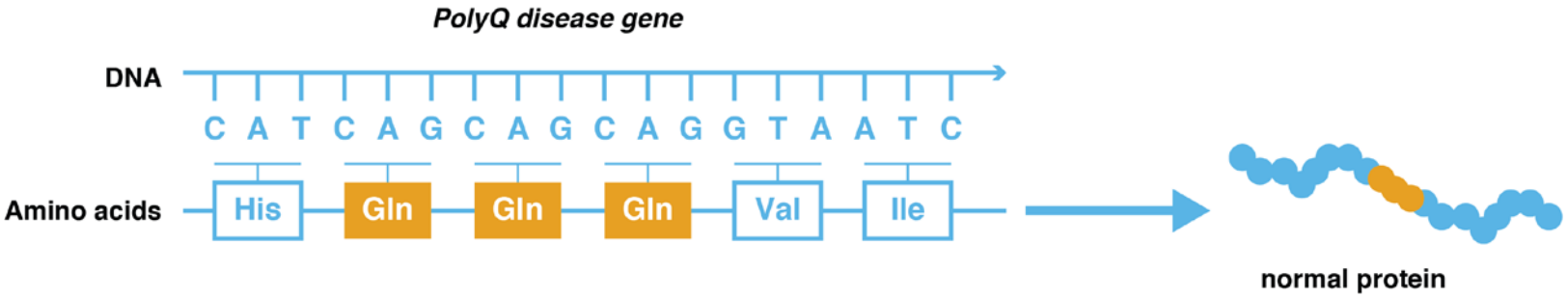
Brain Center
Rudolf Magnus

ATXN1: Expanding the spectrum of polyglutamine repeats in ALS.

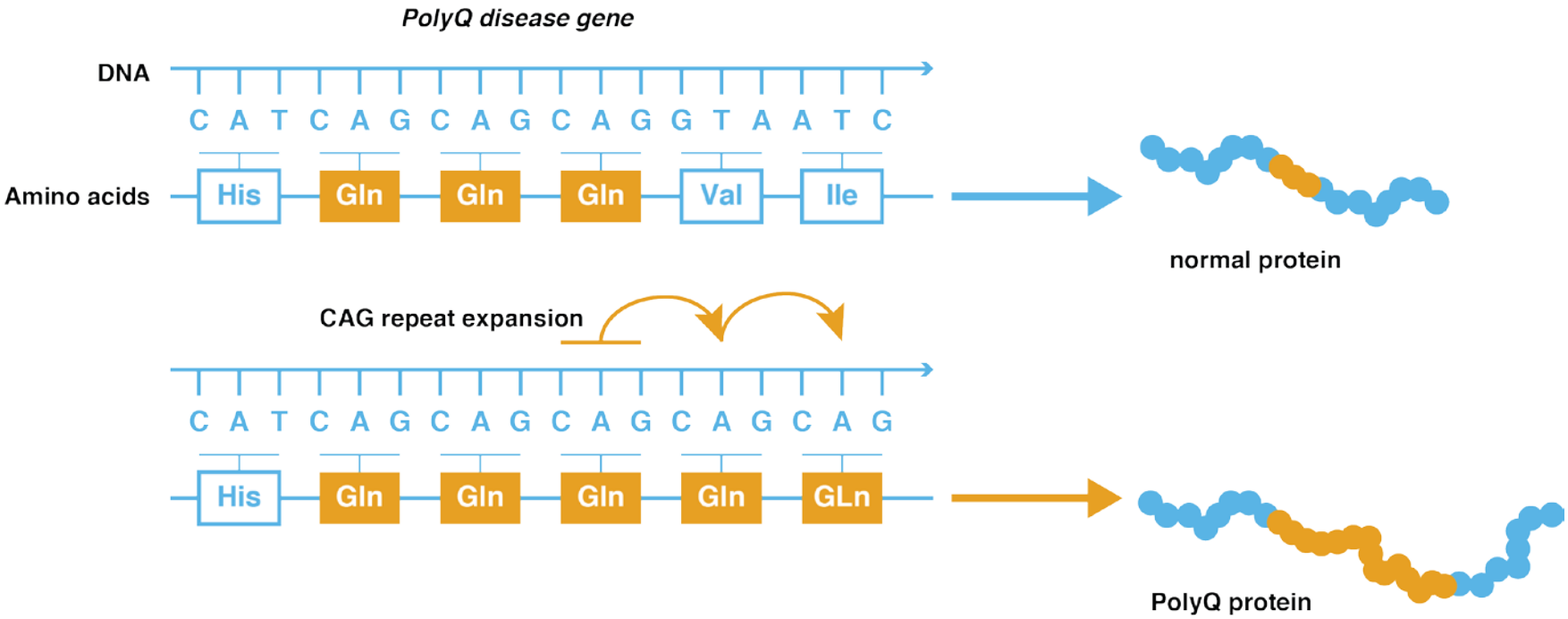
Gijs Tazelaar – UMC Utrecht

ENCALS meeting 2017 Ljubljana

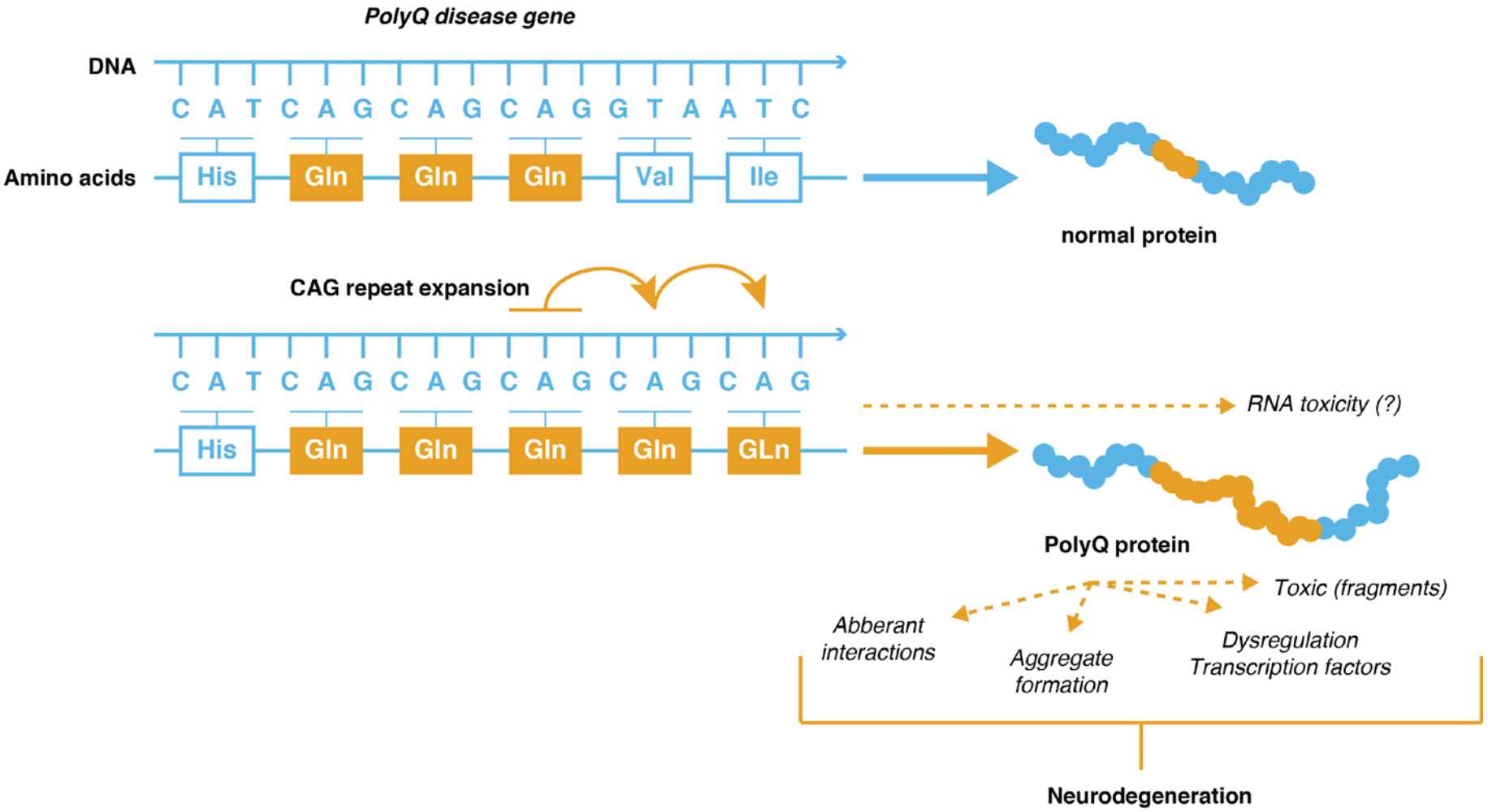
Polyglutamine & PolyQ diseases



Polyglutamine & PolyQ diseases



Polyglutamine & PolyQ diseases



Spinocerebellar ataxias (SCA) & Polyglutamine expansions

Many caused by *CAG*-expansion

- SCA1 (*ATXN1*), SCA2 (*ATXN2*), SCA3 (*ATXN3*), SCA6, SCA7, SCA17

Clinical symptoms:

- Adult onset
- Slow progressive (15-30 yrs)
- Cerebellar symptoms
- Non-cerebellar symptoms: spasticity, hyperreflexia, weakness, cramps, fasciculations, atrophy, cognitive impairment, Parkinsonism, autonomic dysfunction

ALS
& Polyglutamine expansions

Vol 466|26 August 2010|doi:10.1038/nature09320 nature

Ataxin-2 intermediate-length polyglutamine expansions are associated with increased risk for ALS

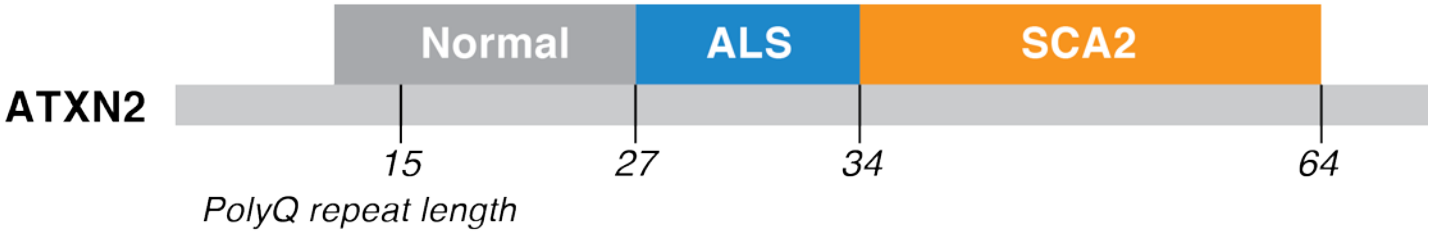
Andrew C. Elden[1]*, Hyung-Jun Kim[2]*, Michael P. Hart[1]*, Alice S. Chen-Plotkin[3,4]*, Brian S. Johnson[1],

Yeast screen for TDP-43 toxicity: Pbp1 (orthologue of Ataxin-2)

Ataxin-2 in ALS

Subjects	Total	≤26 repeats	27–33 repeats	Percentage of 27–33 repeats	*P* value	OR (95% CI)
ALS	915	872	43	4.7%	3.6×10^{-5}	2.80 (1.54–5.12)
Neurologically normal	980	966	14	1.4%		

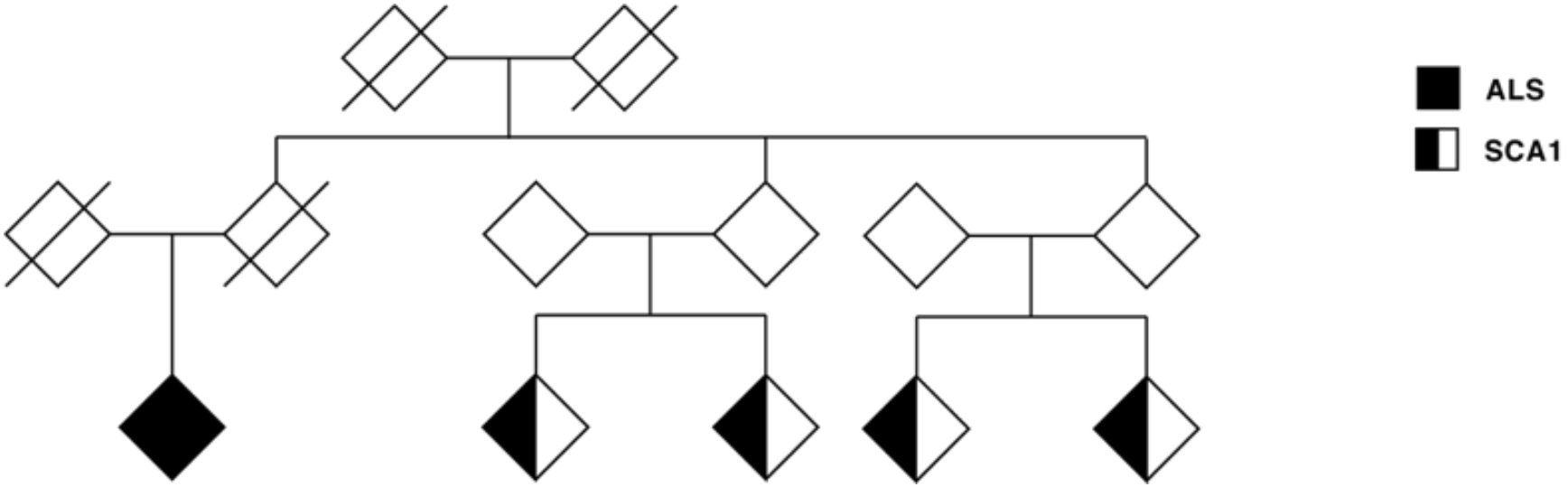
OR, odds ratio; CI, confidence interval



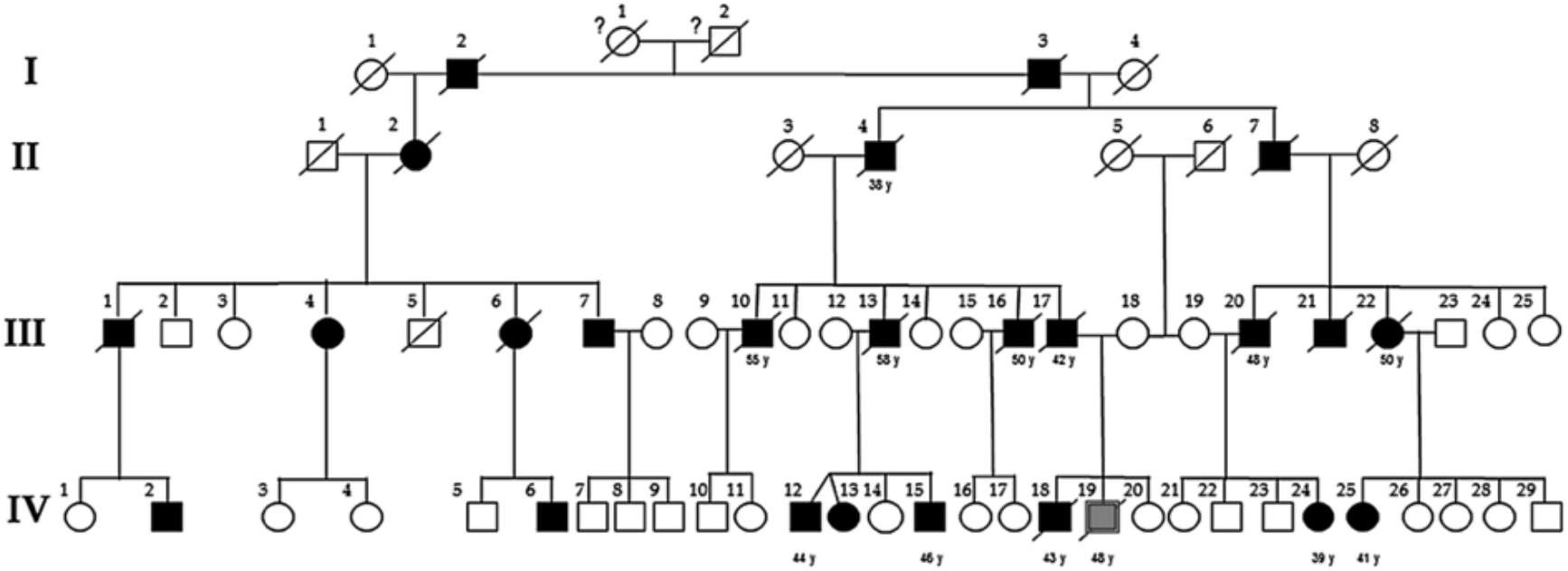
What about other polyQ genes in ALS?

PolyQ gene	Patients with ALS tested	Controls tested	PolyQ repeat lengths[a]	Differences in polyQ length in ALS vs control?
SCA1 (ataxin 1)	526	526	21–37 (27, 28)	No
SCA2 (ataxin 2)[b]	915	980	4.7% Q27–Q33 ALS; 1.4% Q27–Q33 control	Yes; $p = 3.6 \times 10^{-5}$
SCA3 (ataxin 3)	488	623	13–33 (13, 21)	No
SCA6 (*CACNA1A*)	146	167	3–17 (10, 12)	No
SCA7 (ataxin 7)	156	170	1–12 (5)	No
SCA17 (*TBP*)	116	85	25–41 (35, 36)	No
DRPLA (atrophin 1)	120	106	19–36 (27)	No
Huntington disease (huntingtin)	121	115	7–31 (18)	No
AR (androgen receptor)[c]	146	100	Male: 22–26 (23 ALS; 23, 25 control); female: 21–26 (22, 23 ALS; 25 control)	No

ALS case in Dutch SCA1 family (ATXN1 polyQ)



ALS case in Italian SCA1 family



Rossella Spataro & Vincenzo La Bella; J Neurol (2014) 261:1442–1443

ATXN1 & ALS in Italian ALS cohort:

Alleles	sALS	Controls	*p* Value	OR (95% CI)[a]
ATXN-1, n (%)				
≥32 repeats	57 (7.07)	13 (2.4)	0.0001[b]	2.396 (1.26–4.56)
<32 repeats	749 (92.9)	531 (97.6)		

Conforti et al.; Neurology. 2012 Dec 11;79(24):2315-20

ATXN2 vs ATXN1

	Similarities:	Differences:		
			ATXN2	ATXN1
Genetics	Both have variability in CAG repeat size	Normal range:	15-32	27-36
	Both polyglutamine repeats can be interrupted	Interruption:	CAA (Glutamine)	CAT (Histidine)
Cell Biology	Both are TDP-43 RNA targets	TDP-43 toxicity:	Yes	Unknown
Pathology	Neuronal cell loss in overlapping CNS regions	Inclusion bodies :	TDP-43	Intranuclear

SAME SAME

BUT DIFFERENT

Aim(s) of the study

To investigate whether there is an increase in the number of CAG/CAT repeats in ATXN1 in ALS compared to non-ALS controls

Cohort	ALS	Controls
Netherlands	1434	1425
France	496	425
Belgium	537	212
Ireland	205	354
Total	2672	2416

ALS Clinical data (N = 2531)	
Male (%)	1494 (59.0)
Age at onset in years (SD)	61.9 (11.5)
Survival after onset in months (SD)	42.9 (38.8)
Bulbar onset (%)	774 (30.6)
C9ORF72 expansion (%)	211 (8.3)

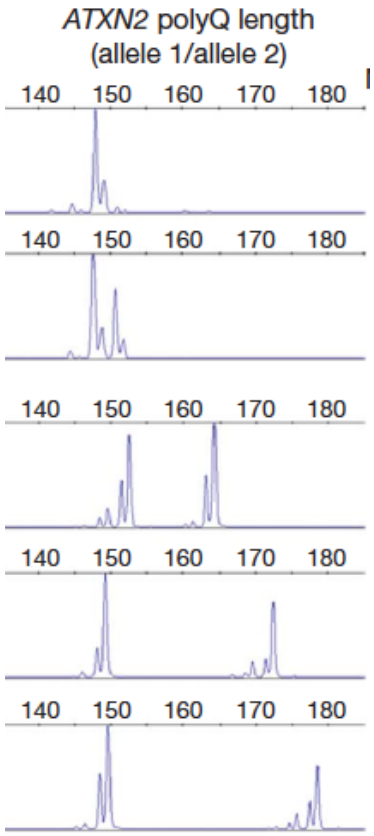
Methods

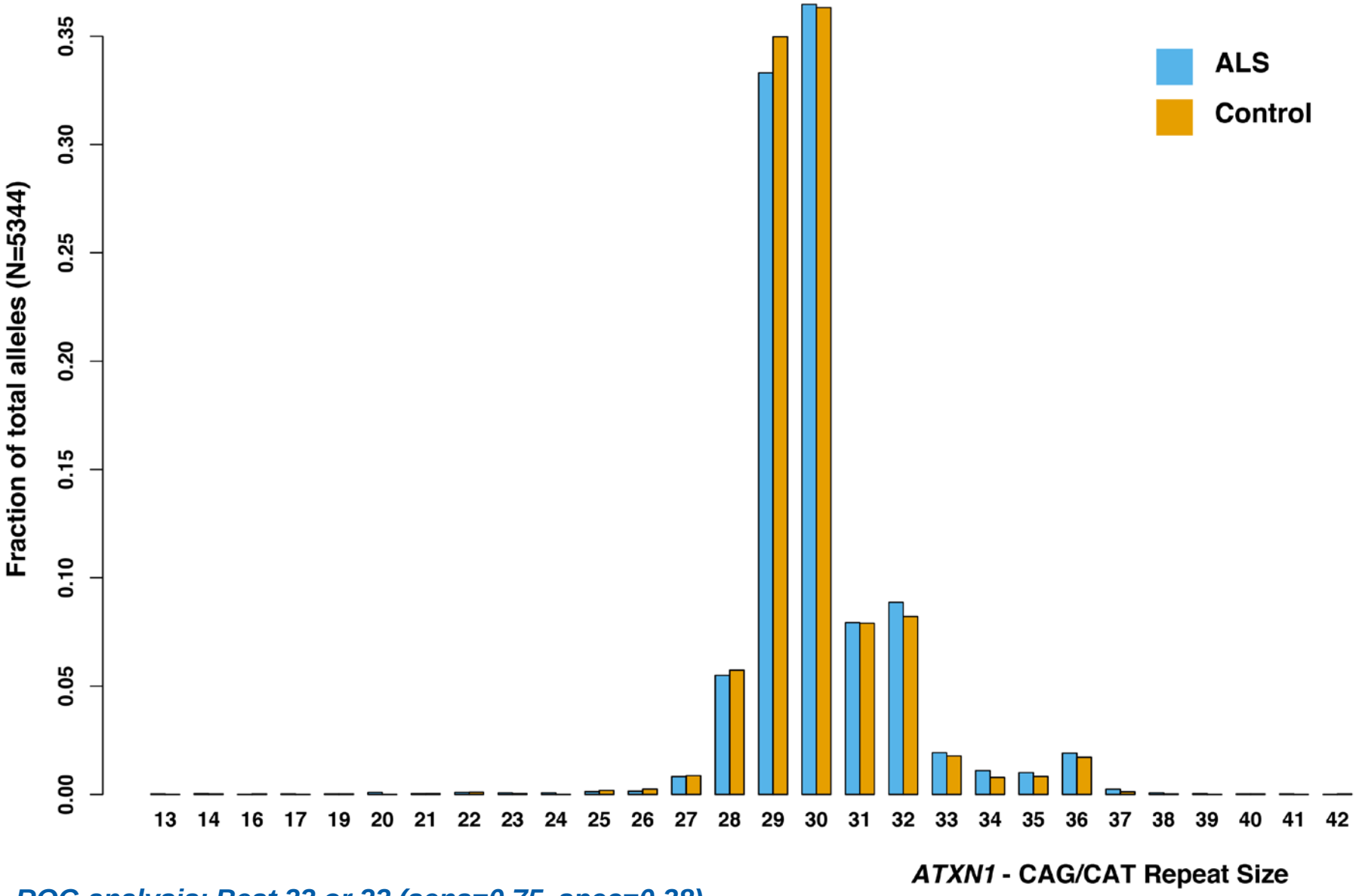
Step 1: Sanger sequencing – 850 samples

- Advantage: The actual length of the repeat
- Disadvantage: Artifacts and Low Call Rates
- Outcome: 29 & 30 Most common alleles

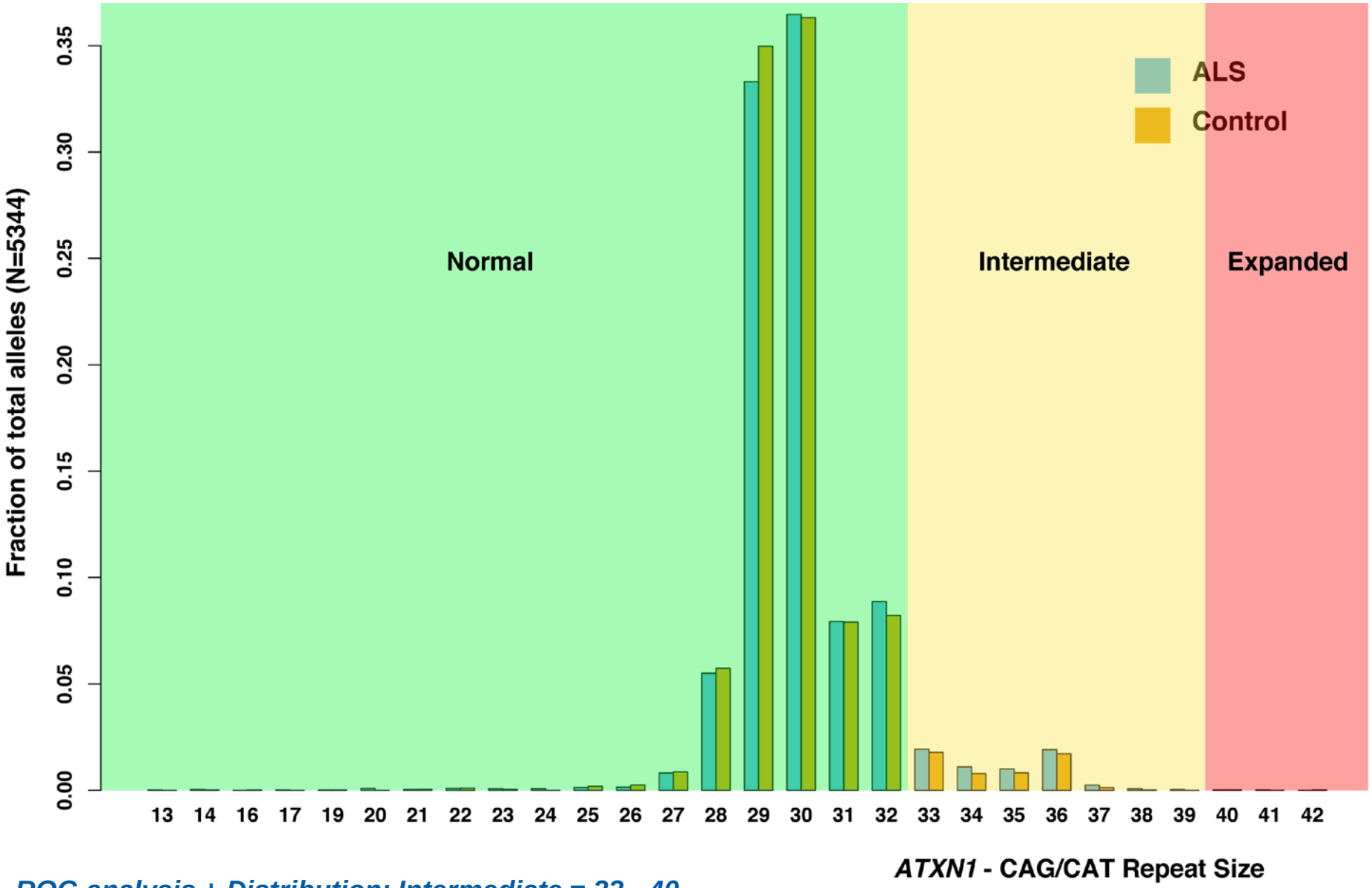
Step 2: Repeat PCR – All samples

- Automated calling





ROC-analysis: Best 32 or 33 (sens=0.75, spec=0.28)



ROC-analysis + Distribution: Intermediate = 33 - 40

ATXN1 & ALS

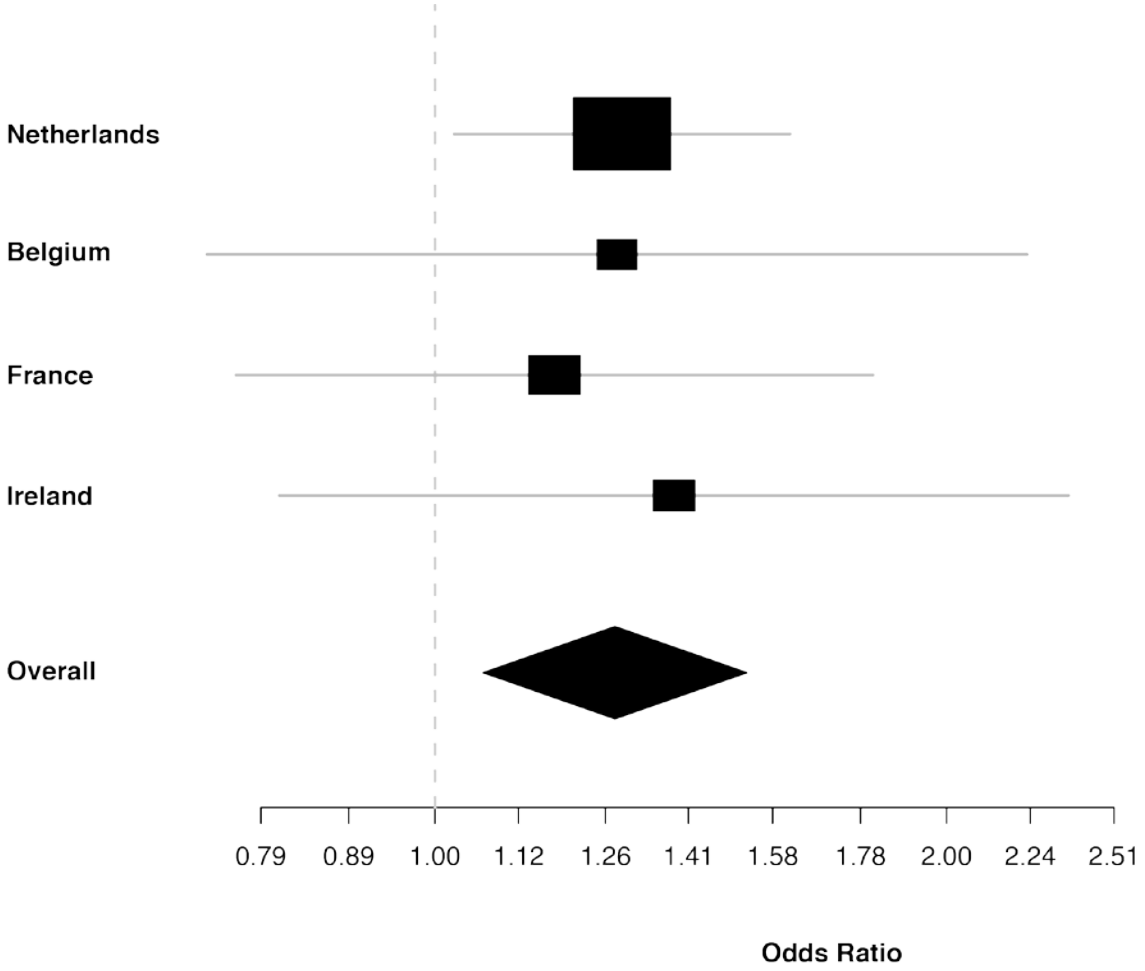
	ATXN1 Repeat Size Genotype (>32 CAG/CAT)	
	normal	expanded (%)
ALS	2344	328 (12.3%)
Controls	2172	244 (10.1%)

ATXN1 & ALS

	ATXN1 Repeat Size Genotype (>32 CAG/CAT)	
	normal	expanded (%)
ALS	2344	328 (12.3%)
Controls	2172	244 (10.1%)

	ATXN1 Repeat Size Genotype (>32 CAG/CAT)	
ALS	normal	expanded (%)
C9ORF72 -	1915	266 (12.2%)
C9ORF72 +	195	16 (7.6%)

Association analysis



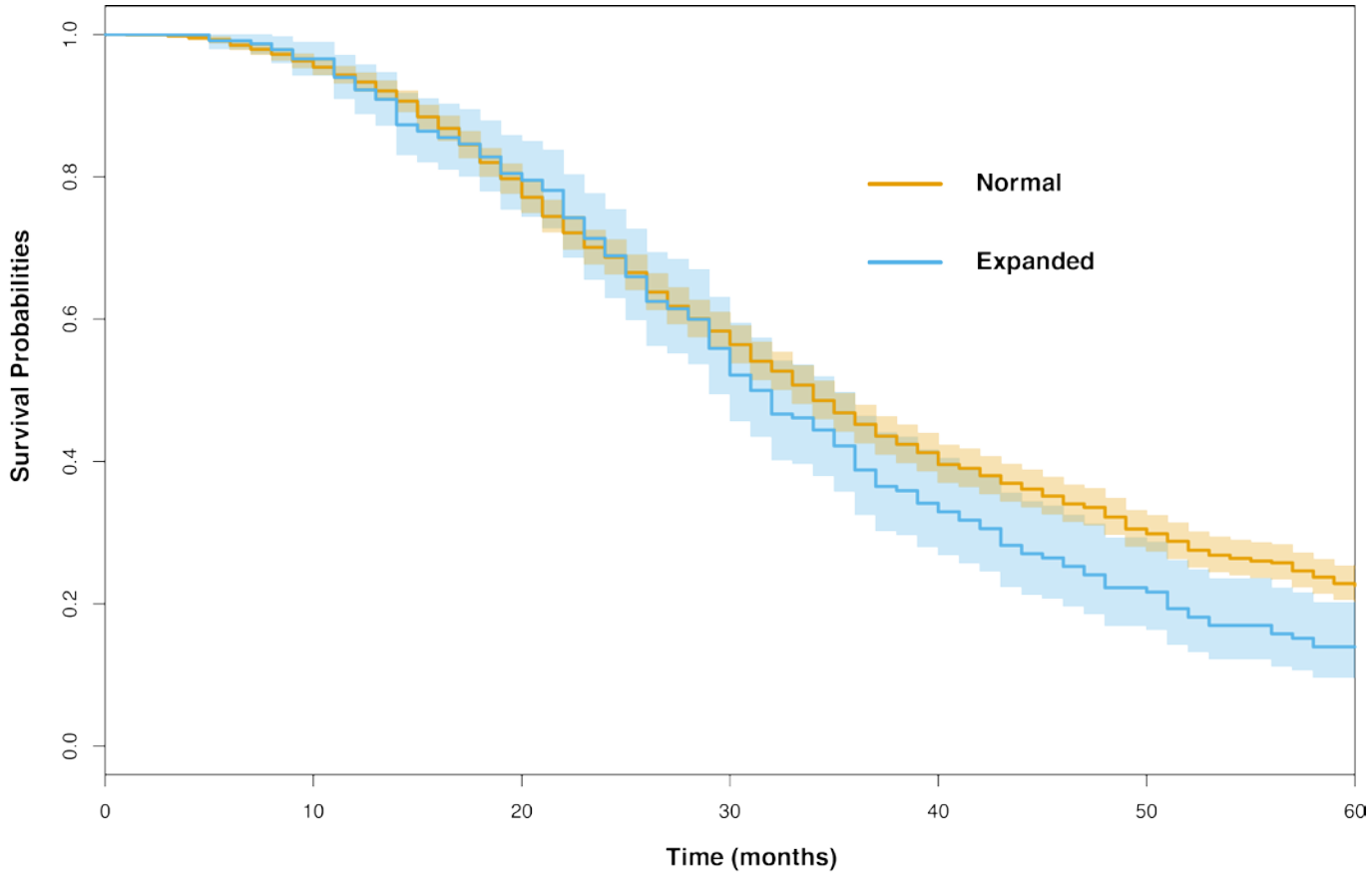
OR = 1.28

95% CI : 1.07-1.53

***p* = 0.0036 (one-sided)**

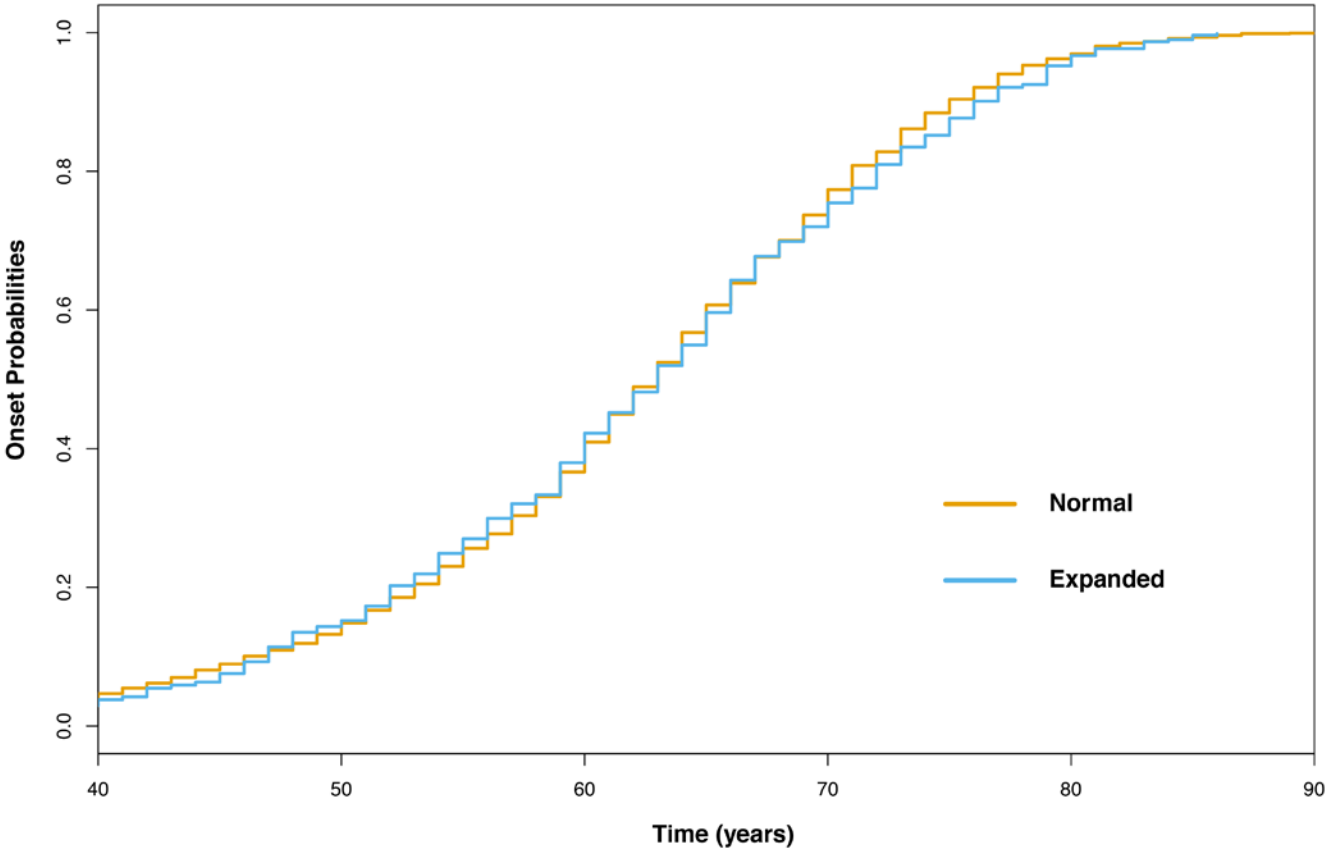
Fixed & Random effects model
Heterogeneity n.s.

ATXN1 & Survival after onset



Corrected for Sex, Site of onset & C9ORF72 (p = 0.14)

ATXN1 & Age at onset



(Preliminary) Conclusions - Genetics

Significant association with an increased length of CAG(/CAT) trinucleotide repeats in *ATXN1* and ALS

- **Cut-off 33**
- **Dominant model**

No enrichment for intermediate *ATXN1* expansions in C9ORF72 expanded ALS individuals

No significant difference in survival

No difference in age at onset

Discussion

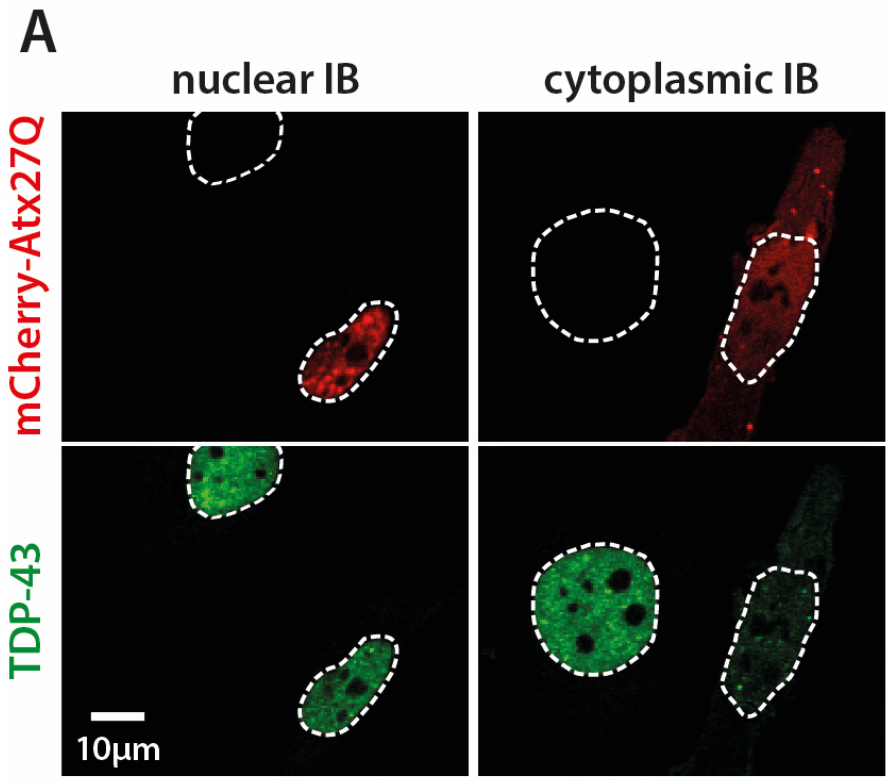
ATXN1 vs ATXN2

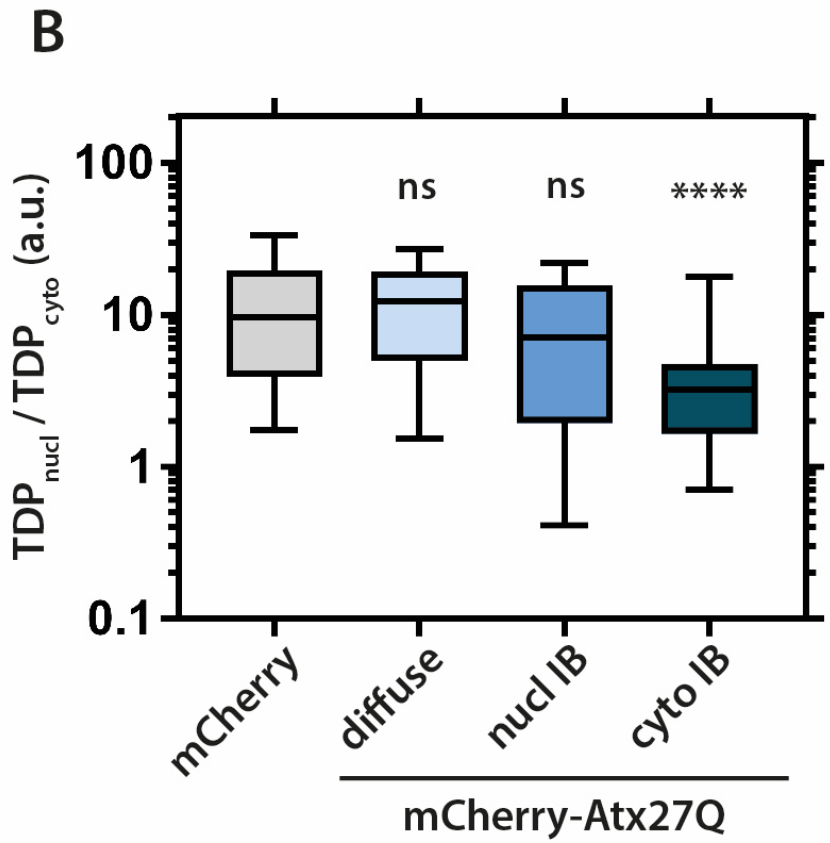
- **Allele frequency intermediate expansion**
  - **ALS: 1-2% in *ATXN2* vs 6% in *ATXN1***
  - **Controls: 0.4% in *ATXN2* vs 5% in *ATXN1***

- **RR: *ATXN2* 3.06 *(pooled, Sproviero et al.)* vs *ATXN1* 1.22 *(p=0.01)***

- **Genetic differences (eg. CAT interruptions)**
- **Biological differences**

Discussion – The next step





Steven Boeynaems – KU Leuven

Acknowledgements

Michael van Es
Jan Veldink
Leonard vd Berg

Lindy Kool
Stephan Goedee
Henk-Jan Westeneng
Abdelilah Assialioui
Joke van Vugt
William Brands
Jelena Medic
Bas Middelkoop
Rick van der Spek
Annelot Dekker

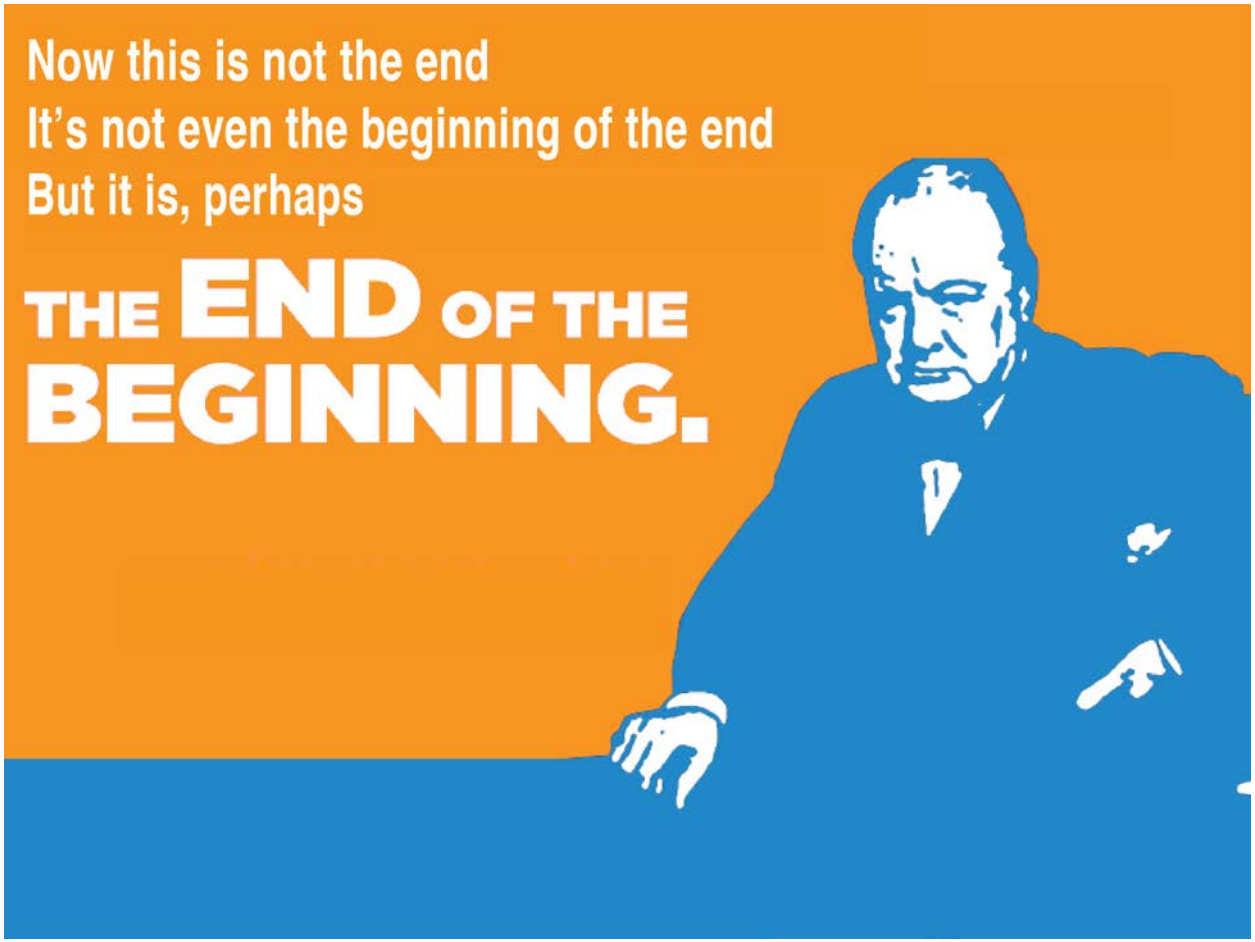
Philippe Corcia
Patrick Vourc'h
Philippe Couratier
Francois Salachas
Stéphanie Millecamp

Steven Boeynaems
Matthieu Moisse
Philip Van Damme

Russell McLaughlin
Orla Hardiman

Stichting ALS Nederland

Brain Center Rudolf Magnus


Now this is not the end
It's not even the beginning of the end
But it is, perhaps
THE END OF THE BEGINNING.