Magnetic resonance spectroscopy (MRS) and neurodevelopment: applications to HIV infection and exposure in children

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As the brain undergoes healthy maturation, significant changes in structure, function and metabolism occur.

Deviations from healthy maturation may indicate neurodevelopmental delays or disorders.
A well-characterized cohort of children: HIV-infected, HIV-exposed, uninfected and HIV-unexposed, uninfected children were scanned longitudinally.
Magnetic resonance spectroscopy (MRS) identifies and quantifies biochemical information about tissues in the form of a spectrum.

Different biochemicals, or metabolites, present unique information about brain health - such as neuronal integrity, cellular density, and neurotransmission - in localized regions.
MRS and neurodevelopment

How is MRS useful in examining questions related to neurodevelopment?

- Metabolite levels have been found to correlate with neurological development and cognitive measures.

- Changes in normal $^1$H MRS spectrum are observed in many neurological disorders - alterations in metabolite levels may precede observable changes to brain structure or cognition.
MRS and neurodevelopment

Metabolite levels as biomarkers of neurodevelopment.

**N-acetylaspartate (NAA)**

NAA levels increase with age in children, with the steepest increases observed in infancy and early childhood.

NAA observed to decrease in disease → indicating loss or damage to neuron populations, axons, dendrites and synaptic terminals.
Choline/phosphocholine/glycerophosphorylcholine (Cho) levels are highest in infancy, and remain relatively constant in childhood.

Cho levels increase/decrease in disease → increased Cho levels imply glial proliferation/inflammation or increased cellular membrane breakdown; decreased Cho levels suggest overall cell loss.
As a result of prevention of mother-to-child transmission programs, mother-to-child transmission rates have declined globally.

In South Africa, 95% of HIV-positive pregnant women and 68% of HIV-exposed infants have been receiving antiretroviral therapy (ART).

New, growing population of HIV-infected children on ART as well as HIV-exposed, uninfected (HEU) children.
What kinds of clinical questions can be investigated with MRS?

1. **HIV-infected children**: How do different ART initiation times affect neurodevelopment?

2. **HIV-exposed, uninfected children**: Are children born HIV uninfected, but exposed to HIV and ART, at risk of neurodevelopmental delays?
Children from Cape Town who are enrolled in the “children with HIV early antiretroviral” (CHER) trial.

**HIV-infected mothers**

- **In utero**: zidovudine antenatally from 28 to 34 weeks
- **At birth**: single dose nevirapine (sd NVP) to the mother, and zidovudine for a week and a sd NVP to the infant

**HIV-infected children (n = 38)**

- Randomised study enrollment at 6 - 8 weeks old

**Arms**

- **Arm 1 - Deferred treatment**
- **Arm 2 - Early treatment (for 40 weeks)**
- **Arm 3 - Early treatment (for 96 weeks)**

Examine relationship between metabolite levels (marker of neurodevelopment) and treatment/clinical measures.
Based on previous studies and CHER findings:

We hypothesized that at age 5 years, the children who initiated ART early (12 weeks or younger) would have improved metabolite levels in the basal ganglia compared to children who received later ART (older than 12 weeks).
HIV-infected children

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<table>
<thead>
<tr>
<th></th>
<th>NAA</th>
<th>Choline</th>
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<tbody>
<tr>
<td>Early ART</td>
<td>5.31mM</td>
<td>1.11mM</td>
</tr>
<tr>
<td>Late ART</td>
<td>5.20mM</td>
<td>1.03mM</td>
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Significantly lower choline levels ($p = 0.05$) in later ART group

Mean choline levels at age 5 indicate an advantage of early ART treatment regimen.
HIV-infected children

Do metabolites levels (as markers of neurodevelopment) at age 5 relate to clinical measures in infancy?

**Measurement in infancy**
- CD4 count at enrollment
- CD8 count at enrollment
- Viral Load at enrollment

**Measurement in basal ganglia at age 5**
- Choline levels — cellular density
- NAA levels — neuronal density
NAA levels at age 5 correlate significantly with CD4/CD8 ratio (a measure of immune system health) in infancy (median age ~ 7 weeks old) — across all treatment regimens. Indicates damage (low CD4/CD8 ratio) in early infancy persists into childhood.
Results indicate advantages - higher mean choline levels - of early ART compared to deferred treatment.

Results suggest damage from HIV infection sustained in early infancy persists into childhood in the basal ganglia - regardless of treatment regimen.
HIV-infected children: How do different ART initiation times affect neurodevelopment?

HIV-exposed, uninfected children: Are children born HIV uninfected, but exposed to HIV and ART, at risk of neurodevelopmental delays?
Follow these children longitudinally to compare neurodevelopment

HEU = HIV exposed, uninfected
HUU = HIV unexposed, uninfected
Hypothesis
We hypothesize that from 5 to 7 years of age, HEU children would exhibit different NAA and choline level trajectories in gray matter compared to HUU children.

Test hypothesis
Measure metabolite levels in gray matter as a marker of typical neurodevelopment in children over time (from age 5 to 7 years).

Constant choline levels
Increased NAA levels
HIV-exposed, uninfected children

Cohort description

21 5-year olds

13 HEU
8 HUU

31 7-year olds

9 re-scanned

9 HEU
22 HUU
Mean NAA levels increase significantly from age 5 to age 7 in HIV-exposed, uninfected children only.
HIV-exposed, uninfected children

HEU children have HIGHER mean choline levels at age 7

$p = 0.003$

Midfrontal gray matter
Conclusions

Results suggest HIV-exposure may affect normal neurological development in young children in gray matter.

Results indicate a disruption or delay in development between preschool age (5 years) and school age (7 years).
HIV-infected and HIV-exposed, uninfected children

Future work

- Currently examining MRS data at age 7
- At present, acquiring 9 year old data.
- Examine neuropsychological and behavioral data in combination with metabolite levels.

Can NAA and choline levels recover? Do differences persist at ages 7 and 9?
Ultimately, we would like to use all modalities in combination to create a more complete picture of neurodevelopment - in terms of brain structure, function and metabolism - within these children at ages 5, 7 and 9 years.
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