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

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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_{ST}
MC1R_1	Melanocortin receptor 1	caagaaccgcaacctgcact/ggccaggaagaggttgaag	PCR–RFLP[a]	Y13965:g.361G>A	Exon	0.074	235	0.1378	0.0213	0.128345	0.8163
MC1R_2	Melanocortin receptor 1	catgcctgcgccgacatttgt/aagcagaggctggacaccat	PCR–RFLP[a]	Y13965:g.218T>A	Exon	0.074	235	0.1378	0.0213	0.128345	0.8172
SFN	Stratifin	agccagtctgatccagaaagg/gcgatctcatagtggaagacg	Taqman	AF071008:g.408G>C	Exon	0.313	225	0.4303	0.3689	0.337727	0.1423
KRT1	Keratin-1	agagactccctggagaacacc/agtgcctccaagtcaaaagc	Taqman	M23912:g.5279G>C	Exon 6	0.417	234	0.4861	0.4829	0.367959	0.0257
KRTAP6	Keratin-associated protein-6	ccaatggcatgaaggtgt/aaaaagggaagggttggtg	Taqman	M95719:g.1305A>G	3′-UTR	0.174	227	0.2875	0.2511	0.246143	0.1237
TNF_1	Tumor necrosis factor α	gggactcgtatgccaat/tctaaaccaagaaggggatga	Taqman	X56756:g.836delACA[b]	Intron 4	0.201	244	0.3210	0.0000	0.269467	0.3321
TNF_2	Tumor necrosis factor α	gggactcgtatgccaat/tctaaaccaagaaggggatga	Taqman	AY513771:g.244T>C[c]	Exon	0.137	190	0.2362	0.2421	0.20833	0.0362
SERPINA3	Alpha-1 anti-chymotrypsin	gttcaacctcacagagaccc/gcacgctaagatcattgaaga	Taqman	DQ383805:g.134A>G[d]	Exon 2	0.104	201	0.1871	0.1891	0.169616	0.0268
ACVR2B_1	Activin receptor IIB	ttcggttgtgtctggaaggcg/gctttgacatcaggttctgct	Primer extension	U57707:g.826+13C>T	Intron 4	0.080	175	0.1472	0.1257	0.136366	0.0273
ACVR2B_2	Activin receptor IIB	ttcggttgtgtctggaaggcg/gctttgacatcaggttctgct	Primer extension	U57707:g.826+124G>A	Intron 4	0.500	177	0.5000	0.4463	0.375	0.2269
BMPR_1	Booroola	cagagcaaaagatgttccagcag/cttcagaggatgtgggacaaatgaa	Primer extension	AF357007:g.1797A>C	3′-UTR	0.147	207	0.2513	0.2077	0.219699	0.0328
BMPR_2	Booroola	cagagcaaaagatgttccagcag/cttcagaggatgtgggacaaatgaa	Primer extension	AF357007:g.2256A>G	3′-UTR	0.196	209	0.3154	0.3158	0.265646	0.1247
CAST_1	Calpastatin	ctattctctagttgcgatgtg/atcagaagtgctgctccac	Primer extension	U66320:g.1022+200G>A	Intron 14	0.031	210	0.0600	0.0524	0.058189	0.0432
CAST_2	Calpastatin	ctattctctagttgcgatgtg/atcagaagtgctgctccac	Primer extension	U66320:g.1022+288C/T	Intron 14	0.030	203	0.0574	0.0591	0.055721	0.0985
MEG3	Callipyge	tccgagctccaataatcttc/tccttgacacgtaagcatgg	Taqman	AY017222:g.379G>T[d]	3′-UTR	0.402	205	0.4810	0.4537	0.365301	0.0426
CSN1S1_1	αS1-Casein	taaacaaaaattagcttgg/aaatggaatggcattgttcta	PCR–RFLP	AY534901:g.881A>G	5′-UTR	0.339	214	0.4480	0.3224	0.347659	0.2204
CSN1S1_2	αS1-Casein	ctctctagcttttcagacaa/aagcatttatgtgtcatgct	PCR–SSCP[e]	AY444506:g.137C>T	Exon 17	0.210	205	0.3315	0.3220	0.276565	0.0112
CSN3	k-Casein	ccaacatataaacccaggaatcc/gttgtcttctttgatgtctccttagag	PCR–RFLP	X51822:c.168T>C	Exon 4	0.193	212	0.3120	0.2264	0.26332	0.0126
CTSB	Cathepsin B	ctttctgtctggcctctgag/aggaagtccgagtacacagag	Taqman	AY787747:g.275A>G[d]	Intron 6	0.111	207	0.1975	0.1643	0.178022	0.0534
DES_1	Desmin	gatgagtaccgccaccagat/ttccacataaggcttcatttga	Primer extension	AB011673:g707+8A>G	Intron 3	0.009	172	0.0173	0.0174	0.01714	0.0317
DES_2	Desmin	gatgagtaccgccaccagat/ttccacataaggcttcatttga	Primer extension	AB011673:g.994+211G>T	Intron 5	0.198	172	0.3172	0.2558	0.266891	0.0724
FABP4	Fatty acid binding protein 4	tgctaggtcatccccataatcg/ttccttctcagcattgaagg	Primer extension	X89244:g409+165C>T	Intron 3	0.294	209	0.4153	0.4545	0.329087	−0.0064
GHR	Growth hormone receptor	tatgcccaggtaagcgacat/attgagtacgaggccctgtg	Taqman	AY292283:g.122A>G	Exon 10	0.204	201	0.3247	0.3085	0.272015	0.0397
GHRHR	Growth hormone releasing hormone receptor	gaaactggagccaactcagg/acagcggaatgaggagaagc	Taqman	AY292289:g.339G>T[d]	Intron 10	0.498	206	0.5000	0.4320	0.374994	0.1167
IGF1	Insulin-like growth factor 1	cacacacccttgttgcactcc/agagcatccaccaactcagc	Taqman	AY737509:g.211C>T[d]	Exon 3	0.454	207	0.4958	0.4348	0.372885	0.0567
IL2_1	Interleukin 2	aagagtcatcagaagaggaaa/aaccttgggcatgtagaagt	PCR–SSCP[f]	AF287479:g.318A>G	Exon 1	0.460	211	0.4968	0.4171	0.373372	0.0578
IL2_2	Interleukin 2	ggtgacaaattgtggatctgg/gaggatgcaggcaaatgaca	PCR–SSCP[g]	AF287479:g.3605C>T	Intron 3	0.275	213	0.3984	0.3615	0.319058	0.043
IL4	Interleukin 4	agagatcatcaaaacgctgaa/gtctgctacaggcagctc	Taqman	DQ384928:g.160C>T	Intron 4	0.169	192	0.2812	0.2552	0.241689	0.118
ITGB1	Integrin B1	gggagacactgtgaatgtagc/cggtgtagttaggggttgcact	Taqman	AY787745:g.203C>T	Intron 8	0.078	206	0.1433	0.1456	0.133011	0.0318
LEP_1	Leptin	ccctctcctgagtttgtc/gcctatgtggggcatcctta	Primer extension	U43943:g.314A>G	Exon 3	0.012	207	0.0239	0.0242	0.023578	0.1308
LEP_2	Leptin	ccctctcctgagtttgtc/gcctatgtggggcatcctta	Primer extension	U43943:g.476″>G	Exon 3	0.024	209	0.0467	0.0478	0.045612	0.0895
GDF8	Myostatin	ccctcccttactgtcatcc/atcaagcccaaaatctctcc	Taqman	AY032689:g.2156C>T[d]	Exon 3	0.054	202	0.1030	0.0792	0.097678	0.101
MYH1	Myosin 1	cagaccaggaaatggctgtt/tagatcatccaggtgcata	Taqman	AY737517:g.295A>G[d]	Intron 1	0.073	184	0.1360	0.1250	0.126729	0.0058
PRNP_1	Prionprotein	gcaggagctgctgcagct/cacaaagttgttctggttactatc	PCR–RFLP[b]	U67922:g.22684C>T	Exon 3	0.027	202	0.0530	0.0347	0.05157	0.0089
PRNP_2	Prionprotein	atgatctcagcacctaccttg/ataagaggcctgctcatggca	PCR–RFLP	U67922:22226C>T	Intron 2	0.252	206	0.3774	0.3107	0.306194	0.0946
TYRP1	Tyrosinase-related protein 1	gctccaggcagaatgaaatc/ctgtgagaaccctctggtcac	Taqman	AY737511:g.216C>T	Exon 2	0.163	200	0.2722	0.2550	0.235144	0.0977
ZP2	Zona pellucida glycoprotein 2	ccatctctacatggtgcctct/ttgtttgaggagagtttgct	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_{ST}, 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.

[a] Våge et al. (1999).

[b] Nash et al. (1991).

[c] Alvarez-Busto et al. (2004).

[d] Pariset et al. (2006).

[e] Prinzenberg et al. (2003).

[f] Lühken et al. (2000).

[g] Lühken et al. (2002).

[b] Lühken et al. (2004).

Table 2. Genetic diversity parameters in individual breeds

Locus	Akkaraman (Turkey) Gene diversity	F_{IS}	Rare allele frequency	Bergamasca (Italy) Gene diversity	F_{IS}	Rare allele frequency	Karagouniko (Greece) Gene diversity	F_{IS}	Rare allele frequency	Rhönsheep (Germany) Gene diversity	F_{IS}	Rare allele frequency	Rubia del Molar (Spain) Gene diversity	F_{IS}	Rare allele frequency	Turcana (Romania) Gene diversity	F_{IS}	Rare allele frequency	Welsh Mountain (GB) Gene diversity	F_{IS}	Rare allele frequency	Zelazna. (Poland) Gene diversity	F_{IS}	Rare allele frequency
MC1R_1	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
MC1R_2	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
SFN	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
KRT1	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
KRTAP6	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
TNF_1	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
TNF_2	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
SERPINA3	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
ACVR2B_1	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
ACVR2B_2	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
BMPR_1	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
BMPR_2	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
CAST_1	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
CAST_2	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
MEG3	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
CSN1S1_1	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
CSN1S1_2	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
CSN3	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
CTSB	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
DES_1	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
DES_2	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
FABP4	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
GHR	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
GHRHR	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
IGF1	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
IL2_1	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
IL2_2	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
IL4	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
ITGB1	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
LEP_1	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
LEP_2	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
GDF8	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
MYH1	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
PRNP_1	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
PRNP_2	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
TYRP1	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
ZP2	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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