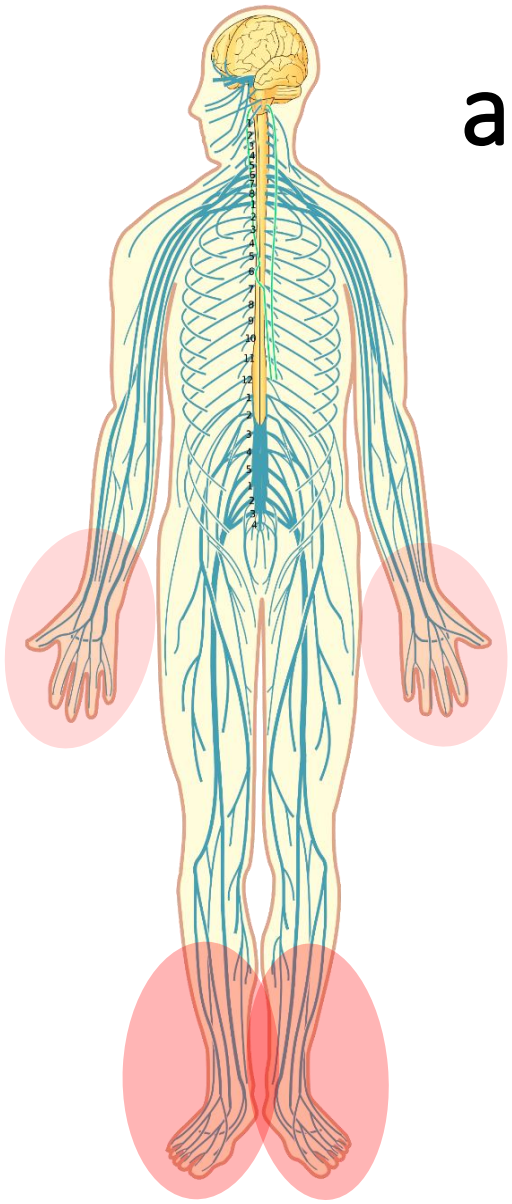
# Sensory neuropathy affects 40% of HIV+ South Africans and 46% of risk can be predicted by one genotype plus demographic factors!

Jessica Gaff, Prinisha Pillay, Huguette Gaelle Ngassa Mbenda, Simon Laws, Catherine Cherry, Peter Kamerman and Patricia Price

jessica.gaff@postgrad.curtin.edu.au



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

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

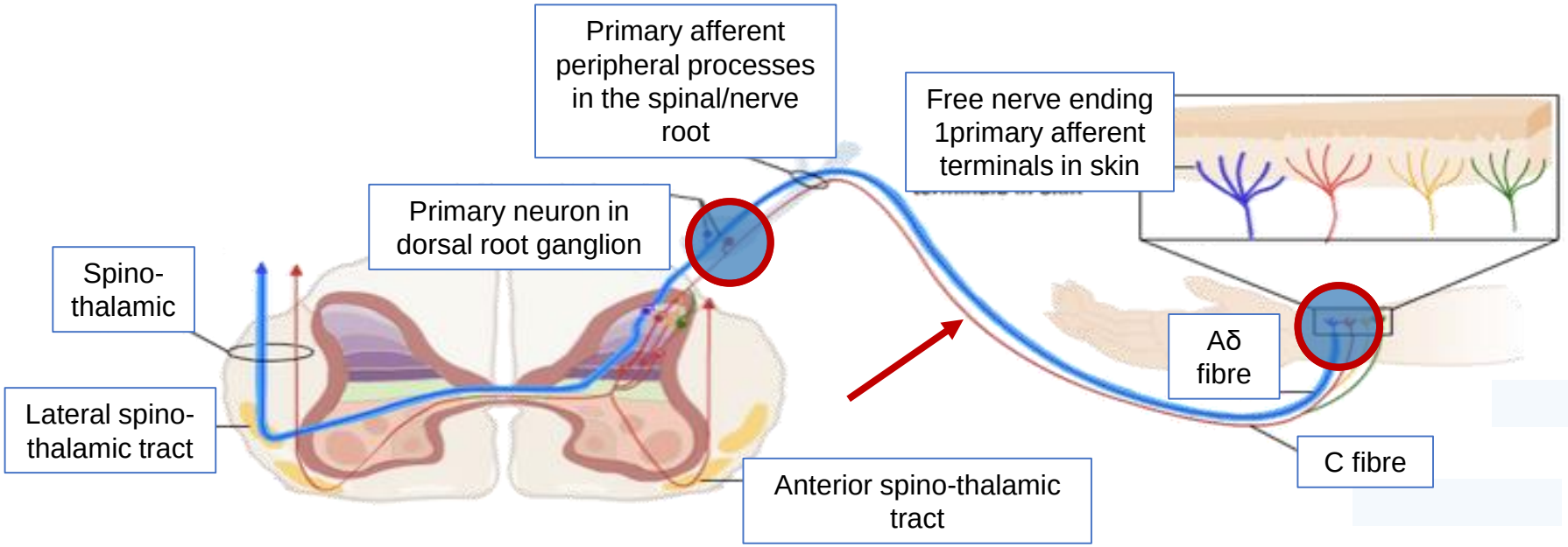
Symptoms may include:

- Burning or numbness
- Pins & needles
- Pain hypersensitivity
- Pain without painful stimulus
- Reduced ankle reflexes

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

Kamerman et al 2012, Cherry et al 2012, Pillay et al 2018, Phillips et al 2014

# Clinical pathology of HIV-SN




Neuronal loss in the dorsal root ganglion

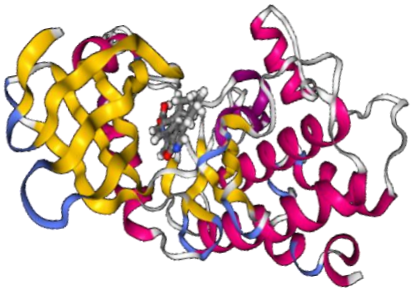
Dieback degeneration of long axons

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

# CaMKK2 is a candidate!



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

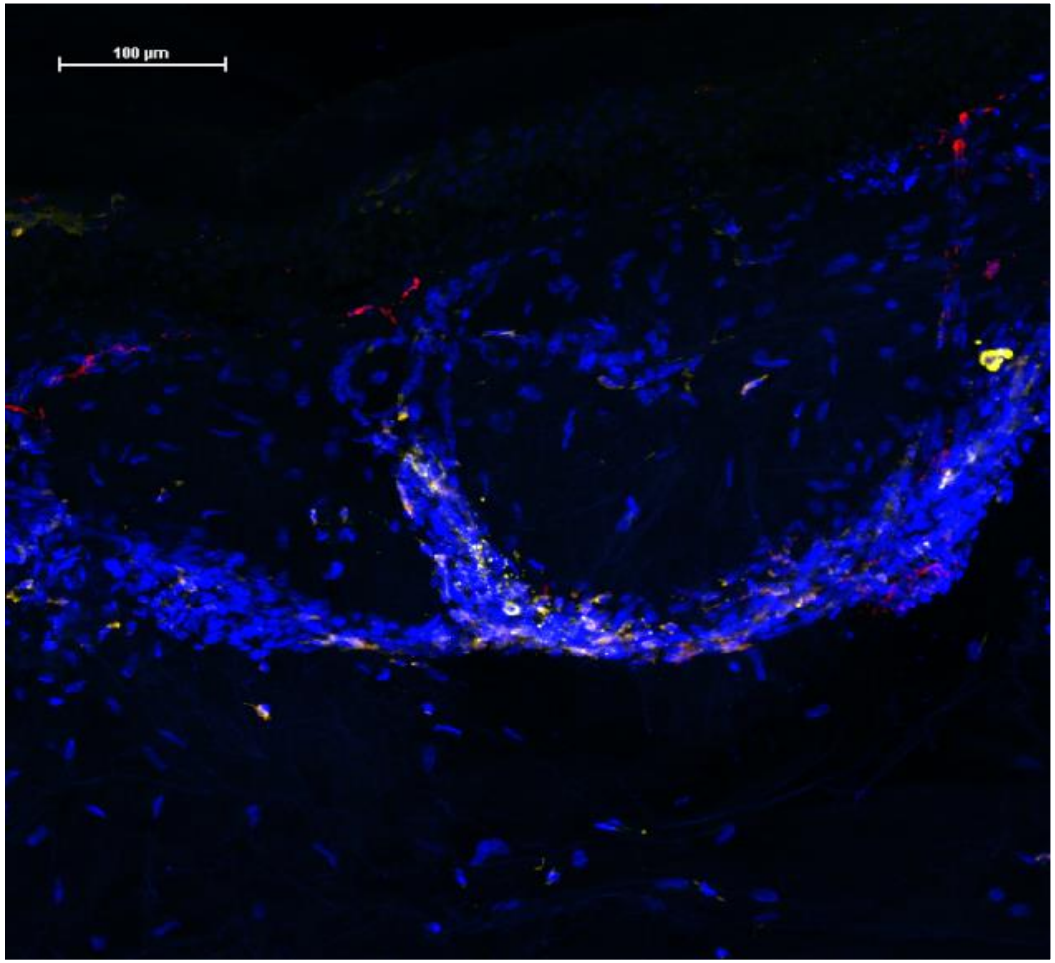
**CAMKI**
- Axonal elongation
- Memory formation

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

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

Raccioppi and Means 2012, Wen et al 2013, Hawley et al 2005

# We can visualise CaMKK2 in biopsies using fluorescent microscopy!




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

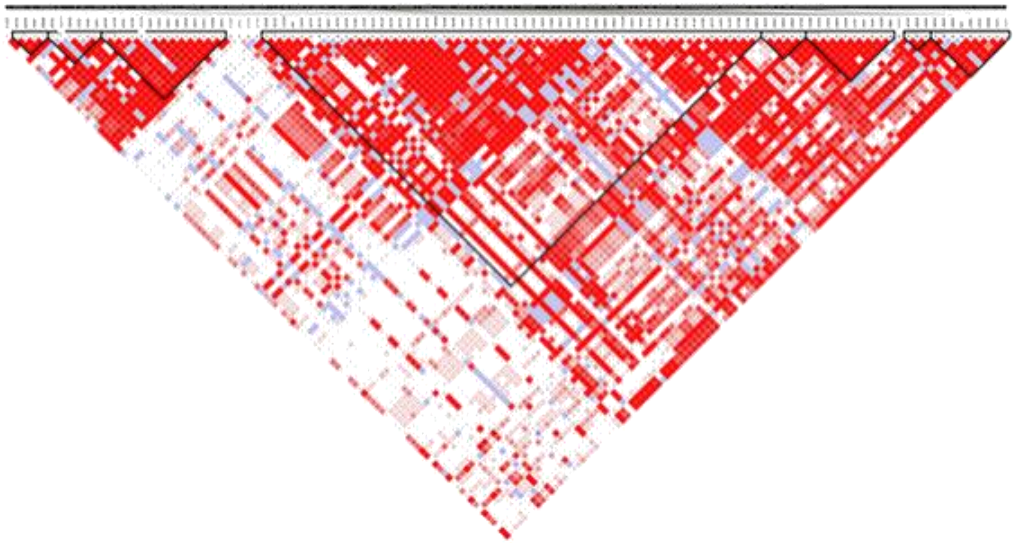
We were able to visualise CaMKK2

- 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



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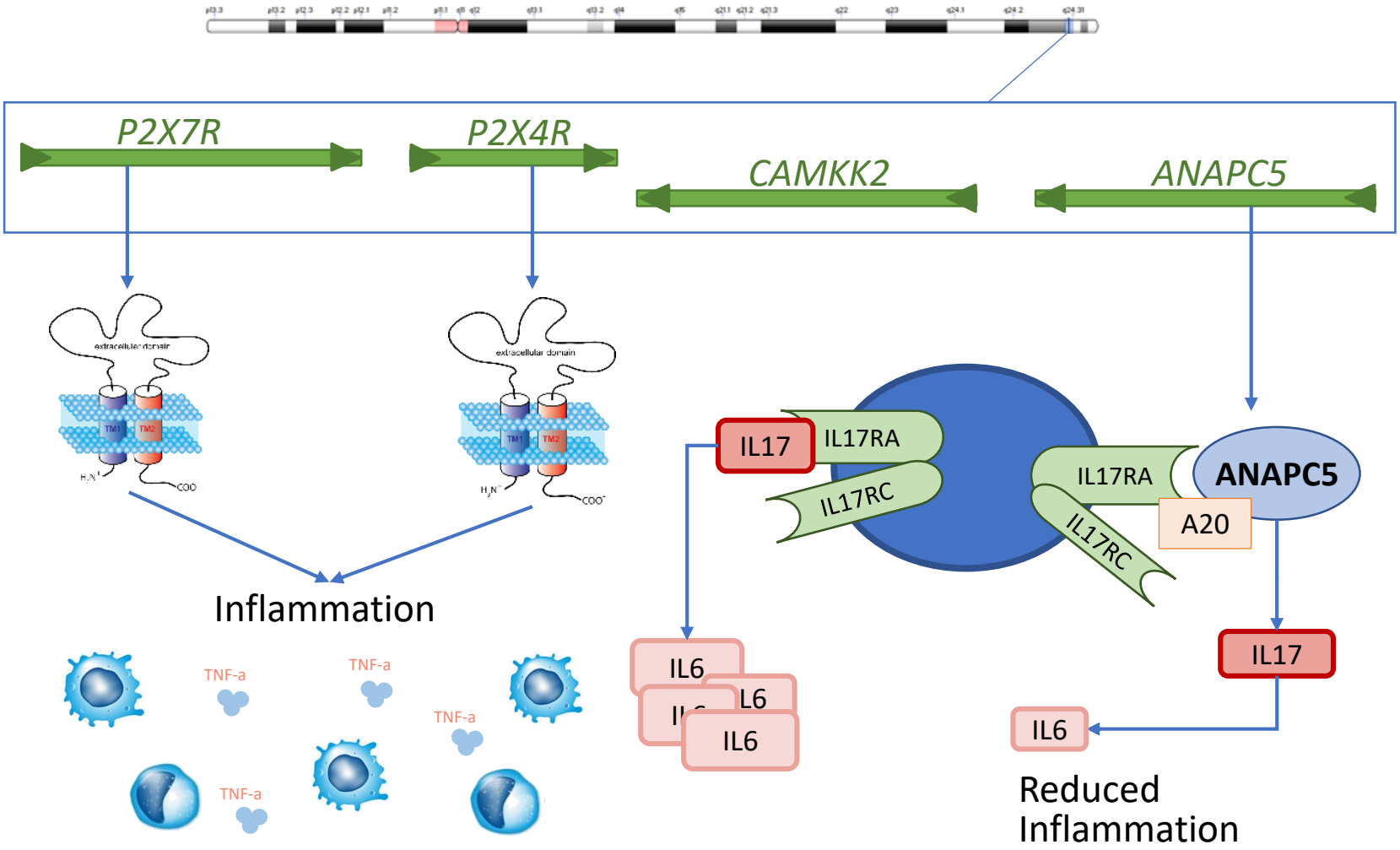
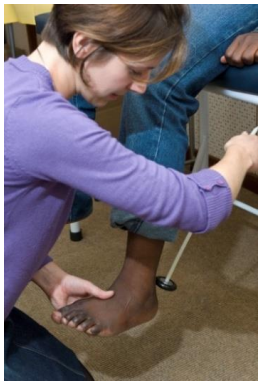
# *CAMKK2* is linked with neighbouring genes



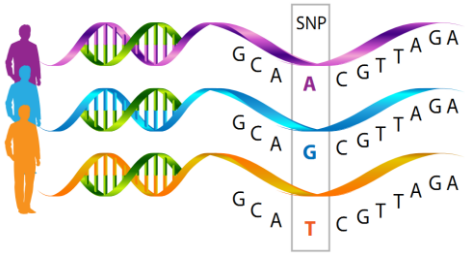

Kawasaki et al. 2008; Tsuda et al. 2003; Lin et al. 2006; Ho et al. 2013

# Participants were genotyped for polymorphisms in CAMKK2

75 HIV+ Africans
Stavudine-free ART

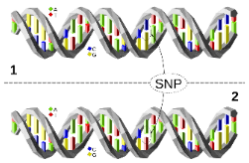


Demographic and clinical records collected. Assessed for HIV-SN using the BPNS




DNA genotyped for 48 polymorphisms across *P2X7R*, *P2X4R*, *CAMKK2* & *ANAPC5*

Haplotypes derived using fastPHASE




Bivariate & multivariate analyses

STATA

*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

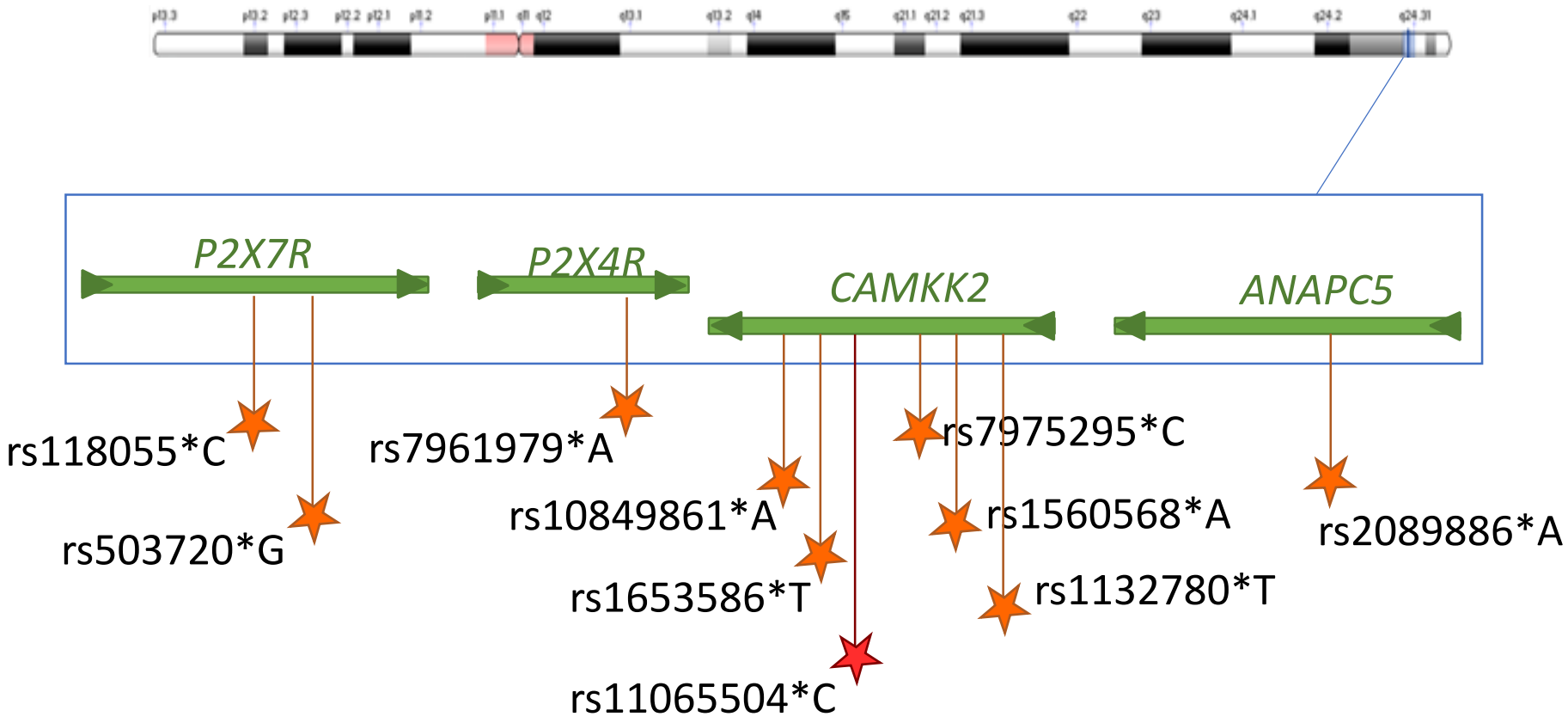
# Demographic and clinical variables are risk factors of HIV-SN

| South African HIV-SN | | | |
|---|---|---|---|
| **Variable** | **+ve (n=29)** | **-ve (n=46)** | **P** |
| Age (years) | 40 (24-60) | 37 (19-58) | 0.11 |
| Height (cm) | 168 (147-179) | 163 (135-186) n=45 | 0.03 |
| Weight (kg) | 66 (45-112) | 55 (35-110) n=44 | 0.03 |
| Current CD4 T-cells/µl | 221 (22-685) | 300 (8-832) | 0.06 |
| Nadir CD4 T-cells/µl | 107 (4-575) | 223 (8-771) | 0.002 |
| HIV RNA >500 copies/ml | 21/29 (72%) | 25/46 (54%) | 0.12 |
| History of Tuberculosis | 6/28 (29%) | 3/45 (7%) | 0.08 |

# Demographic and clinical factors

| Model $p<0.0000$, $n=71$, Pseudo $R^2=0.18$ | | | |
|---|---|---|---|
| **Variable** | **Odds Ratio** | **p Value** | **95% Confidence Interval** |
| Body Weight | 1.04 | 0.029 | 1.00-1.08 |
| Nadir CD4 T-cells | 1.00 | 0.027 | 0.99-1.00 |
| Prior Tuberculosis | 4.26 | 0.077 | 0.90-20.03 |

# *CAMKK2* polymorphisms associate with HIV-SN




p<0.20

p<0.05

# Optimal model considering demographics and polymorphisms

| Model p<0.0000, n=69, Pseudo $R^2$=0.46 | | | |
|---|---|---|---|
| **Variable** | **Odds Ratio** | **p Value** | **95% Confidence Interval** |
| Body Weight | 1.07 | 0.031 | 1.01-1.13 |
| Prior Tuberculosis | 11.23 | 0.071 | 0.81-156.58 |
| rs503720*G (*P2X7R*) | 133.57 | 0.002 | 6.47-2757.01 |
| rs10849861*A (*CAMKK2*) | 5.99 | 0.050 | 1.00-35.87 |
| rs1653586*T (*CAMKK2*) | 0.02 | 0.006 | 0.001-0.31 |
| rs11065504*C (*CAMKK2*) | 6.68 | 0.088 | 0.76-58.92 |

Pseudo $R^2$=0.46

**Accounts for 46% of the risk of HIV-SN in this group!**

Stata Corp, TX

# 7 haplotypes associate with HIV-SN

| Haplotype | Freq | P value |
|---|---|---|
| | | |
| P2X4R-4 | 12% | 0.14 |
| CAMKK2-3 | 16% | 0.13 |
| | | |
| | | |
| ANAPC5 -8 | 9% | 0.10 |
| | | |

2x perfectly predict protection

**Only in individuals <u>without</u> HIV-SN**

2x perfectly predict risk

**Only in individuals <u>with</u> HIV-SN**

# Optimal model considering demographics and haplotypes

| Model p=0.0005, n=71, Pseudo $R^2$=0.21 | | | |
|---|---|---|---|
| **Variable** | **Odds Ratio** | **p Value** | **95% Confidence Interval** |
| Body Weight | 1.04 | 0.032 | 1.00-1.08 |
| Nadir CD4 T-cells | 0.99 | 0.023 | 0.99-1.00 |
| Prior Tuberculosis | 11.28 | 0.126 | 0.71-16.60 |
| *P2X4R* Haplotype 4 | 133.57 | 0.132 | 0.18-1.69 |

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# 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

| Polymorphism | Gene | Location |
|---|---|---|
| rs503720 | *P2X7R* | Intronic |
| rs10849861 | *CAMKK2* | intergenic |
| rs1653586 | *CAMKK2* | 3’ UTR |
| rs11065504 | *CAMKK2* | intronic |

# 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

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Curtin University
CURTIN HEALTH INNOVATION RESEARCH INSTITUTE
UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
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