Drug resistant clones of the *P. chabaudi* AS lineage

1. Outline, nomenclature and general information

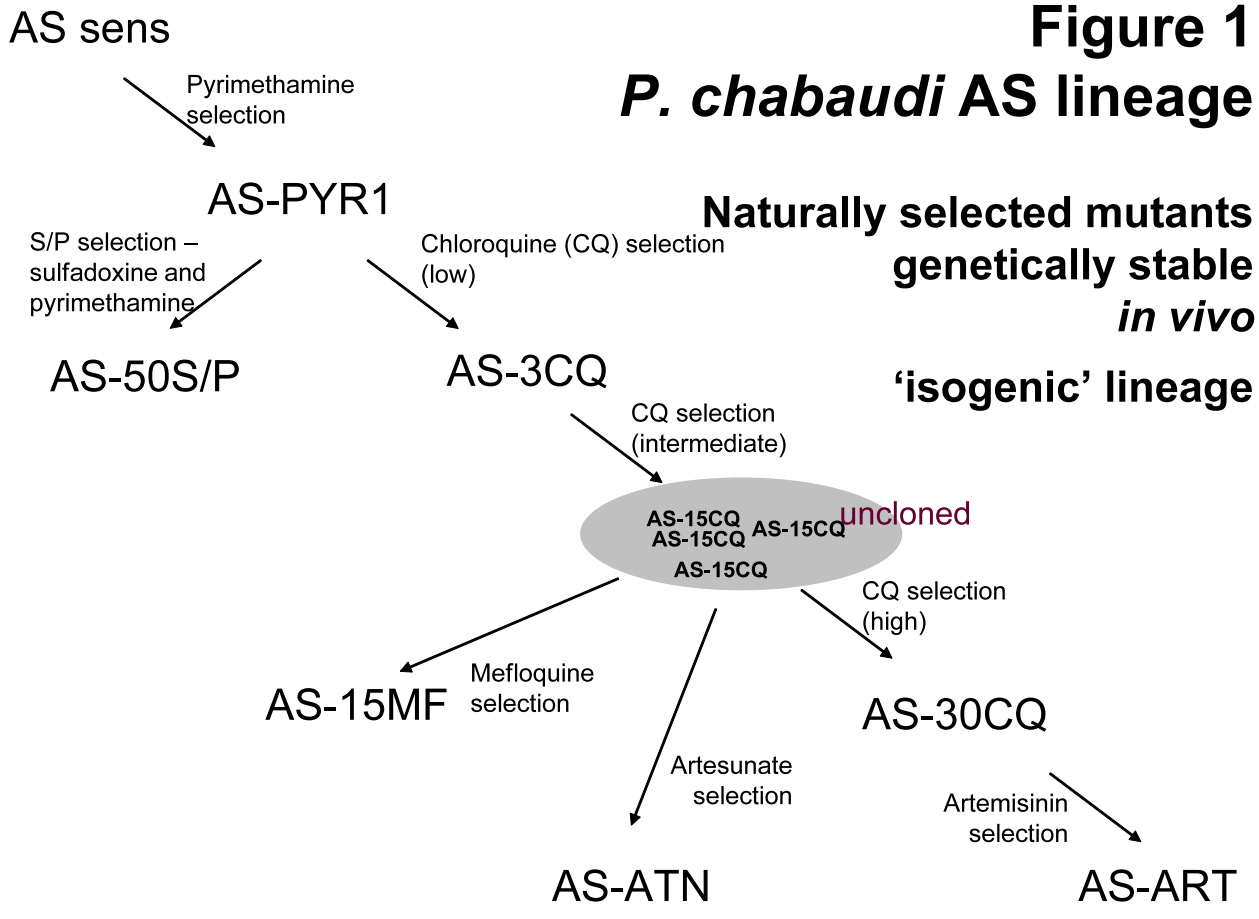
Please also consult the following files, as required

> "Drug resistant clones AS lineage – 2 Published papers" and "Drug resistant clones AS lineage – 3 Clone information"

General

Almost all of the drug resistance work in *P. chabaudi* has been carried out on the AS strain. This strain is the same as that sequenced at the Wellcome Trust Sanger Institute. The original AS strain was cloned (limiting dilution) from a specific thicket rat along with other clones such as AD, AJ, AQ etc. Other clones (BC, BW, CB, ER etc) originated from different rats. These rats were brought back from Central African Republic (I Landau, 1969).

All of the clones are phenotypically and genotypically distinct (from each other and from AS). They have different growth and virulence phenotypes, for example. For genotype, all clones differ, for example, in their *dhfr* and *dhps* nucleotide and aminoacid sequences (*unpublished*). The genetic diversity of AS and AJ is the most intensely characterised. Illumina sequencing identifies about 100,000 (AS v AJ) SNPs. Similarly over 600 (AS v AJ) AFLPs have been characterised. Both technologies give similar approximate mean SNP frequencies of 1 SNP/~180 base pairs. All cloned strains may be verified genotypically.



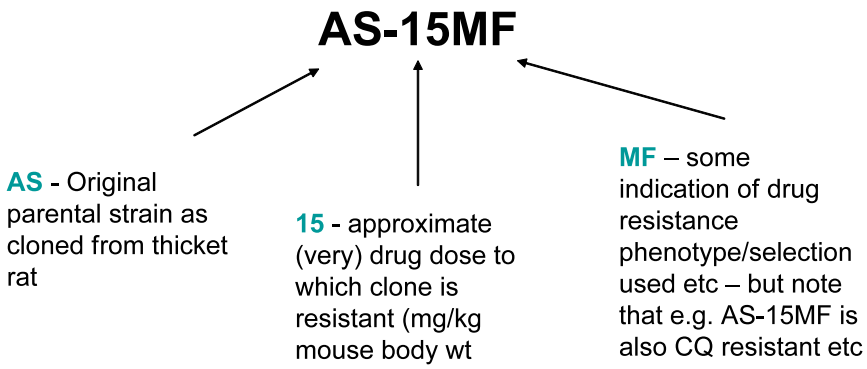
The AS drug resistance lineage

The clones in the AS drug resistance lineage (Figure 1) are all produced by passaging a previous drug-sensitive or drug-resistant strain (progenitor) through mice treated with a particular drug. Most clones were produced after extensive passage in the presence of initially low but increasing doses. In contrast, AS-PYR1 is the result of cloning parasites surviving one passage in the presence of pyrimethamine. Resistant parasites survive and may, eventually, be cloned. These clones are, as expected, almost isogenic. The clones are produced *serially* more than in parallel. We have Illumina sequenced most clones of the lineage (AS, AS-50S/P, AS-30CQ, AS-15MF, AS-ART). A very small and consistent set of point mutations accumulated in the lineage. For example, eight point mutations differentiate AS-sens and AS-ART, see below. A small number of indel/CNV mutations have also been characterised. The resistance phenotypes are all genetically stable. All clones in the lineage may now be positively identified by sequencing only two genes.

Nomenclature

The clone nomenclature indicates the starting strain name (usually AS) and a hyphenated suffix which usually gives some clue to either the drug used in the last selection step or the resistance phenotype or the history of selection.

Figure 2
Nomenclature of drug resistant clones - 1



For example AS-PYR clone was produced by selection in the presence of pyrimethamine and is resistant to pyrimethamine.

Numbers in front of the drug abbreviation element of the clone name (see Figure 2) indicate a very approximate level of drug resistance (drug concentration in mg/kg body weight)

AS-15MF was produced by selection in the presence of mefloquine (MF).

Thus AS-30CQ is more highly resistant to CQ than either AS-15CQ or AS-3CQ

Multi- and Cross-resistance

A model of a simple direct relationship between a variant of a specific gene (genotype) and a drug-response (phenotype) predicts that a clone will tend to retain the phenotype of its progenitors. Later clones will tend to exhibit mult-drug resistance phenotypes. This is mostly the case here. Most clones retain the resistance phenotypes of their progenitor clone. For example, AS-15MF retains pyrimethamine resistance and an intermediate level of chloroquine resistance. Similarly AS-ART (resistant to ART) retains resistance to high levels of chloroquine (CQ) and to pyrimethamine.

Note that resistance to a particular drug may appear in the lineage *before* parasites were exposed to the drug. For example, AS-15MF is resistant to lumefantrine, even though lumefantrine had not been used previously. This is not altogether surprising since resistance to mefloquine and lumefantrine may share a similar mechanism (CNV of *mdr1*, overexpression of *MDR1*) and AS-15MF indeed carries a CNV of this type.

Artemisinin resistance is another very important example, as follows. Surprisingly, AS-15MF and AS-30CQ have ART-R phenotypes (relative to AS-3CQ, AS-PYR1 and AS-sens) even though they

had never been exposed to artemisinin (see Hunt *et al*. 2010). This happens because of the *ubp1* mutation which appears (under CQ selection) in the AS-15CQ uncloned population and presumably gets selected by CQ in AS-30CQ and by MF in AS-15MF. Artemisinin selection of AS-30CQ produces an extended ART-R phenotype in AS-ART which we believe is linked to an additional mutation (AP2 μ-chain, Henriques *et al*. 2013).

Further nomenclature

Numbers after the drug abbreviation element indicate a specific clone, when more than one clone from a selected uncloned population was obtained. Thus AS-PYR1 is *one* clone amongst many clones obtained from that population. Another example is AS-ATNMF1. It may be interesting to identify the mutations in more than one clone.

Genetics

The genetic architecture/determinants of the drug resistance phenotypes has been studied by linkage analysis of genetic crosses between these AS drug resistant clones and AJ (the genetically diverse, drug sensitive) clone. Different genetic linkage paradigms have been employed – classical linkage analysis, QTL analysis, Linkage Group Selection etc. Along with genome sequencing, this has been remarkably successful in identifying the determinants of drug resistance.

Genome re-sequencing

Using AS-WTSI genome sequence (Sept 2009 assembly) as reference sequence, we have sequenced AS-sens, AS-50S/P, AS-15MF, AS-30CQ, AS-ART. By identifying mutations in AS-50S/P, AS15-MF, AS-30CQ and AS-ART it has been possible to confirm and identify where these mutations originated in the lineage by di-deoxy sequencing of intervening clones (AS-PYR1, AS-3CQ)

For example, in the main artery of the lineage (AS-sens to AS-ART), there are

8 Point mutations

- 1 intergenic, non-coding mutation (chr14) – not associated with drug responses (first appears in AS-PYR1)
- 7 non-synonymous mutations (6 confer drug resistance phenotypes, 1 does not)
  - S106N *dhfr* (chr07) confers pyrimethamine resistance (in AS-PYR1)
  - A173E *aat1* (chr11, 10 TM aminoacid transporter) confers CQ-R (in AS-3CQ)
  - T709N PCHAS_030200 ?? confers CQ intermediate resistance (in AS-30CQ)
  - T719N PCHAS_031370 (12 TM transporter) confers CQ intermediate resistance (in AS-30CQ)
  - V2728F *ubp1* (chr02, de-ubiquitinating enzyme) confers ART-R and CQ-hiR (in AS-30CQ)
  - I568T *ap2μ* (chr14, AP2 mu-chain) confers ART-hiR (in AS-ART)

  - Y162H PCHAS_101550 may not confer drug response phenotype (in AS-30CQ)

2 known deletions namely, a 34 bp deletion on chr07 (in AS-PYR1) and a ~1.5 kb deletion on chr05 (in AS-3CQ)

Overall conclusion and perspective

The AS lineage of drug resistant clones comprises parasites with a wide range of drug and multi-drug responses. These responses depend upon a small number of point mutations and one CNV (duplication/translocation) event. In fact there are only a very small number of mutations which are fixed in the lineage (by selection and cloning) and most of these are related to a drug resistance phenotype.

There is a complex series of papers which define the phenotypes, genotypes and their relationships extending through 1971 – 2013. Please refer to “Drug resistant clones AS lineage – 2 Published papers” and “Drug resistant clones AS lineage – 3 Clone information” for details.