Trachoma strains in Indigenous Australian populations are variants of urogenital Chlamydia trachomatis.

Phil Giffard

Genesis of study

- Approach: from Northern Territory Government, Sexual Assault Referral Centre (SARC)
  - SARC responsibilities include:
    - Clinical examination of possible abuse victims
    - Advise child protection and law enforcement authorities

SARC’s problem:
- Instances of Chlamydia trachomatis detection in children with no other evidence indicating sexual abuse e.g. disclosure.
  - What does one conclude?
  - Local guidelines: “STI, (the presence of an STI in a preadolescent is most likely the result of sexual abuse and formal assessment should always be initiated)”
  - What does “most likely” mean, numerically?

- Consequences of wrong call are serious.
  - Other conceivable explanations besides sexual abuse? Opinions differ.

The sociopolitical context

- 2007 ‘Little Children are Sacred” report
- 2007 “National Emergency Response” from Australian Commonwealth Government:
  - ‘The Intervention
- Subsequent ‘Stronger Futures” program:
  - More police
  - More community services
  - Controls on pornography and alcohol
  - Welfare quarantining measures
  - Suspension of racial discrimination act

- Highly controversial and polarizing
- Real extent of sexual abuse unclear
- Investigation of possible sexual abuse in Indigenous communities: socially and politically highly charged

Basis of overarching experimental design

- If child sexual abuse is inferred from presence of STI, then….
  - an STI test is a diagnostic test for sexual contact
  - Positive diagnostic test in absence of sexual contact
    - False positive

- Conceivable mechanisms of false positivity tested experimentally to determine frequency.

- Outputs: confidence limits on false positive frequencies
  - Positive predictive value: needs abuse prevalence in tested population, and sensitivity of STI diagnosis for detecting sexual contact.

Knowledge gaps

A C. trachomatis positive urine specimen could arise from autoinoculation from an ocular infection to the urogenital site.

- Knowledge gap: Are “trachoma strains” of C. trachomatis ever seen in urogenital specimens?
  - [Study complete, but not being presented here]
  - This question generates another....

- Knowledge gap: So, just what is a trachoma strain in Australia?
  - Nearly all evidence regarding ocular strain tropism is from overseas, primarily Africa.

- This presentation: first genome analysis of Australian trachoma strains of C. trachomatis.

One conceivable event that could give rise to false positives

Autoinoculation/contamination/infection of the urogenital site with C. trachomatis material from ocular infection.

This is seen as plausible in areas in which trachoma remains endemic.
C. trachomatis tropism

- Serovars defined by Momp/ompA
- Immunodominant cell surface protein
  - Trachoma: Serovars A, B, Ba, C
  - STIs, non-trachoma ocular infections: Serovars D, E, F, G, H, Ia, I, K
  - Invasive STIs: Serovars L1, L2, L3
  - Most or all of non-trachoma serovars able to cause conjunctivitis (adult or perinatal)

- MLST and whole genome studies to date have indicated that the “trachoma strains” form a monophyletic lineage.

We set out to revisit this

- Ensure we were looking for the right strains
  - Previous studies were quite small

- Readily available material
  - Mother-Child Study

Mother-child study

- Performed in 1980s-90 by Menzies researchers
- Unique C. trachomatis survey of children’s eyes and mothers’ UGT, in Top End communities
  - Snap shot of co-existing ocular and UG C. trachomatis serovar proportions

<table>
<thead>
<tr>
<th>Sample</th>
<th>UG serotype</th>
<th>Age (years)</th>
<th>Trachoma grading</th>
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</thead>
<tbody>
<tr>
<td>Aus25</td>
<td>Ba</td>
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<td>F, F, C</td>
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<td>B</td>
<td>9.35</td>
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</table>

Five frozen Mother-child study isolates were revived into culture.

Grown in Ian Clarke’s lab, University of Southampton.
So, where do they fit in the C. trachomatis phylogeny?

THE AUSTRALIAN TRACHOMA ISOLATES ARE NOT IN THE CLASSICAL TRACHOMA LINEAGE

Recombination boundaries near ompA defined:
Events leading to “B” and “C” lineages involve ~“ompA only”

Recombination of “Ba” ompA variant involves a larger piece of DNA.

Can recombination between the Australian isolates and the classical trachoma lineage be identified anywhere else in the genome?

- Searched for where our isolates more similar to ocular lineage than other lineages.
- 1000 bp window

- Only one locus identified: pmpEFGH
  - Novel sequence in Ba and C isolates
  - Elevated similarity with classical ocular lineage.
  - Suggests recombination involving unknown strain allied to classical ocular lineage.

- No non-ompA recombined loci identified in the “B” Australian isolates.

OmpA sequences

<table>
<thead>
<tr>
<th>OmpA genotype</th>
<th>Closest GenBank match</th>
<th>Nucleotide change</th>
<th>Amino acid change</th>
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<td>G586A</td>
<td>Val 196 Ile</td>
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</table>

- Correspond with ompA genotype H
- Observed previously in trachoma strain from Australia
Evidence for recombination at pmpEFGH locus

Genome wide association study on orthologous SNPs failed to identify additional loci associated with ocular tropism.

B-Jali reference

Genome wide association study on orthologous SNPs failed to identify additional loci associated with ocular tropism.

pmpE

pmpF

pmpG

pmpH
Trp operons of Australian isolates:
Typical for UGT strains

Colour of rectangle indicates
the operon sequence

AusB identical to most T2 genotype H
isolates and diverse T1 UGT isolates

AusBa and AusC identical to F_70.

Tarp genes also, consistent with
geno-m-wide phylogeny

Closest relatives to Aus B isolates at TarP

Closest relatives to Aus Ba and AusC isolates at TarP

A hint of involvement of pmpEFGH in
tropism has been seen before...

- Isolate TW-448: "trachoma" isolate from Taiwan
  - Genotype Da
  - Arguably only convincing non-A,Ba,C,D trachoma isolate ever

- Subjected to expanded MLST (Nunes, A., Borrego, M.J. & Gomes, J.P. Genomic features beyond Chlamydia trachomatis phenotypes: what do we think we know? Infect Genet Evol 16, 392-400 (2013).)

- Has identical pmpEFGH locus to TW-3: "Genotype C" trachoma isolate from Taiwan

- We think that both ompA and pmpEFGH can contribute to anatomical tropism.

Conclusions

- The model of a monophyletic ocular lineage of C. trachomatis is disproved
  - Lineage appears to be sampling artefact
  - Australian genotype Ba and Genotype C:
    - Closest relatives, in T1 lineage: F_SotonF3, F_Sw6, F_Sw4, D_SotonD1, Ds_2923
    - Appearance of having acquired ompA and pmpEFGH from "classical ocular lineage"
    - Separate ompA recombination events for Ba and C.

- Australian genotype B:
  - Closest relatives, in T2 lineage: G_SotonG1, D_UW3_Cx, D_SotonD5, D_SotonD6, H_R31975, K_SotonK1
  - Appearance of having acquired ompA from classical ocular lineage

- The association between ompA allelic state and tropism is not disproved
  - ompA based genotyping can be used to look for trachoma serovars in UGT samples in Australia

- Ockham’s Razor suggests that ompA and pmpEFGH confer/assist tropism.

- No sign of selection for mutation in trp operon or Tarp gene in Australian isolates.

Stop Press: Mother child study UGT B’s
virtually identical to trachoma B

Red = trachoma isolate or isolate of unknown anatomical source
Blue = UGT isolate

Is this intermediate tropism consistent with acquiring ocular ompA and not acquiring ocular pmpEFGH?

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