

13th Annual Meeting of the TSE EURL
Rome, Italy
7nd-8th May 2026

EU-wide collaboration on PrP genetics in sheep with AS: state of play and next steps

Why does AS occur?

- No temporal trends, outbreaks or clustering (Fediaevsky et al., 2008)
- EFSA 2021: more likely that AS is a non-contagious, rather than a contagious disease.....but major knowledge gaps still remain
- PrP genotype is a major risk factor for AS: 141 (L/F) and 154 (R/H) are highly associated with AS cases

4-codon PrP genotype of AS cases in Italy

- 85% (111/130) have F141 or H154
- ALRR does not confer resistance (8,5% are ALRR/ALRR)
- Only 2,5% (3/123) ALRQ/ALRQ

	n°	%
ALRQ/ALRQ	3	2,31%
ALRQ/AFRQ	27	20,77%
ALRQ/ALHQ	19	14,62%
ALRQ/ALRR	4	3,08%
AFRQ/AFRQ	19	14,62%
AFRQ/ALRR	16	12,31%
AFRQ/ALHQ	3	2,31%
ALRR/ALRR	11	8,46%
ALRR/ALHQ	21	16,15%
ALRR/ALRH	1	0,77%
ALHQ/ALHQ	3	2,31%
ALHQ/ALRH	2	1,54%
ALHQ/ALRK	1	0,77%
TOT	130	100,0%

Full sequencing of PrP from AS cases in Italy

- **No AS cases in wt/wt sheep**
- Rare PrP mutations in AS (R159H, Y172D, E203K)

Sequence	n°	%
ALRQ/ALRQ (wt/wt)	0	0,0%
ALRQ/ALRH ¹⁵⁹ Q	1	0,8%
ALRQ/ALRQD ¹⁷²	1	0,8%
ALRQ/ALRQK ²⁰³	1	0,8%
ALRQ/AFRQ	25	19,2%
ALH ¹⁴³ RQ/AFRQ	2	1,5%
ALRQK ¹⁷⁶ /AFRQ	1	0,8%
ALRQ/ALHQ	18	13,8%
ALS ¹⁴⁶ RQ/ALHQ	1	0,8%
ALRQ/ALRR	4	3,1%
AFRQ/AFRQ	19	14,6%
AFRQ/ALRR	16	12,3%
AFRQ/ALHQ	2	1,5%
ALRR/ALRR	11	8,5%
ALRR/ALHQ	21	16,2%
ALRR/ALRH	1	0,8%
ALHQ/ALHQ	3	2,3%
ALHQ/ALRH	2	1,5%
ALHQ/ALRK	1	0,8%
	130	100,0%

Do wt/wt sheep develop AS?

- It could be hypothesized that the necessary condition to develop AS is PrP variation (mutations/polymorphisms)
- This would possibly support a genetic, non-contagious, etiology for AS
 - genetic TSEs never described in animals
 - genetic CJD in humans, autosomal dominant, caused by spontaneous misfolding of mutated PrP; >40 PRNP mutations that confer lifetime risks ranging from <0.1 to ~100%

EU-wide collaboration

Aims:

- to determine if there are AS cases in wt/wt sheep (and how many)
- to gain insight into the causal role of rare PrP mutations

Strategy:

- to achieve full sequence data of ALRQ/ALRQ AS cases detected in the different MS

State of play

- ✓ 15 NRLs provided:
 - 4-codon data of AS cases (requirement by EC 999/2001)
 - if available, full sequence data of ALRQ/ALRQ cases
 - if available, tissue or DNA of ALRQ/ALRQ cases

- ✓ The EURL collected data and assisted in PrP sequencing of AS cases

AS cases 2002-2023 (TSE annual reports)

EU	AT	17
	BE	11
	BG	6
	CY	
	CZ	8
	DE	157
	DK	15
	EE	2
	EL	33
	ES	278
	FI	25
	FR	595
	HR	6
	HU	234
	IE	53
	IT	129
	LT	
	LU	
	LV	
	MT	
	NL	18
	PL	87
	PT	790
	RO	1
	SE	56
	SI	14
	SK	60
	Total EU27	2.595
	XI ^(a)	3
	Total EU27+XI	2.598
Other non-EU	CH	
	IS	9
	ME	
	MK	
	NO	212
	RS	
	TR	
	United Kingdom	382
	Total other non-EU	603
TOTAL	Total	3.201

Participating NRLs

Country	Data	N° cases 4-codon	N° ALRQ/ALRQ	Sequence ALRQ/ALRQ	Tissue /DNA	Control population
Austria	Y	17	1	N (1 to do)	Y	Y
Belgium	Y	4	0	/	N	Y
Germany	Y	248	15	Y (n=10)	Y	?
Spain	Y	189	6	Y (n=3)	Y	N
Finland	Y	17	14	Y (n=6; + 6 pending)	Y	Y
Croatia	Y	5	0	/	N	N
Italy	Y	130	3	Y* (n=3)	Y	Y
Netherlands	Y	19	0			
Poland	Y	108	3	Y* (n=3)	Y	Y
Portugal	Y	668	21	Y (90-222)	?	Y
Slovenia	Y	15	0	/	Y	Y
Slovakia	Y	59	0	/*	N	N
Iceland	Y	11	0	/	N	Y
Norway	Y	224	5	Y (n=2)	Y	N
UK	Y	407	0	/*	Y	Y
TOT		2121	68 (3,2%)	27		

In red those to complete (aim: 55/68)

* full sequences available for all cases

PrP genotypes in AS (4 codons)

ALRR/ALHQ	368	17,35%
ALRQ/ALHQ	264	12,45%
AFRQ/ALRR	248	11,69%
ALRQ/AFRQ	233	10,99%
ALRR/ALRR	230	10,84%
AFRQ/AFRQ	227	10,70%
ALHQ/ALHQ	161	7,59%
ALRQ/ALRR	126	5,94%
AFRQ/ALHQ	111	5,23%
ALRQ/ALRQ	68	3,21%

ALHQ/ALRH	19	0,90%
AFRQ/ALRH	18	0,85%
ALRR/ALRH	9	0,42%
AFRQ/VLRQ	8	0,38%
ALRQ/VLRQ	8	0,38%
ALRQ/ALRH	6	0,28%
ALHQ/VLRQ	4	0,19%
ALRR/VLRQ	2	0,09%
ALRR/ALRK	2	0,09%
ALRH/ALRH	2	0,09%
AFRQ/ALRK	1	0,05%
ALHQ/ALRK	1	0,05%
ALRQ/ALRK	1	0,05%
ALRQ/ALLQ	1	0,05%
AFRQ/VFRQ	1	0,05%
AFHQ/AFRR	1	0,05%
ALRR/AFRR	1	0,05%

TOTAL 2121

ALRR/ALHQ	368	17,4%
ALRQ/ALHQ	264	12,4%
AFRQ/ALRR	248	11,7%
ALRQ/AFRQ	233	11,0%
ALRR/ALRR	230	10,8%
AFRQ/AFRQ	227	10,7%
ALHQ/ALHQ	161	7,6%
ALRQ/ALRR	126	5,9%
AFRQ/ALHQ	111	5,2%
ALRQ/ALRQ	68	3,2%

Are these ALRQ/ALRQ really wt/wt?

87 out of 2121 (4,2%) with no AFRQ, ALHQ or ALRR

Aim 1: to determine if there are AS cases in wt/wt sheep (and how many)

68 ALRQ/ALRQ detected:

- ✓ 27 have been sequenced:
 - ✓ 23 (85%) with additional variations
 - ✓ **4 wt/wt (all from Germany; tissues sent to EURL)**
- ✓ 21 with partial sequence (92-222; do not include 224)
 - ✓ 13 (60%) have additional mutations/polymorphisms
- ✓ **At least 36/48 (75%) ALRQ/ALRQ are not wt/wt**
- ✓ We aim to have 50-55 sequenced out of 68 ALRQ/ALRQ detected among 2121 AS cases

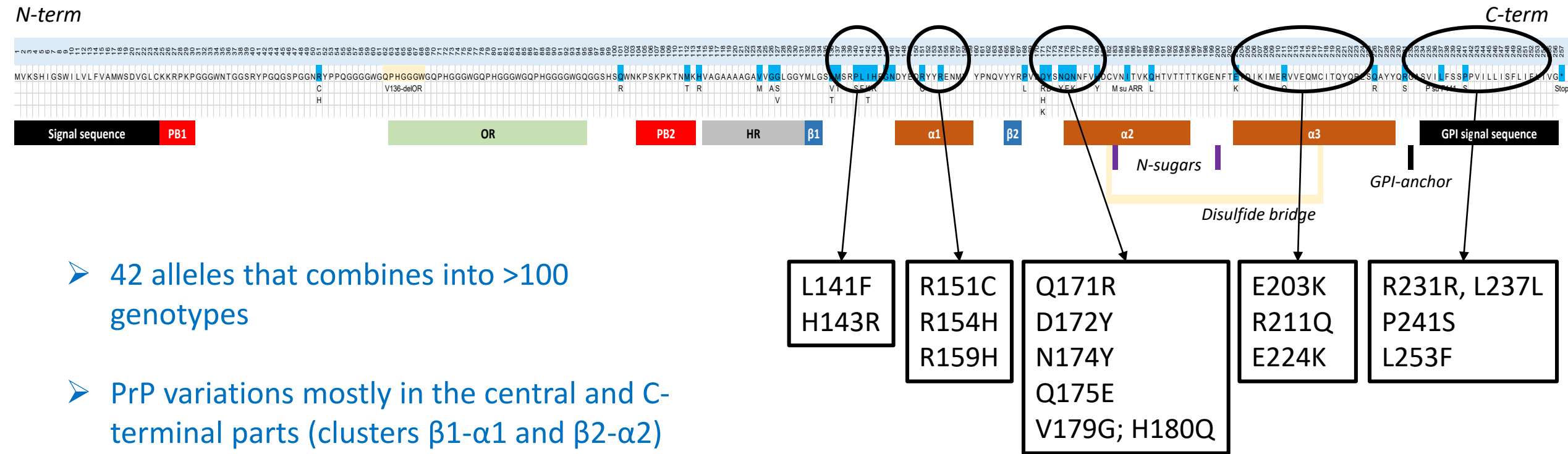
Aim 2: to gain insight into the causal role of rare PrP mutations

	Heterozygous	Homozygous	With other variations		Origin
H143R	3		1	D172Y	Germany, Spain
R151C	1	1	1	E224K	Finland
R159H	1				Italy
D172Y	2		1	H143R	Spain, Italy
N174Y	1				Germany
Q175E	1				Germany
V179G; H180Q	1				Germany
E203K	1				Italy
R211Q		1			Germany
E224K	5	3	2	R151C; R231R+L237L	Poland, Norway, Finland
R231R, L237L	2		1	E224K	Poland, Spain
P241S	2				Poland, Norway
L253F	1				Germany

- ✓ 13 different PrP variations detected
- ✓ 5 cases homozygotes (151CC; 211QQ; 224KK)
- ✓ 5 cases with >1 PrP variation
- ✓ H143R and Y172 detected also in Portugal (partial 90-222 sequences)

Full sequencing of PrP in the Italian sheep population (2009-2022; n=9035)

PrP polymorphisms/mutations in AS in EU (2005-2023)



- ✓ Clusters of PrP variations potentially associated with AS
- ✓ GPI-anchoring!

New PrP polymorphisms/mutations potentially associated with AS

- Italy: R159H, Y172D, E203K
 - Very rare mutations in the general population in Italy (collectively, 0.02% of all alleles)
- Finland: E224K and R151C (both heterozygous and homozygous!)
 - Both are frequent polymorphisms in Finnish Landrace sheep (Hautaniemi et al. BMC Veterinary Research 2012)
- Poland: E224K, P241S
 - E224K is a polymorphism associated to AS in Romanov sheep in Poland
 - P241S is probably rare in Poland (Piestrzyńska-Kajtoch et al., Mol Biol Rep 2012); it can be however a frequent polymorphism in some breed (Goldman et al., Journal of Applied Microbiology 2005)

Next steps

- Complete the dataset of AS cases (4-codons data and ALRQ/ALRQ sequences)
 - NRLs having ALRQ/ALRQ will be contacted to share tissues/DNA
- Controls
 - 4-codons datasets: descriptive, but we will discuss with Giuseppe on the possible use of control populations
 - sequenced ALRQ/ALRQ: control negative samples? (frequency of other variations in healthy sheep)
 - NRLs will be contacted to understand the feasibility

Controls for the sequenced ALRQ/ALRQ

	ALRQ/ALRQ		
	tot	with variations	% ALRQ/ALRQ with variations
AS Italy (n=130)	3	3	100%
Population Italy (n=9035)	2603	567	22%
CS Italy (n=3109)	2572	111	4%

PrP variations detected in ALRQ/ALRQ AS cases are different from those detected in CS cases!

Working hypothesis: to collect sequences from sheep with the ALRQ/ALRQ four-codon genotype 1–4 times the number of sequenced ALRQ/ALRQ AS cases that you are contributing

Aknowledgments

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