Published in final edited form as:

Nat Genet. 2007 June ; 39(6): 721–723. doi:10.1038/ng2046.

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

Manuela Fanciulli^{1,11}, Penny J Norsworthy^{1,11}, Enrico Petretto^{1,11}, Rong Dong¹, Lorraine Harper², Lavanya Kamesh², Joanne M Heward³, Stephen C L Gough³, Adam de Smith⁴, Alexandra I F Blakemore⁴, Philippe Froguel^{4,5}, Catherine J Owen⁶, Simon H S Pearce⁶, Luis Teixeira⁷, Loïc Guillevin⁷, Deborah S Cunninghame Graham⁸, Charles D Pusey⁹, H Terence Cook¹⁰, Timothy J Vyse⁸, and Timothy J Aitman^{1,8}

¹Physiological Genomics and Medicine Group, UK Medical Research Council (MRC) Clinical Sciences Centre, Imperial College, London W12 0NN, UK

²Renal Immunobiology, Division of Immunity and Infection, Medical School, University of Birmingham, Birmingham B15 277, UK

³Division of Medical Sciences, Medical School, University of Birmingham, Birmingham B15 277, UK

⁴Section of Genomic Medicine, Division of Medicine, Imperial College London, Hammersmith Hospital, Du Cane Road, London W12 0NN, UK

⁵Centre National de la Recherche Scientifique (CNRS), UMR 8090-Institute of Biology, Pasteur Institute, Lille, 59800 France

⁶Institute of Human Genetics, International Centre for Life, Central Parkway, Newcastle upon Tyne NE1 3BZ, UK

⁷Department of Internal Medicine, Reference Center for Vasculitis and Systemic Sclerosis, Cochin Hospital, Assistance Publique-Hôpitaux de Paris, Paris-Descartes University 27, rue du Faubourg Saint-Jacques, 75679 Paris Cedex 14, France

⁸Section of Molecular Genetics and Rheumatology, Imperial College London, London W12 0NN, UK

⁹Renal Medicine, Imperial College London, London W12 0NN, UK

¹⁰Department of Histopathology, Imperial College London, London W12 0NN, UK

Abstract

Naturally occurring variation in gene copy number is increasingly recognized as a heritable source of susceptibility to genetically complex diseases. Here we report strong association between *FCGR3B* copy number and risk of systemic lupus erythematosus ($P = 2.7 \times 10^{-8}$), microscopic

© 2007 Nature Publishing Group

Correspondence should be addressed to T.J.A. (t.aitman@csc.mrc.ac.uk).

¹¹These authors contributed equally to this work.

AUTHOR CONTRIBUTIONS

M.F., E.P., H.T.C., T.J.V. and T.J.A. designed the study. M.F., P.J.N. and R.D. performed PCR-based experiments. A.d.S., A.I.F.B. and P.F. performed oligonucleotide array comparative genomic hybridization analysis. M.F. and E.P. performed statistical and data analysis. L.H., L.K., J.M.H., S.C.L.G., P.F., C.J.O., S.H.S.P., L.T., L.G., D.S.G.G., C.D.P. and T.J.V. provided material and contributed to the collection of the case-control studies. The manuscript was written by M.F., E.P., H.T.C., T.J.V. and T.J.A.

COMPETING INTERESTS STATEMENT

The authors declare no competing financial interests.

Note: Supplementary information is available on the Nature Genetics website

polyangiitis ($P = 2.9 \times 10^{-4}$) and Wegener's granulomatosis in two independent cohorts from the UK ($P = 3 \times 10^{-3}$) and France ($P = 1.1 \times 10^{-4}$). We did not observe this association in the organ-specific Graves' disease or Addison's disease. Our findings suggest that low *FCGR3B* copy number, and in particular complete *FCGR3B* deficiency, has a key role in the development of systemic autoimmunity.

Structural variation is now recognized as a rich source of genetic diversity in the human genome, and during the past 3 years thousands of common copy number variants (CNVs) have been described¹⁻⁴. Although rare CNVs have been established as a cause of mental retardation and single-gene disorders^{5,6}, the role of more common, transmissible CNVs in evolutionary selection and disease susceptibility is largely unknown.

We recently reported an association between low *FCGR3B* copy number and autoimmune glomerulonephritis in a sample of individuals with systemic lupus erythematosus (SLE) from UK nuclear families⁷. In the present study, the previously reported association ($P = 1 \times 10^{-3}$) was confirmed and strengthened ($P = 1.4 \times 10^{-8}$, 95% c.i. = 0 to 2.9×10^{-5} ; Fig. 1a) in a larger sample comprising 161 SLE patients with glomerulonephritis from the UK and in 312 independent controls from the UK 1958 birth cohort (**Supplementary Methods and Supplementary Table 1** online). Compared with individuals possessing two copies of *FCGR3B* (the median copy number in the control population), individuals with fewer than two copies had a significantly higher risk of glomerulonephritis (odds ratio (OR) = 2.43, $P = 1 \times 10^{-3}$, Table 1).

We estimated gene copy number as described in **Supplementary Methods and Supplementary Table 2** online and as previously reported⁷. Additionally, we validated the quantitative PCR assay by semiquantitative PCR (**Supplementary Fig. 1** online) and by array comparative genomic hybridization analysis (**Supplementary Fig. 2** online). Statistical analyses are described in **Supplementary Methods**.

We then investigated the involvement of CNV at *FCGR3B* in the development of SLE with no known renal involvement in a set of 375 unrelated cases recruited from the UK (**Supplementary Methods and Supplementary Table 1**). We found a new, strong association ($P = 1.4 \times 10^{-5}$; 95% c.i. = 0 to 2.9×10^{-5} ; Fig. 1b and Table 1) and an increased risk for development of SLE in individuals with fewer than two copies of *FCGR3B* (OR = 2.21, $P = 3 \times 10^{-4}$, Table 1). When we considered all SLE cases together ($n = 536$), regardless of renal involvement, the P value of association was 2.7×10^{-8} (95% c.i. = 0 to 2.9×10^{-5}). However, we did not observe any significantly higher risk of acquiring glomerulonephritis in individuals with SLE who possessed fewer than two copies of *FCGR3B* (OR = 1.09; 95% c.i. = 0.73-1.62, $P = 0.660$).

Given these findings and the key role of Fc receptors in regulation of inflammatory and immune responses⁸⁻¹⁰, we hypothesized that CNV in *FCGR3B* may confer susceptibility to a range of autoimmune disorders, with or without glomerular involvement. Therefore, we first studied anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis, a systemic autoimmune disorder in which renal disease occurs commonly. Individuals with ANCA-associated vasculitis typically present with one of two clinical syndromes: Wegener's granulomatosis or microscopic polyangiitis (MPA); we considered these two subtypes separately (**Supplementary Methods and Supplementary Table 1**).

In two independent cohorts of individuals with Wegener's granulomatosis from the University of Birmingham and from the Cochin Hospital in Paris, we found association between *FCGR3B* copy number and susceptibility to Wegener's granulomatosis (Birmingham cohort, $P = 3 \times 10^{-3}$ and 95% c.i. = 2.5×10^{-3} to 3.1×10^{-3} ; Paris cohort, $P =$

1.1×10^{-4} and 95% c.i. = 4.5×10^{-5} to 1.7×10^{-4} ; Fig. 1c,d). Similarly, we observed a significant association between *FCGR3B* copy number and MPA in a separate Birmingham cohort ($P = 2.9 \times 10^{-4}$; 95% c.i. = 1.8×10^{-4} to 3.9×10^{-4} ; Fig. 1e).

We also studied individuals with Graves' disease and isolated autoimmune Addison's disease, as examples of organ-specific autoimmune diseases with no renal involvement (**Supplementary Methods** and **Supplementary Table 1**). We did not see any significant association between *FCGR3B* copy number and either Graves' disease (in a large cohort that included 429 cases and 190 controls; $P = 0.65$; 95% c.i. = 0.64-0.65; Fig. 1f) or Addison's disease (74 cases, 179 controls; $P = 0.057$; 95% c.i. = 0.056-0.059; Fig. 1g).

Logistic regression models indicated an increased risk of Wegener's granulomatosis and MPA in UK individuals with a low *FCGR3B* copy number, with OR = 2.46 ($P = 0.015$) and OR = 2.56 ($P = 0.013$), respectively. Individuals in the French Wegener's granulomatosis cohort did not show significantly increased risk with low copy number, although we saw a reduced risk in those with high copy number (Table 1). Consistent with the association results, logistic regression models did not show any increased risk in individuals with low *FCGR3B* copy numbers for either Graves' or Addison's disease (Table 1).

The frequency of individuals with zero copies of *FCGR3B* (indicating complete genomic absence of *FCGR3B* and total deficiency of FCGR3B protein^{11,12}) differed markedly between cases and controls. We observed zero copies of *FCGR3B* in 25 out of 1,279 (2%) individuals with autoimmunity (7 SLE patients with glomerulonephritis, 14 with SLE, one with MPA, 1 with Wegener's granulomatosis and 2 with Graves' disease) but in only 1 of 862 controls (0.1%). This suggests that complete FCGR3B deficiency confers a very high risk of autoimmunity, particularly for SLE.

Our data show an association between low *FCGR3B* copy number and risk of development of SLE and ANCA-associated vasculitis, both of which have systemic inflammation as a key feature and commonly involve the glomerulus. In contrast, we did not find any significant association with Graves' or Addison's diseases, both of which are organ-specific autoimmune disorders, suggesting a different role for *FCGR3B* CNV in development of systemic and organ-specific autoimmunity.

FCGR3B is expressed by neutrophils and eosinophils and is important in tethering of neutrophils to immune complexes and in clearance of immune complexes¹³. We hypothesize that in individuals with SLE, reduced clearance of immune complexes predisposes to disease in glomeruli and at other sites. In ANCA-associated vasculitis, in which immune complexes seem not to have a central pathogenic role¹⁴, our data suggest that reduced or absent expression of FCGR3B may be associated with enhanced neutrophil activation by ANCA.

These findings suggest that low *FCGR3B* copy number may be a common genomic mechanism in the development of SLE, Wegener's granulomatosis and MPA. The data also support the hypothesis that frequent, heritable CNV in the human genome confers risk to a range of common human diseases. As CNVs have been reported frequently in genes that act in inflammation and immune response pathways^{3,4,15}, structural variation in such genes may contribute to the evolution of predisposition to a range of infectious and inflammatory disorders.

All study participants gave written informed consent. Ethics committee approval was obtained for all studies as detailed in the **Supplementary Methods**.

Acknowledgments

We thank C. Neuwirth and Y. Tan (Clinical Sciences Centre); A. Wong (Imperial College); J. Cedric and M. Delpuch (INSERM U567); M. Marre (INSERM U695) and B. Balkau (INSERM U780-IFR69) for their role in collecting patients and controls. We thank A. Tsalenko, A. Scheffer, N. Sampas, P. Tsang and L. Bruhn from Agilent Laboratories for contributions to the array comparative genomic hybridization results. We acknowledge intramural funding from the Clinical Sciences Centre (to T.J.A.) and support from the Wellcome Trust Cardiovascular Functional Genomics award (T.J.A.), from the FP6 EURATools (European Union contract number LSHG-CT-2005-019015) award (T.J.A.), from the UK MRC (T.J.A., H.T.C.) and from a Wellcome Trust Senior Fellowship (T.J.V.). We acknowledge use of genotype data from the British 1958 Birth Cohort DNA collection, funded by the UK Medical Research Council grant G0000934 and the Wellcome Trust grant 068545/Z/02.

References

1. Sebat J, et al. *Science*. 2004; 305:525–528. [PubMed: 15273396]
2. Iafrate AJ, et al. *Nat. Genet.* 2004; 36:949–951. [PubMed: 15286789]
3. Tuzun E, et al. *Nat. Genet.* 2005; 37:727–732. [PubMed: 15895083]
4. Redon R, et al. *Nature*. 2006; 444:444–454. [PubMed: 17122850]
5. Stankiewicz P, et al. *Trends Genet.* 2002; 18:74–82. [PubMed: 11818139]
6. Lupski JR. *Nat. Genet.* 2006; 38:974–976. [PubMed: 16941003]
7. Aitman TJ, et al. *Nature*. 2006; 439:851–855. [PubMed: 16482158]
8. Salmon JE, et al. *Arthritis Rheum.* 2001; 44:739–750. [PubMed: 11315912]
9. Takai T. *J. Clin. Immunol.* 2005; 25:1–18. [PubMed: 15742153]
10. Nimmerjahn F, et al. *Immunity*. 2006; 24:19–28. [PubMed: 16413920]
11. de Haas M, et al. *Blood*. 1995; 86:2403–2413. [PubMed: 7662988]
12. Clark MR, et al. *J. Clin. Invest.* 1990; 86:341–346. [PubMed: 1694867]
13. Coxon A, et al. *Immunity*. 2001; 14:693–704. [PubMed: 11420040]
14. Harper L, et al. *J. Pathol.* 2000; 190:349–359. [PubMed: 10685069]
15. Gonzalez E, et al. *Science*. 2005; 307:1434–1440. [PubMed: 15637236]

Figure 1.

Distribution of *FCGR3B* copy number in different groups of affected individuals (filled bars) versus unaffected controls (open bars). (a) SLE with nephritis. (b) SLE without nephritis. (c) Wegener's granulomatosis (UK). (d) Wegener's granulomatosis (France). (e) Microscopic polyangiitis. (f) Graves' disease. (g) Addison's disease. A nonparametric Mann-Whitney *U* test was applied to assess association with gene copy number at *FCGR3B*. Two-tailed *P* values for significance were estimated by 100,000 Monte Carlo simulations. *n*, number of subjects in each group.

Table 1
Estimated risk of several autoimmune disorders according to *FCGR3B* gene copy number

Autoimmune disease	Cases/controls ^a	Copy number	OR	95% c.i.	P value ^b
SLE with nephritis	152/312	<2	2.43	1.47-4.03	0.001
		>2	0.40	0.15-1.07	0.07
SLE without nephritis	359/312	<2	2.21	1.13-3.40	0.0003
		>2	1.13	0.63-2.00	0.684
Wegener's granulomatosis (UK)	80/188	<2	2.46	1.19-5.10	0.015
		>2	0.76	0.32-1.79	0.525
Wegener's granulomatosis (France)	77/181	<2	1.58	0.76-3.27	0.220
		>2	0.28	0.12-0.62	0.002
Microscopic polyangiitis	76/188	<2	2.56	1.22-5.39	0.013
		>2	0.52	0.19-1.42	0.204
Graves' disease	278/188	<2	0.87	0.51-1.49	0.621
		>2	0.88	0.56-1.40	0.599
Addison's disease	71/179	<2	0.69	0.33-1.43	0.327
		>2	2.89	1.15-7.28	0.023

SLE, systemic lupus erythematosus; OR, odds ratio (risk of acquiring disease); c.i., confidence interval.

^aSubjects with missing age or gender data were discarded from the logistic regression analysis

^bPvalue: level of significance of the OR.