Sensory neuropathy affects 40% of HIV+ South Africans and 46% of risk can be predicted by one genotype plus demographic factors!

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HIV-SN can severely impair ability to work & quality of life!

Affects 60% of HIV+ Africans receiving stavudine in their treatment regimens

Symptoms may include:
• Burning or numbness
• Pins & needles
• Pain hypersensitivity
• Pain without painful stimulus
• Reduced ankle reflexes

There is no prevention, no cure & very few effective therapeutics!

Clinical pathology of HIV-SN

Neuronal loss in the dorsal root ganglion
Dieback degeneration of long axons
Loss of primary afferent terminals in the skin
Macrophage & cytokine infiltration of the DRG and skin

Mountford et al. 2018; Polydefkis et al. 2002; Shikuma et al. 2015
CaMKI is a candidate!

- **SIRT1**
  - Neuronal DNA repair
  - Axonal regeneration
  - Dendrite arborisation

- **AMPK**
  - Neuronal metabolism, proliferation, differentiation
  - Synapse connectivity
  - Neuronal survival

- **CAMKI**
  - Axonal elongation
  - Memory formation

- **CAMKIV**
  - Synapse formation
  - Dendrite arborisation
  - Excitatory synaptic strength
  - Memory formation

We can visualise CaMKK2 in biopsies using fluorescent microscopy!

Biopsies were donated from Indonesian individuals with and without HIV-SN

We were able to visualise CaMKK2
- Quantity
- Location
- Interactions
We can investigate the genetic signature of CAMKK2 in HIV-SN

CAMKK2 is located on chromosome 12 in a region of linkage disequilibrium

Polymorphisms in CAMKK2 may be co-inherited with polymorphisms in neighbouring genes

https://ldlink.nci.nih.gov/?tab=home
CAMKK2 is linked with neighbouring genes

Inflammation

Kawasaki et al. 2008; Tsuda et al. 2003; Lin et al. 2006; Ho et al. 2013
Participants were genotyped for polymorphisms in CAMKK2.

Demographic and clinical records collected. Assessed for HIV-SN using the BPNS.


Haplotypes derived using fastPHASE.

Bivariate & multivariate analyses.

75 HIV+ Africans Stavudine-free ART.

*BPNS = AIDS clinical trials group Brief Peripheral Neuropathy Screen
38% of patients developed HIV-SN

9 patients were diagnosed with HIV-SN prior to starting ART

20 patients developed HIV-SN between starting ART and follow-up at 6-8 months

Total = 29/75
Demographic and clinical variables are risk factors of HIV-SN

<table>
<thead>
<tr>
<th>Variable</th>
<th>+ve (n=29)</th>
<th>-ve (n=46)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>40 (24-60)</td>
<td>37 (19-58)</td>
<td>0.11</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>168 (147-179)</td>
<td>163 (135-186)</td>
<td>0.03</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>66 (45-112)</td>
<td>55 (35-110)</td>
<td>0.03</td>
</tr>
<tr>
<td>Current CD4 T-cells/µl</td>
<td>221 (22-685)</td>
<td>300 (8-832)</td>
<td>0.06</td>
</tr>
<tr>
<td>Nadir CD4 T-cells/µl</td>
<td>107 (4-575)</td>
<td>223 (8-771)</td>
<td>0.002</td>
</tr>
<tr>
<td>HIV RNA &gt;500 copies/ml</td>
<td>21/29 (72%)</td>
<td>25/46 (54%)</td>
<td>0.12</td>
</tr>
<tr>
<td>History of Tuberculosis</td>
<td>6/28 (29%)</td>
<td>3/45 (7%)</td>
<td>0.08</td>
</tr>
</tbody>
</table>
Demographic and clinical factors

Model p<0.0000, n=71, Pseudo $R^2=0.18$

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds Ratio</th>
<th>p Value</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight</td>
<td>1.04</td>
<td>0.029</td>
<td>1.00-1.08</td>
</tr>
<tr>
<td>Nadir CD4 T-cells</td>
<td>1.00</td>
<td>0.027</td>
<td>0.99-1.00</td>
</tr>
<tr>
<td>Prior Tuberculosis</td>
<td>4.26</td>
<td>0.077</td>
<td>0.90-20.03</td>
</tr>
</tbody>
</table>
CAMKK2 polymorphisms associate with HIV-SN

- P2X7R: rs118055*C, rs503720*G
- P2X4R: rs7961979*A, rs10849861*A, rs1653586*T
- CAMKK2: rs7975295*C, rs10849861*A, rs11065504*C
- ANAPC5: rs1560568*A, rs1132780*T, rs2089886*A

**Significance Levels:**
- p<0.20
- p<0.05
**Optimal model considering demographics and polymorphisms**

Model p<0.0000, n=69, Pseudo R²=0.46

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds Ratio</th>
<th>p Value</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight</td>
<td>1.07</td>
<td>0.031</td>
<td>1.01-1.13</td>
</tr>
<tr>
<td>Prior Tuberculosis</td>
<td>11.28</td>
<td>0.071</td>
<td>3.1-156.38</td>
</tr>
<tr>
<td>rs503720*G (P2X7R)</td>
<td>133.57</td>
<td>0.002</td>
<td>6.47-2757.01</td>
</tr>
<tr>
<td>rs10849861*A (CAMKK2)</td>
<td>5.99</td>
<td>0.050</td>
<td>1.0-35.87</td>
</tr>
<tr>
<td>rs1653586*T (CAMKK2)</td>
<td>0.02</td>
<td>0.006</td>
<td>0.001-0.31</td>
</tr>
<tr>
<td>rs11065504*C (CAMKK2)</td>
<td>6.68</td>
<td>0.088</td>
<td>0.76-58.92</td>
</tr>
</tbody>
</table>

Accounts for 46% of the risk of HIV-SN in this group!
7 haplotypes associate with HIV-SN

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Freq</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>P2X4R-4</td>
<td>12%</td>
<td>0.14</td>
</tr>
<tr>
<td>CAMKK2-3</td>
<td>16%</td>
<td>0.13</td>
</tr>
<tr>
<td>ANAPC5 -8</td>
<td>9%</td>
<td>0.10</td>
</tr>
</tbody>
</table>

2x perfectly predict protection
Only in individuals **without** HIV-SN

2x perfectly predict risk
Only in individuals **with** HIV-SN
Optimal model considering demographics and haplotypes

Model $p=0.0005$, $n=71$, Pseudo $R^2=0.21$

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds Ratio</th>
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<td>0.99</td>
<td>0.023</td>
<td>0.99-1.00</td>
</tr>
<tr>
<td>Prior Tuberculosis</td>
<td>11.28</td>
<td>0.126</td>
<td>0.71-16.60</td>
</tr>
<tr>
<td>$P2X4R$ Haplotype 4</td>
<td>133.57</td>
<td>0.132</td>
<td>0.18-1.69</td>
</tr>
</tbody>
</table>
Why are polymorphisms more strongly associated with HIV-SN than haplotypes?

Small cohort and genetic diversity – there may be rarer haplotypes which are not analysed in a small cohort

Linkage disequilibrium – the polymorphisms we identified may be linked with polymorphisms outside our panel

The polymorphisms may contribute directly?
Associating polymorphisms may play a direct role in HIV-SN

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Gene</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs503720</td>
<td>\textit{P2X7R}</td>
<td>Intronic</td>
</tr>
<tr>
<td>rs10849861</td>
<td>\textit{CAMKK2}</td>
<td>Intergenic</td>
</tr>
<tr>
<td>rs1653586</td>
<td>\textit{CAMKK2}</td>
<td>3’ UTR</td>
</tr>
<tr>
<td>rs11065504</td>
<td>\textit{CAMKK2}</td>
<td>Intronic</td>
</tr>
</tbody>
</table>
Study conclusions!

*CAMKK2* polymorphisms are a strong marker of HIV-SN in Africans

The polymorphisms associated with HIV-SN are non-coding. So may play a role via the regulation of expression of *CaMKK2* or neighbouring genes.

This study implicates a role for *CAMKK2* in HIV-SN and further investigation is warranted!
Significance

If we can identify genetic markers we can offer customised HIV care for those at risk

Identifying the mechanisms leading to HIV-SN may allow the development of therapeutics to prevent, treat and cure HIV-SN
Acknowledgments

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Special thanks to the people living with HIV who have participated in this research

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