Molecular Epidemiology of Full-Length Hepatitis C Virus Genomes in Recent Infection: The Inc3 Study

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Disclosures

• No conflicts of interests
• Funding of InC3 cohorts
  – NHMRC, Australia
  – NIH
Introduction

• Limitations in phylogenetic studies in HCV
  – Acute vs. late infection
  – Within a limited geographical region
  – Using segments of the genome

• This study
  – Acute infections (within 180 days)
  – Cross-continental
  – Full genome
InC3

• The International Collaboration of Incident HIV and Hepatitis C in Injecting Cohorts (InC$^3$)

  – Four countries
  – Nine cohorts
  – Over 30,000 blood samples
  – Longitudinal behavioural data of approximately 4,900 recruited subjects
Objectives

• To establish a cross-continental repository of full length deep sequenced early infection HCV sequences
• To establish the behavioural and demographic associations with phylogenetic clustering
• To compare the phylogenetic signal of full length genome vs. segments of the genome
Methods

• Selection of early viraemic samples
  – Within 180 days since estimated date of infection
    AND
  – Quantitative viral load > 1000 IU/ml
  OR
  – Qualitative RNA positivity
Experimental protocol – Wet Lab

RNA extraction

- qPCR
  - Genotyping if necessary
- cDNA
  - Full genome in one step
    - Yes: End protocol, purification and sequencing
    - No: Full genome in three steps
      - Yes: End protocol, purification and sequencing
      - No: Re-start protocol with ultracentrifugation of all remaining serum to precipitate the virus
Total number of recruited individuals: 4880

Total number of eligible participants: 543

Available number of samples: 368

Amplification of Partial genome: 23

Amplification of full genome: 192

Amplification failed: 153

Genotype 1a, 2b and 3a sequences used in cluster analysis: 180
Experimental protocol – Dry Lab

1. Generation of consensus sequences
2. ML phylogenetic trees with 1000 bootstrap replicates
3. Cluster definition with Phylopart
4. Behavioural and demographic associations with clustering
Results - Sequence repository

• Largest early HCV sequence repository in the world
• Largest genotype 3a full genome sequence repository in the world
• Largest genotype 1a and 3a full genome sequence repository for Australia
ML consensus tree for genotype 1a sequences with 1000 bootstrap replicates

Australia
North America
Combination
# Associations with clustering

<table>
<thead>
<tr>
<th>Did show a significant association</th>
<th>Did not show a significant association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age*</td>
<td>Gender</td>
</tr>
<tr>
<td>Continent of origin</td>
<td>Sexual orientation</td>
</tr>
<tr>
<td>Being imprisoned at the time of enrolment</td>
<td>Level of education</td>
</tr>
<tr>
<td>Ethnicity*</td>
<td>Past history of imprisonment</td>
</tr>
<tr>
<td></td>
<td>Type of drug injected</td>
</tr>
<tr>
<td></td>
<td>Being on opioid replacement therapy</td>
</tr>
<tr>
<td></td>
<td>HIV co-infection</td>
</tr>
</tbody>
</table>

*association persisted with binary logistic regression
Discussion

• Micro-epidemics in geographically isolated communities
  – Implications on a universal vaccine
• Phylogenetic diversity is added by few areas of the genome
  – Presence of immune epitopes in critical areas
Conclusions

• Established world’s largest early infection deep sequenced HCV sequence repository
• HCV probably evolves independently within geographically isolated communities
• Full genome offers a better phylogenetic signal than any of the genomic segments
Acknowledgements

• InC3 collaborators
  – Andrew Lloyd
  – Kimberly Page
  – Naglaa Shoukry
  – Julie Brunaueu
  – Gregory Dore
  – Jason Grebely
  – Lisa Maher
  – Andrea Cox
  – Janke Schinkel
  – Maria Prins
  – Georg Lauer
  – Arthur Kim
  – Mageret Hellard

• Institutions
  – School of Medical Sciences, UNSW
  – Kirby Institute, UNSW
  – ASHM

• Supervisors
  – Andrew Lloyd
  – Rowena Bull
  – Fabio Luciani
  – Auda Etahla

• InC3 participants

• Staff and students of Viral Immunology Systems Programme, Kirby Institute, UNSW