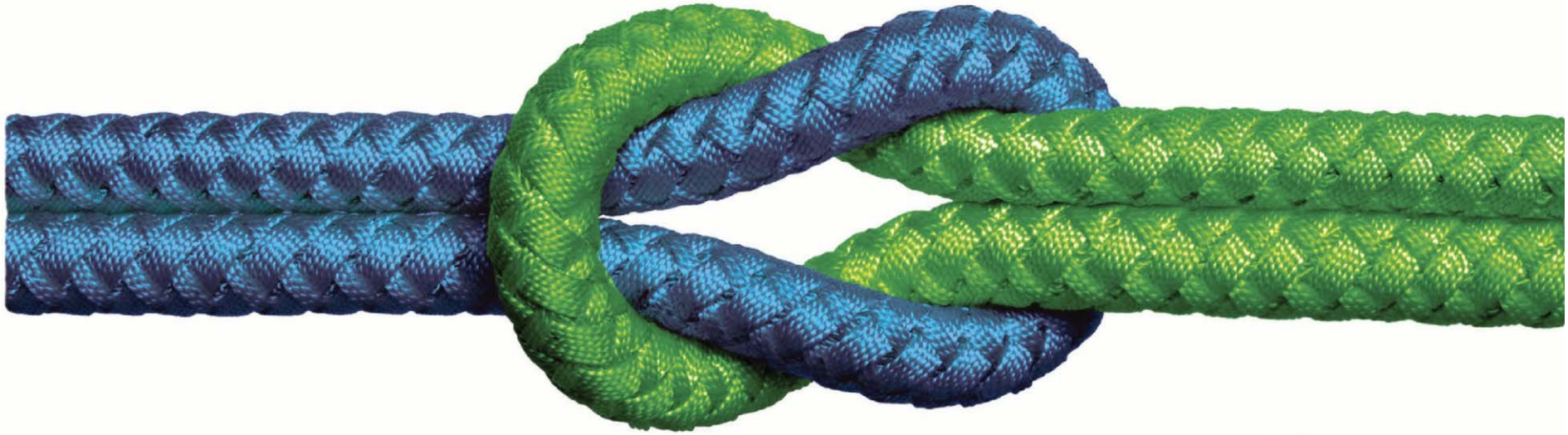
EUROPEAN **HUNTINGTON'S DISEASE** NETWORK

Exciting times - HD research and clinical trials in HD: an overview

G. Bernhard Landwehrmeyer, MD, FRCP
Professor of Neurology, Ulm University, Germany
Principal Investigator Enroll-HD

EHA 2017



STRONGER together

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We experience exciting times for HD

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Gene silencing has reached HD patients: three clinical trials of gene silencing compounds (ASO) in HD are currently ongoing, the first (IONIS-HTT_{Rx} trial) is almost completed

Overview

- Exciting science:
  - Can you go further than ‘shooting the messenger’ and correct the primary DNA defect? Gene editing as a next frontier – hype and hope
  - Changing CAG sizes through the DNA repair machinery – a new therapeutic target?
- The HD clinical trial landscape
- Hopes for tomorrow & help for today

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Exciting science

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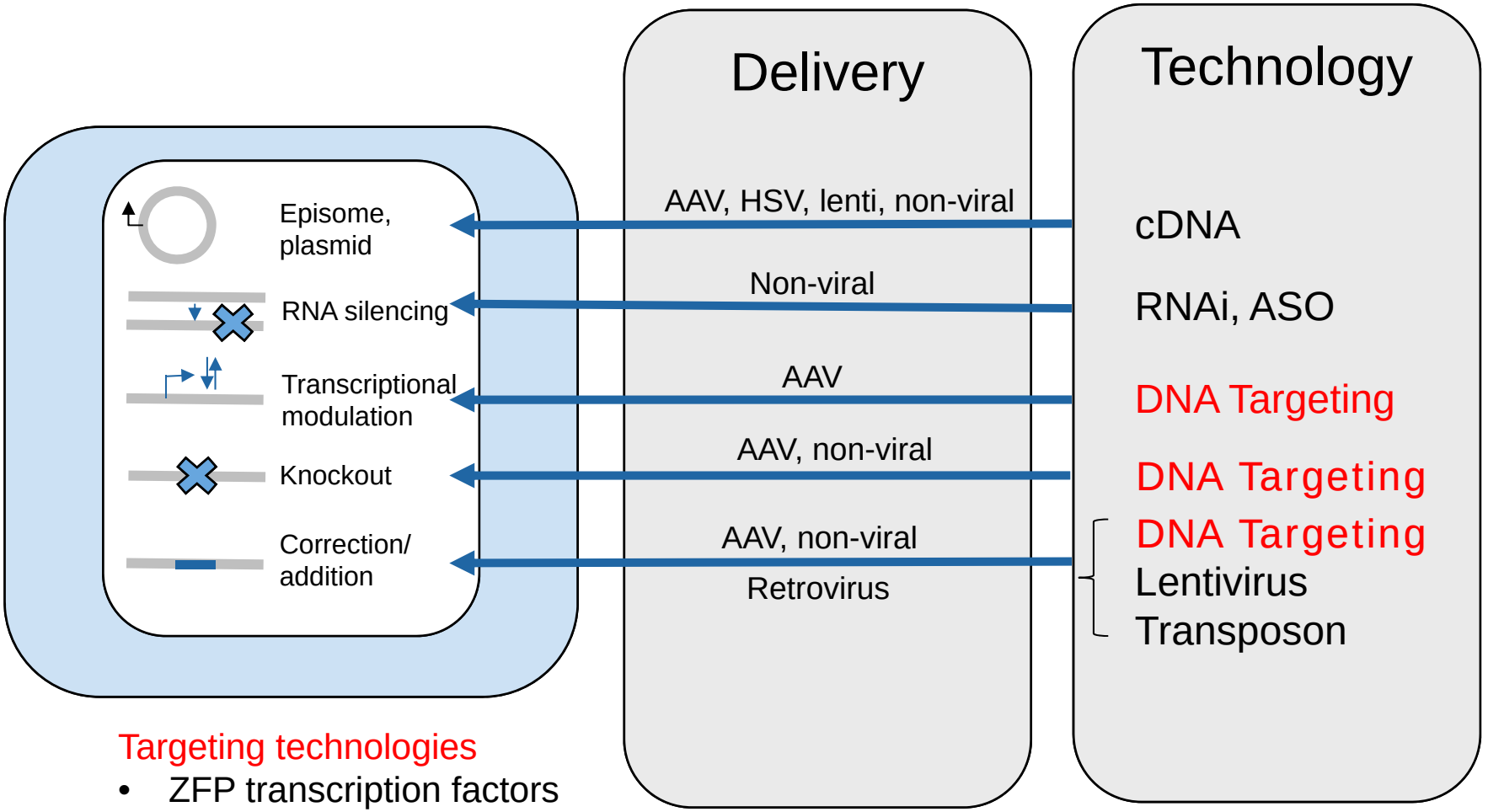
Approaches to gene silencing

Gene-Therapy



Targeting technologies

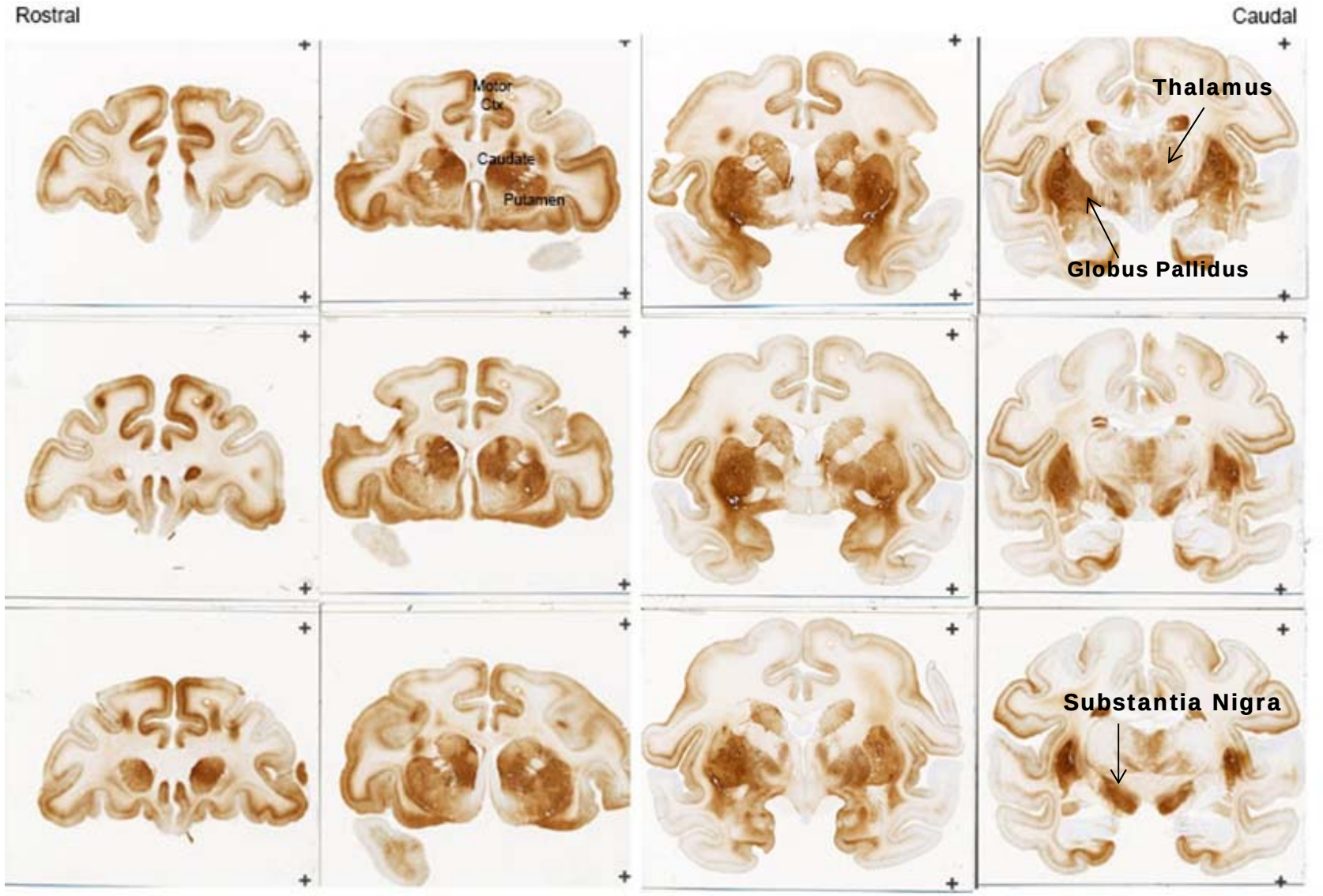
- ZFP transcription factors
- ZF nuclease
- CRISPr/Cas9
- TALENs/meganuclease

rAAV as a Gene Delivery Vector for CNS Disorders

Adeno-Associated Virus (AAV)

- Replication defective parvovirus
- Transduce non-dividing cells
- Nonpathogenic
- Vector production and purification methods have been established for clinical use
- A single intracranial administration AAV could provide long term suppression of Htt

AAV1-eGFP May Produced Widespread Transduction in the Striatum and Cortex



Courtesy of Lisa M. Stanek, Genzyme

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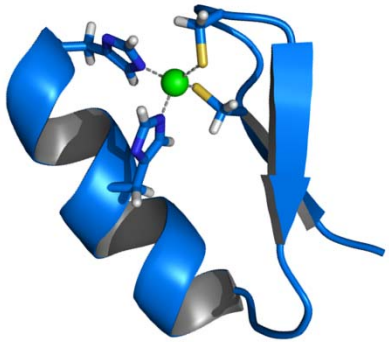
Suppressing the mutant htt gene copy by targeting DNA

Zinc finger DNA binding domains can be engineered to recognize specific target sequences

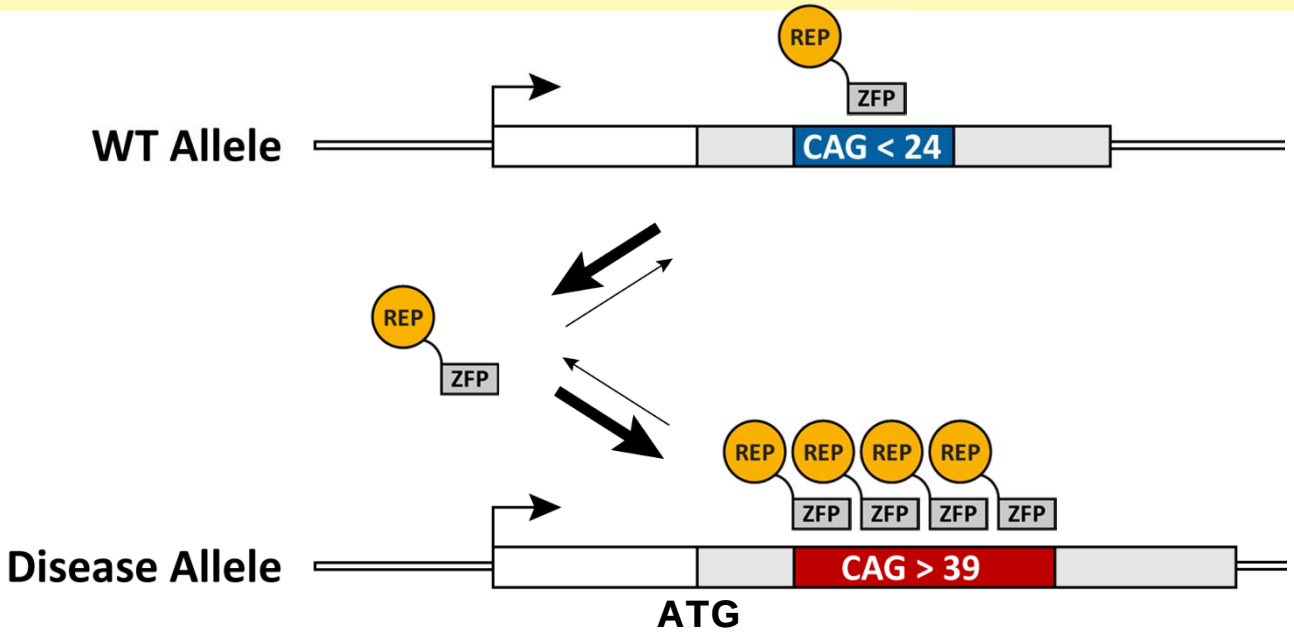


5′ C A G 3′

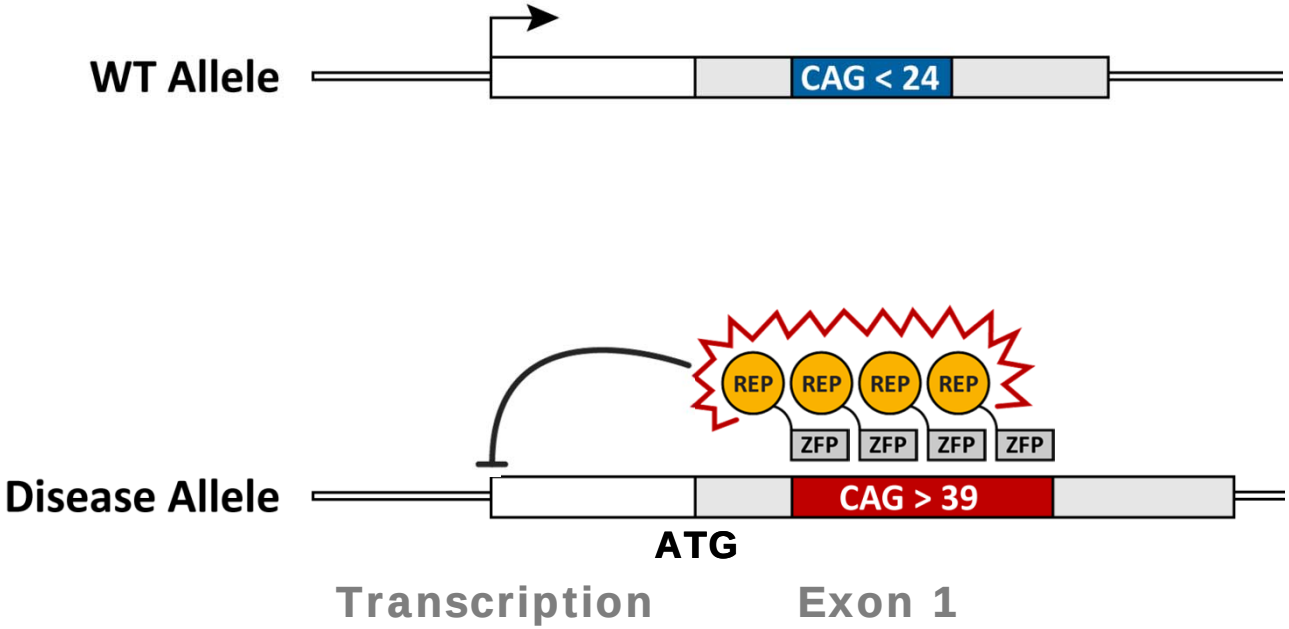
Sangamo BioSciences

Shire

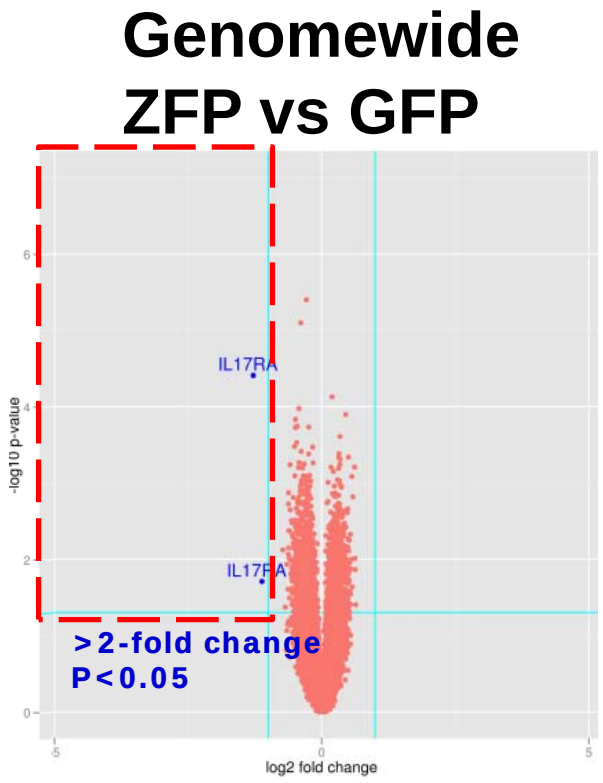
Engineered ZFPs for allele-specific repression of mutant Htt

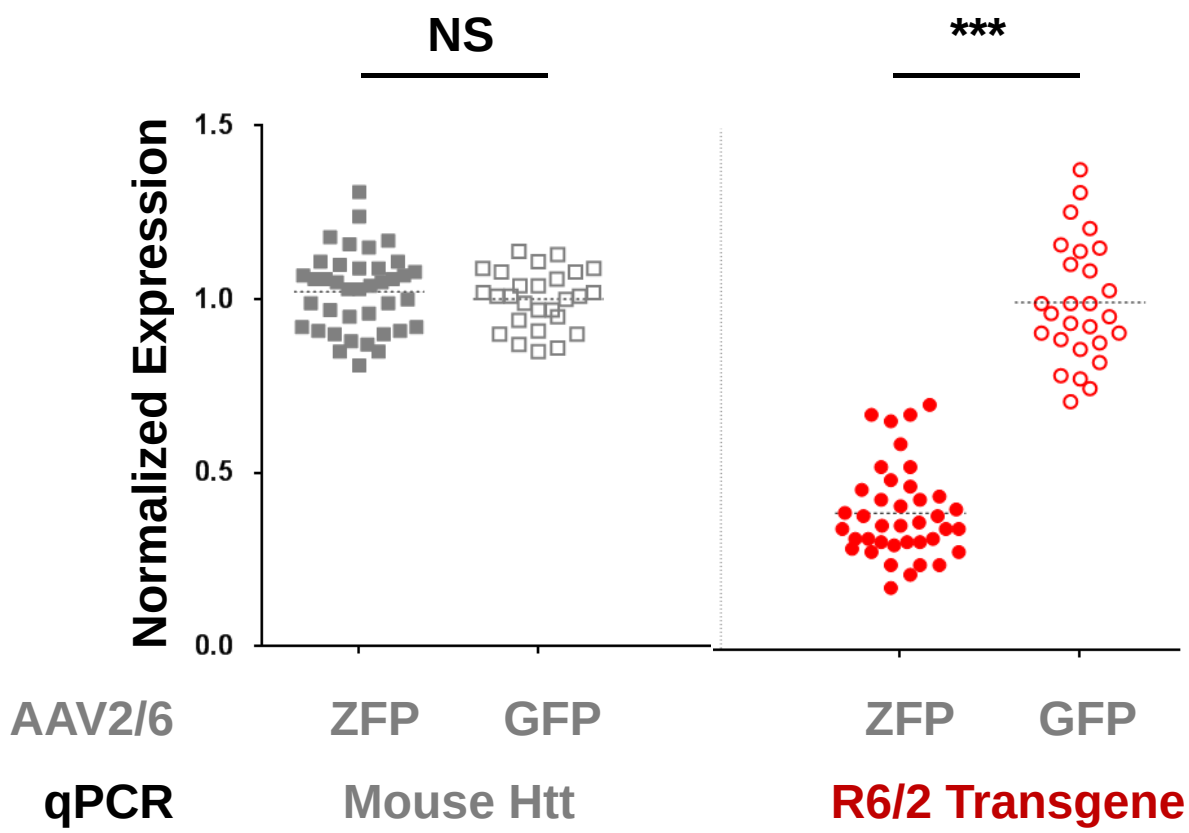


Engineered ZFPs for allele-specific repression of mutant Htt

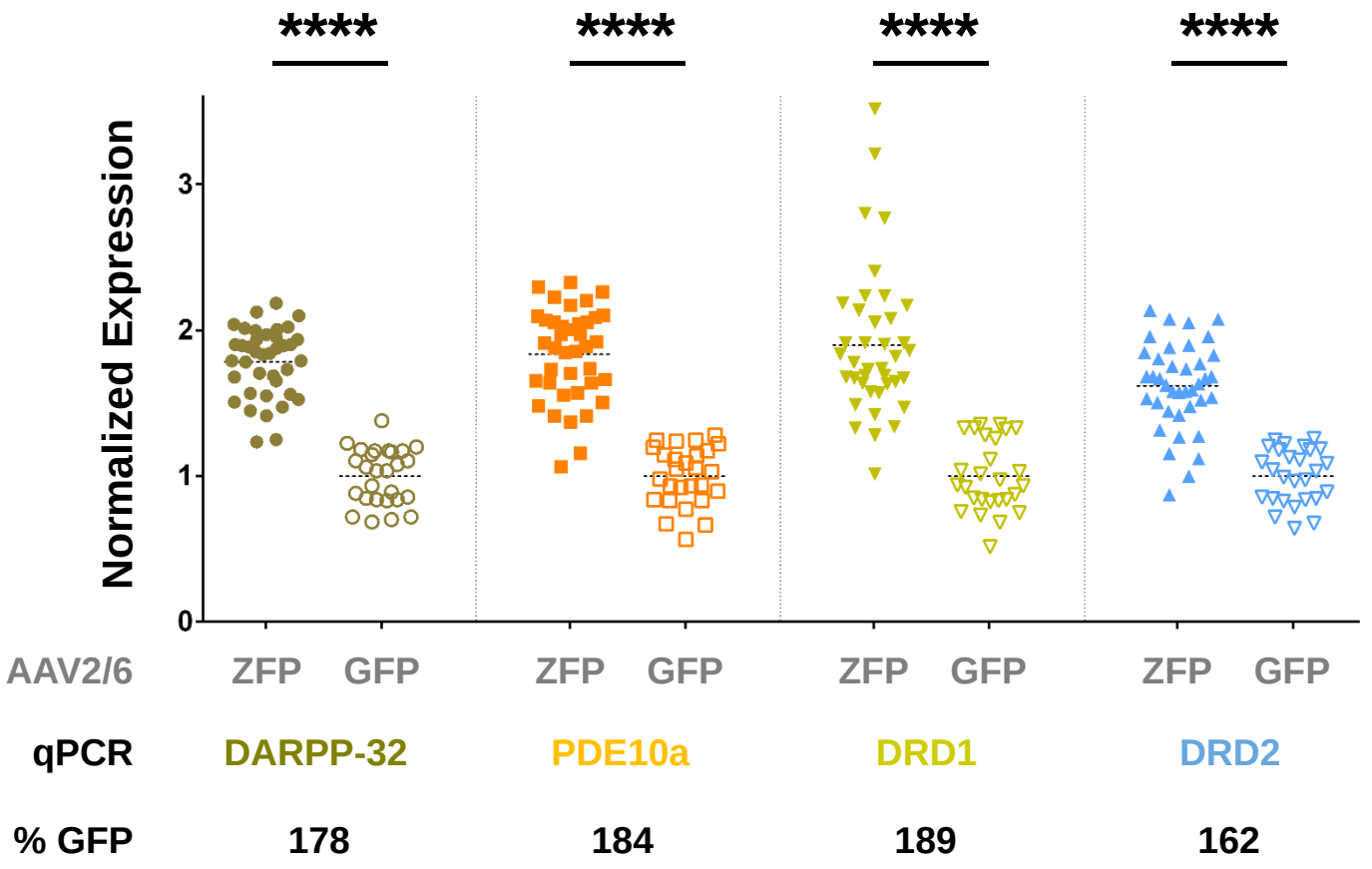


Engineered ZFPs selectively repress mutant HD-alleles





ZFP expression rescues expression of medium spiny neuron markers in R6/2 mice



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Correcting the primary genetic alteration at the DNA level: the promise of gene editing

Excitement in the science community

"I set a high bar for writing about mouse studies. I don't include them in my textbooks or news articles, and only rarely blog about them. But when experiments in mice shine a glimmer of hope on a horrific illness with a long history of failed treatments, I pay attention."

Recent scientific articles

The Journal of Clinical Investigation

BRIEF REPORT

Molecular Therapy
Original Article

AMERICAN SOCIETY of GENE & CELL THERAPY

ORIGINAL ARTICLE

Article

Cell

Elimination of Toxic Microsatellite Repeat Expansion RNA by RNA-Targeting Cas9

Ranjan Batra,[1,2,3,10] David A. Nelles,[1,2,3,10] Elaine Pirie,[1,2,3] Steven M. Blue,[1,2,3] Ryan J. Marina,[1,2,3] Harrison Wang,[1,2,3] Isaac A. Chaim,[1,2,3] James D. Thomas,[4] Nigel Zhang,[1,2,3] Vu Nguyen,[1,2,3] Stefan Aigner,[1,2,3] Sebastian Markmiller,[1,2,3] Guangbin Xia,[5] Kevin D. Corbett,[1,6,7] Maurice S. Swanson,[4] and Gene W. Yeo[1,2,3,8,9,11,*]

Gene editing for HD – the idea

- In people destined to develop HD there is a string of C-A-G letters that goes on…and on and on and on and on and on and on and on and on and on and on and on and on and on and on and on and on…many times more than necessary
- The idea is deleting all those extra “on-and-on” repeats

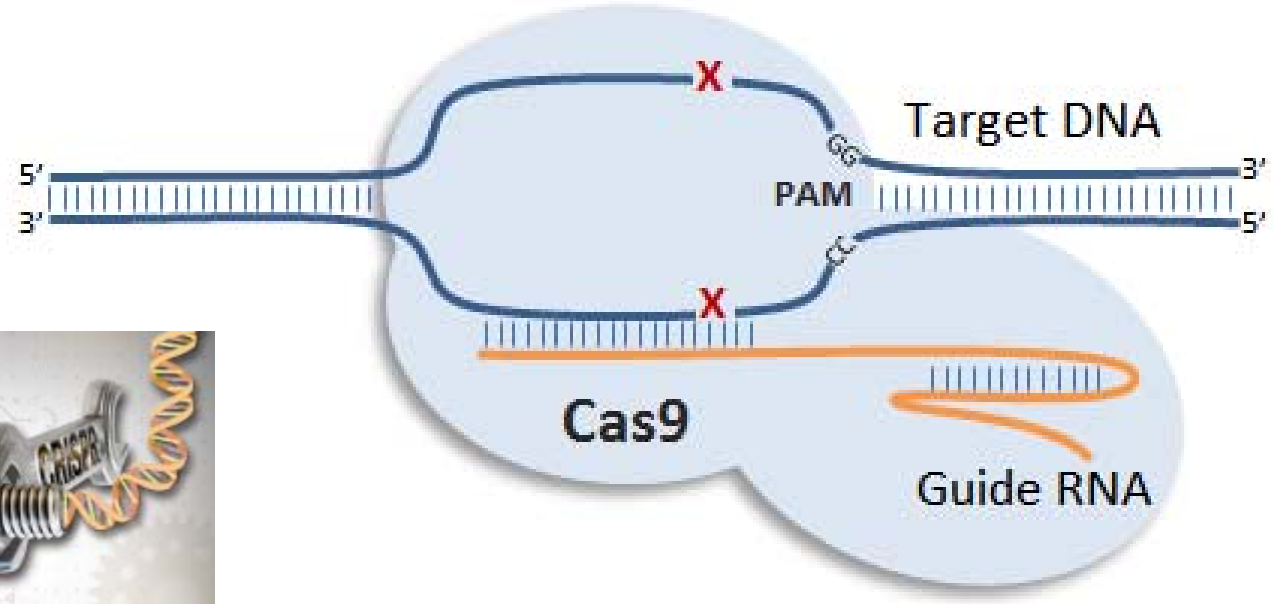
Gene editing versus gene silencing

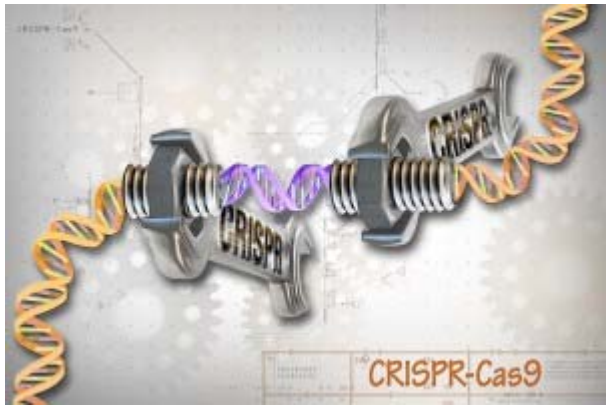
- The hope: “While RNAi and antisense oligonucleotides can dampen expression of the extended gene, the effect isn’t permanent in the way that snipping out the repeat or even the entire gene would be. And a one-time or few-times editing out is preferable to a regular need for treatment.” (Ricky Lewis, PhD)

Genome Editing Technology Platforms Comparative Analysis

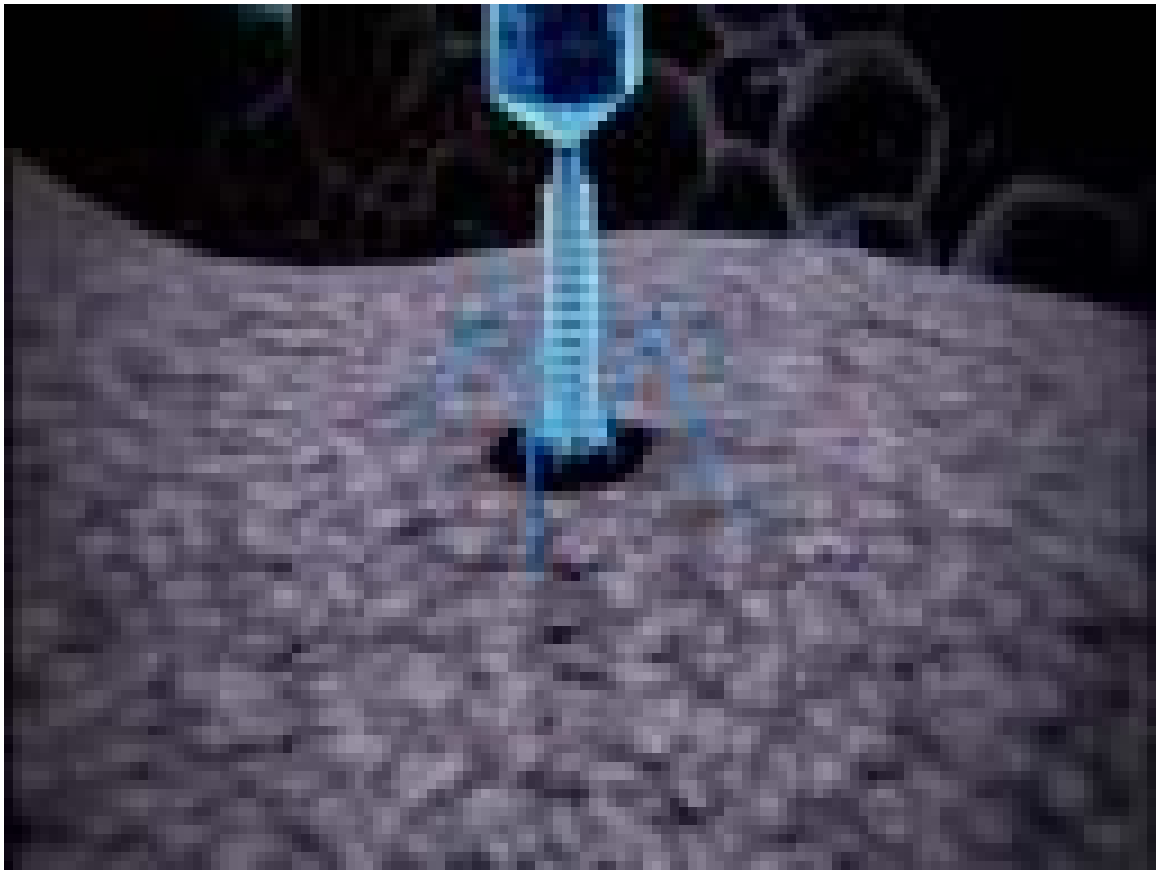
	ZFNs	TALENs	CRISPR	MegaN/MegaTal
Size	• 1kb x 2	• 3kb x 2	• 4.2kb +0.1kb	• 1- 2.5 kb x1
Design Density	• Every ~1.7 bp	• Similar to ZFNs	• Every 11-50 bp • (PAM constrained)	• Central 4bp constraint
Specificity	• Ability to optimize for on-target/off-target activity	• Single nucleotide binding • No ability to optimize	• Watson-Crick bp binding • Specificity for any particular guide is fixed	• Difficult to design (MN) • Optimization possible through protein design (MT)
ex vivo	• Multiple programs translated into the clinic (over 80 subjects treated)	• 1 program moving to clinic – 2016 (2 subjects treated)	• Nascent clinical programs	• No clinical programs
in vivo	• Low immunogenicity risk (ZFP is human protein; ZFNs non-immunogenic in vivo)	• Immunogenicity risk (bacterial origin) • Size constrained	• Immunogenicity risk (bacterial origin) • Size impacts delivery options (2 parts)	• Immunogenicity risk (bacterial origin)
				Modified courtesy Geoff Nichol

Genome editing – CRISPR-Cas9





CRISPR-Cas9 – how it was found



CRISPR-Cas9 – how it works

3 steps (think about editing a spelling error in a text using your computer)

1. Find the mistake by moving your cursor to the right spot where the wrong letters are
2. Delete the mistake, cutting out the wrong letters
3. Correct the mistake, filling in the right letters

CRISPR-Cas9 – how it works

1. Find the mistake by moving your cursor to the right spot where the wrong letters are

   RNA guide strand (‚cursor')

2. Delete the mistake, cutting out the wrong letters

   CRSPR-Cas9 (‚scissor')

3. Correct the mistake, filling in the right letter

   DNA repair machinery (‚retype')

The issues

- The cursor moves to wrong spots
- The scissors cut at the wrong places
- The scissors are recognized as foreign and attacked
- The repair goes wrong
- You have to get all things DELIVERED

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Correction of single cells or cell cultures is a reality, treatment of entire living organisms (100 billion cells in CNS alone) is fiction

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Realistically it will take at least a decade before gene silencing therapeutics become a prescribe-able therapeutic option for HD expansion mutation carriers

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Exciting science: changing CAG sizes through the DNA repair machinery – a new therapeutic target?

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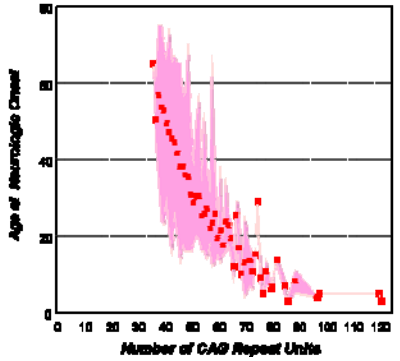
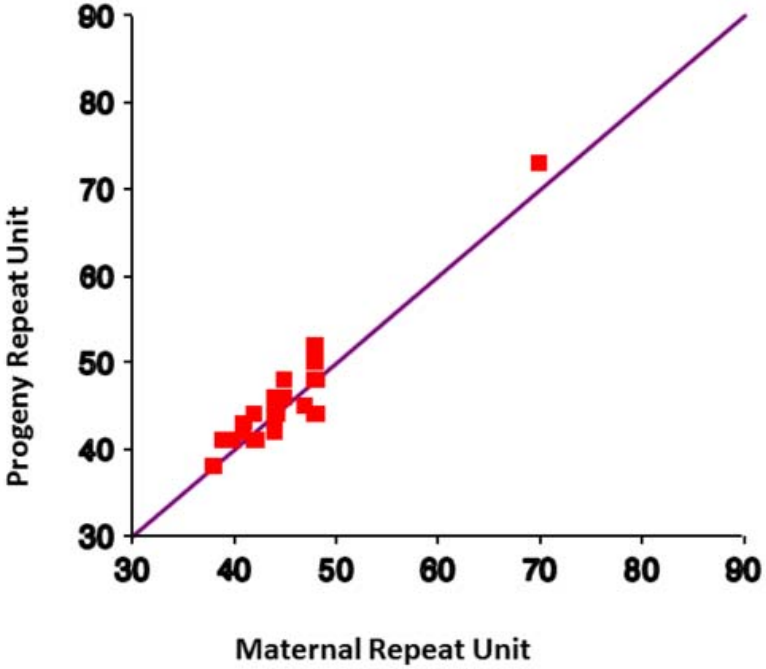
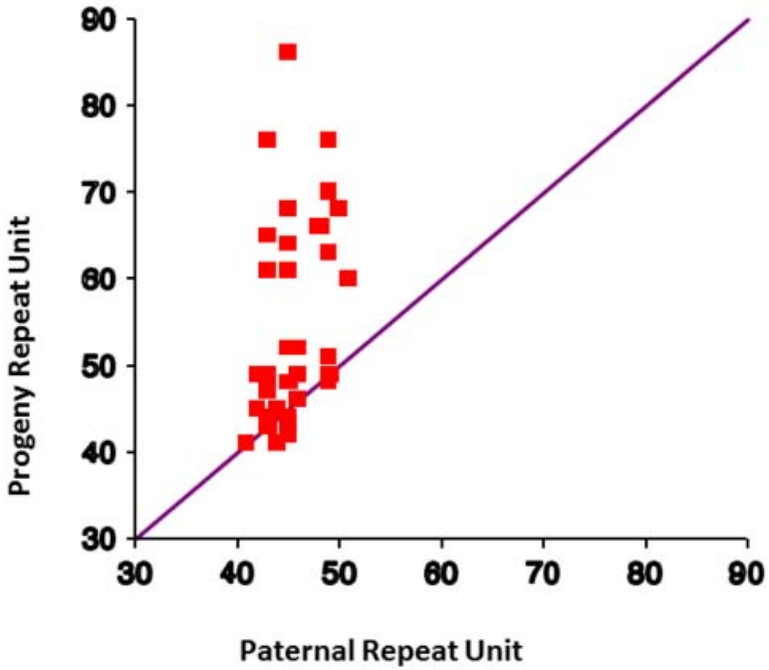
At the core of HD is the CAG expansion mutation

The CAG repeat expansion is intergenerationally instable







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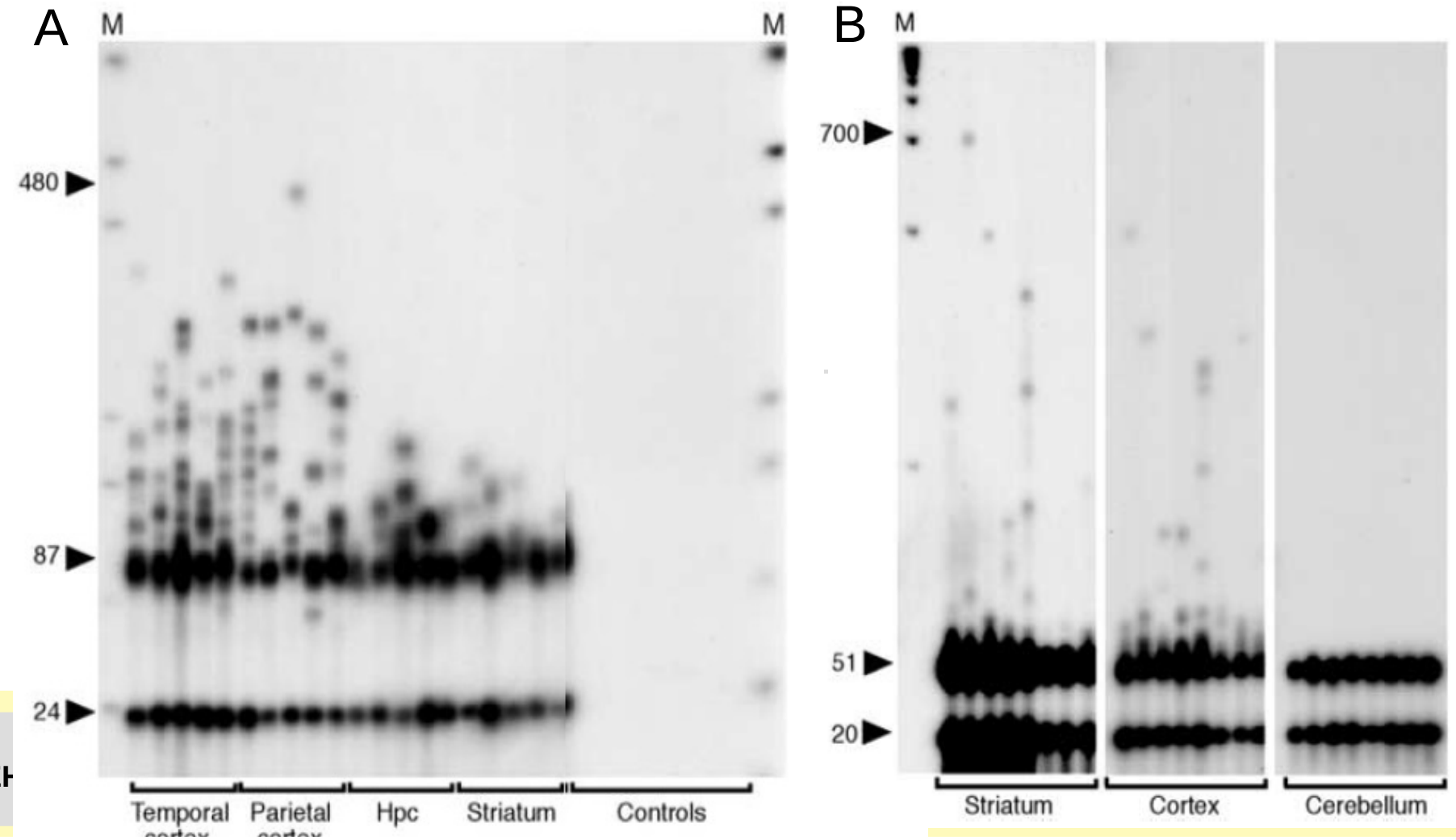
Is an ongoing, further increase of the dynamic CAG-expansion during the life of HD expansion carriers an important driver of clinical onset and progression?

Enourmous expansions can happen in brain of HD patients



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Clues from experiments of nature – genetic modifier studies

Cell

Article

Identification of Genetic Factors that Modify Clinical Onset of Huntington's Disease

Genetic Modifiers of Huntington's Disease (GeM-HD) Consortium*
*Correspondence: gusella@helix.mgh.harvard.edu
http://dx.doi.org/10.1016/j.cell.2015.07.003

Cell 162, 516–526, July 30, 2015

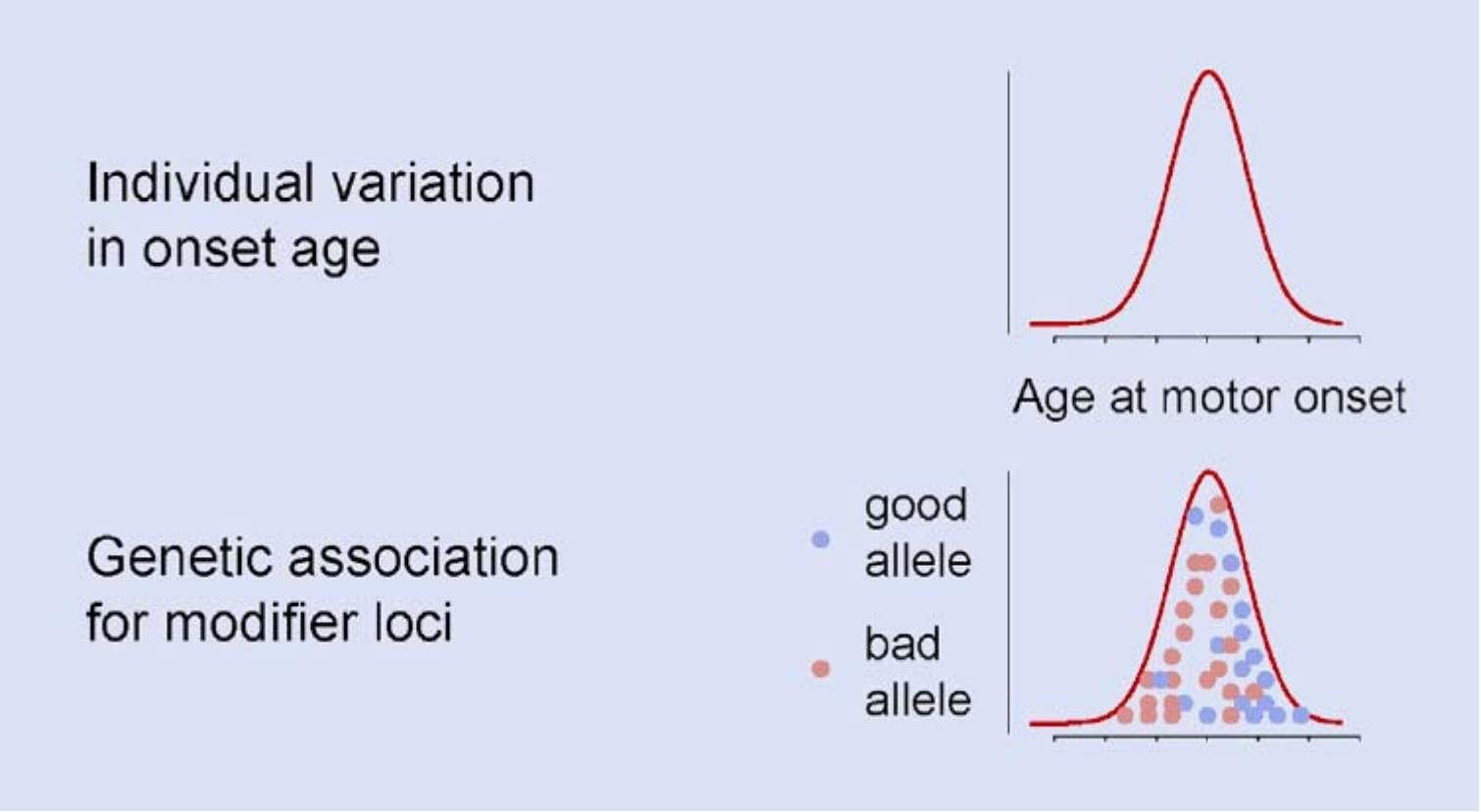
The Genetic Modifiers of Huntington's Disease (GeM-HD) Consortium was organized into the following groups: GeM Group 1: Jong-Min Lee, Vanessa C. Wheeler, Michael J. Chao, Jean Paul G. Vonsattel, Ricardo Mouro Pinto, Diane Lucente, Kawther Abu-Elneel, Eliana Marisa Ramos, Jayalakshmi Srinidhi Mysore, Tammy Gillis, Marcy E. MacDonald, and James F. Gusella; GeM Group 2: Denise Harold, Timothy C. Stone, Valentina Escott-Price, Jun Han, Alexey Vedernikov, Peter Holmans, and Lesley Jones; GeM Group 3: Seung Kwak and Mithra Mahmoudi; GeM Group 4: Michael Orth and G. Bernhard Landwehrmeyer; Registry Investigators: Jane S. Paulsen; PREDICT-HD Investigators: E. Ray Dorsey and Ira Shoulson; COHORT, PHAROS, and TREND-HD Investigators; Richard H. Myers; and HD-MAPS Investigators.

The concept of genetic modifiers is straightforward to understand

Comparing two groups of beer lovers
Beer is CAG expansion in the *HTT* gene
Beer belly caused by drinking beer is HD
TV time is a modifier of HD

Jong-Min Lee, Ph.D

Individual variation of onset age is in part (50%?) due to genetic variability in modifier genes



Human Genetics: *HD Community of HDGEC and Clinicians*

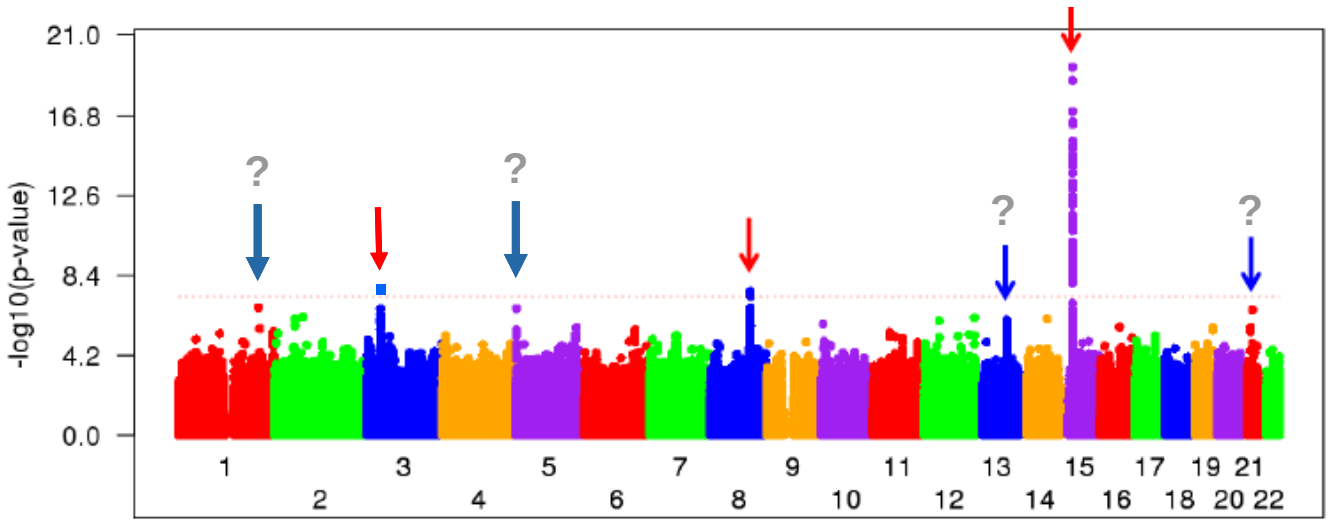
GeM-HD Consortium

GWAS 4:

Number	4030 (all with MAOO data)
Platform	OmniExpress Exome Array
Sample origin	EHDN Registry
Clinical dataset	Onset, HDCC, repeat visits

GWAS 5: Enroll-HD

Number	3400 (HDGEC) 60(IAC)
Platform	OmniExpress Exome Array
Sample origin	ENROLL-HD
Clinical dataset	Onset, HDCC, yr2 visits



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DNA repair machinery plays and inportant role as genetic modifier of the onset of HD

Human Genetic Modifiers of Clinical Onset

Candidate Genes:

Chr3: *MLH1*- mismatch repair

Chr15: *FAN1*- inter-strand crosslink repair

Chr8: RRM2B- DNA repair; UBR5-DNA damage response

Annals of NEUROLOGY
An Official Journal of the American Neurological Association and the Child Neurology Society

RESEARCH ARTICLE

DNA Repair Pathways Underlie a Common Genetic Mechanism Modulating Onset in Polyglutamine Diseases

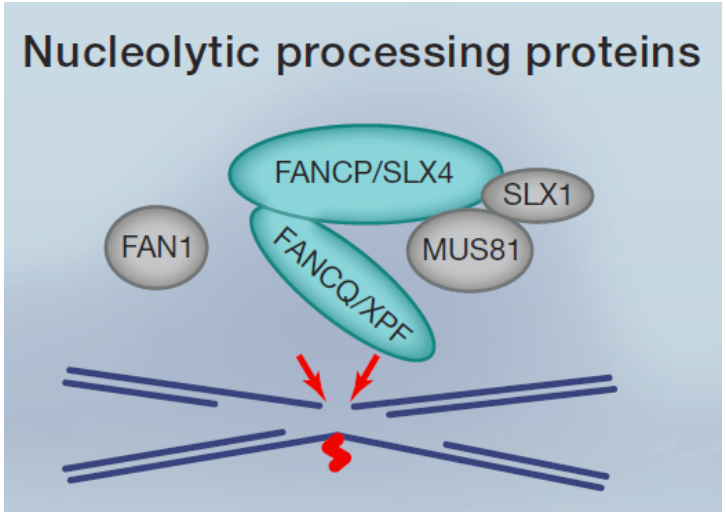
Conceição Bettencourt, PhD,[1,2] Davina Hensman-Moss, MD,[3] Michael Flower, MD,[3] Sarah Wiethoff, MD,[1,4] Alexis Brice, MD,[5,6] Cyril Goizet, MD,[7,8] Giovanni Stevanin, PhD,[5,9] Georgios Koutsis, MD,[10] Georgia Karadima, MD,[10] Marios Panas, MD,[10] Petra Yescas-Gómez, MD,[11] Lizbeth Esmeralda García-Velázquez, MSc,[11] María Elisa Alonso-Vilatela, MD,[11] Manuela Lima, PhD,[12,13,14] Mafalda Raposo, BSc,[12,13,14] Bryan Traynor, MD,[15] Mary Sweeney, BSc,[16] Nicholas Wood, MD,[1] Paola Giunti, MD,[1,17] The SPATAX Network, Alexandra Durr, MD,[5,6] Peter Holmans, PhD,[18] Henry Houlden, MD,[1,16] Sarah J. Tabrizi, MD,[3] and Lesley Jones, PhD[18]

Results: Significance of individual SNPs

Significant (Bonferroni for 22 SNPs,8 disease groups ($p<2.84x10^{-4}$))

SNP	Gene	Disease Group	2-sided p	Direction in GeM-HD?
rs3512	*FAN1*	All (HD+SCAs)	$1.52x10^{-5}$	Yes
rs1805323	*PMS2*	All (HD+SCAs)	$3.14x10^{-5}$	Yes
rs3512	*FAN1*	All SCAs	$2.22x10^{-4}$	Yes
Significant (Bonferroni for 22 SNPs ($p<2.27x10^{-3}$))				
rs1805323	*PMS2*	HD	$3.14x10^{-5}$	Yes
rs1805323	*PMS2*	SCA1	$1.67x10^{-3}$	Yes
rs1037699	*RRM2B*	SCA6	$4.86x10^{-4}$	Yes
rs1037700	*RRM2B*	SCA6	$5.47x10^{-4}$	Yes
rs5893603	*RRM2B*	SCA6	$2.13x10^{-3}$	Yes

Bettencourt et al. 2016



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Observations in HD patients suggest that a loss of FAN1 function leads to earlier onset; a FAN1 activator therefore would be required to modify HD onset pharmacologically

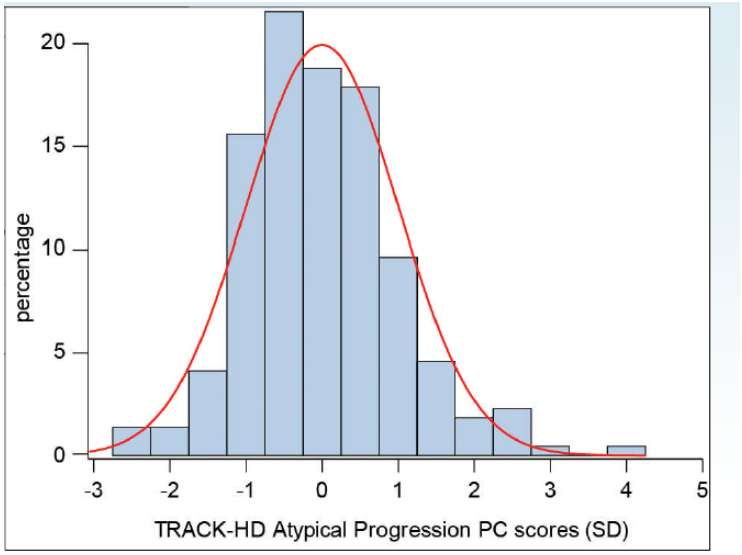
Identification of genetic variants associated with Huntington's disease progression

Dr Davina Hensman Moss (UCL)

Supervised by Prof Sarah J Tabrizi (UCL)
and Prof Lesley Jones (Cardiff)

Defining multimodal scores as QTs

- A multimodal progression score was defined for Track-HD and REGISTRY participants and used as quantitative trait

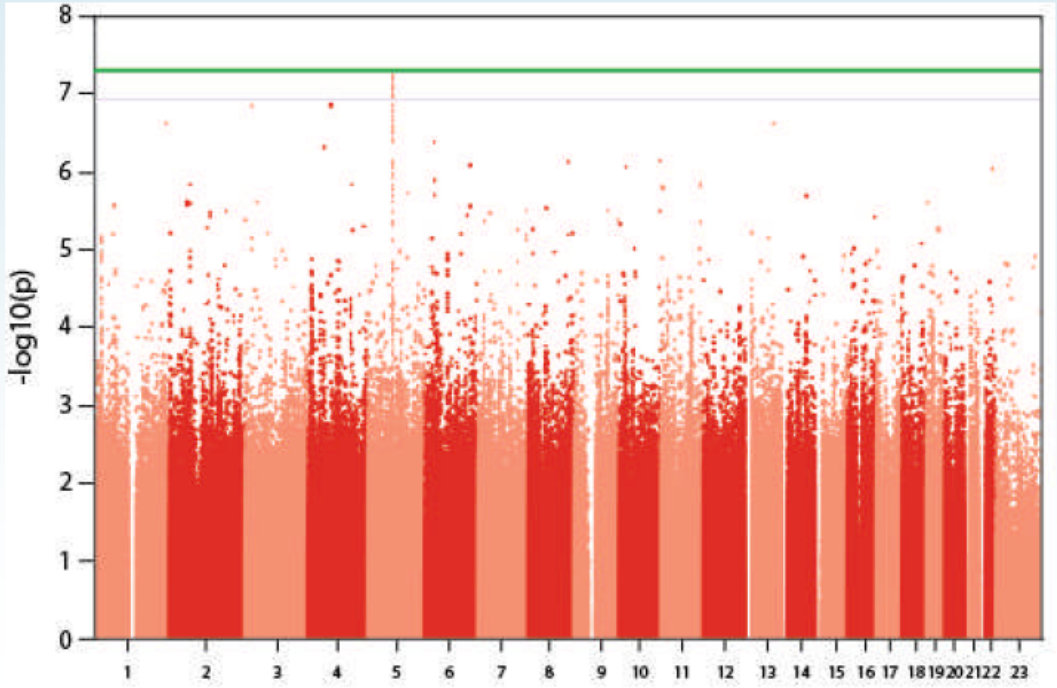


Hensman Moss *et al*, Lancet Neurology in press

Locus on chromosome 5 spanning *DHFR*, *MSH3* and *MTRNR2LR*

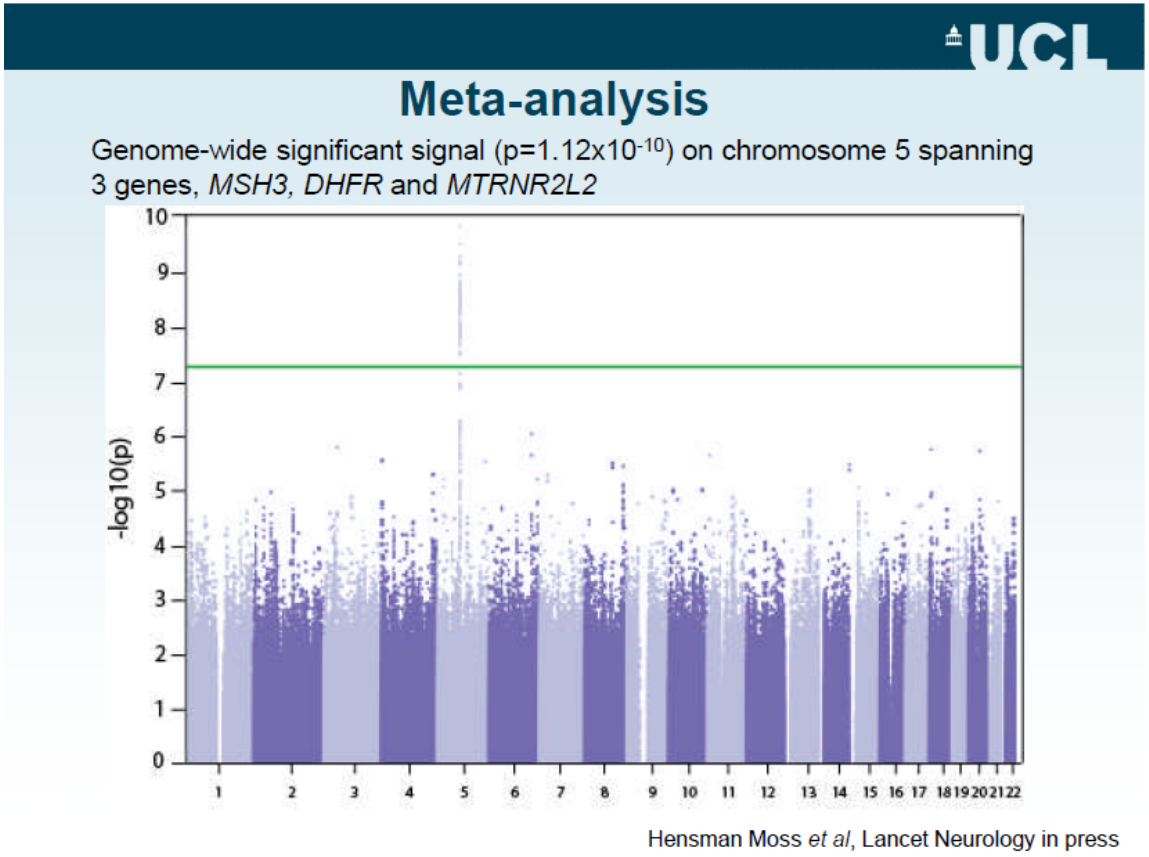
UCL

GWAS using TRACK-HD progression measure as a quantitative trait



Hensman Moss *et al*, Lancet Neurology in press

MSH3 is likely a modifier of rate of progression in HD



- Previously implicated in model systems
- Operating likely through effects on somatic instability

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Can the genetic modifiers identified be used as new drug targets?

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The HD clinical trial landscape: a look back

Cochrane review I: symptom relief

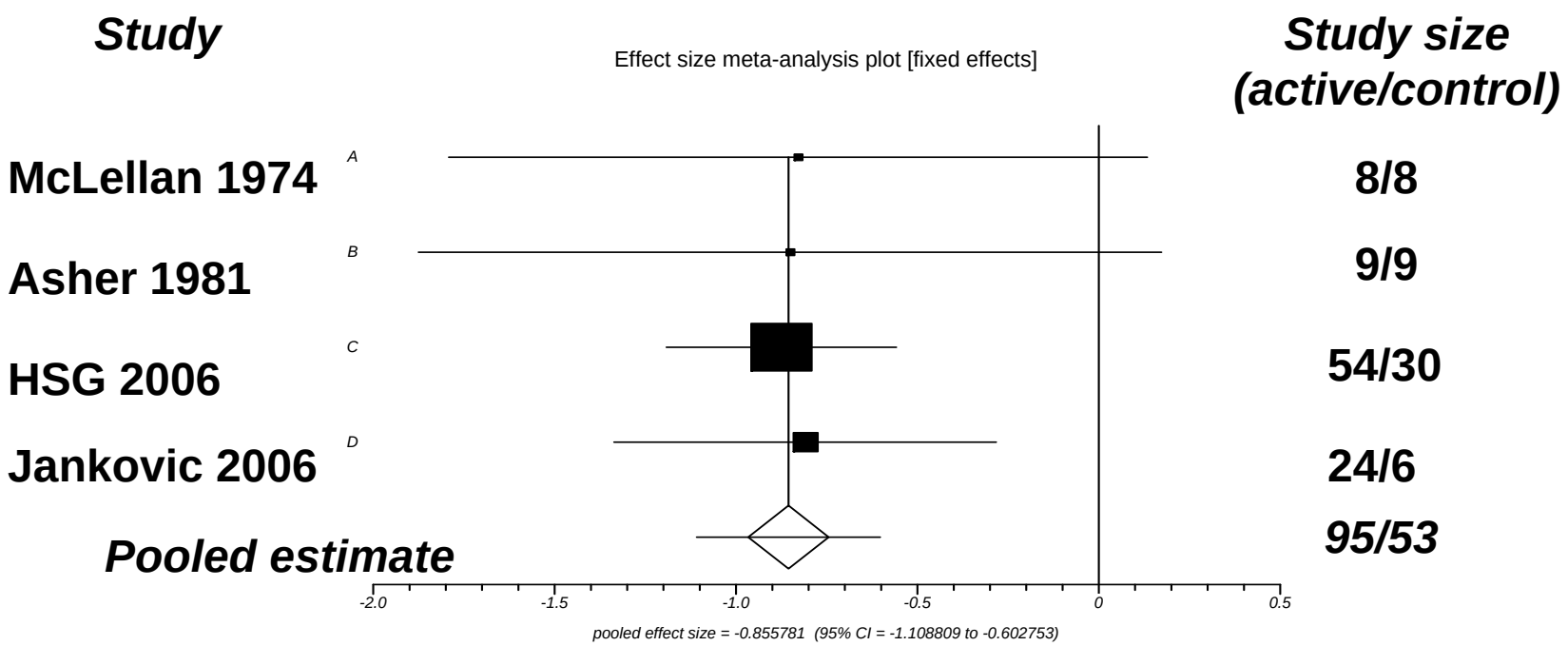
Therapeutic interventions for symptomatic treatment in Huntington's disease (Review)

Mestre T, Ferreira J, Coelho MM, Rosa M, Sampaio C

THE COCHRANE COLLABORATION®

22 trials
(1254 participants)

A treatment to ameliorate chorea Tetrabenazine: RCTs - Forrest Plots



Gaps in knowledge

- Selective serotonin reuptake inhibitors (SSRIs) are the most commonly prescriped drugs in HD worldwide
- Not a single RCT supports this clinical practice
- So far no treatment is available to improve declining cognitive abilities

Cochrane review II: disease progression

Therapeutic interventions for disease progression in Huntington's disease (Review)

Mestre T, Ferreira J, Coelho MM, Rosa M, Sampaio C

THE COCHRANE COLLABORATION®

8 trials
(1366 patients)

Compounds studied

- Riluzol
- Remacemide
- Lamotrigene
- Co-Q10
- Creatine
- Ethyl-EPA

No study met the primary endpoint

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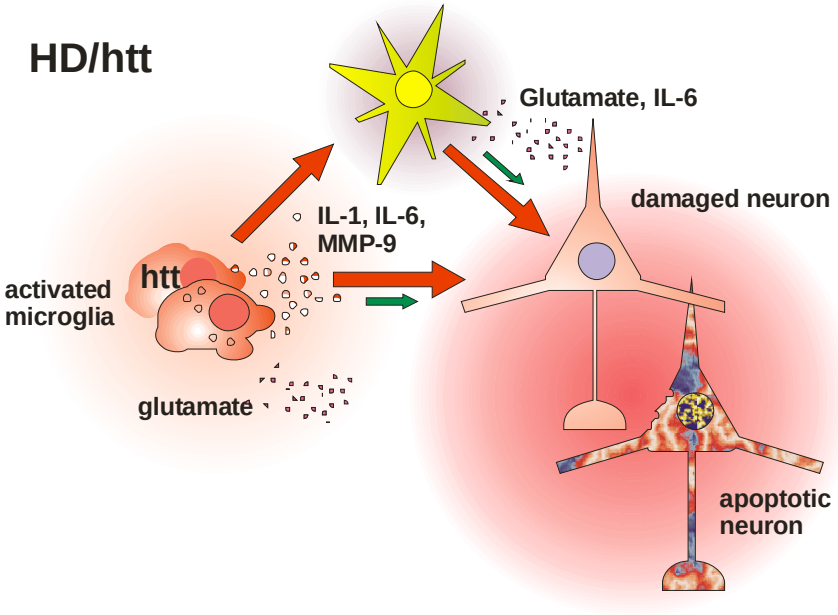
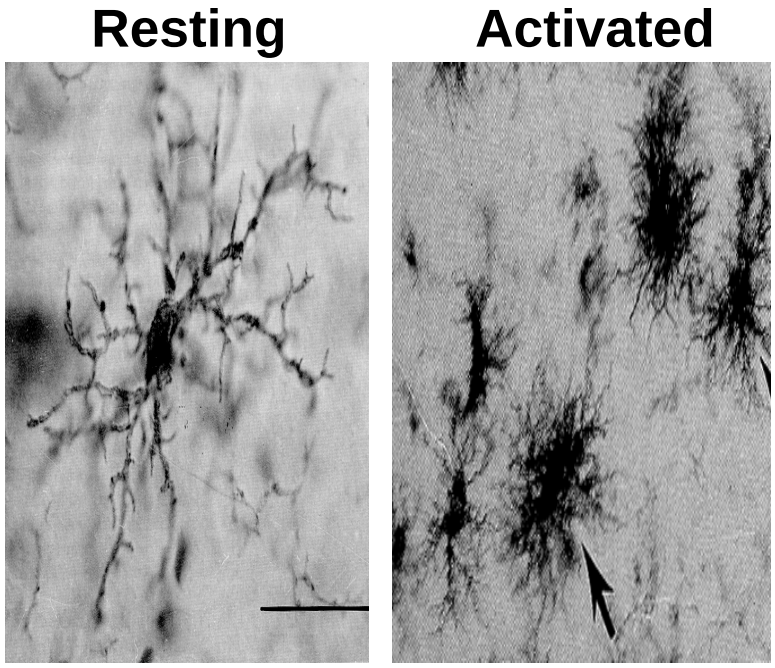
‚The Route to Success is to Go from Failure to Failure with Undiminshed Enthusiasm‘

Winston Churchill

Targeting neuro-inflammation

- Neuroinflammation – a player in HD pathophysio-logy?





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Legato-HD: a Multicenter, Multinational, Randomized, Double Blind, Placebo Controlled, Parallel Group Study to Evaluate the Efficacy and Safety of Laquinimod (0.5 and 1.0 mg/day) as Treatment in Patients with HD

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Pride-HD: a Dose-Range Finding Study Evaluating the Efficacy and Safety of Pridopidine

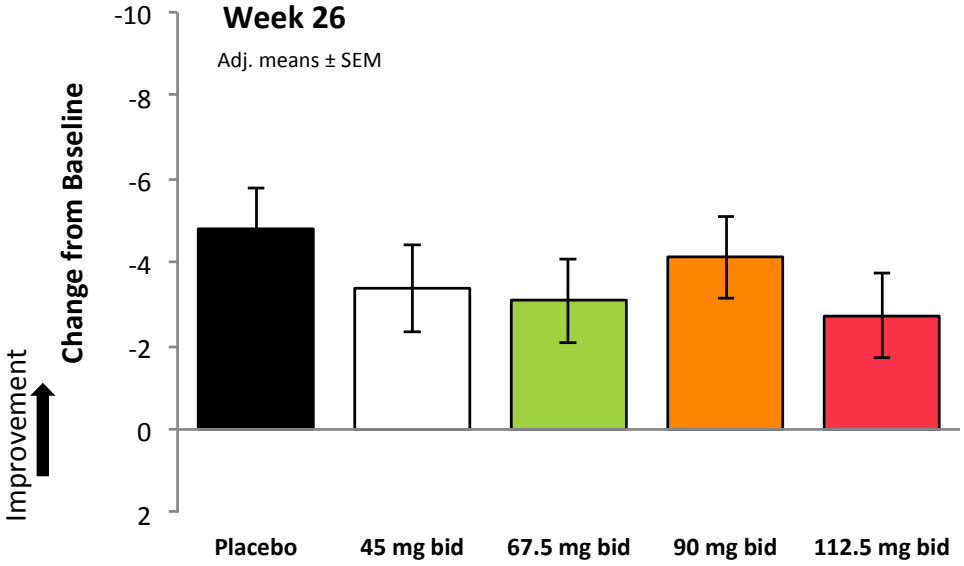
Pride-HD – the questions

- Do higher dosages of pridopidine result in larger effect sizes?
- Are higher dosages well tolerated?
- Can a real-life benefit be demonstrated?
- Is it important how long patients are treated (6 vs 12 months)?

Pride-HD – the answers

- Do higher dosages of pridopidine result in larger effect sizes? NO
- Are higher dosages well tolerated? YES
- Can a real-life benefit be demonstrated? MAYBE
- Is it important how long patients are treated (6 vs 12 months)? YES

Pride-HD - results



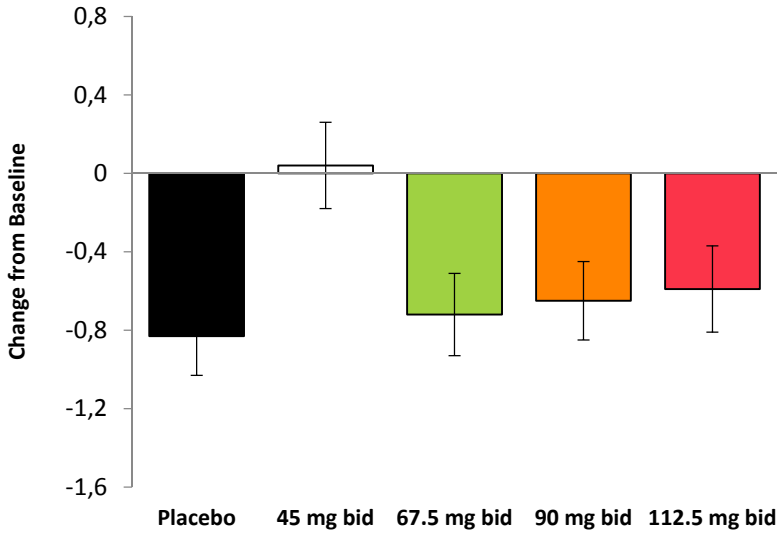
	45 mg bid	67.5 mg bid	90 mg bid	112.5 mg bid
N	75	79	81	81
Wk26 Δ to placebo	1.42	1.71	0.67	2.1
p value	0.3199	0.2235	0.6282	0.1337

Total motor scores (TMS) improved at all dosages INCLUDING placebo

Does the numeric improvement in TMS have functional impact?

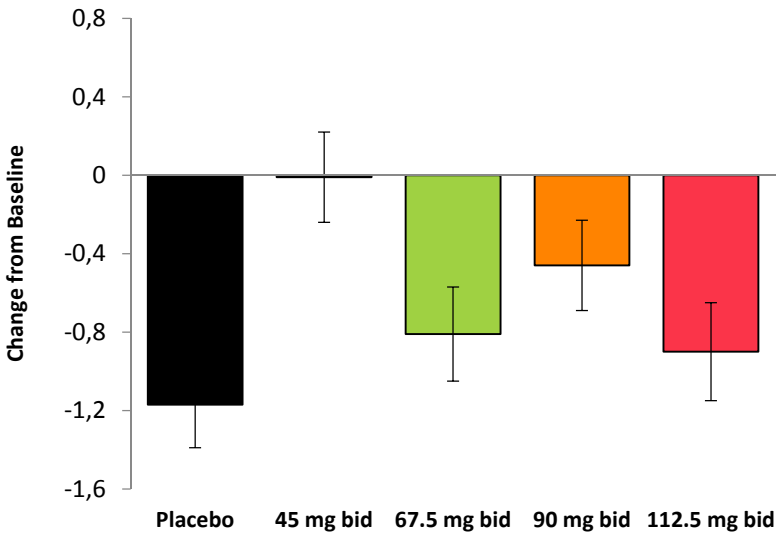
Pride-HD functional impact – TFC after 12 months of treatment

TFC – all participants



	45 mg bid	67.5 mg bid	90 mg bid	112.5 mg bid
N	75	79	81	81
Wk52 Δ to placebo	0.87	0.11	0.19	0.24
p value	0.0032	0.7042	0.5099	0.4061

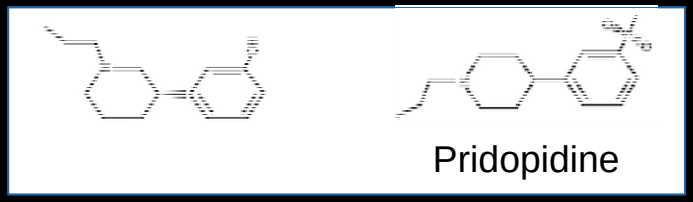
TFC – early stage participants



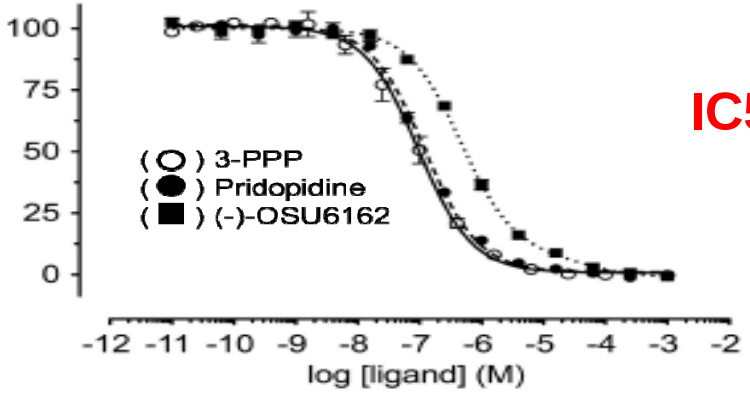
	45 mg bid	67.5 mg bid	90 mg bid	112.5 mg bid
N	59	54	56	58
Wk52 Δ to placebo	1.16	0.36	0.71	0.27
p value	0.0003	0.2704	0.0239	0.4144

Pridopidine is a Sigma-1 receptor (S1R) ligand

Similar in structure to the known S1R agonist (+)-3-PPP



Inhibition of [^{3}H](+)-pentazocine binding

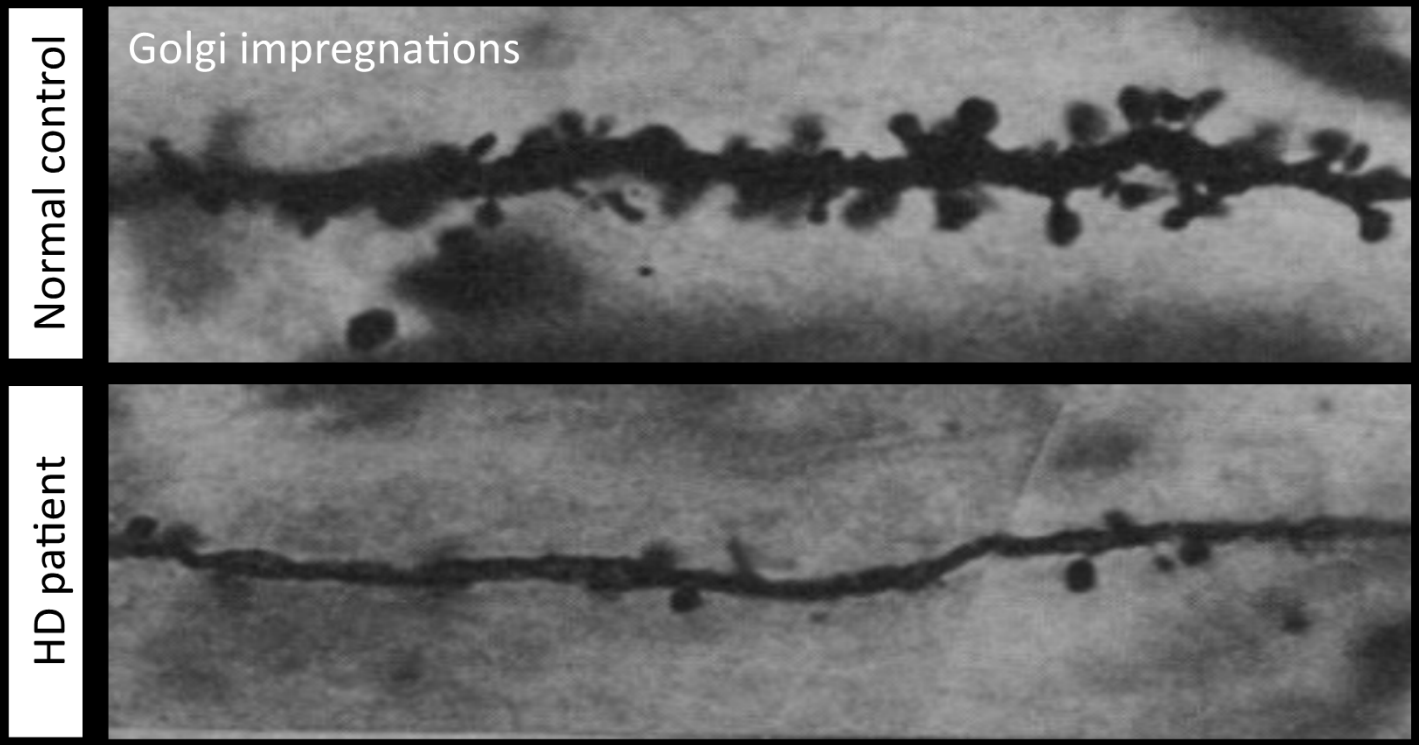


IC50 ~ 100 nM

~100-fold higher affinity for S1R than for D2R (10 µM)

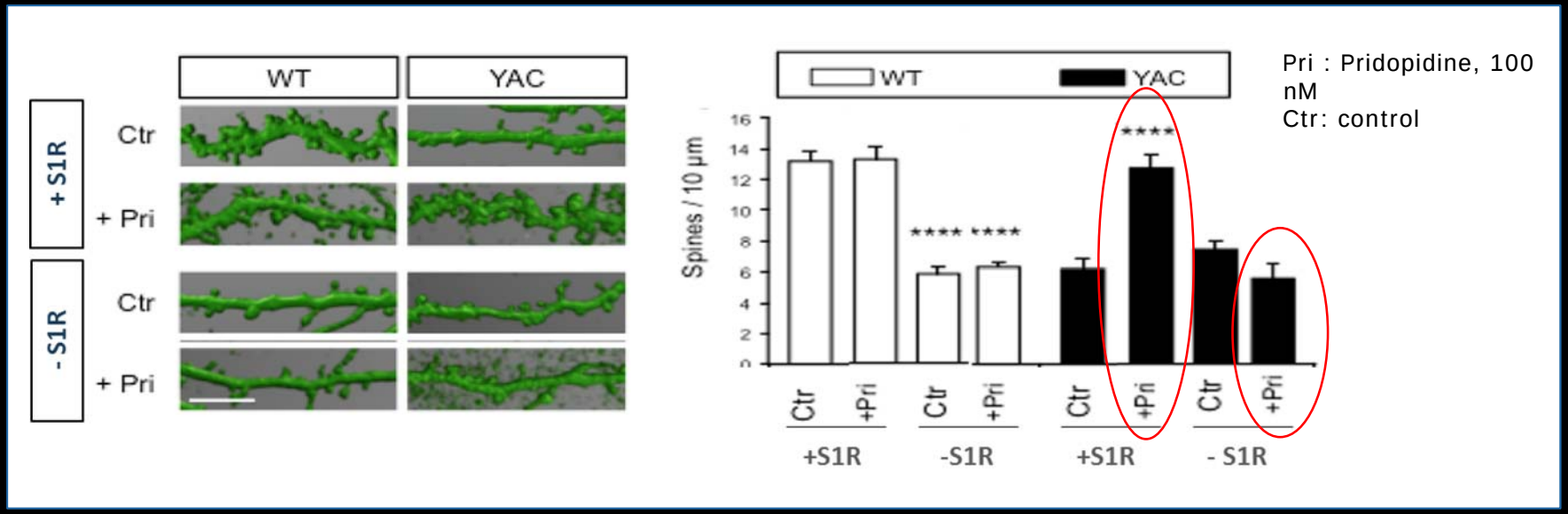
Source: Sahlholm et al Molecular Psychiatry,2013 (2-14), and Teva internal report

Medium spiny neurons (MSNs) have synaptic abnormalities in HD



Source: Graveland et al., (1985) Science

Pridopidine may prevent the loss of spines acting on S1R



- ✓ Pridopidine rescues spine loss in a YAC128 cellular model of HD
- ✓ Knock down of S1R abolished pridopidine's rescue effect

Source: Ilya Bezprozvanny lab (unpublished data)

EUROPEAN **HUNTINGTON'S DISEASE** NETWORK

A phase III trial may start in 2018

EUROPEAN **HUNTINGTON'S DISEASE** NETWORK

A continued effort: reducing the burden of HD by ameliorating disease signs and symptoms

EUROPEAN **HUNTINGTON'S DISEASE** NETWORK

HD is a disease of families – families need social and psychological support to be able to cope with an objective difficult situation

EUROPEAN **HUNTINGTON'S DISEASE** NETWORK

Case managers are crucial to help families to navigate the complex system to get real life access to help and assistance in principle available

EUROPEAN **HUNTINGTON'S DISEASE** NETWORK

Can a HD patient get better by currently available medical treatment?

EUROPEAN **HUNTINGTON'S DISEASE** NETWORK

An unequivocal 'YES'

What we can do

- Low mood can be improved
- Irritability and aggression can get better
- Sleep problems can be fixed
- Lost weight can be regained
- Chorea can be suppressed (to some extent)
- The ability to move around can be improved

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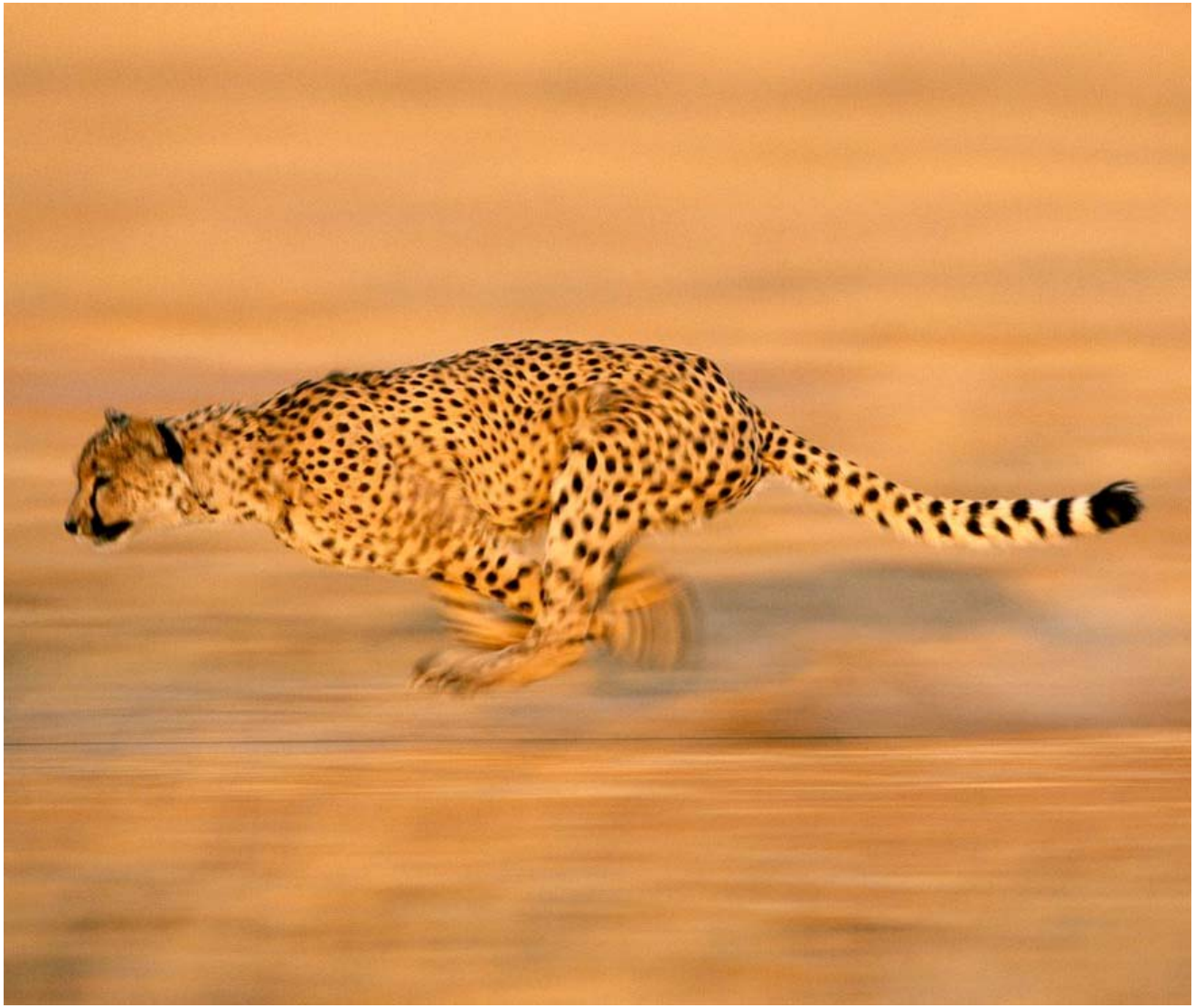
However, there are limits:
all improvements do not last forever
and new problems emerge

EUROPEAN **HUNTINGTON'S DISEASE** NETWORK

To make real advances in HD treatment options we need HD research


STRONGER together

We want to bring HD research up to speed



Working together worldwide to address a disease that effects people all over the world: Enroll-HD

EUROPEAN **HUNTINGTON'S DISEASE** NETWORK

We need to get the balance right:
HOPE & HELP
For today (and tomorrow)

Thank you for your attention!

G. Bernhard Landwehrmeyer