



Variation in cytochrome P450 *CYP2C19/CYP2C9* and efficacy of chlorproguanil/dapsone

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Study participants

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Objectives



- ❖ We showed previously that fast and slow metabolizing alleles of *CYP2C19/CYP2C9* affect pharmacokinetics of dapson and the antimalarial biguanide chlorcycloguanil in Gambian adults
- ❖ Chlorproguanil and dapson were co-formulated in Lapdap as an affordable antimalarial therapy
- ❖ Lapdap was withdrawn from the market and further development terminated due to anaemia thought to be associated with the dapson component
- ❖ We assessed the effects of *CYP2C19/CYP2C9* metabolizing alleles on anaemia/severe anaemia following treatment of uncomplicated malaria in Gambian children



Methods (1)



- ❖ Six hundred and twenty four children, 6 months to 10 years old (47% female) participated in a randomised trial of safety and effectiveness of Chlorproguanil-dapsone and Co-artemether for uncomplicated malaria (ClinicalTrials.gov Identifier: [NCT00118794](https://clinicaltrials.gov/ct2/show/study/NCT00118794)). The Chlorproguanil-dapsone arm of the clinical trial was the subject of our pharmacogenetic studies
- ❖ Inclusion criteria were: clinical symptoms of acute malaria; axillary temperature greater than or equal to 37.5 °C; positive blood smear; parasite density range at screening of 2000-200000 parasites/ μ L of blood; packed cell volume (PCV) greater than or equal to 20 %



Methods (2)



- ❖ *CYP2C19*17* which significantly increased AUC and C_{\max} of chlorcycloguanil, *CYP2C19*2* which tended to decrease chlorcycloguanil AUC and *CYP2C9*8* which significantly decreased T_{\max} for chlorcycloguanil in adults were genotyped in the children treated with Lapdap
- ❖ Indices of anaemia and severe anaemia were measured in the children after treatment
- ❖ Associations of the polymorphisms with anaemia and severe anaemia and interaction effects with G6PD deficiency and parasite density at enrolment were estimated using Stata 9.0

Results (1)

Table 1: Incidence of severe anaemia and haemoglobin level by *CYP2C* carrier status at day 3.

		Severe anaemia by day 3			Haemoglobin at day 3			Change in haemoglobin from day 0 to day 3		
		Incidence	OR (95% CI)	p	Mean (SD)	Difference (95% CI)*	p*	Mean (SD)	Difference (95% CI)	p
<i>CYP2C19*2</i>	Non-carrier	11/402 (2.7%)	0.81 (0.26-2.59)	0.73	8.48 (1.78)	-0.10 (-0.34,0.15)	0.44	1.60 (1.63)	0.10 (-0.18,0.38)	0.49
	Carrier	4/179 (2.2%)			8.39 (1.70)			1.70 (1.46)		
<i>CYP2C19*17</i>	Non-carrier	13/367 (3.5%)	0.26 (0.06-1.18)	0.08	8.37 (1.82)	0.08 (-0.16,0.32)	0.50	1.63 (1.63)	0.01 (-0.26,0.28)	0.93
	Carrier	2/208 (1.0%)			8.59 (1.65)			1.64 (1.49)		
<i>CYP2C9*8</i>	Non-carrier	14/546 (2.6%)	1.23 (0.16-9.63)	0.85	8.43 (1.73)	0.48 (-0.02,0.97)	0.06	1.66 (1.56)	-0.55 (-1.11,-0.01)	0.06
	Carrier	1/32 (3.1%)			8.80 (2.14)			1.11 (1.75)		

- ❖ *CYP2C19*17* tended to reduce risk of severe anaemia at day 3
- ❖ *CYP2C9*8* variant carriers had higher haemoglobin (Hb) levels at day 3 after adjusting for Hb level at day 0 and had a lower drop in Hb from day 0 to day 3 compared with noncarriers

Results (2)

Table 2: Effects of G6PD-deficiency on severe anaemia at day 3

G6PD	SNP	Severe anaemia by day 3			
		Incidence	OR (95% CI)	p	p interaction
	CYP2C19*2				
Normal	Non-carrier	10/348 (2.9%)	0.23 (0.03-1.78)	0.16	
	Carrier	1/151 (0.7%)			
Deficient	Non-carrier	1/16 (6.3%)	5.00 (0.38-66.01)	0.22	0.05
	Carrier	2/8 (25.0%)			
	CYP2C19*17				
Normal	Non-carrier	9/310 (2.9%)	0.37 (0.08-1.73)	0.32	
	Carrier	2/183 (1.1%)			
Deficient	Non-carrier	3/15 (20.0%)	--	--	--
	Carrier	0/9 (0%)			
	CYP2C9*8				
Normal	Non-carrier	10/470 (2.1%)	1.84 (0.23-19.95)	0.57	
	Carrier	1/26 (3.9%)			
Deficient	Non-carrier	3/24 (12.5%)	--	--	--
	Carrier	0/0			

- ❖ G6PD deficiency modified the effect of *CYP2C19*2* on severe anaemia at day 3 causing a higher incidence in carriers vs. noncarriers

Results (3)

Table 3: Effects of parasite density on haemoglobin level at day 3 by *CYP2C* genotype

Parasite density at baseline	SNP	Mean (std dev)	Difference (95% CI)*	p	p interaction
	CYP2C19*2				
Low	Non-carrier	8.89 (1.79)	-0.23 (-0.58,0.12)	0.20	
	Carrier	8.53 (1.87)			
High	Non-carrier	8.08 (1.69)	0.02 (-0.30,0.35)	0.88	0.30
	Carrier	8.24 (1.51)			
	CYP2C19*17				
Low	Non-carrier	8.78 (1.92)	-0.23 (-0.57,0.12)	0.19	
	Carrier	8.76 (1.67)			
High	Non-carrier	7.97 (1.62)	0.36 (0.05,0.67)	0.02	0.02
	Carrier	8.41 (1.63)			
	CYP2C9*8				
Low	Non-carrier	8.74 (1.78)	0.64 (-0.03,1.31)	0.06	
	Carrier	9.24 (2.41)			
High	Non-carrier	8.13 (1.64)	0.17 (-0.52,0.87)	0.63	0.36
	Carrier	8.24 (1.65)			

❖ Parasite density at baseline modified the effect of *CYP2C19*17* on Hb level at day 3, which was significantly higher in carriers versus noncarriers when baseline parasitaemia was high



Discussion & Conclusions



- ❖ *CYP2C19*17* which rapidly activates antimalarial biguanides confers some protection from anaemia on chlorproguanil/dapsone treatment and the effect is stronger in children with high initial parasite density.
- ❖ The *CYP2C19*2* low activation allele increases risk of anaemia only in those with G6PD deficiency.
- ❖ *CYP2C9*8* may give some protection from anaemia either by increasing therapeutic effect of dapsone or protecting against dapsone related haemolysis.
- ❖ These genetic markers may modify the outcome of treatment of antimalarial drugs and should be typed in any future trials of antimalarial biguanides and sulphones.



Future perspectives



- ❖ Most clinical trials of chlorproguanil-dapsone in Africa reported anaemia as an adverse effect
- ❖ The role of the drug metabolising alleles in adverse side reactions studied here should be investigated further to produce a panel of markers together with G6PD that could be screened to optimize therapy in future clinical trials involving biguanide/dapsone to improve the success of the trials
- ❖ The markers in *CYP2C19/CYP2C9* may govern to some extent the predisposition to adverse side reactions of dapsone
- ❖ *CYP2C19/C9* profile could be used to personalise treatment with antimalarials.