The Role of Brain Development in Huntington’s Disease

Peg Nopoulos, M.D.

Paul W. Penningroth Professor of Psychiatry
Professor of Psychiatry, Neurology and Pediatrics
DEO and Department Chair, Department of Psychiatry
Carver College of Medicine
University of Iowa, Iowa City, IA
Take home points

• The Huntington Gene, Huntingtin (HTT) is important for human brain development
  ➢ For repeats UP TO roughly 45, this is advantageous meaning the more repeats, the better the brain development
  ➢ For repeats greater than 45, this is detrimental meaning the more repeats the worse the brain development

• Human brain development is a prolonged process
  ➢ Maturational processes occur through 30 years of age

• Implications for our community: gene therapy is at our doorstep
  • Currently designed for evaluating those who are already manifest, slowing disease progression
  • If successful, next step will be to administer therapy to preHD subjects to prevent disease

• Knocking down a gene involved in brain development needs to be considered with an abundance of caution

• The most important concept is conceptualization of the effects of HTT on a spectrum – from 10 through 100, from below to above disease threshold
Outline

• Effects of the Huntington Gene (HTT) on Normal Brain Development
  - Implications for understanding the evolution of the human brain

• Effects of the HTT on Disease Pathology
  - Developmental aspects of a degenerative disorder
  - What happens in Juvenile Onset or Pediatric HD?
  - Distinction between development and degeneration
  - Implications for Gene Therapy

• Next Steps
Outline

- **Effects of the Huntington Gene (HTT) on Normal Brain Development**
  - Implications for understanding the evolution of the human brain

- **Effects of the HTT on Disease Pathology**
  - Developmental aspects of a degenerative disorder
  - What happens in Juvenile Onset or Pediatric HD
  - Distinction between development and degeneration
  - Implications for Gene Therapy

- **Next Steps**
The Amazing Human Brain
What were the genetic mechanisms that drove this evolutionary change?

- Human brain 3 times the size of the chimpanzee – nearest living relative
- *Increase in intelligence*
The genes governing intelligence (IQ) are likely the genes that were key to human brain evolution.

General IQ is considered to be one of the most heritable behavioral traits.

- NO STUDIES to date of identified and replicated any gene, or gene variant that significantly relates to intellect (Chabris et al, 2012).
- One GWAS report showed gene variants related to educational attainment (a proxy for IQ) but the effect size was tiny – accounting for 0.02% of the variance in IQ (Rietveld et al, 2013).

So – the search for genes involved in human brain evolution continue.
Evolution: Two Primary Concepts

• Positive selection
  ➢ Genes that lead to ‘advantageous’ traits are more likely to get passed on to next generation (survival of the fittest)

• Variation of Phenotype is needed
  ➢ With more variation, it is more likely that there are certain individuals that are more ‘adaptive’ to change, are more fit, are more likely to survive
• Spontaneous Mutation
  ➢ Binary change – mutated vs. not mutated; 2 phenotypes

• A better way to create variation?
  ➢ Over ½ of the genome involved repetitive DNA sequences. Called *The Repeatome*
    ➢ Considered the “dark matter” of the genome
      ➢ Under-studied, poorly annotated, functionally mysterious
    ➢ One component = Short tandem repeats or STRs
      ➢ 1-6 base pair motifs of DNA
      ➢ Make of 3% of genome
      ➢ Genes with TRIPLET REPEATS have become of high interest
• **Evolutionary Biologists**: Genes with triplet repeats may be ‘advantageous mutators’ that can serve as evolutionary tuning knobs
  - Provide a wide range of genetic (phenotypic variance) by which natural selection can act
But what about the normal variation. **Is this variation meaningful?**

*Could triplet repeat genes BELOW DISEASE THRESHOLD act as digital genetic modulators, increasing the levels of phenotypic variability in a population?*

**Huntington’s Gene: Huntingtin (HTT)**

- Develop a good brain
- Develop a higher functioning brain
- Develop the highest functioning brain

Never before studied in humans
Expected Relationship

Develop a good brain

Develop a higher functioning brain

Develop the highest functioning brain

IQ

# of CAG Repeats
Outline

• **Effects of the Huntington Gene (HTT) on Normal Brain Development**
  - Implications for understanding the evolution of the human brain

• **Effects of the HTT on Disease Pathology**
  - Developmental aspects of a degenerative disorder
  - What happens in Juvenile Onset or Pediatric HD?
  - Distinction between development and degeneration
  - Implications for Gene Therapy

• **Next Steps**
Developmental mechanisms in the pathogenesis of neurodegenerative diseases

Mark F. Mehler *, Solen Gokhan

Laboratory of Developmental and Molecular Neuroscience, Departments of Neurology, Neuroscience and Psychiatry,
Rose F. Kennedy Center for Research in Mental Retardation and Developmental Disabilities, Einstein Comprehensive Cancer Center,
Divisions of Growth Control and Neurooncology, Albert Einstein College of Medicine, Bronx, NY 10461, USA
**Classical Concept: Gain of Function**

Normal Neuron → Disease process (toxic mHTT) → Degeneration → Cell Death

**Developmental Concept: Loss of Function**

Disease process is abnormal development → mutant steady state made possible by compensation → Degeneration → Cell Death

- Maturational Processes
- Aging
- mHTT
What about ABOVE Disease Threshold

- Below disease threshold – possible advantageous effects of CAG
- Could mutant HTT (CAG>40) have adverse effects on brain development?

CAG repeat length

Possible advantage of increasing repeats

Possible disadvantage of increasing repeats?
Above disease threshold is a "dose effect"

Longer CAG repeat → earlier HD onset

Longer repeat = more severe mutation
Effects of *mHTT* Above Disease Threshold

- **Minor adverse effects on development**
  - Repeat = 40 = mild developmental aberration
    - Excellent compensation through childhood

- **Moderate adverse effects on development**
  - Repeat = 50 = moderate developmental aberration
    - Compensation ok but there are some signs of poorer function (i.e. lower IQ)
    - Compensation fails earlier (earlier onset)

- **Major impact on brain development**
  - Repeat = 60 = severe developmental aberration
    - Even lower IQ
    - Earliest onset – in childhood

<table>
<thead>
<tr>
<th># of CAG Repeats</th>
<th>IQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td></td>
</tr>
</tbody>
</table>
What to expect for ENTIRE SPECTRUM

Hypothesis of Effect of CAG Repeats on IQ

Below Disease Threshold
- Develop a good brain

Above Disease Threshold
- Minor adverse effects on development
- Moderate adverse effects on development
- Major impact on brain development

What to Expect for ENTIRE SPECTRUM

IQ

# of CAG Repeats

Brain Function

Deficient

Superior

Advantage

Disadvantage

Number of Repeats

10 100
The Kids-HD Program

• Study of children at risk for HD
  • Funded by NINDS 2009-2019 (CHDI 2010-2015)

• Subject 6-18 years of age (a small sample 19-25)

• At risk for HD: must have a parent (or grandparent) with HD

• HD families flown in from all over the country

• Healthy Controls (HC) with no family history of HD

• Brain Function = cognitive and motor tasks / Brain Structure = MRI

• All subjects donate DNA for genotype
  ➢ *For research purposes ONLY*
  ➢ CAG <39 = gene non-expanded (GNE)
  ➢ CAG ≥ 40 = gene expanded (GE)

• No juvenile Onset or Pediatric HD
# The Kids-HD Program

## Table 1: Demographics of Sample

<table>
<thead>
<tr>
<th></th>
<th>At Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gene Expanded (GE)</td>
</tr>
<tr>
<td>Number of individuals</td>
<td>81</td>
</tr>
<tr>
<td>Additional (return) visits</td>
<td>58</td>
</tr>
<tr>
<td>Total observations</td>
<td>139</td>
</tr>
<tr>
<td>Female/Male</td>
<td>48/33</td>
</tr>
<tr>
<td></td>
<td>Mean (s.d.)</td>
</tr>
<tr>
<td></td>
<td>Range</td>
</tr>
<tr>
<td>Age (years)</td>
<td>13.35 (4.45)</td>
</tr>
<tr>
<td></td>
<td>6-25</td>
</tr>
<tr>
<td>CAG repeat</td>
<td>46.42 (5.54)</td>
</tr>
<tr>
<td></td>
<td>40 - 60</td>
</tr>
</tbody>
</table>
Study Sample

Our sample has high % of ‘high CAG’ (>50)
- PREDICT = 1.5% > 50, 19% > 45
- Clinic = 14% > 50, 28% > 45
- Kids-HD = 25% > 50, 52% > 45

Modeling Development (Not degeneration)
- Mean Estimated Years to onset is >40 years

Motor scores same in GE and GNE
- UHDRS shows normal motor development
Effects of *HTT* on IQ over entire spectrum

### Intelligence

- Number of CAG repeats directly predicts intelligence (IQ) in a non-linear fashion.
- CAG repeats are beneficial up to a certain point, around 40.
- After this zenith, increasing repeats have a negative effect on intelligence with the longest repeat lengths having the lowest IQ scores
  - NOT due to degeneration

---

**Results: IQ**

F = 6.63, p = 0.01 CAG*CAG

Model controls for age, sex

No Sex*CAG interaction

---

Lee et al, 2017 EBiomedicine
Replication in Adults

N=657 preHD

- Age <30, TMS <10; Estimated YTO >15
- No history of depression

### Results

**Symbol Digit Modalities**

- **p < 0.001**

**Trails B**

- **p < 0.01**

**Stroop Color Naming**

- **p < 0.05**

**Stroop Word Reading**

- **p > 0.001**
Replication in Adults

N=657 preHD
- Age <30, TMS <10; Estimated YTO >15
- No history of depression

**POSTER #78**
How do we Model Brain Development?

Primer on normal brain development

Post-natal ‘school age’ 6-25 years of age

- Total brain volume by 6 years of age is 95% of adult
- Very little change in brain volume between 6 and 30
How do we Model Brain Development?

Primer on normal brain development

Post-natal ‘school age’ 6-25 years of age

• However, there is SUBSTANTIAL tissue remodeling
  ➢ Gray Matter pruning – programmed synaptic elimination
  ➢ White matter volume growth – myelination of tracks
Trajectories of Development

- Accelerated Longitudinal Design
- Gold Standard for evaluating brain development
- NOT a typical time 1-time 2 longitudinal study
- All observations used to model the trajectory of change over the age range
  - Model accounts for the fact that some visits are repeats of the same person
Developmental Trajectory Verbal Fluency

- Name all of the words you can think of starting with letter ‘C’
- PEAKS near age 30
- Frontal lobe function
  ➢ Brain develops from posterior to anterior

Verbal Fluency Score

Age (yrs)
Developmental Trajectory Verbal Fluency

- CAG repeat effect
- Repeats between 38 and 42 show continued development of this skill
- Repeats 44 and above show developmental blunting of this skill

Verbal Fluency

Age (yrs)

Jordan Schultz
Trajectory of Development: Striatum

**Green line:** normal striatal development in GNE children. Slow growth in volume until peak around 14, then decrease with puberty (pruning).

**Red Line:** GE children begin with striatal hypertrophy. Likely due to earlier development in infancy. Steady decline in volume. Markedly different developmental trajectory.

Van der Plas et al, Neurology 2019
• Above 50 repeats, there was a significant CAG repeat effect where the longer the repeat, the higher the volume early in life and the steeper the slope of change.

Van der Plas et al, Neurology 2019
The Kids-HD Program

- So if the striatum is abnormal, why are these kids not having any symptoms?

What other parts of the brain might be responsible?
Invokes one of the most important advances in neuroscience: circuitry

Disease process is abnormal development

Maturational Processes
Aging
mHTT

mutant steady state
made possible by compensation

Degeneration

Cell Death
• Motor abnormality in HD is CHOREA
  • Involuntary movements
• Direct pathway
  • Promotes movement
• Indirect pathway
  • Inhibits movement
• Primary pathology is in the indirect pathway
  • Loss of inhibition = involuntary movements
• Work by Peter Strick
  • Cerebellum is connected to the striatum through the indirect pathway
  • Work done in monkeys using virus as tracers

• Soooooo
  – If the indirect pathway is ‘sick’ or under-developed and is vulnerable to becoming disinhibited = chorea
  – Could the cerebellum – through its integration with this pathway – compensate?

Integration with Cerebellum

Direct: promotes movements
Indirect: inhibits movement

Motor Cortex

Direct Pathway
Indirect Pathway

Striatum

Globus Pallidus (GP)
Sub-Thalamic Nucleus (STN)

Thalamus

Globus Pallidus (GP)

Dentate Nucleus

Cerebellum

Pons
• Theory – abnormal growth of the striatum is compensated for by the cerebellum
• The abnormal growth of the striatum is compensated for by the cerebellum.

• Whhaaaa? The cerebellum involved in HD?
  - the one area of the brain discussed the least in all literature
  - considered to be ‘spared’ by HD disease pathology
Evaluation of Circuitry

- Resting State Magnetic Resonance Imaging – rsMRI
  - Subject rests in the scanner for several minutes
  - BOLD signal = ‘blood flow’ or activity

- Areas that ‘oscillate together’ are considered to be functionally connected
Evaluation of Circuitry

- Is the cerebellum circuitry stronger in GE than GNE?

Seed the Dentate Nucleus (output of Cerebellum) In GNE

Seed the Dentate Nucleus (output of Cerebellum) In GE

Subtract the images – if blue color is left, then the GE connections are stronger than the GNE.
Evaluation of Circuitry

• Group analysis: seed placed in right dentate

• Blue = GE has STRONGER connection compared to GNE
Evaluation of Circuitry

- Group analysis: seed placed in right dentate
- Blue = GE has STRONGER connection compared to GNE
- Target areas shown in red

The cerebellum is hyper-connected in GE children
Evaluation of Circuitry

- Group analysis: seed placed in right dentate
- *Blue = GE has STRONGER connection compared to CC*
- Target areas shown in red

The cerebellum is hyper-connected in GE children
Trajectory of Development: Striatum

- Trajectory of Striatal-cerebellar connectivity
- Like hypertrophy of striatum, the hyper-connectivity is highest early in life in the GE subjects and declines over time
- CAG repeat effect also recapitulates what is seen in striatal volume -
  - the higher the repeat, the higher the connectivity early in life and the steeper the slope of change
Trajectory of Development: Striatum

- Trajectory of Striatal-cerebellar connectivity
- Like hypertrophy of striatum, the hyper-connectivity is highest early in life in the GE subjects and declines over time
- CAG repeat effect also recapitulates what is seen in striatal volume -
  - the higher the repeat, the higher the connectivity early in life and the steeper the slope of change
Outline

• **Effects of the Huntington’s Gene (HTT) on Normal Brain Development**
  ➢ Implications for understanding the evolution of the human brain

• **Effects of the HTT on Disease Pathology**
  ➢ Developmental aspects of a degenerative disorder
  ➢ *What happens in Juvenile Onset or Pediatric HD?*
  ➢ Distinction between development and degeneration
  ➢ Implications for Gene Therapy

• **Ability paired with Liability**
Neurodevelopmental Approach

A. Classic Concept

- Classic concept of HD is a neurodegenerative disease of the striatum
  - Normal development, toxic gain of function/mutant protein

B. Developmental Concept of Adult Onset Disease

- Developmental theory AOHD
  - *HTT* is vital for brain development
  - *mHTT* causes abnormal development; initial compensation
    - Mutant steady state through childhood
    - AOHD when compensation fails

C. Developmental Concept of Juvenile Onset Disease

- Developmental concept JOHD
  - Severe developmental aberration
  - Unable to compensate
  - Symptoms present as child

- Juvenile Onset HD (JOHD)
  - Diagnosis of HD made prior to 20 years of age
  - Pediatric HD = children up to age 18 who are currently diagnosed with HD
### JOHD Sample

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=234)</th>
<th></th>
<th>JHD (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (s.d.)</td>
<td></td>
<td>Mean (s.d.)</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td></td>
<td>Range</td>
</tr>
<tr>
<td>M:F</td>
<td>114:120</td>
<td></td>
<td>6:10</td>
</tr>
<tr>
<td>Age</td>
<td>12.5 (3.8)</td>
<td>6-23</td>
<td>14.0 (5.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5-23</td>
<td>5-23</td>
</tr>
<tr>
<td>CAG</td>
<td>20.2 (4.0)</td>
<td>11-34</td>
<td>73.9 (11.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11-34</td>
<td>54-96</td>
</tr>
<tr>
<td>Disease Duration</td>
<td>-</td>
<td></td>
<td>2.8 (2.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.2-8.4</td>
</tr>
</tbody>
</table>

Expressed as ICV ratio. Linear model corrected for age, sex and scanner.
JOHD Brain Structure

Expressed as ICV ratio. Linear model corrected for age, sex and scanner.

Z-score

Larger than HC

Smaller than HC

ICV

Cerebral White

Cortical Gray

Caudate

Putamen

Globus Pallidus

Thalamus

Cerebellum

CONTROL MEAN

Sig.

*** <0.001

**  <0.01

*  <0.05
Theoretical Construct of Abnormal Growth in Circuitry

Integrated Circuitry

CAG = 10 - 39
Normal Function

CAG = 40 - 59 (AOHD)
Abnormal development with initial cerebellar compensation, onset of symptoms in adulthood

CAG > 60 (JOHD)
- Severe abnormal development;
- Cerebellum proportionately enlarged
- Compensation fails early – childhood onset
• Effects of the Huntington Gene (HTT) on Normal Brain Development
  ➢ Implications for understanding the evolution of the human brain
• Effects of the HTT on Disease Pathology
  ➢ Developmental aspects of a degenerative disorder
  ➢ What happens in Juvenile Onset or Pediatric HD?
  ➢ Distinction between development and degeneration
  ➢ Implications for Gene Therapy
• Next Steps
Neurofilament Light (NfL)

- NfL is NOT elevated in GE children until roughly 15-20 years prior to disease onset

- NfL is elevated in JOHD

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Plasma NfL, mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>GNE</td>
<td>1.45 ± 0.10</td>
</tr>
<tr>
<td>40-60 YTO</td>
<td>1.48 ± 0.16</td>
</tr>
<tr>
<td>30-40 YTO</td>
<td>1.38 ± 0.13</td>
</tr>
<tr>
<td>20-30 YTO</td>
<td>1.45 ± 0.20</td>
</tr>
<tr>
<td>15-20 YTO</td>
<td>1.79 ± 0.14</td>
</tr>
<tr>
<td>&lt;15 YTO</td>
<td>2.26 ± 0.27</td>
</tr>
<tr>
<td>JHD</td>
<td>3.29 ± 0.18</td>
</tr>
</tbody>
</table>
Outline

• Effects of the Huntington Gene (HTT) on Normal Brain Development
  ➢ Implications for understanding the evolution of the human brain

• Effects of the HTT on Disease Pathology
  ➢ Developmental aspects of a degenerative disorder
  ➢ What happens in Juvenile Onset or Pediatric HD?
  ➢ Distinction between development and degeneration
  ➢ Implications for Gene Therapy

• Next Steps
Developmental Trajectory
Verbal Fluency

- Knock-down of genes for prevention of disease
- Important to consider
  - CAG repeat
  - Age at time of intervention
Outline

• Effects of the Huntington Gene (HTT) on Normal Brain Development
  ➢ Implications for understanding the evolution of the human brain

• Effects of the HTT on Disease Pathology
  ➢ Developmental aspects of a degenerative disorder
  ➢ What happens in Juvenile Onset or Pediatric HD?
  ➢ Distinction between development and degeneration
  ➢ Implications for Gene Therapy

• Next Steps
Kids HD

ChANGE HD
SUCCESSFUL COMPETITIVE RENEWAL OF KIDS-HD

Funded by the National Institute of Neurologic Disease and Stroke (NINDS)

Approved project period 12/1/2019 - 6/30/2024

Eligible participants:
- Young people at risk for HD
- Ages 6-30

Rationale: Replicate findings with larger sample AND model development through age 30
New for ChANGE HD

• Computerized neurocognitive assessment (NIH Toolbox)
• Harmonized MRI protocol across sites
• Blood sample for analysis and biobanking
• Sensitive fine motor evaluation using Q-Motor and Q-Cog technologies (Ralf Reilmann, Germany)
• Collaboration in research on Neurofilament Light (NfL) as a biomarker in pre-manifest HD (Ed Wild, UK)
• At-risk young people ages 6 to 30, annual visits
New for **ChANGE HD**

- The University of Iowa – Iowa City, IA
- Penn Medicine and the Children's Hospital of Philadelphia – Philadelphia, PA
- Columbia University Medical Center – New York, NY
- The University of Texas Health Science Center at Houston – Houston, TX
- UC Davis Medical Center – Sacramento, CA

Five sites!
Philadelphia
University of Pennsylvania
Children’s Hospital of Philadelphia (CHOP)

Pedro Gonzalez-Alegre, MD, PhD
Jeffrey Berman, PhD
Jillian Lebus, MA
Lisa Blakely, PhD

Timothy P.L. Roberts, PhD
New York: Columbia

Hiral Shah, MD

Sachin Jambawlikar, PhD
University of Texas Houston

Erin Stimming, MD

Refaat Gabr, PhD
THANK YOU: Nopoulos Lab

Special Thank you to All the kids and families that have participated in the past decade!!!
THANK YOU: Nopoulos Lab

QUESTIONS?

Special Thank you to All the kids and families that have participated in the past decade!!!
### Table 2: Predictors of Age of Motor Onset (AMO)

<table>
<thead>
<tr>
<th>Predictor</th>
<th>β</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caudate</td>
<td>+0.334</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cerebellum*</td>
<td>+0.205</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

N=213 participants who converted from preHD to motor onset during the study

Linear regression model used to predict Age of Motor Onset (controlling for total brain volume, age, sex, and CAP (CAG*age Product)

Volume of the caudate predicted AMO after controlling for baseline caudate volume, cerebellum volume remained significant in predicting AMO, suggesting that larger cerebellar volumes are a predictor of AMO above and beyond what the caudate predicts — the larger the cerebellum, the later the AMO.

This suggests that the cerebellus is compensatory, neuroprotective, only only when this compensation fails, symptoms begin
Circuitry

- Theory – abnormal growth of the striatum is compensated for by the cerebellum

Mutant Steady State (GE)
Cerebellum input to indirect pathway quells movement

Cerebellar compensation fails – indirect pathway now unable to balance direct pathway....involuntary movements, motor onset
Trajectory of Development: Striatum
Huntingtin ($HTT$)

- Possible mechanism for variation of phenotype
  - PolyQ acts as a flexible hinge allowing the flanking domains to come into close spatial proximity*
  - Conformational changes are directly related to degree of flexibility

PolyQ acts as a FLEXIBLE HINGE where increasing length confers increasing optimization of protein conformation

Above disease threshold, PolyQ is a RUSTY HINGE

*Caron et al., PNAS 2013