A glucocorticoid receptor gene haplotype is associated with increased risk for low birth weight infants among Kenyan mothers

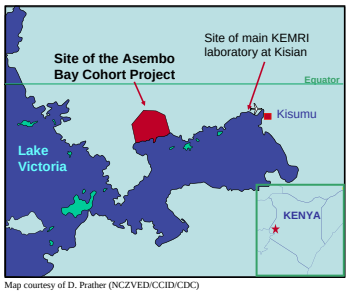
D. Smelser[1], A. Grant[2], C. Bean[3], G. Satten[3], S. Kariuki[5], L. Zhang[1,4], A.A. Lai[4], Y.P. Shi[4], L. Slutsker[4], B. Nahlen[4], F. ter Kuile[4], V. Udhayakumar[4].

1) National Office of Public Health Genomics; 2) National Center on Birth Defects and Developmental Disabilities; 3) Division of Reproductive Health; 4) Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA; 5) Kenya Medical Research Institute, Kisumu, Kenya.

Background:

Inflammatory pathway components play critical roles in mediating preterm and low birth weight births in response to malaria infection. The glucocorticoid receptor gene, also known as *NR3C1*, mediates cross-talk between the inflammatory response and endocrine pathways. Glucocorticoid receptor polymorphisms in this study were selected based on their previous association with glucocorticoid activity, which may be a factor contributing to low birth weight. The gene is located on chromosome 5q31.3.

Figure 1: Location of Asembo Bay Cohort and CDC/ Kenya Medical Research Institute (KEMRI) Field Station in Western Kenya



Map courtesy of D. Prather (NCZVED/CCID/CDC)

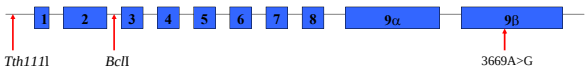
Methods:

Cohort Description: This study utilized the retrospective (1992-1999) longitudinal population-based cohort study of mothers within the Asembo Bay Cohort Project (ABCP) of Western Kenya, a malaria endemic area (>95% Luo ethnic group). Capillary blood samples were taken by finger or heel prick, cord blood and placental smears were also taken. Aliquots of peripheral blood were stored in liquid nitrogen for a source of DNA. Genomic DNA was obtained from whole blood (Bloland et al, 1999, Aidoo et al, 2001). **735 mothers with genotype data for all 3 SNPs were included in this study: 61 delivered a low birth weight (<2500g) infant (cases) and 674 delivered a normal weight (≥2500g) infant (controls).**

Genotypic Analysis: Three SNPs with published evidence of altering glucocorticoid receptor gene expression were included in this study design: 3669A>G (rs6198), *Bcl*I (intron 2) and *Tth111*I (5' flanking region, rs10052957). Genotyping was performed with the Sequenom high throughput iPLEX MALDI-TOF mass spectrometry single base primer extension assay.

Statistical Analysis: Using SAS 9.1 for Windows, biologically significant covariates [sex of child, parity, height of mother, PNPL (asexual malaria parasites present in placental smear) and PNMD (asexual malaria parasites present in mother's peripheral smear)] as well as the three SNP genotypes were examined univariately for an association with low birth weight. Statistically significant variables as well as those with biological significance were later used in the multivariate analysis. Haploview 4.0 was used to construct and determine population haplotypes. PHASE 2.1 was used to generate individual haplotypes which went into a logistic regression model along with the significant covariates. All crude and adjusted odds ratios and 95% confidence intervals were calculated using SAS 9.1 for Windows.

Figure 2: Glucocorticoid Receptor SNPs



Results:

- Among the glucocorticoid receptor polymorphisms analyzed, only the 3669A>G SNP increased the odds for delivering a low birth weight infant. (Table 2)
- GGA was the only haplotype that showed a significant association with having a low birth weight infant in univariate analysis. (Table 3)
- After adjusting for potential confounders of parity, PNPL and height, the GG/GA genotype of the 3669A>G polymorphism was no longer significantly associated with the odds of delivering of a low birth weight infant. (Table 4)
- The GGA haplotype remained significantly associated with increased odds for delivering a low birth weight infant after adjusting for parity, placental malaria parasitemia, and height. (Table 4)

Table 1: Characteristics of Kenya Study Population

	Cases (<2500g) N (%)	Controls (≥2500g) N (%)	p value
Sex of child			
Male	19 (31)	270 (40)	0.52
Female	29 (48)	275 (41)	
>1, same	7 (11)	59 (9)	
>1, different	6 (10)	70 (10)	
Parity†			
1-3	38 (62)	231 (34)	**<0.0001**
≥4	23 (38)	443 (66)	
Height of mother†			
<158cm	12 (23)	134 (22)	0.93
≥158cm	41 (77)	471 (78)	
PNPL (Asexual malaria parasites present in placental smear) †			
positive	20 (41)	171 (31)	0.18
negative	29 (59)	373 (69)	
PNMD (Asexual malaria parasites present in mother's peripheral smear)			
positive	22 (36)	228 (35)	0.82
negative	39 (64)	431 (65)	

†parity, PNPL (placental malaria parasitemia) and maternal height included in the multivariate analysis due to statistical and/or biological significance.

Table 2: Crude Odds Ratios for the three glucocorticoid receptor SNPs and low birth weight

Genotype	Case N (%)	Control N (%)	Total N (%)	p value	OR (95% CI)
3669A>G[1]					
AA	52 (85)	645 (96)	697 (95)	**0.0004**	**3.85 (1.73, 8.56)**
AG & GG	9 (15)	29 (4)	38 (5)		
***Bcl*I**[2]					
CC & CG	20 (33)	230 (34)	250 (34)	0.83	0.94 (0.54, 1.65)
GG	41 (67)	444 (66)	485 (66)		
***Tth111*I**					
AA	8 (13)	43 (6)	51 (7)	0.13	2.32 (0.99, 5.39)
AG	24 (39)	270 (40)	294 (40)		1.10 (0.63, 1.94)
GG	29 (48)	361 (54)	390 (53)		Reference

[1]AG & GG combined since only 2 GGs in controls, 0 in cases. Low frequency of the G allele is common in African populations. [2]CC & CG combined since only 1 CC in cases.

Table 3: Association of haplotypes of the three glucocorticoid receptor SNPs with low birth weight

Haplotype	Population Frequency	Cases N (%)	Controls N (%)	p value
AGG	0.691	40 (0.652)	468 (0.7)	0.34
ACA	0.143	9 (0.152)	96 (0.1)	0.76
AGA	0.100	6 (0.102)	67 (0.1)	0.95
ACG	0.040	1 (0.20)	28 (0.04)	0.24
GGA	**0.026**	**5 (0.07)**	**15 (0.02)**	**>0.0001**

Table 4: Adjusted odds ratios of the significant 3669A>G genotype and the significant GGA haplotype with low birth weight

	p value	OR (95% CI)
Genotype AG/GG*	0.2996	0.34 (0.05, 2.60)
Haplotype GGA*	**0.0005**	**4.58 (1.94, 10.82)**

*Adjusted for Parity, PNPL, Maternal height

Conclusions:

- In this Kenyan maternal population, having the GGA haplotype of the glucocorticoid receptor gene SNPs 3669A>G, *Bcl*I (intron 2) and *Tth111*I increases the odds of delivering a low birth weight infant. [**OR=4.58 (1.94, 10.82)**]
- These results may suggest that endocrine pathway genes are associated with low birth weight. Further research is necessary to determine the mechanisms of these genes and other pathways might involved in low birth weight.