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Caprine Prion Gene Polymorphisms in Main Northern Cyprus Goat Breeds

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ABSTRACT

The administration of genetic breeding programs to eradicate transmissible spongiform encephalopathies in goat and sheep is crucial for animal breeding, health and welfare, together with food safety and security. Goat production and indigenous goat breeds (Cyprus Native hair Goat, Damascus) are important for the Cyprus's agricultural diversity Therefore, the allele frequencies of Caprine Prnp gene variants should be well understood for goat breeding programs in Northern Cyprus. This current study was designed to determine the putative allele frequencies and genotype distributions of Caprine Prnp gene variations in Northern Cyprus Damascus, Cyprus Native Hair and hybrid goats. Four different locations in the Northern Cyprus were selected for targeted gene sequencing. Blood samples were collected from 50 Northern Cyprus goats. Genomic DNA was extracted from goats' whole blood. As a result, ten different Caprine Prnp polymorphisms were detected including P42= (CCG>CCA), G127S (GGC>AGC), S138= (AGT>AGC), N146S (AAT>GAT), R154H (CGT>CAT), Gln172Arg (CAG>CGG), G172H (CAG>CAT), G172R (CAG>CGT), V179V (GTG>GTT), V187 (CAG>CAT). The three most common genetic markers were P42= 16%), R154H (12%), N146S (8%), respectively. Two different polymorphism, P42= and R154H, were detected in Cyprus native hair goat whereas P42=, G127S and N146S were observed in the Damascus breed and P42=, G127S, R154H and N146S in Hybrid goats. The predisposition genotype, 146NN homozygosity, was found at the same ratio as the Northern Cyprus Native Hair Goat. There were no statistically significant differences between the breeds. The use of Cyprus Cyprus native hair goat in animal husbandry has a critical importance in terms of species genetic diversity We believe that this breed will play a key role in the reduction of scrapie cases, based on its resistance genotype H154 ratio. Larger studies are needed to gain a complete understanding with regards to the impacts of the 146D, 146S, and 154H alleles in Damascus, Cyprus Native Hair Goat and Damascus-Saanen hybrids.

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MB extracted DNA. MCE and MB did sequence analysis. MCE, DA and MB wrote the manuscript. DA presented the idea of the research.

Key words
Prnp, Prion diseases, goat, Northern cyprus

INTRODUCTION

Prion diseases or transmissible spongiform encephalopathies (TSEs) are fatal infectious neurodegenerative diseases which can affect both humans and animals (Medyan et al., 2017; Madsen-Bouterse *et al.*, 2021; Marín-Moreno *et al.*, 2017). Prion diseases have generally been characterized by neurodegenerative symptoms in brain tissues whereas based on the conformational changes from the normal prion protein (PrP^C) to the destructive isoform of the prion protein (PrP^{Sc}), which causes distributional changes in the secondary structure of PrP^C (Sakaguchi, 2007). The small ruminants, including goats and sheep, are natural hosts of scrapie and are related to the group of transmissible spongiform encephalopathies (TSEs), which also includes bovine spongiform encephalopathy (BSE) in cattle, chronic wasting disease (CWD) in deer, fatal familial insomnia (FFI), Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome (GSS) in humans (Lühken *et al.*, 2007).

Classical scrapie indicates similar epidemiological peculiarities in sheep and goats. Even though the incidence in goats is much lower than in sheep, milk, and placenta of contaminated goats may serve as possible sources of infection in sheep (Anomymous, 2019; Konold *et*

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al., 2016). In goats, scrapie was first described in 1942 (Migliore *et al*., 2020). After which clinical cases have been reported across Europe. Animal transactions among herds and environmental spread are notable risk factors (Anomymous, 2019).

In both goats and sheep, genotypes of the prion protein gene (*Prnp*) lead to an individual's susceptibility to scrapie. The polymorphic codons in sheep are know well. Genetic selection and breeding plans have been implemented in Europe, towards reducing the prevalence of sensitive genotypes to scrapie (Torricelli *et al*., 2021). As already applied in sheep, single nucleotide polymorphisms (SNPs) of the Caprine *Prnp* gene could provide an opportunity to categorize caprine genotypes which are susceptible or resistant to the scrapie disease (Aguilar-Calvo *et al*., 2014). Caprine PrP^{C} includes 256 amino acids which are arranged into two domains so that only the C-terminal spherical domain (position 102 - 234) is necessary for the conversion into pathogenic PrP^{Sc} (Meydan *et al*., 2017).

The caprine *Prnp* gene has revealed a considerable number of polymorphisms regarding resistance and specifically 28 amino acid replacements have been determined in common and domestic European goat breeds (Medyan et al., 2017; Zhou *et al*., 2008). Among species and populations from outside of Europe, novel *Prnp* polymorphisms have been detected (Table I). Table I shows *Prnp* polymorphism in different countries. Concisely, it shows the majority of amino acid polymorphisms emerge in only a few countries. Even so, seven out of the 29 reported amino acid changes appear to have a global distribution by haplotype gradation, from most prevalent: R143, P240, S127, K222, H154, S146, Q211, M142 and a minimum five of these have been offered to be associated with TSE sensitivity (Vaccari *et al*., 2009).

Alternatively, the Caprine *Prnp* gene polymorphisms at codons I142M, H143R, N146S, R154H and Q222K have been associated with a scrapie protective effect against prion disease in United Kingdom, Greece, Southern Cyprus and Italy (Papasavva-Stylianou *et al*., 2011a; Kdidi *et al*., 2021). Furthermore, in Cyprus, S146 or D146 genotypes have been associated with a high level of resistance (Kdidi *et al*., 2021). In goats, heterozygosity at codons I142M, H143R, N146S, R211Q, and Q222K confers reduced sensitivity to scrapie development (Kim *et al*., 2019). Table II shows susceptibility and resistant alleles in the Caprine Prnp gene.

The influence of *Prnp* genetics on sheep and goat scrapie in Europe over the last decade has been successfully applied including the southern part of Cyprus (Republic of Cyprus) according to the most recent up - to - date EU 999/2001 regulation (Regulation EU 772/2020) which is based on all available study outcomes by the European Commission (EU). The EU regulation suggested K222, D146 and S146 polymorphisms could be used as scrapie resistance alleles in genetic management both in scrapie outbreaks and in disease prevention (Georgiadou *et al*., 2017). Boer (England and Netherlands), Nubian (Netherlands), Native/hybrid, Damascus and related breeds (Cyprus) are goat breeds with D146 or 146S haplotype. Toggenburg (England and Netherlands), and Saanen (Netherlands, France, Spain, Italy) are goat breeds with the K22 marker (Georgiadou *et al*., 2017; Anonymous, 2012). Goats with the 146N/N, 222 Q/Q genotype are susceptible to classical scrapie because they lack copies of the protective polymorphisms (Table I) (Goldmann *et al*., 2011; Georgiadou *et al*., 2017).

Goat breeding in the Northern Cyprus holds an important place in animal husbandry. Consumption of goat meat and dairy products very importantly traditional Cypriot cheese known as hellim/halloumi have a significant place in the local cultural heritage. Dairy products have the largest share of exported products (58%) (Northern Cyprus Veterinary Services, n.d.). However, wild goat, Cyprus mouflon (*Ovis orientalis ophion*), are also found in the mountain ranges of the island.

Table I. *Prnp* polymorphisms in different countries.

Countries	Prnp polymorphisms
China (Zhou *et al*., 2008)	W102G, S240P, G127S, Q222K, H143R, R211G, N146S, T219I, R154H, R211Q, I218L
Cyprus (South part) (Papasavva-Stylianou *et al*., 2011)	W102G, T110P, I142M, H143R, N146N, N146D, N146S, R151H, R154H, Q163Q, P168Q, I208T, P240S
Japan (Babar *et al*., 2009)	I142M, G127S, H143R, S240P, N146S, R211Q
Italia (Torricelli *et al*., 2021)	G37V, M137I, R139S, I142M, H143R, N146D, R154H, R211Q, Q222K, P240S
Greecy (Bouzalas *et al*., 2010)	Q171R, R211Q, Q222K, S240P
Pakistan (Babar *et al*., 2009)	S240P
USA (Babar *et al*., 2009; White *et al*., 2008; Meydan *et al*., 2017)	I142M, G127S, H143R, N146S, Q222K, R154H, R211Q, S240P
Turkey (Akis *et al*., 2020)	H143R, N146S,146D, R154H, Q171R, R211Q, P240S

In the current study, we aimed to determine the putative allele frequency as well as the genotype distribution of caprine *Prnp* gene variations in Northern Cyprus by targeted gene sequencing.

MATERIALS AND METHODS

Study design, data and biological sample collection

A total 50 different goat blood samples were collected from four different locations (Dikmen, Düzova, Akdeniz and Büyük Kaymaklı) in Northern Cyprus. Sampling was carried on clinically healthy goats belonging to Cyprus native hair goat (n = 20), Damascus (n = 16) and Damascus and Saanen hybrids (n = 14). DNA isolation was performed from whole blood and EDTA - treated tubes for each examined goat using Invitrogen DNA isolation kit (Thermo Scientific, UK) and DNA concentrations have been measured using nanodrop (NanoDrop™ 2000/2000c, Thermo, UK). The study protocol was approved by the ethical review board of Near East University Local Ethics Committee of Animal Experiments (application no: YDU/2019/05-75).

Caprine Prnp *gene sequencing*

The *Capra hircus Prpn* gene reference sequence was used to compare and align DNA sequences (GenBank: HM038415.1). Based on codons I142M, H143R, N146S, R154H and Q222K whole open reading frame was searched for amino acid polymorphisms to determine the Caprine *Prnp* gene markers.

DNA samples were used as a template in PCR amplification (740 bp) of the entire open reading frame of the *Prnp* gene with forward 5'-ATGGTGAAAAGCCACATAGG-3' and revers 5'-TATCCTACTATGAGAAAAATGAGG-3' primers. Sequencing was performed by Macrogene (Netherlands) using 50 ng/l DNA (A260/280 = ~1.8) and the target region was sequenced with Forward F5'-AACCAACATGAAGCATGTGG-3' and Reverse 5'-GAT- AGTAACGGTCCTCATAG-3' primers.

Statistical analysis

Sequencing analysis was performed on SnapGene software (GSL Biotech LLC, USA). An ANOVA tests non-parametric Friedman test was applied to check each locus.

RESULTS

Fifty Northern Cyprus goats DNA was sequenced to investigate the possible polymorphisms that show resistance or predisposition to scrapie. Ten different genetic variations were detected in targeted gene location including P42= (CCG>CCA), G127S (GGC>AGC), S138= (AGT>AGC), N146S (AAT>GAT), R154H (CGT>CAT), Gln172Arg (CAG>CGG), G172H (CAG>CAT), G172R (CAG>CGT), V179V (GTG>GTT), V187 (CAG>CAT). The most common genetic marker were P42= (14%) detected at three locations: Düzova, Akdeniz (Cyprus native goats) and Küçük Kaymaklı, respectively. The second most detected single nucleotide polymorphism was R154H (12%), which was found at two different locations: Düzova and Akdeniz, respectively. N146S was the third most common (16%) and was only in detected in Düzova. Also, G127S was only detected in Düzova. Cyprus native goats showed two different *Prnp* gene polymorphism including P42= and R154H, whereas P42= G127S and N146S were detected in Damascus breed and P42 = G127S, R154H and N146S in hybrid goats (Table III).

In this study, the N146S heterozygous genotype was detected in 14% of Goats' *Prpn* gene (18,8% Damascus and 28,6% Hybrid). Additionally, the N146N genotype was also found at the same ratio. These findings were consistent with previous research, which also found a decreased vulnerability in both N146D and N146S genotypes to natural and artificial scrapie infections in field trials, as well as a higher genotype for 146NN (Medyan et al., 2017; Windig *et al*., 2016; Kim *et al*., 2019; Konold *et al*., 2020). As a result of the statistical analysis, no significant difference was found between the groups (Aguilar- Calvo et al., 2014).

Table II. Susceptibility and resistant alleles in the Caprine *Prnp* gene (Georgiadou et al., 2017;Castañeda-Bustos et al., 2014).

Aminoacid Position	Genotype	Susceptibility	Resistance
142	MM		++
	IM		+
	II	+	
146	SS		+++
	NS		++
	NN	+++	
	ND		
	DD		++
	DS		+++
154	HH		++
	RH		+
	RR	+	
211	QQ		++
	RQ		+
	RR	+	
222	KK		++++
	KQ		+
	QQ	+	
240	PP		+++
	PS		+
	SS	+	

Table III. Genotype distributions of the genetic polymorphisms in the goat *Prnp* gene among goat breeds (N, number; %, frequency).

Polymorhisms				**Damascus (N=16)**		**Akdeniz (Cyprus Native hair Goat) (N=20)**		**Hybrid (N=14)**		**All (N=50)**
p.(P42P)	p.(G127G)	p.(N146N)	p.(R154R)	N	%	N	%	N	%	%
p.(P42=)	-	-	-	1	6,25	6	30	1	14,29	16
-	p.(G127S)	-	-	1	6,25	-	-	1	14,29	4
	-	p. (N146S)	-	3	18,75	-	-	4	28,57	14
			p.(R154H)	-	-	2	10	2	14,29	8

DISCUSSION

In sheep, European Union countries and the United Kingdom (UK) recorded 688 scrapie incidents in 2020, 309 cases less than in 2019. In all, 65 instances of scrapie in sheep were reported by two of the four non-EU countries (Iceland and Norway). Classical scrapie (CS) was reported by seven EU and one non-EU country, namely Bulgaria, Cyprus, Greece, Italy, Portugal, Romania, Spain, and Iceland; whereas atypical scrapie (AS) was reported in Belgium, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Poland, Portugal, Romania, Slovakia, Spain, and Sweden; plus the UK and another non-EU countries (Norway). Italy also reported a total of twelve inconclusive scrapie cases in sheep, which are not included in the country's total scrapie caseload (Anonymous, 2021).

The caprine *Prnp* gene S146 and D146 polymorphisms naturally confer considerable protection against the illness according to previous studies performed in Cyprus. High Genetic Merit Folks (HGMF) or Breeding Program Classical Scrapie (BP-SC) standards have been applied to breed herds across the world. However, only BP-SC standards have been used in Republic of Cyprus, but not in the northern part of the island (Anonymous, 2014). According to EU27 and UK report in 2020, a total of 328 Scrapie cases were documented, with nine asymptomatic scrapie and 319 classical scrapie cases in Cyprus (Cyprus accounts for 74% of classical scrapie cases in European countries) (Anonymous, 2021).

In 2017, based on a combination of the weight of evidence and the strength of resistance, the European Food Safety Authority (EFSA) provided a ranking of resistance to classical scrapie as follows: K222 > D146 = S146 > Q211 = H154 = M142 wild type (Migliore *et al.*, 2020). H154 was linked to increased natural scrapie resistance in Greek and Ionica breed goats as well as S146 or D146 in Cypriot goats. Cyprus native hair goat is important agricultural diversity for Cyprus and significantly resistant genotype 154 HH homozygosity was found as 4% (Colussi *et al.*, 2008).

Furthermore, investigations have shown that specific codons such as A42G, S138S, V179V and S240P within the Caprine *Prnp* gene are strongly resistant to the scrapie disease (Papasavva-Stylianou *et al.*, 2007; Zhou *et al.*, 2008; Goldmann *et al.*, 2011; Anonymous, 2012; Windig *et al.*, 2016). In the marker assisted selection (MAS) breeding program, genetic variability at different codons of the coding region in the *Prnp* gene have been a focus of investigation into the relationship between genetic variation and scrapie infection and production attributes (Lan *et al.*, 2012). The bias of synonymous codons, on the other hand, was a global arrangement for maximizing the growth and development efficiency of different types of cells, which relates to a similar bias of tRNA abundance. Theoretically, there is codon bias in the P42= and it is strongly associated with codon S138S of the goat *Prnp* gene (Barillet *et al.*, 2009; Anonymous, 2022)· These synonymous SNPs within the goat *Prnp* gene may control the expression of genes linked to production attributes, based on codon bias and a significant correlation between codons A42G, C138T and new polymorphisms at codons G172R and V179V have been discovered (Vaccari *et al.*, 2009).

In this study, P42= G127S, R154H and N146S SNPs were detected in three different goat breeds including Damascus, Cyprus native and Hybrid. The most valuable finding of the study was revealing the allele frequencies of Caprine *Prnp* gene polymorphisms in Northern Cyprus showing that N146S variant in Damascus goats (18.8%) and hybrid (28.6%) and R154H polymorphism in Cyprus Native Hair Goat (10%) and hybrid (14.29). Due to the lack of, or very low frequencies of D146 and S146 alleles across Europe, with the exception of Cyprus, no data from field investigations on genotypes at codon 146 resistance to European scrapie strains have been released to date (Lan *et al.*, 2012; Papasavva-Stylianou *et al.*, 2007; Georgiadou *et al.*, 2017; Kim *et al.*, 2019).

In our investigation, allele 154H was also associated to natural scrapie resistance. In Greek and Cypriot goats, where this gene appears to be somewhat protective against scrapie, a similar effect has previously been observed (Papasavva-Stylianou *et al*., 2011). Histidine at codon 154 is also strongly related to the condition in goats (Barillet *et al*., 2009; Belt *et al*., 1995; Konold *et al*., 2020). The haplotype 154H was 8% at two different farms in the Duzova region and 4% in the Cyprus native goats in the Akdeniz region. The 154H polymorphism was discovered in 12% of the participants in this investigation. Considering the results obtained, it has been determined that a strict breeding improvement policy is needed throughout Cyprus.

CONCLUSION

As a result, selecting regionally appropriate genes for scrapie resistance breeding in goats remains a difficult task (Acín *et al*., 2021; Castañeda-Bustos *et al*., 2014; Georgiadou *et al*., 2017). Based on the selection of animals containing the amino acids aspartic acid (D) and serine (S) at this position, the findings from this case-control research clearly implies that polymorphisms at codon 146 may provide a tool for preventing scrapie disease in goats in Northern Cyprus. Additional research into the impact of the alleles 146D, 146S, and 154H on animals carrying these alleles, as well as the impact of these alleles on different TSE agents, may provide accurate and reliable information on the efficacy of breeding for these alleles as a means to control and eradicate classical scrapie in goats. In order to avoid a Scrapie pandemic in Northern Cyprus, more stringent precautions should be applied. Clinical Veterinary Genetics should be given more attention across the island. Damascus, Native and hybrid breeds can be used to combat future scrapie outbreaks. The use of the Cyprus native hair goat is very important in terms of diversity. Considering the H154 ratio, we think that this breed will play an important role in the fight against scrapie. The fact that Cyprus is an island country and the introduction of other races from outside will not be beneficial for the scrapie resistance.

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Statement of conflicts of interest

The authors have declared no conflict of interest.

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