lead to inaccurate identification or exclusion of expression

477   QTLs. However, the average imputation accuracy of the 1000 bull genome project data used

478   in this study was high (0.926) [21] and there was a good consistency between the imputed

479   sequence genotypes and RNA sequence genotypes (average concordance = 0.943, Additional

480   File 2: Supplementary Figure S3). Stringent thresholds were also imposed to control the false

481   discovery rate of expression QTLs mapping (either FDR<0.01 or FDR approximately < 0.01

482   for sQTLs). In the current study, we did not consider the case where a haplotype can be

483   potentially associated with expression phenotypes. While a haplotype analysis can be

484   informative, it would require a very large sample size to achieve reliable results due to testing

485   a large number of combinations of haplotype blocks. In a human study where over 2,000

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486 individuals were analysed, expression QTLs conditioning on expression levels of

487 transcription factor genes were reported [12]. Finally, our results obtained from genome-wide

488 associations do not necessarily contain causal relationships. However, our findings are

489 important for prioritising informative SNP candidates for future validation of causal

490 relationships.

491

Conclusions
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493 We found that eeQTLs overlapped with both geQTLs, due to polymorphisms affecting the

494 level of expression of the whole gene, and with sQTLs, due to polymorphisms affecting the

495 exon usage within the gene. sQTLs tended to be closer to the transcription start sites more

496 often located in introns than geQTLs. We found the largest number of sQTLs in white blood

497 cells probably because the power to find them was greatest in this dataset. However, many of

498 the sQTLs found in other tissues were also detected in blood cells and many sQTLs found in

499 blood could be detected in other tissues at higher p-values. The genetic correlation between

500 expression QTLs in different tissues was often indistinguishable from 1.0 indicating that

501 many expression QTLs operate in a similar way across tissues. Consequently, combining

502 results from several tissues using the multi-transcriptome meta-analysis increased power to

503 detect all 3 types of expression QTLs. The potential of exon-level QTLs information was

504 demonstrated by the identification of several strong candidates of putative causal mutations

505 for complex traits: sQTL 6:87392580 within *CSN3* for milk production and eeQTL

506 5:93945738 within *MGST1* for milk fat yield.

507

Methods
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509   **Sample collection.** For Experiment I, the sampling of 18 tissues from one lactating Holstein

510   cow followed procedures described by Chamberlain et al [24]. For Experiment II and III, the

511   sampling and processing of all tissues including white blood and milk cells is detailed in

512   Additional file 1: Supplementary Methods. Briefly, animals of Experiment II and III were

513   selected from Agriculture Victoria Research dairy herd at Ellinbank, Victoria, Australia. In

514   Experiment II, milk and mammary tissue samples were taken from six Holstein cows. In the

515   Experiment III, milk and blood samples were originally taken from 112 Holstein and 29

516   Jersey cows, but only RNA sequence data of 105 Holstein and 26 Jersey with > 50 million

517   reads for milk cells or >25 million reads for white blood cells and had aconcordant alignment

518   rate [18] >80% were used. For Experiment IV, the sampling of 41 *semitendinosus* muscle and

519   35 liver from Angus bulls was previously described by [42, 43]. As recommended by

520   ENCODE guidelines (https://www.encodeproject.org/about/experiment-guidelines/)

521   biological replicates were favoured over technical replicates for experiments II-IV. However

522   Chamberlain et al [24] assessed technical replicates for experiment I.

523   **RNA seq data.** For Experiment I, RNA extraction and sequencing followed the procedures

524   described by Chamberlain et al [24]. For Experiment II and III, the RNA extraction and

525   sequencing procedure is detailed in Additional file 1: Supplementary Methods. For

526   Experiment IV, RNA extraction and sequencing is previously described by Khansefid et al.

527   [43]. For all experiments sequence quality was checked and were aligned to the Ensembl

528   UMD3.1 bovine genome assembly using TopHat2 [18]. The RNA sequence data processing

529   and quality checking are detailed in Additional file 1: Supplementary Methods.

530   **Whole genome seq data.** Experiments III and IV had whole genome sequence genotypes

531   imputed from the SNP chip genotypes using FImpute [44] based on the 1000 bull genomes

532   project [19]. The overall imputation accuracy of the most recent genome sequence data

533   ranged from 0.898 to 0.952 depending on chromosomes [21]. 50K Illumina genotypes were

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534   used for imputation for experiment III with previous protocols [27, 45]. For experiment IV,

535   800K and 50K Illumina genotypes were used with procedures following [33]. SNPs were

536   filtered for minor allele frequency > 0.01 and resulted in 14,302,604 and 13,632,145 SNPs

537   used in the analysis in experiment III and IV, respectively. There were 10,242,837 SNPs

538   shared between experiment III and IV.

539   **Gene/exon analysis.** Gene count data were generated by Python package HTSeq [46] using

540   default settings. The exon count data were generated by Bioconductor package featureCounts

541   [47] in R v3.3.2 [48]. The Ensembl based bovine genome reference (UMD3.1) was used to

542   define genes and exons. Genes and exons with count per million >0 in more than 40% of

543   RNA samples were used for all the following analyses. This filtering allowed the analysis to

544   focus on exons or genes with relatively robust expression in many RNA sequencing samples.

545   The exon-based tissue principal components analysis used DEseq2 based on the 250 exons,

546   the expression of which were most variable across studied tissues [49]. The usage of 500 and

547   1,000 exons with the most variable expression across tissue samples were also tested.

548   Consistent with [49], the selection of different numbers of exons had little impact on the

549   clustering patterns (Supplementary Figure S4). The significance of the clustering was

550   determined using ellipse method proposed by [22] and implemented in ggplot2 [50]. The

551   confidence interval was set to 0.95 to which ellipses were drawn based on data categorised by

552   tissue types or by experiments. The separation of ellipses indicated independence of

553   categories of data. The phenotype of exon inclusion was calculated as the exon to gene

554   expression ratio. The phenotype of intron excision was estimated using the publically

555   available software leafcutter [9, 10]. Briefly, leafcutter used RNA seq BAM files as input and

556   generated ratios of reads supporting each alternatively excised intron as the intron excision

557   phenotype [10](http://davidaknowles.github.io/leafcutter/). Those exons and introns with

558   ratio values <0.001 were removed and the remaining ratio values were transformed to $\log_2$

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559   scale, then underwent exon/intron –wise quantile normalisation and individual –wise zscore

560   standardisation [51].

561   **Gene differential splicing.** Both exon inclusions and intron excisions were analysed and

562   used in combination for gene differential splicing for (1) the overall tissue effects and (2) the

563   breed effects. Primarily, differential splicing was defined for the gene containing exons

564   whose variation in inclusion ratios were significantly (FDR<0.1) associated with the tissue or

565   breed variable. To be called as significantly spliced exons, they were required to have at least

566   one adjacent intron whose variation in excision ratios were also significantly (FDR<0.1)

567   associated with the tissue or breed variable. The tissue effects were analysed in a linear mixed

568   model in lme4 [52] in R as:

569     $y_{ijk} = b_i + x_j + t_k + e$ (1)

570   Where y = exon inclusion or intron excision ratios, b_i = the animal random effects (i=214),

571   x_j= the experiments (j=4), t_k = tissue type (k=19), e= random residual term. The fitting of the

572   animal random effects accounted for the fact that only 1 animal was used in experiment I.

573   The P values of F tests were calculated using Satterthwaite approximation implemented in

574   lmerTest [53]. The breed effects for the milk transcriptome data were analysed in a linear

575   model in R as:

576     $y_l = breed_l + e$ (2)

577   Where y = exon inclusion or intron excision ratios in the milk cell transcriptome, $breed_l$ =

578   breeds (l=2, Holstein and Jersey). p values of exons/introns for the tissue effects in equation

579   (1) or the breed effects in equation (2) were used to calculate the false discovery rates (FDR)

580   using qvalue [54] in R. The FDR threshold of such detected exon/intron group was

581   considered as approximately $0.1 \times 0.1 = 0.01$, as a combination of FDR thresholds of exon

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582   and intron analyses to reflect our selection criteria for significant splicing events. For genes

583   showing significant differential splicing for (1) the overall tissue effects and (2) the breed

584   effects as described above, enrichments of biological pathway were tested using GOrilla [55].

585   As many genes had differential splicing events associated with tissue differences, top 10% of

586   the genes with significant differential splicing were selected based on the approximate FDR

587   with combined FDR values of both exon and intron analyses.

588   **cis expression splicing QTLs**. Only transcriptomic data of experiment III and IV were used

589   in sQTLs mapping. Similar to differential splicing analysis described above, a significant

590   (FDR<0.01) cis splicing QTLs was expected to satisfy two conditions simultaneously: (1) a

591   SNP, within or up to $\pm$ 1Mb away from the exon, was significantly (FDR<0.1) associated

592   with the variation in the exon inclusion ratio and (2) the same SNP was significantly

593   (FDR<0.1) associated with at least one event of the excision of the intron next to the same

594   exon at the same significance level. Both individual exon inclusion and intron excision values

595   were used as phenotype to map associated QTLs with widely used [8] Matrix eQTL[56]

596   package in R. For each cell type of the experiment III (white blood and milk cells) and

597   experiment IV (liver and muscle), SNPs $\pm$ 1Mb from the exon or intron were tested for

598   regressions with the exon inclusion or intron excision phenotype. For milk cell transcriptome,

599   breed was fitted as a covariate.

600   To compare cis sQTLs with exon expression cis eeQTLs and cis gene expression geQTLs,

601   the expression count data were normalised by voom [57] estimating mean-variance

602   relationship to calculate observation-level weighted expression values. Normalised

603   expression values of exons and genes were used as phenotype to map cis expression QTLs

604   (within $\pm$ 1Mb) at FDR <0.01 level as described above.

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605   **SNP annotation**. The gene transcription start site coordinates were downloaded from

606   Ensembl (http://www.ensembl.org) and the absolute difference between the position of a SNP

607   and the transcription start site of the gene were calculated for the SNP with significant cis

608   effects. The SNP functional categories were generated using predictions from Ensembl

609   Variant Effect Predictor [28] in conjunction with NGS-SNP [29]. All analysed SNPs were

610   assigned a functional category.

611   **Dairy and beef cattle pleiotropic QTL.** To test the significance of overlap between cis

612   expression QTLs and SNPs associated with cattle phenotype, meta-analyses of dairy and beef

613   cattle pleiotropy were performed using single-trait GWAS results from Xiang et al [32] and

614   Bolormaa et al [33]. HD 800K SNP chip genotypes were used for trait GWAS. 24 dairy cattle

615   traits with matching phenotype in 9,662 bulls and cows and 16 beef cattle traits with animal

616   numbers >2,000 were selected. Briefly, the multi-trait χ^2 statistic for the ith SNP was

617   calculated based on its signed t values generated from each single trait GWAS [33]:

618     $\chi^2 = t'_i V^{-1} t_i$ (3)

619   For dairy cattle, the meta-analysis was based on the weighted SNP effects t_w combining SNP

620   effects calculated separately in bulls and cows. The t_w accounting for the phenotypic error

621   differences between bulls and cows [27] was calculated as:

622     $$t_w = \frac{B_w}{se_w} = \frac{\dfrac{\dfrac{B_{bull}}{se_{bull}^2} + \dfrac{B_{cow}}{se_{cow}^2}}{\dfrac{1}{se_{bull}^2} + \dfrac{1}{se_{cow}^2}}}{\sqrt{\dfrac{1}{\dfrac{1}{se_{bull}^2} + \dfrac{1}{se_{cow}^2}}}} \quad (4)$$

623   Where the weighted SNP t value t_w was the quotient of the weighted SNP effects B_w and the

624   weighted effect error se_w. B_{bull} and se_{bull} were the SNP effects and error obtained from single-

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625   trait GWAS in bulls and B_{cow} and se_{cow} were the SNP effects and error of cows. Those SNPs

626   which had meta-analysis FDR<0.01 were chosen to be compared with cis expression QTLs.

627   The lead SNP loci were defined as ±1 Mb from the lead SNPs identified in the previous

628   analysis [32] and [33].

629   **The significance of overlaps.** The significance of overlaps were compared with the expected

630   number using the Fisher's exact test (*p*) implemented in GeneOverlap [58] in R. This analysis

631   required four types of counts: the size of overlap between set A (e.g., SNPs that were blood

632   sQTLs) and set B (e.g., SNPs that were milk sQTLs), the size of set A, the size of set B and

633   the size of background. The union number of whole genome sequence SNPs with MAF>0.01

634   in each breed and the bovine high density chip SNPs were used as the background. Where

635   expression QTL categories from different breeds of dairy and beef cattle were tested for

636   overlap, the number of common SNPs between breeds was used.

637   **Genetic correlations using local genomic relationship matrices.** The cross-tissue sharing

638   of SNPs were confirmed by bivariate GREML analysis using GCTA [59]. For an exon or a

639   gene of interest, its inclusion ratios or expression levels in two different tissues were treated

640   as two different phenotype, tr_1 and tr_2. The SNPs within 1Mb of this exon or gene were used

641   to make a local genomic relationship matrix, i.e., LGRM, representing the local polygenic

642   component a with potential associations with the variation in the splicing or expression level

643   of the exon or gene. This allowed linear mixed modelling of the local additive genetic

644   variances of tr_1, $\mathrm{var}_{lg}(tr_1)$ and of tr_2, $\mathrm{var}_{lg}(tr_2)$ and the local additive genetic covariance

645   between t_1 and t_2, $\mathrm{cov}_{lg}(tr_1,tr_2)$ using GREML [59]. This approach agreed with the definition

646   of *cis* expression QTLs defined in this study (also within 1Mb distance to the exon or gene)

647   and allowed the estimation of genetic correlation $r_{lg} = \dfrac{cov_{lg}(tr_1,tr_2)}{\sqrt{var_{lg}(tr_1)var_{lg}(tr_2)}}$ (5). Genetic

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648   correlations were also tested for their significance of being different from 0 or 1, by fixing the

649   correlation value to 0 and 1 using GCTA [59].

650   **Validation by multi-transcriptome meta-analysis.** The validation based on expression QTL

651   effect commonality across tissues was conducted by comparing the combined expression

652   QTL effects from white blood cells (experiment III), milk cells (experiment III) and muscle

653   (experiment IV) transcriptomes with their effects in the liver transcriptome (experiment IV).

654   The standardised expression QTL effects, b/se, signed t values were calculated from single-

655   transcriptome results of white blood cells (t_1), milk cells (t_2) and muscle (t_3). The significance

656   of multi-transcriptome effects of an expression QTL was tested by χ^2 distribution with 1

657   degree of freedom:

658     $$\chi_{(1)}^2 = \left[\sum_{n=1}^{N} \frac{t_n}{\sqrt{N}} \right]^2 \quad (6)$$

659   N = the number of studied tissues (N=3 in this case) where the original SNP t values were

660   estimated. Provided the individual t-values followed a t-distribution under the null hypothesis,

661   the properties of the average t value in the current study was a simple mathematical result

662   which approximated the chi square distribution with 1 degree of freedom, the null hypothesis

663   of which was that the SNP does not have any significant associations in any of the 3 tissue

664   types. Previously, the concept of meta-analysis combining SNP t values estimated from

665   different datasets has been also applied to analyse multiple quantitative phenotypic traits in

666   large animals to increase power (see [33, 60] and equation (3)). The expression QTLs that

667   participated in the validation analysis had single-transcriptome effect $p < 0.05$ in each tissue

668   and the significance of the multi-transcriptome effects was defined as $p < 1e\text{-}05$. Significant

669   multi-transcriptome expression QTLs were compared with the liver single-transcriptome

670   effects at $p < 0.05$ level. We chose to combine two tissues which appeared to display strong

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671   power (white blood and milk cells, experiment III) with the third tissue from a different

672   experiment with relatively weak power (muscle, experiment IV). The single tissue left to be

673   compared with was liver, a tissue which also appeared to show weak power and was from

674   experiment IV. These choices intended to create enough differences for the meta-analysis to

675   combine the SNP effects and for the combined SNP effects to be compared with the SNP

676   effects in the single tissue.

677

List of abbreviations

679   sQTLs: splicing quantitative trait loci;

680   geQTLs: gene expression quantitative trait loci;

681   eeQTLs: exon expression quantitative trait loci.

682

Declarations

Ethics approval and consent to participate

685   For experiment I-III, the animal ethics was approved by the Victoria Animal Ethics

686   Committee (application number 2013-14), Australia. For experiments IV, the animal ethics

687   was approved by the University of New England Animal Ethics Committee (AEC 06/123,

688   NSW, Australia) and Orange Agriculture Institute Ethics Committee (ORA09/015, NSW,

689   Australia).

690

Consent for publication

692   Not applicable.

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693

Availability of data and materials

695 The RNA sequence data for experiment I was published [24] (NCBI Sequence Read Archive,

696 SRA, accession SRP042639); For experiment II: SRA accession SRP111067; For experiment

697 III: SRA accession PRJNA305942; For experiment IV: SRA accession PRJNA392196; The

698 imputed whole genome sequence data was part of the 1000 bull genome project [19].

699

Competing interests

701 The authors declare that they have no competing interests.

702

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709

Author contributions

711 A.J.C., R.X., M.E.G., B.J.H and H.D.D. conceived the experiments. C.P.P., C.M.R., B.A.M.,

712 J.B.G., L.C.M., Y.C. and A.J.C. performed sample collections and RNA sequencing

713 experiments. S.B., I.M.M., M.K. and H.D.D. provided data and assisted with study design.

33

714   R.X., B.J.H., C.J.V., A.J.C., M.E.G, P.J.B and Z.Y. analysed data. R.X. and M.E.G. wrote the

715   paper. R.X., M.E.G., B.J.H, A.J.C., I.M.M and H.D.D.. revised the paper. All authors read

716   and approved the final manuscript.

717

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Reference

723   1.   Bourneuf E, Otz P, Pausch H, Jagannathan V, Michot P, Grohs C, Piton G, Ammermüller S,
724   Deloche M-C, Fritz S: **Rapid Discovery of De Novo Deleterious Mutations in Cattle**
725   **Enhances the Value of Livestock as Model Species.** *Sci Rep* 2017, **7**.
726   2.   Hayes BJ, Lewin HA, Goddard ME: **The future of livestock breeding: genomic selection for**
727   **efficiency, reduced emissions intensity, and adaptation.** *Trends Genet* 2013, **29:**206-214.
728   3.   Meuwissen T, Hayes B, Goddard M: **Genomic selection: A paradigm shift in animal**
729   **breeding.** *Animal frontiers* 2016, **6:**6-14.
730   4.   Andersson L, Archibald AL, Bottema CD, Brauning R, Burgess SC, Burt DW: **Coordinated**
731   **international action to accelerate genome-to-phenome with FAANG, the Functional**
732   **Annotation of Animal Genomes project.** *Genome Biol* 2015, **16**.
733   5.   Albert FW, Kruglyak L: **The role of regulatory variation in complex traits and disease.**
734   *Nature reviews Genetics* 2015, **16:**197.
735   6.   Schaub MA, Boyle AP, Kundaje A, Batzoglou S, Snyder M: **Linking disease associations**
736   **with regulatory information in the human genome.** *Genome Res* 2012, **22:**1748-1759.
737   7.   Bouwman AC, Daetwyler HD, Chamberlain AJ, Ponce CH, Sargolzaei M, Schenkel FS,
738   Sahana G, Govignon-Gion A, Boitard S, Dolezal M, et al: **Meta-analysis of genome-wide**
739   **association studies for cattle stature identifies common genes that regulate body size in**
740   **mammals.** *Nat Genet* 2018, **50:**362-367.
741   8.   Consortium G: **The Genotype-Tissue Expression (GTEx) pilot analysis: Multitissue gene**
742   **regulation in humans.** *Science* 2015, **348:**648-660.
743   9.   Li YI, van de Geijn B, Raj A, Knowles DA, Petti AA, Golan D, Gilad Y, Pritchard JK: **RNA**
744   **splicing is a primary link between genetic variation and disease.** *Science* 2016, **352:**600-604.
745   10.   Li YI, Knowles DA, Humphrey J, Barbeira AN, Dickinson SP, Im HK, Pritchard JK:
746   **Annotation-free quantification of RNA splicing using LeafCutter.** *Nat Genet* 2018, **50:**151.
747   11.   Consortium G: **Genetic effects on gene expression across human tissues.** *Nature* 2017,
748   **550:**204.
749   12.   Zhernakova DV, Deelen P, Vermaat M, van Iterson M, van Galen M, Arindrarto W, van't Hof P,
750   Mei H, van Dijk F, Westra H-J: **Identification of context-dependent expression quantitative**
751   **trait loci in whole blood.** *Nat Genet* 2017, **49:**139-145.

752   13.   Mazzoni G, Kadarmideen HN: **Computational Methods for Quality Check, Preprocessing**
753   **and Normalization of RNA-Seq Data for Systems Biology and Analysis.** In *Systems Biology*
754   *in Animal Production and Health, Vol 2.* Springer; 2016: 61-77

755   14.   Okonechnikov K, Conesa A, García-Alcalde F: **Qualimap 2: advanced multi-sample quality**
756   **control for high-throughput sequencing data.** *Bioinformatics* 2015, **32:**292-294.

757   15.   Wang L, Wang S, Li W: **RSeQC: quality control of RNA-seq experiments.** *Bioinformatics*
758   2012, **28:**2184-2185.

759   16.   Kim D, Langmead B, Salzberg SL: **HISAT: a fast spliced aligner with low memory**
760   **requirements.** *Nat Methods* 2015, **12:**357.

761   17.   Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M,
762   Gingeras TR: **STAR: ultrafast universal RNA-seq aligner.** *Bioinformatics* 2013, **29:**15-21.

763   18.   Kim D, Pertea G, Trapnell C, Pimentel H, Kelley R, Salzberg SL: **TopHat2: accurate**
764   **alignment of transcriptomes in the presence of insertions, deletions and gene fusions.**
765   *Genome Biol* 2013, **14:**R36.

766   19.   Daetwyler HD, Capitan A, Pausch H, Stothard P, Van Binsbergen R, Brøndum RF, Liao X,
767   Djari A, Rodriguez SC, Grohs C: **Whole-genome sequencing of 234 bulls facilitates mapping**
768   **of monogenic and complex traits in cattle.** *Nat Genet* 2014, **46:**858-865.

769   20.   Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R:
770   **The Sequence Alignment/Map format and SAMtools.** *Bioinformatics* 2009, **25:**2078-2079.

771   21.   Pausch H, MacLeod IM, Fries R, Emmerling R, Bowman PJ, Daetwyler HD, Goddard ME:
772   **Evaluation of the accuracy of imputed sequence variant genotypes and their utility for**
773   **causal variant detection in cattle.** *Genet Sel Evol* 2017, **49:**24.

774   22.   Fox J, Weisberg S: *An R companion to applied regression.* Sage Publications; 2011.

775   23.   Cánovas A, Rincón G, Bevilacqua C, Islas-Trejo A, Brenaut P, Hovey RC, Boutinaud M,
776   Morgenthaler C, VanKlompenberg MK, Martin P: **Comparison of five different RNA sources**
777   **to examine the lactating bovine mammary gland transcriptome using RNA-Sequencing.**
778   *Sci Rep* 2014, **4**.

779   24.   Chamberlain AJ, Vander Jagt CJ, Hayes BJ, Khansefid M, Marett LC, Millen CA, Nguyen TTT,
780   Goddard ME: **Extensive variation between tissues in allele specific expression in an**
781   **outbred mammal.** *BMC Genomics* 2015, **16:**993.

782   25.   Li YI, Knowles DA, Pritchard JK: **LeafCutter: Annotation-free quantification of RNA**
783   **splicing.** *bioRxiv* 2016:044107.

784   26.   MacLeod I, Bowman P, Vander Jagt C, Haile-Mariam M, Kemper K, Chamberlain A,
785   Schrooten C, Hayes B, Goddard M: **Exploiting biological priors and sequence variants**
786   **enhances QTL discovery and genomic prediction of complex traits.** *BMC Genomics* 2016,
787   **17:**1.

788   27.   Kemper KE, Reich CM, Bowman PJ, vander Jagt CJ, Chamberlain AJ, Mason BA, Hayes BJ,
789   Goddard ME: **Improved precision of QTL mapping using a nonlinear Bayesian method in**
790   **a multi-breed population leads to greater accuracy of across-breed genomic predictions.**
791   *Genet Sel Evol* 2015, **47:**1.

792   28.   McLaren W, Gil L, Hunt SE, Riat HS, Ritchie GR, Thormann A, Flicek P, Cunningham F: **The**
793   **Ensembl Variant Effect Predictor.** *Genome Biol* 2016, **17:**1.

794   29.   Grant JR, Arantes AS, Liao X, Stothard P: **In-depth annotation of SNPs arising from**
795   **resequencing projects using NGS-SNP.** *Bioinformatics* 2011, **27:**2300-2301.

796   30.   **National Center for Biotechnology Information (NCBI)** [https://www.ncbi.nlm.nih.gov/]

797   31.   Littlejohn MD, Tiplady K, Fink TA, Lehnert K, Lopdell T, Johnson T, Couldrey C, Keehan M,
798   Sherlock RG, Harland C: **Sequence-based Association Analysis Reveals an MGST1 eQTL**
799   **with Pleiotropic Effects on Bovine Milk Composition.** *Sci Rep* 2016, **6**.

800   32.   Xiang R, MacLeod I, Bolormaa S, Goddard M: **Genome-wide comparative analyses of**
801   **correlated and uncorrelated phenotypes identify major pleiotropic variants in dairy cattle.**
802   *Sci Rep* 2017, **7:**9248.

803   33.   Bolormaa S, Pryce JE, Reverter A, Zhang Y, Barendse W, Kemper K, Tier B, Savin K, Hayes
804   BJ, Goddard ME: **A multi-trait, meta-analysis for detecting pleiotropic polymorphisms for**
805   **stature, fatness and reproduction in beef cattle.** *PLoS Genet* 2014, **10:**e1004198.

35

806   34.   McClure M, Ramey H, Rolf M, McKay S, Decker J, Chapple R, Kim J, Taxis T, Weaber R,
807   Schnabel R: **Genome-wide association analysis for quantitative trait loci influencing**
808   **Warner−Bratzler shear force in five taurine cattle breeds.** *Anim Genet* 2012, **43:**662-673.
809   35.   Kühn C, Freyer G, Weikard R, Goldammer T, Schwerin M: **Detection of QTL for milk**
810   **production traits in cattle by application of a specifically developed marker map of BTA6.**
811   *Anim Genet* 1999, **30:**333-339.
812   36.   Glantz M, Gustavsson F, Bertelsen HP, Stålhammar H, Lindmark-Månsson H, Paulsson M,
813   Bendixen C, Gregersen VR: **Bovine chromosomal regions affecting rheological traits in**
814   **acid-induced skim milk gels.** *J Dairy Sci* 2015, **98:**1273-1285.
815   37.   Takata A, Matsumoto N, Kato T: **Genome-wide identification of splicing QTLs in the**
816   **human brain and their enrichment among schizophrenia-associated loci.** *Nature*
817   *Communications* 2017, **8:**14519.
818   38.   Guan L, Yang Q, Gu M, Chen L, Zhang X: **Exon expression QTL (eeQTL) analysis**
819   **highlights distant genomic variations associated with splicing regulation.** *Quantitative*
820   *Biology* 2014, **2:**71-79.
821   39.   Flutre T, Wen X, Pritchard J, Stephens M: **A statistical framework for joint eQTL analysis**
822   **in multiple tissues.** *PLoS Genet* 2013, **9:**e1003486.
823   40.   Thompson JA, Tan J, Greene CS: **Cross-platform normalization of microarray and RNA-**
824   **seq data for machine learning applications.** *PeerJ* 2016, **4:**e1621.
825   41.   Ongen H, Dermitzakis ET: **Alternative Splicing QTLs in European and African**
826   **Populations.** *Am J Hum Genet* 2015, **97:**567-575.
827   42.   Chen Y, Gondro C, Quinn K, Herd R, Parnell P, Vanselow B: **Global gene expression**
828   **profiling reveals genes expressed differentially in cattle with high and low residual feed**
829   **intake.** *Anim Genet* 2011, **42:**475-490.
830   43.   Khansefid M, Millen CA, Chen Y, Pryce JE, Chamberlain AJ, Vander Jagt CJ, Gondro C,
831   Goddard ME: **Gene expression analysis of blood, liver, and muscle in cattle divergently**
832   **selected for high and low residual feed intake.** *J Anim Sci* 2017, **95:**4764-4775.
833   44.   Sargolzaei M, Chesnais JP, Schenkel FS: **A new approach for efficient genotype imputation**
834   **using information from relatives.** *BMC Genomics* 2014, **15**.
835   45.   Erbe M, Hayes B, Matukumalli L, Goswami S, Bowman P, Reich C, Mason B, Goddard M:
836   **Improving accuracy of genomic predictions within and between dairy cattle breeds with**
837   **imputed high-density single nucleotide polymorphism panels.** *J Dairy Sci* 2012, **95:**4114-
838   4129.
839   46.   Anders S, Pyl PT, Huber W: **HTSeq—a Python framework to work with high-throughput**
840   **sequencing data.** *Bioinformatics* 2015, **31:**166-169.
841   47.   Liao Y, Smyth GK, Shi W: **featureCounts: an efficient general purpose program for**
842   **assigning sequence reads to genomic features.** *Bioinformatics* 2013, **30:**923-930.
843   48.   Team RC: **R: A language and environment for statistical computing.** 2013.
844   49.   Love MI, Huber W, Anders S: **Moderated estimation of fold change and dispersion for**
845   **RNA-seq data with DESeq2.** *Genome Biol* 2014, **15:**550.
846   50.   Wickham H: *ggplot2: elegant graphics for data analysis.* Springer; 2016.
847   51.   Degner JF, Pai AA, Pique-Regi R, Veyrieras J-B, Gaffney DJ, Pickrell JK, de Leon S,
848   Michelini K, Lewellen N, Crawford GE: **DNase I sensitivity QTLs are a major determinant**
849   **of human expression variation.** *Nature* 2012, **482:**390-394.
850   52.   Bates D, Mächler M, Bolker B, Walker S: **Fitting Linear Mixed-Effects Models Using lme4.**
851   *Journal of Statistical Software* 2015, **67:**48.
852   53.   Kuznetsova A, Brockhoff PB, Christensen RHB: **lmerTest Package: Tests in Linear Mixed**
853   **Effects Models.** *Journal of Statistical Software* 2017, **82:**26.
854   54.   Bass AJ, Dabney A, Robinson D: **qvalue: Q-value estimation for false discovery rate control.**
855   **R package version 2.9.0, http://github.com/jdstorey/qvalue.**, vol. 2017; 2015.
856   55.   Eden E, Navon R, Steinfeld I, Lipson D, Yakhini Z: **GOrilla: a tool for discovery and**
857   **visualization of enriched GO terms in ranked gene lists.** *BMC Bioinformatics* 2009, **10:**48.
858   56.   Shabalin AA: **Matrix eQTL: ultra fast eQTL analysis via large matrix operations.**
859   *Bioinformatics* 2012, **28:**1353-1358.

36

860   57.   Law CW, Chen Y, Shi W, Smyth GK: **Voom: precision weights unlock linear model analysis**
861   **tools for RNA-seq read counts.** *Genome Biol* 2014, **15:**R29.
862   58.   Shen L: **GeneOverlap: An R package to test and visualize gene overlaps.** 2014.
863   59.   Yang J, Lee SH, Goddard ME, Visscher PM: **GCTA: a tool for genome-wide complex trait**
864   **analysis.** *Am J Hum Genet* 2011, **88:**76-82.
865   60.   Bolormaa S, Hayes BJ, van der Werf JH, Pethick D, Goddard ME, Daetwyler HD: **Detailed**
866   **phenotyping identifies genes with pleiotropic effects on body composition.** *BMC Genomics*
867   2016, **17:**1.

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Additional files:

Additional file 1 (DOCX): Supplementary Methods.

Additional file 2 (PDF): Supplementary Figure S1-7.

Additional file 3 (XLSX): Supplementary Table S1. RNA-seq reads mapped to different bovine genome origins.

Additional file 4 (XLSX): Supplementary Table S2. Splicing junction annotation analysis using BAM files generated by different alignment software.

Additional file 5 (XLSX): Supplementary Table S3. 2x2 tables for the overlap between exon and intron analyses.

Additional file 6 (XLSX): Supplementary Table S4. Genes that display significant differential splicing cross tissues.

Additional file 7 (XLSX): Supplementary Table S5. Genes that display significant differential splicing between breeds.

Additional file 8 (XLSX): Supplementary Table S6. Summary of significant cis splicing sQTLs (within 1Mb distance to the exon).

Additional file 9 (XLSX): Supplementary Table S7. Summary table for count values of sQTLs and eeQTLs in white blood and milk cells.

Additional file 10 (XLSX): Supplementary Table S8. SNP overlap between expression QTLs and SNPs with pleiotropic effects on conventional traits of dairy cattle.

Additional file 11 (XLSX): Supplementary Table S9. SNP overlap between expression QTLs and SNPs with pleiotropic effects on conventional traits of beef cattle.

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Additional file 12 (XLSX): Supplementary Table S10. SNP overlap between blood eeQTLs and putative causal variants identified by Littlejohn et al (2016).

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Figure Legends:

Figure 1. **a**: Sample principal components clustering based on exon expression. Circles on the plot were ellipses drawn based on tissue types at the confidence interval = 0.95. Tissue types with which the non-overlapping ellipses were drawn were emphasised with underscored text labelling. Ellipses that were drawn based on experiments can be found in Supplementary Figure S4. **b**: The significant splicing events between breeds and between genotypes (cis splicing quantitative trait loci, sQTLs) for *CSN3* in the milk cell transcriptome. In the upper panel, from left to right: the 1st pair of bars are the least square means of normalised expression level of the gene (ENSBTAG00000039787) in Holstein and Jersey breeds; the 2nd pair of bars are the normalised expression level of the 5th exon (6:87392578-87392750) in Holstein and Jersey breeds; the 3rd pair of bars are the normalised inclusion ratio of the 5th exon in Holstein and Jersey breeds; and 4th pair of bars is the frequency of the B allele of the sQTL (Chr6:87392580) for *CSN3* in Holstein and Jersey breeds. The standard errors bars are indicated. In the lower panel, from left to right: the 1st bar is the effects (signed t values, b/se) of the sQTL (Chr6:87392580) B allele on the normalised expression of the gene; the 2nd bar is the sQTL B allele effect on the normalised expression of the 5th exon; and the 3rd bar was the sQTL B allele effects on the normalised inclusion ratio of the 5th exon.

Figure 2. Manhattan plots of significant cis splicing quantitative trait loci (sQTLs, approximate FDR<0.01 and within 1Mb of the exon) in white blood cells (**a**), milk cells (**b**), liver tissue (**c**) and muscle tissue (**d**). A significant sQTLs was defined as a SNP associated with the variation in the exon inclusion ratio and also variation in at least one excision of an adjacent intron at the same significance level. The input SNPs had significance p<0.0001.

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sQTLs in all tissues with their associated genes and significance are given in Supplementary Table S6.

Figure 3. Features of cis splicing quantitative trait loci (sQTLs) compared to exon expression QTL (eeQTLs) and gene expression QTL (geQTLs). **a**: The distance between the transcription start site (TSS) and the expression QTLs. TSS information was downloaded from Ensembl (bovine reference UMD3.1). **b**: The proportion of expression QTLs annotated as splice, UTR, gene_end, synonymous, missense, intron, intergenic or other. SNP annotations were based on Variant Effect Predictor. 'Splice' included all SNP annotations containing the word 'splice'. 'UTR' included 3' and 5' untranslated region. 'Gene_end' included upstream and downstream.

Figure 4. Overlaps of different expression QTL types for white blood cells (**a**), milk cells (**b**), liver(**c**) and muscle (**d**). Within each panel, y-axis was the number of significant expression QTLs; from left to right as guided by the green dots, the 1st bar indicated the number of significant cis splicing QTL (sQTLs); the 2nd bar indicated the number of significant exon expression QTL (eeQTLs); the 3rd bar indicated the number of significant gene expression QTL (geQTLs); the 4th bar indicated the number of SNPs identified as both geQTL and eeQTL; the 5th bar indicated the number of SNPs identified as both geQTL and sQTL; the 6th bar indicated the number of SNPs identified as both eeQTL and sQTL; and the 7th bar indicated the number of SNPs identified as geQTL and eeQTL and sQTL. The red colour indicates that the overlap between categories of expression QTLs was significantly more than expected by random chance based on Fisher's exact test.

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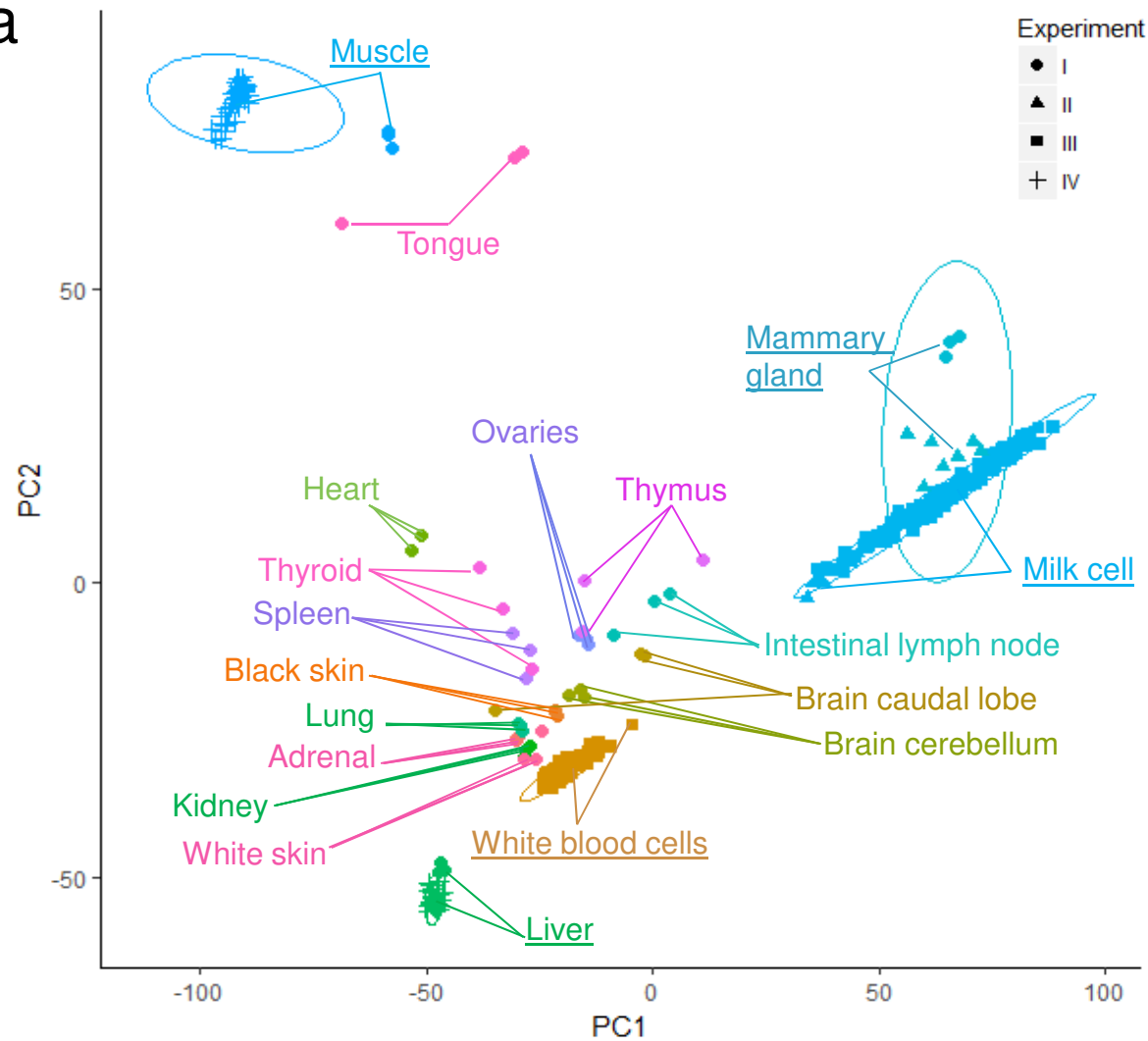**Figure 5.** Shared genetic influence on the splicing, exon and gene expression between tissues. Blood refers to white blood cells and milk refers to milk cells. **a**: Each matrix shows the pair wise comparison of the numbers of significant SNP and the total number of significant SNPs detected for each analysis shown in parentheses. The significance of each overlap was tested by Fisher' exact test, given the total number of SNP analysed and the total number of significant SNP, the result of which is represented by the colour of that position in the matrix. **b**: Each matrix shows the pair wise comparison of the numbers of exon/gene with significant associations and the total number of exon/gene detected with significant associations for each analysis shown in parentheses. In panel **b**, the numbers were either exon numbers for sQTLs (splicing quantitative trait loci) and eeQTLs (exon expression quantitative trait loci) or gene numbers for geQTLs (gene expression quantitative trait loci. **c**: Between tissue genetic correlations of either the inclusion ratio of the exons, the expression of the exons or the expression of the genes that had significant sharing of expression QTLs in panel **a.** Dot size and transparency were negatively correlated with p value of the significance of the genetic correlation being different from 0. The error bars of the genetic correlation were shown in vertical lines of each dot. Some genes of interests were highlighted.

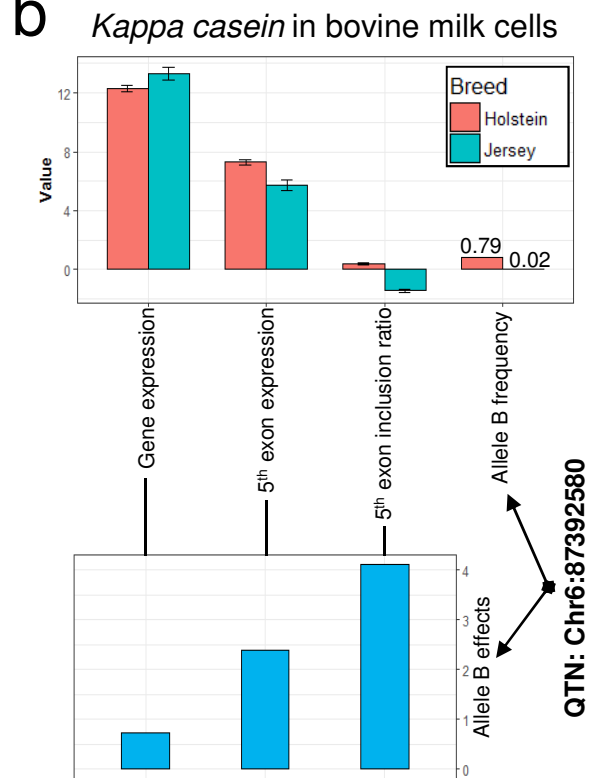**Figure 6.** Multi-transcriptome meta-analysis (blood, milk and muscle) for cis splicing sQTLs (**a**), exon expression eeQTLs (**b**) and gene expression geQTLs (**c**). In each panel, the significance of multi-transcriptome effects were tested against a χ^2 with 1 degree of freedom for combined expression QTLs effects (dots in blue and orange). These multi-transcriptome effects were shown together with the single-transcriptome effects in liver of the same expression QTLs (dots in green).
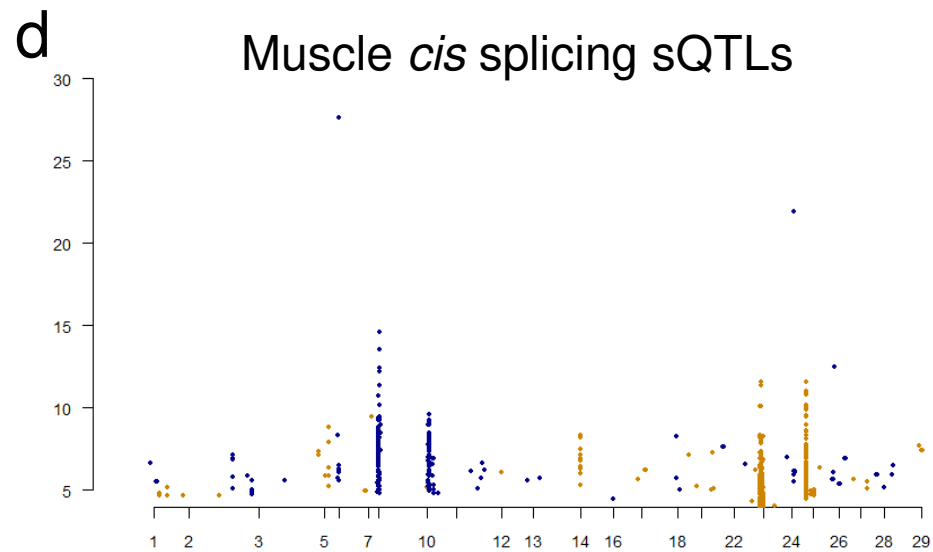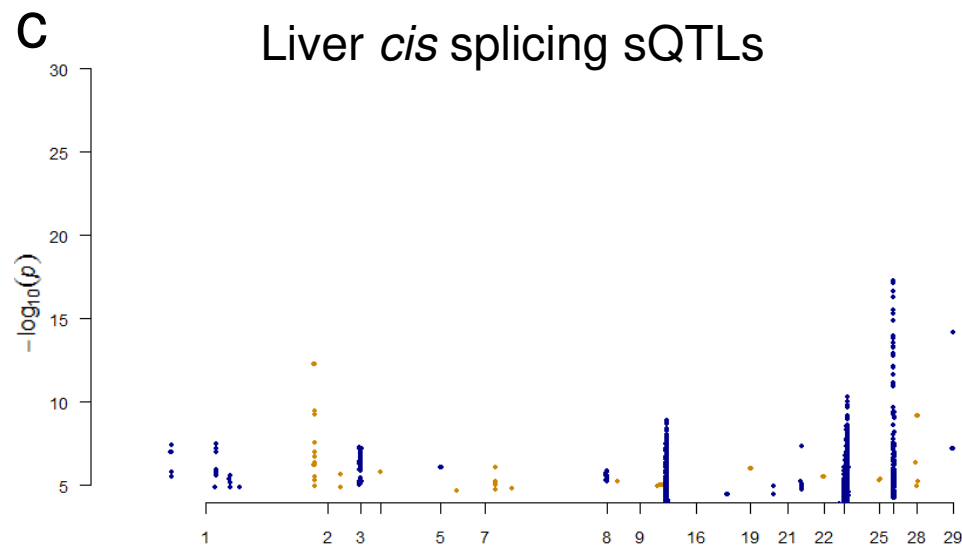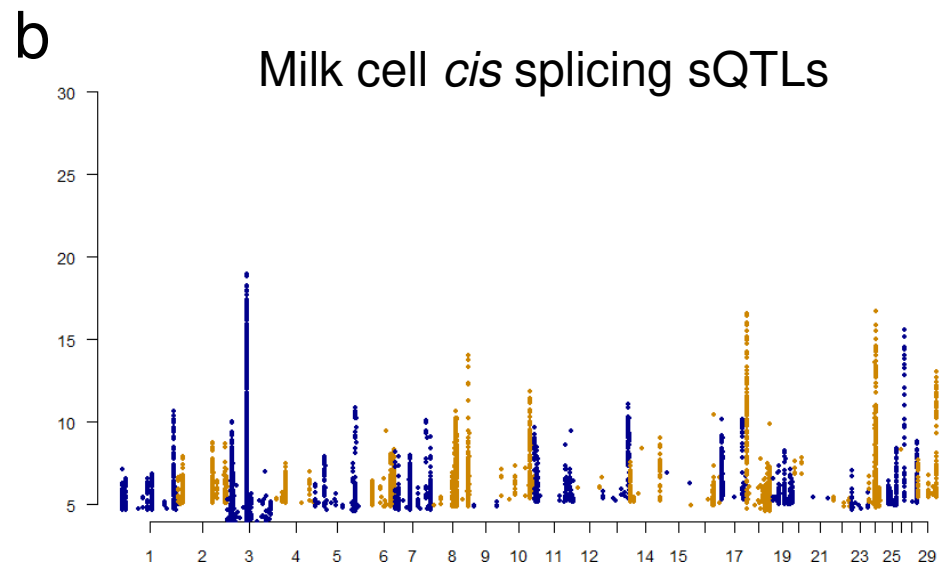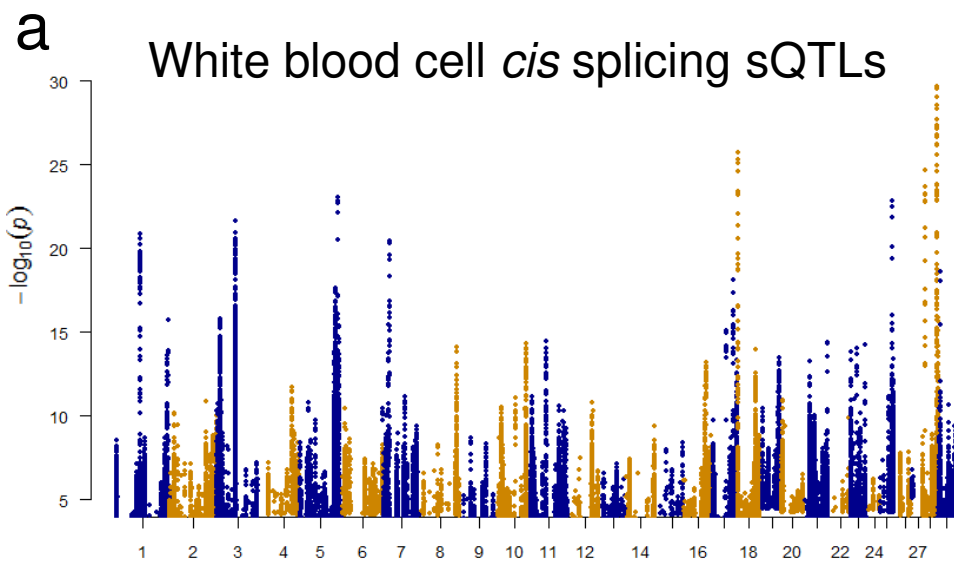
Figure 7. Significance of the overlap, based on the Fisher's exact test, between pleiotropic

QTL for a range of traits in cattle for dairy(**a**) and beef (**b**) and cis splicing quantitative trait

loci (sQTLs), exon expression QTL (eeQTLs) and gene expression QTL (geQTLs) in all

tissues, where the colour represents the significance of the overlap. Where blood refers to

white blood cells and milk refers to milk cells. Significance of the overlap was based on the

Fisher's exact test. Only chromosomes containing overlapping SNPs are shown. **c**) An

example of *MGST1* showing the relationship between QTL effects on exon expression in

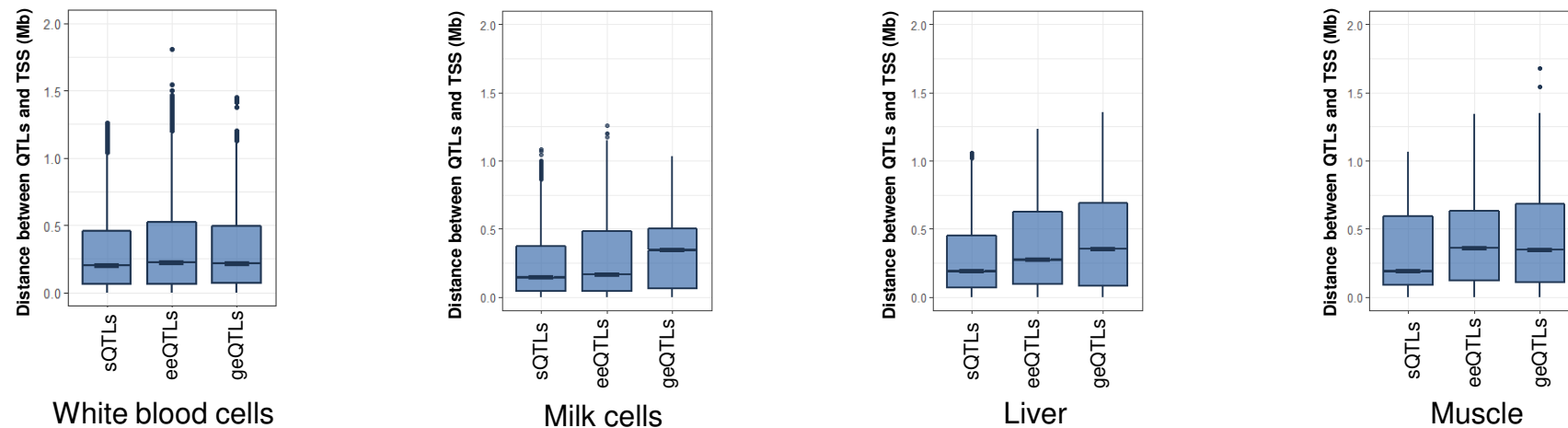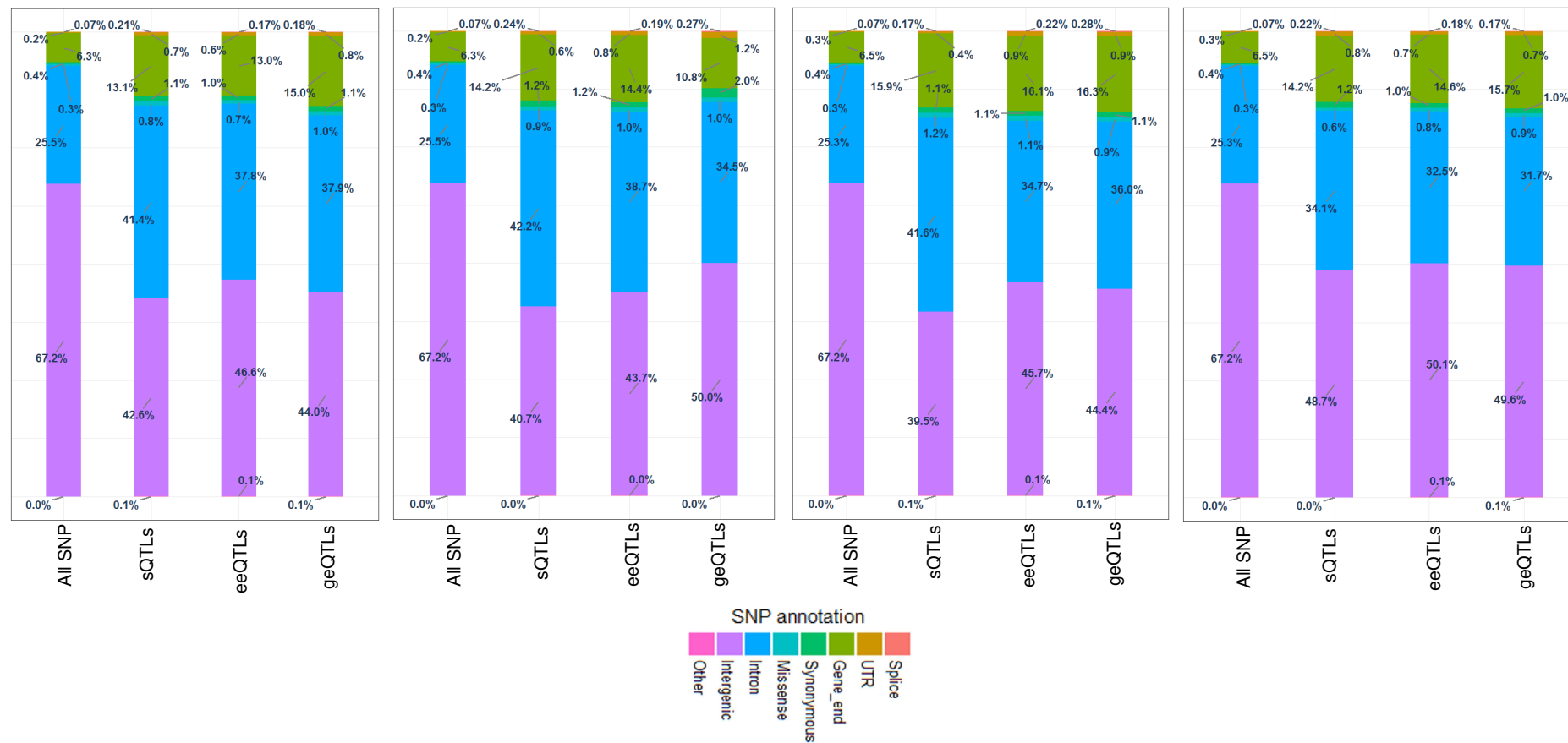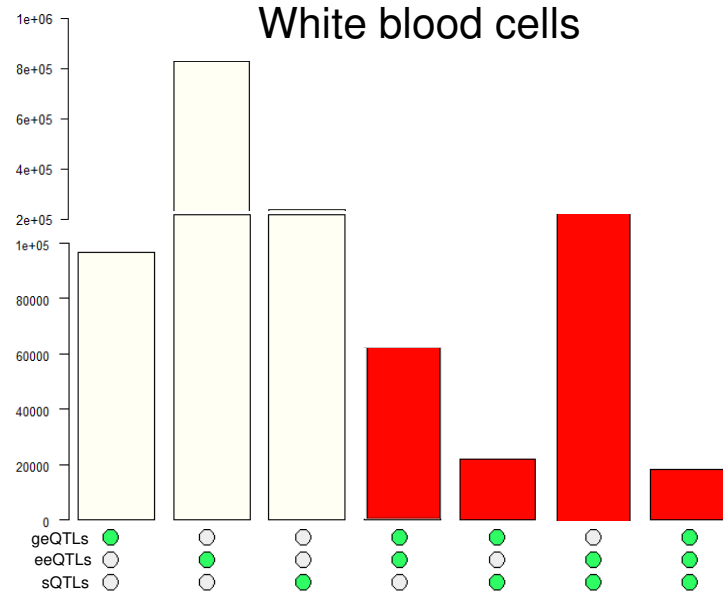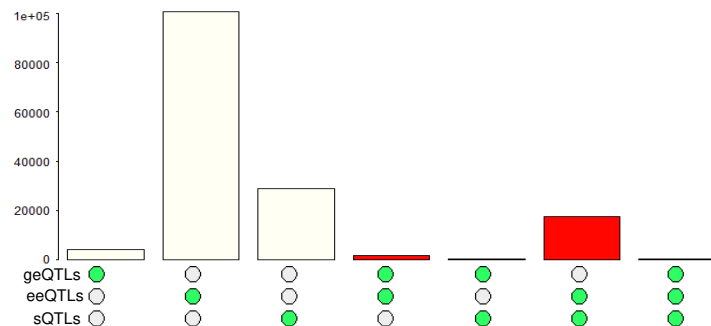milk cells and their effects on dairy cattle milk fat yield.

a   White blood cell *cis* splicing sQTLs

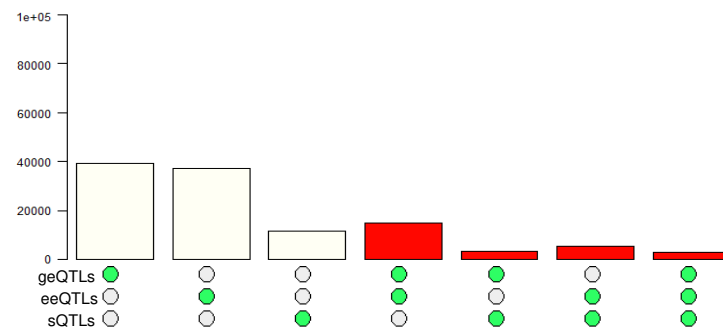b   Milk cell *cis* splicing sQTLs

c   Liver *cis* splicing sQTLs

d   Muscle *cis* splicing sQTLs

a. White blood cells

b. Milk cells

c. Liver

d. Muscle

a   sQTLs

→ Multi-transcriptome effects
→ Single-transcriptome effects

b   eeQTLs

→ Multi-transcriptome effects
→ Single-transcriptome effects

c   geQTLs

→ Multi-transcriptome effects
→ Single-transcriptome effects

a

Chr	2	3	5	6	7	9	10	11	13	14	15	17	18	19	20	21	23	25	29	
																				Blood.sQTLs, p=2e-17
																				Blood.eeQTLs, p=7e-15
																				Blood.geQTLs, p=1e-20
																				Milk.sQTLs, p=2e-07
																				Milk.eeQTLs, p=4e-17
																				Milk.geQTLs, p=1e+00
																				Liver.sQTLs, p=1e+00
																				Liver.eeQTLs, p=1e-03
																				Liver.geQTLs, p=1e+00
																				Muscle.sQTLs, p=1e+00
																				Muscle.eeQTLs, p=1e+00
																				Muscle.geQTLs, p=1e+00

Dairy cattle pleiotropic SNPs

b

Chr:	2	3	5	6	7	8	11	13	14	16	17	18	19	20	23	25	29	
																		Blood.sQTLs, p=1e+00
																		Blood.eeQTLs, p=3e-02
																		Blood.geQTLs, p=1e+00
																		Milk.sQTLs, p=1e-01
																		Milk.eeQTLs, p=4e-01
																		Milk.geQTLs, p=1e+00
																		Liver.sQTLs, p=1e+00
																		Liver.eeQTLs, p=1e+00
																		Liver.geQTLs, p=1e+00
																		Muscle.sQTLs, p=1e+00
																		Muscle.eeQTLs, p=8e-01
																		Muscle.geQTLs, p=1e+00

Beef cattle pleiotropic SNPs

c

MGST1 eeQTLs in milk

Effects on milk fat yield (y-axis)
Effects on exon expression in milk (x-axis)

exon1
exon2
exon3
exon4

$r = 0.72$
$p < 2.2e\text{-}16$

log2 SNP count
8
6
4
2
0