# CHLAMYDIA DIAGNOSTICS AND ISSUES OF TREATMENT FAILURE

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## *ACTS INVESTIGATORS: THIS AIM*
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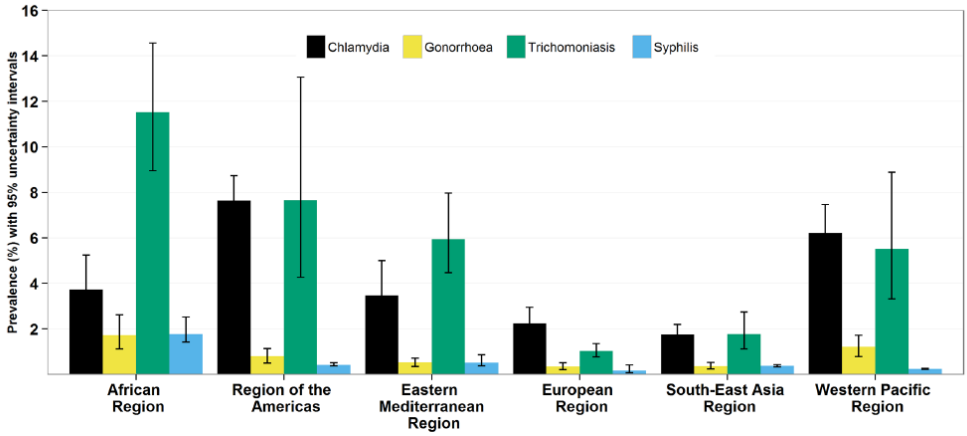
Samuel Kroon

# *CHLAMYDIA TRACHOMATIS* – MOST COMMONLY REPORTED BACTERIAL STI WORLDWIDE




Newman *et al.*, PLoS One 2012

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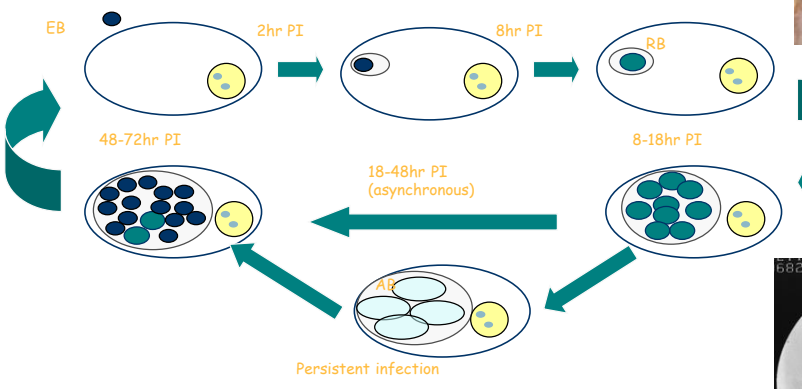
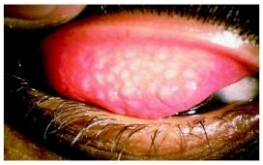
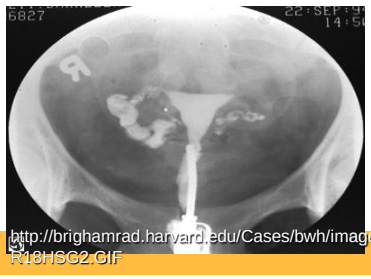
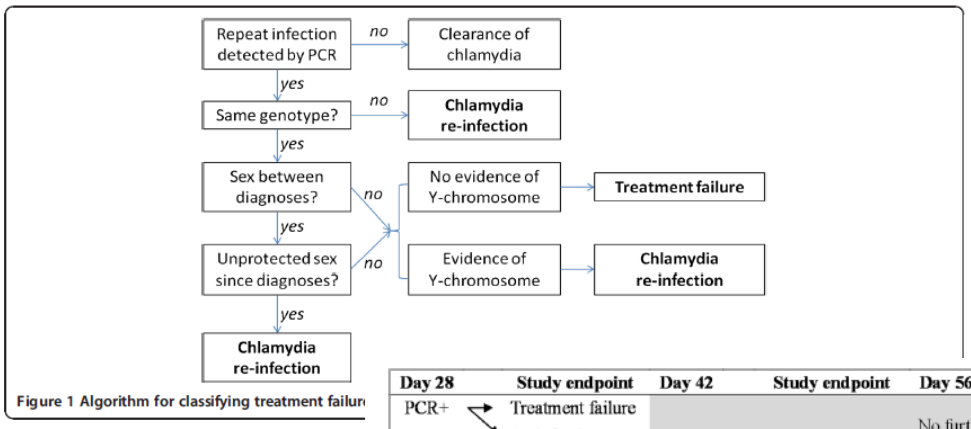


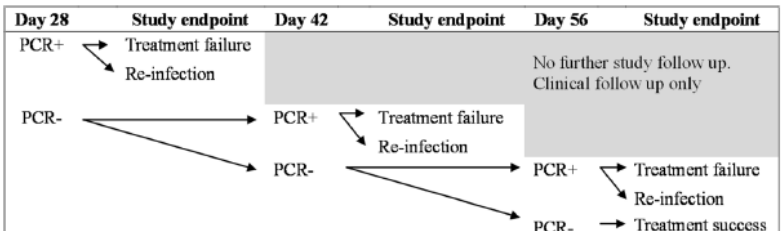


http://brighamrad.harvard.edu/Cases/bwh/images/5R18HSG2.GIF

# AUSTRALIAN CHLAMYDIA TREATMENT STUDY




Hocking *et al*., 2012




# MECHANISMS OF TREATMENT FAILURE – IS ANY OF THIS DRIVEN BY THE GENOTYPE OR PHENOTYPE OF THE ISOLATES?

1. Do treatment failure isolates have resistance?
2. Is there a certain serovar that treatment failure comes from?
3. Do treatment failure isolates fail treatment by persistence?
4. Is resistance mediated by genotypic heterogeneity that we can only see *in vivo*?

# TREATMENT FAILURE GENOTYPE?

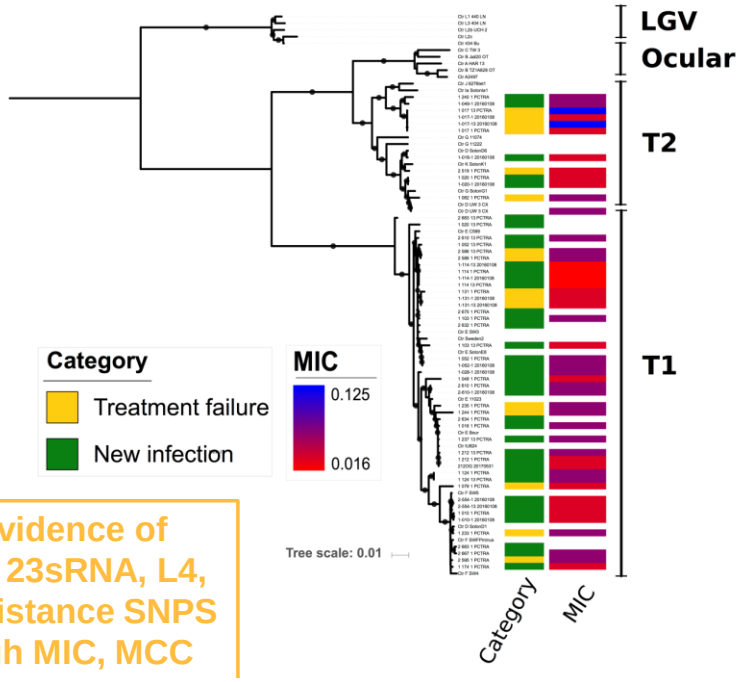
Cultured isolates

Enriched and extracted chlamydial

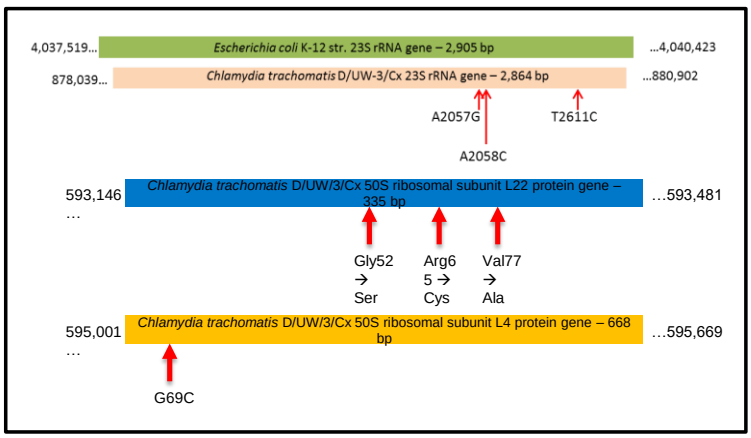
Or direct from swabs- seq capture

Illumina miseq Assembled genomes

- phylogeny,
- Resistance SNPS
- MIC




**No evidence of classic 23sRNA, L4, Lon resistance SNPS No high MIC, MCC**



## TREATMENT FAILURE
## GENOME WIDE ASSOCIATION ANALYSES

- 74 genomes (43 NI (32 Pcap); 25 TF (24 Pcap);
- 25 early (1) and late (13) pairs were sequenced
- 6 single samples (pair was not sequenced)
- In general the late isolate (13) had lower average reads.

3 Polymorphisms associated with Treatment failure

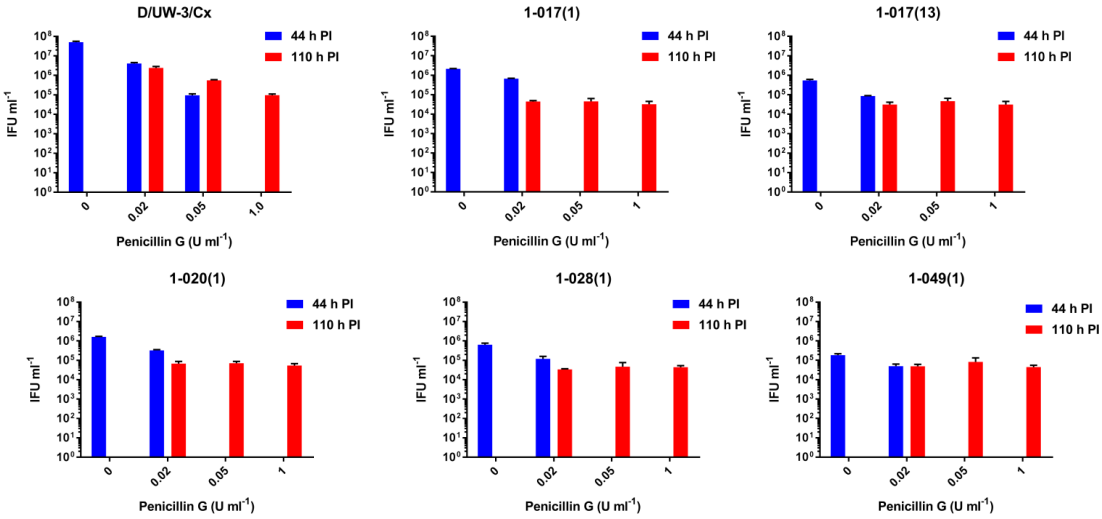
CT_328 – T3SS effector- 50SrRNA bp 308

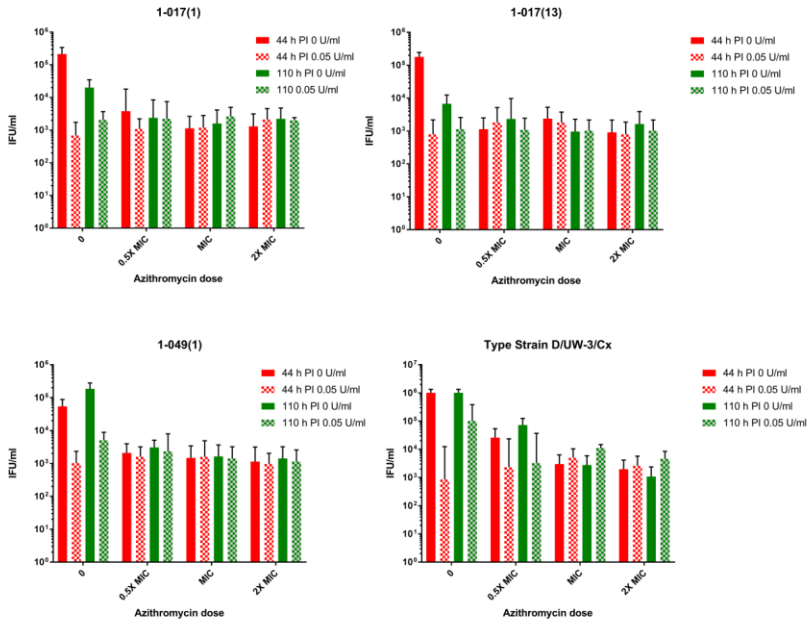
CT_619 base 1161 G (A) – Serine – Serine

CT_108 base 608 C (T) Valine to alanine

**Heterogeneity?????? Population Dynamics??**

## IS TREATMENT FAILURE ACTUALLY CHLAMYDIAL PERSISTENCE?



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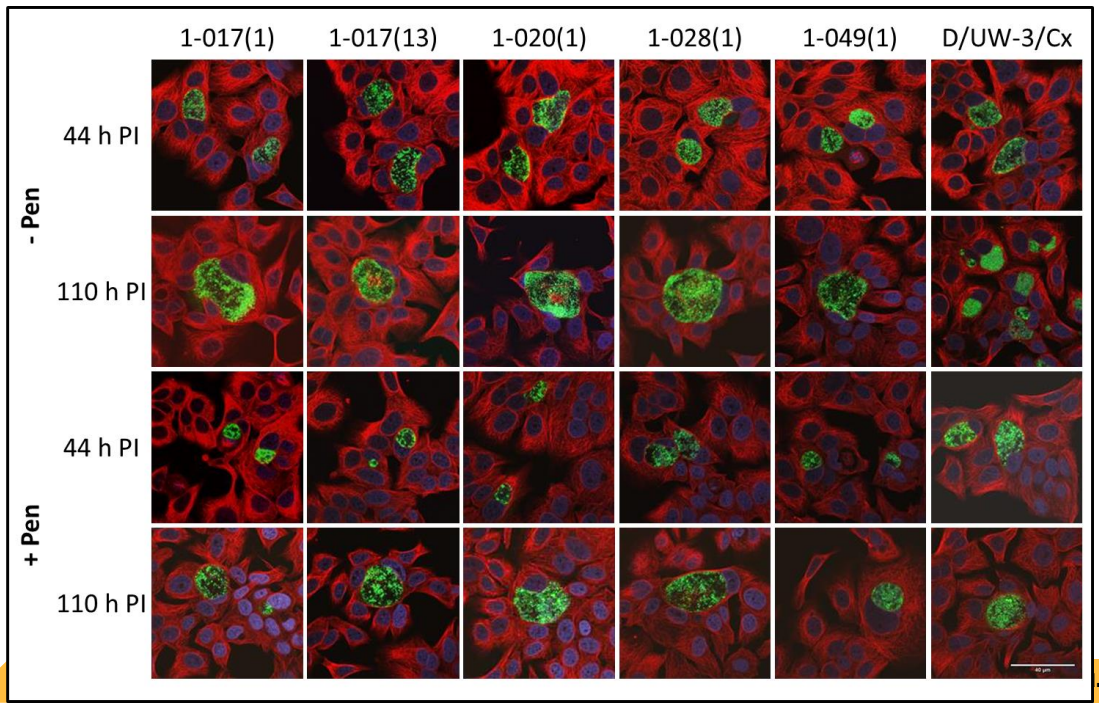


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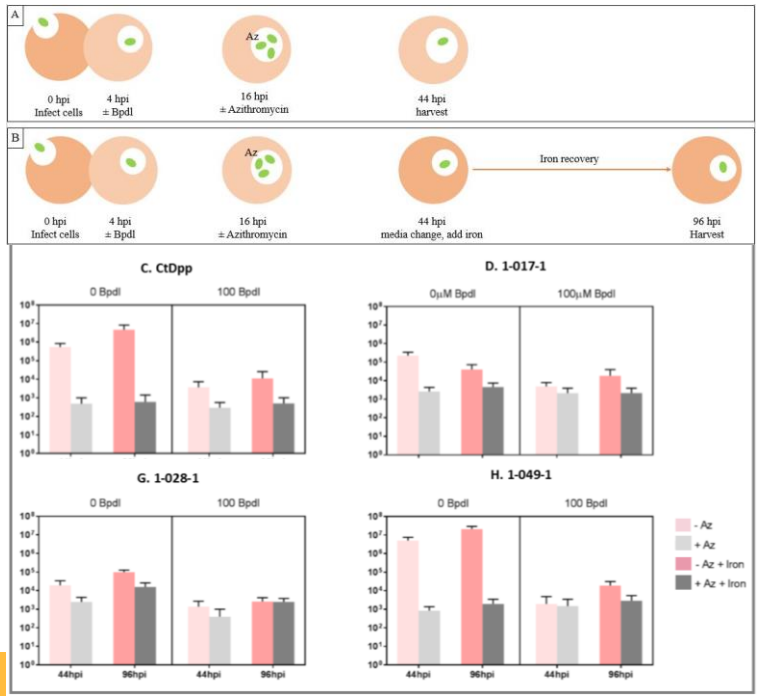
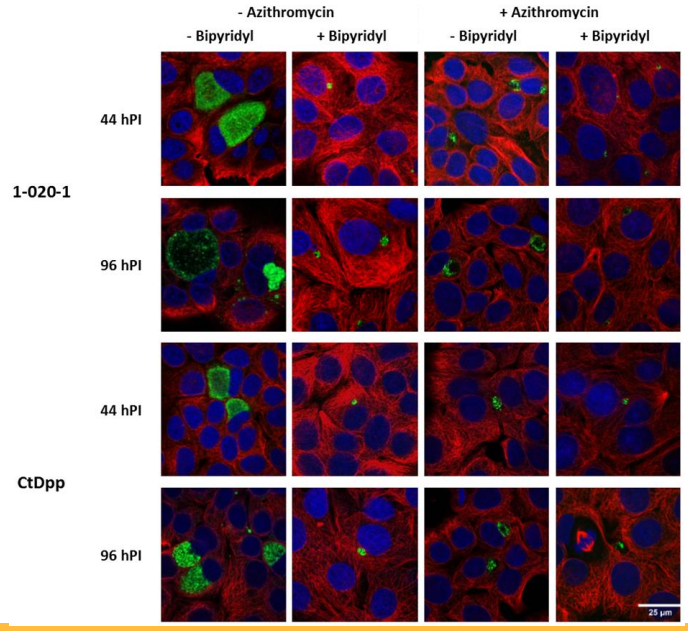
# IRON DEPRIVATION PERSISTENCE?

Iron deprivation persistence showed

High variability in clinical isolates

Azithromycin efficacy not impacted



## Chlamydial treatment failure is occurring in low frequency in women

- No evidence of resistance (direct or indirect)
- Little evidence of genotypic correlates
- Little evidence of persistence correlates
  - Either iron deprivation or penicillin
  - BUT persistence thresholds show huge variation

**THANK YOU**



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Genomic and phenotypic characterisation of Chlamydial isolates from women that failed to respond to azithromycin treatment.

Chlamydia trachomatis is an obligate intracellular pathogen with approximately 131 million cases of sexually transmitted infection each year. The infection can result in serious sequelae such as pelvic inflammatory disease, infertility, or ectopic pregnancy. Recently, using a cohort study design the investigators on the Australian Chlamydia Treatment Study have identified that more women than originally thought fail to resolve the infection after treatment with azithromycin. The isolates have been cultured and characterised and after culture no evidence of a higher MIC to azithromycin was detected. However, sequence capture analysis and immediate culture in the presence of azithromycin from the swab suggests that the in vivo chlamydial population is more dynamic and distinct than what we can culture in the laboratory. The possible role of chlamydial persistence, specific genomic loci in members of the population and population dynamics all appear to differ in the Chlamydia from the women who failed treatment compared to women who resolved the infection after azithromycin treatment. Here, we will present the first genomic analysis of chlamydia in women who failed treatment, and the first characterisation of persistence and growth phenotypes of isolates associated with treatment failure and present our model for how treatment failure occurs in Chlamydia.

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## GENOME WIDE ASSOCIATION ANALYSES

**GWAS**

Performed using a pipeline called **SEER** (Lees et al. 2016)

Identifies sequences (*k*-mers) of 10-120bp that are enriched in groups of strains with a phenotype of interest.

Population structure is taken into account.

- 74 genomes (43 NI (32 Pcap); 23 TF (24 Pcap);
- 25 early (1) and late (13) pairs were sequenced
- 6 single samples (pair was not sequenced)
- In general the late isolate (13) had lower average reads.

Settings used in final run:

**120bp long k-mers**
**11 TF isolates vs 61 NI isolates**
**Assemblies generated using metaSPAdes.**
**Where the TF isolates suggested an ambiguous phylogeny, I selected the late (13) isolate.**
**Enriched *k*-mers have to be present in at least 5% of the dataset (i.e at least 4 genomes)**

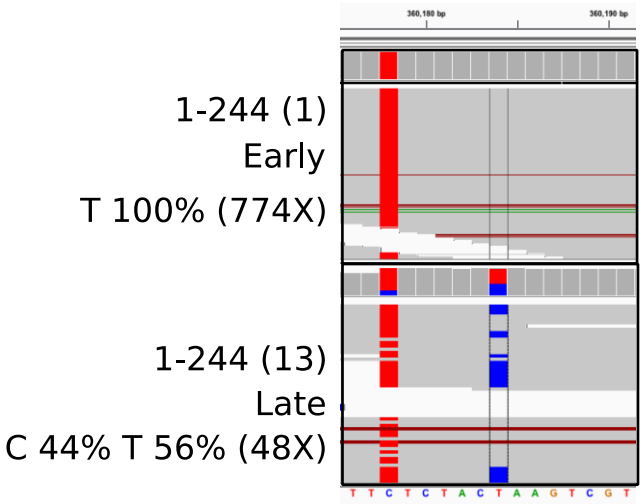
## SEER HIT 1

CT_328

Annotation: 50S ribosomal protein L1

Enriched sequence found in:

**1_079_13 (TF)**
**1_082_13 (TF)**
**1_202_13 (TF)**
**1_235_13 (TF)**
**1_244_13 (TF)**



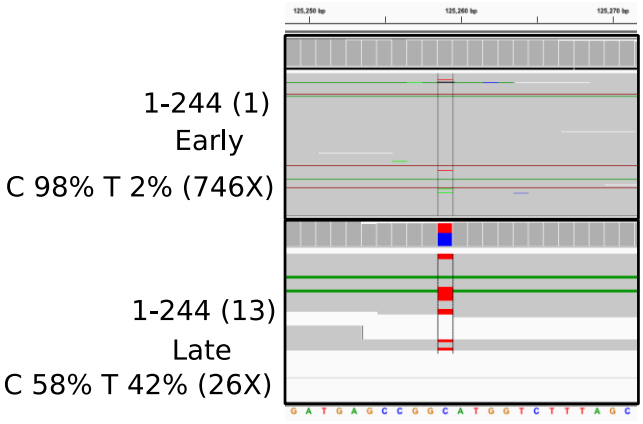

## SEER HIT 2

CT_108

Annotation: Nif3-like dinuclear metal center hexameric protein

Enriched sequence found in

**1_082_13 (TF)**
**1_233_13 (TF)**
**1_235_13 (TF)**
**1_244_13 (TF)**
**2_614_13 (NI)**



# SEER HIT 3

CT_619

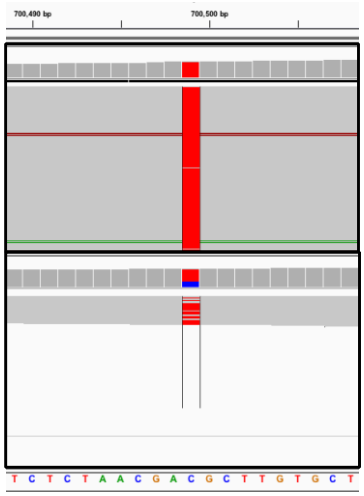
Annotation: T3SS effector

Enriched sequence found in:

**1_131_13 (TF)**
**1_233_13 (TF)**
**1_235_13 (TF)**
**1_244_13 (TF)**

1-244 (1)
Early
C 2% T 98% (571X)

1-244 (13)
Late
C 33% T 67% (36X)




Ah yes, of course. I forgot to check the effect. Unfortunately the first 2 are synonymous and only the last one is non-syn but to a similar amino acid. .

For CT_318 it is base 300 in the gene - coding for a G when the major variant is an A. That would be a synonymous Leucine to Leucine, im afraid.

For CT_619 base 1161 G, major variant is an A. Serine to serine

And CT_108 base 608. C, major variant T. Changes codon from Valine to alanine.

Hmm... might just be an association by chance then. Bummer.

Cheers,