



Novel polymorphisms in ovine *prion protein* gene

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Summary

The aim of this study was to identify the *PRNP* polymorphisms outside the standard codons 136, 154 and 171 in 1110 sheep with no clinical sign of scrapie from all 18 Turkish native sheep breeds and compare our results with published data on ovine *PRNP* polymorphism from other regions of the world. Among the 22 amino acid polymorphisms and three silent mutations, 10 were novel for ovine *PRNP*: p.Gly94Gly, p.Leu128Ile, p.Met132Leu, p.Ser135Arg, p.Met137Val, p.Asn146Lys, p.Arg159Arg, p.Tyr160Asn, p.Gln163His and p.Thr193Ser. These data reveal that sheep breeds close to the historic center of small ruminant domestication have remained highly diverse in the prion gene locus, with distinctive genetic similarities to both Asian and European sheep breeds.

Keywords gene variability, *PRNP* gene, Turkish native sheep

Scrapie is a fatal neurodegenerative disease that affects small ruminants and is the prototype of transmissible spongiform encephalopathies (TSE) or prion diseases that also include Creutzfeldt–Jakob disease in humans and bovine spongiform encephalopathy (BSE) in cattle. It is characterized by the accumulation of an abnormal, protease-resistant isoform of the cellular prion protein in brain and some other tissues (for review see, Prusiner 1998). In sheep, susceptibility to scrapie has been proven to be largely controlled by polymorphisms in the amino acid sequence of the *PRNP* gene. To date, more than 30 amino acid polymorphisms have been described in the ovine *PRNP* open-reading frame (Table S1). Originally it was found that three of these polymorphisms have a major effect on scrapie susceptibility in sheep, these were p.Ala136Val, p.Arg154His and p.Gln171Arg (for review, see Goldmann 2008), but it is has become clear over the past years that amino acid replacements in other codons can have significant association with susceptibility. For example, association with resistance to natural scrapie has been suggested for p.Met137Thr and p.Asn176Lys in Italian sheep (Vaccari *et al.* 2009) and in goats with p.Asn146Ser or p.Asn146Asp (Papasavva-Stylianou *et al.* 2011). Resistance to BSE is associated with p.Met112Thr in British sheep (Saunders *et al.* 2009), whereas increased susceptibility to atypical scrapie is associated with the ovine p.Leu141Phe polymorphism (Moum *et al.* 2005).

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Accepted for publication 10 October 2012

Here, we identified *PRNP* polymorphisms outside of codons 136, 154 and 171 in 1110 sheep from 18 native Turkish breeds and compared our results with published data on ovine *PRNP* alleles. The sample origin, sequencing method and standard three-codon *PRNP* genotypes have been presented in Meydan *et al.* (2012). Twenty-two DNA sequence variations leading to amino acid polymorphisms were detected in total and deposited in GenBank with accession numbers JX187517–JX187539. Eight were novel changes (p.Leu128Ile, p.Met132Leu, p.Ser135Arg, p.Met137Val, p.Asn146Lys, p.Tyr160Asn, p.Gln163His and p.Thr193Ser), whereas the other 14 changes have all been observed before: p.Gln101Arg, p.Met112Thr, p.His114Arg, p.Gly127Val, p.Gly127Ser, p.Leu141Phe, p.His143Arg, p.Gly145Ser, p.Gly145Val, p.Asn146Ser, p.Tyr172Asp, p.Gln175Glu, p.Ile185Thr and p.Gln189Leu (see Table S1 for references). Three silent mutations also were found at codons p.Gly94Gly (GGT/GGC), p.Arg159Arg (CGT/CGC) and p.Leu237Leu (CTG/CTC). The distribution of these polymorphisms in Turkish native sheep breeds is shown in Table S2. Most but not all of the additional amino acid polymorphisms were found in linkage with the ARQ allele, for example, p.Leu128Ile. However, two polymorphisms, p.Asn146Lys and Gln163His, were found in novel allele combinations with ARR and ARH respectively, supporting the view that not only the ARQ allele acquires further mutations (Fig. 1). This finding is not particular for the Turkish breeds, as we have sequenced in British sheep polymorphisms p.Gly89Ser and p.Asp170Gly on the VRQ and AHQ alleles respectively (W. Goldmann, unpublished data). One quarter of all ARQ alleles (340/1374 alleles) had an additional polymorphism, and on average each breed showed four to five additional polymorphisms in the ARQ

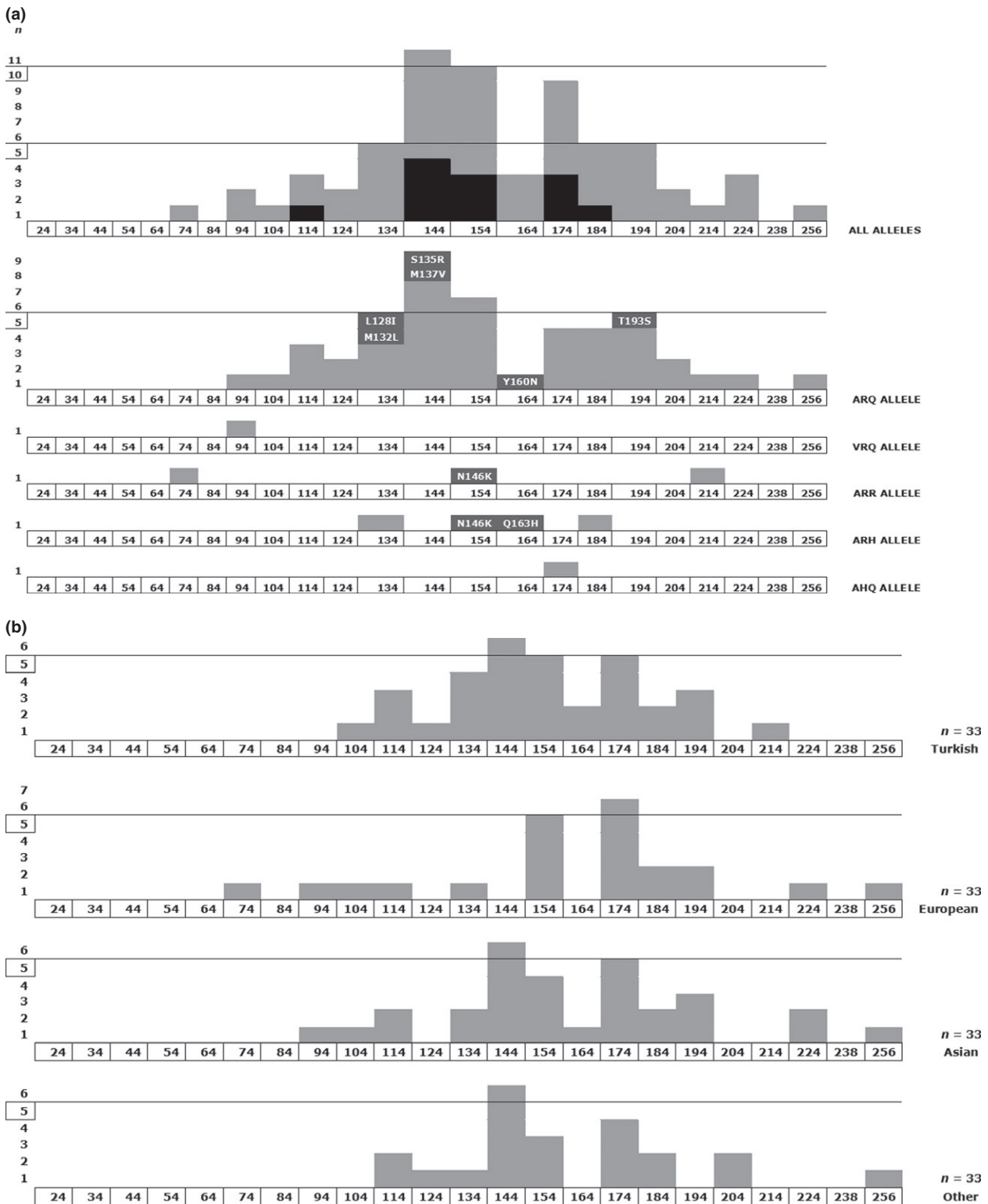


Figure 1 Histogram showing the number (*n*) of polymorphisms in light gray (*y*-axis) on the *PRNP* sequence (*x*-axis) shown here in consecutive 10 amino acid blocks with the exception of the first block (amino acids 1–24, signal peptide) and last block (239–256, GPI-signal peptide). The base sequence in the ALL ALLELES histogram is the ARQ allele. Amino acid polymorphisms reported here for the first time are highlighted in dark gray. Positions of amino acid polymorphisms that are linked with scrapie susceptibility are shown in black. Details of polymorphisms and their publication details are given in full in Table S1, Supporting Information. (a) Polymorphisms by allelic linkage (not all polymorphisms have unambiguously defined 136, 154, 171 linkage). (b) Polymorphisms as defined by changes to the ARQ sequence arranged by country/world region.

background, but all appeared to be mutually exclusive. In one breed, the Gökçeada, very low *PRNP* variability was observed with only 1% of ARQ carrying an additional polymorphism. In contrast, the highest genetic variability was seen in Ivesi and Akkaraman, both showing nine additional polymorphisms in 30% of their ARQ alleles. The phylogenetic tree was constructed as a graphic representation of the genetic relationship among the Turkish native sheep breeds at the *PRNP* locus (Fig. S1).

The most common polymorphisms in Turkish native breeds were p.Met112Thr, p.Gly127Ser, p.Gly127Val, p.Asn146Ser and p.Gln189Leu (Ün *et al.* 2008; Alvarez *et al.* 2011; Frootan *et al.* 2011; Öner *et al.* 2011; this study). The ARQ alleles with p.Gly127Ser, p.Asn146Ser and p.Gln189Leu are indicators of the Asian breed background (Gombojav *et al.* 2003; Hussain *et al.* 2011; Karami *et al.* 2011).

The p.Met112Thr polymorphism was found in two-thirds of the breeds and would be expected to give increased protection from scrapie (Laegreid *et al.* 2008; Saunders *et al.* 2009). Based on goat studies, we expect the p.Asn146Ser substitution to also protect sheep. From the eight novel polymorphisms, three (p.Met132Leu, p.Met137Val and p.Asn146Lys) are likely to produce some scrapie survival advantage based on their position within *PRNP* and knowledge from other species (Goldmann 2008; Vaccari *et al.* 2009; Papasavva-Stylianou *et al.* 2011). Whether any of the other polymorphisms effect scrapie cannot yet be concluded with confidence, but it has been observed that heterozygous *PRNP* genotypes can benefit the host (Goldmann *et al.* 1994; Kobayashi *et al.* 2009), and a large proportion of these sheep were *PRNP* gene heterozygotes. The apparently low frequency of the p.Leu141Phe polymorphism reduces the susceptibility of Turkish breeds to atypical scrapie (Benestad *et al.* 2008).

The earliest evidence of sheep domestication was found in the Near East, with Turkey as an area of major importance (Peters *et al.* 1999; Zeder 2008). Thus, it is likely that some of the Turkish native sheep breeds of today are some of the oldest living descendants of their first domesticated ancestors, and Anatolian (Turkish) native breeds may be special in maintaining very valuable genetic diversity. Recently, it was suggested by Kijas *et al.* (2012) that 'domestication occurred from a broad genetic base' and 'frequent genetic exchange has occurred during the development of modern breeds'. Our *PRNP* gene survey supports this view by showing that Turkish breeds have high genetic diversity (Fig. 1b) and that a considerable number of *PRNP* polymorphisms are shared by the breeds of the world. However, there appeared to be some separation between polymorphisms that can be found in breeds of European and Turkish/Asian origin, and we speculate that these regional differences in *PRNP* variation may contribute among other factors to the apparent differences in the occurrence of TSE outbreaks in European and Turkish/Asian sheep.

Acknowledgments

Authors would like to thank Dr. B. Yüceer and R. Değirmenci for their assistance in the collection of samples. This study was partially supported by a grant from Scientific Research Projects (BAP) of Ankara University (Project No: BAP-09B4347007; Project coordinator: Dr. M.M. ÖZKAN).

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Supporting information

Additional supporting information may be found in the online version of this article.

Figure S1 Neighbor joining (NJ) dendrogram for Turkish native sheep breeds based on pairwise F_{ST} values calculated from all *PRNP* haplotype frequencies.

Table S1 A comparison of *PRNP* gene amino acid substitutions among Turkish, European, Asian and other sheep breeds.

Table S2 The distribution and number of various *PRNP* gene haplotypes in Turkish native sheep breeds.

Table S1. A comparison of *PRNP* gene amino acid substitutions among Turkish, European, Asian and other sheep breeds.

Codons 136/154/171	Polymorphism	Sequence change	References †				N1	NT	NW	N
			Turkish	European	Asian	Others				
<i>(A) Variation of ARQ, VRQ, AHQ, ARR, ARH alleles</i>										
ARQ	G 85 R	GGA / AGA				22			1	1
VRQ	G 89 S	GGT / AGT		12					1	1
ARQ	Q 101 R	CAG / CGG	T	1		17, 22			1	28 29
ARQ	M 112 T	ATG / ACG	T, 4, 10, 34	1, 8, 11, 13, 28, 37		14, 17, 22, 35	18, 30	76 18	716	809
ARQ	M 112 I	ATG / ATT	34			38			3	66 69
ARQ	H 114 R	CAT / CGT	T				30	1		5 6
ARQ	A 116 E	GCA / GAA	34						6	6
ARQ	A 116 P	GCA / CCA					29, 33			10 10
ARQ	G 127 S	GGC / AGC	T, 4, 10, 25			14, 15, 17, 20, 22, 35, 38		65 12	238	315
ARQ	G 127 V	GGC / GTC	T, 4, 10, 25	37		14, 15, 21, 22, 35		24 5	36	65
ARQ	G 127 A	GGC / GCC					18			2 2
ARQ	L 128 I *	CTT / ATT	T					1		1
ARQ	M 132 L *	ATG / CTG	T					5		5
ARQ	S 135 R *	AGT / AGG	T					1		1
ARQ	M 137 V *	ATG / GTG	T					6		6
ARQ	M 137 T	ATG / ACG		7, 8, 11, 13, 28, 36, 37		17	9, 18, 19, 31		5	188 193
ARQ	S 138 N	AGC / AAC		32, 8			9, 31			43 43
ARQ	S 138 R	AGC / AGA				17				16 16
ARQ	L 141 F	CTT / TTT	T, 4	1, 6–8, 11, 13, 23, 28, 32, 36, 37		22, 35	9, 18, 19, 30	1 2	2121	2124
ARQ	I 142 K	ATA / AAA		37						1 1
ARQ	I 142 T	ATA / ACA					19			2 2
ARQ	H 143 R	CAT / CGT	T, 4, 10, 25	1, 11, 37		35, 38	9, 18, 33	14 3	239	256
ARQ	G 145 S	GGC / AGC	T, 25					3 1		4
ARQ	G 145 V	GGC / GTC	T, 10					1 27		28
ARQ	N 146 S	AAT / AGT	T, 4, 10, 34			21, 22		89 25	8	122
ARR	N 146 K *	AAT / AAG	T					2		2
ARR	N 146 K *	AAT / AAG	T					3		3
ARQ	R 151 H	CGT / CAT		1						4 4
ARQ	R 151 C	CGT / TGT		1, 8, 13, 32		20	18, 31			42 42
ARQ	R 151 G	CGT / GGT					33			1 1
ARX	Y 152 F	TAC / TTC				17, 38				10 10
ARX	M 157 I	ATG / ATA				35				1 1
ARQ	Y 160 N *	TAC / AAC	T					1		1
ARR	Q 163 H *	CAA / CAC	T					1		1

ARQ	R 167 S	AGA / AGT				9			16	16		
ARQ	P 168 L	CCA / CTA		13					50	50		
AHQ	D 170 G	GAT / GGT		12					4	4		
ARQ	Y 172 D	TAT / GAT	T, 4, 25, 34	1		21		33	11	3	41	55
ARQ	S 173 N	AGT / AAT		34		21				2	7	9
ARQ	Q 175 E	CAG / GAG		T	1, 13				2		46	48
ARQ	N 176 K	AAC / AAA			1, 36, 37			19, 30, 33			24	24
ARQ	V 179 E	GTG / GAG		34		21				8	18	26
ARQ	H 180 Y	CAT / TAT						9, 18			21	21
ARQ	I 185 T	ATC / ACC		T	26					2	1	3
ARQ	Q 189 L	CAA / CTA		T, 4, 25		14, 15, 20, 22, 35, 38			20	4	152	176
ARQ	Q 189 R	CAA / CGA				14, 15					3	3
ARQ	T 191 I	ACA / ATA				20					2	2
ARQ	T 193 S*	ACC / TCC		T						16		16
ARQ	T 195 S	ACC / TCC						9			4	4
ARQ	T 196 S	ACC / TCC						9			2	2
ARQ	R 211 Q	CGA / CAA			8						1	1
ARR	V 213 E	GTG / GAG		4						2		2
ARQ	V 213 E	GTG / GAG		4						3		3
ARX	Q 220 H	CAG / CAC				35					3	3
ARX	R 223 K	AGA / AAA				35					5	5
ARQ	S 240 P	TCC / CCC						33			1	1
ARQ	P 241 S	CCT / TCT			13, 28	38		18			586	586
ARR	PHGGGWGQ octapeptide repeat				28						2	2

(B) Other alleles in codons 136, 154 and 171

TRR				24		17			1		2	3
TRQ				4, 10, 24, 25, 34	6, 27	17		9	46	28	73	147
TRH				24					2			2
TRK						17					1	1
ARK				4, 10, 24, 25	2, 6, 23	14–17, 20, 35, 38		9	21	8	206	235
ALQ					3						4	4
ARN						20					1	1

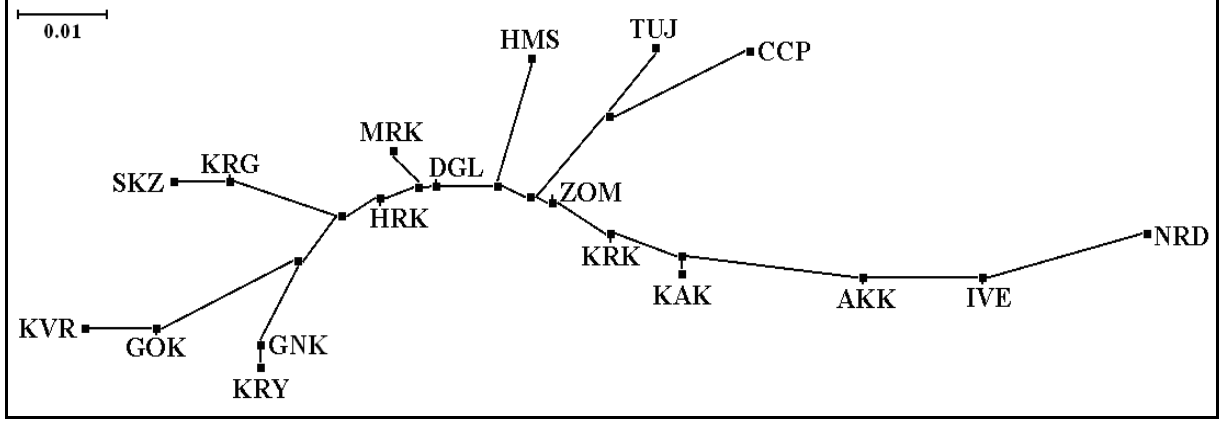
* Polymorphisms not previously reported.; these were confirmed by sequencing twice and in both directions on DNA from different PCR reactions. DnaSP software was used for haplotype analysis. † References were listed below. N1: Number of allele observations in this study; NT: Previously recorded observations in Turkey; NW: Previously recorded observations worldwide; N: Total observations (N1+NT+NW).

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Figure S1: Neighbor joining (*NJ*) dendrogram for Turkish native sheep breeds based on pairwise F_{ST} values calculated from all *PRNP* haplotype frequencies.



AKK, Akkaraman; CCP, Çine Çaparı; DGL, Dağlıç; GNK, Güneykaraman; GOK, Gökçeada (Imroz); HMS, Hemşin; HRK, Herik; IVE, İvesi (Awassi); KAK, Kangal Akkaraman; KRG, Karagül (Karakul); KRK, Karakaş; KRY, Karayaka; KVR, Kıvırcık; MRK, Morkaraman; NRD, Norduz; SKZ, Sakız (Chios); TUJ, Tuj (Tushin); ZOM, Zom.

Table S2: The distribution and number of various *PRNP* gene haplotypes in Turkish native sheep breeds.

	AKK n=100	CCP n=40	DGL n=40	GNK n=30	GOK n=50	HMS n=55	HRK n=45	IVE n=100	KAK n=100	KRG n=50	KRK n=35	KRY n=45	KVR n=140	MRK n=100	NRD n=35	SKZ n=50	TUJ n=45	ZOM n=50	Total n=1110
Polymorphisms																			
G 94 G									1										1
Q 101 R	1																		1
M 112 T	9	15	2	5		5	2		3		5	5	6	5	2	3	6	3	76
H 114 R								1											1
G 127 S	1	9	3			2	7	10		6	2			14		1	7	3	65
G 127 V						2	3	1			1		1	3			11	2	24
L 128 I			1																1
M 132 L	2						3												5
S 135 R	1																		1
M 137 V			1					5											6
L 141 F								1											1
H 143 R	6							1	3	1							1	2	14
G 145 S						2		1											3
G 145 V																1			1
N 146 S	2		7			21	2	17	5	3	2	7		6	2	6	5	4	89
N 146 K									5										5
R 159 R			1																1
Y 160 N			1																1
Q 163 H									1										1
Y 172 D			5								5							1	11
Q 175 E													2						2
I 185 T						2													2
Q 189 L	2				1			5	9		1						1	1	20
T 193 S										7							9		16
L 237 L	10																		10
Total	34	24	21	5	1	34	17	42	27	17	16	12	9	28	4	11	40	16	358

n: number of analyzed samples

AKK, Akkaraman; CCP, Çine Çaparı; DGL, Dağlıç; GNK, Güneykaraman; GOK, Gökçeada (Imroz); HMS, Hemşin; HRK, Herik; IVE, İvesi (Awassi); KAK, Kangal Akkaraman; KRG, Karagül (Karakul); KRK, Karakaş; KRY, Karayaka; KVR, Kıvrıcık; MRK, Morkaraman; NRD, Norduz; SKZ, Sakız (Chios); TUJ, Tuj (Tushin); ZOM, Zom.