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SNP rs403212791 in exon 2 of the *MTNR1A* gene is associated with reproductive seasonality in the Rasa aragonesa sheep breed.

Running title: *MTNR1A* in Reproductive Seasonality

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Abstract

The aim of this study was to characterize and identify causative SNPs in the *MTNR1A* gene responsible for the reproductive seasonality traits in the Rasa aragonesa sheep breed. A total of 290 ewes (155, 84 and 51 mature, young and ewe lambs, respectively) from one flock were controlled from January to August. The following three reproductive seasonality traits were considered: the total days of anoestrus (TDA) and the progesterone cycling months (P4CM); both ovarian function seasonality traits based on blood progesterone levels; and the oestrus cycling months (OCM) based on oestrous detection, which indicate behavioural signs of oestrous. We have sequenced the total coding region plus 733 and 251 bp from the promoter and 3'-UTR regions, respectively, from the gene in 268 ewes. We found 9 and 4 SNPs associated with seasonality traits in the promoter (for TDA and P4CM) and exon 2 (for the three traits), respectively. The SNPs located in the gene promoter modify the putative binding sites for various trans-acting factors. In exon 2, two synonymous SNPs affect RFLP sites, rs406779174/RsaI (for the three traits) and rs430181568/MnlI (for OCM), and they have been related with seasonal reproductive activity in previous association studies with other breeds. SNP rs400830807, which is located in the 3'-UTR, was associated with the three traits, but this did not modify the putative target sites for ovine miRNAs according to *in silico* predictions. Finally, the SNP rs403212791 (NW_014639035.1: g.15099004G>A), which is also associated with the three seasonality phenotypes, was the most significant SNP detected in this study and was a non-synonymous polymorphism, leading a

change from an Arginine to a Cysteine (R336C). Haplotype analyses confirmed the association results and showed that the effects found for the seasonality traits were caused by the SNPs located in exon 2. We have demonstrated that the T allele in the SNP rs403212791 in the *MNTR1A* gene is associated with a lower TDA and higher P4CM and OCM values in the Rasa Aragonesa breed.

Keywords: sheep**,** oestrous behaviour, ovulatory activity, *MTNR1A*, SNP

1. Introduction

Rasa Aragonesa is an autochthonous Mediterranean sheep breed from northeastern Spain that is mainly reared in extensive or semi-extensive farming systems and oriented for meat production. Improvements in efficiency on farms are made possible by exploiting changes in genetics, nutrition and the management of an ewe flock. In this sense, the Cooperative Oviaragon-Grupo Pastores carries out a selection programme for prolificacy in Rasa Aragonesa sheep that began in 1994, currently includes 490,337 ewes because the number of lambs born per ewe plays a key role in the efficiency and viability of these farms [1]. The annual lambing rate can also be increased by getting a higher proportion of ewes to breed out-of-season. Sheep breeds from the Mediterranean area have a marked seasonality for breeding activities, showing seasonal oestrous behaviours and ovulation patterns. The maximal reproductive activity is associated with short days, with the highest percentage of ewes exhibiting ovulatory activities from August to March. This reproductive seasonality causes a seasonal fluctuation in lamb market prices, with the lowest

prices being when the lamb supply is the highest (late spring to early fall). Several methods to control the reproduction of sheep have been used such as environmental manipulation (light control), the sudden introduction of rams, which induces oestrus in ewes (ram effect), or other methods based on the administration of exogenous hormones. However, the increasing demand for hormone-free products and the evolution of European rules and directives towards a reduction in or even complete cessation of the use of exogenous hormones has led to the search for alternative methods, such as light control, the ram effect or the use of genetic markers. Spring ovulatory activities have heritability and repeatability values of 0.20 and 0.30, respectively [2], but they are only measured in females, are exhibited relatively late in an ewe's life and are only present in some management systems. Thus, the use of genetic markers would be a powerful tool in selection programmes. Only two candidate genes related to reproductive seasonality traits have been successfully identified, including *melatonin receptor subtype 1A* (*MTNR1A*) [3-13], and the *arylalkylamine N-acetyltransferase* (*AANAT*) [14]. Arylalkylamine N-acetyltransferase is involved in the biosynthesis of melatonin and controls daily changes in melatonin production. Melatonin acts through high-affinity G-protein coupled receptors, one of which is melatonin receptor 1A encoded by the *MTNR1A* gene. *MTNR1A* has been characterized in several breeds as a candidate gene and appears to play a key role in the control of photoperiod-induced seasonality mediated by circadian melatonin concentrations [15-16]. However, *MTNR1A* genotypes did not show an association with reproductive seasonality in Ile de France and Cornell flocks (composed of East Friesian Cross, Dorset, Finnsheep × Dorset, and Finnsheep ewes) [17-18], suggesting

99 that the effects of *MTNR1A* polymorphisms may depend on the genetic
100 background and/or environmental conditions. Furthermore, previous studies
101 have associated two polymorphic RFLP sites (606/RsaI and 612/MnlI) within the
102 *MTNR1A* coding sequence with reproductive activity. However, since these
103 SNPs are synonymous, they are not causative mutations, indicating that other
104 polymorphisms in linkage disequilibrium or regulatory sequences in the
105 *MNTR1A* gene could be influencing the ability to breed out of season. Genetic
106 mapping of quantitative trait loci (QTL) and a genome-wide association study
107 (GWAS) for aseasonal reproduction in sheep using microsatellites and SNPs,
108 respectively, revealed several chromosomes and SNPs that could be implicated
109 in this trait [19-20].
110 Therefore, the main objective of this study was to identify causative SNPs
111 responsible for reproductive seasonality traits in the Rasa aragonesa sheep
112 breed. Polymorphisms were detected and characterized from the entire
113 *MTNR1A* gene coding region and promoter. Then, an association study was
114 performed between all the polymorphisms detected and the three reproductive
115 seasonality traits.
116

117 **2. Material and Methods**

118 *2.1 Ethics statement*

119 All experimental procedures were performed in accordance with the guidelines
120 of the European Union (2003/65/CE) and Spanish regulations (RD 1201/2005,
121 BOE 252/34367-91) for the use and care of animals in research. No hormonal
122 treatments were applied to ewes during the study.

123 *2.2 Animal resources*

124 Phenotypic seasonality data were obtained from a Rasa aragonesa sheep flock
125 managed at an experimental farm (“Pardina de Ayés”) owned by Oviaragón
126 S.C.L., located in the Pre-Pyrenees (Ayés, Sabiñánigo, Huesca; North-Eastern
127 Spain, 42° 29' 48.55" N 0° 23' 37.54", 790 m above sea level) and described in
128 Martinez-Royo et al. [20]. The experimental period lasted from January to
129 August in 2012. The flock was composed of 290 ewes in the following three age
130 groups: mature (5.2-7.2 y, n=155; 5.5 ± 0.5; mean ± SD), young (all: 1.9 y,
131 n=84) and, ewe lambs (all: 0.94 y; n=51) at the beginning of the experiment.
132 Their individual live weight (LW) and body condition score (BCS) on a 1 to 5
133 scale [21] were assessed every three weeks. Mean LW and BCS values were
134 similar in mature and young ewes age groups. Pooled overall means and
135 standard deviations for the entire experimental period were 52.5 ± 7.7 kg and
136 2.9 ± 0.3 for LW and BCS, respectively, for mature and young ewes. However,
137 ewe lambs had an LW and BCS of 40.6 ± 3.8 kg and 2.8 ± 0.1, respectively.
138 Management of the ewes is described in Martinez-Royo et al.[20]. The ewes
139 were handled in a single lot and subjected to the same management, nutrition
140 and environmental conditions.

141 *2.3 Measurement of reproductive seasonality traits*

142 Three reproductive seasonality traits were considered and described in
143 Martinez-Royo et al. [20]. Briefly, the first was the total days of anoestrus (TDA)
144 based on weekly individual plasma progesterone levels. TDA was the sum of
145 the days in anoestrus, considering anoestrus as periods with three or more
146 consecutive progesterone concentrations lower than 0.5 ng/ml. Likewise, ewes
147 were not considered for this study if they were not cycling in the preceding

148 breeding season (based on three samples one week apart taken in October),
149 had progesterone levels below the threshold in all samples taken in January,
150 and had more than 4 consecutive samples higher than or equal to the threshold
151 (possible pathological ewes).
152 The second reproductive seasonality trait was the progesterone cycling months
153 (P4CM), which was defined for each ewe as the rate of cycling months based
154 on progesterone determination. When progesterone levels were higher than or
155 equal to 0.5 ng/ml in at least one blood sample in the month, an ewe was
156 considered cyclic.
157 Finally, the third reproductive seasonality trait considered was the oestrus
158 cycling months (OCM), which is defined as the rate of months cycling based on
159 daily oestrous records for each ewe. Eight vasectomised rams fitted with
160 harnesses and marking crayons were mixed with the ewes, and daily oestrous
161 detection was examined [22]. Thus, after natural mating, oestrus was recorded
162 as a coloured mark on the ewes' rump.

163 *2.3 Sampling and genotyping*
164 Genomic DNA was extracted from blood samples from 268 ewes (138, 79 and
165 51 mature, young and ewe lambs, respectively) from the total ewes in the flock
166 (n=290) using the FlavorPrep Genomic DNA mini kit (Flavorgen, Ibian,
167 Zaragoza, Spain). Twenty-two ewes were not considered because of missing
168 data for some variables. Direct Sanger sequencing of the PCR products from all
169 the ewes (n=268) was used to search for genotype polymorphisms in the
170 experimental population. Primers were used to amplify the total coding, 5'-UTR,
171 partial 3'-UTR, and promoter genomic regions in the *MNTR1A* gene using three
172 different PCR reactions (Supplementary Table 1). Genomic DNA (50 ng) was

amplified in a final PCR volume of 25 µl containing 5 pmol of each primer, 200 nM dNTPs, 2.0 mM MgCl2, 50 mM KCl, 10 mM Tris–HCl, 0.1% Triton X-100 and 1 U Taq polymerase (Biotools, Madrid, Spain). The following cycling conditions were used: an initial denaturation step at 94 °C for 3 min; 35 cycles of 94 °C for 1 min, the annealing temperature for 1 min, and 72 °C for 1 min and 30 s for fragments 1-3 and 2, respectively; and a final extension step at 72 °C for 10 min. The annealing temperatures for each fragment are indicated in Supplementary Table 1. All PCR amplifications of genomic DNA were performed in a MyCycler thermal cycler (BioRad, Madrid, Spain). The PCR products were purified using the FlavorPrep Gel/PCR purification mini kit (Flavorgen, Ibian, Zaragoza, Spain), according to the manufacturer's instructions. The PCR products were sequenced in both directions by STAB Vida Lda. (Caparica, Portugal) using an ABI 3730XL sequencer (Applied Biosystems, CA, USA).

The homology searches were performed using BLAST (National Centre for Biotechnology Information: https://blast.ncbi.nlm.nih.gov/Blast.cgi). To align the sequences, the CLUSTAL Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/) software was used. The Variant Effect Predictor software (VEP: http://www.ensembl.org/Ovis aries/Tools/VEP?db=core), which predicts the possible impact of an amino acid substitution on the structure and function of a protein, was used. The degree of the amino acid change was also assessed using the BLOSUM 62 scoring matrix (http://www.ncbi.nlm.nih. Gov/Class/FieldGuide/BLOSUM62.txt), where low values indicate more drastic changes. Antisense matches for individual miRNAs in the 3′-UTR sequences of the isolated ovine *MTNR1A* gene were determined using MirTarget

198 (http://mirdb.org/) and TargetScan (http://www.targetscan.org/vert_71/)
199 programs. The locations of the SNPs and gene clusters were identified based
200 on the latest sheep genome version for Ovis aries (Oar_v4.0,
201 https://www.ncbi.nlm.nih.gov/genome/?term=ovis+aries).
202
203 *2.4 Linkage disequilibrium (LD)*
204 The gametic LD among SNPs (D' and r^2) within the *MTNR1A* gene was
205 calculated and visualized using the Haploview v4.2 program [23]. LD blocks
206 were defined based on the four-gamete rule algorithm [24].

207 *2.5 Statistical analysis*
208 2.5.1. SNP association studies
209 The Hardy–Weinberg equilibrium exact test values and observed and expected
210 heterozygosities and minor allele frequency (MAF) for each SNP were
211 calculated using the PLINK 1.9 software [25].
212 The associations between the *MTNR1A* gene polymorphisms and reproductive
213 seasonality traits (TDA, P4CM, and OCM) were determined by fitting a Linear
214 Mixed Model using the Mixed procedure (MIXED) in the SAS statistical
215 package. The model included the genotype of the SNPs (S), the age (mature,
216 young and ewe lambs) (A), and the interaction between the age × genotype of
217 the SNPs (A × S) as fixed effects; the live weight (LW) and body condition score
218 (BCS) as covariates; and the animal (A) and residual I as random effects.
219 Homogeneous variance was considered for the residual (e ~N (0, σ2)). The
220 equation for the model was as follows:

221 $$Y = \mu + S + A + (A \times S) + LW + BCS + A + e$$

To test the differences between genotypes for each breed, the least square means (LSMs) for each pair-wise comparison were estimated. A Bonferroni correction was fitted to take into account the multiple tests. All SNPs were independently analysed with the same statistical model.

2.5.2. Haplotype association studies

SNPs were phased with PLINK1.9 [23] considering the blocks defined by Haploview and using the expectation–maximization (E–M) algorithm to assign individual haplotypes. We considered diplotypes with a posterior probability higher than 0.8.

Associations between the haplotypes and reproductive seasonality traits were performed using a Linear Mixed Model using the Mixed procedure (MIXED) in SAS. The fitted model was similar to that used for the SNP association studies but included the haplotype (H) effect and the interaction between the age × haplotype (A × H). Haplotypes for each individual were codified as 0, 1 or 2, indicating the number of copies of each haplotype. The equation for the model was as follows:

$$Y = \mu + H + A + (A \times H) + LW + BCS + A + e$$

Only haplotypes with a frequency greater than or equal to 1% were considered. To test the differences between the haplotypes, the LSMs for each pair-wise comparison were estimated. The Bonferroni correction was applied to take into account multiple tests.

3. Results and discussion.

3.1. Structural characterization and linkage disequilibrium

All of exon 1, 1120 bp from exon 2 (869 bp from the protein coding region plus 251 bp from the 3'-UTR) and 733 bp from the promoter region were sequenced. Exons were identified by comparison with ovine sequences (GenBank sequence NM 001009725 and AF078545). Sequences from the total population (n=268) revealed 35 polymorphisms, including 13 SNPs in the promoter region, 1 SNP in exon 1 and 21 SNPs in exon 2 (17 and 4 SNPs in the coding and 3'-UTR regions, respectively) (Table 1). Table 1 shows the location, alias (nomenclature in this manuscript), dbSNP identifiers, amino acid substitution effect, and genotypic and allelic frequencies of the SNPs. *MTNR1A* is in reverse orientation on the genome, and SNPs are ordered according to their position in the latest genome version (Oar4.0: GenBank acc. number NW_014639035.1). All SNPs were in Hardy-Weinberg equilibrium, though exon 1 (snp_22) showed a reduced number of heterozygous animals. In the promoter region, we sequenced the same fragment as in Martinez-Royo et al. [12], which is located 536 bp upstream from the transcription start site (TSS). We did not find any new SNPs in this region. As described in Martinez-Royo et al. [12], six of these polymorphisms modify putative binding sites for various trans-acting factors, including snp_26 (Brn-2 and Oct-1 consensus sites), snp_27 (SRY), snp_31 (SRY), snp_32 (Nkx-2), snp_33 (SRY), and snp_34 (EF2). In exon 1, a synonymous polymorphism was detected; in contrast, 9 non-synonymous SNPs were found in exon 2. Four of the SNPs had not been previously described in other sheep breeds, with two of these being non-synonymous polymorphisms, one that changes from a Lysine to Threonine (K335T) (snp_9) and the other a non-conservative change from a Glycine to Valine (G123V) (snp_20), and both

272 substitutions were predicted as deleterious (with a SIFT value of 0.04) by the
273 VEP software. However, we did not find homozygous animals for the predicted
274 deleterious allele (G and A for snp_9 and snp_20, respectively), and only one
275 heterozygous animal for each SNP was found in two different animals. None of
276 the other non-synonymous substitutions were predicted as deleterious; all were
277 considered tolerated with SIFT values ranging from 0.37 (snp_10) to 0.05
278 (snp_12).
279 Fig. 1 shows the linkage disequilibrium plot among *the* SNPs in *MTNR1A*. Four
280 LD blocks were predicted (Fig. 1). One block included all the promoter SNPs
281 except snp_23, snp_24, snp_25 and snp_27 (Block 4). These two last SNPs
282 (snp_23 and snp_24) constituted Block 3. Finally, two different blocks were
283 predicted in exon 2. Polymorphisms in the promoter and exon 2 showed low
284 linkage disequilibrium. The maximum value of D' and r^2 between the promoter
285 and exon 2 SNPs were 0.82 and 0.14 (Figure 1), respectively, being separated
286 by approximately 24 Kb.
287
288 3.2. SNP association studies
289 The median values and 5th–95th percentiles for the TDA, P4CM and OCM traits
290 for the population are shown in Supplementary Table 2. Thirty-seven of the
291 ewes (29, 6 and 2 mature, young and ewe lambs, respectively) did not present
292 anoestrus during the experiment (TDA=0). Similarly, 87 (60, 17 and 10 mature,
293 young and ewe lambs, respectively) and 9 (7 and 2 mature and young ewes,
294 respectively) ewes were cycling throughout the experiment when considering
295 the P4CM and OCM traits, respectively. TDA and P4CM had a negative
296 correlation because P4CM was a less strict trait for ovarian function than TDA in

297 the three age groups studied. The phenotypic correlations among the three
298 traits are shown in Supplementary Table 3.
299
300 3.2.1. Promoter region
301 Results from the association studies are shown in Supplementary Table 4.
302 SNPs snp_9 and snp_20 were not considered in the association analysis
303 because only one heterozygous animal was found (MAF<0.01). The interaction
304 between the SNP and age affected the TDA and P4CM traits in the promoter
305 region (Table 2 and Supplementary Table 4). The two blocks associated with
306 these SNPs were in complete linkage disequilibrium (D'=1 and r^2=1) ([snp_29,
307 snp_30 and snp_34] and [snp_24, snp_26, snp_32 and snp_33]) (Figure 1).
308 After Bonferroni correction, only the TDA phenotype differed among the SNPs
309 (P<0.05) at ewe lamb age (SNP x age fixed effect), showing significant
310 differences between the homozygous alternative genotypes. However, this
311 association may be spurious as it relies upon an unbalanced distribution of
312 genotypes and a small number of ewe lambs (n = 51) for each SNP. In this
313 sense, only 3 homozygous animals for the MAF allele were found for snp_24,
314 snp_26, snp_29, snp_30, snp_32, snp_33 and snp_34 among the ewe lambs.
315 Considering all ages (SNP fixed effect), the SNPs were significantly associated
316 with the TDA and P4MC traits, showing a balanced distribution of genotypes
317 (Table 2 and Supplementary Table 4), with a homozygous animal frequency for
318 the MAF allele of approximately 17% (Table 1). The present results are in
319 agreement with those described by Martinez-Royo et al. [12] in that SNPs in the
320 *MNTR1A* gene promoter are associated with reproductive seasonality.
321 However, our results are slightly different because only the OCM trait was

examined in that study. Martinez-Royo et al. [12] found 5 associated SNPs, two of which were also associated with TDA and P4CM in the present study, snp_28 (called 677 in Martinez-Royo et al. [12]) and snp_34 (called 422 in Martinez-Royo et al. [12]). The C (snp_26), A (snp_32), A (snp_33), and A (snp_34) alleles were associated with lower TDA and higher P4CM values and created putative binding sites for various trans-acting factors, such as EF-2, SRY, Nkx-2 and Brn-2, respectively. These SNPs showed strong linkage disequilibrium (Fig. 1). These allelic variants may play a role in gene regulation by increasing the *MTNR1A* gene expression level (mRNA) and thus the final protein contents. Therefore, these variations may enhance the ability of MNTR1A to mediate the physiological function of melatonin, decreasing TDA and increasing P4CM.

3.2.2. Coding region

The SNP found in exon 1 was synonymous and not associated with reproductive seasonality traits. Furthermore, this SNP was not in Hardy-Weinberg equilibrium. For any SNP, the interaction between the SNP and age was not significantly associated with reproductive seasonality traits, though four SNP effect associations were found (Table 3 and Supplementary Table 4). Two of them were at the snp_17 (612/MnlI) and snp_18 (606/RsaI) polymorphic RFLP sites that have been previously associated with seasonality traits in Rasa aragonesa and other breeds [3-13]. These results are in agreement with those from Martinez-Royo et al. [12], in which snp_18 was associated with the OCM trait in the Rasa aragonesa sheep breed. In that study, snp_17 was not associated, but here, we found a significant association with the OCM trait.

However, these SNPs are synonymous polymorphisms and therefore not causative mutations. One of the other two significant SNPs is a non-synonymous polymorphism (snp_8), which led to a change of an Arginine to a Cysteine (R336C); the other one is located in the 3'-UTR region (snp_2). Snp_8 was the most significant SNP and was predicted as tolerated (with a SIFT value of 0.22) by the VEP software. The GG genotype had greater value than the AA genotype, whereas the AG genotype presented intermediate values for the TDA (73.08±7.25, 53.57±7.36 and 20.85±13.13 for the GG, AG and AA genotypes, respectively) and P4CM (0.80±0.03, 0.86±0.03 and 0.94±0.05 for the GG, AG and AA genotypes, respectively) traits. However, only significant differences were observed between the GG and AA genotypes for the OCM trait (0.49±0.03, 0.54±0.03 and 0.64±0.06 for the GG, AG and AA genotypes, respectively). This SNP has been previously described in two populations from Iran and Morocco (NEXTGEN project, http://nextgen.epfl.ch/). Similar results were also found for snp_2, as the two SNPs showed high linkage disequilibrium (D'=1, r^2=0.94). Snp_2 was located in the 3'-UTR region; this SNP could modify putative ovine miRNA target sites. However, this SNP did not modify a miRNA target sequence according to the MirTarget and Targetscan software predictions. Snp_8 could be the causative mutation for the observed effects in the reproductive seasonality traits because it was the most significant SNP and produces an amino acid change. According to the BLOSUM 62 scoring matrix, the substitution generated by snp_8 (arginine to cysteine) is rated -3, with the most severe rating (i.e., introduction of a premature stop codon) being -4. Arginine is a positively charged amino acid, whereas cysteine is polar in nature. Moreover, cysteine is important for the generation of cysteine knots and

372 disulfide linkages between subunits in some proteins and could potentially affect
373 the binding of melatonin to its receptor.
374
375 3.3. Haplotype association studies
376 Haplotype association studies were performed considering the four LD blocks
377 predicted with Haploview (Figure 1 and Supplementary Table 5) and a block
378 containing all the significant SNPs (Block 5; Supplementary Table 5). Totals of
379 5, 22, 3, 6 and 21 haplotypes were found for blocks 1, 2, 3, 4 and 5,
380 respectively. Because we only considered diplotypes with a posterior probability
381 higher than 0.8, we performed the haplotype analysis with 245, 243, 268, 268
382 and 233 ewes for blocks 1, 2, 3, 4 and 5, respectively.
383 3.3.1. Promoter region.
384 The statistically significant SNP associations were confirmed by *MTNR1A*-
385 specific haplotype analyses. The haplotype and haplotype x age effects affected
386 the TDA and P4CM traits (Table 4 and Supplementary Table 5). Haplotypes 3
387 and 1 for blocks 3 and 4, respectively, showed significant associations with the
388 TDA and P4CM traits. In this sense, homozygous h3/h3 animals (n= 102) had
389 higher and lower values for TDA and P4CM, respectively, than animals with one
390 copy (n= 119) or no h3 copies (n= 47) for block 3. However, only significant
391 differences were found between having 0 and 2 copies of the haplotype. For
392 this haplotype, only snp_24 was associated with TDA and P4CM, as having the
393 G allele showed higher and lower values for these traits (Table 2), respectively,
394 but without modifying any trans-acting factor putative binding sites. Thus, the
395 SNPs in block 3 could not be the responsible for the observed effects. Similar
396 results were found for haplotype 1 in block 4 because the of the high degree of

LD between the two blocks (D'=1), as homozygous h1/h1 animals (n= 103) had higher values than animals with one copy (n= 117) or no h1 copies (n= 48) for block 4 (Table 4). For this haplotype, the T, C, A, C, G, G and G alleles for SNPs snp_26, snp_28, snp_29, snp_30, snp_32, snp_33 and snp_34, respectively, were also associated with higher and lower TDA and P4CM values, respectively, in the SNP association studies (Table 2). *In silico* analysis showed that the T (snp_26), G (snp_32), G (snp_33), and G (snp_34) alleles were associated with higher and lower TDA and P4CM, respectively, and could destroy the putative binding sites for various trans-acting factors (EF-2, SRY, Nkx-2 and Brn-2, respectively), demonstrating that the SNPs may play a role in regulating gene expression. However, the LSMs for the haplotype x age effect were only significant for the TDA trait in the SNP association studies, while for P4CM, a trend was only observed between having 0 or 2 copies for haplotypes at an ewe lamb age after Bonferroni correction. In this sense, only three ewe lambs had 0 copies of the h3 (block 3) and h1 (block 4) haplotypes for the TDA trait, and even then, these associations were not very confident.

3.3.2. Coding region.

In exon 2, the haplotype affected the TDA, P4CM and OCM traits (Table 5 and Supplementary Table 5), but the interaction between the haplotype and the age was not significant. Homozygous h3/h3 animals (n= 15) had lower values for TDA than animals with one copy (n= 97) or no h3 copies (n= 133) for block 1. For the P4CM and OCM traits, homozygous h3/h3 animals had higher values than animals with one copy or no h3 copies. Significant differences were found between animals carrying 0 copies and 1 or 2 copies of the haplotype for the three traits. A trend was also observed between 1 and 2 copies (P=0.064) for

the TDA trait. Regarding block 2, results similar to those found for block 1 were observed due to the high degree of LD between the two blocks (D'=0.97). Significant differences were found between the three diplotypes for haplotype h2, showing that homozygous h2/h2 animals (n= 13) had lower values than animals with no h2 copies (n= 131). An intermediate TDA value was observed for the heterozygous diplotype (n= 99). For the P4CM and OCM traits, homozygous h2/h2 animals had higher values than animals with one copy or no h2 copies, showing the significant differences between having 0 and 2 copies for the haplotype for both traits.

For these haplotypes, the A, A, C and A alleles in snp_2 (block 1), snp_8 (block 2), snp_17/MnlI (block 2) and snp_18/RsaI (block 2), respectively, were associated with lower TDA and higher P4CM and OCM values in the SNP associations studies (Table 3). Only snp_8 produces a putative functional effect (a change of an Arginine to a Cysteine) (see section 3.2.2). The association results obtained for snp_2, snp_17 and snp_18 could be due to the high linkage disequilibrium between these SNPs and snp_8, as the causative mutation of the traits studied in this work.

3.3.3. Block with significant SNPs.

To determine whether the phenotype effects observed were caused by snp_8 in exon 2 or SNPs in the promoter, we created another haplotype block with all the significant SNPs associated with the seasonality traits (block 5; Supplementary Table 5). We found 3 haplotypes associated with TDA and P4CM and two associated with OCM (Table 6 and Supplementary Table 5). One copy of the h4 (**AACAACCTCTAAA**) and h13 (**AACA**GTTCACGGG) haplotypes showed lower TDA values than having no copies of this haplotype (alleles significantly

associated with lower TDA and higher P4CM and OCM values for each SNP in each haplotype according to the SNP association results are shown in bold). These haplotypes had the alleles from exon 2 (**A**, **A**, and **A** for snp_2, snp_8, and snp_18/RsaI, respectively), but not those from the promoter region associated with lower TDA values in the SNP association studies (Tables 2 and 3). In the same way, the h12 haplotype (GG**C**G**A**T**CTCTAAA**), which was associated with higher TDA values, showed that the alleles from the SNPs in exon 2 were associated with an increase in this trait (G, G, and G for snp_2, snp_8, and snp_18/RsaI, respectively). However, SNPs in the promoter region were associated with lower instead of higher TDA values (the **A**, T, **C**, **T**, **C**, **T**, **A**, **A**, and **A** alleles for snp_24, snp_25, snp_26, snp_28, snp_29, snp_30, snp_32, snp_33 and snp_34, respectively). Similar results were found for P4CM (the same significant haplotypes were found in the TDA phenotype). For the OCM trait, the two haplotypes associated with higher OCM values (h10, **AACA**G**C**TCACGGG and h13, **AACA**GTTCACGGG) had the alleles from exon 2 (**A**, **A**, and **A** for snp_2, snp_8, and snp_18/RsaI, respectively), but not those from the promoter region associated with higher OCM values in the SNP association studies (Tables 2 and 3). These results indicated that the effect is due to SNPs in exon 2 and not from those in the promoter region. As discussed in sections 3.2.2 and 3.3.2, snp_8 located in exon 2 might be the causative mutation of the effect found in this study because this SNP leads to a change in an amino acid. Despite the long distance between the SNPs located in the promoter and exon 2 (approximately 24 kb) and the low linkage disequilibrium found between them (the maximum values of r^2 and D' between snp_8 and the significant SNPs in promoter region for snp_29 snp_30, and snp_34 were 0.06

and 0.33, respectively), it appears that the effects observed for the SNPs in the promoter region are due to the linkage disequilibrium with snp_8.

4. Conclusions

We have sequenced the entire coding region plus 733 and 251 bp from the promoter and 3'-UTR regions, respectively, in the *MTNR1A* gene in 268 Rasa aragonesa sheep breed ewes, finding 13 SNPs associated with seasonality traits in the promoter and exon 2 regions. Haplotype analyses confirmed these results and allowed an assessment of the impact of the SNPs located in the promoter and exon 2 regions on the effects observed for the seasonality values. In this sense, alleles associated with lower TDA values (or higher values for P4CM) in the promoter are in linkage disequilibrium with those in exon 2 responsible for the effects found in this study, and they can be segregated together. Thus, the observed effects could be due to SNPs in exon 2 but not the SNPs in the promoter. We have demonstrated that the snp_8 T allele in the *MNTR1A* gene is associated with lower and higher TDA and P4CM values, respectively, both of which are seasonality traits for ovarian function based on blood progesterone levels; this allele is also associated with higher OCM values, which indicates behavioural signs of oestrous in the Rasa Aragonesa breed. This SNP is in linkage disequilibrium with snp_17 (612/MnlI) and snp_18 (606/RsaI), which have been related with seasonal reproductive activities in several association studies with other breeds. However, these SNPs are synonymous polymorphisms and do not appear to be the causative mutations. In this sense, snp_8 could be the causative mutation for the observed effects in

496 the reproductive seasonality traits because it produces an amino acid change
497 from an Arginine to a Cysteine (R336C).

498

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505

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Figure captions

Fig. 1. Schematic representation of ovine *MTNR1A* and the linkage disequilibrium plot for the SNPs in *MTNR1A* for the Rasa aragonesa population using Haploview. *MTNR1A* is in a reverse orientation on the genome, and the SNPs are ordered according to their position in the latest genome version (Oar4.0: GenBank acc. number NW_014639035.1). The linkage disequilibrium colour scheme corresponds with the *D′* parameter, while the linkage disequilibrium values correspond to the r^2 parameter. Haplotype blocks are indicated by dark lines. A strong LD (D′ = 1, LOD ≥ 2) is indicated by red, and lighter shades of pink indicate varying degrees of LD, with lighter shades displaying less than darker shades (D′ < 1, LOD ≥ 2). White indicates low LD (D′ < 1, LOD < 2).

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Table 1. Information about the location and amino acid substitution effect of the identified SNPs in the *MTNR1A* gene and their genotypic and allelic frequencies. *MTNR1A* is in a reverse orientation on the genome, and the SNPs are ordered according to their positions in the latest genome version (Oar4.0: GenBank acc. number NW_014639035.1).

Location	Alias[1]	dbSNPs	Oar 4.0 (NW_014639035.1)	Amino acid substitution	Genotype	Genotype frequencies	Allele	Allele frequencies
exon 2	snp_1	rs420016236	g.15098857C>T	3' UTR	CC	0.597	C	0.78
				variant	CT	0.366	T	0.22
					TT	0.037		
	snp_2	rs400830807	g.15098860G>A	3' UTR	GG	0.544	G	0.742
				variant	GA	0.392	A	0.258
					AA	0.064		
	snp_3	rs414185743	g.15098868T>C	3' UTR	TT	0.322	T	0.568
				variant	TC	0.493	C	0.432
					CC	0.185		
	snp_4	rs423194759	g.15098916T>C	3' UTR	TT	0.322	T	0.568
				variant	TC	0.493	C	0.432
					CC	0.185		
	snp_5	rs403826495	g.15098968T>C	I348V	TT	0.322	T	0.568
				(ATA/GTA)	TC	0.493	C	0.432
					CC	0.185		
	snp_6	rs413084140	g.15098976T>C	H345R	TT	0.322	T	0.568
				(CAT/CGT)	TC	0.493	C	0.432
					CC	0.185		
	snp_7	rs426523476	g.15098996A>G	P338P	AA	0.182	G	0.57
				(CCC/CCT)	AG	0.496	A	0.43
					GG	0.322		
	snp_8	rs403212791	g.15099004G>A	R336C	GG	0.511	G	0.724
				(CGC/TGC)	GA	0.425	A	0.276
					AA	0.064		
	snp_9	ss2137144054	g.15099006T>G	K335T	TT	0.996	T	0.998
				(AAA/ACA)	GT	0.004	G	0.002
					GG	0		

snp_10	rs416266900	g.15099204G>T	A269D	GG	0.613	G	0.786
			(GCC/GAC)	GT	0.346	T	0.214
				TT	0.041		
snp_11	rs429718221	g.15099206G>A	P268P	GG	0.333	G	0.576
			(CCC/CCT)	GA	0.486	A	0.424
				AA	0.181		
snp_12	rs404378206	g.15099223C>T	V263I	CC	0.909	C	0.955
			(GTT/ATT)	CT	0.091	T	0.045
				TT	0		
snp_13	rs417800445	g.15099296C>T	R238R	CC	0.624	C	0.796
			(AGG/AGA)	CT	0.342	T	0.204
				TT	0.034		
snp_14	rs427019119	g.15099314C>T	L232L	CC	0.617	C	0.792
			(CTG/CTA)	CT	0.35	T	0.208
				TT	0.033		
snp_15	rs407388227	g.15099391C>T	V207I	CC	0.634	C	0.794
			(GTC/ATC)	CT	0.321	T	0.206
				TT	0.045		
snp_16	rs420819884	g.15099422C>T	V196V	CC	0.918	C	0.955
			(GTG/GTA)	CT	0.074	T	0.045
				TT	0.008		
snp_17	rs430181568	g.15099485C>T	P175P	CC	0.634	C	0.792
			(CCG/CCA)	CT	0.317	T	0.208
				TT	0.049		
snp_18	rs406779174	g.15099491G>A	Y173Y	GG	0.28	G	0.525
			(TAC/TAT)	GA	0.49	A	0.475
				AA	0.23		
snp_19	ss2137144055	g.15099575C>T	T145T	CC	0.922	C	0.959
			(ACG/ACA)	CT	0.074	T	0.041
				TT	0.004		
snp_20	ss2137144056	g.15099642C>A	G123V	CC	0.996	C	0.998
			(GGA/GTA)	CA	0.004	A	0.002
				AA	0		
snp_21	rs419680097	g.15099644C>A	T122T	CC	0.638	C	0.79
			(ACG/ACT)	CA	0.305	A	0.21

					AA	0.057		
exon 1	snp_22	ss2137144057	g.15121956G>A	N16N	GG	0.696	G	0.761
				(AAT/AAC)	GA	0.13	A	0.239
					AA	0.174		
Promoter	snp_23	rs406334919	g.15122684T>C		TT	0.699	T	0.831
					TC	0.265	C	0.169
					CC	0.036		
	snp_24	rs419743392	g.15122766G>A		GG	0.379	G	0.601
					GA	0.445	A	0.399
					AA	0.176		
	snp_25	rs400561563	g.15122788C>T		CC	0.544	C	0.735
					CT	0.382	T	0.265
					TT	0.074		
	snp_26	rs399461430	g.15122829T>C		TT	0.379	T	0.601
					TC	0.445	C	0.399
					CC	0.176		
	snp_27	rs412826644	g.15122893G>A		GG	0.915	G	0.956
					GA	0.081	A	0.044
					AA	0.004		
	snp_28	rs426266687	g.15122902C>T		CC	0.382	C	0.605
					CT	0.445	T	0.395
					TT	0.173		
	snp_29	rs402949406	g.15122931C>A		CC	0.386	C	0.607
					CA	0.441	A	0.393
					AA	0.173		
	snp_30	rs411931887	g.15122934C>T		CC	0.387	C	0.607
					CT	0.441	T	0.393
					TT	0.173		
	snp_31	rs406184829	g.15123052T>C		TT	0.695	T	0.829
					TC	0.268	C	0.171
					CC	0.037		
	snp_32	rs415456480	g.15123097G>A		GG	0.379	G	0.601
					GA	0.445	A	0.399
					AA	0.176		
	snp_33	rs428880789	g.15123143G>A		GG	0.379	G	0.601

			GA	0.445	A	0.399
			AA	0.176		
snp_34	rs405080439	g.15123157G>A	GG	0.386	G	0.607
			GA	0.441	A	0.393
			AA	0.173		
snp_35	rs429917252	g.15123238C>T	CC	0.699	C	0.831
			CT	0.265	T	0.169
			TT	0.036		

[1]Nomeclature used for each SNP in this work.

Table 2. Type III test for the SNP and SNP x age effects for the *MTNR1A* polymorphisms using the seasonality phenotype data from Rasa aragonesa ewes. The least square means and standard errors of the *MTNR1A* polymorphisms in the seasonality phenotype data in Rasa aragonesa ewes are also shown. Only significant SNPs after Bonferroni correction are shown. M=mature; Y=young; L=ewe lambs. snp_29-snp_30-snp_34 and snp_24-snp_26-snp_32-snp_33 were linked (r^2=1). Different letters indicate significant differences: a, b: P<0.05; c, d: P<0.01; and e, f: P<0.001.

SNP	Trait	P-value SNP	LSmeans SNP			P-value SNPxAge	Age	LSmeans SNPxAge		
			AA/CC/GG	**AC/CT/AG**	**CC/TT/AA**			**AA/CC/GG**	**AC/CT/AG**	**CC/TT/AA**
snp_29	TDA	0.01	68.72±7.33c	58.84±7.13cd	37.05±11.29d	0.012	**M**	54.74±9.96	46.61±9.05	61.56±11.44
snp_30							**Y**	63.45±10.55	63.67±10.13	49.51±14.24
snp_34							**L**	87.96±10.96a	66.23±10.94ab	0.09±25.91b
	P4CM	0.015	0.81±0.03a	0.85±0.03ab	0.93±0.04b	0.03	**M**	0.85±0.03	0.88±0.03	0.83±0.04
							Y	0.82±0.04	0.82±0.04	0.88±0.05
							L	0.76±0.04	0.83±0.04	1.08±0.1
			GG/TT/GG/GG	**AG/AG/CT/AG**	**AA/CC/AA/AA**			**GG/TT/GG/GG**	**AG/AG/CT/AG**	**AA/CC/AA/AA**
snp_24	TDA	0.008	68.86+7.32c	58.48+7.15cd	36.58+11.26d	0.013	**M**	55.83±10.04	46.21±9.03	90.81±11.37
snp_26							**Y**	63.16±10.55	63.37±10.13	49.19±14.23
snp_32							**L**	87.58±10.78a	65.84±10.76ab	-0.25±25.11b
snp_33	P4CM	0.013	0.81+0.03a	0.85+0.03ab	0.93+0.04b	0.033	**M**	0.85±0.04	0.89±0.03	0.84±0.04
							Y	0.82±0.04	0.83±0.04	0.88±0.04
							L	0.73±0.04	0.86±0.04	1.08±0.1
			TT	**CT**	**CC**			**TT**	**CT**	**CC**
snp_28	TDA	0.008	37.02+11.27c	58.50+7.13cd	69.10+7.31c	0.012	**M**	61.60±11.42	45.77±9.06	56.00±9.93
							Y	49.44±14.22	63.61±10.12	63.41±10.54
							L	0.00±25.59a	66.13±10.74ab	87.87±10.77b
	P4CM	0.013	0.93+0.04a	0.85+0.03ab	0.81+0.03b	0.03	**M**	0.83±0.04	0.89±0.03	0.85±0.04
							Y	0.88±0.05	0.83±0.04	0.82±0.04
							L	1.08±0.04	0.82±0.04	0.76±0.1
			CC	**CT**	**TT**			**CC**	**CT**	**TT**
snp_25	TDA	0.025	52.40+7.15b	65.52+7.54a	68.99+11.93ab	0.104	**M**	47.50±9.49	52.83±9.35	46.69±18.14
							Y	56.84±9.58	66.05±10.86	54.57±22.70
							L	52.85±10.60	86.66±12.09	105.7±16.09
	P4CM	0.005	0.88+0.03d	0.80+0.03c	0.80+0.04cd	0.089	**M**	0.88±0.04	0.86±0.04	0.88±0.07
							Y	0.85±0.04	0.82±0.04	0.83±0.09
							L	0.90±0.04	0.73±0.05	0.71±0.6

Table 3. Type III test for the SNP effects on the *MTNR1A* gene using the seasonality phenotype data from Rasa aragonesa ewes. The least square means and standard errors for the SNP effects on the *MTNR1A* polymorphisms are also shown. Only significant SNPs after Bonferroni correction are shown. Different letters indicate significant differences: a, b: P<0.05; c, d: P<0.01; and e, f: P<0.001.

SNP	Trait	P-value SNP	LSmeans SNP		
snp_2			GG	GA	AA
	TDA	0.0001	66.62±7.91a,c	48.79±7.88b	16.25±15.15d
	P4CM	0.003	0.81±0.03a	0.87±0.03b	0.96±0.06b
	OCM	0.015	0.49±0.03a	0.55±0.03ab	0.64±0.07b
snp_8			GG	GA	AA
	TDA	<0.0001	73.08±7.25c,e	53.57±7.36a,d	20.85±13.13b,f
	P4CM	0.001	0.80±0.03a,c	0.86±0.03b	0.94±0.05b,d
	OCM	0.022	0.49±0.03a	0.54±0.03ab	0.64±0.06b
snp_17			TT	TC	CC
	TDA	0.037	78.41±14.37	59.38±8.40	50.71±7.94
	P4CM	0.073	0.76±0.06	0.83±0.03	0.86±0.03
	OCM	0.018	0.39±0.06a	0.50±0.04ab	0.55±0.03b
snp_18		0.0002	72.25±9.01e	58.05±7.78c	33.28±9.69f,d
	TDA	0.003	0.79±0.03c	0.84±0.03a	0.92±0.04b,d
	P4CM	0.02	0.46±0.04a	0.50±0.03ab	0.57±0.04b
	OCM	0.0002	72.25±9.01e	58.05±7.78c	33.28±9.69f,d

Table 4. Type III test for the haplotype and haplotype x age effects for blocks 3 and 4 on the *MTNR1A* gene promoter region using the seasonality phenotype data from Rasa aragonesa ewes. The least square means and standard errors for the haplotype and haplotype x age effects for the *MTNR1A* haplotypes are also shown. Only significant SNPs after Bonferroni correction are shown. Different letters indicate significant differences: a, b: P<0.05; c, d: P<0.01; and e, f: P<0.001.

				SNP effect[2]				SNP x age effect[2]				
Trait	**Block[1]**	**Haplotype**	**Freq.**	**P-value**	**0 copy**	**1 copy**	**2 copies**	**P-value**	**Age**	**0 copy**	**1 copy**	**2 copies**
TDA	3	h3 (TG)	0.61	0.010	35.52±11.35c	57.61±7.29cd	67.32±7.43d	0.013	M	58.98±11.51	44.94±9.23	52.56±10.22
									Y	48.2±14.35	62.36±10.26	62.15±10.67
									L	-0.61±25.7a	65.53±10.87ab	87.27±10.89b
	4	h1 (TCACTGGGC)	0.61	0.011	35.1±11.32c	57.64±7.29cd	66.94±7.41d	0.016	M	58.10 ±11.34	44.38±9.35	52.86±10.23
									Y	47.96±14.32	62.13 ±10.23	61.91±10.64
									L	-0.76 ±25.7a	66.40±10.78ab	86.94±10.89b
P4CM	3	h3 (TG)	0.61	0.016	0.93±0.04a	0.85±0.03ab	0.082±0.03b	0.036	M	0.84±0.04	0.89±0.03	0.86±0.04
									Y	0.88±0.05	0.83±0.04	0.83±0.04
									L	1.08±0.10	0.83±0.04	0.76±0.04
	4	h1 (TCACTGGGC)	0.61	0.015	0.93±0.04a	0.85±0.03ab	0.82±0.03b	0.031	M	0.84±0.04	0.89±0.04	0.86±0.04
									Y	0.88±0.05	0.83±0.04	0.83±0.05
									L	1.08±0.10	0.83±0.04	0.69±0.07

[1] Block 3: snp_23 - snp_24 and Block4: snp_26 - snp_28 - snp_29 - snp_30 - snp_31 - snp_32 - snp_33 - snp_34 - snp_35.

[2] M=mature; Y=young; L=ewe lambs. 0 copy: LSmeans and SE for 0 copy of the haplotype; 1 copy: LSmeans and SE for 1 copy of the haplotype; and 2 copies: LSmeans and SE for 2 copies of the haplotype.

Table 5. Type III test for the haplotype effects of blocks 1 and 2 in exon 2 on the *MTNR1A* gene using the seasonality phenotype data from Rasa aragonesa ewes. The least square means and standard errors for the haplotype effect for the *MTNR1A* gene are also shown. Only significant SNPs after Bonferroni correction are shown. Different letters indicate significant differences: a, b: P<0.05; c, d: P<0.01; and e, f: P<0.001

				SNP effect[2]			
Trait	Block[1]	Haplotype	Freq.	P-value	0 copy	1 copy	2 copies
TDA	1	h3 (CATCTTG)	0.26	0.0001	66.62±7.91a,e	48.79±7.88b	16.25±15.15b,f
	2	h2 (AGGCCCCAC)	0.26	0.0004	65.13±7.85a,e	50.33±7.96b	13.78±15.42a,f
P4CM	1	h3 (CATCTTG)	0.26	0.0029	0.81±0.03a	0.87±0.03b	0.96±0.06b
	2	h2 (AGGCCCCAC)	0.26	0.014	0.82±0.03a	0.86±0.03ab	0.97±0.06b
OMC	1	h3 (CATCTTG)	0.26	0.015	0.49±0.03a	055±0.03b	0.64±0.06b
	2	h2 (AGGCCCCAC)	0.26	0.023	0.49±0.03a	0.54±0.03ab	0.64±0.07b

[1] Block1: snp_1 - snp_2 - snp_3 - snp_4 - snp_5 - snp_6 - snp_7; Block2: snp_8 - snp_10 - snp_11 - snp_13 - snp_14 - snp_15 - snp_17 - snp_18 - snp_21

[2] 0 copy: LSmeans and SE for 0 copy of the haplotype; 1 copy: LSmeans and SE for 1 copy of the haplotype; and 2 copies: LSmeans and SE for 2 copies of the haplotype.

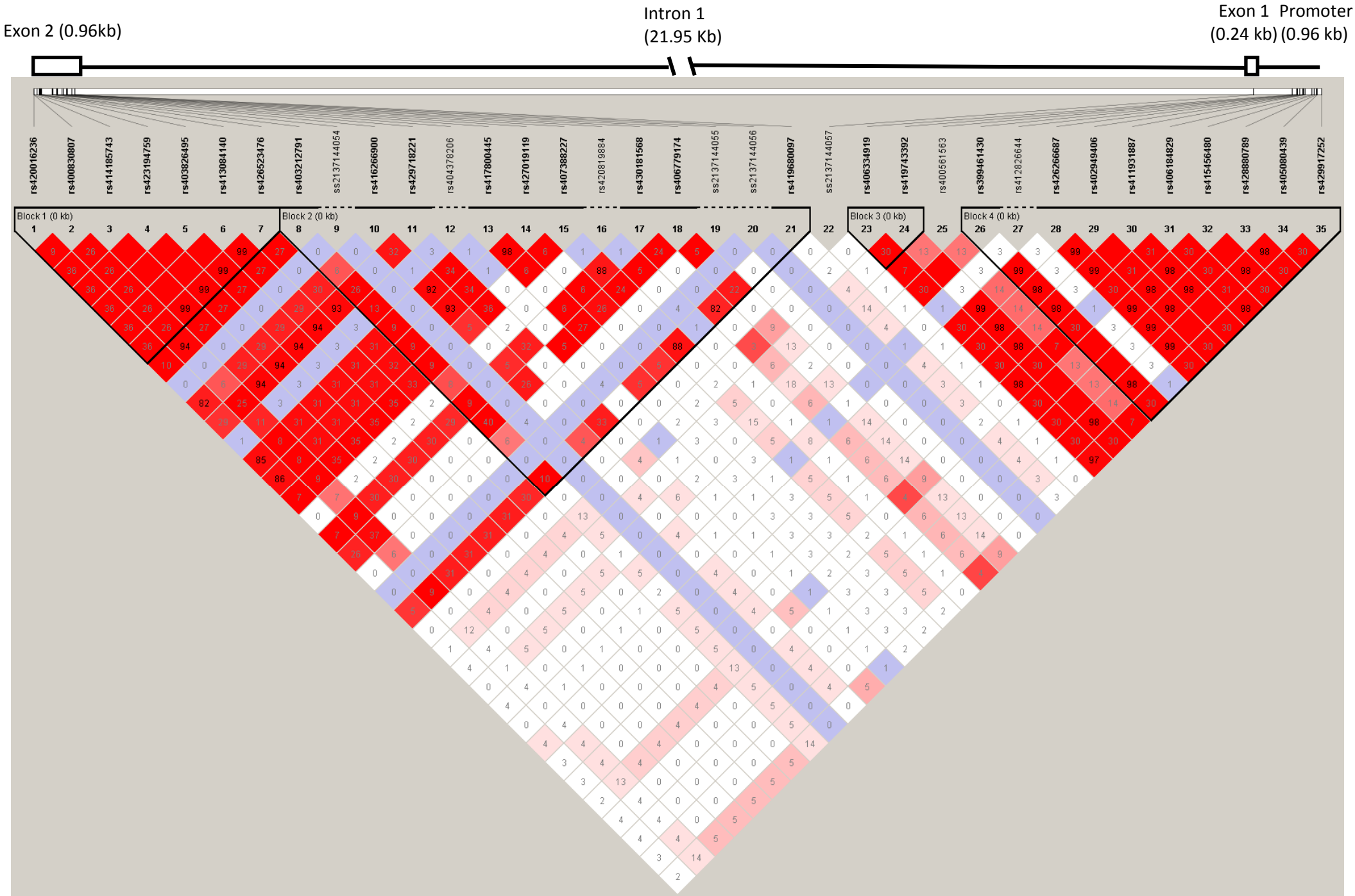
Table 6. Type III test for the haplotype effects of block 5 on the *MTNR1A* gene using the seasonality phenotype data from Rasa aragonesa ewes. The least square means and standard errors for the haplotype effect on the *MTNR1A* gene are also shown. Only significant haplotypes after Bonferroni correction are shown. Different letters indicate significant differences: a, b: P<0.05; c, d: P<0.01; and e, f: P<0.001. Alleles associated with lower TDA and higher P4CM and OMC values for each SNP according to SNP association results are shown in bold.

			SNP effect[2]			
Trait	Haplotype	Freq.	P-value	0 copy	1 copy	2 copies
TDA	h4(**AACAACCTCTAAA**)	0.17	0.0387	63.17±7.88a	48.77±8.44b	37.25±21.15ab
	h12 (GG**C**G**A**T**CTCTAAA**)	0.02	0.029	56.83±7.42a	98.48±17.77b	--
	h13 (**AACA**GTTCACGGG)	0.05	0.0014	59.20±7.45c	30.74±11.94d	--
P4CM	h4(**AACAACCTCTAAA**)	0.17	0.014	0.81±0.03a	0.87±0.03b	0.97±0.08b
	h12 (GG**C**G**A**T**CTCTAAA**)	0.02	0.040	0.83±0.03a	0.71±0.07b	--
	h13 (**AACA**GTTCACGGG)	0.05	0.026	0.83±0.03a	0.92±0.04b	
OMC	h10 (**AACA**G**C**TCACGGG)	0.003	0.048	0.50±0.03a	0.61±0.03b	--
	h13 (**AACA**GTTCACGGG)	0.05	0.0014	0.50±0.03c	0.65±0.05ad	--

[1] Block 5: snp_2 - snp_8 - snp_17 - snp_18 - snp_24 - snp_25 - snp_26 - snp_28 - snp_29 - snp_30 - snp_32 - snp_33 - snp_34

[2] 0 copy: LSmeans and SE for 0 copy of the haplotype; 1 copy: LSmeans and SE for 1 copy of the haplotype; and 2 copies: LSmeans and SE for 2 copies of the haplotype.

Fig.1.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Highlights

- Significant SNPs in promoter and exon 2 of the MTNR1A were found.
- Haplotype analyses showed that the effects found were caused by the SNPs in exon 2.
- SNP rs403212791 in exon 2 gene is associated to reproductive seasonality
- The SNP rs403212791 leads a change of an Arginine to Cysteine (R336C).
- The SNP rs403212791 was not previously reported associated for reproductive seasonality.