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FCGR3B copy number variation is associated with susceptibility to systemic but not organ-specific autoimmunity

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Abstract

Naturally occurring variation in gene copy number is increasingly recognized as a heritable source of susceptibility to genetically complex diseases. Here we report robust association between *FCGR3B* copy number and risk of SLE (P=1.4*10^{-5}), microscopic polyangiitis (P=2.9*10^{-4}), and in two independent cohorts of Wegener's granulomatosis from the UK (P=3*10^{-3}) and France (P=1.1*10^{-4}). We did not observe this association in the organ specific Grave's and Addison's diseases. Our findings suggest that *FCGR3B* copy number plays a key role in the pathogenesis of systemic autoimmune diseases.

Structural variation is now recognised as a rich source of genetic diversity in the human genome and during the past five years, thousands of common copy number variants (CNV) have been described[1]. Although the association of rare CNVs in single gene disorders and in mental retardation has been established[2], the role of more common, transmissible CNVs in evolutionary selection and disease susceptibility is largely unknown.

Analyses of the functional attributes of genes reported to show CNV have revealed enrichment for genetic determinants involved in immune responses, inflammation and drug detoxification[3]. We recently reported an association between low copy number of the *FCGR3B* gene and autoimmune glomerulonephritis in a sample of patients with systemic lupus erythematosus (SLE) from UK nuclear families[4]. Since Fc gamma receptors play a key role in the initiation and regulation of the humoral-induced inflammatory response[5-7], we hypothesized that CNV in *FCGR3B* may confer susceptibility to a range of autoimmune disorders.

Gene copy number was estimated by quantitative real-time PCR assay as previously described[4], additionally validated by semi-quantitative PCR (**Supplementary Figure 1**). Significance of the differences in *FCGR3B* copy number between cases and controls was estimated by nonparametric Mann-Whitney *U*-test and risk ratios were estimated by multivariate logistic regression models[4].

First, we confirmed and strengthened the association between glomerulonephritis in SLE and CNV at *FCGR3B* (Mann-Whitney *U*–test $P = 1.4*10^{-8}$, 95% CI 0-$2.9*10^{-5}$, **Fig. 1a**) in a larger sample including 161 SLE patients with glomerulonephritis from the UK and 312 independent controls from the UK 1958 birth cohort[8]. Compared with possession of two copies of *FCGR3B*, individuals possessing fewer than two copies had significantly higher risk of glomerulonephritis (odds ratio (OR) = 2.431, 95% CI 1.47-4.0, $P = 1*10^{-3}$)(**Table 1a**). We then investigated the involvement of CNV at *FCGR3B* in the development of SLE with no known renal involvement in an expanded set of 375 unrelated cases from the UK. All phenotypes were defined according to ACR criteria[9]. We found a strong association (Mann-Whitney *U*-test, $P = 1.4*10^{-5}$, 95% CI 0-$2.9*10^{-5}$, **Fig. 1b; Table 1a**) and increased risk for development of SLE with <2 *FCGR3B* copy number (OR = 2.2, 95% CI 1.13-3.40, $P = 3*10^{-4}$) (**Table 1a**). However, no significant increased risk of acquiring glomerulonephritis was observed in SLE subjects who possessed <2 copy number at *FCGR3B* (OR = 1.093, 95% CI 0.735-1.625, $P = 0.660$). These data suggest that CNV at *FCGR3B* is an independent risk factor for both SLE and glomerulonephritis. In our previous report[4] we did not find a significant association with SLE using discordant sib pair analysis ($P = 0.083$, n=187 sib-pairs), although we recognized that the shared genetic background in sib-pairs decreased the power to detect the genetic effect of *FCGR3B* copy number. Here we use a larger (n=312) and independent control population in the association analysis which provides higher power to detect independent association with SLE ($P = 1.4*10^{-5}$) and glomerulonephritis ($P = 1.4*10^{-8}$).

These findings led us to investigate whether *FCGR3B* may be a common susceptibility gene for other autoimmune diseases with or without glomerular involvement. We therefore studied ANCA-associated vasculitis, a systemic autoimmune disorder in which renal disease occurs commonly. Patients with ANCA-associated vasculitis typically present with one of two clinical syndromes – microscopic polyangiitis (MPA) or Wegener's granulomatosis (WG); these two subtypes were considered separately. We also studied Grave's disease (GD) and a uniform cohort of Addison (ADD) patients with adrenal failure, as examples of organ-specific autoimmune diseases with no renal involvement.

In two independent cohorts of patients with WG, from the University of Birmingham (80 cases and 190 unrelated controls), and from the Necker Hospital in Paris (84 cases and 181 unrelated controls), we found association between low *FCGR3B* copy number and susceptibility to WG (Birmingham cohort, Mann-Whitney *U*-test, $P = 3*10^{-3}$, 95% CI $2.5*10^{-3}$-$3.1*10^{-3}$; Paris cohort, Mann-Whitney *U*-test, $P = 1.1*10^{-4}$, 95% CI $4.5*10^{-5}$-$1.7*10^{-4}$) (**Fig. 1c, 1d**). In a separate Birmingham cohort of patients with MPA (76 cases) compared to the 190 Birmingham controls, we found significant association between MPA and *FCGR3B* copy number (Mann-Whitney *U*-test, $P = 2.9*10^{-4}$, 95% CI $1.8*10^{-4}$-$3.9*10^{-4}$, **Fig. 1e**). In contrast to the association of MPA and WG with *FCGR3B* copy number, no association was seen between *FCGR3B* and either Grave's disease (429 cases from Birmingham, Mann-Whitney *U*-test, $P = 0.65$, 95% CI 0.64-0.65, **Fig.**

1f) or Addison's disease (74 cases vs. 179 controls from Newcastle, Mann-Whitney *U*-test, *P* = 0.057, 95% CI 0.056-0.059, **Fig. 1g**). Logistic regression models indicated an increased risk of WG and MPA in patients carrying low *FCGR3B* copy number, with OR = 2.464 (95% CI 1.19-5.1, *P* = 0.015) and OR = 2.561 (95% CI = 1.217-5.389, *P* = 0.013) respectively (**Table 1a**) but did not show significant increased risk with low copy number in the French WG cohort. Logistic regression showed no association between low *FCGR3B* copy number and both Grave's and Addison's disease (**Table 1a**). The genetic effect of high copy number in these populations is less clear. We observed a protective effect for WG in the French cohort (OR = 0.277, *P* = 0.002, **Table 1a**) and for development of glomerulonephritis in UK SLE cases (OR = 0.309, *P* = 0.01, data not shown), and an increased risk of disease in patients carrying >2 copies of *FCGR3B* in the ADD cohort (OR = 3.438, *P* = 0.014, **Table 1a**).

A striking association was seen between frequency of zero *FCGR3B* copy number and development of autoimmunity in SLE, glomerulonephritis and ANCA associated vasculitis (**Table 1b**). Zero copy number, which suggests complete genomic absence of *FCGR3B* and likely total deficiency of FCGR3B protein[10], was seen in 25 out of 1279 cases of autoimmunity but in only one of 862 healthy controls. This suggests that complete *FCGR3B* deficiency is a, newly defined, highly penetrant risk factor for development of systemic autoimmunity. However, we did not observe a significant correlation between possession of zero *FCGR3B* copy number and early age of onset in the autoimmune diseases considered in this study.

These results show an association between low *FCGR3B* copy number and risk of development of both SLE and ANCA-associated vasculitis both of which are associated with systemic inflammation and commonly involve the glomerulus. In contrast we found no association with GD and ADD, both organ-specific autoimmune diseases, suggesting different mechanisms leading to autoimmunity in these disorders. *FCGR3B* is expressed by neutrophils and eosinophils. It plays an important role in tethering of neutrophils to immune complexes and may play a role in clearance of immune complexes[11]. We hypothesize that, in patients with SLE, reduced clearance of immune complexes predisposes to disease in glomeruli and at other sites. In ANCA-associated vasculitis Fc-receptor mediated activation of neutrophils leads to inflammation[12,13] and it may appear paradoxical that low copy number of *FCGR3B* should be protective. However, recent work (Tsuboi N, JASN 2006; abstract# SA-FC063) in transgenic animals shows that human FCGR3B is not pro-inflammatory in glomerular inflammation whereas the other human neutrophil Fcgamma receptor FCGR2A is pro-inflammatory. We therefore speculate that the balance between expression of FCGR3B and FCGR2A on neutrophils determines their relative efficiency in immune complex removal and inflammatory activation and may explain the effect of low copy number of *FCGR3B* in both SLE and ANCA vasculitis.

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Figure 1. Histograms showing the distribution of *FCGR3B* copy number in different groups of cases (filled columns) versus unaffected controls (open columns). **a**, lupus glomerulonephritis; **b**, systemic lupus erythematosus; **c**, UK Wegener's granulomatosis; **d**, French Wegener's granulomatosis; **e**, microscopic polyangiitis; **f** Grave's disease, **g** Addison's disease. Nonparametric Mann-Whitney *U*-test was applied to assess association with gene copy number at *FCGR3B;* 2-tailed *P*-values for significance were estimated by 100,000 Monte Carlo simulations. *n,* number of subjects in each group.

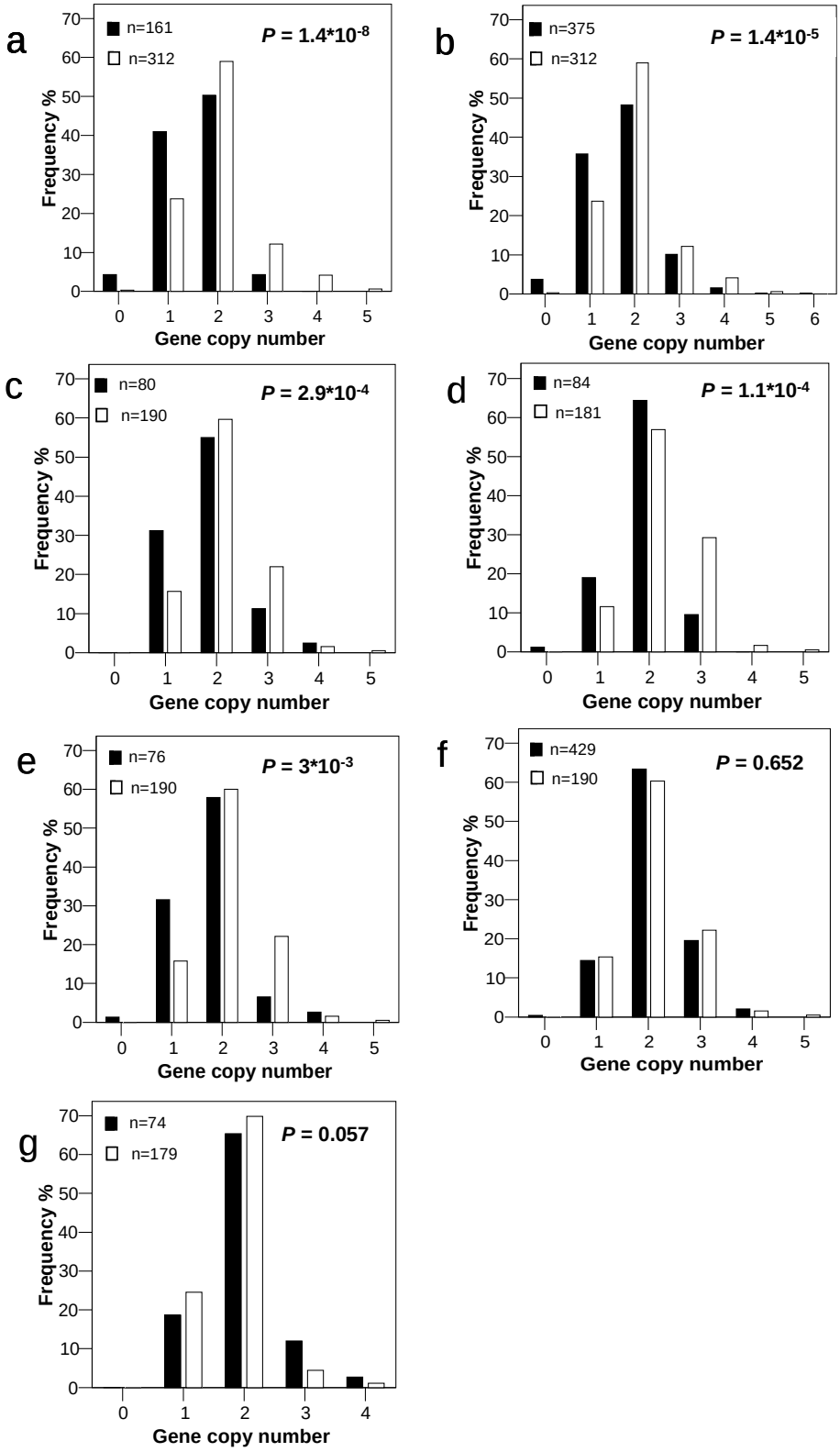

Table 1. a, Logistic regression results and risk estimates for lupus glomerulonephritis, SLE, MPA, WG in UK and French population samples, and GD. OR*,* odds ratio, risk of acquiring disease relative to the population-specific median (CNV = 2). An odds ratio >1 indicates a detrimental effect of *FCGR3B* copy number, and <1 indicates a protective effect of *FCGR3B* copy number. In all logistic regression models gender and age were included as covariates. CI, confidence interval; *P*-value*,* level of significance of OR. **b***,* Clinical features of affected individuals carrying zero *FCGR3B* copy number. nk, not known.

a

Autoimmune disease	Cases/ controls	Copy number categories	OR	95% CI	*P*-value
Lupus Glomerulonephritis	152/312	< 2	2.431	1.467-4.028	0.001
		> 2	0.406	0.153-1.075	0.07
SLE	359/312	< 2	2.207	1.132-3.400	0.0003
		> 2	1.127	0.634-2.001	0.684
WG (UK)	80/188	< 2	2.464	1.190-5.100	0.015
		> 2	0.756	0.319-1.792	0.525
WG (France)	77/181	< 2	1.577	0.762-3.267	0.220
		> 2	0.277	0.123-0.624	0.002
MPA	76/188	< 2	2.561	1.217-5.389	0.013
		> 2	0.525	0.194-1.421	0.204
GD	278/188	< 2	0.874	0.512-1.491	0.621
		> 2	0.883	0.556-1.404	0.599
ADD	71/185	< 2	0.673	0.316-1.433	0.304
		> 2	3.438	1.283-9.219	0.014

b

	Gender	Clinical Diagnosis	Current Age	Age at Diagnosis	Renal Involvement
Cases					
	Male	Lupus Glomerulonephritis	53	nk	+
	Male	Lupus Glomerulonephritis	36	28	+
	Female	Lupus Glomerulonephritis	nk	nk	+
	Female	Lupus Glomerulonephritis	40	34	+
	Female	Lupus Glomerulonephritis	21	nk	+
	Female	Lupus Glomerulonephritis	27	nk	+
	Female	Lupus Glomerulonephritis	31	27	+
	Male	SLE	32	18	-
	Female	SLE	39	nk	-
	Female	SLE	36	nk	-
	Female	SLE	47	nk	-
	Female	SLE	40	34	-
	Female	SLE	20	nk	-
	Female	SLE	54	nk	-
	Female	SLE	51	nk	-
	Male	SLE	22	nk	-
	Female	SLE	28	19	-
	Male	SLE	52	44	-
	Female	SLE	60	nk	-
	Female	SLE	53	nk	-
	Female	SLE	38	20	-
	Female	MPA	68	67	+
	Female	WG	84	nk	nk
	Male	GD	60	51	-
	Female	GD	62	49	-
Controls					
	Female		48		

Supplementary Figure 1. Copy number variation of *FCGR3B* gene was confirmed by semi-quantitative PCR using human genomic DNA from ANCA vasculitis patients in which copy number has been determined by quantitative PCR. (**a**)The top bands indicate 0, 1, 2 and 4 copies of *FCGR3B* gene; bottom bands show 2 copies of *FOXP2* control gene. *FCGR3B* gene amplification products were sequence verified. (**b**) Quantification of amplicons by densitometry. After normalization to FOXP2 band intensities, the ratios of intensities between subjects with different *FCGR3B* copy number (CN) were calculated. Ratios were as follows: 2.5 for CN 2 vs. CN 1; 3.8 for CN 4 vs. CN 1.