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***FCGR3B* copy number variation is associated with systemic lupus erythematosus risk in Afro-Caribbeans**

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Abstract

Objectives—To evaluate *FCGR3B* copy number variation (CNV) in African and European populations and to determine if *FCGR3B* copy number is associated with SLE and SLE nephritis risk in Afro-Caribbeans, adjusting for African genetic ancestry.

Methods—We estimated *FCGR3B* to determine if there were ethnic variations in CNV (unrelated unadmixed Europeans and Africans). We then examined CNV at *FCGR3B* in relation to SLE and SLE nephritis within a case–control collection of 134 cases of SLE (37 with SLE nephritis) and 589 population controls of mainly Afro-Caribbean descent resident in Trinidad.

Results—We found a significant difference in copy number *FCGR3B* distribution between unadmixed African and European UK cohorts, with 27 (29%) vs 3 (5%) for those with low (0 or 1) copy *FCGR3B*, respectively, $P = 0.002$. In a Trinidadian SLE case–control study, low *FCGR3B* CNV was associated with SLE risk 1.7 (95% CI 1.1, 2.8), $P = 0.02$, which remained after adjustment for African genetic ancestry; odds ratios (ORs) 1.7 (95% CI 1.0, 2.8), $P = 0.04$.

Conclusion—Our studies suggest that *FCGR3B* low copy number is associated with SLE risk in Afro-Caribbean populations independently of CNV due to African ancestry.

Keywords

SLE; Admixture; *FCGR3B*; Copy number variation; African

Introduction

Emerging analyses of copy number variation (CNV) across the genome has highlighted the role that structural variation may play in the aetiology of genetic disease susceptibility. CNV has been reported in a number of recent studies [1], with increasing reports of individual gene CNV and association of CNV with specific diseases [2–13], including an association between low *FCGR3B* CNV and systemic immunity in a number of European cohorts [14–16] and C4 gene deletions and SLE risk [13]. There is also evidence for regional CNV variation between different ethnic groups [1]. However, association of low *FCGR3B* CNV was not confirmed in a Chinese lupus nephritis cohort of 202 individuals, compared with 146 controls [17]. Further studies on Columbian patients of Spanish (Paisa) origin have confirmed association of low and high *FCGR3B* CNV in 146 subjects with SLE, and identified possible epistasis with *CCL3L1* (CC chemokine ligand 3-like 1) [18], which is encoded by a variable copy number gene that may additionally enhance inflammatory responses and increase the chance of autoimmune disease [10].

SLE and SLE nephritis show large ethnic differences in disease risk, with prevalence rates 6–8 times higher in African-Americans and in Caribbean migrants of West African descent than in Europeans, but little is known about *FCGR3B* CNV in ethnically diverse African cohorts with SLE. We decided to investigate the association of low CNV in unadmixed Africans and Europeans, and subsequently in a case–control study of SLE within a Trinidadian Afro-Caribbean population.

Methods

CNV was measured in this study by paralogue ratio test (PRT) assay as previously described and validated [19]. First, we established *FCGR3B* CNV in a population-based control sample of unrelated unadmixed Europeans and Africans from the UK with no known renal disease. Individuals were selected via primary care clinics if they claimed all four grandparents were of either European or African descent. Additionally, data were validated from data collected on country of origin and mother's main language. Finally, these individuals were genotyped using independent genome-wide ancestry informative markers (AIMs) as described previously [20], five-way admixture estimated using ADMIXMAP software (admixture mapping software; open source software developed at London School of Hygiene & Tropical Medicine) and those included with >97.5% African ancestry. All study participants gave written informed consent. The study was approved by London School of Hygiene & Tropical Medicine ethics board and the Trinidad and Tobago Ministry of Health.

We then examined CNV at *FCGR3B* (as described earlier by PRT methods) in relation to SLE and SLE nephritis within a case–control collection of 134 cases of SLE (37 with SLE nephritis) and 589 population controls of mainly Afro-Caribbean descent resident in Trinidad [21]. Novel aspects of this study included the use of five-way admixture in this population to examine separately the effects of admixture on SLE and CNV. Analysis of admixture was based on a panel of AIMs chosen to have large allele frequency differentials between West African, European, Chinese, Indian and Native American populations. A Bayesian model for population admixture, individual admixture and locus ancestry was fitted by Markov chain simulation [20].

All analyses were carried out using Stata statistical software (release 10.0, 2008; Stata Corporation, College Station, TX, USA), and adjusted for five-way admixture, age and gender. The association between the relevant outcome and CNV status at time of survey was

explored using logistic regression analysis—effects on risk being estimated by odds ratios (ORs) with 95% CIs.

Results

We were able to obtain *FCGR3B* CNV results on ~96% of the samples using the PRT assay, which comprised: (i) UK unadmixed European and African control cohort; (ii) SLE Afro-Caribbean Trinidad cohort; and (iii) non-SLE Afro-Caribbean Trinidad controls.

FCGR3B

In the West African unadmixed control population, 27/93 (29%) also had significantly higher proportions of low (0, 1) *FCGR3B* CNV compared with European control 3/56 (5%) populations, with $P = 0.002$. Similar results were found for low *FCGR3B* in a Trinidadian Afro-Caribbean control population, $P < 0.05$ compared with European controls. Prevalence of *FCGR3B* CNV in European controls was similar when validated with results from a secondary European cohort (MRC 1946 European cohort). In Fig. 1, One Trinidadian Afro-Caribbean control (0 cases), two African controls living in the UK and no Europeans had zero copies of *FCGR3B*. Numbers of low *FCGR3B* CNV were significantly higher in SLE cases compared with Afro-Caribbean controls, $P = 0.01$. The Afro-Caribbean control with zero *FCGR3B* CNV had no evidence of other autoimmune disease or high ANA status identified from medical records. Trinidadian Afro-Caribbean SLE cases had significantly higher proportions of low (0, 1) *FCGR3B* CNV [32/126 (25%)] compared with non-SLE Afro-Caribbean control [79/479 (16%)] populations, with $P = 0.01$.

Table 1 shows summary characteristics across three groups of Afro-Caribbean patients in Trinidad: (i) Afro-Caribbean non-SLE controls; (ii) Afro-Caribbean SLE cases; and (iii) Afro-Caribbean SLE nephritis cases. Estimation of African ($P = 0.0004$) and European ancestry ($P = 0.002$) significantly differed between SLE cases and non-SLE controls. Estimates of Chinese ($P = 0.0001$) and Native American ancestry ($P = 0.02$) were significantly higher in SLE cases than in non-SLE controls. Of those with low *FCGR3B* CNV with genetic estimates of ancestry (admixture), SLE cases did not have significantly lower mean African ancestry than non-SLE controls (67 vs 68%, respectively), $P = 0.7$.

Table 2 shows the association of low (0 or 1) CNV at *FCGR3B* with SLE from a case–control study of SLE and SLE nephritis in Trinidad Afro-Caribbeans. The crude OR adjusted for age group and gender was 1.7 (95% CI 1.1, 2.8), $P = 0.02$. The OR for low *FCGR3B* CNV associated with SLE risk remained similar after adjustment for five-way ancestry including African admixture; OR 1.7 (95% CI 1.0, 2.8), $P = 0.04$. For SLE nephritis, the crude OR for low *FCGR3B* adjusted for age group and gender was 2.3 (95% CI 1.1, 4.8), and adjusted for five-way ancestry including African admixture OR was 2.4 (95% CI 1.1, 5.5), $P = 0.03$.

We confirmed that the association with low CNV was independent of five-way admixture for SLE and SLE nephritis. There was no additional risk for SLE nephritis compared with SLE patients without nephritis in either the adjusted or unadjusted models. ANA status was strongly associated with SLE diagnosis but was not associated with African admixture.

Discussion

The most significant results are the distributions of *FCGR3B* number between African and European populations. This is statistically significant and present in several populations of West African descent (West Africans in the UK, and Trinidadian Afro-Caribbeans).

This study suggests an association of low *FCGR3B* CNV with SLE and SLE nephritis in an Afro-Caribbean cohort, independent of ethnic background and is the first study to include five-way admixture. Calculations based on the actual numbers genotyped indicate our study was 80% powered to detect ORs of 1.7 and 2.4, respectively, for association of *FCGR3B* with SLE and lupus nephritis. Recent studies have identified that *FCGR3B* may be involved in immune complex clearance, as low *FCGR3B* is associated with SLE [12, 14, 15]. Willcocks *et al.* [12] showed that both in a family with Fc gamma RIIIb deficiency and in the normal population, *FCGR3B* CNV correlates with protein expression, with neutrophil uptake of and adherence to immune complexes, and with soluble serum Fc gamma RIIIb. The authors suggested that reduced Fc gamma RIIIb expression may contribute to the impaired clearance of immune complexes, a recognized feature of SLE, which may support the association between low *FCGR3B* CNV and SLE found in European (and other) populations [12]. Further recent studies have identified association between high and low *FCGR3B* CNV with SLE (and primary SS) in a Spanish Paisa Columbian population, stated as 85% European and 15% Amerindian with possible epistatic interactions in those SLE individuals with two copies of CCL3L1, although no formal adjustment for admixture was undertaken [18]. No association of *FCGR3B* was found with SLE nephritis in 202 Chinese individuals lupus nephritis cohort [17].

Further examination showed *FCGR3B* was not in linkage disequilibrium (LD) with any of the AIMs selected to estimate admixture. Although Afro-Caribbeans are at substantially higher risk of SLE and SLE nephritis, the risk of low *FCGR3B* CNV with SLE remains after adjusting for African ancestry. As in previous studies, there was no additional risk for low CNV with organ-specific immunity such as SLE nephritis, within SLE cases. Further studies will determine if the effect of *FCGR3B* CNV is associated with SLE risk in other ethnic groups.

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Rheumatology key messages

- Our studies suggest *FCGR3B* low copy number is associated with SLE risk in Afro-Caribbean populations independently of CNV due to African ancestry.
- We found significant differences in distribution of *FCGR3B* CNV between African and European populations.

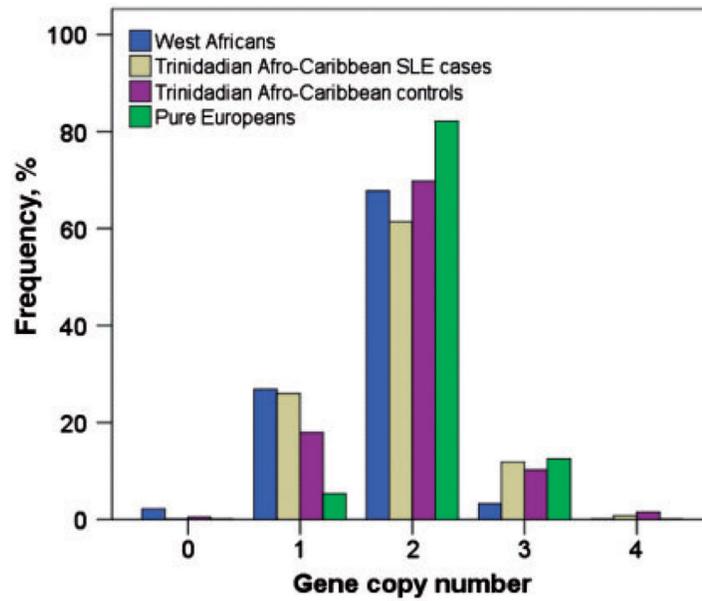


Fig. 1. Distribution of *FCGR3B* by PRT CNV in West African (unadmixed), Afro-Caribbean and European populations. Totals (*N*): Trinidadian Afro-Caribbean SLE cases (*n* = 126); Trinidadian Afro-Caribbean non-SLE controls (*n* = 479); West Africans (*n* = 93); and Europeans (*n* = 56).

Table 1

SLE and SLE nephritis in relation to *FCGR3B* copy number and African ancestry: characteristics at survey of participants

| Risk factor | Afro-Caribbean controls, n (%) n = 589 | Afro-Caribbean SLE cases, n (%) n = 134 | Afro-Caribbean SLE nephritis subset of SLE cases, n (%) n = 37 | P-value All SLE cases vs non-SLE controls |
|-----------------------------------|---|--|---|--|
| Age at survey, years ^a | | | | 0.2 |
| 15–24 | 16 (3) | 1 (1) | 0 (0) | |
| 25–34 | 76 (13) | 17 (13) | 7 (19) | |
| 35–44 | 163 (28) | 43 (32) | 12 (32) | |
| 45–54 | 148 (25) | 43 (32) | 12 (32) | |
| 55–64 | 117 (20) | 20 (15) | 3 (8) | |
| >65 | 64 (11) | 10 (7) | 3 (8) | |
| Education after 18 years | NA | 25 (19) | 7 (19) | |
| Lack of running water at 12 years | NA | 50 (37) | 15 (41) | |

| Genetic ancestry Mean (SD) | n = 558 (five-way admixture) | n = 119 (five-way admixture) | n = 32 (five-way admixture) | |
|-------------------------------|------------------------------|------------------------------|-----------------------------|--------|
| African | 0.68 (0.17) | 0.62 (0.24) | 0.61 (0.26) | 0.0004 |
| European | 0.12 (0.11) | 0.10 (0.07) | 0.09 (0.07) | 0.002 |
| Native American | 0.05 (0.05) | 0.07 (0.05) | 0.07 (0.06) | 0.02 |
| Indian | 0.08 (0.08) | 0.09 (0.07) | 0.09 (0.08) | 0.12 |
| Chinese | 0.08 (0.07) | 0.12 (0.12) | 0.14 (0.14) | 0.0001 |

| Proportions of African ancestry per copy category | n = 450 | n = 113 | n = 31 | |
|---|-------------|-------------|-------------|-------|
| 0, 1 copies <i>FCGR3B</i> | 75 (17) | 28 (25) | 10 (32) | |
| Mean African ancestry (s.d.), % | 0.68 (0.18) | 0.67 (0.25) | 0.69 (0.21) | 0.7 |
| 2 copies <i>FCGR3B</i> | 342 (76) | 70 (62) | 19 (61) | |
| Mean African ancestry (s.d.), % | 0.69 (0.18) | 0.59 (0.25) | 0.54 (0.29) | 0.001 |
| 3 copies <i>FCGR3B</i> | 33 (7) | 15 (13) | 2 (6) | |
| Mean African ancestry (s.d.), % | 0.65 (0.22) | 0.65 (0.21) | 0.85 (0.02) | 1.0 |

^aFive missing age data. N/A: not available.

Table 2

Adjusted regression models for SLE and SLE nephritis risk with *FCGR3B*; controlling for African genetic ancestry

| | SLE cases vs non-SLE controls | | SLE nephritis vs non-SLE controls | | SLE nephritis vs non-renal SLE | |
|---------------------------|--|---|--------------------------------------|---|------------------------------------|---|
| | <i>n</i> = 562 (prevalence %), <i>P</i> -value | Adjusted OR ^a (95% CI) genotypic | <i>n</i> = 480, <i>P</i> -value | Adjusted OR ^a (95% CI) genotypic | <i>n</i> = 113, <i>P</i> -value | Adjusted OR ^a (95% CI) genotypic |
| 2 copies <i>FCGR3B</i> | | 1.0 (95% CI) | | 1.0 (95% CI) | | 1.0 (95% CI) |
| 0, 1 copies <i>FCGR3B</i> | 0.04 | 1.7 (1.0, 2.8) | 0.03 | 2.4 (1.1, 5.5) | 0.3 | 1.7 (0.7, 4.5) |

^aOR adjusted for age, gender and African genetic ancestry.