Ethnic differences in genetic risk factors for painful HIV-associated sensory neuropathy

Peter Kamerman
Outline

I. HIV-SN background

II. Risk factors
   - Environmental
   - Polymorphisms in mitochondrial DNA
   - Polymorphisms in the “TNF Block”

III. Genetics of pain in HIV-SN
   - Polymorphisms in GCH1, KCNS1, “TNF Block”
Peripheral neuropathies in HIV infection

HIV disease course

**Seroconversion** → **Asymptomatic** → **AIDS**

**Rare**
- Inflammatory demyelinating polyneuropathies (AIDP/CIDP)
- Mononeuritis multiplex
- Polyradiculopathy

**Common**
- HIV-associated sensory neuropathy (HIV-SN)

Power et al., 2009
Prevalence of HIV-SN

Australia: Smyth et al., 2007; Malawi: Beadles et al., 2009; van Oosterhout et al., 2005; South Africa: Hitchcock et al., 2008; Maritz et al., 2010; Wadley et al., 2011; SE Asia: Affandi et al., 2008; Sithinamsuwan et al., 2008; Vivithanaporn et al., 2010; Wright et al., 2008; Uganda: Nakasujja et al., 2005; USA: Ellis et al., 2010; Simpson et al., 2006
Clinical characteristics

**Signs**
- Reduced:
  - Pin-prick sensitivity
  - Vibration sense
  - Reflexes
  - Temperature sense

**Symptoms**
- Pain
- Numbness
- Paraesthesias
Effect of painful HIV-SN on QoL

Ellis et al., 2010
## Treatment of painful HIV-SN

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pain relief superior to placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Topical lidocaine gel (5%)</td>
<td>Yes</td>
</tr>
<tr>
<td>Capsaicin cream (0.075%)</td>
<td>Yes</td>
</tr>
<tr>
<td>Capsaicin patch (8%)</td>
<td>?</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>Yes</td>
</tr>
<tr>
<td>Lamotrigine</td>
<td>Yes</td>
</tr>
<tr>
<td>Gabapentin / Pregabalin</td>
<td>Yes</td>
</tr>
<tr>
<td>Mexilitine</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Clifford et al., 2012; Phillips et al., 2010
SUMMARY

• HIV-SN is common

• It commonly is painful, and the pain can cause significant disability.

• The pain is difficult to treat
Risk of developing HIV-SN

- Phenotype
  - HIV-SN
  - Painful HIV-SN

Genes → Phenotype → Environment
Risk of developing HIV-SN

- Worsening HIV
- Treatment with d-drugs
- ↑ Age

Phenotype
- HIV-SN
- Painful HIV-SN
Risk of developing HIV-SN

Environment
- Worsening HIV
- Treatment with d-drugs
- ↑ Age

Phenotype
- HIV-SN
- Painful HIV-SN
Continued High Prevalence and Adverse Clinical Impact of Human Immunodeficiency Virus–Associated Sensory Neuropathy in the Era of Combination Antiretroviral Therapy

The CHARTER Study

Ronald J. Ellis, MD, PhD; Debralee Rosario, MPH; David B. Clifford, MD; Justin C. McArthur, MBBS, MPH; David Simpson, MD; Terry Alexander, RN; Benjamin B. Gelman, MD, PhD; Florin Vaida, PhD; Ann Collier, MD; Christina M. Marra, MD; Beau Ances, MD, PhD; J. Hampton Atkinson, MD; Robert H. Dworkin, PhD; Susan Morgello, MD; Igor Grant, MD; for the CHARTER Study Group

Arch Neurol. 2010;67(5):552-558

<table>
<thead>
<tr>
<th>Whole cohort (n = 1539)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-SN*</td>
<td>57%</td>
</tr>
<tr>
<td>Painful HIV-SN**</td>
<td>22%</td>
</tr>
</tbody>
</table>

* At least one sign of nerve deficit
** At least one sign and painful symptoms
Continued High Prevalence and Adverse Clinical Impact of Human Immunodeficiency Virus–Associated Sensory Neuropathy in the Era of Combination Antiretroviral Therapy

**The CHARTER Study**

Ronald J. Ellis, MD, PhD; Debralee Rosario, MPH; David B. Clifford, MD; Justin C. McArthur, MBBS, MPH; David Simpson, MD; Terry Alexander, RN; Benjamin B. Gelman, MD, PhD; Florin Vaida, PhD; Ann Collier, MD; Christina M. Marra, MD; Beau Ances, MD, PhD; J. Hampton Atkinson, MD; Robert H. Dworkin, PhD; Susan Morgello, MD; Igor Grant, MD; for the CHARTER Study

Arch Neurol. 2010;67(5):552-558

<table>
<thead>
<tr>
<th></th>
<th>Whole cohort (n = 1539)</th>
<th>No d-drug exposure (n = 741)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-SN*</td>
<td>57%</td>
<td>45%</td>
</tr>
<tr>
<td>Painful HIV-SN**</td>
<td>22%</td>
<td>15%</td>
</tr>
</tbody>
</table>

* At least one sign of nerve deficit

** At least one sign and painful symptoms
Risk of developing HIV-SN

- Worsening HIV
- Treatment with d-drugs
- ↑ Age

Genes

Environment

? -> Phenotype

• HIV-SN
• Painful HIV-SN
Ethnic differences in genetic risk factors for HIV-SN
Genetic association studies

Phenotypic variability

Genotyped polymorphism
(e.g., single nucleotide polymorphisms - SNPs)

Causative polymorphisms

Marker polymorphisms
Genetic association studies

Linkage disequilibrium (LD)

Measured marker SNP

Unidentified causative mutation

LD
Genetic association studies

Linkage disequilibrium (LD)

Recombination

Measured marker SNP

Unidentified causative mutation

LD weakened
Genetic association studies

Population-based genetic variation
Genetic association studies

*Population-based genetic variation*

Adapted from: Li et al., 2008
Genetic association studies

SNPs

Haplotypes

Adapted from Tegeder et al., 2006
Risk of developing HIV-SN

Pathogenesis of HIV-SN
- Mitochondrial dysfunction
- Immune dysfunction

Phenotype
- HIV-SN
- Painful HIV-SN

Hypothesis-driven
Candidate gene studies

Genes

Environment
- Worsening HIV
- Treatment with d-drugs
- ↑ Age
Mitochondrial dysfunction

Genetic studies

HIV-SN

Dysfunctional electron transport chain

- Reduced energy production
- Increased ROS synthesis
Mitochondrial dysfunction

Genetic studies

244 HIV+ non-Hispanic white patients on HAART


HIV-SN (Symptoms OR Signs)

Hulgan et al., 2005
- Adjusted OR for $T = 2.9$ (95%CI: 1.2-7.1), $p = 0.03$ [Freq: Cases = 17.1%, Controls = 6.7%]
- Adjusted for: age, sex, ddi + d4T use, protease inhibitor use, viral load, CD4 T-cell count
- No correction for multiple comparisons

Hulgan et al., 2005
Mitochondrial dysfunction

Genetic studies

156 HIV+ non-Hispanic African American patients on HAART

Genotyped mtDNA haplotypes:
L1a, L1b, L1c, L1/L2, L2a, L2b, L3, L3b, L3e

HIV-SN
(Symptoms OR Signs)

Canter et al., 2010
Mitochondrial dysfunction

- Adjusted OR for L1c = 3.7 (95%CI: 1.1-12.0), p = 0.03 [Freq: Cases = 17.7%, Controls = 6.7%]
- Adjusted for: age, sex, ddI + d4T use, protease inhibitor use, viral load, CD4 T-cell count
- No correction for multiple comparisons

Canter et al., 2010
Mitochondrial dysfunction

171 Black Africans
- Assessed for HIV-SN (at least one bilateral sign)
- mtDNA haplotypes
< 2% had L1 haplotype

Sinxadi et al., in press
SUMMARY

• Variations in mtDNA are associated with altered risk for developing HIV-SN

• There are significant ethnic differences in the markers that have been identified.
A possible role for TNF-α in HIV-SN?

Nagano et al., 1996; Pardo et al., 2001
A possible role for TNF-α in HIV-SN?

Perineural gp120 induces TNF-α expression in rats

Zheng et al., 2011a
A possible role for TNF-α in HIV-SN?

Systemic ddC induces TNF-α expression in rats

Zheng et al., 2011a
Immune dysfunction: *TNF Block*

Figure: Valente, 2008
Immune function: *TNF Block*

*Carriage of the rs1799964*^*2* and *rs9281523*^*2* increase risk of HIV-SN in **WHITES**

- SN ascertainment: at least one sign and one symptom
- n = 55, 16 with HIV-SN
- Adjusted for: age, height, sex, plasma lactate, viral load, CD4 T-cell count, HCV, Protease inhibitor use, duration of infection,

Cherry et al., 2008
Immune function: **TNF Block**

*Carriage of the rs1799964*2 increases risk of HIV-SN in **MALAY**

- SN ascertainment: at least one sign and one symptom
- $n = 99$, 33 with HIV-SN
- Adjusted for: age, height, sex, viral load, CD4 T-cell count, HCV, protease inhibitor use, efavirenz use, isoniazid use

Affandi et al., 2008
Immune function: *TNF Block*

*rs1799964*\(^{*}2\) is common to both **WHITES** and **MALAY**
Immune function: TNF Block

5 x 6-SNP TNF Block haplotypes include rs1799964*2

Valente et al., 2008; Chew et al., 2010
Immune function: **TNF Block**

TNFA haplotype FVa6,7,8 increases risk of HIV-SN in *MALAY* and *CHINESE* but not *WHITES*

- SN ascertainment: at least one sign and one symptom
- Adjusted for: age, height

Chew et al., 2010
What about Africans?
Immune function: **TNF Block**

404 Black African HIV+ out-patients
- Screened for HIV-SN (bilateral signs + symptoms)
- On ART > 6 months
- 100% exposure to d4T

64 excluded from genetic analysis
- Not of Southern African ancestry
- Other potential causes of SN
- Insufficient DNA extraction
- Failed genotyping

Risk factors:
- ↑ Age
- ↑ Height

n = 340 patients included in genetic analysis
Immune function: **TNF Block**

- Literature-based SNP selection
- TagSNP selection
  \((HapMap \#27, \text{YRI, pairwise tagging, } r^2 = 1, MAF > 0.01)\)

Corrected for:
- Age
- Height
- Multiple comparisons
Immune function: *TNF Block*

*No association between* rs1799964*2* or *rs9281523*2* and HIV-SN in AFRICANS*

<table>
<thead>
<tr>
<th>Gene</th>
<th>Marker</th>
<th>Minor allele freq (%)</th>
<th>HIV-SN</th>
<th>Control</th>
<th>P &gt; 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNFA</td>
<td>rs1799964</td>
<td>19</td>
<td>21</td>
<td>P &gt; 0.05</td>
<td></td>
</tr>
<tr>
<td>BAT1</td>
<td>rs9281523</td>
<td>5</td>
<td>3</td>
<td>P &gt; 0.05</td>
<td></td>
</tr>
</tbody>
</table>
Immune function: *TNF Block*

**Gene** | **SNP** | **Minor allele freq (%)** | **Odds ratio (dominant model)** | **p-value (multivariate)*** | **Carriage of minor allele is protective**
---|---|---|---|---|---
*NFKBIL1* | rs3130059 | 31 | 0.53 | 0.026 | ✓
*BAT1* | rs2071592 | 31 | 0.49 | 0.002 | ✓

* Corrected for age, height and multiple comparisons

A Wadley & L Hendry, *manuscript in preparation*
Immune function: *TNF Block*

Haplotype analysis indicates a 14kbp region around rs2071592

A Wadley & L Hendry, *manuscript in preparation*
SUMMARY

• Polymorphisms in the TNF Block are associated with altered risk of having HIV-SN

• There are significant ethnic differences in the markers identified.
Genetics risk factors for painful HIV-SN
Burden of painful HIV-SN

HIGH PAIN PREVALENCE

HIV sensory neuropathy

% patients reporting pain

HIV-SN: Ellis et al., 2010; Maritz et al., 2010; Smyth et al., 2007; Tagliati et al., 1999; Wadley et al., 2011.
Risk factors for painful HIV-SN

<table>
<thead>
<tr>
<th>Risk of pain</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑ risk</td>
</tr>
<tr>
<td>Previous d-drug use</td>
</tr>
<tr>
<td>↑ nadir CD4 T-cell count</td>
</tr>
<tr>
<td>Current major depressive disorder</td>
</tr>
<tr>
<td>↓ intra-epidermal nerve fibre density</td>
</tr>
<tr>
<td>↓ risk</td>
</tr>
<tr>
<td>Current d-drug use</td>
</tr>
</tbody>
</table>

Ellis et al., 2010

Figure: http://www.absolutefootcare.ca/
Risk factors for painful HIV-SN

Viral load: Simpson et al., 2002
IENFD: Polydefkis et al., 2002; Zhou et al., 2007
Catastrophizing: Lucey et al., 2011

Figure: http://www.absolutefootcare.ca/
Genetic risk factors for painful HIV-SN

404 Black African HIV+ out-patients
- Screened for HIV-SN (bilateral signs + symptoms)
- On ART > 6 months
- 100% exposure to d4T

64 excluded from genetic analysis
- Not of Southern African ancestry
- Other potential causes of SN
- Insufficient DNA extraction
- Failed genotyping

n = 340 patients included in genetic analysis
n = 333 patients included in genetic analysis

- n = 182 excluded
  - No neuropathy
  - Failed genotyping

n = 182 patients with HIV-SN
- 143 with pain [mean intensity: 5.2 (3.2) on 11-point NRS]

n = 340 patients included in genetic analysis

404 Black African HIV+ out-patients
- Screened for HIV-SN (bilateral signs + symptoms)
- On ART > 6 months
- 100% exposure to d4T

54 excluded from genetic analysis
- Not of Southern African ancestry
- Other potential causes of SN
- Insufficient DNA extraction
- Failed genotyping
Genetic risk factors for painful HIV-SN

n = 158 patients with HIV-SN

- Literature-based SNP selection
- TagSNP selection (HapMap #27, YRI, pairwise tagging, $r^2 = 1$, MAF > 0.01)

Corrected for:
- Age
- Height
- Sex
- CD4 T-cell count
- Multiple comparisons

Genotyping

SNP & haplotype analysis
Genetic risk factors for painful HIV-SN

- GCH1
- KCNS1
- TNF Block
Genetic risk factors for painful HIV-SN

GTP cyclohydrolase and tetrahydrobiopterin regulate pain sensitivity and persistence


Tegeder et al., 2006
Genetic risk factors for painful HIV-SN

GTP cyclohdyrolase and tetrahydrobiopterin regulate pain sensitivity and persistence

Irmgard Tegeder^{1,2,10}, Michael Costigan^{1,10}, Robert S Griffin^{1}, Andrea Abele^{2}, Inna Belfer^{3,4}, Helmut Schmidt^{2}, Corina Ehnhart^{1}, Jemiel Nejim^{5,9}, Claudiu Marian^{2}, Joachim Scholz^{1}, Tianxia Wu^{4}, Andrew Alchorne^{1}, Luda Dichteck{2}, Alexander M Binshtok^{1}, David Goldman^{1}, Jan Adolph^{1}, Swetha Sama^{5}, Steven J Atlas^{7}, William A Carlezon^{1}, Aram Parsegian^{8}, Jörn Lötsch^{2}, Roger B Fillingim^{6}, William Maixner^{2}, Gerd Geisslinger^{2}, Mitchell B Max^{4} & Clifford J Woolf^{1}

Polymorphisms in the GTP cyclohydrolase gene (GCH1) are associated with ratings of capsaicin pain

Claudia M. Campbell^{2}, Robert R. Edwards^{a,b}, Cheryl Carmona^{c}, Magdalena Uhart^{d}, Gary Wand^{d}, Alene Carteret^{c}, Yu Kyeong Kim^{6}, James Frost^{1}, James N. Campbell^{c,*}

Reduced hyperalgesia in homozygous carriers of a GTP cyclohydrolase 1 haplotype

Irmgard Tegeder^{a}, Jan Adolph^{a}, Helmut Schmidt^{a}, Clifford J. Woolf^{b}, Gerd Geisslinger^{a}, Jörn Lötsch^{a,*}


Cross-sectional Assessment of the Consequences of a GTP Cyclohydrolase 1 Haplotype for Specialized Tertiary Outpatient Pain Care

Alexandra Dochring, Ph.D.*, Rainer Freynhagen, MD,† Norbert Griessinger, MD,‡ Michael Zimmermann, MD,* Reinhard Stitt, MD,§ Nils von Heintz, MD,* Gerd Geisslinger, MD, PhD,* and Jörn Lötsch, MD*


Association of Guanosine Triphosphate Cyclohydrolase 1 Gene Polymorphisms with Fibromyalgia Syndrome in a Korean Population

Seong-Kyu Kim, Seong-Ho Kim, Seong-Su Nah, Ji Hyun Lee, Seung-Jae Hong, Hyun-Sook Kim, Hye-Soon Lee, Hyoun Ah Kim, Chung-Il Joung, Jisuk Bae, Jung-Yoon Choe, and Shin-Seok Lee

The Journal of Rheumatology 2013; 40:3
Genetic risk factors for painful HIV-SN

Molecular Pain

Research
Lack of influence of GTP cyclohydrolase gene (GCH1) variations on pain sensitivity in humans
Hyungsuk Kim and Raymond A Dionne*

Molecular Pain

Research
Does the pain-protective GTP cyclohydrolase haplotype significantly alter the pattern or severity of pain in humans with chronic pancreatitis?
Mark Lazarev¹, Janette Lamb¹, M Michael Baranda², Feng Dai³, Michelle A Anderson⁵, Mitchell B Max¹,³, David C Whitcomb *¹,²,⁴,⁶ for the NAPS2 Consortium
Genetic risk factors for painful HIV-SN

GCH1

Chromosome 14

55308726 bp 3’

rs10483639
rs7142517

5’ 55369570 bp

rs8007267

30 SNPs
- 8 literature-based SNPs (RED)
- 14 tagSNPs (PURPLE)
- 4 Literature + tagSNP (GREEN)

Wadley et al., 2012; Hendry et al., in press
Genetic risk factors for painful HIV-SN

30 SNPs
- 8 literature-based SNPs (RED)
- 14 TagSNPs (PURPLE)
- 4 Literature + TagSNP (GREEN)

Chromosome 14

No association with pain intensity

Wadley et al., 2012; Hendry et al., in press
Genetic risk factors for painful HIV-SN

Multiple chronic pain states are associated with a common amino acid–changing allele in KCNS1

Michael Costigan,¹,*  Inna Belfer,²,*  Robert S. Griffin,¹,*  Feng Dai,²  Lee B. Barrett,¹  Giovanni Coppola,³  Tianxia Wu,⁴  Carly Kiselycznyk,⁵  Minakshi Poddar,²  Yan Lu,⁶  Luda Diatchenko,⁷  Shad Smith,⁷  Enrique J. Cobos,¹  Dmitri Zaykin,⁸  Andrew Allchorne,¹  Pei-Hong Shen,⁵  Lone Nikolajsen,⁹  Jaro Karppinen,¹⁰  Minna Männikkö,¹⁰  Anthi Kelempisioti,¹⁰  David Goldman,⁵  William Maixner,⁷  Daniel H. Geschwind,³  Mitchell B. Max,²,†  Ze'ev Seltzer⁶,†  and Clifford J. Woolf¹,†
Genetic risk factors for painful HIV-SN

4 SNPs
- 3 TagSNPs (PURPLE)
- 1 Literature + TagSNP (GREEN)

Hendry et al., in press
Genetic risk factors for painful HIV-SN

Only taqSNP haplotypes associate with changes in pain intensity

<table>
<thead>
<tr>
<th>Haplotypes</th>
<th>rs4499491</th>
<th>rs6017486</th>
<th>rs6073643</th>
<th>Multivariate</th>
<th>Pain intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P-value*</td>
<td>P-value**</td>
</tr>
<tr>
<td>C</td>
<td>G</td>
<td>T</td>
<td></td>
<td>0.02</td>
<td>0.14</td>
</tr>
<tr>
<td>C</td>
<td>G</td>
<td></td>
<td></td>
<td>0.01</td>
<td>0.11</td>
</tr>
<tr>
<td>A</td>
<td>A</td>
<td></td>
<td></td>
<td>0.01</td>
<td>0.11</td>
</tr>
<tr>
<td>A</td>
<td>A</td>
<td>T</td>
<td></td>
<td>0.02</td>
<td>0.10</td>
</tr>
</tbody>
</table>

* Corrected for age and height
** Corrected for age, height, sex, CD4 and multiple comparisons

Hendry et al., in press
Genetic risk factors for painful HIV-SN

**TNF Block**

63 SNPs
- 33 Literature-based SNPs
- 30 tagSNPs
Genetic risk factors for painful HIV-SN

rs28445017*2 associates with increased pain intensity

<table>
<thead>
<tr>
<th>SNP</th>
<th>β-coefficient</th>
<th>p-value (multivariate)*</th>
<th>Increases pain intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rs28445017*2</td>
<td>2.08</td>
<td>0.04</td>
<td>✓</td>
</tr>
</tbody>
</table>

* Corrected for age, height, sex, CD4 and multiple comparisons
Genetic risk factors for painful HIV-SN

rs28445017*2 (A allele) associates with increased pain intensity
Genetic risk factors for painful HIV-SN

**TNF Block**

*rs28445017 is monomorphic in Whites*

<table>
<thead>
<tr>
<th>SNP</th>
<th>β-coefficient</th>
<th>p-value (multivariate)*</th>
<th>Increases pain intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs28445017</td>
<td>2.08</td>
<td>0.046</td>
<td>✓</td>
</tr>
</tbody>
</table>

* Corrected for age, height, sex, CD4 and multiple comparisons
SUMMARY

• Using a cohort of Black Africans, with HIV-SN, we have identified some novel markers of altered pain sensitivity
Conclusions

• Candidate gene studies of HIV-SN support the proposed mechanisms of HIV-SN

• Hypothesis-free GWAS studies may help identify novel contributing factors

• Further investigation of the genetics of HIV-SN-related pain is required to explain the high prevalence of pain in this neuropathy
Thanks to

University of the Witwatersrand (Johannesburg, South Africa)
Liesl Hendry
Antonia Wadley

Burnet Institute (Melbourne, Australia)
Kate Cherry

University of Western Australia (Perth, Australia)
Patricia Price

Funding:
National Research Foundation of South Africa
University of the Witwatersrand