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Abstract	The Valdostana goat is an alpine breed, raised only in the northern Italian region of the Aosta Valley. This breed's main purpose is to produce milk and meat, but is peculiar for its involvement in the "Batailles de Chèvres," a recent tradition of non-cruel fight tournaments. At both the genetic and genomic levels, only a very limited number of studies have been performed with this breed and there are no studies about the genomic signatures left by selection. In this work, 24 unrelated Valdostana animals were screened for runs of homozygosity to identify highly homozygous regions. Then, six different approaches (ROH comparison, Fst single SNPs and windows based, Bayesian, Rsb, and XP-EHH) were applied comparing the Valdostana dataset with 14 other Italian goat breeds to confirm regions that were different among the comparisons. A total of three regions of selection that were also unique among the Valdostana were identified and located on chromosomes 1, 7, and 12 and contained 144 genes. Enrichment analyses detected genes such as cytokines and lymphocyte/leukocyte proliferation genes involved in the regulation of the immune system. A genetic link between an aggressive challenge, cytokines, and immunity has been hypothesized in many studies both in humans and in other species. Possible hypotheses associated with the signals of selection detected could be therefore related to immune-related factors as well as with the peculiar battle competition, or other breed-specific traits, and provided insights for further investigation of these unique regions, for the understanding and safeguard of the Valdostana breed.

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The Valdostana goat: a genome-wide investigation of the distinctiveness of its selective sweep regions

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Abstract The Valdostana goat is an alpine breed, raised only in the northern Italian region of the Aosta Valley. This breed's main purpose is to produce milk and meat, but is peculiar for its involvement in the "Batailles de Chèvres," a recent tradition of non-cruel fight tournaments. At both the genetic and genomic levels, only a very limited number of studies have been performed with this breed and there are no studies about the genomic signatures left by selection. In this work, 24 unrelated Valdostana animals were screened for runs of homozygosity to identify highly homozygous regions. Then, six different approaches (ROH comparison, Fst single SNPs and windows based, Bayesian, Rsb, and XP-EHH) were applied comparing the Valdostana dataset with 14 other Italian goat breeds to confirm regions that were different among the comparisons. A total of three regions of selection that were also unique among the Valdostana were identified and located on chromosomes 1, 7, and 12 and contained 144 genes. Enrichment analyses detected genes such as cytokines and lymphocyte/leukocyte proliferation genes involved in the regulation of the immune system. A genetic link between an aggressive challenge, cytokines, and immunity has been hypothesized in many studies both in humans and in other species. Possible hypotheses associated with the signals of selection detected could be therefore related to immune-related factors as well as with the peculiar battle competition, or other breed-specific traits, and provided insights for further investigation of these unique regions, for the understanding and safeguard of the Valdostana breed.

Introduction

Over the past several years, the increase of genomic technologies and molecular information has given researchers the chance of developing useful tools for genome-wide analyses in livestock. Since 2008, a series of single-nucleotide polymorphism (SNP) chips of medium and high density have been developed and assessed for the major livestock species (Nicolazzi et al. 2015). These tools have provided the opportunity to investigate the underlying structure of genomes for several purposes such as detection of selective sweeps, breed differentiation, genome-wide association studies (GWAS), and genomic selection in cattle, pigs, sheep, horses, and chickens (Meuwissen et al. 2013; Nicolazzi et al. 2015).

The selective sweep can be defined as a reduction or elimination of variation among the nucleotides in genomic regions adjacent to a mutation that become fixed from natural or artificial selective pressure. This selection tends to cause changes not only in the pattern of variation among selected loci, but also neutral loci linked to them via the

Talenti Andrea and Francesca Bertolini have contributed equally to the work.

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well-known hitch-hiking effect. The effect due to selective pressure can affect different traits, from aesthetic to economical variants, and they could also be associated with deleterious phenotypes as well as behavioral traits. These regions of lower variability could be therefore seen as "genomic footprints" that allow identification of loci subjected to that selective pressure (de Simoni Gouveia et al. 2014). Several approaches have been used to detect these regions, such as run of homozygosity (ROH; Zhao et al. 2012; Fleming et al. 2016), fixation index analysis (Fst; Kijas et al. 2012; Porto-Neto et al. 2013), and haplotype-based analyses (e.g., de Simoni Gouveia et al. 2014). Other approaches, such as Bayesian methods, have also been successfully used on some occasions to detect selective sweeps as well (e.g., Druet et al. 2014).

Compared with the other major livestock species, the goat was one of the last for which medium-density SNP chips became available. In 2012, through the international goat genome consortium, the first medium-density Goat 52 K SNP chip was designed and released (Tosser-Klopp et al. 2014). The first goat genome of a Yunnan black female goat was completely assembled and officially released about one year before in 2013 (Du et al. 2012; Tosser-Klopp et al. 2012; Dong et al. 2013). Since the Caprine 52 K SNP chip was recently developed, only a limited number of studies have been reported but they encompass a wide variety of aspects including (i) linkage disequilibrium, population distribution, and structure analyses in several goat breeds (Kijas et al. 2013; Nicoloso et al. 2015; Lashmar et al. 2015); (ii) implementation and development of marker-assisted breeding scheme strategies (Brito et al. 2015; Lashmar et al. 2015); (iii) development of SNP chip-based caprine parentage tests (Talenti et al. 2016); and (iv) signatures of selection and GWAS analyses for phenotypic traits and adaptation (Becker et al. 2015; Kim et al. 2015; Reber et al. 2015). Italy is a country that can be considered an important reservoir of genetic resources for goat species in Europe. Nowadays, 36 breeds are officially recognized by the National Goat and Sheep Breeder Association and 14 of them are localized in the Alpine regions (ASSONAPA, http://www.assonapa.it). The Valdostana goat is an alpine breed, raised in the northern Italian region of the Aosta valley in the extreme north-west corner of the Alpine area, a natural border of Northern Italy. The Valdostana has been primarily used for the production of cheese (in 125 days of lactation, the production is around 249 Kg) and meat and for the production of traditional and seasoned products (e.g., the Mocetta). While this breed is from the alpine region, it differs from the other breeds of the same area primarily because of its larger size, and for the presence of well-developed horns in females (ASSONAPA). The Valdostana characteristics have been influenced by the natural selection of the mountain environment, but also by the selection of farmers for the maintenance of the recent traditional fighting tournaments that are organized in Valle d'Aosta. These non-cruel fights, called "*Batailles de* Chèvres," are a recent event of fight tournaments that take place in the valley (Association Comité Régional Batailles des Chèvres). The current status of this population is 640 registered animals and this breed is considered at risk with a declining number of animals reared (Nicoloso et al. 2015). At both genetic and genomic levels, only a very limited number of studies have been performed on this breed (Colussi et al. 2008; Nicoloso et al. 2015) and there is no information about the genomic signatures left by selection. AQ1

The aim of this work was to identify unique selective sweep regions in the Valdostana goat genome resulting from man-made artificial selection and natural/environmental selection. These genomic regions could govern phenotypic traits of interest and may be linked to peculiar phenotypic characteristics of this breed. To accomplish this task, we used the medium-density Goat 52 K SNP chip to detect ROH and we compared the Valdostana genome with those of 14 other Italian breeds using ROH comparisons, Fst, haplotype-based, and Bayesian analyses. AQ2

Materials and methods

Goat sampling, genotyping, and multidimensional scaling analysis

Animals belonging to 15 different breeds were collected in Italy from different farms (approximately three from each farm) to collect animals as much unrelated as possible. For each animal, blood samples were collected following the European rules (Council of Europe 1986) for animal care and DNA extraction was performed using a commercial kit (NucleoSpin Blood, Macherey–Nagel) according to the manufacturer's instructions. Then, DNA samples were genotyped using the CaprineSNP50 BeadChip (Illumina Inc., San Diego, CA; Tosser-Klopp et al. 2014). For further details, see Nicoloso et al. (2015). Goats ($N = 369$) and breeds ($N = 15$) included in this study are listed in Table 1. In addition to Valdostana ($n = 24$; 15 females and 9 males), a group of 14 other breeds (Argentata dell'Etna, Dell'Aspromonte, Ciociara Grigia, Girgentana, Maltese, Nicastrese, Sarda, Di Teramo, Bionda dell'Adamello, Camosciata delle Alpi, Nera di Verzasca, Orobica, Saanen, Valpassiria) was investigated in order to find the most unique and divergent genomic regions across the Valdostana genome. To further confirm the unrelatedness of the animals within the dataset, above all among the Valdostana goats, an in-house script was used for calculating the number of discordant homozygotes at each locus between all pairs of individuals in the dataset. A pair is

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Table 1 Name of breeds and number of animals for each breed considered for the analyses

Breed name	No.
Valdostana	24
Argentata dell'Etna	24
Dell'Aspromonte	24
Bionda dell'Adamello	24
Camosciata delle Alpi	30
Ciociara Grigia	19
Girgentana	24
Maltese	31
Nicastrese	24
Nera di Verzasca	19
Orobica	23
Saanen	24
Sarda	32
Di Teramo	23
Valpassiria	24

All animals except the Nera di Verzasca are already generally described in Nicoloso et al. 2015

defined related if the total number of discordant homozygotes is lower than 100 (<0.5%). Out of a total of 67,896 comparisons among individuals, only 32 pairs had a number of discordant homozygotes below the given threshold of 100 and were considered closely related, and none of them were individuals of the Valdostana breed (data not shown).

SNPs with a call rate <90%, monomorphic SNPs, and variants not mapped to the assembly or on the X chromosome were excluded from subsequent analyses using Plink v1.9 (Chang et al. 2015). Monomorphic SNPs can be considered fixed across all breeds, so they were not considered informative for the purpose of the analyses. After the SNP marker quality check, animals with an individual call rate < 0.95% as performed by Nicoloso et al. (2015) were removed from the dataset. The filtered dataset was then phased and imputed breed by breed for the missing genotypes using Beagle v3.3.2 (Browning and Browning 2007, 2008; Browning 2011). Multidimensional scaling (MDS) was performed in two dimensions using the cluster algorithm of Plink v1.9 (Chang et al. 2015).

Runs of homozygosity in Valdostana goats and enrichment analyses of regions under selection

Analyses of high-homozygosity regions across the genome were conducted with the --homozyg command in Plink v1.9 (Chang et al. 2015), including in each window 20, 25, or 30 SNPs with the command --homozyg-snp, and allowing no heterozygotes (--het 0). The output files (.summary) contained for each SNP a raw value that indicated the number of animals and was normalized by dividing that number by the total number of animals included in the analysis, obtaining a locus homozygosity (H) range from 0 (0) to 1 (100%) as performed in Bertolini et al. (2016). Regions with $H \geq 0.62$ at each SNP site, equivalent to the top 0.2% of the empirical distribution of all the SNPs, were considered as regions of higher homozygosity.

Annotation of all highly homozygous regions was obtained downloading the complete list of genes available for the *Capra hircus* genome CHIR_1.0 available in the CoGe (Comparative Genomics) database (Lyons and Freeling 2008, https://genomevolution.org/coge/). Then the list of genes was screened at the desired positions using the BEDTools software (Quinlan and Hall 2010). Enrichment analysis was performed using the web-based tool Enrichr (Chen et al. 2013; Kuleshov et al. 2016; http://amp.pharm.mssm.edu/Enrichr/), where "Wiki pathway" and "Gene Ontology biological processes" were investigated.

Valdostana vs other goat breeds

A total of six different analyses were performed comparing the Valdostana to the 14 other breeds considered separately (ROH comparison) or comparing the Valdostana to the 14 other breeds as a whole (Fst, haplotype-based, and Bayesian analysis) in order to investigate whether the most homozygous regions detected in Valdostana could be considered as unique to the breed.

ROH comparison

For each of the remaining 14 breeds, homozygosity was determined as described above for the Valdostana and the results were separately H transformed. Summary statistics were calculated modifying the approach suggested by Akey et al. (2010) to compare the locus-specific divergence for each goat breed based on H scores:

$$SHD_i = \sum_{i \neq j} \frac{HDij - E(HDij)}{sd(HDij)}, \quad (1)$$

where HD^{ij} is the difference of H between two breeds i and j, and $E(HD^{ij})$ and $sd(HD^{ij})$ are the expected value and standard deviation of HD between ith and jth breeds, respectively. An SHD value >6 was considered as the threshold which indicates the highest divergence at each locus, equivalent to approximately the top 0.2% of the empirical distribution.

Fst analysis

Fst analysis between Valdostana compared to all the 14 other goat breeds of the dataset was performed for each

SNP, using the formula reported by Karlsson et al. (2007). Then, a mean Fst value (mFst) was calculated in 1 Mb sliding windows with 500 Kb overlapping using an in-house script. The window size was chosen to be consistent with the ROH, according to SNP density (20 SNP * 50,000 bp/SNP = 1,000,000 Mb). Values >0.31 for the mFst and >0.56 for the single-SNP Fst represented approximately 0.2 and 0.05%, respectively, of the empirical distribution of all the values, and were the most divergent between the two groups and were therefore considered.

Bayesian analysis

A Bayesian approach called Bayes B implemented in GenSel software (Fernando and Garrick 2009) was used to obtain the variance explained by SNPs in every genomic non-overlapping window of 1 Mb each, using categorical traits. Valdostana goats were treated as "case" and all the other breeds together were treated as "controls"; the comparison was performed between these two groups, with no fixed effects or covariates being added in the model. A prior probability (p_i) of 0.992 was used to fit 250–300 markers per iteration of the Markov chain in a mixture model for the estimation of individual SNP effects (Dekkers 2012; Onteru et al. 2013), with VarG = 123.383, VarR = 1. Windows that explained more that 1% of the variance were considered.

Haplotype-based analysis

Two analyses, Rsb and XP-EHH, were performed. Rsb was defined as the standardized log-ratio of the integrated extended haplotype homozygosity (EHH) between pairs of populations (Tang et al. 2007), while Cross-population Extended Haplotype Homozygosity (XP-EHH) compares the integrated EHH profiles between two populations at the same SNP (Sabeti et al. 2007). The Rsb statistic compares EHH for the same SNP in two different populations and can provide evidence of selection given the presence of high-frequency or fixed alleles in one population but not on the other (Tang et al. 2007). Similarly, the XP-EHH detected selective sweeps in which one allele had undergone strong directional selection in one population while remaining polymorphic in the population as a whole (Sabeti et al. 2007).

The *rehh* R package was used to compute *Rsb* values with default parameters (Gautier and Vitalis 2012), whereas the selscan software was then used to compute XP-EHH (Szpiech and Hernandez 2014). XP-EHH values were then normalized using the norm tool included in the selscan package. Ancestral allele information, which is important for this analysis, was identified starting from a dataset composed of eight Ibexes (data not shown) and seven Bezoars (produced by the NEXTGEN project, 2009) that were genotyped with the same GoatSNP50 BeadChip, in a manner similar to what has been previously performed in cattle (Matukumalli et al. 2009). These two caprine species are known to be geographically close (Alpine Ibex) or the closest ancestors of the modern goat (Bezoar, Colli et al. 2015). Values >8 and >4.5 that represented around 0.2% of the empirical distribution of all the normalized values for Rsb and XP-EHH, respectively, were considered as biologically relevant.

Results

The GoatSNP50 BeadChip contains 53,347 SNPs, and a total of 3,404 SNPs were mapped to the X chromosome or were unmapped, and 1,051 SNPs did not pass the quality-filtering step. All of these were excluded from further analyses. Therefore, the working dataset included 48,892 autosomal SNPs. All animals had a genotyping rate >0.95%. The MDS plot shown in Fig. 1 demonstrates a clear separation between breeds raised in the north and in the south of Italy, with the Valdostana (black dots) clearly belonging to the cluster of northern breeds, as already reported by Nicoloso et al. (2015), with some animals overlapping the Alpine and Nera di Verzasca breeds.

Runs of homozygosity

For the runs of homozygosity, three SNP windows were considered. The window of 20 SNPs identified three peaks above the threshold (Fig. 2), while using 25 and 30 SNPs showed a decay of one of the peaks (Fig. S1 and S2). Therefore, the window with 20 SNPs was chosen for the following analysis. For the selected threshold, three regions with H ≥ 0.62 were detected (Fig. 2). One region was detected on chromosome 1 (from 112,414,563 to 113,060,421 bp), with the highest H value of 0.63 (Fig. S3) and a length of 645 Kb. A second region located on chromosome 7 (from 15,057,327 and 19,670,982 bp) had the highest H value of 0.83 and was 4.6 Mb in length (Fig. S4), and a third smaller region on chromosome 12 (from 28,544,783 to 28,664,628 bp) showed the highest H value of 0.63 (Fig. S5) with 120 kb length. The list of the 129 annotated genes located in the three high-homozygosity regions is reported in Table 2. The region on chromosome 1 contained 4 genes and the second region on chromosome 7 had 116 genes. A total of 37 genes were included in the subregion on chromosome 7 within the region on the top of the peak, with all the SNPs having H = 0.83. These regions included the *MAP2K2, APBA3*, and *ATCAY* genes. The third region on chromosome 12 contained 1 annotated gene.

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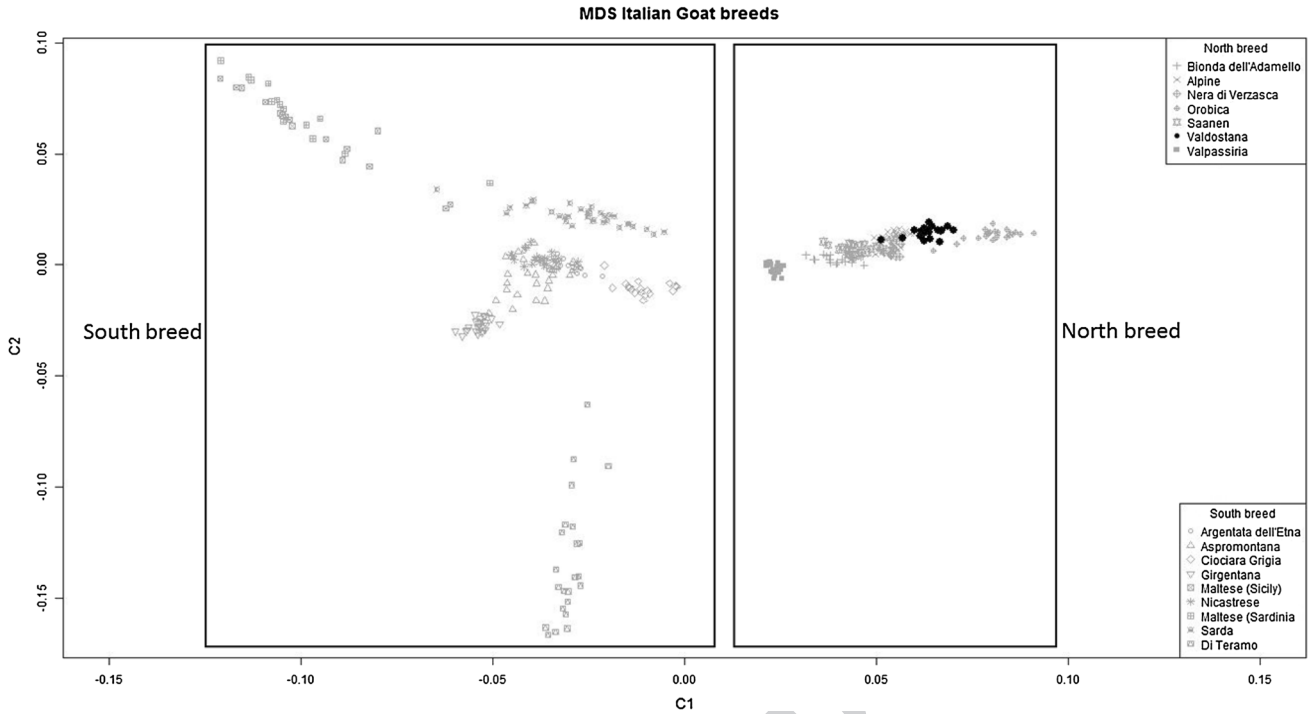

Fig. 1 Multidimensional scaling of Italian goat breeds and populations including Valdostana. The two clusters indicate breeds raised in the south and the north of Italy. Valdostana is *black colored*

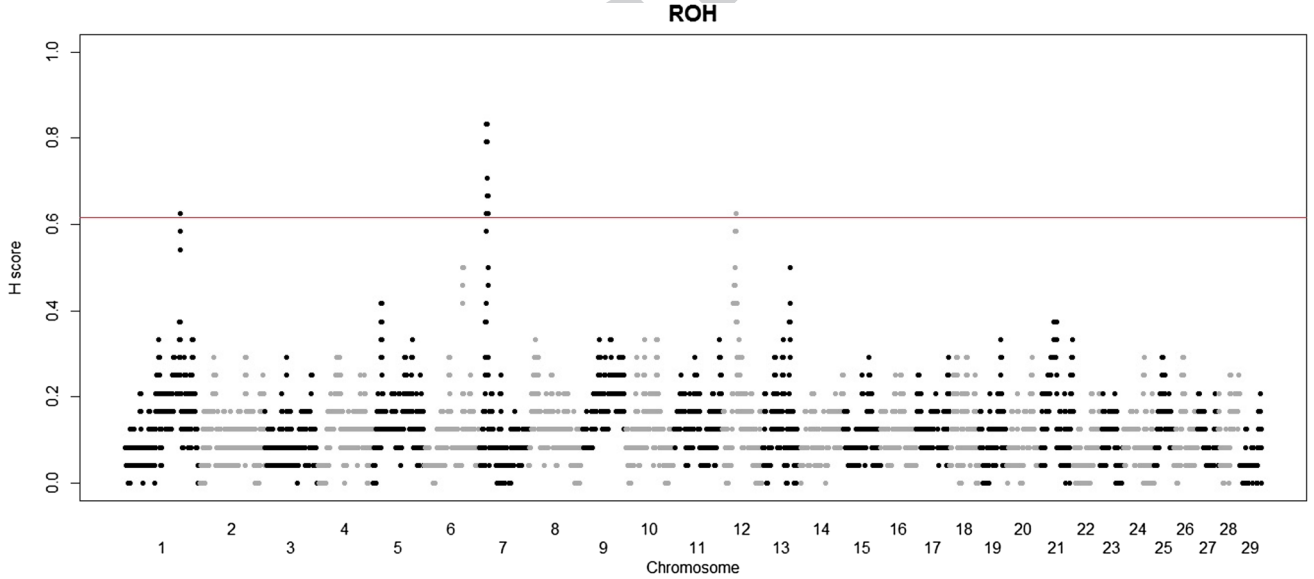

Fig. 2 Regions of homozygosity in the Valdostana dataset. The raw values obtained were normalized according to the number of animals used in the analysis. A threshold of H score = 0.62 was chosen to detect the regions with low heterozygosity (indicated with the *red line*)

The enrichment analyses of the genes reported clusters of genes (*adjusted P value* <0.05) that are involved in activities related to the immune system such as regulation of immunoglobulin production, lymphocyte, T cells, mononuclear and leukocyte proliferation, as well as regulation of the JAK–STAT cascade (Table 3).

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Table 2 List of genes included in the highly homozygous regions

Chr	Start	End	Gene symbol	Gene name	Chr	Start	End	Gene symbol	Gene name
1	675002	123987664	TRNAG-UCC	transfer RNA glycine (anticodon UCC)	7	16863564	16866964	C7H19orf77	–
1	7624399	148400517	TRNAV-CAC	transfer RNA valine (anticodon CAC)	7	16878586	16930540	NFIC	Nucleus factor I/C (CCAAT-binding protein L54)
1	112490894	112492829	RAP2B	RAP2B, member of RAS oncogene family	7	17004688	17022568	CELF5	CUGBP, Elav-like family member 5
1	112845294	112849567	P2RY1	purinergic receptor P2Y, G-protein coupled, 1	7	17063113	17082730	NCLN	Nicalin
7	15053932	15058909	HSD11B1L	hydroxysteroid (11-beta) dehydrogenase 1-like	7	17081246	17083566	S1PR4	Sphingosine-1-phosphate receptor 4
7	15060184	15062136	C7H19orf70	–	7	17095410	17116109	GNA15	Guanine nucleotide-binding protein, alpha 15
7	15065044	15069283	LOC102169378	–	7	17126314	17137531	GNA11	Guanine nucleotide-binding protein, alpha 11
7	15069451	15075296	SAFB	scaffold attachment factor B1-like	7	17167056	17167370	LOC102177616	pseudogene
7	15077037	15126429	LOC102169953	–	7	17169117	17182692	AES	Amino-terminal enhancer of split
7	15126550	15154442	SAFB2	scaffold attachment factor B2	7	17191762	17218405	TLE2	Transducin-like enhancer of split 2
7	15277239	15278880	LOC102183866	–	7	17219310	17234875	TLE6	Transducin-like enhancer of Split 6
7	15420303	15542566	PTPRS	Protein tyrosine phosphatase, receptor Type, S	7	17247811	17267239	ZNF77	Zinc finger 77
7	15541392	15647804	KDM4B	Lysine (K)-specific Demethylase 4B	7	17273536	17300375	ZNF555	Zinc finger 555
7	15669170	15703813	UHRF1	Ubiquitin-like with PHD and ring finger domains 1	7	17316607	17328730	ZNF554	Zinc finger 554
7	15708356	15722205	ARRDC5	Arrestin domain-containing 5	7	17333511	17350262	THOP1	Thimet oligopeptidase 1
7	15739749	15761331	PLIN3	Perilipin 3	7	17357031	17364911	SGTA	Small glutamine-rich tetratricopeptide repeat-containing, alpha
7	15770780	15786827	TICAM1	Toll-like receptor adaptor molecule 1	7	17377647	17384058	SLC39A3	Solute carrier family 39 (zinc transporter), member 3
7	15802328	15807760	FEM1A	Fem-1 Homolog A	7	17394483	17395184	DIRAS1	DIRAS family, GTP-binding RAS-like 1
7	15879539	15915689	DPP9*	Dipeptidil-peptidase 9	7	17444465	17541139	GNG7	Guanine nucleotide-binding protein
7	15920677	15932753	C7H19orf10*		7	17561663	17563284	GADD45B	Growth arrest and DNA damage-inducible, Beta
7	15936585	15953655	TNFAIP8L1*	Tumor necrosis factor, alpha-induced protein 8-Like 1	7	17587771	17597983	LMNB2	Lamin B2

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Table 2 (continued)

Chr	Start	End	Gene symbol	Gene name	Chr	Start	End	Gene symbol	Gene name
7	16015285	16040053	SEMA6B*	Sema domain, transmembrane domain (TM), And cytoplasmic domain, (Semaphorin) 6B	7	17600811	17601233	TIMM13	Translocase of inner mitochondrial membrane 13 Homolog
7	16041644	16044715	LRG1*	Leucine-rich repeat	7	17608098	17641028	TMPRSS9	Transmembrane protease, serine 2
7	16046725	16057405	PLIN5*	Perilipin 5	7	17669940	17680121	SPPL2B	Signal peptide peptidase-like 2B
7	16061550	16071410	PLIN4*	Perilipin 4	7	17679041	17687505	LSM7	LSM7 homolog, U6 small nuclear RNA and mRNA degradation associated
7	16075055	16099421	HDGFRP2*	hepatoma-derived growth factor-related protein 2	7	17689919	17711175	LINGO3	Leucine-rich repeat and Ig domain-containing 3
7	16114145	16123589	UBXN6*	UBX domain protein 6	7	17719888	17725516	C7H19orf35	-
7	16125311	16148160	CHAF1A*	Chromatin assembly factor I	7	17727496	17778465	OAZ1	Ornithine decarboxylase Antizyme 1
7	16166455	16180027	SH3GL1*	SH3 Domain GRB2-Like 1	7	17783151	17822107	DOT1L	DOT1-Like histone H3K79 methyltransferase
7	16180192	16189526	MPND*	MPN Domain-containing	7	17827659	17831621	PLEKHJ1	Pleckstrin Homology domain-containing J1
7	16195347	16205742	STAP2*	Signal-transducing adaptor family member 2	7	17831541	17840494	SF3A2	Splicing factor 3a, subunit 2, 66 kDa
7	16206199	16221966	FSD1*	Fibronectin type III and SPRY domain-containing 1	7	17841203	17842810	AMH	Anti-mullerian hormone
7	16222367	16227311	TMIGD2*	Transmembrane and immunoglobulin domain-containing 2	7	17844234	17848014	JSRP1	Junctional Sarcoplasmic reticulum protein 1
7	16228061	16236382	SHD*	Src Homolog 2	7	17853923	17886867	AP3D1	Adaptor-related protein complex 3, Delta 1 Subunit
7	16243123	16257235	CCDC94*	Coiled-Coil domain-containing 94	7	17889329	17892234	IZUMO4	IZUMO Family member 4
7	16259166	16265047	EBI3*	Epstein–Barr virus-induced 3	7	17903199	17911300	MOB3A	MOB kinase activator 3 A
7	16265767	16283544	ANKRD24*	Ankyrin repeat domain 24	7	17929169	17940970	MKNK2	MAP kinase-interacting Serine/Threonine Kinase 2
7	16296333	16305024	SIRT6*	Sirtuin 6	7	18017834	18025614	SEPT8	Septin 8
7	16305157	16316543	CREB3L3*	CAMP Responsive element-binding protein 3-like 3	7	18029881	18036074	CCNI2	Cyclin I family, member 2
7	16349231	16366609	MAP2K2*	Mitogen-activated protein kinase 2	7	18044228	18120787	KIF3A	Kinesin family member 3 A
7	16399643	16403577	ZBTB7A*	Zinc finger and BTB domain-containing 7 A	7	18128067	18135921	IL4	interleukin 4
7	16416362	16447151	PIAS4*	Protein inhibitor of activated STAT, 4	7	18152706	18155442	IL13	interleukin 13

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Table 2 (continued)

Chr	Start	End	Gene symbol	Gene name	Chr	Start	End	Gene symbol	Gene name
7	16462232	16471129	EEF2*	Eukaryotic translation elongation factor 2	7	18198088	18310565	RAD50	RAD50 homolog, double-strand break repair protein
7	16474787	16486621	DAPK3*	Death-associated protein kinase 3	7	18326235	18328495	IL5	interleukin 5
7	16499574	16,503,730	NMRK2*	Nicotinamide riboside kinase 2	7	18360575	18360980	LOC102188306	pseudogene
7	16515642	16550762	ATCAY*	Ataxia, cerebellar, Cayman type	7	18375,010	18382102	IRF1	interferon regulatory factor 1
7	16587868	16611947	ZFR2*	Zinc finger RNA-binding protein 2	7	18474665	18500421	SLC22A5	solute carrier family 22 (organic cation/carnitine transporter), member 5
7	16629035	16636141	MATK*	Megakaryocyte-associated tyrosine kinase	7	18524881	18567090	SLC22A4	Solute carrier family 22 (organic cation/zwitterion transporter), member 4
7	16640063	16641228	RAX2*	Retina and anterior neural fold homeobox	7	18584900	18605166	PDLIM4	PDZ and LIM domain 4
7	16644654	16646660	MRPL54*	Mitochondrial ribosomal protein L54	7	18623324	18655016	P4HA2	prolyl 4-hydroxylase, alpha polypeptide II
7	16648979	16656398	APBA3*	Amyloid beta (A4) precursor protein-binding, family A, member 3	7	18678180	18823311	LOC102182028	pseudogene
7	16656486	16686391	TJP3*	Tight junction protein 3	7	18847086	18849440	GM-CSF	–
7	16694357	16737658	PIP5K1C*	Phosphatidylinositol-4-Phosphate 5-Kinase, Type I, Gamma	7	18862567	18864359	IL3	interleukin 3
7	16745649	16757967	CACTIN*	Spliceosome C complex subunit	7	18922344	18985869	ACSL6	acyl-CoA synthetase long-chain family member 6
7	16762240	16773455	TBXA2R*	Thromboxane A2 receptor	7	18991713	19128723	MEIKIN	meiotic kinetochore factor
7	16776308	16779707	GIPC3	GIPC PDZ domain-containing family, member 3	7	19162923	19262640	FNIP1	folliculin-interacting protein 1
7	16782955	16788034	HMG20B	high-mobility group 20B	7	19268176	19485469	RAPGEF6	Rap guanine nucleotide exchange factor 6
7	16795853	16809504	MFSD12	Major facilitator superfamily domain-containing 12	7	19503530	19596261	CDC42SE2	CDC42 small effector 2
7	16809268	16815892	C7H19orf71	–	7	19670441	19693055	LYRM7	LYR motif-containing 7
7	16816243	16828319	FZR1	Fizzy/cell division cycle 20-related 1	12	28572340	28620141	UBL3	ubiquitin-like 3
7	16847991	16857677	DOHH	Deoxyhypusine hydroxylase/monooxygenase					

Genes located in the region with the highest H value ($H=0.83$) were indicated with the * symbol

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Table 3 Gene enrichment for genes inside the genomic window on chromosome 7

Biological process name	*P* value	Adjusted *P* value
Regulation of lymphocyte proliferation (GO:0050670)	0.0001429	0.02775
Regulation of mononuclear cell proliferation (GO:0032944)	0.0001474	0.02775
Regulation of leukocyte proliferation (GO:0070663)	0.0001716	0.02775
Positive regulation of lymphocyte proliferation (GO:0050671)	0.0002095	0.02775
Positive regulation of mononuclear cell proliferation (GO:0032946)	0.0002178	0.02775
Positive regulation of JAK–STAT cascade (GO:0046427)	0.0001554	0.02775
Positive regulation of leukocyte proliferation (GO:0070665)	0.0002441	0.02775
Regulation of JAK–STAT cascade (GO:0046425)	0.00003227	0.02775
Regulation of alpha–beta T-cell activation (GO:0046634)	0.0002385	0.02775
Positive regulation of immunoglobulin production (GO:0002639)	0.0003314	0.0339

Terms and related metrics are reported from Gene Ontology for Biological Processes (**Biological process name**) and Wiki pathway. Only terms with adjusted P value <0.05 were considered

Comparison of the Valdostana breed with the other breeds

Six different approaches were tested to find regions across the genome that differentiated the Valdostana from the other goat breeds.

ROH comparisons, shown in Fig. S6, identified three regions of highest divergence between the ROH of Valdostana and the ROH of the other breeds examined separately with the same parameters. The first region was located on chromosome 1 (from 112,301,140 to 113,060,421 bp), the second on chromosome 7 (from 15,057,327 to 19,670,982), and the last on chromosome 12 (from 27,763,600, to 28,664,628). These regions included the windows of high homozygosity detected analyzing the Valdostana separately, and is shown in detail in Fig. S3-S5.

The results of the single-SNP Fst analysis are shown in Fig. 3a and identified SNPs on 4 chromosomes: chromosome 1 (8 SNPs from 110,663,697 to 124,748,543 bp), chromosome 7 (12 SNPs from 15,992,536 to 19,504,658 bp), chromosome 9 (1 SNP 61,687,558 bp), and chromosome 12 (3 SNPs from 25,743,128 to 28,327,291 bp). These results were confirmed also performing the Fst analysis in 1 Mb partially overlapping windows and is shown in Fig. 3b. The analysis identified nine windows that had values higher than the selected threshold of 0.31. This included two overlapping windows located on chromosome 1 (from 112 Mb to 113.5 Mb bp) and seven continuous and mainly overlapping windows located on chromosome 7 (from 15 Mb to 19.5 Mb). The window that included the markers identified with the single-SNP approach on chromosome 12 was right under/below the established threshold. Considering the two approaches, the windows detected with the Fst analyses were overlapping the three homozygous regions detected on chromosomes 1, 7, and 12 through runs of homozygosity.

GenSel analysis identified two 1 Mb windows that explained more than 1% of the variance. One window was located on chromosome 7 (from 16,043,582 to 16,974,423 bp) and explained 8.86% of the total variance. This window was included in the highly homozygous subregion and in the Fst analysis. The second window was located on chromosome 13 (61,006,494 to 61,971,928 bp) that explained 1.58% of the variance (Fig. 4).

The region on chromosome 7 was also confirmed by the Rsb analysis that identified 24 SNPs in the range of 15,221,110−20,065,201 bp above the threshold. A total of 13 SNPs were continuous from 15,221,110 to 15,948,105 bp. 1 SNP was located at position 17,028,582, and 8 and 2 SNPs were continuous in the ranges of 18,446,344−18,816,632 bp and 19,718,859−20,065,201 bp, respectively. Another non-continuous region was detected on chromosome 12 from 22,054,337 to 29,826,735 bp and contained 64 SNPs above the threshold. The XP-EHH analysis was concordant for the region on chromosome 7, with 54 non-continuous SNPs above the threshold that span from 14,464,313 to 20,737,623 bp and chromosome 12, with 10 SNPs from 24,467,948 to 28,489,734 bp. A continuous region was identified on chromosome 1, from 112,270,731 to 113,060,421 bp, which was therefore concordant with the ROH and Fst analyses. Two other SNPs on chromosome 13 (60,072,974 and 60,128,943 bp) were also above the threshold (Fig.5).

Discussion

Selective sweep analysis is a useful tool to investigate regions under selection in livestock, not only in animals under strong selection such as cattle, but also in those species that are reared for human consumption without a specific breeding scheme, such as goats (e.g., Andersson and

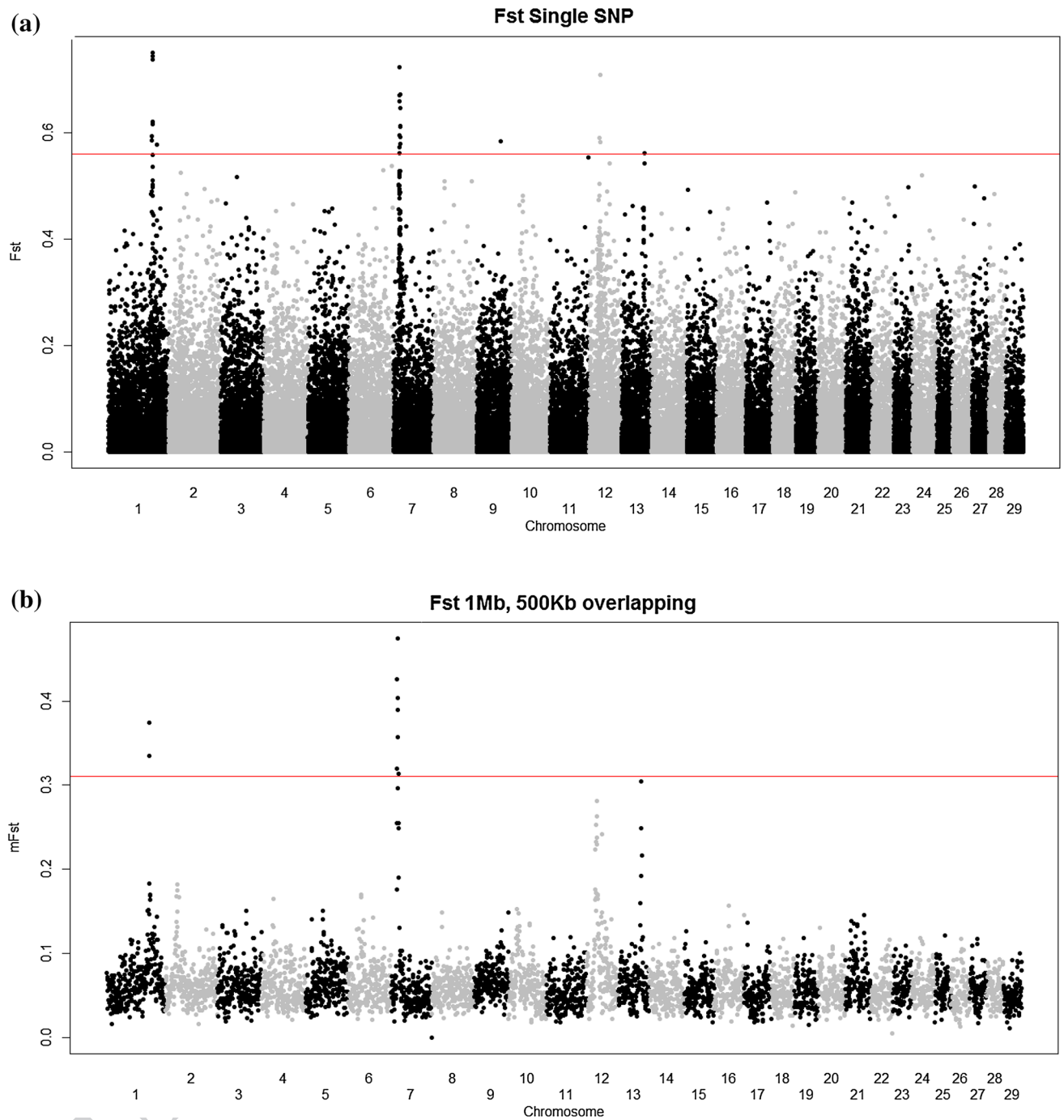

Fig. 3 Fst plot considering the single SNPs **a** and 1 Mb, 500 Kb overlapping window **b**. On the Y-axis, mean Fst (mFst) values are plotted, while on the X-axis chromosomes are plotted. The *red line* across the plot indicated the fixed threshold of 0.56 for the single SNPs **a** and 0.32 for the mFst **b**

Georges 2004; Kim et al. 2015). Among the 36 officially recognized Italian breeds (http://www.assonapa.it), 21 are considered not to be at risk (number of registered animals > 1200 registered head), 11 are endangered (number of registered animals < 1200 with a declining trend), and four are classified as in critical status (number of animals < 100), as reported by FAO (2013). With 600 officially registered animals, the Valdostana could therefore be considered an endangered breed.

The multidimensional scaling (MDS) plot confirmed the division between breeds raised in the north and those in the south of Italy (Nicoloso et al. 2015). This division

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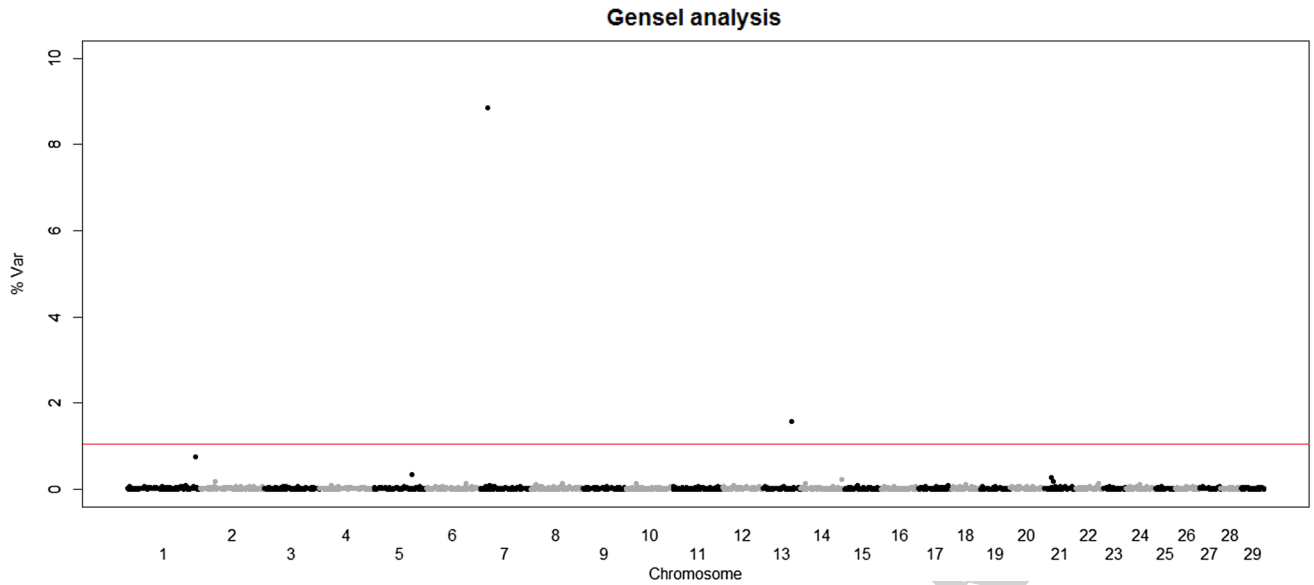

Fig. 4 1 Mb non-overlapping window plot of Bayes B analysis. The percentage of the overall explained variance is plotted on the Y-axis and chromosomes are plotted on the X-axis. The *red line* indicates the threshold of 1% of explained variance

is probably due to several factors, such as the climate difference between the two Italian regions, where the north is colder and humid and the south is generally hotter and arid. Despite these conditions, some breeds can be raised in both parts, but this climatic difference facilitates the selection of more specific breeds in the different regions. As expected, the Valdostana breed fits in the northern cluster, with some animals that overlap the Alpine and Nera di Verzasca breeds. This fact is probably due to some gene flow that occurred between these three breeds, because they have always been reared free-range on pastures in the same regions. In the case of the Alpine breed, these two breeds also share the same coat color and pattern.

To consider a region as highly homozygous, a threshold of H>0.62 was chosen. This value was chosen also considering the presence of possible genotyping errors and the possibility that some of the Valdostana goats analyzed may have a few recent non-Valdostana ancestors. All these factors could reduce the number of animals that share a common homozygous region. The runs of homozygosity analyses revealed the presence of a long region of about 4 Mb located on chromosome 7 and two other shorter regions (645 and 120 Kb) located on chromosomes 1 and 12, respectively.

The uniqueness of the region on chromosome 7 in the Valdostana breed was demonstrated by all five different analyses that compared the Valdostana genome with a group of 14 non-Valdostana goat breeds sampled across Italy. Despite a slightly different number of regions detected, all the five statistical analyses were concordant in showing the region on chromosome 7 as the most divergent between Valdostana and the other breeds. The regions identified on chromosomes 1 and 12 were also found divergent in almost all the comparisons, except for the Bayesian analysis.

Three of the genes within the highest homozygous H score on chromosome 7 (H=0.85) were the *MAP2K2* (Mitogen-Activated Protein Kinase Kinase 2) gene, the *APBA3* gene (Amyloid Beta (A4) Precursor Protein-Binding, Family A, Member 3), and the *ATCAY* gene (Ataxia, cerebellar, Cayman type). These genes could be directly or indirectly involved in modulating scrapie or Yersinia Pseudotuberculosis, two widespread diseases of sheep and goat (Tanahashi and Tabira 1999; King and Turner 2004; Nordström et al. 2005; Gossner and Hopkins 2015). It has been observed that Valdostana goats have a difference in several alleles of *PRNP* (Prion Protein gene: the major gene involved in scrapie) compared to the other breeds of northern Italy even if this difference was not significant (Colussi et al. 2008). The uniqueness of the region in Valdostana may provide interesting insights for future studies directed in this direction.

The enrichment analysis revealed that several of the genes within the region are linked to the development/regulation of several components of the immune system. It is interesting to underline that a genetic link between behavior and immunity systems has been hypothesized (Petitto et al. 1994). These authors showed that cytokines and T-cell proliferation were higher in mice bred for high aggression than in mice bred for low aggression. Since that initial research, the association between immune cell activity and various measures of aggressive behavior has been described in

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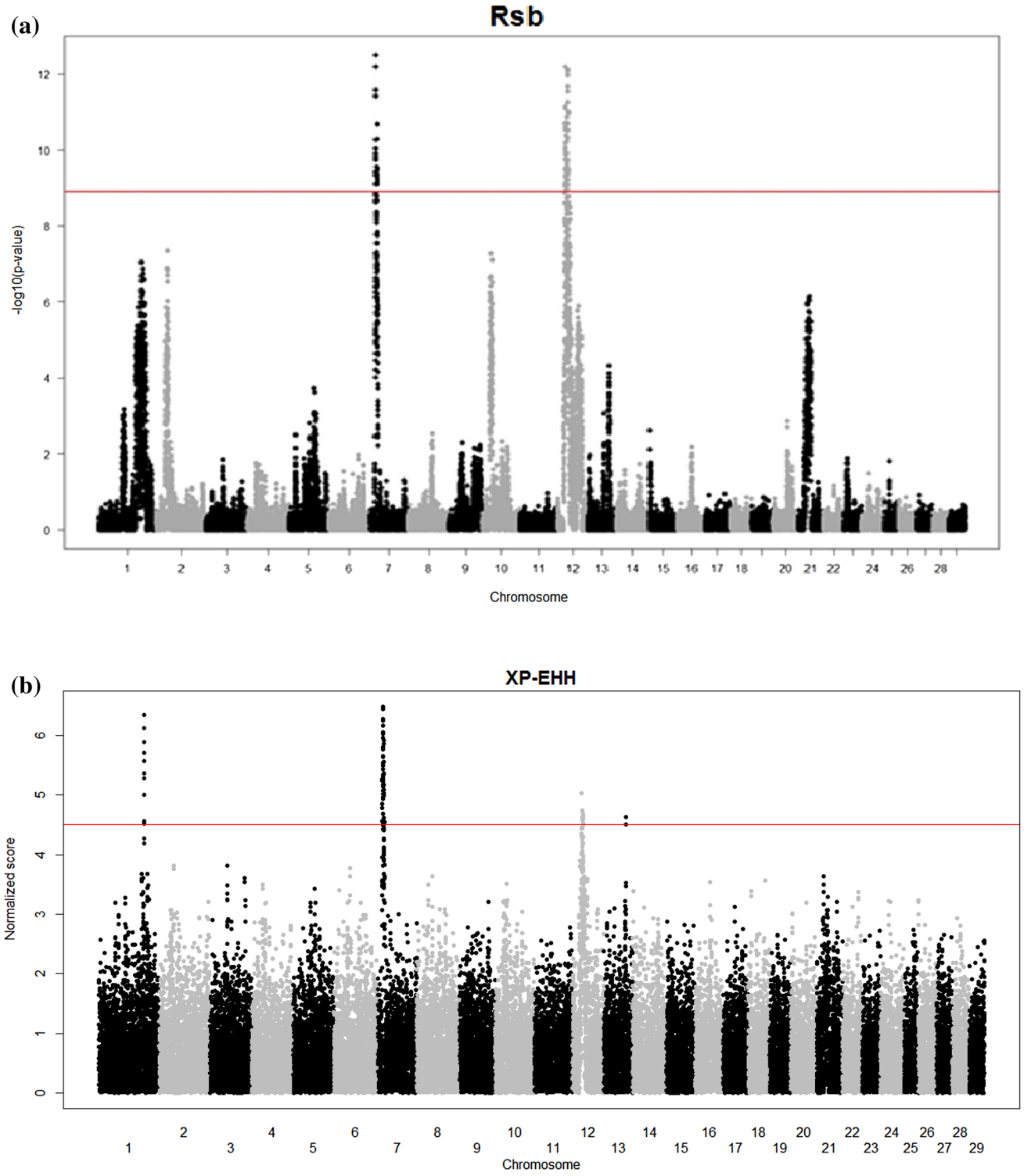

Fig. 5 Rsb **a** and XP-EHH **b** analyses. The normalized score for each SNP locus is plotted on the Y-axis and chromosomes are plotted on the X-axis. The *red lines* indicate the threshold values of 8 and 4.5 for Rsb and XP-EHH, respectively

several studies and documented in humans, mice, and cats. The factors that have been found in these studies include pathways that mainly involved inflammatory cytokines and T cells (reviewed by Zalcman and Siegel 2006). Interleukins modulate neurotransmitters and neurocrine activity influencing the individual's behavioral response to

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Fig. 6 Two Valdostana goats during the "Batailles des Chevres." The image was provided by Cinzia Finotto from the "Associazione Regionale Allevatori Valdostani"

potentially threatening environmental stimuli (Bhatt and Siegel 2006).

These findings may be linked with the peculiar activity, battle competition, for which the Valdostana has been employed. This characteristic non-cruel "*Batailles de Chèvres*" has a recent origin and is officially recognized, with the first competition having taken place in 1981. In addition, with the Valdostana cow traditional battle, *Bataille de Reine*, these bloodless competitions use the animal's natural behavior to fight (Fig. 6). Each match ends when one of the two competitors recognizes the superiority of the other. This event represents an attraction for the tourists and an economic opportunity for the farmers that own the strongest animals. Even if directed selection for the traits related to this competition were not performed, a recent estimation of heritability of the "fighting ability" trait in Valdostana cattle showed that selection for battle performance would be successful (Sartori and Mantovani 2010). The large region on chromosome 7 is probably an event of recent selection, and maybe it can be partially explained by the new fighting activity of this breed of goat.

In conclusion, we found evidence of selective sweep regions on three different chromosomes in the Valdostana goat breed. These regions showed a high level of homozygosity unique when compared to a wide representation of the Italian goat breeds. Interestingly, these regions contained genes involved in the immune system development/regulation. Our findings suggest that this region could be linked with the very recent, non-cruel battle events that are uniquely involved with these breeds. Further analyses will need to be performed to investigate in detail the three regions that could also be related to other breed-specific traits. All these are insights for further investigations of these unique genomic regions, for the understanding and safeguard of the Valdostana breed.

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