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NEW POLYMORPHISMS AT MTNR1A GENE AND THEIR ASSOCIATION WITH REPRODUCTIVE RESUMPTION IN SARDA BREED SHEEP

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ABSTRACT

The aim of this study was to characterize the MTNR1A locus in Sarda sheep breed, in order to identify potential single nucleotide polymorphisms (SNPs) associated with reproductive resumption. The reproductive performance of 200 lactating ewes, aged 3e5 years, with body condition score (BCS) 2.5 e4.0, at least at their third lambing were monitored for two consecutive years. In both year the enrolled ewes were exposed for 100 days to 10 adult, fertile rams. Mating, pregnancy and lambing for each ewe were recorded in order to evaluate differences in reproductive performance according to the analysed genotypes. From individual blood samples, DNA was extracted to amplify and to sequence promotor, the coding region, a part of intron and of 30 Untranslated region (30 UTR) of the MTNR1A gene. A total number of 29 SNPs were found (named SNP1 to SNP29), five of which caused also amino acid changes. The polymorphic sites found at positions g.17355452C > T (SNP16, rs430181568) and g.17355358C > T (SNP17, rs407388227) were linked (D’ = 1 and r2 = 1) and showed a significant association to DRIL trait (distance in days from ram introduction to lambing). In both years, the ewes carrying C/C genotype in both these polymorphic sites showed the lowest DRIL compared to the other genotypes ($P < 0.05$). The ewes carrying C/C and T/C genotype exhibited the lambing peak at 170 days, and approximately 60% of the total lambing at 180 days from the ram introduction. Instead, ewes carrying T/T genotype showed the lambing peak around 200 days after ram introduction. Six haplotypes have been identified and the most frequent haplotype was also associated with lower DRIL ($P < 0.05$). Litter size displayed no statistical significance either among genotypes or among haplotypes. This study provided the major part of the MTNR1A gene in Sarda sheep breed and evidenced that SNP17 is associated with a shorter DRIL. The obtained results underlined the role of this polymorphism in improving reproductive efficiency in Sarda sheep and provides a suitable information for improving genetic selection.

Introduction

The photoperiodic pattern drives seasonal reproductive activity of several sheep breeds in the Mediterranean areas [1,2]. Photo-periodic information is received by the retina and transmitted, via a multi-step neural pathway, to the pineal gland, where the message modulates the rhythm of melatonin secretion [3]. Reproductive activity in sheep naturally occur when the circulating melatonin levels are high as a result ofa prolonged melatonin secretion during short photoperiods [4,5]. Melatonin exerts a fundamental function in this process, as its circulating levels vary on the basis of the daily light/dark alternation, with higher values during dark hours (during short day season) and lower values during light hours (during long days season) [6]. Receptors for melatonin are located in several organs and tissues, including male and female reproductive tract and premammillary hypothalamic area (PMH), that have a crucial role in ovine seasonal reproduction [7]. The melatonin receptor subtype 1A (MTNR1A) gene, is currently recognized as a candidate gene related to the seasonal reproductive activity in sheep [8e10]. In the MTNR1A gene exon II, different sheep breeds exhibit nucleotide variations. These are assumed to change the reproductive response to seasonal variations and to improve, in general, the reproductive performance, even in out of the breeding season [11e14]. Two SNPs, within the exon II (rs406779174 and rs430181568, classic RsaI and MnlI restriction fragment length polymorphism site, respectively), were associated with reproductive seasonality traits. These polymorphisms were not causative, so that it was difficult to explain how they influence the reproductive activity [14]. Subsequently, another SNP, rs407388227, was identified as causative by means of a Val > Ile substitution in the amino acid chain and it was always linked with rs430181568 [6]. Currently, the rs407388227 SNP, causing an alteration of the transduction of the melatonin signal, explains its role played in the regulation of the reproductive seasonality [15]. Recently, Calvo et al. [10] in Rasa Aragonesa sheep identified another causative SNP, rs403212791, that improves the reproductive activity. Based on the above consideration, the aim of this study was to detect and characterize new SNPs from the MTNR1A gene sequence in Sarda breed, highlighting other possible polymorphisms associated with reproductive resumption.

Materials and methods

The care and use of animals were under the control of the veterinarians of the National Veterinary Service, in accordance with the Animal Welfare Act. Blood samples were collected by the above veterinarians during routine health assessments.

Animals and management

An ovine farm located in North Sardinia (40.80° N, 8.26° E), has been used for the research. The ewes farmed were approximately 1000 Sarda sheep, kept under natural photoperiods since birth. The animals grazed on leguminous and gramineous grasses during the day and received 300 g per head/daily of concentrate commercial feed (crude protein 20.4% and 12.5 MJ ME/kg DM) at the time of milking. The sheep were penned at night and received hay (crude protein 11.1% and 7.2 MJ ME/kg DM) and water ad libitum. On April 10, 2017, 200 lactating ewes (157.5 ± 8.5 days from previous lambing), aged 3e5 years (3.9 ± 0.8 years), with body condition score (BCS) 2.5e4.0 (3.3 ± 0.4), at least at their third lambing were chosen. The exclusion of ewes at first and second lambing agreed with Mura et al. [6]. Briefly, in the Sarda breed, the first lambing generally occurs between January and April. This breed exhibits high milk yield and, consequently, its reproductive activity is poor for approximately at least 2 months after lambing. For that reason, there is a delay in the second lambing compared to adult ewes and only multiparous ewes were selected for the present study. The ewes were identified by individual ruminal bolus. Their (BCS) on a 1 to 5 scale [16] were assessed on April 8. Of these ewes 2 subsequent reproductive seasons, from April 2017 to December 2018 were studied. In the second reproductive season we followed 196 ewes, from the starting number of 200, as four ewes were excluded for health problems.

Reproductive data collection

The enrolled ewes were exposed to 10 adult, fertile rams from April 20 to July 31, both in 2017 and 2018. This management ensures that lambings occur between late September and the end of December. Lambing in this period guarantees an optimal lactation length, considering that in Sardinia the climate condition allows the growth of green grass in autumn, winter and spring, while the high summer environmental temperature desiccates pastures, leading ewes to dry period. Rams were clinically healthy and of proven fertility, as they had previously produced progeny. Matings were registered daily, as the rams were provided with marker harnesses, that were changed every 10 days, in order to obtain more precise information on the mounting activity. After 100 days of cohabitation with females, all the rams were removed. Pregnancy diagnosis was performed from 40 days after ram introduction to 40 days after ram removal, through trans-vaginal echography. From 150 to 250 days after ram introduction, lambing dates and litter size were registered. From the recorded data, fertility rate (number of lambed ewes per ewe exposed to the ram), litter size (number of newborn lambs per lambed ewes) and distance in days from ram introduction to lambing (DRIL), were calculated.

Genotyping

Blood sample was collected from each ewe from jugular vein using a sterile vacuum tube (BD Vacuntainer System, Belliver Industrial Estate, Plymouth, UK) with EDTA (ethylenediamine tetracetic acid) as an anticoagulant. Subsequently, five aliquots of 200 ml from each sample were obtained and stored at 20° C until analyses. DNA was extracted from an aliquot of whole blood using a commercial DNA extraction kit (NucleoSpinRBlood, MachereyeNagel Postfach 101,352 D-52313, Duren Neumann Neander Str. 6e8D-52355 Duren, Germany). The primers set used to amplify the promoter genomic region (fragment 1 Fw: GCACAAAAAGAAGCCAAGGA Rv: TCAGGTGTCGCACTGTAACC, and fragment 2 Fw: TGTTCCTGGGAGAAGTCTGG Rv: CATGCATCAAACCTGGACTG) in the MNTR1A gene were those reported by Martinez-Rojo et al. [17]. To amplify the exon 1 (fragment 3), the exon 2 and partial 3' UTR (fragment 4), were used respectively the primers (Fw: CTCGACGCTCTGGGGAT and Rv: CCGAACAGGGAAGAGGTTG, Fw: GGCCCTAACCCATGTTTTCT and Rv: CTCCCACTCTGTTCCCTGAA) according to Calvo et al. [10]. All the fragments were amplified in a final volume of 50 μl by PCR (polymerase chain reaction) in a reaction mix containing 5.0 μl of 10x PCR buffer (50% glycerol (v/v), 20 mM Tris-HCl (pH 8.7), 100 mM KCl, 0.1 mM EDTA, stabilizers), 3.0 μl of 1.5 mMMgCl2, 8 μl of 1.25 mMofeachdNTPs, 10pMofeachprimer and 0.25 μl of5U Taq DNA polymerase (HOTFIREPol DNA Polymerase Solis BioDyne, Teaduspargi, Tartu, Estonia) and 2.5 μl of template DNA (100 ng). Ten ml of 10x Solution S (additive that facilitates amplification of templates) were used to amplify the fragment 3. The PCR conditions for the 1 and 2 fragments were as follows: initial denaturation step at 94° C for 3 min, followed by 35 circles of denaturation at 94° C for 1 min, annealing at 60° C and 61° C for the fragment 1 and 2 respectively for 1 min, elongation 72° C for 1 min; final extension at 72° C for 10 min. The PCR reaction for the fragments 3 and 4 was conducted according to Calvo et al. [10]. All PCR amplifications of genomic DNA were conducted with use of the MAXYGENE II Thermal Cycler (Axygen® Tewksbury, MA, USA). The PCR products were confirmed by electrophoresis in 1% TAE buffer on the 1.5% (w/v) agarose gel containing 9 ml of RedSafe Dna Stain 20,000X (iNtROn Biotechnology) parallel with a 100 bpmarker (Solis BioDyne, Teaduspargi, Tartu, Estonia) at a constant voltage of 110 V for 40 min and then displayed using a UV light trans-illuminator (UVItec, Cambridge, UK). All PCR products were purified and sequenced in both directions by a commercial sequencing service. The Blast algorithm (www.ncbi.nlm.nih.gov/blast/) was used to compare the obtained sequences with the genome version Oar_rambouillet_v1.0 e GenBank assembly accession number: GCF_002742125.1. Nucleotide sequence alignments were carried out using the BioEdit Sequence Alignment Editor software (http://www.mbio.ncsu.edu/BioEdit/BioEdit.html). To predict the impact of amissense variation on the structure and function of a protein, the Variant Effect Predictor (VEP), a tool of Ensembl genome browser, was used (http://www.ensembl.org/Tools/VEP). The SIFT (Sorting Intolerant From Tolerant) as algorithm score effect was considered [18]. To evaluate the linkage disequilibrium by D' and r^2 among genotypes, the Haploview v4.2 software was used [19].

Statistical analysis

Allelic frequencies were determined by direct counting of the observed genotypes. The chi-squared test was used to determine Hardy-Weinberg equilibrium of the mutation. The statistical analysis was performed using R statistical software (Version 4.0.0 R Core Team 2020 R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/). The R package lme4 [20] was used to analyse the associations between each genotype and reproductive traits measured as fertility rate, litter size and DRIL. The R Package Haplo.stats was used to build haplotypes and to evaluate association between reproductive performances and found haplotypes [21].

The same Linear Mixed Model was used for genotypes and haplotypes:

$$Y_{jkl} = \mu + G_j + A_k + G_jA_k + B_m + S_n + e_{jkmnl}$$

Where Y_{jkl} is the trait measured for each animal, μ is the overall mean, G_j is the fixed effect of the genotype or haplotype (3 or 6 levels), A_k is the fixed effect of age (3, 4 and 5 years old), G_jA_k is the fixed effect of interaction between genotype or haplotype and age, B_m (BCS) is a covariate, S_n is the random effect of each animal, and e_{jkmnl} is the random residual effect of each observation. The data are expressed as least square means (LSmeans). Statistical significance was set at $P < 0.05$.

Results

We amplified and sequenced 4 fragments, correspondent to the entire coding region, and, partially, promoter, intron and 30 UTR of the MTRN1A gene. A total number of 29 single nucleotide polymorphisms (SNPs) were found (Table 1) and they were distributed as follows: 11 in the promoter region, 1 in the intron, 13 in the exon 2, and 4 in the 3' UTR. In the exon 1 no variation was found. The allele and genotype frequency for each found SNP are shown in Table 1, together with P values of Hardy Weinberg equilibrium. All genotype positions are ordered according to the last sheep genome assembly version (Oar_Rambouillet_v1.0, with accession RefSeq GCF_002742125.1). Eight of the SNPs found in exon 2, were no sense, while the other five were missense. All SNPs that change amino acid, were predicted as tolerated by VEP, in fact, their SIFT score were as following: SNP17 = 0.27, SNP21 =0.37, SNP22 = 0.22, SNP24 = 0.12, SNP25 = 1. The linkage (D' and r2) between all SNPs was calculated and the results are reported in supplementary Table 1. Each SNP was analysed to verify its relationship with fertility rate, litter size and DRIL in the 2 considered years. The SNP16 and SNP17 were totally linked (D' = 1 and r2 = 1), so that they were considered as a unique SNP, and from now on we refer only to SNP17. In both years, the ewes carrying C/C genotype at this locus showed the lowest DRIL compared to the other genotypes for P< 0.05 (Table 2). In 2017 and 2018, only C/C and T/C animals mated within the first 10 days and the mating peak for these genotypes was recorded in the first 20 days after ram introduction. Instead the T/T ewes, in both years, started mating 20 days after ram introduction with mating peak at 50 days from ram introduction (Fig. 1). Furthermore, in both years from the lambing trend, that overlap with the mating and pregnancy trend, the ewes carrying C/C and T/C genotype exhibited approximately 60% of the total lambing at 180 days and the lambing peak at 170 days from the rams introduction (Fig. 2). In T/T ewes, on the other hand, the first mating occurs 30 days after rams introduction and the lambing peak occurred at 200 days. None of the other SNPs exhibited any association with the analysed reproductive traits. In 2018, 196 ewes were considered as 2 C/C and 2 T/C ewes were excluded from the study for health problems, but the reproductive data did not differ between the considered years. By the data processing, we have considered all the haplotypes having frequency greater than 0.05, for a total of 6 haplotypes, identified with number code H1e H6 as reported in Table 3. The haplotype H1 and H6 were associated with lower DRIL ($P < 0.05$) (Table 4). Litter size did not display statistical significance in the six haplotypes. Since no difference were found in reproductive data between the 2 years only the 2017 haplotype analysis were considered.

Discussion

The sequence analysis of the MTNR1A gene revealed 29 SNPs distributed in the coding sequence, intron, promoter and 3' UTR. The exon 2 is the most examined because, within its nucleotide sequence, two polymorphic sites are found to be involved in the regulation of reproductive seasonality [22,23]. In our previous studies on Sarda breed 8 SNPs were already found in exon 2 and these are confirmed in this research [6]. Almost the same variations were also detected in different breeds in many countries [22,24,25]. In the present study, the entire coding sequence of exon 2 has been analysed and 5 additional SNPs were found, according to Calvo et al. [10] surely thanks to the increased number of the analysed nucleotides. In the Raza Aragonesa exon 2 sequence, moreover, other 5 SNPs have been recognized [10] which are missing in Sarda breed. Furthermore, only in exon 2 of the MTNR1A gene, we found 5 substitutions that cause an amino acid change; among them 3 were identified in Sarda breed for the first time. In the Raza Aragonesa other 4 SNPs causing amino acid changes were identified [10], which are not found in the Sarda breed. In this research the nucleotide sequence of the exon 1 showed no variations as also reported by Martinez-Royo et al. [17], while Calvo et al. [10] in this region detected only one SNP. In the promoter region, we identified 11 SNPs and 9 of which are common with those found by Calvo et al. [10]. The latter authors, in the Raza Aragonesa, also identify 4 other SNPs that we did not find in the Sarda breed. Analysis of the data showed that the SNP16 (612/MnlI) is always linked to SNP17 and these were associated with an advance of reproductive recovery in both years. Until now we always referred to the SNP in position rs430181568 (SNP16 in this study) but, since the mutation in position rs407388227 (SNP17 in this study) determines an amino acid change, it is logical to take this last SNP into consideration for the effects on the reproductive activity recorded. This amino acid change (Val > Ile) occurs in position 220 of the protein chain (GenBank accession number AAB17721.1), the fifth transmembrane domain (TM5) [26]. TM5 is a crucial site for the melatonin receptor 1a function. Mutations, within this domain, that lead to amino acid change, could determine important variation in receptor signalling [27]. Indeed, modifications in amino acid chain at positions 195, 208 and 211 were reported as responsible for modifying the binding capacity of the melatonin receptor [28]. In addition, significant differences in cAMP inhibition were observed between ovine MT1 Val 220 and Ile 220 [15], thus suggesting a potential modification in the interpretation of the melatonin signal in sheep with different genotypes at SNP17. These differences of melatonin signal perception could participate to the phenotypic differences in reproductive resumption observed in this study. In fact, ewes carrying genotype T/T delayed the onset of the reproductive activity compared to C/C and T/C genotypes. This fact, as evidenced by the different DRIL we recorded in the two years in the different genotypes, can be linked to a different sensitivity to photoperiod. Also considering the mating trend in both the years the genotypes affected the trigger of reproductive activity. Indeed, the highest number of the ewes carrying C/C genotype mated in the first twenty days after rams introduction. Still ewes with the T/C genotype have more than half of the mating within 30 days from the ram introduction.

Melatonin synthesis by the ovary has been found confirming the importance of this hormone in the ovarian activity [29,30]. Furthermore, melatonin receptors have been detected in the granulosa cells, that are in direct contact with oocytes, suggesting that this hormone may influence the follicle development [29]. Follicles collected by melatonin treated ewes displayed a greater development in culture than those from untreated sheep [31]. In addition, melatonin modulates the expression of genes involved in steroidogenesis, in the conversion of progesterone to androgens, and in the luteinisation of granulosa cells [32,33]. Reducing free radicals in the theca, granulosa and corpus luteum cells, melatonin displayed a clear antioxidant effect also maintaining healthy follicles and corpora lutea [34,35]. Considering that the different MTNR1A genotypes affect the melatonin signal transmission it can be hypothesized that C/C ewes were less sensitive to photoperiod thus considerably advancing their reproductive recovery compared to T/T genotype. In fact, the sheep carrying the C/C or T/C genotype responded earlier to the ram effect as if they were in a less deep state of anoestrous compared to those of the T/T genotype. Haplotype 1 showed the highest frequency and together with H6 exhibited an association with the shortest DRIL. This fact, is linked to the presence of the C allele in SNP16 and SNP17. These SNPs, which as mentioned above, are totally linked and are considered responsible of the improved reproductive performance.

At the end of the observation (250 days from ram introduction), the total fertility rate registered showed no association with different polymorphisms and haplotypes in both years. However, evaluating the lambing trend, we can evidence considerable difference according to genotypes in the number of lambed ewes during the observed periods. In fact, in both years,160 and 170 days after ram introduction, a markedly higher number of ewes carrying the C/C and T/C genotypes lambed compared to those T/T. This means that the largest number of C/C and T/C ewes mated in the first 20 days from ram introduction suggesting that their anoestrous state was shallower than in T/T ewes. Instead, ewes with the T/T genotype showed the mating peak 30 days later than other genotypes, indicating that T/T ewes needed of a longer stimulus to onset the reproductive activity, so that they surely were in a deeper anoestrous state. In fact, one of the most important factors responsible for the response to ram effect is the "depth of anoestrous" that influence the proportion of cycling ewes at ram introduction [36]. It should be considered that the presence of cyclic ewes is capable of inducing ovulation in the other ewes of the flock advancing the onset of the reproductive resumption and this effect is known as "female-female effect" [36]. Therefore, all these factors could influence the final fertility rate hiding the differences among genotypes because the considered period is long enough so that also T/T ewes, although later than the other genotypes, mated and lambed.

Conclusion

This study provided the most important part of the nucleotide sequence of the MTNR1A gene in Sarda sheep breed. Twenty-nine polymorphic sites were identified and two of them, SNP16 and SNP17, were totally linked. Moreover, these SNPs displayed a relationship with reproductive resumption as the C/C ewes showed a shorter DRIL compared to the other genotypes. These data highlight the crucial role of this polymorphism in regulating the reproductive recovery in Sarda sheep and provides a valid tool to improve genetic selection.

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Figures

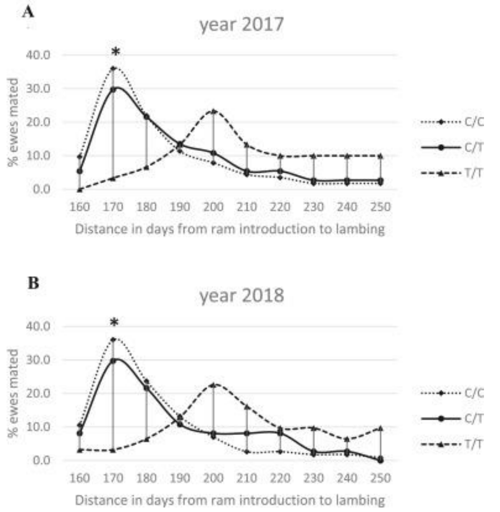

Fig. 1. Graphical representation of the percentage of mated ewes, measured at 10 days intervals, from 150 to 250 days after ram introduction according to genotypes at position g.17,355,358 (Oar_Rambouillet_v1.0, RefSeq assembly accession GCF_002742125.1). Box A referred to year 2017; Box B referred to year 2018. ∗ Indicates statistical differences for P < 0.05.

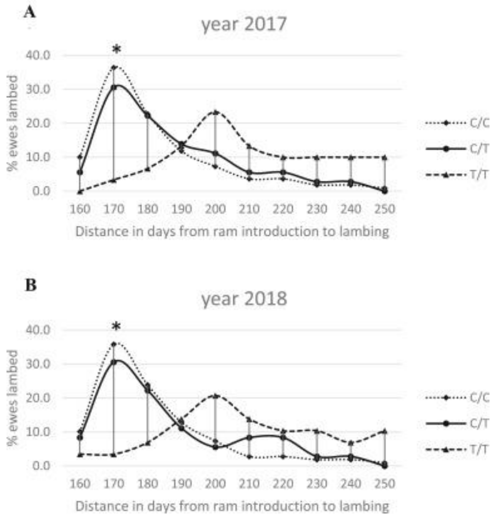

Fig. 2. Graphical representation of the percentage of lambed ewes, measured at 10 days intervals, from 150 to 250 days after ram introduction according to genotypes at position g.17,355,358 (Oar_Rambouillet_v1.0, RefSeq assembly accession GCF_002742125.1).

Tables

Table 1. SNPs, genotype and allele frequency, Hardy Weinberg equilibrium, locus and amino acid (AA) change in MTNR1A gene. The sequence is in a reverse orientation on the genome, and the SNPs are ordered according to their positions in the latest sheep genome version (Oar_Rambouillet_v1.0, GCF_002742125.1).

Alias	SNP ID	Position on Oar_rambouillet_v1.0	Genotype	Genotype frequency	Allele	Allelic frequency	Hardy Weinberg	MTNR1A	AA change
SNP1	rs409468184	17379432	GG	65.2	G	0.787	0.052	Promoter	-
			GA	27.0	A	0.213			
			AA	7.8					
SNP2	rs428941001	17379252	CC	66.7	C	0.80	0.096	Promoter	-
			CA	26.7	A	0.20			
			AA	6.7					
SNP3	rs405080439	17379097	GG	67.0	G	0.80	0.092	Promoter	-
			GA	26.4	A	0.20			
			AA	6.6					
SNP4	rs428880789	17379083	GG	64.2	G	0.79	0.150	Promoter	-
			GA	28.8	A	0.21			
			AA	7.0					
SNP5	rs415456480	17379037	GG	63.9	G	0.78	0.051	Promoter	-
			GA	27.8	A	0.22			
			AA	8.3					
SNP6	rs411931887	17378874	CC	69.1	C	0.81	0.061	Promoter	-
			CT	24.6	T	0.19			
			TT	6.3					
SNP7	rs402949406	17378871	CC	6.8	C	0.20	0.086	Promoter	-
			CA	26.6	A	0.80			
			AA	66.6					
SNP8	rs426266687	17378842	CC	67.5	C	0.80	0.077	Promoter	-
			CT	25.9	T	0.20			
			TT	6.6					
SNP9	rs399461430	17378769	CC	6.7	C	0.23	0.494	Promoter	-
			CT	33.3	T	0.77			
			TT	60.0					
SNP10	rs400561563	17378728	CC	26.7	C	0.53	0.475	Promoter	-
			CT	53.3	T	0.47			
			TT	20.0					
SNP11	rs419743392	17378706	GG	63.0	G	0.78	0.264	Promoter	-
			GA	30.3	A	0.22			
			AA	6.7					
SNP12	-	17377677	GG	65.0	G	0.80	0.270	Intron	-
			GA	29.0	A	0.21			
			AA	6.0					
SNP13	rs419680097	17355611	CC	26.7	C	0.50	0.505	Exon 2	-
			CA	46.7	A	0.50			
			AA	26.7					
SNP14	ss2137144055	17355542	CC	86.7	C	0.93	0.475	Exon 2	-
			CT	13.3	T	0.07			
			TT	0.0					

SNP15	rs406779174	17355458	GG	53.3	G	0.73	0.820	Exon 2	-
			GA	40.0	A	0.27			
			AA	6.7					
SNP16	rs430181568	17355452	CC	62.1	C	0.72	0.000001	Exon 2	-
			CT	20.2	T	0.28			
			TT	17.7					
SNP17	rs407388227	17355358	CC	62.1	C	0.72	0.000001	Exon 2	Ile/Val
			CT	20.2	T	0.28			
			TT	17.7					
SNP18	rs427019119	17355281	CC	68.0	C	0.82	0.295	Exon 2	-
			CT	27.0	T	0.19			
			TT	5.0					
SNP19	rs417800445	17355263	CC	66.6	C	0.80	0.078	Exon 2	-
			CT	26.5	T	0.20			
			TT	6.9					
SNP20	rs429718221	17355173	GG	11.4	G	0.29	0.146	Exon 2	-
			GA	35.2	A	0.71			
			AA	53.4					
SNP21	rs416266900	17355171	GG	61.0	G	0.76	0.134	Exon 2	Ala/Asp
			GT	30.7	T	0.24			
			TT	8.3					
SNP22	rs403212791	17354971	GG	86.7	G	0.93	0.475	Exon 2	Cys/Arg
			GA	13.3	A	0.07			
			AA	0.0					
SNP23	rs426523476	17354963	GG	9.4	G	0.28	0.447	Exon 2	-
			GA	37.3	A	0.72			
			AA	53.3					
SNP24	rs413084140	17354943	CC	54.0	C	0.71	0.053	Exon 2	His/Arg
			CT	33.4	T	0.29			
			TT	12.6					
SNP25	rs403826495	17354935	CC	55.5	C	0.73	0.262	Exon 2	Ile/Val
			CT	35.0	T	0.27			
			TT	9.5					
SNP26	rs423194759	17354883	CC	10.0	C	0.26	0.102	3’Utr	-
			CT	32.3	T	0.74			
			TT	57.7					
SNP27	rs414185743	17354835	CC	52.0	C	0.70	0.153	3’Utr	-
			CT	36.0	T	0.30			
			TT	12.0					
SNP28	rs400830807	17354827	GG	85.4	G	0.93	0.431	3’Utr	-
			GA	14.6	A	0.07			
			AA	0.0					
SNP29	rs420016236	17354824	CC	68.0	C	0.81	0.060	3’Utr	-
			CT	25.3	T	0.19			
			TT	6.7					

Table 2. Litter size, fertility and DRIL for each genotype during the two years analysed. The data are expressed as LSmeans

Year	**Genotype**	**Litter size**	**Fertility rate**	**DRIL**
2017	C/C	1.2	0.88	171+19.1[a]
	C/T	1.1	0.87	185+19.2[b]
	T/T	1.1	0.87	201+22.4[b]
2018	C/C	1.3	0.89	170+18.5[a]
	C/T	1.2	0.88	183+20.6[b]
	T/T	1.1	0.87	202+23.9[b]

DRIL= distance in days from ram introduction to lambing;
Different lower-case letters (a, b) indicate differences for *P* ≤ 0.05

Table 3 - Haplotypes and their frequency

Haplocode	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**haplotype frequency**
H1	G	C	G	G	G	C	A	C	T	T	G	G	A	C	G	C	C	C	C	A	G	G	A	C	C	T	C	G	C	0.496
H2	G	C	G	G	G	C	A	C	T	C	G	G	C	C	G	T	T	C	C	G	G	G	G	T	T	C	T	G	C	0.203
H3	A	A	A	A	A	T	C	T	C	C	A	G	C	C	A	T	T	T	T	A	T	G	A	C	C	T	C	G	T	0.133
H4	G	C	G	G	G	C	A	C	T	C	G	G	C	T	A	T	T	C	C	G	G	A	G	T	T	C	T	A	C	0.066
H5	A	A	A	A	A	T	C	T	C	C	A	A	C	C	A	T	T	T	T	A	T	G	A	C	C	T	C	G	T	0.066
H6	G	C	G	G	G	C	A	C	C	C	A	G	A	C	G	C	C	C	C	A	G	G	A	C	C	T	C	G	C	0.063

Supplementary Table 1

Linkage disequilibrium values between the different SNPs obtained by Haploview software:
L1 and L2 are the two compared loci
D' is the value of LD between the two compared SNPs.
LOD is the log of the likelihood odds ratio, a measure of confidence in the value of D'
r2 is the correlation coefficient between the two compared SNPs
CIlow is 95% confidence lower bound on D'
CIhi is the 95% confidence upper bound on D'
Dist is the distance (in bases) between the compared SNPs
T-int is a statistic used by the HapMap Project to measure the completeness of information represented by a set of markers in a region

L1	L2	D'	LOD	r^2	CIlow	CIhi	Dist	T-int
SNP29	SNP28	1.0	5.32	1.0	0.71	1.0	3	26.6
SNP29	SNP27	1.0	5.32	1.0	0.71	1.0	11	-
SNP29	SNP26	1.0	5.32	1.0	0.71	1.0	59	-
SNP29	SNP25	1.0	5.32	1.0	0.71	1.0	111	-
SNP29	SNP24	1.0	5.32	1.0	0.71	1.0	119	-
SNP29	SNP23	1.0	5.32	1.0	0.71	1.0	139	-
SNP29	SNP22	1.0	5.32	1.0	0.71	1.0	147	-
SNP29	SNP21	1.0	4.07	0.821	0.62	1.0	347	-
SNP29	SNP20	1.0	1.02	0.219	0.16	0.99	349	-
SNP29	SNP19	1.0	4.07	0.821	0.62	1.0	439	-
SNP29	SNP18	1.0	3.79	0.8	0.58	1.0	457	-
SNP29	SNP17	1.0	1.23	0.25	0.2	1.0	534	-
SNP29	SNP16	1.0	0.2	0.018	0.05	0.97	628	-
SNP29	SNP15	1.0	3.36	0.687	0.56	1.0	634	-
SNP29	SNP14	1.0	1.23	0.25	0.2	1.0	718	-
SNP29	SNP13	1.0	1.23	0.25	0.2	1.0	787	-
SNP29	SNP12	1.0	5.32	1.0	0.71	1.0	22853	-
SNP29	SNP11	1.0	5.32	1.0	0.71	1.0	23882	-
SNP29	SNP10	1.0	0.76	0.107	0.11	0.99	23904	-
SNP29	SNP9	1.0	5.32	1.0	0.71	1.0	23945	-
SNP29	SNP8	1.0	0.2	0.018	0.05	0.97	24018	-
SNP29	SNP7	1.0	0.76	0.107	0.11	0.99	24047	-
SNP29	SNP6	1.0	0.76	0.107	0.11	0.99	24050	-
SNP29	SNP5	1.0	0.76	0.107	0.11	0.99	24213	-
SNP29	SNP4	1.0	0.76	0.107	0.11	0.99	24259	-
SNP29	SNP3	1.0	0.76	0.107	0.11	0.99	24273	-
SNP29	SNP2	1.0	0.2	0.018	0.05	0.97	24428	-
SNP29	SNP1	1.0	5.32	1.0	0.71	1.0	24608	-
SNP28	SNP27	1.0	5.32	1.0	0.71	1.0	8	53.2
SNP28	SNP26	1.0	5.32	1.0	0.71	1.0	56	-
SNP28	SNP25	1.0	5.32	1.0	0.71	1.0	108	-
SNP28	SNP24	1.0	5.32	1.0	0.71	1.0	116	-
SNP28	SNP23	1.0	5.32	1.0	0.71	1.0	136	-
SNP28	SNP22	1.0	5.32	1.0	0.71	1.0	144	-
SNP28	SNP21	1.0	4.07	0.821	0.62	1.0	344	-
SNP28	SNP20	1.0	1.02	0.219	0.16	0.99	346	-
SNP28	SNP19	1.0	4.07	0.821	0.62	1.0	436	-
SNP28	SNP18	1.0	3.79	0.8	0.58	1.0	454	-
SNP28	SNP17	1.0	1.23	0.25	0.2	1.0	531	-
SNP28	SNP16	1.0	0.2	0.018	0.05	0.97	625	-
SNP28	SNP15	1.0	3.36	0.687	0.56	1.0	631	-
SNP28	SNP14	1.0	1.23	0.25	0.2	1.0	715	-
SNP28	SNP13	1.0	1.23	0.25	0.2	1.0	784	-
SNP28	SNP12	1.0	5.32	1.0	0.71	1.0	22850	-
SNP28	SNP11	1.0	5.32	1.0	0.71	1.0	23879	-
SNP28	SNP10	1.0	0.76	0.107	0.11	0.99	23901	-
SNP28	SNP9	1.0	5.32	1.0	0.71	1.0	23942	-
SNP28	SNP8	1.0	0.2	0.018	0.05	0.97	24015	-
SNP28	SNP7	1.0	0.76	0.107	0.11	0.99	24044	-
SNP28	SNP6	1.0	0.76	0.107	0.11	0.99	24047	-

SNP28	SNP5	1.0	0.76	0.107	0.11	0.99	24210	-
SNP28	SNP4	1.0	0.76	0.107	0.11	0.99	24256	-
SNP28	SNP3	1.0	0.76	0.107	0.11	0.99	24270	-
SNP28	SNP2	1.0	0.2	0.018	0.05	0.97	24425	-
SNP28	SNP1	1.0	5.32	1.0	0.71	1.0	24605	-
SNP27	SNP26	1.0	5.32	1.0	0.71	1.0	48	79.8
SNP27	SNP25	1.0	5.32	1.0	0.71	1.0	100	-
SNP27	SNP24	1.0	5.32	1.0	0.71	1.0	108	-
SNP27	SNP23	1.0	5.32	1.0	0.71	1.0	128	-
SNP27	SNP22	1.0	5.32	1.0	0.71	1.0	136	-
SNP27	SNP21	1.0	4.07	0.821	0.62	1.0	336	-
SNP27	SNP20	1.0	1.02	0.219	0.16	0.99	338	-
SNP27	SNP19	1.0	4.07	0.821	0.62	1.0	428	-
SNP27	SNP18	1.0	3.79	0.8	0.58	1.0	446	-
SNP27	SNP17	1.0	1.23	0.25	0.2	1.0	523	-
SNP27	SNP16	1.0	0.2	0.018	0.05	0.97	617	-
SNP27	SNP15	1.0	3.36	0.687	0.56	1.0	623	-
SNP27	SNP14	1.0	1.23	0.25	0.2	1.0	707	-
SNP27	SNP13	1.0	1.23	0.25	0.2	1.0	776	-
SNP27	SNP12	1.0	5.32	1.0	0.71	1.0	22842	-
SNP27	SNP11	1.0	5.32	1.0	0.71	1.0	23871	-
SNP27	SNP10	1.0	0.76	0.107	0.11	0.99	23893	-
SNP27	SNP9	1.0	5.32	1.0	0.71	1.0	23934	-
SNP27	SNP8	1.0	0.2	0.018	0.05	0.97	24007	-
SNP27	SNP7	1.0	0.76	0.107	0.11	0.99	24036	-
SNP27	SNP6	1.0	0.76	0.107	0.11	0.99	24039	-
SNP27	SNP5	1.0	0.76	0.107	0.11	0.99	24202	-
SNP27	SNP4	1.0	0.76	0.107	0.11	0.99	24248	-
SNP27	SNP3	1.0	0.76	0.107	0.11	0.99	24262	-
SNP27	SNP2	1.0	0.2	0.018	0.05	0.97	24417	-
SNP27	SNP1	1.0	5.32	1.0	0.71	1.0	24597	-
SNP26	SNP25	1.0	5.32	1.0	0.71	1.0	52	101.4
SNP26	SNP24	1.0	5.32	1.0	0.71	1.0	60	-
SNP26	SNP23	1.0	5.32	1.0	0.71	1.0	80	-
SNP26	SNP22	1.0	5.32	1.0	0.71	1.0	88	-
SNP26	SNP21	1.0	4.07	0.821	0.62	1.0	288	-
SNP26	SNP20	1.0	1.02	0.219	0.16	0.99	290	-
SNP26	SNP19	1.0	4.07	0.821	0.62	1.0	380	-
SNP26	SNP18	1.0	3.79	0.8	0.58	1.0	398	-
SNP26	SNP17	1.0	1.23	0.25	0.2	1.0	475	-
SNP26	SNP16	1.0	0.2	0.018	0.05	0.97	569	-
SNP26	SNP15	1.0	3.36	0.687	0.56	1.0	575	-
SNP26	SNP14	1.0	1.23	0.25	0.2	1.0	659	-
SNP26	SNP13	1.0	1.23	0.25	0.2	1.0	728	-
SNP26	SNP12	1.0	5.32	1.0	0.71	1.0	22794	-
SNP26	SNP11	1.0	5.32	1.0	0.71	1.0	23823	-
SNP26	SNP10	1.0	0.76	0.107	0.11	0.99	23845	-
SNP26	SNP9	1.0	5.32	1.0	0.71	1.0	23886	-
SNP26	SNP8	1.0	0.2	0.018	0.05	0.97	23959	-
SNP26	SNP7	1.0	0.76	0.107	0.11	0.99	23988	-
SNP26	SNP6	1.0	0.76	0.107	0.11	0.99	23991	-
SNP26	SNP5	1.0	0.76	0.107	0.11	0.99	24154	-
SNP26	SNP4	1.0	0.76	0.107	0.11	0.99	24200	-
SNP26	SNP3	1.0	0.76	0.107	0.11	0.99	24214	-
SNP26	SNP2	1.0	0.2	0.018	0.05	0.97	24369	-
SNP26	SNP1	1.0	5.32	1.0	0.71	1.0	24549	-
SNP25	SNP24	1.0	5.32	1.0	0.71	1.0	8	105.25
SNP25	SNP23	1.0	5.32	1.0	0.71	1.0	28	-
SNP25	SNP22	1.0	5.32	1.0	0.71	1.0	36	-
SNP25	SNP21	1.0	4.07	0.821	0.62	1.0	236	-
SNP25	SNP20	1.0	1.02	0.219	0.16	0.99	238	-
SNP25	SNP19	1.0	4.07	0.821	0.62	1.0	328	-
SNP25	SNP18	1.0	3.79	0.8	0.58	1.0	346	-
SNP25	SNP17	1.0	1.23	0.25	0.2	1.0	423	-
SNP25	SNP16	1.0	0.2	0.018	0.05	0.97	517	-

SNP25	SNP15	1.0	3.36	0.687	0.56	1.0	523	-
SNP25	SNP14	1.0	1.23	0.25	0.2	1.0	607	-
SNP25	SNP13	1.0	1.23	0.25	0.2	1.0	676	-
SNP25	SNP12	1.0	5.32	1.0	0.71	1.0	22742	-
SNP25	SNP11	1.0	5.32	1.0	0.71	1.0	23771	-
SNP25	SNP10	1.0	0.76	0.107	0.11	0.99	23793	-
SNP25	SNP9	1.0	5.32	1.0	0.71	1.0	23834	-
SNP25	SNP8	1.0	0.2	0.018	0.05	0.97	23907	-
SNP25	SNP7	1.0	0.76	0.107	0.11	0.99	23936	-
SNP25	SNP6	1.0	0.76	0.107	0.11	0.99	23939	-
SNP25	SNP5	1.0	0.76	0.107	0.11	0.99	24102	-
SNP25	SNP4	1.0	0.76	0.107	0.11	0.99	24148	-
SNP25	SNP3	1.0	0.76	0.107	0.11	0.99	24162	-
SNP25	SNP2	1.0	0.2	0.018	0.05	0.97	24317	-
SNP25	SNP1	1.0	5.32	1.0	0.71	1.0	24497	-
SNP24	SNP23	1.0	5.32	1.0	0.71	1.0	20	99.0
SNP24	SNP22	1.0	5.32	1.0	0.71	1.0	28	-
SNP24	SNP21	1.0	4.07	0.821	0.62	1.0	228	-
SNP24	SNP20	1.0	1.02	0.219	0.16	0.99	230	-
SNP24	SNP19	1.0	4.07	0.821	0.62	1.0	320	-
SNP24	SNP18	1.0	3.79	0.8	0.58	1.0	338	-
SNP24	SNP17	1.0	1.23	0.25	0.2	1.0	415	-
SNP24	SNP16	1.0	0.2	0.018	0.05	0.97	509	-
SNP24	SNP15	1.0	3.36	0.687	0.56	1.0	515	-
SNP24	SNP14	1.0	1.23	0.25	0.2	1.0	599	-
SNP24	SNP13	1.0	1.23	0.25	0.2	1.0	668	-
SNP24	SNP12	1.0	5.32	1.0	0.71	1.0	22734	-
SNP24	SNP11	1.0	5.32	1.0	0.71	1.0	23763	-
SNP24	SNP10	1.0	0.76	0.107	0.11	0.99	23785	-
SNP24	SNP9	1.0	5.32	1.0	0.71	1.0	23826	-
SNP24	SNP8	1.0	0.2	0.018	0.05	0.97	23899	-
SNP24	SNP7	1.0	0.76	0.107	0.11	0.99	23928	-
SNP24	SNP6	1.0	0.76	0.107	0.11	0.99	23931	-
SNP24	SNP5	1.0	0.76	0.107	0.11	0.99	24094	-
SNP24	SNP4	1.0	0.76	0.107	0.11	0.99	24140	-
SNP24	SNP3	1.0	0.76	0.107	0.11	0.99	24154	-
SNP24	SNP2	1.0	0.2	0.018	0.05	0.97	24309	-
SNP24	SNP1	1.0	5.32	1.0	0.71	1.0	24489	-
SNP23	SNP22	1.0	5.32	1.0	0.71	1.0	8	91.35
SNP23	SNP21	1.0	4.07	0.821	0.62	1.0	208	-
SNP23	SNP20	1.0	1.02	0.219	0.16	0.99	210	-
SNP23	SNP19	1.0	4.07	0.821	0.62	1.0	300	-
SNP23	SNP18	1.0	3.79	0.8	0.58	1.0	318	-
SNP23	SNP17	1.0	1.23	0.25	0.2	1.0	395	-
SNP23	SNP16	1.0	0.2	0.018	0.05	0.97	489	-
SNP23	SNP15	1.0	3.36	0.687	0.56	1.0	495	-
SNP23	SNP14	1.0	1.23	0.25	0.2	1.0	579	-
SNP23	SNP13	1.0	1.23	0.25	0.2	1.0	648	-
SNP23	SNP12	1.0	5.32	1.0	0.71	1.0	22714	-
SNP23	SNP11	1.0	5.32	1.0	0.71	1.0	23743	-
SNP23	SNP10	1.0	0.76	0.107	0.11	0.99	23765	-
SNP23	SNP9	1.0	5.32	1.0	0.71	1.0	23806	-
SNP23	SNP8	1.0	0.2	0.018	0.05	0.97	23879	-
SNP23	SNP7	1.0	0.76	0.107	0.11	0.99	23908	-
SNP23	SNP6	1.0	0.76	0.107	0.11	0.99	23911	-
SNP23	SNP5	1.0	0.76	0.107	0.11	0.99	24074	-
SNP23	SNP4	1.0	0.76	0.107	0.11	0.99	24120	-
SNP23	SNP3	1.0	0.76	0.107	0.11	0.99	24134	-
SNP23	SNP2	1.0	0.2	0.018	0.05	0.97	24289	-
SNP23	SNP1	1.0	5.32	1.0	0.71	1.0	24469	-
SNP22	SNP21	1.0	4.07	0.821	0.62	1.0	200	70.9
SNP22	SNP20	1.0	1.02	0.219	0.16	0.99	202	-
SNP22	SNP19	1.0	4.07	0.821	0.62	1.0	292	-
SNP22	SNP18	1.0	3.79	0.8	0.58	1.0	310	-
SNP22	SNP17	1.0	1.23	0.25	0.2	1.0	387	-

SNP22	SNP16	1.0	0.2	0.018	0.05	0.97	481	-
SNP22	SNP15	1.0	3.36	0.687	0.56	1.0	487	-
SNP22	SNP14	1.0	1.23	0.25	0.2	1.0	571	-
SNP22	SNP13	1.0	1.23	0.25	0.2	1.0	640	-
SNP22	SNP12	1.0	5.32	1.0	0.71	1.0	22706	-
SNP22	SNP11	1.0	5.32	1.0	0.71	1.0	23735	-
SNP22	SNP10	1.0	0.76	0.107	0.11	0.99	23757	-
SNP22	SNP9	1.0	5.32	1.0	0.71	1.0	23798	-
SNP22	SNP8	1.0	0.2	0.018	0.05	0.97	23871	-
SNP22	SNP7	1.0	0.76	0.107	0.11	0.99	23900	-
SNP22	SNP6	1.0	0.76	0.107	0.11	0.99	23903	-
SNP22	SNP5	1.0	0.76	0.107	0.11	0.99	24066	-
SNP22	SNP4	1.0	0.76	0.107	0.11	0.99	24112	-
SNP22	SNP3	1.0	0.76	0.107	0.11	0.99	24126	-
SNP22	SNP2	1.0	0.2	0.018	0.05	0.97	24281	-
SNP22	SNP1	1.0	5.32	1.0	0.71	1.0	24461	-
SNP21	SNP20	1.0	1.11	0.266	0.18	1.0	2	51.67
SNP21	SNP19	1.0	5.57	1.0	0.74	1.0	92	-
SNP21	SNP18	1.0	3.15	0.657	0.52	1.0	110	-
SNP21	SNP17	0.58	0.36	0.102	0.06	0.89	187	-
SNP21	SNP16	1.0	0.24	0.022	0.05	0.98	281	-
SNP21	SNP15	0.792	2.18	0.524	0.36	0.94	287	-
SNP21	SNP14	0.58	0.36	0.102	0.06	0.89	371	-
SNP21	SNP13	0.58	0.36	0.102	0.06	0.89	440	-
SNP21	SNP12	1.0	4.07	0.821	0.62	1.0	22506	-
SNP21	SNP11	1.0	4.07	0.821	0.62	1.0	23535	-
SNP21	SNP10	1.0	0.97	0.13	0.15	0.99	23557	-
SNP21	SNP9	1.0	4.07	0.821	0.62	1.0	23598	-
SNP21	SNP8	1.0	0.24	0.022	0.05	0.98	23671	-
SNP21	SNP7	1.0	0.97	0.13	0.15	0.99	23700	-
SNP21	SNP6	1.0	0.97	0.13	0.15	0.99	23703	-
SNP21	SNP5	1.0	0.97	0.13	0.15	0.99	23866	-
SNP21	SNP4	1.0	0.97	0.13	0.15	0.99	23912	-
SNP21	SNP3	1.0	0.97	0.13	0.15	0.99	23926	-
SNP21	SNP2	1.0	0.24	0.022	0.05	0.98	24081	-
SNP21	SNP1	1.0	4.07	0.821	0.62	1.0	24261	-
SNP20	SNP19	1.0	1.11	0.266	0.18	1.0	90	58.32
SNP20	SNP18	1.0	0.65	0.175	0.1	0.99	108	-
SNP20	SNP17	1.0	5.3	0.875	0.73	1.0	185	-
SNP20	SNP16	1.0	0.27	0.062	0.06	0.98	279	-
SNP20	SNP15	1.0	1.54	0.318	0.27	1.0	285	-
SNP20	SNP14	1.0	5.3	0.875	0.73	1.0	369	-
SNP20	SNP13	1.0	5.3	0.875	0.73	1.0	438	-
SNP20	SNP12	1.0	1.02	0.219	0.16	0.99	22504	-
SNP20	SNP11	1.0	1.02	0.219	0.16	0.99	23533	-
SNP20	SNP10	1.0	1.99	0.375	0.37	1.0	23555	-
SNP20	SNP9	1.0	1.02	0.219	0.16	0.99	23596	-
SNP20	SNP8	1.0	0.27	0.062	0.06	0.98	23669	-
SNP20	SNP7	1.0	1.99	0.375	0.37	1.0	23698	-
SNP20	SNP6	1.0	1.99	0.375	0.37	1.0	23701	-
SNP20	SNP5	1.0	1.99	0.375	0.37	1.0	23864	-
SNP20	SNP4	1.0	1.99	0.375	0.37	1.0	23910	-
SNP20	SNP3	1.0	1.99	0.375	0.37	1.0	23924	-
SNP20	SNP2	1.0	0.27	0.062	0.06	0.98	24079	-
SNP20	SNP1	1.0	1.02	0.219	0.16	0.99	24259	-
SNP19	SNP18	1.0	3.15	0.657	0.52	1.0	18	45.26
SNP19	SNP17	0.58	0.36	0.102	0.06	0.89	95	-
SNP19	SNP16	1.0	0.24	0.022	0.05	0.98	189	-
SNP19	SNP15	0.792	2.18	0.524	0.36	0.94	195	-
SNP19	SNP14	0.58	0.36	0.102	0.06	0.89	279	-
SNP19	SNP13	0.58	0.36	0.102	0.06	0.89	348	-
SNP19	SNP12	1.0	4.07	0.821	0.62	1.0	22414	-
SNP19	SNP11	1.0	4.07	0.821	0.62	1.0	23443	-
SNP19	SNP10	1.0	0.97	0.13	0.15	0.99	23465	-
SNP19	SNP9	1.0	4.07	0.821	0.62	1.0	23506	-

SNP19	SNP8	1.0	0.24	0.022	0.05	0.98	23579	-
SNP19	SNP7	1.0	0.97	0.13	0.15	0.99	23608	-
SNP19	SNP6	1.0	0.97	0.13	0.15	0.99	23611	-
SNP19	SNP5	1.0	0.97	0.13	0.15	0.99	23774	-
SNP19	SNP4	1.0	0.97	0.13	0.15	0.99	23820	-
SNP19	SNP3	1.0	0.97	0.13	0.15	0.99	23834	-
SNP19	SNP2	1.0	0.24	0.022	0.05	0.98	23989	-
SNP19	SNP1	1.0	4.07	0.821	0.62	1.0	24169	-
SNP18	SNP17	1.0	0.82	0.2	0.12	0.99	77	37.25
SNP18	SNP16	1.0	0.16	0.014	0.05	0.97	171	-
SNP18	SNP15	1.0	2.67	0.55	0.47	1.0	177	-
SNP18	SNP14	1.0	0.82	0.2	0.12	0.99	261	-
SNP18	SNP13	1.0	0.82	0.2	0.12	0.99	330	-
SNP18	SNP12	1.0	3.79	0.8	0.58	1.0	22396	-
SNP18	SNP11	1.0	3.79	0.8	0.58	1.0	23425	-
SNP18	SNP10	1.0	0.86	0.086	0.13	0.99	23447	-
SNP18	SNP9	1.0	3.79	0.8	0.58	1.0	23488	-
SNP18	SNP8	1.0	0.16	0.014	0.05	0.97	23561	-
SNP18	SNP7	1.0	0.86	0.086	0.13	0.99	23590	-
SNP18	SNP6	1.0	0.86	0.086	0.13	0.99	23593	-
SNP18	SNP5	1.0	0.86	0.086	0.13	0.99	23756	-
SNP18	SNP4	1.0	0.86	0.086	0.13	0.99	23802	-
SNP18	SNP3	1.0	0.86	0.086	0.13	0.99	23816	-
SNP18	SNP2	1.0	0.16	0.014	0.05	0.97	23971	-
SNP18	SNP1	1.0	3.79	0.8	0.58	1.0	24151	-
SNP17	SNP16	1.0	0.33	0.071	0.06	0.98	94	53.36
SNP17	SNP15	1.0	1.85	0.364	0.34	1.0	100	-
SNP17	SNP14	1.0	6.92	1.0	0.8	1.0	184	-
SNP17	SNP13	1.0	6.92	1.0	0.8	1.0	253	-
SNP17	SNP12	1.0	1.23	0.25	0.2	1.0	22319	-
SNP17	SNP11	1.0	1.23	0.25	0.2	1.0	23348	-
SNP17	SNP10	1.0	2.37	0.429	0.44	1.0	23370	-
SNP17	SNP9	1.0	1.23	0.25	0.2	1.0	23411	-
SNP17	SNP8	1.0	0.33	0.071	0.06	0.98	23484	-
SNP17	SNP7	1.0	2.37	0.429	0.44	1.0	23513	-
SNP17	SNP6	1.0	2.37	0.429	0.44	1.0	23516	-
SNP17	SNP5	1.0	2.37	0.429	0.44	1.0	23679	-
SNP17	SNP4	1.0	2.37	0.429	0.44	1.0	23725	-
SNP17	SNP3	1.0	2.37	0.429	0.44	1.0	23739	-
SNP17	SNP2	1.0	0.33	0.071	0.06	0.98	23894	-
SNP17	SNP1	1.0	1.23	0.25	0.2	1.0	24074	-
SNP16	SNP15	1.0	0.64	0.196	0.1	0.99	6	56.96
SNP16	SNP14	1.0	0.33	0.071	0.06	0.98	90	-
SNP16	SNP13	1.0	0.33	0.071	0.06	0.98	159	-
SNP16	SNP12	1.0	0.2	0.018	0.05	0.97	22225	-
SNP16	SNP11	1.0	0.2	0.018	0.05	0.97	23254	-
SNP16	SNP10	1.0	0.82	0.167	0.13	0.99	23276	-
SNP16	SNP9	1.0	0.2	0.018	0.05	0.97	23317	-
SNP16	SNP8	1.0	2.59	1.0	0.42	1.0	23390	-
SNP16	SNP7	1.0	0.82	0.167	0.13	0.99	23419	-
SNP16	SNP6	1.0	0.82	0.167	0.13	0.99	23422	-
SNP16	SNP5	1.0	0.82	0.167	0.13	0.99	23585	-
SNP16	SNP4	1.0	0.82	0.167	0.13	0.99	23631	-
SNP16	SNP3	1.0	0.82	0.167	0.13	0.99	23645	-
SNP16	SNP2	1.0	2.59	1.0	0.42	1.0	23800	-
SNP16	SNP1	1.0	0.2	0.018	0.05	0.97	23980	-
SNP15	SNP14	1.0	1.85	0.364	0.34	1.0	84	50.93
SNP15	SNP13	1.0	1.85	0.364	0.34	1.0	153	-
SNP15	SNP12	1.0	3.36	0.687	0.56	1.0	22219	-
SNP15	SNP11	1.0	3.36	0.687	0.56	1.0	23248	-
SNP15	SNP10	0.289	0.05	0.013	0.03	0.84	23270	-
SNP15	SNP9	1.0	3.36	0.687	0.56	1.0	23311	-
SNP15	SNP8	1.0	0.64	0.196	0.1	0.99	23384	-
SNP15	SNP7	0.289	0.05	0.013	0.03	0.84	23413	-
SNP15	SNP6	0.289	0.05	0.013	0.03	0.84	23416	-

SNP15	SNP5	0.289	0.05	0.013	0.03	0.84	23579	-
SNP15	SNP4	0.289	0.05	0.013	0.03	0.84	23625	-
SNP15	SNP3	0.289	0.05	0.013	0.03	0.84	23639	-
SNP15	SNP2	1.0	0.64	0.196	0.1	0.99	23794	-
SNP15	SNP1	1.0	3.36	0.687	0.56	1.0	23974	-
SNP14	SNP13	1.0	6.92	1.0	0.8	1.0	69	52.74
SNP14	SNP12	1.0	1.23	0.25	0.2	1.0	22135	-
SNP14	SNP11	1.0	1.23	0.25	0.2	1.0	23164	-
SNP14	SNP10	1.0	2.37	0.429	0.44	1.0	23186	-
SNP14	SNP9	1.0	1.23	0.25	0.2	1.0	23227	-
SNP14	SNP8	1.0	0.33	0.071	0.06	0.98	23300	-
SNP14	SNP7	1.0	2.37	0.429	0.44	1.0	23329	-
SNP14	SNP6	1.0	2.37	0.429	0.44	1.0	23332	-
SNP14	SNP5	1.0	2.37	0.429	0.44	1.0	23495	-
SNP14	SNP4	1.0	2.37	0.429	0.44	1.0	23541	-
SNP14	SNP3	1.0	2.37	0.429	0.44	1.0	23555	-
SNP14	SNP2	1.0	0.33	0.071	0.06	0.98	23710	-
SNP14	SNP1	1.0	1.23	0.25	0.2	1.0	23890	-
SNP13	SNP12	1.0	1.23	0.25	0.2	1.0	22066	33.95
SNP13	SNP11	1.0	1.23	0.25	0.2	1.0	23095	-
SNP13	SNP10	1.0	2.37	0.429	0.44	1.0	23117	-
SNP13	SNP9	1.0	1.23	0.25	0.2	1.0	23158	-
SNP13	SNP8	1.0	0.33	0.071	0.06	0.98	23231	-
SNP13	SNP7	1.0	2.37	0.429	0.44	1.0	23260	-
SNP13	SNP6	1.0	2.37	0.429	0.44	1.0	23263	-
SNP13	SNP5	1.0	2.37	0.429	0.44	1.0	23426	-
SNP13	SNP4	1.0	2.37	0.429	0.44	1.0	23472	-
SNP13	SNP3	1.0	2.37	0.429	0.44	1.0	23486	-
SNP13	SNP2	1.0	0.33	0.071	0.06	0.98	23641	-
SNP13	SNP1	1.0	1.23	0.25	0.2	1.0	23821	-
SNP12	SNP11	1.0	5.32	1.0	0.71	1.0	1029	39.51
SNP12	SNP10	1.0	0.76	0.107	0.11	0.99	1051	-
SNP12	SNP9	1.0	5.32	1.0	0.71	1.0	1092	-
SNP12	SNP8	1.0	0.2	0.018	0.05	0.97	1165	-
SNP12	SNP7	1.0	0.76	0.107	0.11	0.99	1194	-
SNP12	SNP6	1.0	0.76	0.107	0.11	0.99	1197	-
SNP12	SNP5	1.0	0.76	0.107	0.11	0.99	1360	-
SNP12	SNP4	1.0	0.76	0.107	0.11	0.99	1406	-
SNP12	SNP3	1.0	0.76	0.107	0.11	0.99	1420	-
SNP12	SNP2	1.0	0.2	0.018	0.05	0.97	1575	-
SNP12	SNP1	1.0	5.32	1.0	0.71	1.0	1755	-
SNP11	SNP10	1.0	0.76	0.107	0.11	0.99	22	37.09
SNP11	SNP9	1.0	5.32	1.0	0.71	1.0	63	-
SNP11	SNP8	1.0	0.2	0.018	0.05	0.97	136	-
SNP11	SNP7	1.0	0.76	0.107	0.11	0.99	165	-
SNP11	SNP6	1.0	0.76	0.107	0.11	0.99	168	-
SNP11	SNP5	1.0	0.76	0.107	0.11	0.99	331	-
SNP11	SNP4	1.0	0.76	0.107	0.11	0.99	377	-
SNP11	SNP3	1.0	0.76	0.107	0.11	0.99	391	-
SNP11	SNP2	1.0	0.2	0.018	0.05	0.97	546	-
SNP11	SNP1	1.0	5.32	1.0	0.71	1.0	726	-
SNP10	SNP9	1.0	0.76	0.107	0.11	0.99	41	53.87
SNP10	SNP8	1.0	0.82	0.167	0.13	0.99	114	-
SNP10	SNP7	1.0	6.45	1.0	0.77	1.0	143	-
SNP10	SNP6	1.0	6.45	1.0	0.77	1.0	146	-
SNP10	SNP5	1.0	6.45	1.0	0.77	1.0	309	-
SNP10	SNP4	1.0	6.45	1.0	0.77	1.0	355	-
SNP10	SNP3	1.0	6.45	1.0	0.77	1.0	369	-
SNP10	SNP2	1.0	0.82	0.167	0.13	0.99	524	-
SNP10	SNP1	1.0	0.76	0.107	0.11	0.99	704	-
SNP9	SNP8	1.0	0.2	0.018	0.05	0.97	73	46.15
SNP9	SNP7	1.0	0.76	0.107	0.11	0.99	102	-
SNP9	SNP6	1.0	0.76	0.107	0.11	0.99	105	-
SNP9	SNP5	1.0	0.76	0.107	0.11	0.99	268	-
SNP9	SNP4	1.0	0.76	0.107	0.11	0.99	314	-

SNP9	SNP3	1.0	0.76	0.107	0.11	0.99	328	-
SNP9	SNP2	1.0	0.2	0.018	0.05	0.97	483	-
SNP9	SNP1	1.0	5.32	1.0	0.71	1.0	663	-
SNP8	SNP7	1.0	0.82	0.167	0.13	0.99	29	47.75
SNP8	SNP6	1.0	0.82	0.167	0.13	0.99	32	-
SNP8	SNP5	1.0	0.82	0.167	0.13	0.99	195	-
SNP8	SNP4	1.0	0.82	0.167	0.13	0.99	241	-
SNP8	SNP3	1.0	0.82	0.167	0.13	0.99	255	-
SNP8	SNP2	1.0	2.59	1.0	0.42	1.0	410	-
SNP8	SNP1	1.0	0.2	0.018	0.05	0.97	590	-
SNP7	SNP6	1.0	6.45	1.0	0.77	1.0	3	65.59
SNP7	SNP5	1.0	6.45	1.0	0.77	1.0	166	-
SNP7	SNP4	1.0	6.45	1.0	0.77	1.0	212	-
SNP7	SNP3	1.0	6.45	1.0	0.77	1.0	226	-
SNP7	SNP2	1.0	0.82	0.167	0.13	0.99	381	-
SNP7	SNP1	1.0	0.76	0.107	0.11	0.99	561	-
SNP6	SNP5	1.0	6.45	1.0	0.77	1.0	163	75.84
SNP6	SNP4	1.0	6.45	1.0	0.77	1.0	209	-
SNP6	SNP3	1.0	6.45	1.0	0.77	1.0	223	-
SNP6	SNP2	1.0	0.82	0.167	0.13	0.99	378	-
SNP6	SNP1	1.0	0.76	0.107	0.11	0.99	558	-
SNP5	SNP4	1.0	6.45	1.0	0.77	1.0	46	54.91
SNP5	SNP3	1.0	6.45	1.0	0.77	1.0	60	-
SNP5	SNP2	1.0	0.82	0.167	0.13	0.99	215	-
SNP5	SNP1	1.0	0.76	0.107	0.11	0.99	395	-
SNP4	SNP3	1.0	6.45	1.0	0.77	1.0	14	35.73
SNP4	SNP2	1.0	0.82	0.167	0.13	0.99	169	-
SNP4	SNP1	1.0	0.76	0.107	0.11	0.99	349	-
SNP3	SNP2	1.0	0.82	0.167	0.13	0.99	155	7.9
SNP3	SNP1	1.0	0.76	0.107	0.11	0.99	335	-
SNP2	SNP1	1.0	0.2	0.018	0.05	0.97	180	3.24