Genome wide association studies of maternal behaviour in sheep

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Summary

Maternal behaviour is vital for the offspring survival. In animal production, improvement of maternal behaviour is becoming increasingly important in order to improve lamb survival particularly in extensive systems. Breed differences in maternal behaviour and genetic variation within breed have previously been described in several farm species. However, no studies have so far investigated genetic loci underlying such behaviour in sheep. The aim of this study was to identify QTL associated with maternal behaviours in sheep using genotypes from the ovineSNP50 beadchip. Ewes reared outside were genotyped and individually phenotyped (n=470) after lambing for maternal behaviours in two standardized behavioural tests. QTL detection was performed by linkage, association and joint linkage and association analyses. Six main QTL regions identified on chromosomes 4, 13, 16, 21, and 23 that showed significant associations with ewe vocalizations. Four main QTL regions identified on chromosomes 4, 9, and 23 showed significant associations with maternal behaviour scores. Some of these QTLs contained interesting candidate genes, previously described to be associated with behaviours, which may explain a substantial amount of the genetic variation in maternal behaviours in sheep.

Keywords: QTL, candidate genes, maternal behaviour score, maternal reactivity, vocalisations, locomotion

Introduction

Genetic selection including behavioural traits could be an advantageous strategy aimed at improving robustness of farm animals in various farming conditions by minimizing unsuitable responses to changes in their social and physical environment, limiting an excessive fear of humans and improving sociability (Mignon-Grasteau et al., 2005). Improvement of maternal behaviour is becoming increasingly important in livestock to improve offspring survival which still remains a major preoccupation in extensive husbandry. Genetic variation between and within breeds have been reported for maternal behaviour in various farming species, such as sheep (Dwyer, 2007; Hazard et al., 2016b). In addition, it has been recently reported in sheep that early social reactivity of lambs is also heritable, and associated with some QTL (Hazard et al., 2014). Such social reactivity of lambs at weaning didn't show genotype by environment interaction and could be used to improve general sheep sociability, including maternal behaviour, independently of the environment (Hazard et al., 2016a). However, no studies have so far reported genetic architecture associated with

maternal behaviours in domestic sheep. Thus, the aim of the present study was to perform QTL detection for maternal behaviour attributes.

Material and methods

Animals and management

The experimental animals were Romane meat sheep, reared at the INRA experimental farm of La Fage (Roquefort sur Soulzon, France) exclusively outdoors under extensive conditions. The flock comprised of about 250 reproductive females reared on 280 ha of rangelands. The farming system and management characteristics have been described previously by Gonzalez-Garcia et al. (2014). All the animals were born outdoors in the spring. First lambing occurred at one or two years old. Multigenerational data were recorded over five years. Pedigree included 1608 individuals over 5 generations.

Behavioural tests and responses

At lambing, ewes were individually exposed successively to two behavioural tests. The approach - follow-up (**AF**) test was performed outdoors approximately 2h after any lambing event which occurred during daylight hours only. After lambing (and AF test), both ewes and lambs were transferred to a shelter and tested a day later in an arena test (**AT**). In the AF test phase 1 (**AF1**) a maternal behaviour score (MBS) was recorded on a 5 point-scale in response to a shepherd approaching the lambing area: 1- ewe flees and doesn't return, 2- ewe retreats and comes back, 3- ewe retreats with lamb and comes back, 4- ewe retreats and returns repeatedly, 5- ewe stays close to the lambing area. In the AF test phase 2 (**AF2**), a second maternal behaviour score (MBS) was recorded on a 4 point-scale in response to the capture of the litter by the shepherd: 1- ewe flees, 2- ewe stays close to the lambing area, 3- ewe follows but keeping a distance, 4- ewe follows staying close to the shepherd. In the AT (Ligout et al., 2011), 1) attraction to the litter, 2) reactivity to a brief separation from the litter, and 3) attraction to the litter with a motionless shepherd was evaluated by measuring locomotor activity (i.e., number of virtual zones crossed in phase of test 1, 2 or 3) (AT1/2/3-LOCOM) and counting vocalizations (high bleats, AT1/2/3-HBLEAT; low bleats, AT1/2/3-LBLEAT).

Statistical handling

Solutions from an animal model, accounting for a non-genetic random effect of the ewe and fixed effects were used as phenotypes for subsequent analyses to consider repeated measures in ewes and variation in MBS and AT traits due to parity, age, litter size and year fixed effects.
We used 470 genotyped ewes as well as their nine respective sires and 459 full- or half-sibs genotyped with the Illumina ovineSNP50 beadchip (i.e. 54241 SNPs). Among them 470 and 342 ewes were phenotyped in AT and AF tests, respectively. Pedigree included 1608 individuals over 5 generations. SNP quality was checked as described by Hazard et al. (2014). The QTLmap software was used to search QTL using linkage, association and joint linkage and association analyses (Gilbert et al., 2008). Data were analysed using linkage analysis (LA) by interval mapping within sire family. Genome wide association (GWAS) analysis was performed in the whole genotyped population using the existing linkage disequilibrium (LD) (Meuwissen and Goddard, 2000). The LD decay linear model developed by Legarra and

Fernando (2009) was fitted to our data. A joint analysis (LDLA) considering simultaneously linkage association and linkage disequilibrium was performed to take advantage of both pedigree and LD (Legarra and Fernando, 2009).

Results and Discussion

Maternal behaviour scores recorded in the AF test indicated that 57% of the ewes showed the highest score either staying on the lambing area when the shepherd approached or following the litter when moved by the shepherd (Table 1). The rest of the ewes exhibited retreating behaviours when the shepherd approached. Approximately 30% of the ewes did not follow the shepherd when he moved off the lambing area with the litter. In the AT test, the highest number of high bleats and locomotion of ewes were recorded in response to the brief separation from the litter (i.e. AT2) while the higher number of low bleats was recorded when the contact between the ewe and litter was possible without a human presence (i.e. AT1) (Table 2). The number of low bleats was lower than number of high bleats whatever the phase of arena test. High bleats and locomotion are generally interpreted as an active behavioural strategy to maintain a social link with the litter (Boissy et al., 2007). Thus, we hypothesized that more maternal ewes exhibited higher levels of high bleating and locomotion in response to separation from their litters.

Using a larger data set of phenotyped sheep including ewes that were or not genotyped, we previously found that maternal behaviour scores and locomotion were moderately heritable (MBS, h^2=0.23 ± 0.06; LOCOM, h^2=0.15 ± 0.06) and vocalizations were highly heritable (HBLEAT, h^2=0.45 ± 0.05; LBLEAT, h^2=0.40 ± 0.04) (unpublished data). Using data from genotyped ewes, both linkage and LD-based analyses also resulted in mapping many QTL involved with maternal behaviour traits. Several regions were of great interest due to several correlated traits affected by a limited region and/or a high level of significance. These QTL were summarized in Table 3. QTL regions on OAR 4, 13, 16, 21 and 23 were related to ewe vocalizations. Interestingly, some of these regions had been previously reported to be associated with similar behavioural traits but measured in lambs at weaning to evaluate social reactivity (OAR13, 16) (Hazard et al., 2014). Other QTL regions were related to maternal behaviour scores (OAR 4, 9 and 23). Overlapping regions were found for high bleats and low bleats behaviours on OAR 23, for high bleats and maternal behaviour score on OAR4, and for both maternal behaviour scores on OAR9. These results were consistent with the high negative genetic correlation found between high bleats and low bleats and the high positive genetic correlation found between both maternal behaviour scores. QTL region on OAR 11 was specifically related to locomotion and an overlap was found on this region for locomotion assessed in arena test 2 and 3.

The present results suggest that the attachment of the dam with her litter is influenced by QTL at different loci. This is consistent with previous studies showing that social reactivity in sheep and temperament-related trait measured in various livestock species, but also anxiety-related behaviour assessed in mice, were each controlled by different underlying genetic causes (Turri et al., 2001; Canario et al., 2013; Hazard et al., 2014).

In a preliminary bioinformatics approach, we looked for annotated genes present close to the QTLs reported. Among genes found, QTL region mapped on OAR16 harbours the gene encoding PRLR (prolactin receptor). The PRLR gene is associated with maternal and social behaviour in sheep (Wang et al., 2015) and in rodents (Leckman and Herman, 2002). On chromosome 4, two candidate genes, KCND2 and FOXP2 were located close to two QTLs associated with maternal behaviour scores. The gene KCND2 (potassium voltage-gated

channel subfamily D member 2) has been described to be involved in locomotor rhythm and sensory perception of pain in human (Singh et al., 2006). The gene FOXP2 (forkhead box P2) is known to be involved in vocal learning in mammals (Webb and Zhang, 2005). Last but not least, QTL mapped on chromosome 23 and associated with maternal behaviour score overlapped with two other candidate genes, MC4R (melanocortin 4 receptor) and GAD1 (glutamate decarboxylase 1). The MC4R gene, is associated with eating behaviour in human (Stutzmann et al., 2009) and bioinformatics annotations suggest MC4R and GAD1 genes could be involved in grooming and social behaviour, respectively.

Table 1. Distribution of maternal behaviour scores in approach – follow-up test (AF).

Class	AF1-MBS	AF2-MBS
1	2.5[1]	17.8
2	6.5	13.0
3	7.8	12.2
4	25.8	57.0
5	57.4	-

[1] Percentage of ewes

Table 2. Summary statistics for behavioural traits recorded in arena test (AT).

	Mean (SD)	min	max
AT1-HBLEAT[1]	6.64 (3.93)	0.00	19.0
AT2-HBLEAT[1]	15.64 (5.49)	0.00	39.0
AT3-HBLEAT[1]	8.85 (5.46)	0.00	28.0
AT1-LBLEAT[1]	3.58 (4.02)	0.00	29.0
AT2-LBLEAT[1]	0.45 (1.51)	0.00	16.0
AT3-LBLEAT[1]	1.95 (3.78)	0.00	28.0
AT1-LOCOM[1]	9.04 (3.07)	2.0	23.0
AT2-LOCOM[1]	20.84 (9.30)	1.0	56.0
AT3-LOCOM[1]	5.91 (5.08)	1.0	30.0

[1] Raw data before transformation.

Table 3. Summary of QTLs detected.

Trait	OAR	Peak position (Mb)	Confidence interval	Significance level[1]	Method	Candidate gene
AT1-HBLEAT	16	39.00	37.79-42.16	GW	LD, LDLA	PRLR, RXFP3
AT1-HBLEAT	23	50.90	50.93-50.94	GW	LDLA	MEX3C
AT2-HBLEAT	13	44.60	43.50-55.90	GW	LA, LDLA	PFK
AT2-HBLEAT	16	50.57	50.54-50.62	GW	LDLA	ESD
AT3-HBLEAT	4	54.80	54.78-54.86	GW	LD, LDLA	NAP1L1
AT1-LBLEAT	21	15.90	15.85-15.93	GW	LD, LDLA	-
AT1-LBLEAT	23	48.45	48.43-48.50	GW	LD, LDLA	CTIF
AT2-LOCOM	11	30.65	30.60-30.70	GW	LD, LDLA	MYOCD
AT3-LOCOM	11	30.43	30.35-30.52	GW	LDLA	MAP2K4
AF1-MBS	4	84.15	84.1-84.2	GW	LD, LDLA	KCND2
AF1-MBS	9	83.57	83.52-83.62	CW	LDLA	BCL2L1, PPIA
AF2-MBS	4	53.00	52.98-53.09	CW	LD, LDLA	FOXP2
AF2-MBS	9	83.45	75.92-83.97	GW	LA, LD, LDLA	BCL2L1, PPIA
AF2-MBS	23	59.20	59.16-62.19	GW	LA, LD, LDLA	MC4R, GAD1

[1] Only the significant QTLs reaching the 1% chromosome-wise (CW) or the genome-wise (GW) thresholds are listed in the table.

In conclusion, the identification of the genes underlying the maternal behaviour may provide opportunities for better understanding the genetic components and metabolic pathways involved in regulation of such behaviour.

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