morbus

Huntington (HD)

- a model disease
  -- molecular biology/genetics
  -- predictive testing
    → genetic counselling
  -- treatment strategies
HD brains

CAG 18/47, ♂ 69y
CAG 20/54, ♀ 45y
CAG 17/48, ♂ 42y
control, ♂ 66y

AO: 42
brain 1320g
VS grade 2-3

AO: 36
brain 1030g
VS grade 3

AO: 26
brain 900g
VS grade 3-4

brain 1380g
♀ neurological disorder

atrophy: caudate (CD), pallidum (GP), putamen (Pu)
enlargement: ventricle (V)
HD and rodent models

Petrasch-Parwez

general atrophy

R6/2

WT

mouse

selective atrophy

tgHD

WT

rat

CAG 17/48
Huntingtin aggregates in HD brain

CAG 17/48, ♂ 42y
intranuclear

CAG 20/54, ♀ 45y
neuropil - associated

vibratome sections, 50µm, EM48-DAB immunohistochemistry, cresyl violet
Huntingtin aggregates in HD brain

HD patient
CAG 20/54

tgHD rat
CAG 51

R6/2 mouse
CAG 115-140

vibratome sections, 50µm, EM48-DAB immunohistochemistry

Petrasch-Parwez
Huntingtin aggregates in HD brain

Huntingtin aggregates in HD brain

electron microcopical EM48 immunohistochemistry
Huntingtin aggregates in HD brain: mitochondrial affection

striatal neuron

electron microscopical EM48 immunohistochemistry
animal models for new therapies
Huntingtin cDNA
Polymerase Chain Reaction (PCR)

original allele

amplification

authentic products
direct DNA test for (CAG)\textsubscript{n} expansion: capillary electrophoresis
Huntingtin gene

NNNNN CAG CAG ... CAG CAG CAG NNNNNNNN

Exon 1 -- Exon 2 -- Exon 67

ATG

HZ NRW
trinucleotide block expansion diseases

Morbus Huntington

(CAG)_n
9 36-39 >200

5’UTR 3’UTR

Oculopharyngeal muscle dystrophy

(GCG)_n
6 7-8 13

5’UTR 3’UTR
trinucleotide block expansion diseases type 2

Myotonic dystrophy

5’UTR

3’UTR

5 27-50 >2000+

(CTG)ₙ

6 50-200 >1000+

(CCG)ₙ

FRAX syndrome

5’UTR

3’UTR

6 50-200 >1000+

(CCG)ₙ

7 29-66 >1400+

(GAA)ₙ

Friedreich ataxia

5’UTR

3’UTR
Huntingtin protein

HEAT repeats: tandemly repeated, 37-47 aa in cytoplasmic proteins (huntingtin, elongation factor 3 (EF3), α regulat. subunit of protein phosphatase 2A (PP2A), yeast PI3-kinase TOR1); arrays of HEAT repeats: 3-36 units form rod-like helical structures; protein-protein interaction surfaces involved in intracellular transport.
Huntingtin aggregates
normal neuron
synaptic transmission
axonal transport
excitotoxicity
Huntingtin aggregates normal neuron
Life with HD

neurobiology

clinical status

early: subtle psychomotor dysfunction

late: manifest progressive disease

functional status

chorea

motor impairment

neurobiology

reversible developmental delay

neuronal dysfunction

neuronal death

birth

motor diagnosis of manifest HD

death
*Htt* alleles

(n >9150; `93-`16)
expanded *Htt* alleles
Htt$^+$ aggregates + 
inclusion bodies

normal neuron

(CAG)$_n$/(Gln)$_n$ block expansion
arcane circles: $\text{Htt}^+ \text{ aggregates } + \text{ Inclusion bodies}$
Htt\(^+\) aggregates in tgMH rats: limbic forebrain

51 CAGs, 18 months

extended Amygdala

ventral Striatum

Petrasch-Parwez
Htt\(^{+}\) aggregates in MH: limbic forebrain

54 CAGs, 45 years

ventral Striatum

extended Amygdala

Petrasch-Parwez
Htt$^+$ aggregates in HD: limbic forebrain

→ psychiatric symptoms?
DD: elderly home resident without relatives

DD: \textit{PRNP, HDL2, SCA17} benign chorea (\textit{TITF1 / NKX2A})
predictive DNA test for HD

- direct test [\(\geq 1993\)] >99% definitive
  < 1% „gray zone“

- 1993-2015: >1000 cases (families)

\(\sim 80\%\) direct test
accompany

human genetic counsellors
DNA testing
psychologists
social workers
M.D.‘s (Huntington ward)
St. Josef-Hospital
human genetic counselling

help for individuals to find their right decision

- in autonomy + non-directivity, in accompany

- but also after confrontation with other views
course of genetic counselling

1. session: reflexion >4 weeks (>6 months), psychological accompany
2. blood: test ~4 weeks
3. test result
   ... further meeting(s)
   [clinical examination]
rationale for genetic counselling

- FA+, asymptomatic
- FA+, sympt.
- relatives
- DNA test result communicated
- clin. suspicion
- DNA test+

FA+, asymptomatic
ok or M. Huntington

50% risk

healthy sick
ok or M. Huntington

50% risk → 100% certainty

[Diagram showing a scale from 0 to 100, with images labeled 'Kontrolle' (healthy) on the left and 'Huntington' (sick) on the right, indicating a transition from 50% risk to 100% certainty.]
reasons...

...against testing

...for testing
incertitude
predictive counselling HD

50-70% of persons at risk refrain from DNA testing (5% refuse to obtain result after DNA test)
asking for predictive HD test
predictive counseling HD

take up of result
special conflicts

- counsellees with 25 % risk

⇒ parent 'suffers' from DNA test result as well

- pregnancy
Huntingtin gene htt

≥40 CAG HD allele
36-39 CAG HD allele with ↓ penetrance
27-35 CAG normal, premutation
<27 CAG normal
HD: $(\text{CAG})_n$ expansion vs. age at onset

- **Penetrance↓**
- **Normal**
- **Complete penetrance**

![Graph showing the relationship between CAG expansion and age at onset.](image)
HD: polyglutamin disease

(CAG)$_n$ \xrightarrow{\text{translation}} (Gln)$_n$

„normal“

„pathogenic“
(intranuclear) inclusion bodies

normal neuron

(CAG)$_n$/((Gln)$_n$ block expansion
Caspase digest + ubiquitination
HD: dysregulated gene expression

Serendina, Luthi-Carter 2012
Huntingtin

González-Couto 2011
Aberrant splicing of *HTT* generates the pathogenic exon 1 protein in Huntington disease

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Fig. 1. Aberrant splicing of *Htt* exon 1 to exon 2 results in a short polyadenylated mRNA in the *Hdh*Q150 knock-in mouse model. (A) Schematic representation of the mouse *Htt* gene and primers used for the RT-PCR analysis. \(\bullet\), cryptic polyadenylation signal; \(\bigcirc\), end of overrepresented intronic sequences. (B) RT-PCR analysis of exon 1, the exon 1-intron 1 boundary, intron 1, and exon 2. (C) 3' RACE product was generated from *Hdh*Q\textsuperscript{150}Q\textsuperscript{150} and *Hdh*\textsuperscript{+/Q150} brain RNA (\(\bullet\)), but not from WT controls, and contained a polyA tail located \(\sim\)700 bp into intron 1. The cryptic polyadenylation signal is underlined, the polyA tail is shown in bold, the primer sequence is in italics, and vector sequence is in lowercase. (D) RNA-Seq reads from cortex of 22 mo *Hdh*Q\textsuperscript{150}Q\textsuperscript{150} and WT mice mapping to the *Htt* exon 1-exon 2 region. M, low-molecular-weight marker (New England Biolabs); W, water.
functional significance of HD-related gene expression changes

synopsis: data integration
modifier genes’ influence course of HD
modifier genes’ influence course of HD

modifying sequence variations

*GRIK2, APOE, TCERG1, UCHL1, TP53, DFFB, GRIN2A, GRIN2B, HAP1, ASK1, MAP2K6, PGC1α, CNR1*
Pathogenesis

- allele-specific HD RNA knock-down

b) Pathological mechanisms

- Caspase antagonists
- NMDA antagonists
- Antioxidants
- HDAC inhibitors

Mutant huntingtin protein

Polyglutamine

- HSPs
- Antibodies

Proposed mechanisms of toxicity of the Huntington's disease (HD) gene and potential therapeutic targets
gene therapy HD

chem. modified ssRNA
unmodified ssRNA
duplex RNA

RNA-induced silencing complex
passenger strand removal
selective suppression of mutated HTT
preserved expression of normal HTT
therapy ?