Effects of genetic variation in *CYP2C* locus on pharmacokinetics of chlorcycloguanil

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Acknowledgements

Study participants
MRC staff
EDCTP

Objectives

- To define the prevalence of increased and decreased activity *CYP2C* alleles relevant to treatment of malaria with antimalarial biguanides in The Gambia, West Africa.

- To assess the effects of both known and newly defined alleles and haplotypes on chlorproguanil and chlorcycloguanil pharmacokinetic parameters such as T_{max}, C_{max} and AUC

Methods (1)

- Bioinformatic analysis of the *CYP2C* gene cluster shows two groups of genetic markers (Figure 1) with a high degree of linkage disequilibrium between markers in each group.

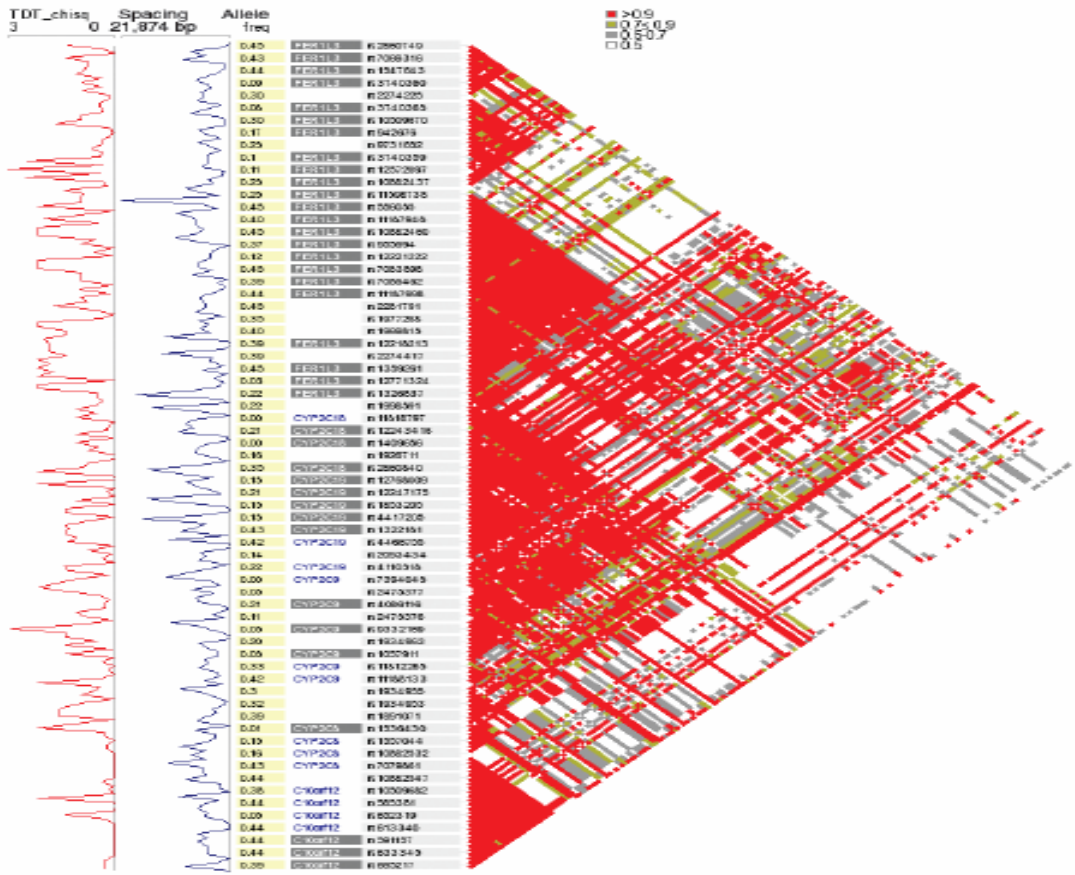


Figure 1 Analysis with marker shows that the C*YP2C* cluster lies in an area of strong linkage disequilibrium (Walton *et al.,* Nature Genetics 2005)

Methods (2)

- We selected 14 loci to genotype by allele specific and real-time PCR

- Study subjects were 43 adult participants who underwent detailed pharmacokinetic studies on Lapdap

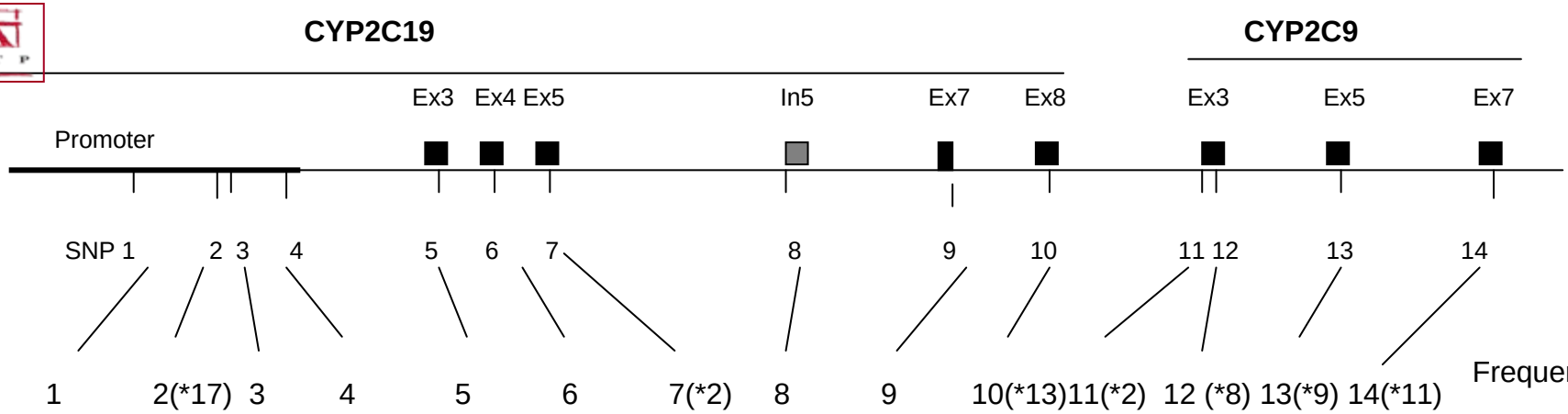
- DNA was extracted and allele specific primers designed using Primer3 software

- Primers tested in a preliminary ARMS PCR run using control DNA samples from Sukuta in the Gambia

- *CYP2C19* SNPs available on the Taqman platform were assessed using ABI 7500 real-time PCR system.

- Haplotypes were assembled using a Bayesian algorithm implemented in the Phase 2.2 computer program and analysed for association using MARKER

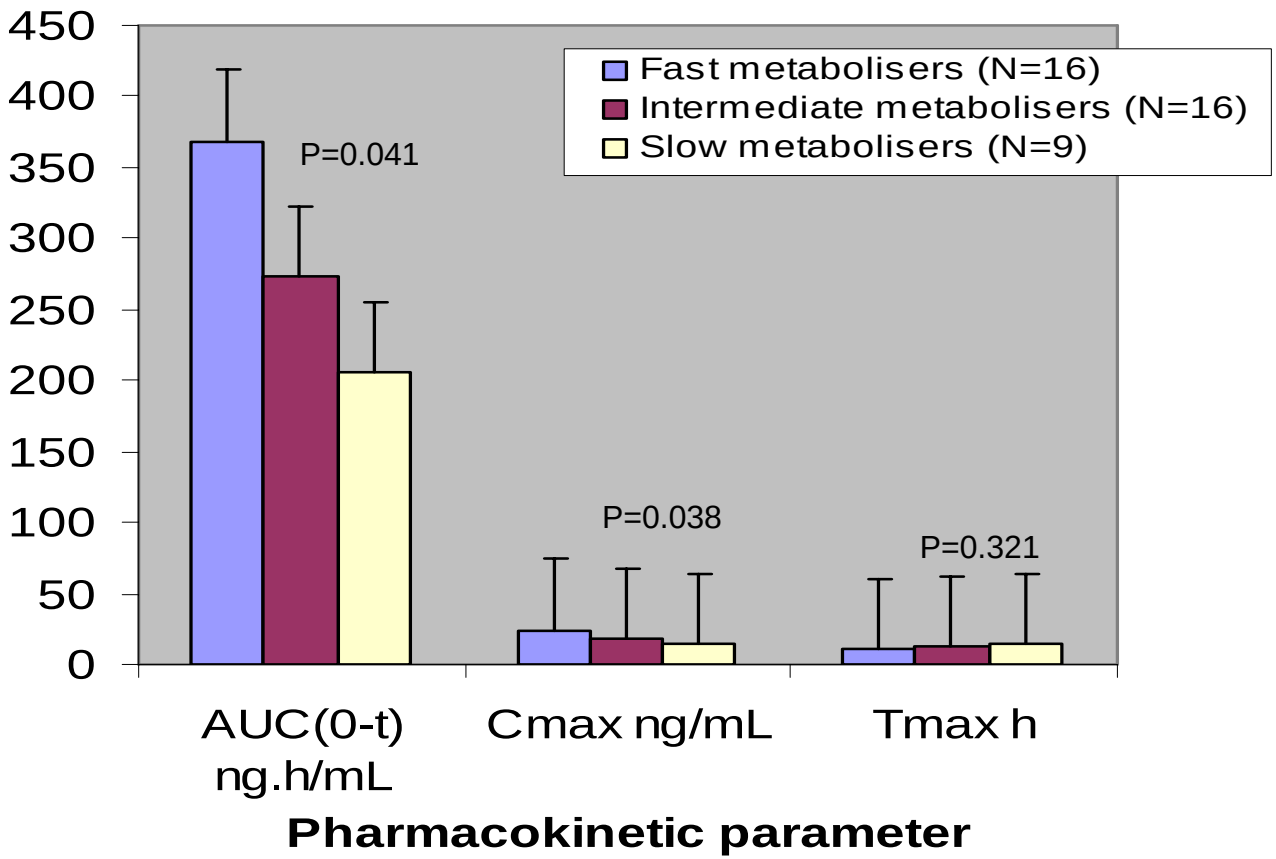
- CYP2C19**17* is a gain of function mutation and the **2* is a loss of function mutation



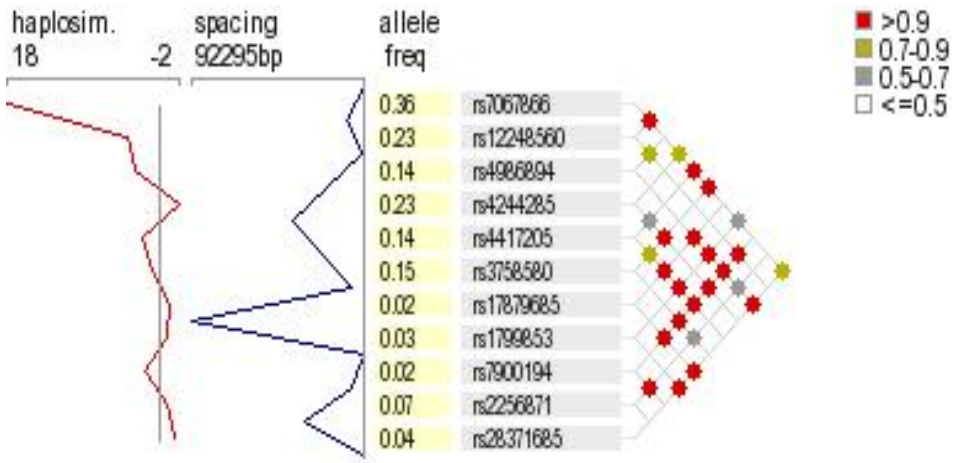
	1	2(*17)	3	4	5	6	7(*2)	8	9	10(*13)	11(*2)	12 (*8)	13(*9)	14(*11)	Frequency
Hap1	G	C	C	T	G	G	G	C	C	C	C	G	C	C	.23
Hap2	T	T	C	T	G	G	G	C	C	C	C	G	C	C	.19
Hap3	T	C	C	T	G	G	G	C	C	C	C	G	C	C	.14
Hap4	T	C	C	C	G	G	A	G	T	C	C	G	C	C	.11
Hap5	G	C	C	T	G	G	G	C	C	C	C	G	T	C	.04
Hap6	G	C	T	T	G	G	G	C	C	C	C	G	C	C	.04
Hap7	T	C	C	T	G	G	G	C	C	C	C	A	C	C	.02
Hap8	T	C	C	T	G	G	G	G	C	C	C	G	C	T	.02
Hap9	T	C	C	T	G	G	G	G	C	C	C	G	C	C	.01
Hap10	T	T	C	T	G	G	G	G	C	C	C	G	C	C	.01
Hap11	G	C	C	T	G	G	G	C	C	C	C	G	T	T	.01
Hap12	G	C	C	T	G	G	G	C	C	C	T	G	C	C	.01
Hap13	G	C	C	T	G	G	G	C	C	T	C	A	C	C	.01
Hap14	G	C	C	T	G	G	G	C	C	T	C	G	C	C	.01
Hap15	G	C	T	T	G	G	A	C	C	C	C	G	C	C	.01
HapMap YRI	.31	.28	-	.14	-	-	.17	.14	.17	.02▲	0	.04▲	.075	.01▲	
Study	.43	.24	.06	.14	-	-	.16	.16	.17	.02	.01	.02	.06	.03	
HW P-value	.28	.71	.67	.75	-	-	.88	.88	.46	.88	.94	.88	.69	.81	

Allele and haplotype frequencies of *CYP2C* alleles in Gambians

Effects of genetic variants of CYP2C19 on antimalarial pharmacokinetics



Haplotype block of *CYP2C19* and *CYP2C9* alleles generated using the program Marker.



The alleles exhibit very strong LD with high D´-values >0.9.

Discussion & Conclusions

❖Slow metabolising alleles and fast metabolising alleles have a frequency of 14% and 24% respectively in the adult population in The Gambia

❖ The allele frequencies in the Gambia are similar to frequencies established in West African Yorubas in the HapMap project and in Europeans

❖ The presence of *fast metabolising alleles* cause significant increase in AUC and Cmax of chlorcycloguanil ($P<0.05$)

❖*CYP2C9* effects cannot be discounted because of strong linkage disequilibrium among *CYP2C* alleles

Future Perspectives

- Work is in progress to select more markers in the *CYP2C* cluster. Novel polymorphisms in the population will be identified by sequencing and the new alleles will be biochemically characterised

- We will determine whether the genetic differences that we have shown in pharmacokinetics of chlorcycloguanil translate to clinical effectiveness of antimalarial treatment in a randomised controlled trial of a chlorproguanil/dapsone combination for mild malaria in children (n=417)