

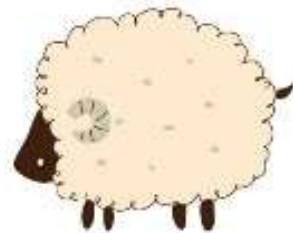


**Prp Gene Based Scrapie Susceptibility in Native Turkish Sheep: Do We Need To Introduce a Breeding Program to Select For Resistance to Scrapie in Turkey?**

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# Scrapie;

- ✓ is an infectious **fatal** disease of sheep and goat.
- ✓ comes in the **family of prion diseases** that include;
  - a. Creutzfeldt-Jakob disease (CJD) in humans,
  - b. Bovine Spongiform Encephalopathy (BSE) or “mad cow” disease in cattle, and
  - c. Chronic Wasting Disease (CWD) in deer and elk.
- ✓ affects the **central nervous system**.





<http://www.avma.org/onlnews/javma/may02/s050102g.asp>



<http://en.wikipedia.org/wiki/Scrapie>



<http://www.teara.govt.nz/en/diseases-of-sheep-cattle-and-deer/4/4/1>

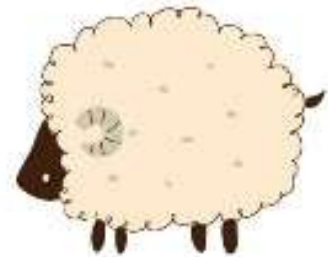


<http://www.teara.govt.nz/en/diseases-of-sheep-cattle-and-deer/4/4/1>

**Figure 1.** Sheep with scrapie.

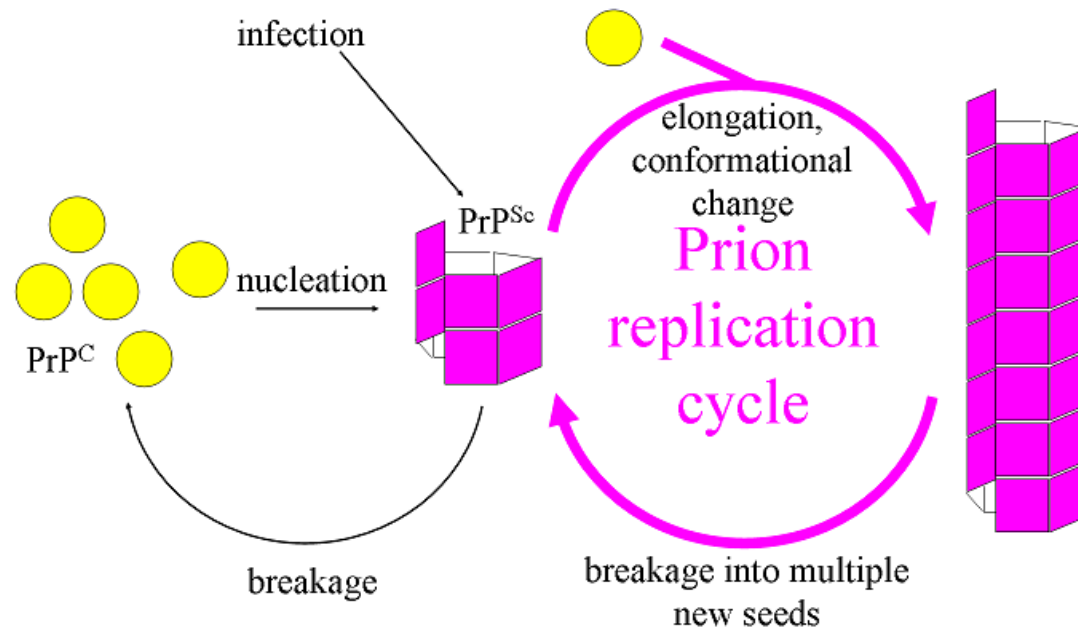
## Its importance;

- ✓ Cattle exposed to tissue affected by scrapie develop **another lethal prion disease (BSE)**
- ✓ They in turn may **transmit the disease to humans** in the form of variant Creutzfeldt-Jakob disease.



# Prion disease;

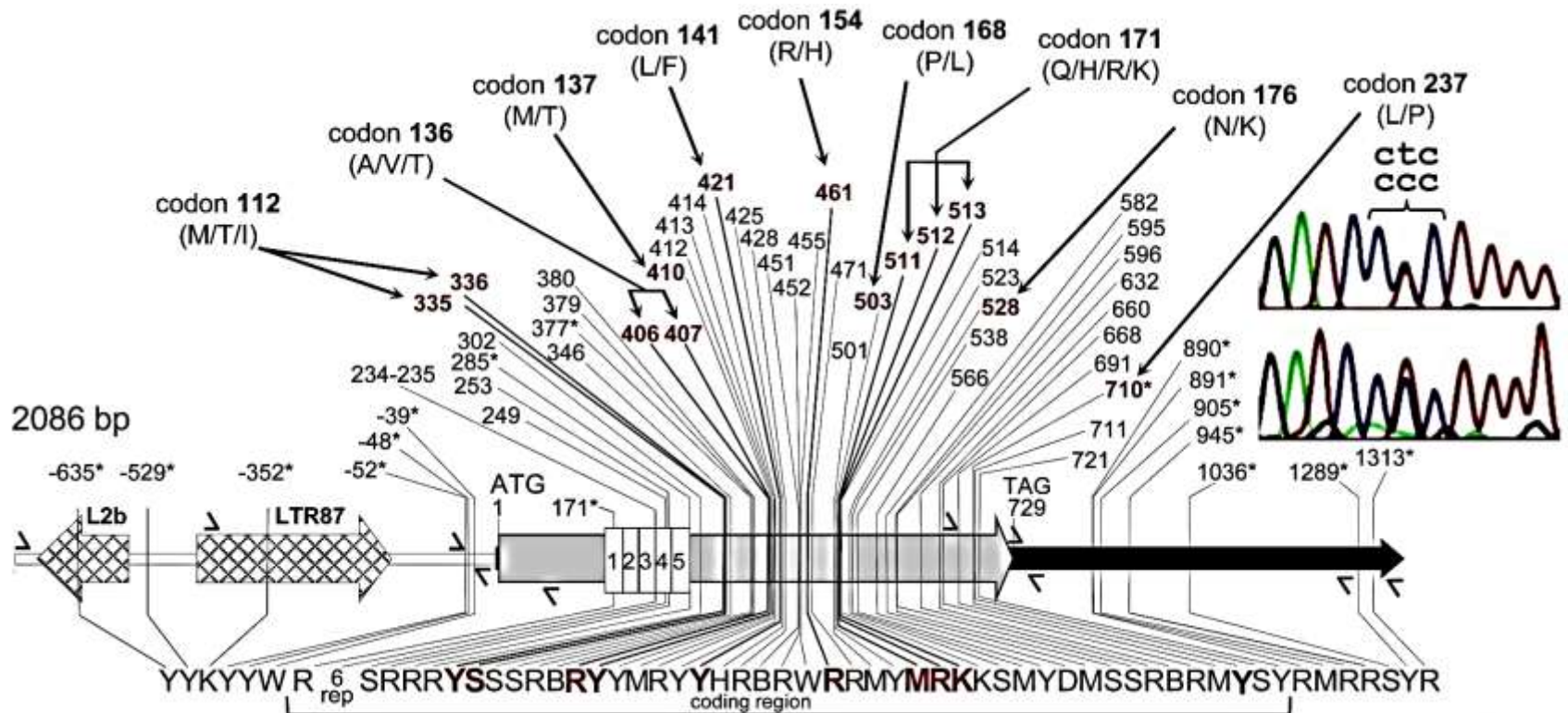
- ✓ are widely believed to be caused by the misfolding of the normal prion protein ( $\text{PrP}^{\text{C}}$ -for *common* or *cellular*) to a pathological isoform ( $\text{PrP}^{\text{Sc}}$ )



# Prion Protein (*PrP*) Gene;

- ✓ is localized on the **13 th chromosome**.
- ✓ It contains **two non-coding** and **one coding exon** with an open reading frame spanning 256 codons.

✓ More than **50 single nucleotide polymorphisms** (SNPs) were observed in the prion protein (PrP) gene of sheep.



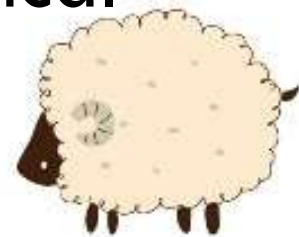
✓ Based on their genotypes **at codons:**

❖ **136:** valine (V) → high scrapie susceptibility  
alanine (A) → low susceptibility

❖ **154:** arginine (R) → susceptibility  
histidine (H) → partial resistance

❖ **171:** glutamine (Q) and histidine (H) → susceptibility  
arginine (R) → resistance of the PrP gene.

sheep show **resistance/susceptibility** to classical scrapie.



**Table 1. Five classes representing different levels of risk**

<b>Genotype Results</b>	<b>Risk Level</b>	<b>Degree of resistance / susceptibility</b>
ARR / ARR	R1	Sheep that are genetically most resistant to scrapie
ARR / AHQ ARR / ARH ARR / ARQ	R2	Sheep that are genetically resistant to scrapie, but will need carefully selection when used for further breeding
AHQ / AHQ AHQ / ARH AHQ / ARQ ARH / ARH ARH / ARQ ARQ / ARQ	R3	Sheep that genetically have little resistance to scrapie and will need careful selection when used for further breeding
ARR / VRQ	R4	Sheep that are genetically susceptible to scrapie and should not be used for breeding unless in the context of a controlled breeding programmed
VRQ / VRQ	R5	Sheep that are highly susceptible to scrapie and should not be used for breeding.

British National Scrapie Plan (NSP), the European Commission  
**(Brussels, 25-11-2002 C (2002) 4279)**



# Atypical scrapie;

- ✓ In 1998 a second type of scrapie was discovered (in Norway).
- ✓ Unfortunately the sheep most resistant to classical scrapie are apparently highly susceptible to atypical scrapie.

# Atypical scrapie

✓ There are still questions on atypical scrapie without any answers.

✓ Causes of the disease ???

✓ Distributions and its frequency ???

✓ Transfer from one species to another ??

**Different**

**from**

**classical**

**scrapie**

# Atypical scrapie

- ✓ Yet the origin is not known.
- ✓ However, researches showed that mutations on 141st and 154th codons may cause the disease.
- ✓ **141** : Leucine(L) / Phenylalanine (F)
- ✓ **154**: Arginine (R) / Histidine (H)

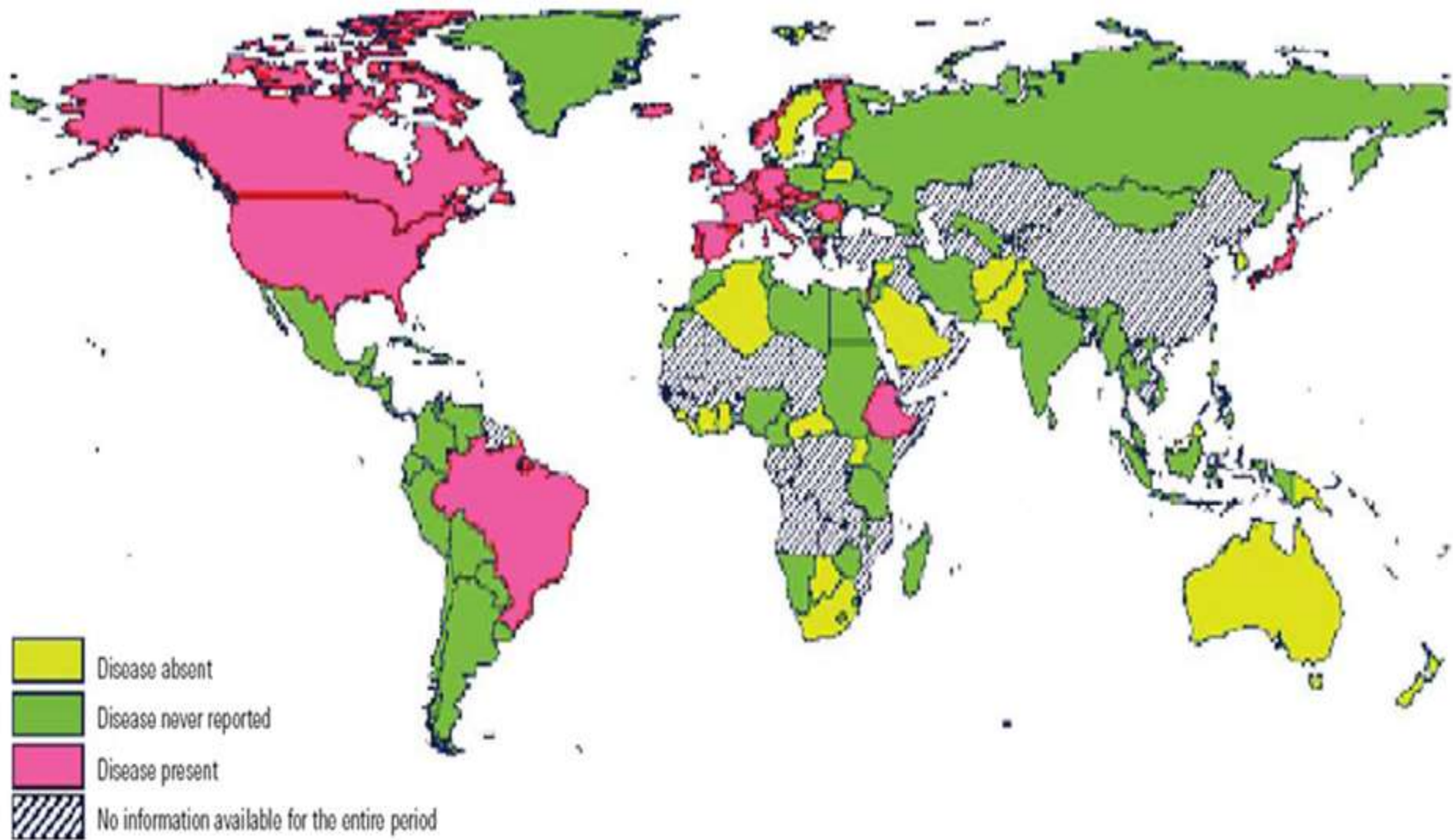
## Atypical Scrapie

**Table 2.** Genotypes grouped by levels of genetic risk for atypical scrapie in sheep, France

Group	Genotypes of <i>PrP</i> gene	Coded Level
1	ALRR/ALRQ, ALRR/VLRQ, ALRQ/ALRQ, ALRQ/ALRH, ALRQ/VLRQ	0
2	ALRR/ALRR, ALRR-ALRH, VLRQ/VLRQ	1
3	ALHQ/ALRH, ALHQ-VLRQ, AFRQ/ALRH, ALRH/ALRH, AFRQ/VLRQ, ALRH-VLRQ	2
4	ALRR/ALHQ, ALRR/AFRQ, ALHQ/ALRQ, AFRQ/ALRQ	3
5	ALHQ/ALHQ, ALHQ/AFRQ, AFRQ/AFRQ	4

## Aims of the National Scrapie Plan are to protect:

- ✓ animal health by reducing and eventually eradicating scrapie,
- and
- ✓ public health from the theoretical risk of BSE, if it was being masked by scrapie.



**Figure 2.** World distribution of scrapie between 1996 – 2004.

## Two studies on scrapie in Turkey (published)

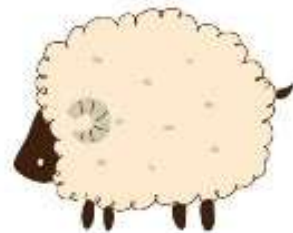
Ün et al., 2008 → 3 different breeds, 109 individuals  
(Kıvırcık, Sakız ve İmroz),

only classical scrapie examined

Alvarez et al., 2011 → 5 different breeds, 100 individuals  
(Akkaraman, Morkaraman, Tuj, Hemşin, Karayaka)

## Aims;

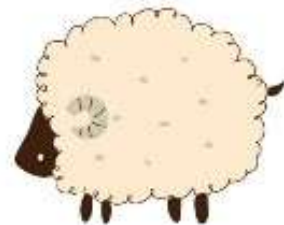
1. To determine sheep *PrP* gene haplotype distribution in Turkish native sheep breeds based on careful and extensive sampling.
  - a) To present this data to:
    - the authorities,
    - breeders associations and
    - university research units in Turkey



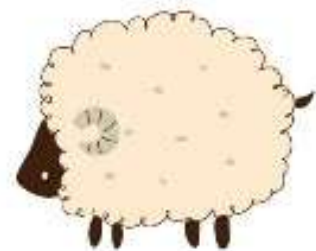
# Materials and Methods



**Figure 3.** Sampling sites of fourteen Turkish native sheep breeds(n=550)



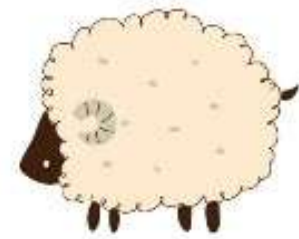
- ✓ Firstly sequencing of 897 bp long region of PrP gene was done as reported in Babar et al.'s (2009)'s study. However, primers of this region didn't work well in Turkish native sheep breeds.
- ✓ Therefore, sequencing of 743 bp long region of genomic DNA containing complete coding sequence in exon 3 of PrP gene was done according to Un et al's (2008) study.



# Sequenced region was edited and aligned.

```
C:\Documents and Settings\lab-user\Desktop\ornekB1-B7_alignment.bio.forward
Courier New 11 B 6 total sequences
Mode: Select / Slide Selection: 571 to 573 Position: Sequence Mask: None Start ruler at: 1
Numbering Mask: None
Scroll speed: slow fast
ornekB1 CAACCAAGTGTACTACAGACCAGTGGATCAGTATAGTAACCAGAACCAACTTTGTGCATGACTGTGTCAACATCACAGTC
ornekB2 CAACCAAGTGTACTACAGACCAGTGGATCAGTATAGTAACCAGAACCAACTTTGTGCATGACTGTGTCAACATCACAGTC
ornekB3 CAACCAAGTGTACTACAGACCAGTGGATCAGTATAGTAACCAGAACCAACTTTGTGCATGACTGTGTCAACATCACAGTC
ornekB4 CAACCAAGTGTACTACAGACCAGTGGAMAGATAGTAACCAGAACCAACTTTGTGCATGACTGTGTCAACATCACAGTC
ornekB5 CAACCAAGTGTACTACAGACCAGTGGACGGATAGTAACCAGAACCAACTTTGTGCATGACTGTGTCAACATCACAGTC
ornekB7 CAACCAAGTGTACTACAGACCAGTGGATCAGTATAGTAACCAGAACCAACTTTGTGCATGACTGTGTCAACATCACAGTC
```

- a. in the form of sequences and
- b. in the collapsed form as haplotypes.



Single nucleotide polymorphisms in codons (136, 141, 154 and 171) were checked directly.

The figure displays four screenshots of a sequence alignment software interface, each showing a different codon position (136, 141, 154, and 171) across 27 different NOR samples. The software interface includes a menu bar (File, Edit, Sequence, Alignment, View), a toolbar with icons for file operations and editing, and a main window showing the sequence alignment. The sequence is displayed in a grid format, with the reference sequence (gi|1778172|g) and the variant sequences (NOR 1 to NOR 27) aligned. The codon positions are highlighted in the sequence, and the corresponding amino acid changes are indicated by different colors (e.g., red for CAG, green for TGG, blue for CAT).

Codon	Reference (gi 1778172 g)	NOR 1	NOR 2	NOR 3	NOR 4	NOR 5	NOR 7	NOR 8	NOR 9	NOR 10	NOR 12	NOR 13	NOR 14	NOR 15	NOR 16	NOR 18	NOR 17	NOR 19	NOR 20	NOR 23	NOR 25	NOR 26	NOR 27	
136.codon	GGGAAGT <b>GCC</b> ATGAGC	GGGAAGT <b>GCC</b> ATGAGC	GGGAAGT <b>GCC</b> ATGAGC	GGGAAGT <b>GCC</b> ATGAGC	GGGAAGT <b>GCC</b> ATGAGC	GGGAAGT <b>GCC</b> ATGAGC	GGGAAGT <b>GCC</b> ATGAGC	GGGAAGT <b>GCC</b> ATGAGC	GGGAAGT <b>GCC</b> ATGAGC	GGGAAGT <b>GCC</b> ATGAGC	GGGAAGT <b>GCC</b> ATGAGC	GGGAAGT <b>GCC</b> ATGAGC	GGGAAGT <b>GCC</b> ATGAGC	GGGAAGT <b>GCC</b> ATGAGC	GGGAAGT <b>GCC</b> ATGAGC	GGGAAGT <b>GCC</b> ATGAGC	GGGAAGT <b>GCC</b> ATGAGC	GGGAAGT <b>GCC</b> ATGAGC	GGGAAGT <b>GCC</b> ATGAGC	GGGAAGT <b>GCC</b> ATGAGC	GGGAAGT <b>GCC</b> ATGAGC	GGGAAGT <b>GCC</b> ATGAGC	GGGAAGT <b>GCC</b> ATGAGC	GGGAAGT <b>GCC</b> ATGAGC
141.codon	:GAAGT <b>GCC</b> ATGAGCAGGCCCTCTT	:GAAGT <b>GCC</b> ATGAGCAGGCCCTCTT	:GAAGT <b>GCC</b> ATGAGCAGGCCCTCTT	:GAAGT <b>GCC</b> ATGAGCAGGCCCTCTT	:GAAGT <b>GCC</b> ATGAGCAGGCCCTCTT	:GAAGT <b>GCC</b> ATGAGCAGGCCCTCTT	:GAAGT <b>GCC</b> ATGAGCAGGCCCTCTT	:GAAGT <b>GCC</b> ATGAGCAGGCCCTCTT	:GAAGT <b>GCC</b> ATGAGCAGGCCCTCTT	:GAAGT <b>GCC</b> ATGAGCAGGCCCTCTT	:GAAGT <b>GCC</b> ATGAGCAGGCCCTCTT	:GAAGT <b>GCC</b> ATGAGCAGGCCCTCTT	:GAAGT <b>GCC</b> ATGAGCAGGCCCTCTT	:GAAGT <b>GCC</b> ATGAGCAGGCCCTCTT	:GAAGT <b>GCC</b> ATGAGCAGGCCCTCTT	:GAAGT <b>GCC</b> ATGAGCAGGCCCTCTT	:GAAGT <b>GCC</b> ATGAGCAGGCCCTCTT	:GAAGT <b>GCC</b> ATGAGCAGGCCCTCTT	:GAAGT <b>GCC</b> ATGAGCAGGCCCTCTT	:GAAGT <b>GCC</b> ATGAGCAGGCCCTCTT	:GAAGT <b>GCC</b> ATGAGCAGGCCCTCTT	:GAAGT <b>GCC</b> ATGAGCAGGCCCTCTT	:GAAGT <b>GCC</b> ATGAGCAGGCCCTCTT	:GAAGT <b>GCC</b> ATGAGCAGGCCCTCTT
154.codon	gi 1778172 g GGACCG <b>T</b> TACTATCGTGAAA	NOR 1 GGACCG <b>T</b> TACTATCGTGAAA	NOR 2 GGACCG <b>T</b> TACTATCGTGAAA	NOR 3 GGACCG <b>T</b> TACTATCGTGAAA	NOR 4 GGACCG <b>T</b> TACTATCGTGAAA	NOR 5 GGACCG <b>T</b> TACTATCGTGAAA	NOR 7 GGACCG <b>T</b> TACTATCGTGAAA	NOR 8 GGACCG <b>T</b> TACTATCGTGAAA	NOR 9 GGACCG <b>T</b> TACTATCGTGAAA	NOR 10 GGACCG <b>T</b> TACTATCGTGAAA	NOR 12 GGACCG <b>T</b> TACTATCGTGAAA	NOR 13 GGACCG <b>T</b> TACTATCGTGAAA	NOR 14 GGACCG <b>T</b> TACTATCGTGAAA	NOR 15 GGACCG <b>T</b> TACTATCGTGAAA	NOR 16 GGACCG <b>T</b> TACTATCGTGAAA	NOR 18 GGACCG <b>T</b> TACTATCGTGAAA	NOR 17 GGACCG <b>T</b> TACTATCGTGAAA	NOR 19 GGACCG <b>T</b> TACTATCGTGAAA	NOR 20 GGACCG <b>T</b> TACTATCGTGAAA	NOR 23 GGACCG <b>T</b> TACTATCGTGAAA	NOR 25 GGACCG <b>T</b> TACTATCGTGAAA	NOR 26 GGACCG <b>T</b> TACTATCGTGAAA	NOR 27 GGACCG <b>T</b> TACTATCGTGAAA	
171.codon	gi 1778172 g GACCAG <b>T</b> GGATCAGTAT	NOR 1 GACCAG <b>T</b> GGATCAGTAT	NOR 2 GACCAG <b>T</b> GGATCAGTAT	NOR 3 GACCAG <b>T</b> GGATCAGTAT	NOR 4 GACCAG <b>T</b> GGATCAGTAT	NOR 5 GACCAG <b>T</b> GGATCAGTAT	NOR 7 GACCAG <b>T</b> GGATCAGTAT	NOR 8 GACCAG <b>T</b> GGATCAGTAT	NOR 9 GACCAG <b>T</b> GGATCAGTAT	NOR 10 GACCAG <b>T</b> GGATCAGTAT	NOR 12 GACCAG <b>T</b> GGATCAGTAT	NOR 13 GACCAG <b>T</b> GGATCAGTAT	NOR 14 GACCAG <b>T</b> GGATCAGTAT	NOR 15 GACCAG <b>T</b> GGATCAGTAT	NOR 16 GACCAG <b>T</b> GGATCAGTAT	NOR 18 GACCAG <b>T</b> GGATCAGTAT	NOR 17 GACCAG <b>T</b> GGATCAGTAT	NOR 19 GACCAG <b>T</b> GGATCAGTAT	NOR 20 GACCAG <b>T</b> GGATCAGTAT	NOR 23 GACCAG <b>T</b> GGATCAGTAT	NOR 25 GACCAG <b>T</b> GGATCAGTAT	NOR 26 GACCAG <b>T</b> GGATCAGTAT	NOR 27 GACCAG <b>T</b> GGATCAGTAT	

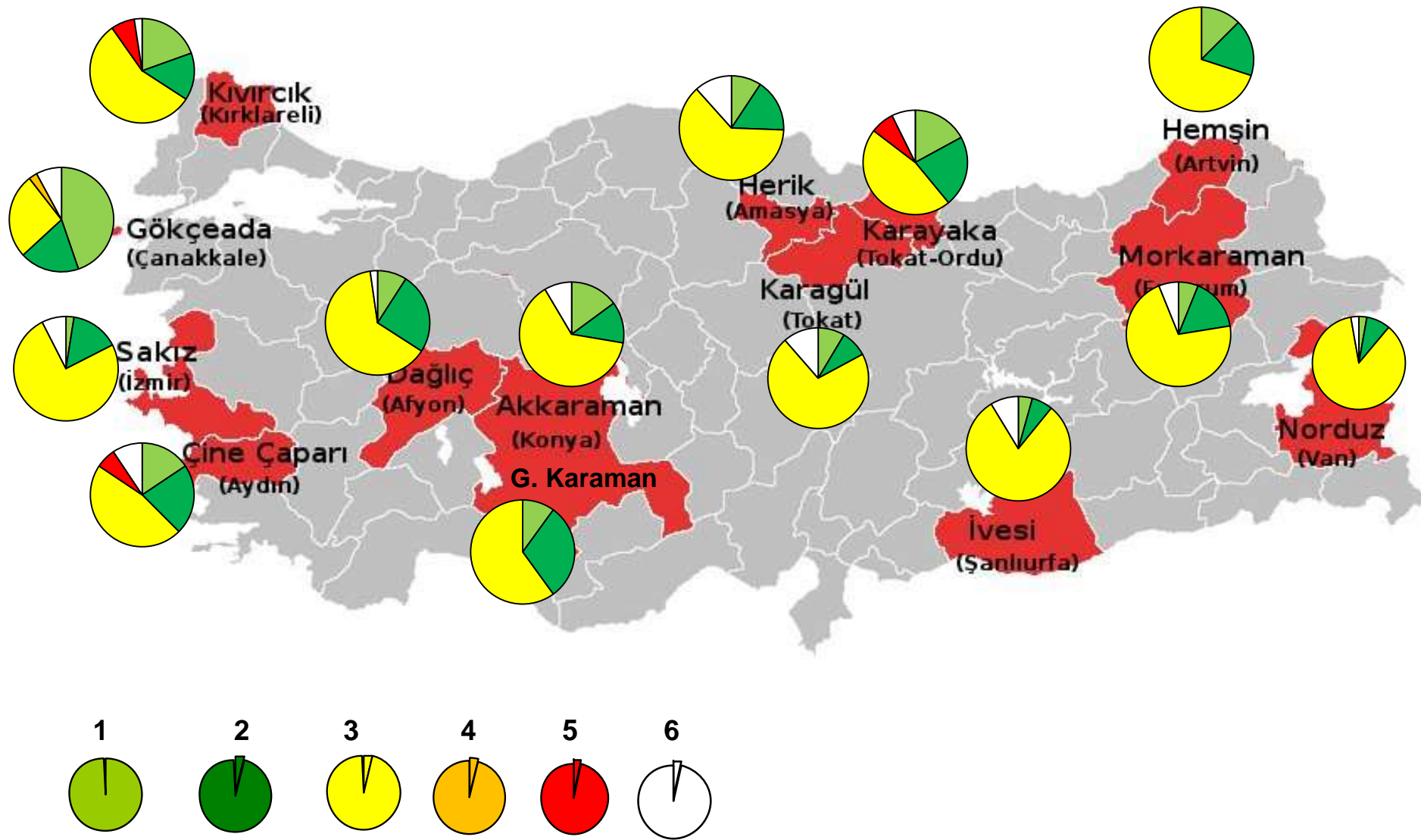
# Results



# Genotypic Frequencies

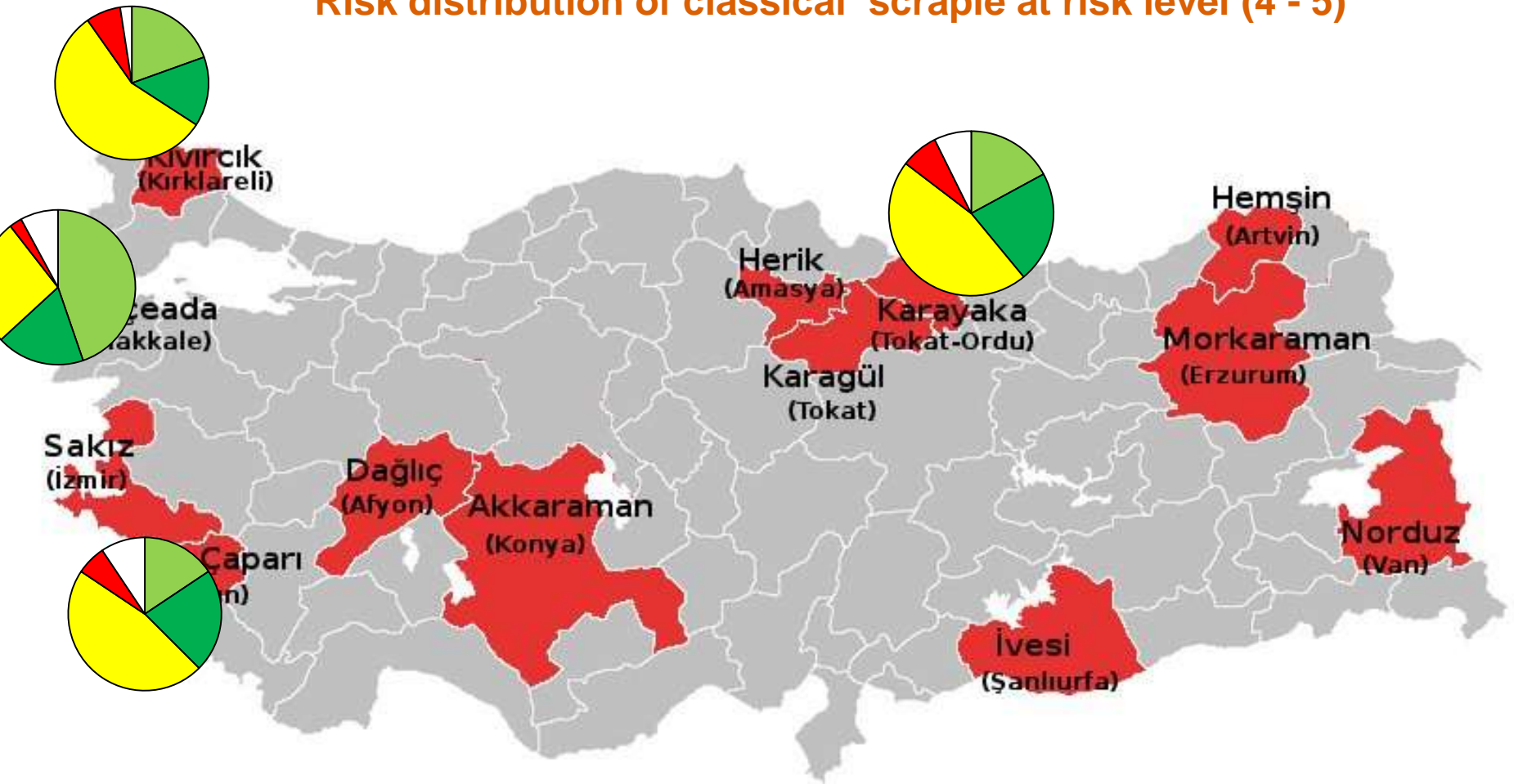
	NOR	CIC	DAG	HER	KIV	AKK	SAK	IVE	MOR	HEM	KRG	KRY	GOK	GKAR
AFRH/AFRQ									0,0204					
ALHQ/ALHQ	0,0278		0,0227		0,04878		0,05							
ALHQ/ALHR													0,0526	
ALRH/ALHH									0,0204					
ALRH/ALRH	0,0833		0,1136		0,04878	0,1489	0,05	0,1739	0,1837	0,1250	0,0857	0,0732	0,0263	0,1500
ALRH/ALRK						0,0213								
ALRH/TLRH												0,0488		
ALRH/VLRH					0,02439									
ALRL/ALRL						0,0213		0,0217	0,0204					
ALRQ/AFRQ					0,02439									
ALRQ/ALHQ			0,0682	0,0444	0,04878		0,1		0,0204					
ALRQ/ALRH	0,0833			0,0444	0,02439			0,0435	0,0204					
ALRQ/ALRK		0,0625	0,0227				0,025	0,0217						
ALRQ/ALRQ	0,6667	0,4688	0,4318	0,5556	0,3659	0,4894	0,55	0,5870	0,4694	0,5750	0,6286	0,3902	0,2368	0,4000
ALRQ/TLHQ							0,025							
ALRQ/TLRQ	0,0278	0,0313		0,0444		0,0213		0,0217	0,0204		0,0571			
ALRQ/VLRQ		0,0313			0,0488							0,0732		
ALRR/ALHQ			0,0227											
ALRR/ALHR													0,0263	
ALRR/ALRH		0,0313				0,0213			0,0204					
ALRR/ALRQ	0,0833	0,1875	0,2273	0,1556	0,1463	0,1064	0,15	0,0652	0,1429	0,1750	0,0857	0,2195	0,1842	0,3000
ALRR/ALRR	0,0278	0,1563	0,0909	0,0889	0,1951	0,1489	0,025	0,0435	0,0612	0,1250	0,0857	0,1707	0,4474	0,1000
ALRR/TLRQ				0,0222			0,025							
ALRR/VLRQ													0,0263	
TLRQ/TLRQ				0,0444		0,0213		0,0217			0,0571			
VLRQ/VLRQ		0,0313			0,0244									
VLRQ/VLRR												0,0244		
ALHQ/ALRH														0,0500
n	36	32	44	45	41	47	40	46	49	40	35	41	38	20

**Table 3.** Genotype frequencies of PrP gene in Turkish native sheep breeds

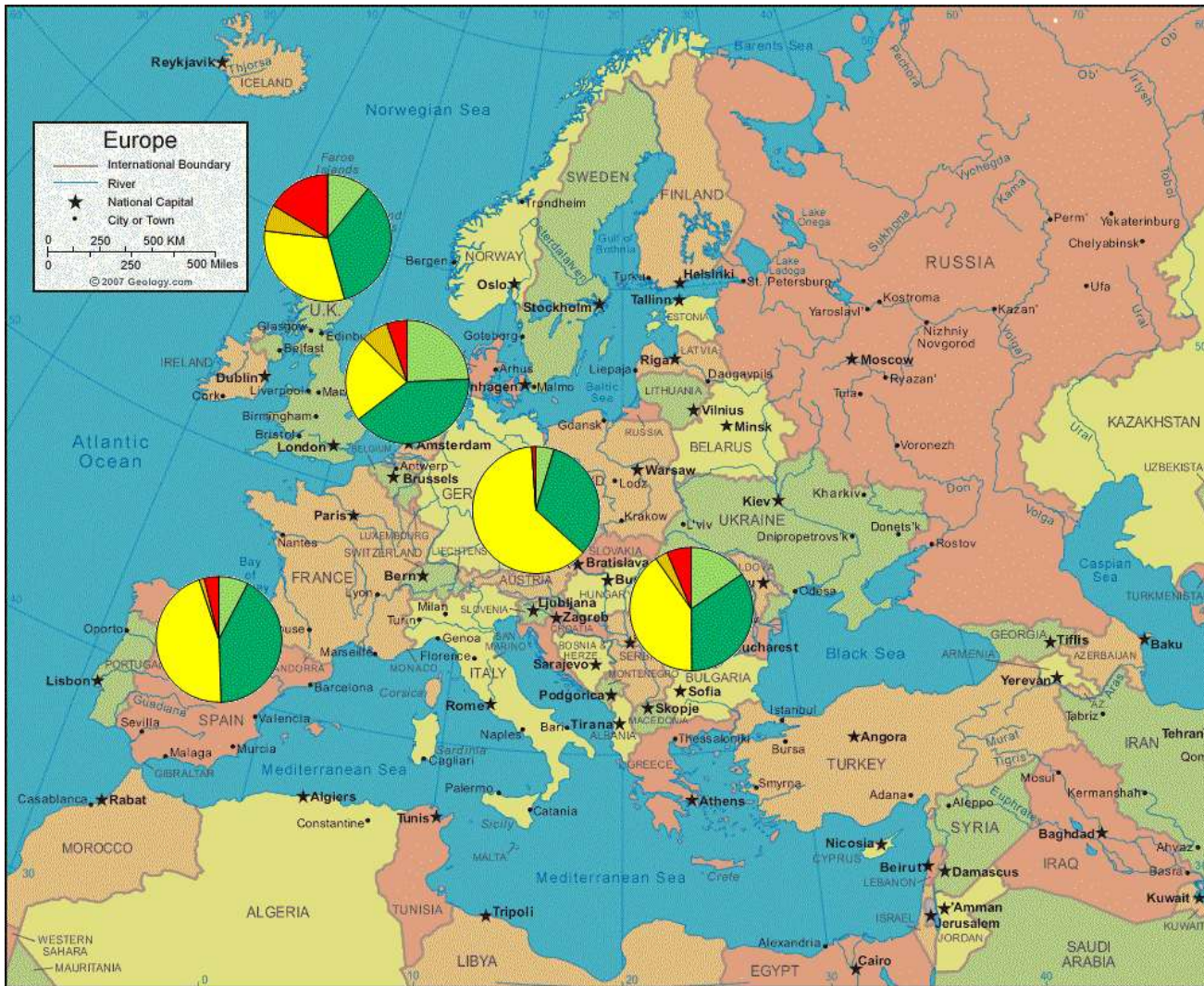


**Figure 3.** Distribution of risk groups in Turkish native sheep breeds for classical scrapie.

## Risk distribution of classical scrapie at risk level (4 - 5)



high risk level of 4 and 5 in Kiv, Gök, Çiç and Kar was observed, but their frequencies are low



Bulgarian (Sirakov, 2011)

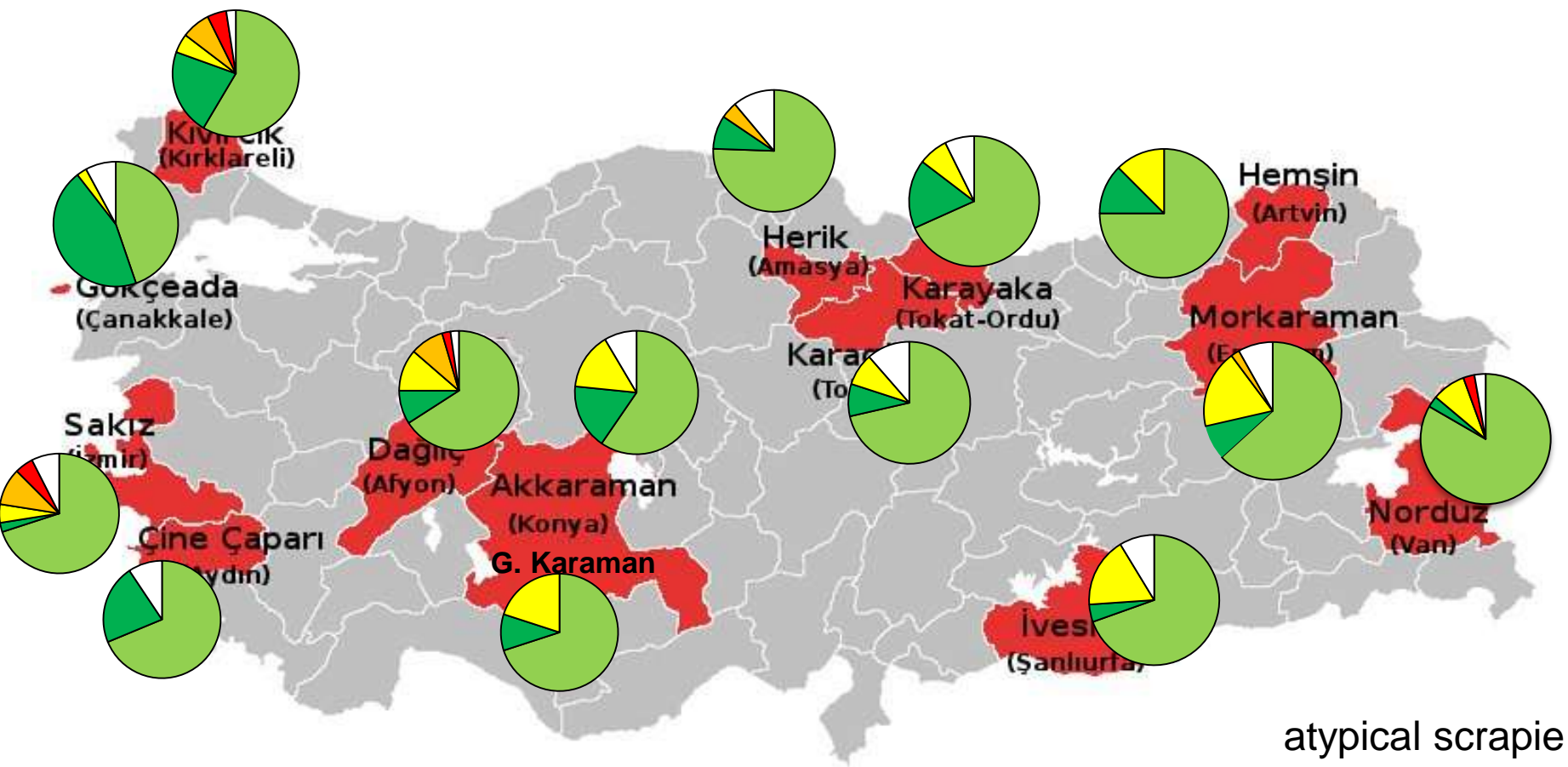
UK(Hoinville et al., 2010)

The Netherlands  
(Hagenaars et al., 2010)

Spanish(Alvarez et al., 2009)

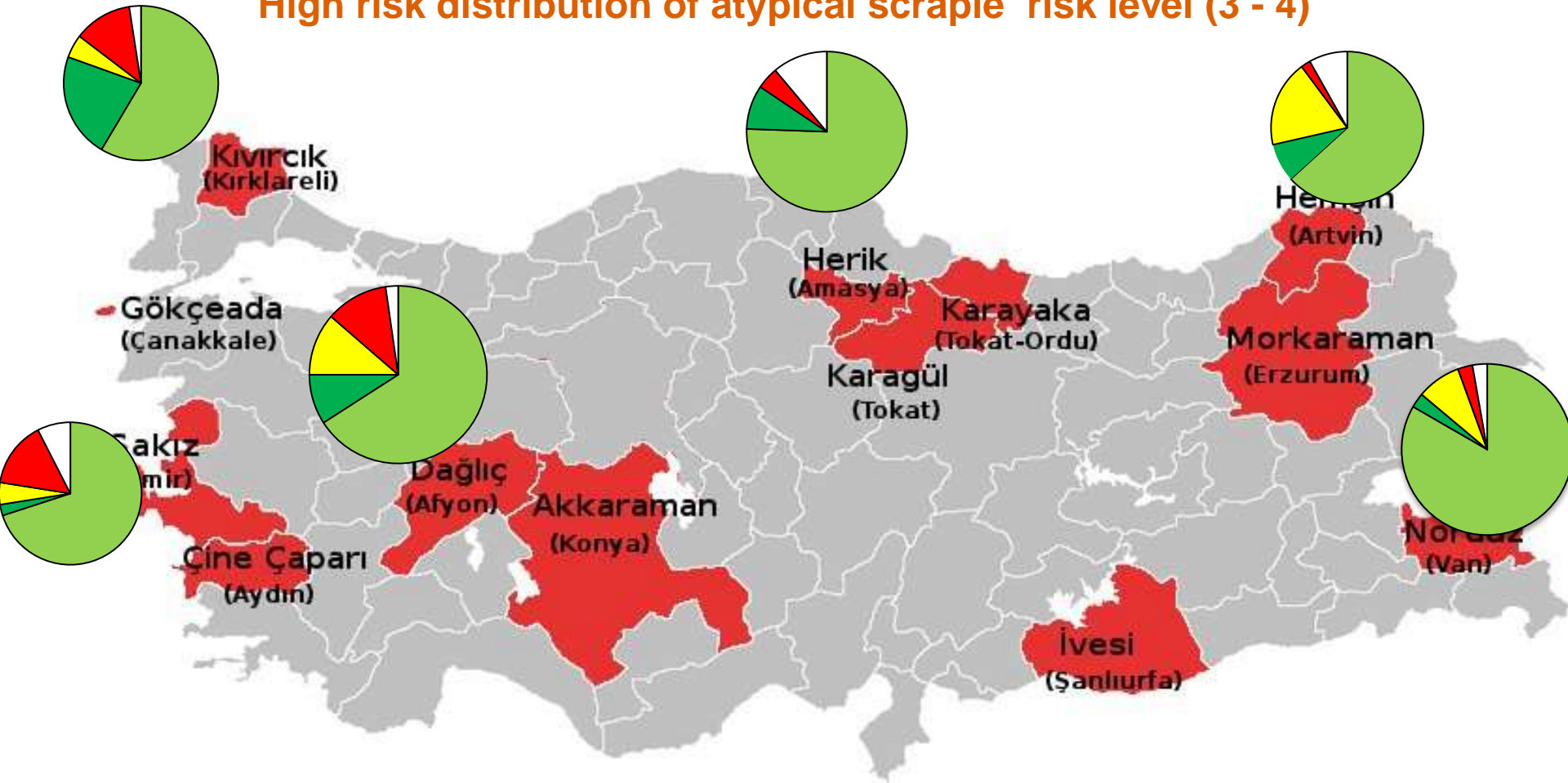
Austrian, Sipos et al., 2002

**In Europe NSP is applied, however frequency for the risk level of 4 and 5 is still high.**



**Figure 4.** Distribution of risk groups in Turkish native sheep breeds for atypical scrapie

## High risk distribution of atypical scrapie risk level (3 - 4)



high risk level of 3 and 4 in Kiv, Sak, Dağ, Her, Hem and Nor was observed,  
but their frequencies are low

**R40S**

**M112T,**

**R114H**

**G127S,**

**G127V**

**G127 A**

**L133M,**

**L133G,**

**M133G,**

**S138N**

**A143H,**

**N146S**

**I185F**

**Q189L**

**N193L**

**R231G**

**L237L**

**S239F**

**R= Arginine**

**S= Serine**

**M= Methionine**

**T= Threonine**

**H= Histidine**

**G= Glycine**

**V= Valine**

**A= Alanine**

**L = Leucine**

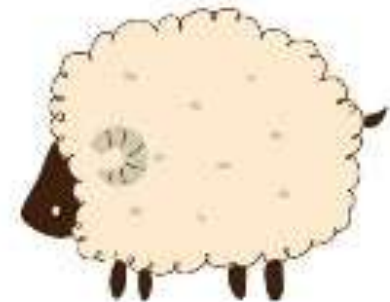
**N=Asparagine**

**I= Isoleucine**

**F = Phenylalanine**

**Q= Glutamine**

**Additional polymorphisms in 14 different codons were observed in Turkish Sheep breeds**

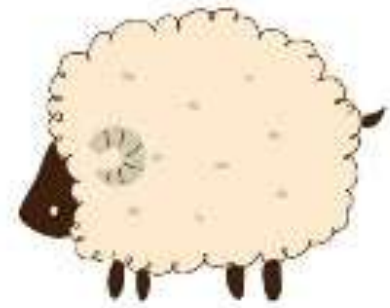


# Discussion

✓ No scrapie cases in Turkey.

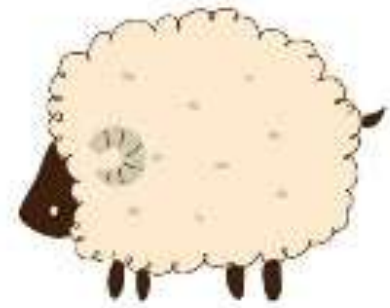
*Possible reasons are;*

1. long incubation period of the disease,
2. lack of suitable screening systems for diagnosis,
3. environmental factors (soil drainage) and flock management (flock size)



✓ **Frequency of VRQ allele** was low in 14 Turkish native sheep breeds (0.0313- 0.0244) CIC-KIV-KRY

✓ Most of the investigated sheep belongs to the low or intermediate risk groups .



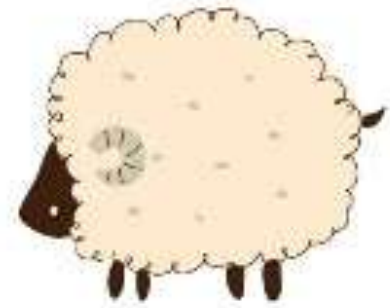
- ✓ The predominant allele in the majority of the breeds is ARQ allele.



ARQ allele is predicted to be wild-type allele of the PrP gene.

Therefore;

- ✓ analysis of fourteen Turkish native sheep supports this prediction.



# As a conclusion;

- ✓ This information may help to establish a fully scrapie susceptibility - free breeds.
- ✓ Turkey will benefit economically.
- ✓ It will be beneficial for the public health in the country.
- ✓ This result may help to establish genetic control programmes of scrapie susceptibility in the future.





**Thank you for your attention!**