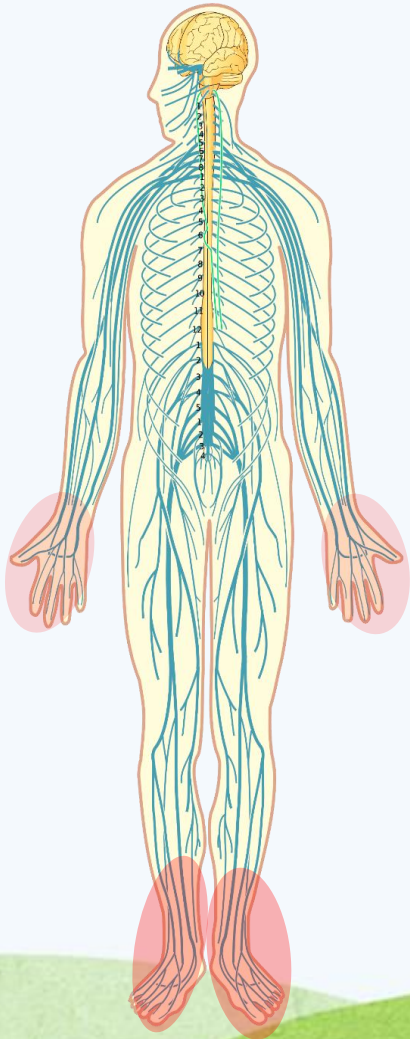
A stylized, colorful illustration of a landscape. The foreground features rolling green hills with dark brown soil patches. On the left, there is a green tree, a purple flower, and an orange flower. A small red bird is flying in the sky above the tree. The background consists of light blue and white wavy bands representing the sky.

CAMKK2 polymorphisms and the encoded protein may contribute to HIV-SN

Jessica Gaff – PhD Candidate – Curtin University

What is HIV-associated Sensory Neuropathy?

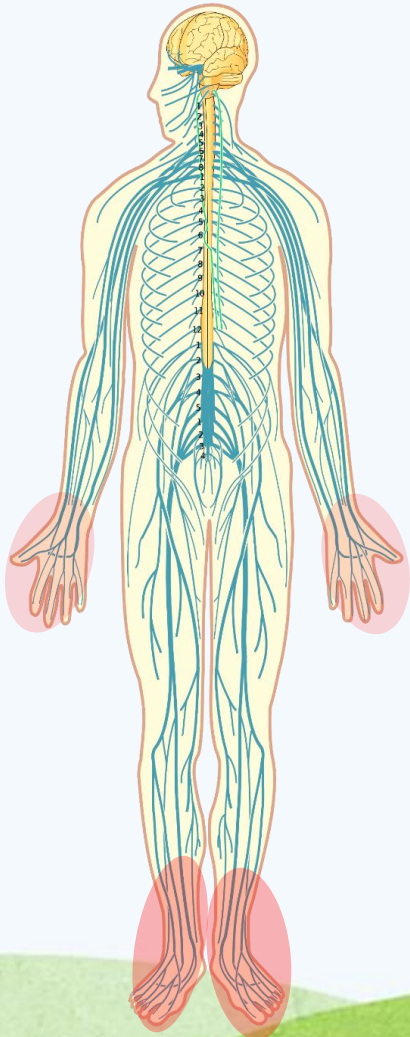


Painful & debilitating neurological condition

Affects up to 60% of people living with HIV

Results from HIV infection directly or from the use of neurotoxic ART – particularly stavudine

What is HIV-associated Sensory Neuropathy?

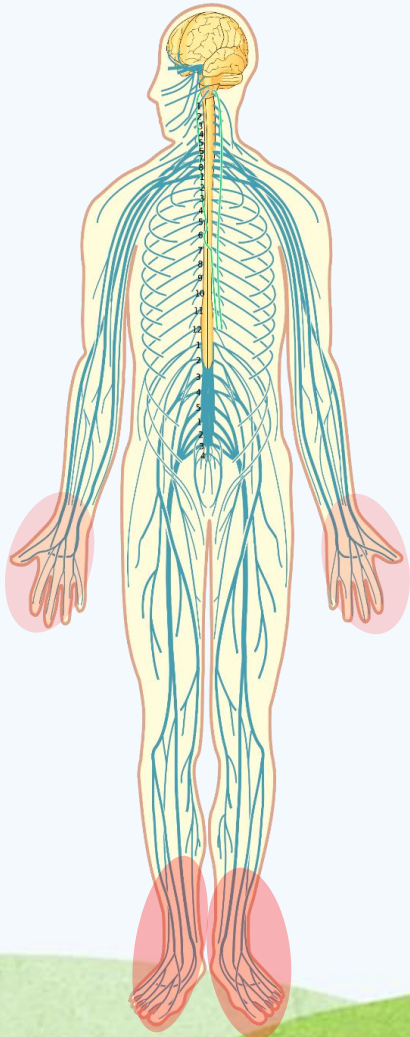


Symptoms may include:

- *reduced ankle reflexes*
- *Burning, numbness, pins & needles*
- *Pain hypersensitivity (Hyperalgesia)*
- *Pain without painful stimuli (Allodynia)*

Impacts ability to work and quality of life!

What is HIV-associated Sensory Neuropathy?



Clinical pathology:

- Neuronal loss in the dorsal root ganglion
- Dieback degeneration of long axons
- Destruction of primary afferent terminals

Underlying inflammatory pathology:

- Infiltration of macrophages
- Increased cytokine and chemokine production

But HIV-SN has a genetic component!

A genetic study by our group investigated 3 genes:

P2X7R: inflammatory signalling

P2X4R: neurotransmission

CAMKK2: neuronal repair & energy homeostasis

153 HIV+ South African patients treated with stavudine with African ancestry and were assessed for neuropathy

Blood collected, DNA extracted, genotyped for 45 SNPs

Haplotypes derived using fastPHASE

SNPs in *CAMKK2* associate with HIV-SN

SNP	GENE	CHI ²
rs1186055	<i>P2X7R</i>	0.17
rs208307	<i>P2X7R</i>	0.15
rs10160951	<i>P2X7R</i>	0.17
rs2230912	<i>P2X7R</i>	0.08
rs2686387	<i>P2X4R</i>	0.15
Rs2668252	<i>P2X4R</i>	0.13
Rs1169719	<i>P2X4R</i>	0.11
rs1653587	<i>CAMKK2</i>	0.12
rs7975295	<i>CAMKK2</i>	0.007
rs2686344	<i>CAMKK2</i>	0.018
rs1560568	<i>CAMKK2</i>	0.023
rs2686367	<i>CAMKK2</i>	0.004

Strong association found with *CAMKK2*

rs2686344 associated with REDUCED risk

rs7975295, *rs1560568* & *rs2686367* associated with INCREASED risk

Weaker effect observed in *P2X7R* and *P2X4R*

HAPLOTYPE (>1% Frequency)	GENE	CHI ²
111111211112121111111	P2X7R	0.18
211121211112121111111	P2X7R	0.12
211111111	P2X4R	0.20
211111212	P2X4R	0.17
212111111	P2X4R	0.13
11111111112	CAMKK2	0.14
11111111121	CAMKK2	0.002
11111112112	CAMKK2	0.14
11112111111	CAMKK2	0.03
11112121111	CAMKK2	0.05
11121212111	CAMKK2	0.014
11121221111	CAMKK2	0.05
11121221112	CAMKK2	0.13
21111111121	CAMKK2	0.03

CAMKK2 haplotypes associate with HIV-SN

Significant association found with 6
CAMKK2 haplotypes

5 haplotype associated with
REDUCED risk

1 haplotype associated with
INCREASED risk

Contains the 2 of the risk SNPs

Weaker effect observed in P2X7R
and P2X4R

The logistic regression model predicts HIV-SN

The optimum model included demographics and genetics:

Age and Height

2x *CAMKK2* SNPs which mark risk significantly independently associated with HIV-SN

Predicted 19% of the risk of HIV-SN

Variable	Odds Ratio	P value	95% confidence interval
n=137 p<0.0001 R ² =0.19			
Age	1.08	0.007	0.02-1.13
Height	1.05	0.03	1.00-1.11
rs208307 (P2X7R)	1.96	0.10	0.86-4.88
rs2668252 (P2X4R)	2.10	0.09	0.90-4.93
rs1169719 (P2X4R)	0.37	0.11	0.11-1.23
rs1560568 (CAMKK2)	2.39	0.03	1.09-5.25
rs2686367 (CAMKK2)	2.85	0.01	1.26-6.42

LIMITATIONS!

Limitation: Patients received stavudine, a risk factor of HIV-SN

*Solution: Assess another cohort without exposure to stavudine
May identify if associations are due to stavudine, HIV or other ART*

Limitation: SNPs vary by ethnicity so may only associate in Africans

*Solution: Assess in a cohort of a different ethnicity.
Associations present in multiple populations may be critical in HIV-SN*

Our Study

Indonesia



COMPLETE:
203 DNA Samples

Indonesian and South African
HIV+ & Stavudine Naïve

Assessed for HIV-SN
using the BPNS

Blood collected &
DNA extracted

Genotyped for 64 SNP across
P2X7R, *P2X4R* & *CAMKK2*

Haplotypes derived using
fastPHASE

South Africa



IN PROGRESS:
83 DNA Samples

CAMKK2 significantly associated with HIV-SN

SNP	GENE	CHI²
rs25644	<i>P2X4R</i>	0.07
rs1718158	<i>CAMKK2</i>	0.18
rs10849861	<i>CAMKK2</i>	0.046
rs1653586	<i>CAMKK2</i>	0.17
rs1653587	<i>CAMKK2</i>	0.17
rs1653587	<i>CAMKK2</i>	0.12
rs7975295	<i>CAMKK2</i>	0.011
rs1560568	<i>CAMKK2</i>	0.011
rs1132780	<i>CAMKK2</i>	0.010

4 CAMKK2 SNPs significantly associated with HIV-SN in Indonesian HIV+ patients

2 SNPs are associated with increased risk

Also associated with increased risk in South Africans – individually and in the risk haplotype

4 CAMKK2 SNPs showed a weaker effect

CAMKK2 haplotypes weakly associate with HIV-SN

HAPLOTYPE (>1% Frequency)	GENE	CHI ²
2211211211121	<i>P2X7R</i>	0.02
1121111211121	<i>P2X7R</i>	0.10
1211121111111	<i>P2X7R</i>	0.14
2212111	<i>P2X4R</i>	0.13
1211112 2 1221111111	<i>CAMKK2</i>	0.09
21221121111111111	<i>CAMKK2</i>	0.06
111121111111112212	<i>CAMKK2</i>	0.19
111112112111111112	<i>CAMKK2</i>	0.14

4 *CAMKK2* haplotypes weakly associated with HIV-SN

1 *CAMKK2* haplotype contains 2 SNPs associated with increased risk in both Indonesians & South Africans

CAMKK2 is implicated in HIV-SN

Logistic regression is yet to be completed...

- ✓ *Associations between CAMKK2 SNPs and HIV-SN are likely due to HIV infection not stavudine*
- ✓ *A role for the expressed protein of CAMKK2 in HIV-SN*

Do the encoded proteins also impact HIV-SN?

Distal leg biopsies collected from:

- HIV-SN patients
- HIV+ patients without SN
- Healthy Individuals



Fixed with 8% paraformaldehyde and cut into 50um sections

Treated with primary, secondary and fluorescently labelled antibodies

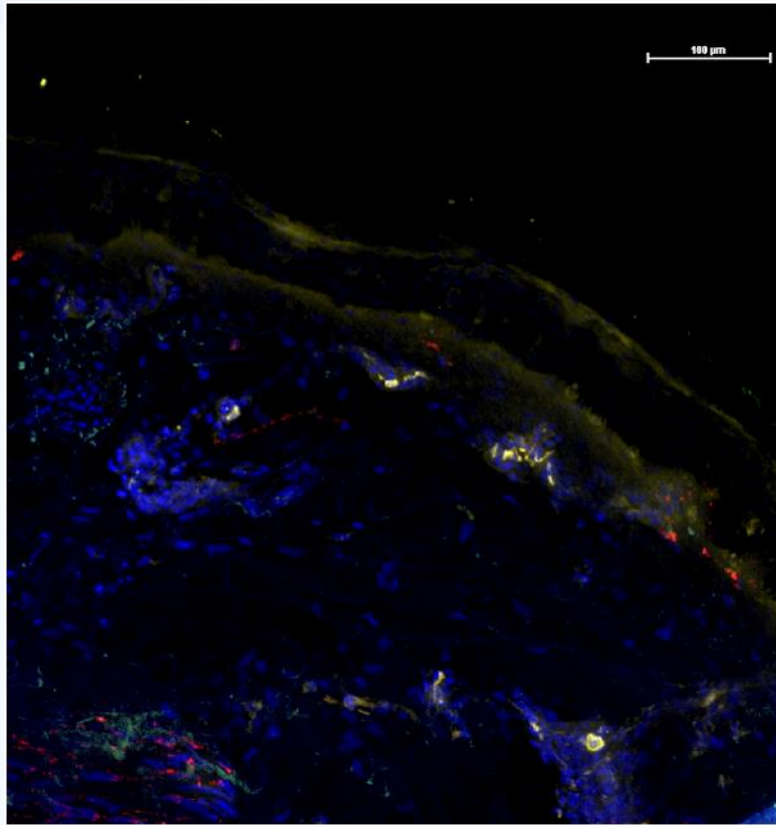
Confocal z-stack images collected and protein expression assessed

DAPI - Cell nuclei

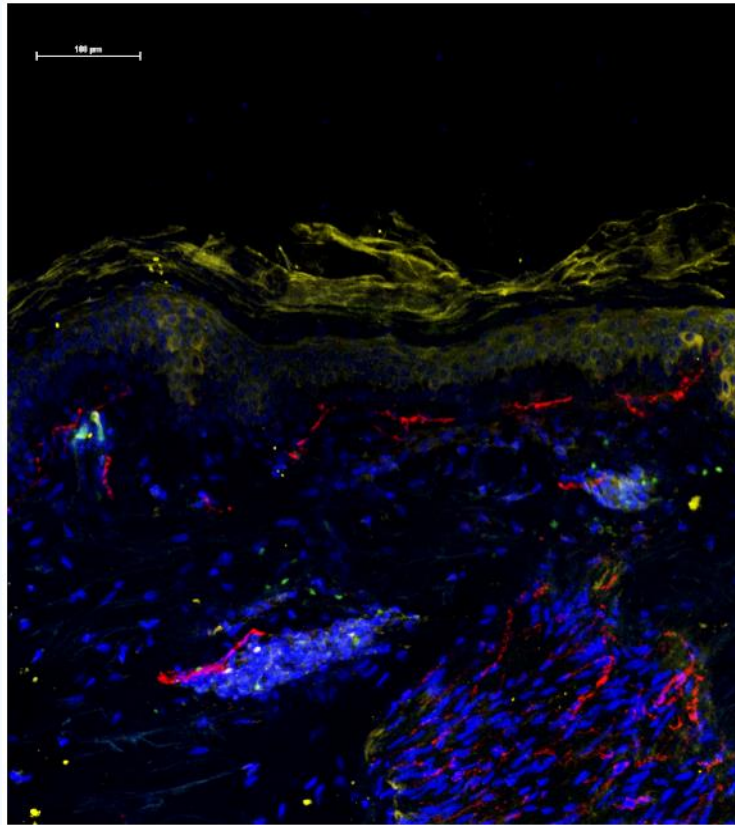
PGP9.5 - Nerves

P2X7R

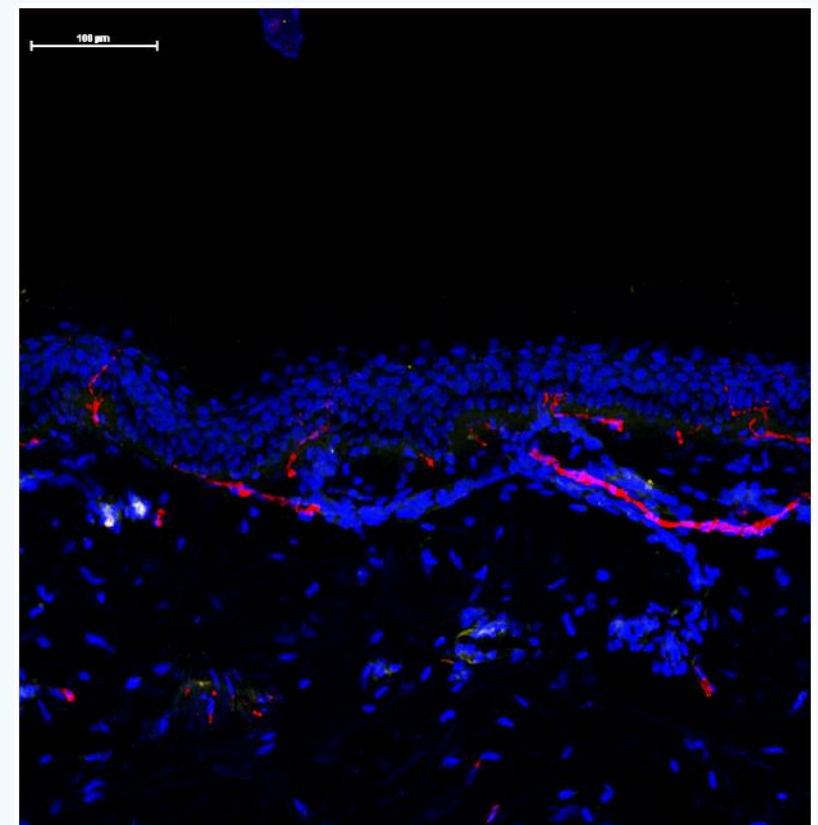
P2X7R is upregulated in HIV patients



HIV+SN+



HIV+SN-



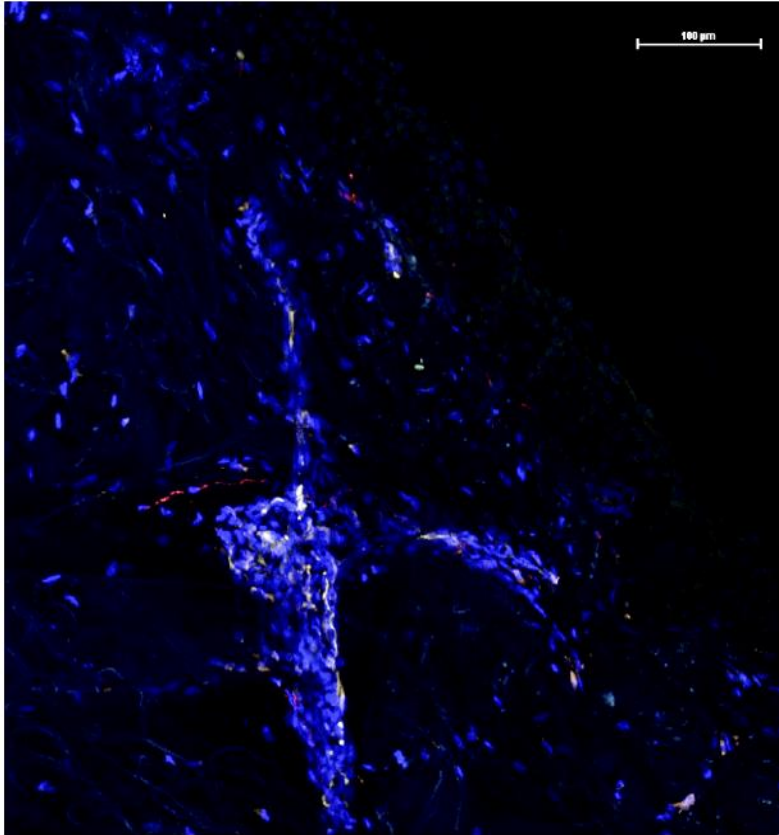
Healthy

■ DAPI – Cell nuclei

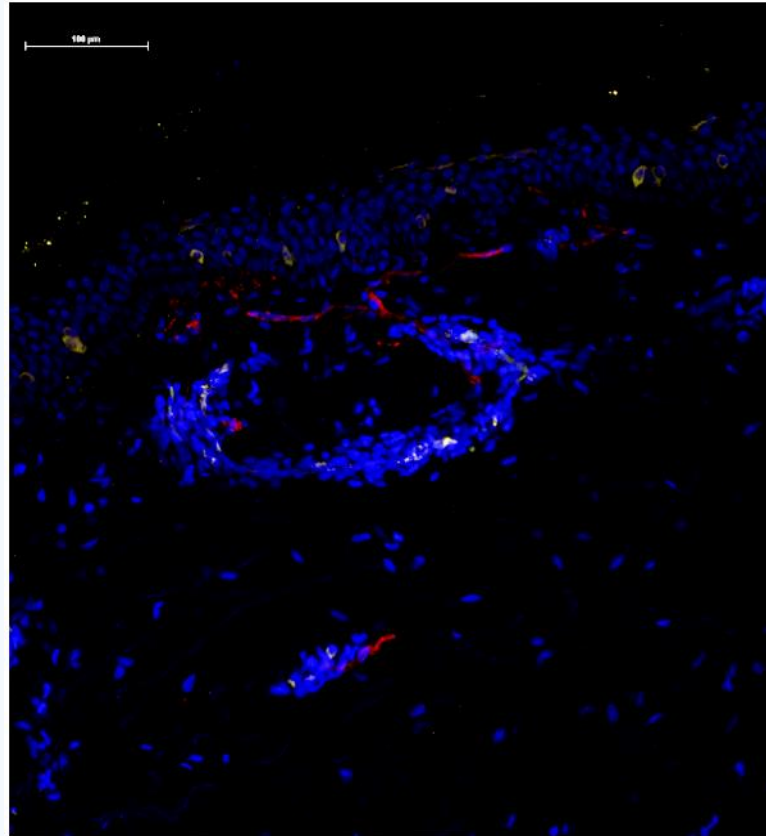
■ PGP9.5 – Nerves

■ P2X4R

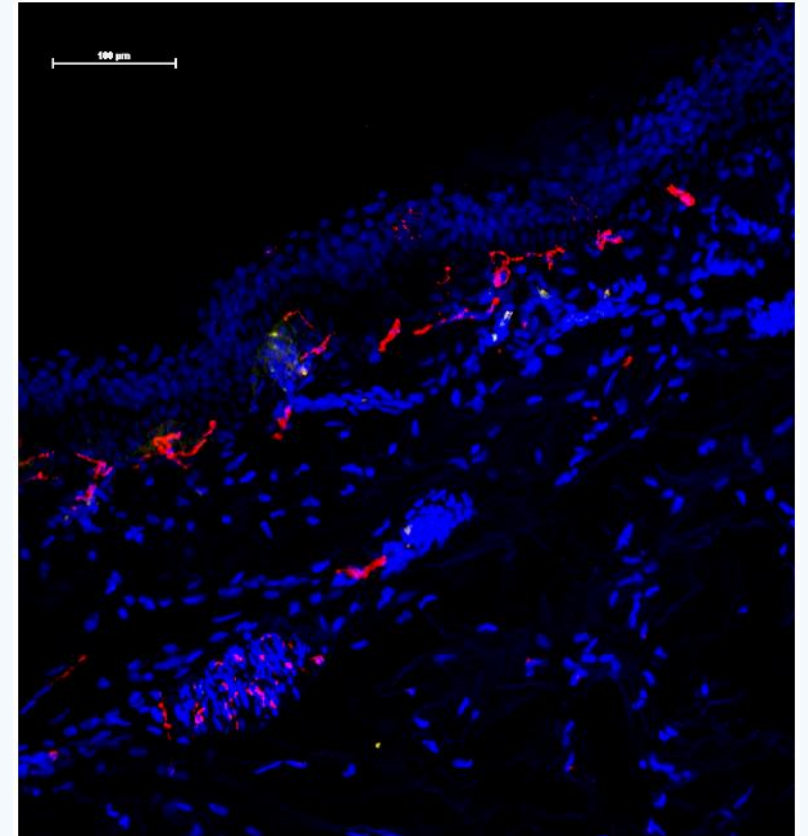
P2X4R is upregulated in HIV-SN patients



HIV+SN+



HIV+SN-



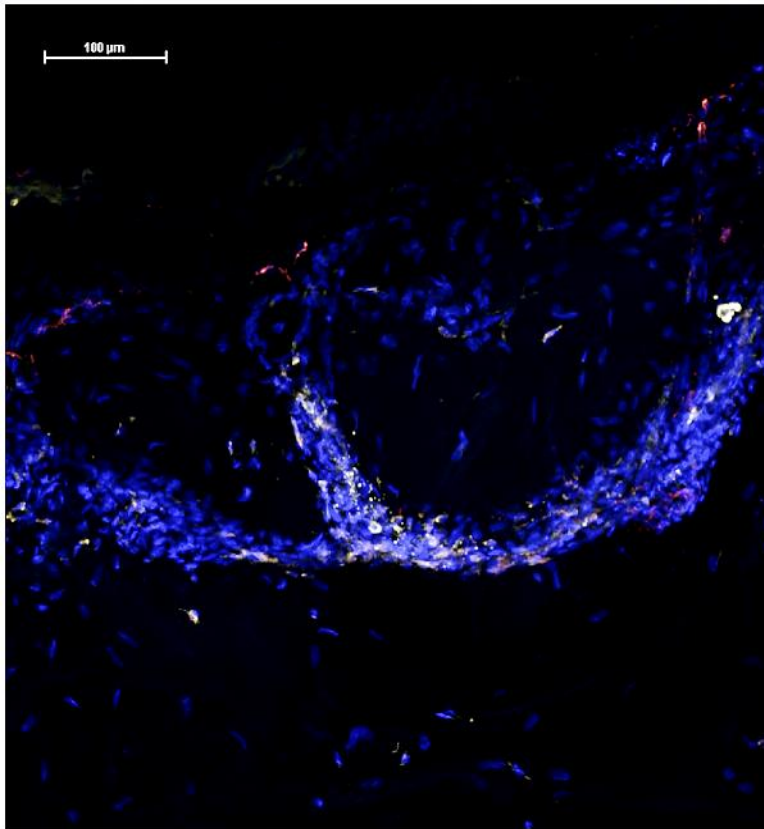
Healthy

DAPI – Cell nuclei

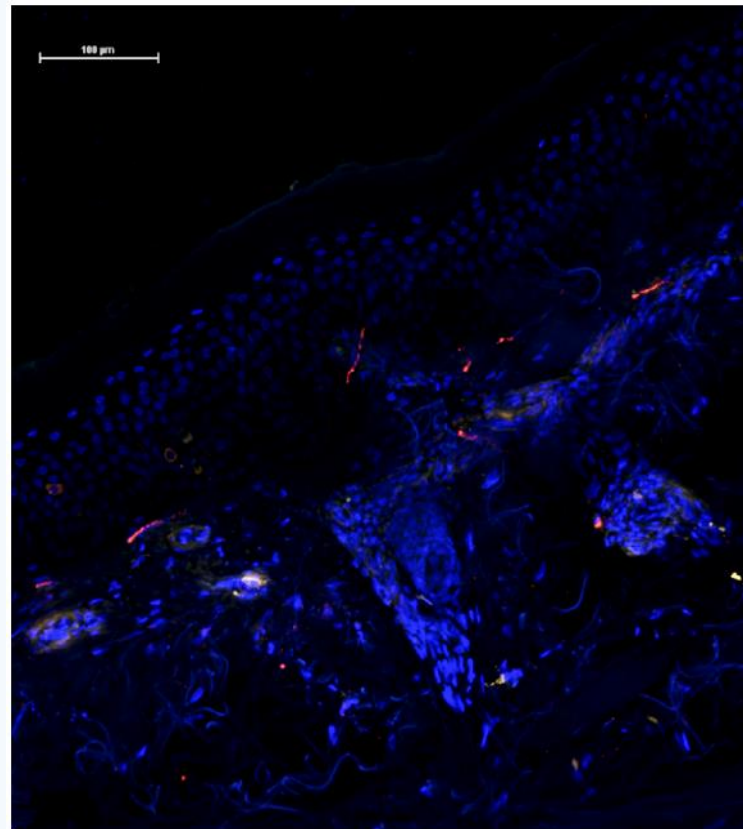
PGP9.5 – Nerves

CaMKK2

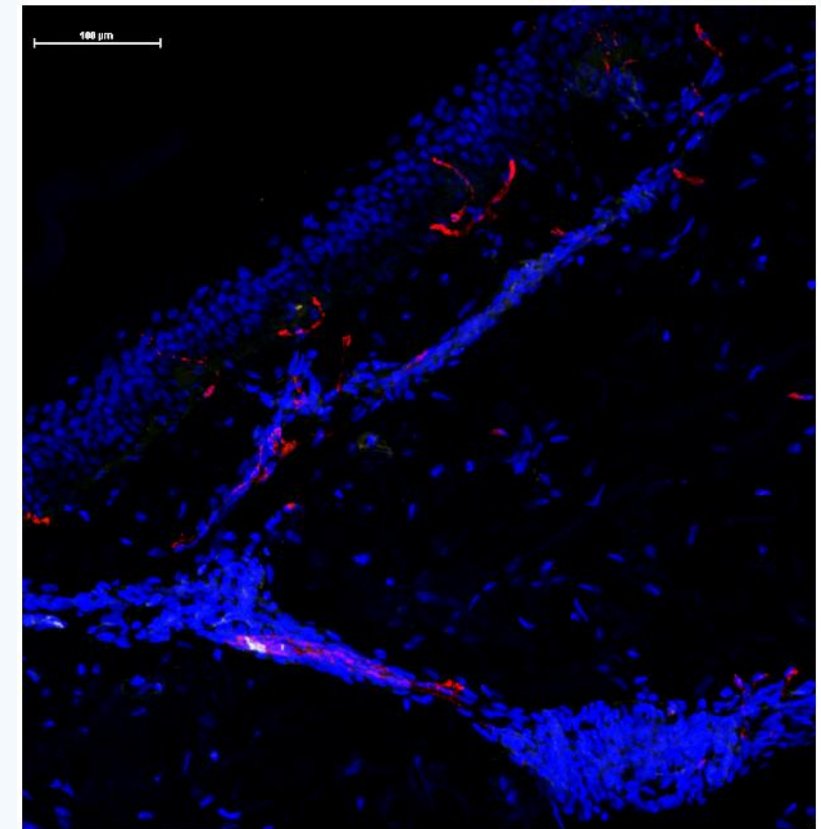
CaMKK2 is upregulated in HIV-SN patients



HIV+SN+



HIV+SN-



Healthy

Summary

SNPs in *CAMKK2* associate with HIV-SN in patients with and without exposure to Stavudine

SNPs in *CAMKK2* associate with HIV-SN in both Indonesian and South African patients

CaMKK2 expression is upregulated in patients with HIV-SN

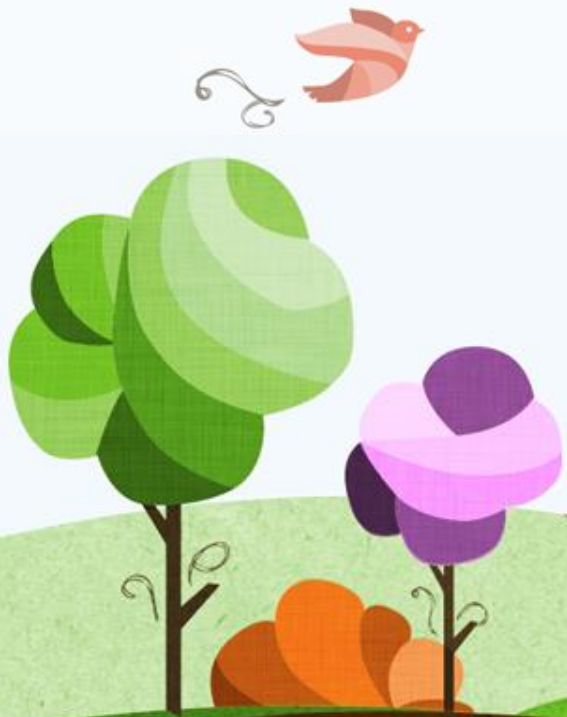
P2X7R and *P2X4R* exhibit a weaker effect

CAMKK2 polymorphisms and the encoded protein may contribute to HIV-SN

CAMKK2 polymorphisms and the encoded protein may contribute to HIV-SN

These results may allow us to develop a prognostic test to identify individuals with increased risk to HIV-SN

and identifies possible mechanisms of the disease, which may provide a therapeutic target in the future!



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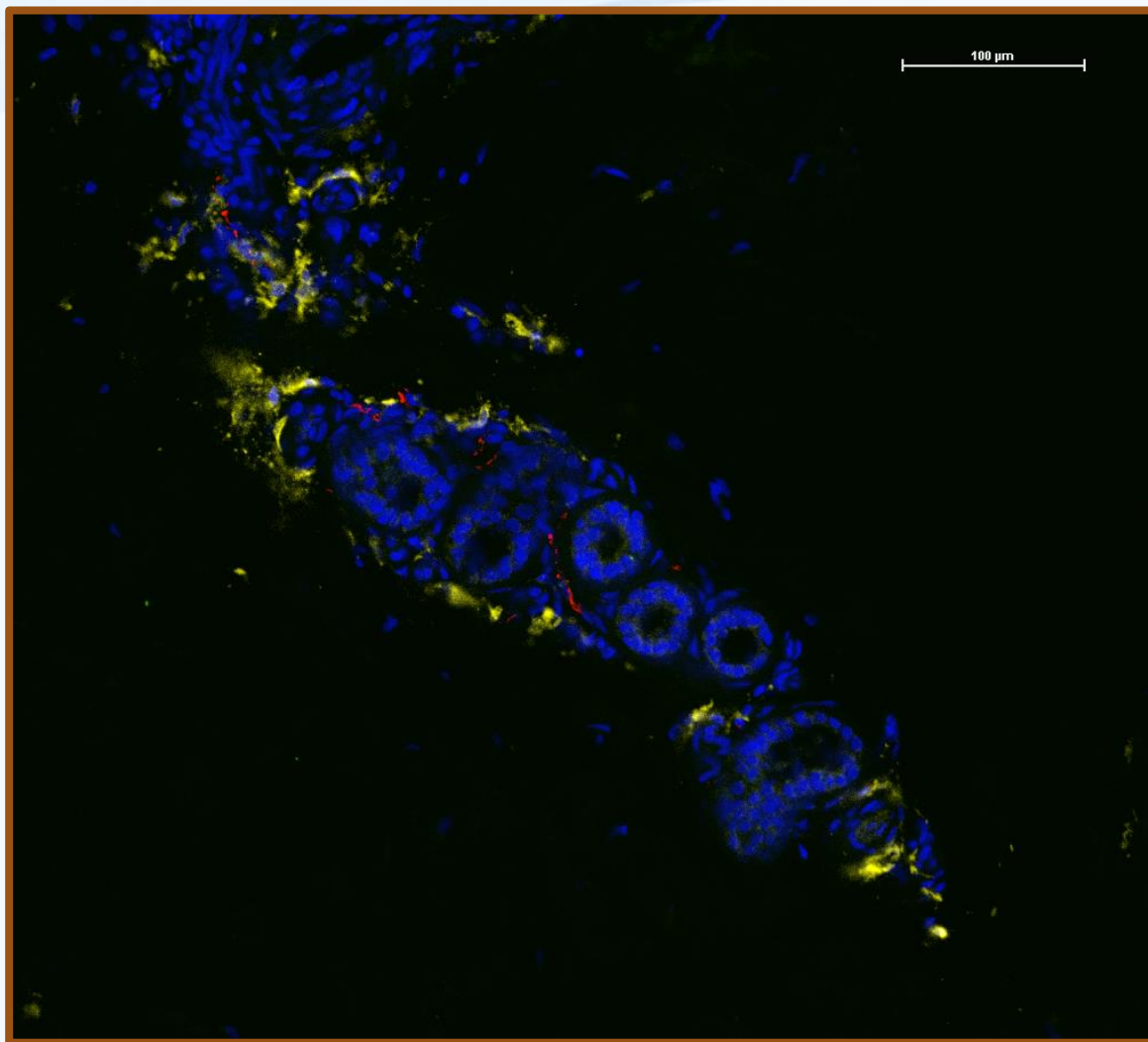
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Thank you!