

# Characterization of 37 Breed-Specific Single-Nucleotide Polymorphisms in Sheep

LORRAINE PARISET, IRENE CAPPUCCIO, PAOLO AJMONE-MARSAN, MICHAEL BRUFORD, SUSANA DUNNER, OSCAR CORTES, GEORG ERHARDT, EVA-MARIA PRINZENBERG, KATJA GUTSCHER, STEPHANE JOOST, GABRIELA PINTO-JUMA, ISAÄC J. NIJMAN, JOHANNES A. LENSTRA, TRINIDAD PEREZ, ALESSIO VALENTINI AND ECONOGENE CONSORTIUM

From the Dipartimento di Produzioni Animali, Università della Tuscia, Viterbo, Italy (Pariset, Cappuccio, and Valentini); the Istituto di Zootecnica, Università Cattolica del Sacro Cuore, Piacenza, Italy (Ajmone-Marsan); the School of Biosciences, University of Wales, Cardiff, UK (Bruford, Pinto-Juma, and Perez); the Departamento de Producción Animal, Universidad Complutense, Madrid, Spain (Dunner and Cortes); the Institut für Tierzucht und Haustiergenetik, Justus-Liebig-Universität, Giessen, Germany (Erhardt, Prinzenberg, and Gutscher); the Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland (Joost); the Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands (Nijman and Lenstra); and <http://www.econogene.eu/> (Econogene Consortium).

Address correspondence to L. Pariset at the address above, or e-mail: [pariset@unitus.it](mailto:pariset@unitus.it).

We identified 37 single-nucleotide polymorphisms (SNPs) in sheep and screened 16 individuals from 8 different sheep breeds selected throughout Europe. Population genetic measures based on the genotyping of about 30 sheep from the same 8 breeds are reported. To date, there are no sheep SNPs documented in the National Center for Biotechnology Information dbSNP database. Therefore, the markers presented here contribute significantly to those currently available.

The several complete-genome projects have led to the emergence of single-nucleotide polymorphisms (SNPs) as most modern genetic markers. SNPs occur frequently in the mammalian genome (Brouillette et al. 2000; Shubitowski et al. 2001) and are useful for rapid, large-scale, and cost-effective genotyping (Schlotterer 2004; Syvanen et al. 2001; Vitalis et al. 2001; Vignal et al. 2002) for ecological and conservation studies (Vignal et al. 2002; Morin et al. 2004; Seddon et al. 2005) and for population and evolutionary studies (Kuhner et al. 2000; Sunnucks 2000; Glaubitz et al. 2003). However, SNPs are still scarce in nonmodel organisms, primarily due to the effort needed to find SNPs in species where little DNA sequence data are available, (Aitken et al. 2004) and their full potential is not yet exploited.

The European Union–sponsored Econogene project led to a collection of samples from sheep breeds from different European and Near-Eastern regions. This allowed an SNP discovery by across-breed comparison of 16 unrelated individuals belonging to 8 breeds representing wide phenotypic

and geographic variation in Western Eurasia: Akkaraman (Turkey), Bergamasca (Italy), Karagouniko (Greece), Rhönsheep (Germany), Rubia del Molar (Spain), Turcana (Romania), Welsh Mountain (Great Britain), and Zelazna (Poland). DNA was isolated after collection of whole blood using standard techniques. Primers designed using sheep sequences (where available) or the consensus sequences of the closest species in GenBank were used both for polymerase chain reaction amplification and sequencing of the corresponding genomic fragment. Sequences were BLAST aligned to establish homology. Thirty-seven SNPs were identified in 27 genes involved in key metabolic pathways or potentially relevant for production traits (Table 1).

Most of these were transitions, although we found 5 transversions and 1 deletion. Subsequently, these SNPs were genotyped in usually 30 individuals from each of the same 8 breeds, sampling no more than 3 individuals per farm. Standard population statistics (Weir 1996; Botstein et al. 1980) for each locus and over all populations were computed using the programs POWERMARKER (Liu and Muse 2001) and GENEPOP 3.3 (Raymond and Rousset 1995) and shown in Table 1. Thirty-two SNPs show an overall frequency of the rare allele higher than 5% and are thus generally applicable to population genetic studies.  $F_{ST}$  values are variable, but several values above 0.2 (*MCR1R*, *TNF\_1*, *ACVR2B*, and *CSN1S1*) suggest that SNPs are indicative for breed-specific selection. The values of 0.82 for both SNPs in the *MC1R* coat color gene are caused by a high frequency of the minor alleles only in the Welsh Mountain sheep.

**Table 1.** SNP genotyping and diversity parameters in sheep genes

Locus	Name	Primers 3'–5' (forward/reverse)	Genotyping method	SNP	Remarks	Rare allele frequency	N	He	Ho	PIC	F <sub>ST</sub>
<i>MC1R_1</i>	Melanocortin receptor 1	caagaaccgcaactgctact/ggccaggaagaggttgaag	PCR–RFLP <sup>a</sup>	Y13965:g.361G>A	Exon	0.074	235	0.1378	0.0213	0.128345	0.8163
<i>MC1R_2</i>	Melanocortin receptor 1	catgctctgcccgcactttgt/aagcagaggtctggaccacat	PCR–RFLP <sup>a</sup>	Y13965:g.218T>A	Exon	0.074	235	0.1378	0.0213	0.128345	0.8172
<i>SFN</i>	Stratifin	agccagctgatccagaagg/gcgatctcatagtggaagacg	Taqman	AF071008:g.408G>A	Exon	0.313	225	0.4303	0.3689	0.337727	0.1423
<i>KRT1</i>	Keratin-1	agagactcctggagaacacc/agtgcctccaagtcacaaagc	Taqman	M23912:g.5279G>C	Exon 6	0.417	234	0.4861	0.4829	0.367959	0.0257
<i>KRTAP6</i>	Keratin-associated protein-6	ccaatggcatgaaggtgt/aaaaaggggaaggggttgggtg	Taqman	M95719:g.1305A>G	3'-UTR	0.174	227	0.2875	0.2511	0.246143	0.1237
<i>TNF_1</i>	Tumor necrosis factor $\alpha$	gggaactcgtatgccaat/tctaaaccaagaaggggatga	Taqman	X56756:g.836delACA <sup>b</sup>	Intron 4	0.201	244	0.3210	0.0000	0.269467	0.3321
<i>TNF_2</i>	Tumor necrosis factor $\alpha$	gggaactcgtatgccaat/tctaaaccaagaaggggatga	Taqman	AY513771:g.244T>C <sup>c</sup>	Exon	0.137	190	0.2362	0.2421	0.20833	0.0362
<i>SERPINA3</i>	Alpha-1 anti-chymotrypsin	gttcaacctcacagagacc/gcagcttaagatcattgaaga	Taqman	DQ383805:g.134A>G <sup>d</sup>	Exon 2	0.104	201	0.1871	0.1891	0.169616	0.0268
<i>ACVR2B_1</i>	Activin receptor IIB	ttcggttgtgtctggaaagggc/gccttgacatcaggttctgct	Primer extension	U57707:g.826+13C>T	Intron 4	0.080	175	0.1472	0.1257	0.136366	0.0273
<i>ACVR2B_2</i>	Activin receptor IIB	ttcggttgtgtctggaaagggc/gccttgacatcaggttctgct	Primer extension	U57707:g.826+124G>A	Intron 4	0.500	177	0.5000	0.4463	0.375	0.2269
<i>BMPR_1</i>	Booroola	cagagcaaaagatgttccagcag/cttcagaggaatgggacaatgaa	Primer extension	AF357007:g.1797A>C	3'-UTR	0.147	207	0.2513	0.2077	0.219699	0.0328
<i>BMPR_2</i>	Booroola	cagagcaaaagatgttccagcag/cttcagaggaatgggacaatgaa	Primer extension	AF357007:g.2256A>G	3'-UTR	0.196	209	0.3154	0.3158	0.265646	0.1247
<i>CAST_1</i>	Calpastatin	ctattctctagtggcagatg/atacagaatgctgtctccac	Primer extension	U66320:g.1022+200G>A	Intron 14	0.031	210	0.0600	0.0524	0.058189	0.0432
<i>CAST_2</i>	Calpastatin	ctattctctagtggcagatg/atacagaatgctgtctccac	Primer extension	U66320:g.1022+288C/T	Intron 14	0.030	203	0.0574	0.0591	0.055721	0.0985
<i>MEG3</i>	Callipyge	tcgagctccaataatctt/cctctgacagctaaagcatgg	Taqman	AY017222:g.379G>T <sup>d</sup>	3'-UTR	0.402	205	0.4810	0.4537	0.365301	0.0426
<i>CYN1S1_1</i>	$\alpha$ S1-Casein	taaacaaaaattagctgtg/aaatggaatggcattgttcta	PCR–RFLP	AY534901:g.881A>G	5'-UTR	0.339	214	0.4480	0.3224	0.347659	0.2204
<i>CYN1S1_2</i>	$\alpha$ S1-Casein	ctctctagctttcagacaa/aagcattatgtgtcatgct	PCR–SSCP <sup>e</sup>	AY444506:g.137C>T	Exon 17	0.210	205	0.3315	0.3220	0.276565	0.0112
<i>CYN3</i>	k-Casein	ccaacataaaaccaggaaatcc/gtggctcttctgtatgctcttagag	PCR–RFLP	X51822:c.168T>C	Exon 4	0.193	212	0.3120	0.2264	0.26332	0.0126
<i>CTSB</i>	Cathepsin B	cttctgtctggcctctgag/aggaagtccagatcacacagag	Taqman	AY787747:g.275A>G <sup>d</sup>	Intron 6	0.111	207	0.1975	0.1643	0.178022	0.0534
<i>DES_1</i>	Desmin	gatgagtaccgccaccagat/ttccacataaggcttcatttga	Primer extension	AB011673:g.707+8A>G	Intron 3	0.009	172	0.0173	0.0174	0.01714	0.0317
<i>DES_2</i>	Desmin	gatgagtaccgccaccagat/ttccacataaggcttcatttga	Primer extension	AB011673:g.994+211G>T	Intron 5	0.198	172	0.3172	0.2558	0.266891	0.0724
<i>FABP4</i>	Fatty acid binding protein 4	tgtcagatccccaatcg/ttctctcagcattgaagg	Primer extension	X89244:g.409+165C>T	Intron 3	0.294	209	0.4153	0.4545	0.329087	-0.0064
<i>GHR</i>	Growth hormone receptor	tatgccaggttaagcagcat/attgagtacgagccctgtg	Taqman	AY292283:g.122A>G	Exon 10	0.204	201	0.3247	0.3085	0.272015	0.0397
<i>GHRHR</i>	Growth hormone releasing hormone receptor	gaaactggagccaactcagg/acagcgggaatgaggagaagc	Taqman	AY292289:g.339G>T <sup>d</sup>	Intron 10	0.498	206	0.5000	0.4320	0.374994	0.1167
<i>IGF1</i>	Insulin-like growth factor 1	cacacaccttggctcctc/agagcatccaccaactcagc	Taqman	AY737509:g.211C>T <sup>d</sup>	Exon 3	0.454	207	0.4958	0.4348	0.372885	0.0567
<i>IL2_1</i>	Interleukin 2	aagatcctcagaagagaaa/aaccttggcctatgagaagt	PCR–SSCP <sup>f</sup>	AF287479:g.318A>G	Exon 1	0.460	211	0.4968	0.4171	0.373372	0.0578
<i>IL2_2</i>	Interleukin 2	gggtgacaaattgtggatctgg/gaggatgcaggcaaatgaca	PCR–SSCP <sup>g</sup>	AF287479:g.3605C>T	Intron 3	0.275	213	0.3984	0.3615	0.319058	0.043
<i>IL4</i>	Interleukin 4	agagatcatcaaaacgctgaa/gtctgctacagccagctc	Taqman	DQ384928:g.160C>T	Intron 4	0.169	192	0.2812	0.2552	0.241689	0.118
<i>ITGB1</i>	Integrin B1	gggagacactgtgaaatgtagc/cgggtgtagttagggttgcact	Taqman	AY787745:g.203C>T	Intron 8	0.078	206	0.1433	0.1456	0.133011	0.0318
<i>LEP_1</i>	Leptin	ccctctcctgagttgtc/gcctatgtggggcatccttta	Primer extension	U43943:g.314A>G	Exon 3	0.012	207	0.0239	0.0242	0.023578	0.1308
<i>LEP_2</i>	Leptin	ccctctcctgagttgtc/gcctatgtggggcatccttta	Primer extension	U43943:g.476 <sup>g</sup> >G	Exon 3	0.024	209	0.0467	0.0478	0.045612	0.0895
<i>GDF8</i>	Myostatin	ccctcccttactgtcctcc/atcaagcccaaaatctctcc	Taqman	AY032689:g.2156C>T <sup>d</sup>	Exon 3	0.054	202	0.1030	0.0792	0.097678	0.101
<i>MYH1</i>	Myosin 1	cagaccaggaatggctgtt/tagatcatccagggtgata	Taqman	AY737517:g.295A>G <sup>d</sup>	Intron 1	0.073	184	0.1360	0.1250	0.126729	0.0058
<i>PRNP_1</i>	Prionprotein	gcaggagctgctgagct/cacaaagttgttctgttactatc	PCR–RFLP <sup>h</sup>	U67922:g.22684C>T	Exon 3	0.027	202	0.0530	0.0347	0.05157	0.0089
<i>PRNP_2</i>	Prionprotein	atgatctcagcacctaccctg/ataagagcctgctcatggca	PCR–RFLP	U67922:22226C>T	Intron 2	0.252	206	0.3774	0.3107	0.306194	0.0946
<i>TYRP1</i>	Tyrosinase-related protein 1	gtctcaggcagaatgaatc/ctgtgagaccctctggtctac	Taqman	AY737511:g.216C>T	Exon 2	0.163	200	0.2722	0.2550	0.235144	0.0977
<i>ZP2</i>	Zona pellucida glycoprotein 2	ccatctctacatggtgctcctt/tgttttgaggagagtttgtct	Taqman	DQ383806:g.105C>T	Exon 8	0.185	203	0.3012	0.2906	0.255845	0.037

N, number of genotyped animals; He, expected heterozygosity of gene diversity; Ho, observed heterozygosity; PIC, polymorphic information content; F<sub>ST</sub>, genetic differentiation parameters of breeds versus total; PCR, polymerase chain reaction; SSCP, single-strand conformation polymorphism; RFLP, restriction fragment length polymorphisms; and UTR, untranslated region.

<sup>a</sup> Våge et al. (1999).

<sup>b</sup> Nash et al. (1991).

<sup>c</sup> Alvarez-Busto et al. (2004).

<sup>d</sup> Pariset et al. (2006).

<sup>e</sup> Prinzenberg et al. (2003).

<sup>f</sup> Lühken et al. (2000).

<sup>g</sup> Lühken et al. (2002).

<sup>h</sup> Lühken et al. (2004).

**Table 2.** Genetic diversity parameters in individual breeds

Locus	Akkaraman (Turkey)			Bergamasca (Italy)			Karagouniko (Greece)			Rhönsheep (Germany)			Rubia del Molar (Spain)			Turcana (Romania)			Welsh Mountain (GB)			Zelazna. (Poland)		
	Gene diversity	$F_{IS}$	Rare allele frequency	Gene diversity	$F_{IS}$	Rare allele frequency	Gene diversity	$F_{IS}$	Rare allele frequency	Gene diversity	$F_{IS}$	Rare allele frequency	Gene diversity	$F_{IS}$	Rare allele frequency	Gene diversity	$F_{IS}$	Rare allele frequency	Gene diversity	$F_{IS}$	Rare allele frequency	Gene diversity	$F_{IS}$	Rare allele frequency
<i>MC1R_1</i>	0	NA	0	0.032	0	0.016	0.032	0	0.016	0	NA	0	0.032	0	0.016	0	NA	0	0.206	0.46	0.889	0	NA	0
<i>MC1R_2</i>	0	NA	0	0.063	-0.017	0.032	0	NA	0	0.032	0	0.016	0	NA	0	0	NA	0	0.206	0.46	0.889	0	NA	0
<i>SFN</i>	0.112	0.658	0.058	0.482	0.1	0.383	0.508	0.213	0.533	0.509	0.149	0.483	0.462	-0.395	0.355	0.454	-0.209	0.339	0.415	0.197	0.278	0.034	0	0.017
<i>KRT1</i>	0.404	-0.037	0.274	0.489	0.011	0.403	0.499	-0.099	0.435	0.503	0.007	0.45	0.417	-0.237	0.29	0.494	-0.176	0.581	0.507	-0.426	0.472	0.509	0.619	0.452
<i>KRTAP6</i>	0.415	-0.032	0.286	0.151	-0.071	0.081	0.178	0.277	0.097	0.503	0.007	0.45	0.183	-0.094	0.1	0.063	-0.017	0.032	0.203	-0.097	0.111	0.364	0.116	0.232
<i>TNF_1</i>	0.437	1	0.694	0.121	1	0.063	0.175	1	0.094	0.28	1	0.161	0.065	1	0.032	0.226	1	0.125	0.515	1	0.421	0.065	1	0.032
<i>TNF_2</i>	0.173	-0.053	0.091	0.303	-0.208	0.183	0.418	-0.011	0.288	0.12	-0.045	0.063	0.26	0.102	0.15	0.188	-0.098	0.103	0.267	-0.125	0.15	0.097	-0.036	0.05
<i>SERPINA3</i>	0.364	-0.250	0.227	0.156	0.36	0.083	0.275	0.063	0.161	0.073	-0.020	0.037	0.068	-0.018	0.034	0.209	-0.115	0.117	0.071	0	0.036	0.289	-0.191	0.172
<i>ACVR2B_1</i>	0	NA	0	0.094	-0.034	0.048	0.032	0	0.016	0.131	-0.057	0.069	0.216	-0.120	0.121	NA	NA	NA	0.338	0.606	0.2	0.217	0.205	0.121
<i>ACVR2B_2</i>	0.511	-0.370	0.45	0.489	0.011	0.597	0.443	-0.165	0.323	0.289	-0.191	0.172	0.462	-0.082	0.65	NA	NA	NA	0	NA	1	0.507	-0.087	0.483
<i>BMPR_1</i>	0	NA	0	0.123	-0.053	0.065	0.319	0.394	0.194	0.16	-0.077	0.086	0.383	0.216	0.25	0.283	0.057	0.167	0.389	0.286	0.25	0.171	-0.083	0.093
<i>BMPR_2</i>	0.364	-0.250	0.227	0.296	-0.200	0.177	0.494	-0.176	0.419	0	NA	0	0.236	0.151	0.133	0.275	0.063	0.161	0.114	-0.032	0.059	0.473	-0.253	0.37
<i>CAST_1</i>	0	NA	0	0	NA	0	0.203	-0.111	0.113	0.132	0.477	0.069	0	NA	0	0.032	0	0.016	0	NA	0	0.034	0	0.017
<i>CAST_2</i>	0	NA	0	0.032	0	0.016	0	NA	0	0	NA	0	0	NA	0	0.266	-0.167	0.155	0.108	-0.030	0.056	0	NA	0
<i>MEG3</i>	0.245	-0.111	0.136	0.505	0.141	0.55	0.389	0.006	0.258	0.471	-0.225	0.365	0.507	-0.184	0.5	0.511	0.231	0.482	0.48	0.422	0.361	0.471	-0.132	0.367
<i>CXN1S1_1</i>	0.255	0.643	0.136	0.274	-0.176	0.161	0.346	0.133	0.217	0.389	0.006	0.742	0.252	-0.154	0.145	0.494	-0.176	0.581	0.363	0.694	0.222	0.447	0.423	0.323
<i>CXN1S1_2</i>	0	NA	0	0.414	0.114	0.283	0.399	0.164	0.267	0.373	0.049	0.242	0.283	-0.179	0.167	0.344	-0.261	0.217	0.245	-0.133	0.139	0.318	0.189	0.194
<i>CXN3</i>	0.5	0.636	0.364	0.178	0.277	0.097	0.262	0.618	0.15	0.346	0.133	0.217	0.42	0.233	0.29	0.338	0.14	0.21	0.257	-0.143	0.147	0.276	0.3	0.161
<i>CTSB</i>	0.464	0.412	0.318	0.032	0	0.016	0.033	0	0.017	0.329	-0.238	0.204	0.177	-0.091	0.097	0.261	0.361	0.15	0.265	0.778	0.147	0.183	-0.094	0.1
<i>DES_1</i>	0	NA	0	0	NA	0	0	NA	0	0.1	-0.037	0.052	0	NA	0	NA	NA	NA	0	NA	0	0	NA	0
<i>DES_2</i>	0.292	-0.143	0.167	0.299	0.044	0.179	0.339	0.333	0.21	0.48	0.138	0.379	0	NA	0	NA	NA	NA	0.324	0.141	0.194	0.373	0.107	0.241
<i>FABP4</i>	0.267	-0.125	0.15	0.415	0.277	0.283	0.404	-0.037	0.274	0.465	-0.111	0.355	0.389	0.006	0.258	0.442	-0.293	0.321	0.454	-0.223	0.333	0.425	-0.254	0.3
<i>GHR</i>	0.518	0.474	0.409	0.346	0.133	0.217	0.097	-0.036	0.05	0.338	-0.250	0.212	0.42	-0.232	0.293	0.398	-0.006	0.267	0.243	0.451	0.133	0.26	0.102	0.15
<i>GHRHR</i>	0.491	0.259	0.636	0.496	0.352	0.411	0.506	-0.055	0.467	0.437	-0.270	0.315	0.465	-0.111	0.355	0.382	0.039	0.75	0.288	0.227	0.833	0.496	0.089	0.419
<i>IGF1</i>	0.373	0.268	0.773	0.472	0.012	0.367	0.485	-0.208	0.397	0.509	-0.020	0.481	0.495	-0.043	0.419	0.463	0.065	0.65	0.392	0.575	0.25	0.498	0.352	0.419
<i>IL2_1</i>	0.245	-0.111	0.864	0.482	-0.071	0.387	0.503	0.47	0.433	0.5	0.067	0.433	0.496	0.089	0.581	0.404	-0.037	0.274	0.517	0.153	0.469	0.51	0.177	0.5
<i>IL2_2</i>	0	NA	0	0.474	-0.020	0.371	0.405	0.125	0.274	0.261	0.361	0.15	0.39	0.174	0.258	0.486	-0.234	0.4	0.324	0.141	0.194	0.456	0.08	0.339
<i>ILA</i>	0	NA	0	0.397	-0.177	0.267	0.414	0.114	0.283	0.15	-0.067	0.08	0.476	0.066	0.37	0.036	0	0.018	0.091	0	0.045	0.183	-0.094	0.1
<i>ITGB1</i>	0.309	-0.176	0.182	0.032	0	0.016	0.032	0	0.016	0.14	-0.061	0.074	0.094	-0.034	0.048	0.283	0.057	0.167	0.175	-0.071	0.094	0.188	-0.098	0.103
<i>LEP_1</i>	0	NA	0	0	NA	0	0	NA	0	0	NA	0	0	NA	0	0	NA	0	0.245	-0.133	0.139	0	NA	0
<i>LEP_2</i>	0	NA	0	0	NA	0	0	NA	0	0	NA	0	0	NA	0	0.165	-0.080	0.089	0.245	-0.133	0.139	0	NA	0
<i>GDF8</i>	0	NA	0	0	NA	0	0.094	-0.034	0.048	0.363	0.153	0.231	0.066	-0.018	0.033	0	NA	0	0	NA	0	0.167	0.357	0.089
<i>MYH1</i>	0.173	-0.053	0.091	0.1	0	0.05	0.098	0.659	0.05	0.073	-0.020	0.037	0.178	0.277	0.097	0.1	-0.037	0.052	0.059	0	0.029	0.266	-0.167	0.155
<i>PRNP_1</i>	0.091	0	0.045	0.036	0	0.018	0.153	0.789	0.081	0.094	-0.034	0.048	0.037	0	0.019	0	NA	0	0	NA	0	0	NA	0
<i>PRNP_2</i>	0.173	-0.053	0.091	0.457	0.224	0.339	0.462	0.253	0.345	0.16	-0.077	0.086	0.462	0.253	0.345	0.131	-0.057	0.069	0.51	-0.308	0.5	0.364	0.085	0.233
<i>TYRP1</i>	0.389	-0.286	0.25	0.151	-0.071	0.081	0.418	-0.080	0.29	0	NA	0	0.353	-0.073	0.224	0.128	0.477	0.067	0.16	0.653	0.083	0.457	-0.139	0.34
<i>ZP2</i>	0.173	-0.053	0.091	0.389	0.006	0.258	0.126	-0.055	0.067	0.372	-0.096	0.241	0.435	-0.110	0.31	0.314	0.231	0.19	0.121	-0.034	0.063	0.26	0.102	0.15

Genetic diversity is calculated as Nei's (1987) unbiased estimator. Inbreeding coefficient  $F_{IS}$  has been calculated according to Weir and Cockerham (1984). NA: not available.

Diversity parameters for individual breeds were calculated using the program FSTAT (Goudet 2000) and listed in Table 2. From the 5 SNPs in 3 genes with overall frequencies below 5%, 4 have appreciable frequencies (0.1–0.266) in specific breeds: CAPN\_1 in Karagouniko and Rhönsheep, CAPN\_2 in Turcana and in Welsh Mountain, LEP\_1 in Welsh Mountain, and LEP\_2 in Turcana and Welsh Mountain. The minor allele of DES\_1 was found only at a low frequency (0.052) in Rhönsheep. Such SNPs may be informative to a reconstruction of the breed history or may be under breed-specific selection.

Our SNP data contribute to the collection of about 100 SNPs identified so far and to the eventual use of these markers for the genetic analysis of breed history of a variety of phenotypes.

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