Recombination in RNA viruses

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Great diversity in viruses

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Mechanisms of viral diversity

3 main mechanisms that work in combination:

• Point mutations
  (frequent in RNA viruses ~1 mutation/10 virions - quasispecies)

• Recombination

• Reassortment

Source: WHO
Recombination in RNA viruses

- Initially RNA viruses were thought to undergo limited recombination

- Frequency ranges from $10^{-8}$ to $10^{-4}$ per site per generation

- Can have a major impact on virus evolution, emergence and epidemiology. Eg. Expand host range, evade host immunity, resistance to antivirals

- Occurs in many RNA virus families
Mechanism: template switch

Requires dual infection

Replication

Recombinant
Mechanisms of recombination

• In principle, recombination can occur in all RNA viruses (segmented and non-segmented)

• Factors influencing template switching include: RNA secondary structure, transcription kinetics and sequence identity between donor and recipient.
Recombination in RNA viruses

- Can have a major impact on virus evolution, emergence and epidemiology. Eg. Expand host range, evade host immunity, resistance to antivirals.
Recombination in Norovirus
Norovirus (NoV) Genome

- 27 - 32 nm
- Non-enveloped virus
- 7,400 - 7,700 nucleotides
Norovirus epidemics and pandemics

Researchers from the University of New South Wales and the Prince of Wales Hospital have warned of a virulent strain of gastroenteritis that has arrived in Australia. Parents have been warned that the disease could spread to childcare centres.

Experts predict hundreds of thousands of Australia could be affected by the virus, which they say is a more contagious form than the one previously circulating.

A gastroenteritis outbreak at a Newcastle hospital is the latest case in an epidemic sweeping Australia and the world, a NSW health expert says.

So far this month, New South Wales, Victoria, Queensland and Tasmania have experienced gastro outbreaks in hospitals, nursing homes and retirement villages.

Yesterday, authorities banned family and friends from Newcastle's Calvary Mater Hospital, where 80 staff and patients have experienced complications associated with the airborne virus.

The hospital was closed to visitors as a precaution and would remain closed until Friday.

WASHINGTON (AFP) - The US government is warning for about three days. No new cases have been reported at the NSW health facility.

Spread by air, water and personal contact, noroviruses are highly contagious and can survive in food, water and the environment for long periods, according to Dr White.
Norovirus epidemics in Sydney

- **Farmington Hills virus (2002)**
- **2006a virus (2006)**
- **2008 virus (2007)**
- **2009 virus (2006)**
- **2009 virus (2007)**
- **2010 virus (2008)**
- **2012 virus (2010)**
- **2012 virus (2008)**

Timeline:
- 1996
- 1998
- 2000
- 2002
- 2004
- 2006
- 2008
- 2010
- 2012
Phylogenetic tree of NoV GII capsid
Investigate Mechanisms of GII.4 Dominance

- Two mechanisms used to evolve:

1. Nucleotide substitution
   - Antigenic Drift

2. Recombination
   - Antigenic Shift
   - different regions of genome originate from different viruses
NoV Rate of Evolution

- Number of changes plotted against time elapsed between that strains detection and the origin strain

- Evolution rate (substitutions/capsid gene/yr) is equivalent to the gradient of the line

- GII.4 = 6.30 ± 0.39, r² = 0.84
- GII.b = 4.03 ± 0.80, r² = 0.68
- GII.3 = 3.09 ± 0.95, r² = 0.49
- GII.7 = 3.82 ± 0.25, r² = 0.99
Evolution Hotspots in the Capsid

- Variation hotspots in the P2 domain
- Greater immune escape in GII.4
NoV recombination common

Polymerase

Saitama U25
  | 100 |
  |     |
  V 207
  | 100 |
  Gwynedd
  |     |
  Saitama U3
  |     |
  Baltimore
  | 100 |
  Saitama U16
  |     |
  Saitama U1
  |     |
  Lordsdale
  |     |
  Bristol
  |     |
  Camberwell
  |     |
  Farmington
  | 85 |
  Miami Beach 326
  |     |
  US95/96
  | 100 |
  Crete
  |     |
  Snow Mountain
  |     |
  OTH 25
  | 100 |
  Toronto
  |     |
  New Orleans 279
  |     |
  Mexico
  |     |
  Lionville
  |     |
  oc98008
  |     |
  Saitama U201
  |     |
  0.1

Capsid

Saitama U25
  | 100 |
  Virginia 207
  | 100 |
  Gwynedd
  |     |
  Saitama U3
  |     |
  Baltimore
  | 100 |
  Saitama U16
  |     |
  Saitama U1
  |     |
  Lordsdale
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  |     |
  Mexico
  |     |
  Lionville
  |     |
  oc98008
  |     |
  Saitama U201
  |     |
  0.1
Simplot – recombination tool

![Graph showing nucleotide identity and genome position with ORF 1 and ORF 2 highlighted.]

- Mc37
- GII.4 prototype
- GII.10 prototype

Nucleotide Identity

Genome Position

ORF 1

ORF 2
Recombination hotspot at ORF1/ORF2 overlap
Does recombination have a role in the evolution of epidemic NoVs?

3 methods
- chi square method
- simplot
- phylogenetic analysis
Maximum likelihood phylogenies of the NoV GII.4 genome.

SimPlots for all putative NoV GII.4 recombinants analyzed in this study.

Recombination breakpoints identified across the full-length genome in the NoV GII.4 lineage.

Model for the emergence and origin of the NoV GII.4 variants.

Recombination analysis in the NGS era
Hepatitis C Virus

- Major cause of hepatitis
- Most common reason for liver transplant (~50% in Australia and USA)
- Predominantly transmitted via intravenous drug use (IDU)
HCV Highly Diverse Genotypes - World View

(a) Map showing the distribution of different genotypes of HCV around the world.

(b) Phylogenetic trees comparing HIV-1 and HCV genotypes.
HCV - Mixed infection cohort

Inc 1M 2M 3M 6M 9 12M 18M 24M 30M 36M 42M 48M 54M 60M 66M 72M

- 300212
- 300001
- 300002
- 300023
- 300031
- 300062
- 300081
- 300086
- 300089
- 300097
- 300101
- 300117
- 300138
- 300144
- 300157
- 300168

Subtype 2a
Subtype 2b
Subtype 6a
Subtype 1a
Subtype 3a

Second infection with same genotype
Third infection with same genotype
Negative timepoint

Third infection with same genotype

Third infection with same genotype

Subtype 3a
NGS powerful tool for detection of multiple infection

HCV genome position (nt)

Coverage (GT1a)
Coverage (GT3a)
Multiple infection or recombination

Reference aligned (against prototype)

De novo aligned
Recombination in HCV

• Intergenotype recombination has been reported to occur within envelope region

• Unknown how frequent

• New antivirals delivered in combination on the market

• Recombination could facilitate antiviral resistance

• Multiple infection common – but is recombination?

• New tools for analysing intrahost recombination needed.
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