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 polarising
•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.

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.

Knowledge gaps

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

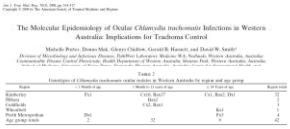
- **Knowledge gap:** Are "trachoma strains" of *C. trachomatis* ever seen in urogenital specimens?
  - (Study complete, but not being presented here)
  - This question generates another…..
- **Knowledge gap:** So, just what is a trachoma strain in Australia?
  - Nearly all evidence regarding ocular strain tropism is from overseas, primarily Africa.
- **This presentation: first genome analysis of Australian trachoma strains of *C. trachomatis.***

C. trachomatis and tropism

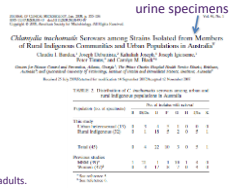
- Serovars defined by Momp/*ompA*
- Immunodominant cell surface protein
  - **Trachoma:** Serovars A, B, Ba, C:
  - **STIs, non-trachoma ocular infections**: Serovars D, E, F, G, H, Ia, J, K
  - **Invasive STIs:** Serovars L1, L2, L3:
  - **Most or all of non-trachoma serovars able to cause conjunctivitis (adult or perinatal)**
- **MLST and whole genome studies to date have indicated that the "trachoma strains" form a monophyletic lineage.**

C. trachomatis tropism in remote Australia looks similar to elsewhere… if you look at ompA


Characterization of *Chlamydia trachomatis ompI* Genotypes Detected in Eye Swab Samples from Remote Australian Communities


The Molecular Epidemiology of Ocular *Chlamydia trachomatis* Infections in Western Australia: Implications for Trachoma Control

urine specimens


Chlamydia trachomatis Serovars among Strains Isolated from Members of Rural Indigenous Communities and Urban Populations in Australia

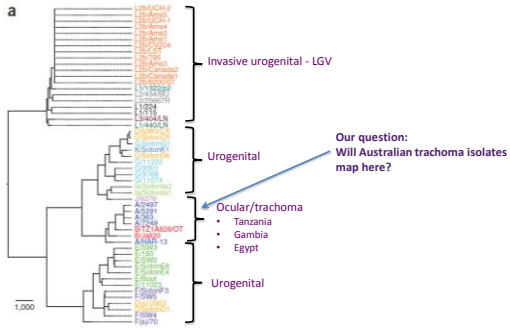
Summary of findings from ocular swabs:
- Serovars C, Ba
- No serovars A or B
- As expected, some urogenital strains in neonates and late teens/adults.

Urogenital specimens: "normal"

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

Phylogeny of C. trachomatis from genome-wide SNPs



Our question:
Will Australian trachoma isolates map here?

Harris SR *et al.* Nature Genetics, 2012

Mother-child study

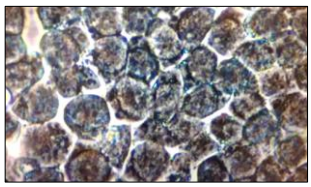
- Performed in 1980s-90 by Menzies researchers
- Unique *C. trachomatis* survey of children's eyes and mothers' UGT, in Top End communities
  - Snap shot of co-existing ocular and UG *C. trachomatis* serovar proportions

cmnty	B eye/naso	B UGT	C eye/naso	C UGT	Ba eye/naso	Ba UGT	UGT serovar, eye/naso	UGT serovar, UGT
1	24 + 4 7 trachoma	2	0	0	11 + 4 4 trachoma	0	3	2
2	0	2	14 8 trachoma	0+1?	0	0	0+1	2
3	0	5+3	0	0	0	0	1	5+3
4	0	4+2	8 3 trachoma	0	0	0	1	10+2
5	0	0	0	0	0	0	0	1
6	0	1	0	0	0	0	0	0

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

Sample no	Orig. serotype	Age (years)	Trachoma grading
Aus25	Ba	0.51	$F_1 P_0 C_0$
Aus28	B	0.73	$F_0 P_1 C_0$
Aus30	C	1.22	$F_1 P_2 C_0$
Aus33	C	1.21	$F_0 P_2 C_0$
Aus36	B	9.25	$F_1 P_2 C_0$

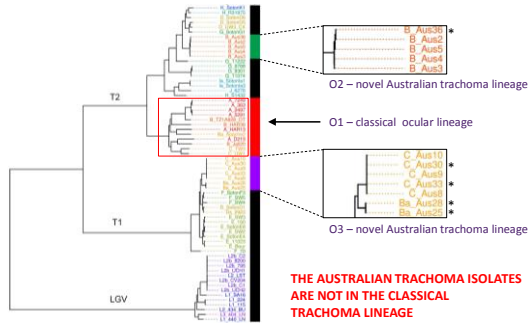
Grown in Ian Clarke's lab, University of Southampton



Genome sequenced at the Wellcome Trust Sanger Institute

So, where do they fit in the *C. trachomatis* phylogeny?

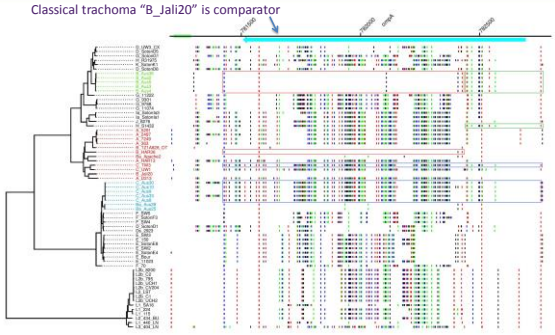
….. Not with other ocular strains



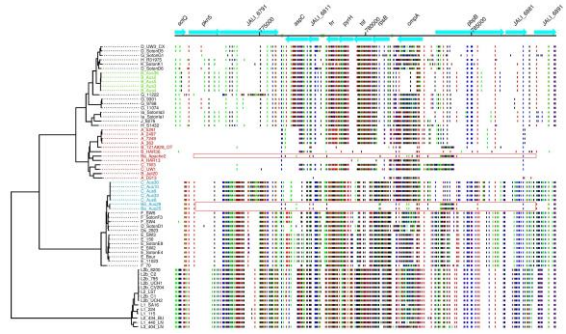
THE AUSTRALIAN TRACHOMA ISOLATES ARE NOT IN THE CLASSICAL TRACHOMA LINEAGE

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

Classical trachoma "B_Jali20" is comparator



Recombination of "Ba" ompA variant involves a larger piece of DNA.



OmpA sequences

ompA genotype (n)	Closest GenBank match	Nucleotide change	Amino acid change
B (5)	B Tunis864 (DQ064280)	C129T#	synonymous
		A154G#	Thr 52 Ala
		A184G#	Met 62 Val
		G186T*	Met 62 Val
		T195C#	synonymous
		T198A#	synonymous
		A228T#	synonymous
		C246T*	synonymous
		A249G#	synonymous
		G586A	Val 196 Ile
Ba (2)	Ba Apache2 (AF063194)	A511G*	Ser 171 Gly
		C662T*	Pro 221 Leu
C (5)	C TW3 (AF352789)	T569C*	Ile 190 Thr
		A571G*	Asn 191 Asp
		G972A*	synonymous
		G1003T*	Ala 335 Ser
		A1063C*	Met 356 Leu

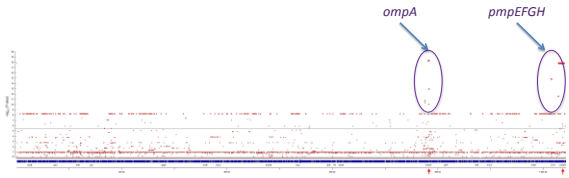
#Correspond with *ompA* genotype H
*Observed previously in trachoma strains from Australia

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

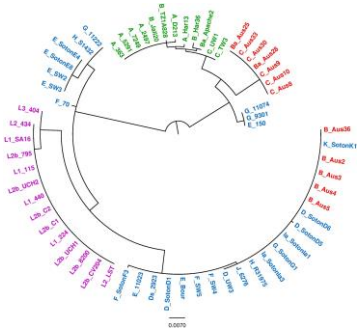
- Searched for where our isolates more similar to ocular lineage than other lineages.
  - 1000 bp window
- **Only one locus identified: *pmpEFGH***
  - Novel sequence in Ba and C isolates
  - Elevated similarity with classical ocular lineage.
  - Suggests recombination involving unknown strain allied to classical ocular lineage
- No non-*ompA* recombined loci identified in the "B" Australian isolates.

Evidence for recombination at pmpEFGH locus

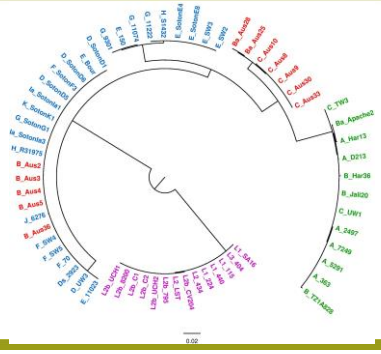
Genome wide association study on orthologous SNPs failed to identify additional loci associated with ocular tropism.



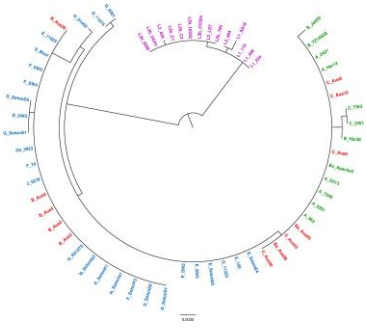
pmpE



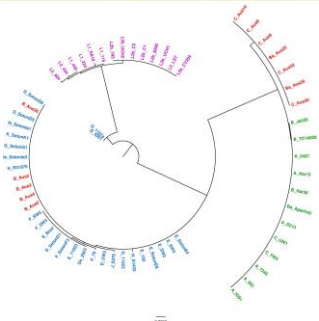
pmpF



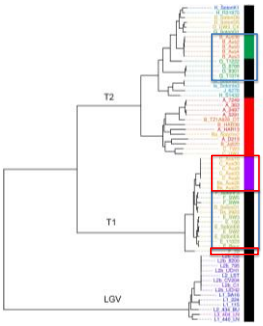
pmpG



pmpH



Trp operons of Australian isolates: Typical for UGT strains

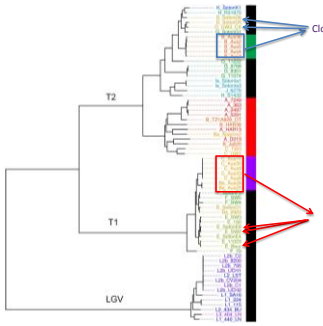


Colour of rectangle indicates *trp* operon sequence

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 genome-wide phylogeny



Closest relatives to Aus B isolates at *TarP*

Closest relatives to Aus Ba and AusC isolates at *TarP*

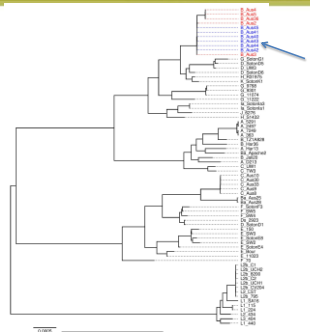
A hint of involvement of pmpEFGH in tropism has been seen before…

- Isolate TW-448: "trachoma" isolate from Taiwan
  - Genotype Da
  - Arguably only convincing non-A,Ba,C,D trachoma isolate… ever
  - Yeh, L.J. et al. Studies of trachoma in families on Taiwan. Zhonghua Min Guo Wei Sheng Wu Xue Za Zhi 8, 120-32 (1975).
- 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:**
    - Form monophyletic group
    - closest relatives, in T1 lineage: F-SotonF3, F_SW-5, F_SW-4, 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*?

ACKNOWLEDGEMENTS

Patiyan Andersson, Susan Hutton, Joanne Bex, Fiona Douglas, Valerie Asche, John Mathews, Steven Tong, Rachael Gorie, Nicole Brenner, Deborah Holt.

Nick Thomson, Simon Harris, Helena Seth Smith , James Hadfield

Ian Clarke, Colette O'Neill , Lesley Cutcliffe

NHMRC project grants 1004123, 1022782
The Wellcome Trust grant 098051

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