Genes and Immunity (2010) 11, 155–160

www.nature.com/gene

ORIGINAL ARTICLE

Association of copy number variation in the FCGR3B gene with risk of autoimmune diseases

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Copy number variation (CNV) in the human genome is an important determinant of susceptibility to autoimmune diseases. Many autoimmune diseases share similar clinical and pathogenic features. Thus, CNVs of genes involved in immunity may serve as shared determinants of multiple autoimmune diseases. Here, we determined the association between CNV in the gene encoding FCGR3B with the risk of developing autoimmune diseases and whether the observed associations are modified by the CNV in CCL3L1 (CC chemokine ligand 3-like 1), a gene encoding a potent chemokine. In a cross-sectional study of 774 subjects, we estimated FCGR3B and CCL3L1 gene copy number in 146, 158 and 61 subjects with systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and primary Sjögren's syndrome (SS), respectively, and 409 healthy controls. The median gene dose of FCGR3B in the study population was two. FCGR3B copy number $<$ or >2 was associated with an increased risk of SLE and primary SS but not RA. This association was mostly evident in subjects who also had two copies of CCL3L1. Thus, our data suggest that epistatic interactions between CNV of FCGR3B and CCL3L1, two immune response genes, may influence phenotypically related autoimmune diseases.
Genes and Immunity (2010) **11,** 155–160; doi:10.1038/gene.2009.71; published online 10 September 2009

Keywords: *copy number variation (CNV); FCGR3B; CCL3L1; systemic lupus erythematosus; Sjögren's syndrome*

Introduction

Copy number variation (CNV) of human DNA segments is an important source of genetic diversity and increasing evidence indicates that CNV may underlie disease susceptibility.[1–4] It is to be noted that there seems to be an enrichment of CNV in immune response genes[1] such that they may contribute to the observed inter-individual variability in susceptibility to infectious diseases, vasculitis and autoimmune diseases. For example, we and others found that the copy number of segmental duplication on chromosome 17q that contains the gene encoding the chemokine CC chemokine ligand 3-like 1 (CCL3L1) influences HIV-AIDS susceptibility,[5–9] and the risk of developing Kawasaki's disease,[10] systemic lupus erythematosus (SLE)[11] and rheumatoid arthritis (RA).[12] CNV in the gene encoding the complement component C4 and *FCGR2C* has been associated with SLE[13] and idiopathic thrombocytopenic purpura,[14] respectively.

The gene *FCGR3B*, at chromosome 1q23, encodes the activatory Fc receptor, and a low copy number of this gene has been associated with increased susceptibility to glomerulonephritis in patients with SLE[15] as well as with anti-neutrophil cytoplasmic antibody-associated vasculitis.[13] FCGR3B is expressed on neutrophils and eosinophils, and has an important role in the regulation of inflammatory and immune responses.[16,17] On the basis of the principle of common variant/multiple diseases (CV/MD), which states that a CV might affect susceptibility to MD,[18–20] here we determined whether the impact of CNV in *FCGR3B* extends beyond SLE and affects the risk of other autoimmune diseases such as RA and primary Sjögren's syndrome (SS). In addition, because the CNV in *CCL3L1* influences the risk of SLE and RA,[11,12] we determined whether this CNV modifies the phenotypic (autoimmune-influencing) effects associated with the CNV in *FCGR3B*.

In addition to the principle of CV/MD, here we considered whether the gene balance hypothesis would be applicable to the CNV in *FCG3RB*. This hypothesis postulates that phenotypic consequences occur both when the gene is under- or overexpressed, that is, in genetic states of haploinsufficiency and high gene dosage.[2] We considered this hypothesis might be applicable to *FCGR3B*, because in our previous studies, we found that a copy number of *CCL3L1* that was lower or greater than the average gene dose found in the study population was associated with an increased risk of developing SLE.[11] The results of the present study validate both the concepts of CV/MD and gene balance,

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Received 4 March 2009; revised and accepted 05 August 2009; published online 10 September 2009

because we found that (i) a copy number of *FCG3RB* that is less than or greater than the population average was associated with an increased risk of developing SLE and primary SS and (ii) the phenotypic effects associated with *FCG3RB* dose differed depending on the coexisting *CCL3L1* gene dose.

Results

The copy number of *FCGR3B* in subjects with SLE, RA and primary SS ranged from zero to five, with an average gene dose of two copies (Figure 1a). The distribution of the copy number of *FCGR3B* differed significantly between controls and SLE or primary SS cases, but not between controls and RA cases (Figure 1a). This suggested that the CNV of *FCGR3B* might be a risk factor for development of SLE and primary SS but not RA.

In an earlier study, we found that a *CCL3L1* copy number that was greater or lower than the average *CCL3L1* gene dose found in the study population (two copies) was associated with an increased risk of developing SLE.[11] We therefore hypothesized that a similar relationship might be applicable to the CNV in *FCGR3B* such that a copy number that was lower or higher than the average gene dose of *FCGR3B* (two copies) might associate with an altered risk of developing SLE, primary SS or RA. Consistent with this possibility, possession of a copy number of *FCGR3B* less than (odds ratio (OR) = 2.41; 95% confidence interval (CI) = 1.41–4.10; $P = 0.001$) or greater than (OR = 2.15; 95% CI = 1.18–3.93; $P = 0.013$) two copies was associated with an increased risk of developing SLE (Figure 1b). Similarly, deviation from the average *FCGR3B* gene dose of two copies was associated with an increased risk of developing primary SS (OR = 2.01; 95% CI = 0.93–4.30; $P = 0.074$ for <2 *FCGR3B* copies and OR = 2.26; 95% CI = 1.01–5.05; $P = 0.048$ for >2 *FCGR3B* copies; Figure 1b). There were no significant differences in the clinical manifestations of patients with less than two copies versus those who had greater than two copies of *FCGR3B* (data not shown). By contrast to its associations with SLE and SS, we did not detect an association between *FCGR3B* CNV and risk of developing RA (Figure 1b).

Possession of a copy number of *FCGR3B* greater than two was also associated with an increased risk (OR = 2.98; 95% CI 0.98–9.06; $P = 0.055$) of lupus nephritis. Although possession of less than two copies of *FCGR3B* was also associated with a trend toward an increased risk of developing lupus nephritis, this association did not reach statistical significance (OR = 1.53; 95% CI = 0.62–3.76; $P = 0.353$). We did not detect an association between *FCGR3B* CNV and levels of anti-DNA, anti-Ro and anti-La autoantibody (data not shown).

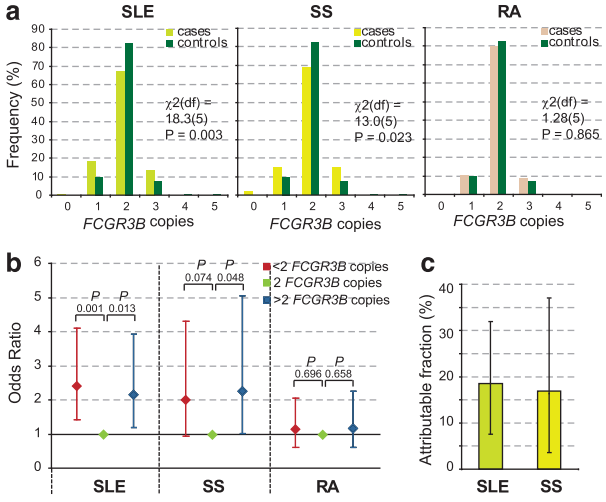

Figure 1 Association of copy number variation (CNV) of *FCGR3B* with risk of developing autoimmune diseases. (**a**) Distribution of copy number of the *FCGR3B* gene in the three study groups. SLE, systemic lupus erythematosus; SS, primary Sjögren's syndrome; RA, rheumatoid arthritis. Statistical significance for a difference in the distribution of the copy number of *FCGR3B* in cases and controls was assessed by the χ^2-test. d.f., degrees of freedom; *P*, significance value. (**b**) Risk of developing SLE, SS or RA assessed by logistic regression analyses. Diamonds and error bars indicate the point and 95% confidence interval estimates of odds ratios. Subjects with two copies of *FCGR3B* were the reference group. (**c**) Attributable fraction of copy number of *FCGR3B* other than two on the risk of developing SLE and SS. The upper limit of the color-coded bars indicates the point estimates and the error bars indicate the 95% confidence intervals for the attributable fraction.

Independent effects of FCGR3B CNV

We next determined whether the copy number of *FCGR3B* is associated with the risk of developing SLE and primary SS independent of the effects of the copy number of *CCL3L1*.[5] To test this, we included *CCL3L1* CNVs (<2 and >2) in the same logistic regression model along with the *FCGR3B* CNVs. After adjusting for the effects of *CCL3L1* CNV, deviation from the average *FCGR3B* gene dose independently associated with the risk of developing SLE (OR = 2.30; 95% CI = 1.35–3.94; $P = 0.002$ for <2 *FCGR3B* copies and OR = 2.25; 95% CI = 1.22–4.13; $P = 0.009$ for >2 *FCGR3B* copies) and primary SS (OR = 1.92; 95% CI = 0.89–4.14; $P = 0.094$ for <2 *FCGR3B* copies and OR = 2.34; 95% CI = 1.04–5.27; $P = 0.041$ for >2 *FCGR3B* copies). Next, we adjusted for the effects of age and gender, and found that the ORs for the association of *FCGR3B* CNV were very similar in multivariate models that included age and gender as covariates (data not shown). Therefore, in all subsequent analyses, we did not include age or gender in the statistical models, and examined the interactive effects of *FCGR3B* and *CCL3L1* CNV on autoimmune diseases.

Modifier effects of CCL3L1 in SLE and primary SS

In previous studies, we found that the copy number of *CCL3L1* modified the Kawasaki disease-, SLE- and HIV-1-disease-influencing effects of *CCR5* haplotypes (Burns *et al*.,[10] Mamtani *et al*.[11] and data not shown). Thus, here we sought to determine whether the underlying copy number of *CCL3L1* modified the SLE- and SS-influencing effects associated with *FCGR3B* CNVs. To accomplish this, we stratified subjects on the basis of their *CCL3L1* copy number (that is, <2, 2 or >2 copies), and determined the association of the copy number of *FCGR3B* before (model 1, Table 1) and after accounting for the three *CCL3L1* genetic backgrounds defined according to copy number (models 2–4, Table 1; <2, 2 or >2 *CCL3L1* copies). The increased risk of SLE associated with $<$ or >2 *FCGR3B* copies was evident mainly in those individuals who also possessed two

Table 1 Modification of the phenotypic effects associated with *FCGR3B* CNV by the copy number of *CCL3L1*

FCGR3B gene copy number	*SLE*			*Primary SS*		
	OR (95% CI)	P-*values*	*AF (%)*[a]	*OR (95% CI)*	P-*values*	*AF (%)*
	Model 1[b] (overall, $N=555$)			Model 1 (overall, $N=470$)		
<2	2.41 (1.41–4.10)	0.001	18.5	2.01 (0.93–4.30)	0.074	16.5
>2	2.15 (1.18–3.93)	0.013		2.26 (1.01–5.05)	0.048	
	Model 2 (<2 *CCL3L1* copies, $N=103$)			Model 2 (<2 *CCL3L1* copies, $N=82$)		
<2	1.77 (0.59–5.30)	0.305	10.9	0.70 (0.08–6.24)	0.752	16.2
>2	1.52 (0.24–9.67)	0.657		6.33 (1.10–36.37)	0.038	
	Model 3 (2 *CCL3L1* copies, $N=206$)			Model 3 (2 *CCL3L1* copies, $N=181$)		
<2	3.7 (1.37–10.10)	0.010	25.8	7.53 (2.30–24.59)	0.001	34.4
>2	2.92 (1.12–7.64)	0.029		1.97 (0.39–9.91)	0.410	
	Model 4 (>2 *CCL3L1* copies, $N=238$)			Model 4 (>2 *CCL3L1* copies, $N=199$)		
<2	1.91 (0.88–4.14)	0.102	16.6	0.88 (0.24–3.20)	0.847	4.1
>2	2.09 (0.87–5.04)	0.098		1.68 (0.51–5.55)	0.397	
	Results of Breslow–Day test			Results of Breslow–Day test		
<2		0.511			0.018	
>2		0.788			0.436	

Abbreviations: AF, attributable fraction; *CCL3L1*, CC chemokine ligand 3-like 1; CI, confidence intervals; CNV, copy number variation; OR, odds ratio; SLE, systemic lupus erythematosus; SS, primary Sjögren's syndrome.
[a]AF estimated for *FCGR3B* copy number that differs from 2 (that is, either $<$ or >2 copies).
[b]Model 1 indicates association between possession of *FCGR3B* copy number and SLE or SS disease susceptibility before accounting for *CCL3L1* gene dose; Models 2–4 indicate association of the *FCGR3B* copy number in the context of different *CCL3L1* gene-dose strata (that is, <2, 2 and >2 *CCL3L1* copies).

copies of *CCL3L1*, the average gene dose of *CCL3L1* (Table 1). In the case of primary SS, the increased risk associated with <2 *FCGR3B* copies was most evident in individuals who also possessed two *CCL3L1* copies, whereas the increased risk associated with >2 *FCGR3B* copies was evident only when these subjects also possessed <2 *CCL3L1* copies (Table 1). The results of the Breslow–Day tests (Table 1) indicated that the heterogeneity of *FCGR3B* ORs across *CCL3L1* strata was statistically significant for subject with SS, but not for those with SLE. For the latter group of patients, *FCGR3B* seemed to be associated with an increased risk independent of the *CCL3L1* copy number.

Attributable fractions for FCGR3B
We calculated attributable fractions to determine the extent to which *FCGR3B* copy number contributed to the susceptibility to SLE or primary SS before and after accounting for the *CCL3L1* genetic background. SLE and primary SS of $\sim$19 and 17%, respectively, could be attributed to a *FCGR3B* copy number that deviated from two (Figure 1c and Table 1). Consistent with the data showing that the associations of $<$ or >2 *FCGR3B* copies is maximal in those with two *CCL3L1* copies, the attributable fractions were also greatest in these subjects.

Discussion

Our data suggests an association between both low- and high *FCGR3B* copy number, and risk of development of SLE and primary SS. These two clinical conditions, although distinct, have some similarities. Both are autoimmune diseases with similar antibody profiles, affect women more frequently than men and similar *HLA* alleles convey an increased risk of developing SLE and primary SS.[21–24] Isenberg[25] has argued that 'both SLE and primary SS are overlapping diseases that cannot be segregated by risk factors, etiology, or clinical or serologic manifestations'. The results of our genetic association study extend these parallels between SLE and primary SS, and in doing so, provide additional credence to the CV/MD hypothesis.[18–20] Previously, we had shown similarities in the genetic determinants that influence HIV-AIDS susceptibility and SLE pathogenesis.[11] This was notable, as SLE and HIV disease share some clinical and immunopathogenic features (reviewed in Mamtani *et al*.[11]). Together, these findings highlight the growing recognition[20] that diseases that may share immunopathogenic features may also share overlapping genetic determinants of disease pathogenesis. In contrast, we did not find an association between *FCGR3B* copy number and RA. These findings throw light on common and specific mechanisms by which the copy number of *FCGR3B* may have a role in the development of different autoimmune diseases.

Another noteworthy feature of our results was that both low- and high *FCGR3B* copy number was associated with an enhanced risk of SLE and primary SS, a feature that is consistent with the gene balance hypothesis, which posits that both haploinsufficiency and overexpression of a gene may influence disease outcomes.[2] Thus, the biological correlates linked to a low- or high *FCGR3B* gene dose might contribute to an autoimmune phenotype. In many mammalian species, Fc receptors provide a functional bridge between the humoral and

cellular branches of the immune system, and have a crucial role in activation and modulation of immune responses.[26] Human FCGR3B is expressed mainly on neutrophils and is essential for tethering of immune complexes to neutrophils.[16] Aitman *et al*.[15] raise the possibility that a reduced expression of FCGR3B in patients with low *FCGR3B* copy number may lead to a diminished clearance of immune complexes in SLE, a finding that has been supported by a recent functional study.[27]

On the other hand, a high *FCGR3B* copy number might be associated with increased expression levels, and consequently highly sensitive neutrophils that may initiate an immune response even when the levels of immune complexes are low. Consistent with this thesis, it has been shown, for example, that allelic variants of *FCGR3B* that are associated with varying density of the cell-surface receptor lead to different levels of phagocytosis of IgG-opsonized erythrocytes.[28] Moreover, when the burden of immune complexes is moderate to high, these sensitive neutrophils may result in a strong cellular immune response, because infiltration of the target tissues with neutrophils sets up a cascade of events beginning with myeloperoxidase deposition and further cellular recruitment of $CD4^+$ T cells.[29]

We found that the risk of SLE and primary SS that can be attributed to a *FCGR3B* copy number other than two copies was both similar and high ($\sim$19 and 17%, respectively). Of note, we had found previously that the deviation from two copies of the *CCL3L1* gene is also associated with an increased risk of SLE in several geographically distinct cohorts.[11] In northwestern Colombians examined herein, deviation of the *CCL3L1* copy number from the average dose contributed to $\sim$28% (95% CI 5.12–46.84) of the risk of developing SLE. A possible explanation for these apparently high estimates of the attributable risk could be that a subset of our study population had a more severe disease phenotype, thereby inflating the strength of association between CNV and SLE. However, considering the wide spectrum of clinical manifestations in the study subjects, a false high estimate of the ORs is unlikely. Therefore, it is possible that the *FCGR3B* and *CCL3L1* CNVs have a key role in modulating the risk of SLE and SS in the study population. Taken together, *FCGR3B* and *CCL3L1* CNV may contribute substantially to SLE susceptibility (attributable fraction for both *FCGR3B* and *CCL3L1* CNV = 45.12% (95% CI 11.52–76.63)). Thus, we propose that a copy number of *FCGR3B* and *CCL3L1* that deviates from a homeostatic diploid state may be an important genomic determinant of risk of autoimmunity.

In our previous work, we found that *CCL3L1* is not only associated with the risk of SLE but it also modified the phenotypic (SLE-modifying) effects of *CCR5* haplotypes.[11] In this light, we sought to investigate whether *CCL3L1* also modifies the disease-influencing effects associated with *FCGR3B*, as CNVs in both of these genes are associated with the risk of SLE. Extending and amplifying these findings, we found that with the exception of the association between possession of >2 *FCGR3B* copies and risk of primary SS, all other associations for $<$ or >2 copies of *FCGR3B* were evident mainly on the genetic background of two copies of *CCL3L1*. This suggests that there might be an epistatic interaction between *FCGR3B* and *CCL3L1*. However, the exact biological mechanisms by which *CCL3L1* CNV might influence the effects of *FCGR3B* are currently unknown. Nevertheless, there is a growing recognition for both the importance of gene–gene interactions in influencing disease phenotype[30,31] and the presence of many well-described and unknown gene networks that may influence different disease states.[32] In this context, we surmise that *CCL3L1-FCGR3B* may participate in key immunopathogenic networks that influence autoimmune diseases. Collectively, these findings underscore that autoimmune and other disease states may be the result of more complex gene–gene interactions than heretofore recognized. In addition, our findings may have translational value as they suggest that FCGR3B might be a potential therapeutic target for autoimmune diseases such as SLE.

Materials and methods

Study population

The study sample comprised of 774 individuals. This included 132 (83.54%) women and 26 (16.46%) men with RA; their mean age ± s.d. (standard deviation) was 48.97 ± 12.24 years, the mean duration of disease was 11 ± 8.1 years and 78.2% tested positive for rheumatoid factor. Extra-articular manifestations were registered in 45.9% of subjects.

There were 143 (97.90%) women and 3 (2.10%) men with SLE; their mean age ± s.d. was 32.77 ± 11.15 years, the mean duration of disease was 7.5 ± 7 years, and antinuclear antibodies, anti-DNA, anti-Sm, anti-RNP, anti-Ro and anti-La antibodies were positive in 99.2, 72.4, 35.4, 63.4, 38.2 and 15.2% subjects, respectively. Lupus nephritis was detected in 41.3% of the SLE patients.

All the 61 patients with primary SS were women; their mean age ± s.d. was 52.42 ± 13.63 years and the mean duration of disease was 6.4 ± 5.2 years. During the course of the disease, all patients presented with sicca symptoms, 59% had vascular-inflammatory involvement and 19.7% had internal-organ manifestations. All primary SS patients had a lymphocytic infiltrate in minor salivary glands with a focus score >1, and anti-Ro and anti-La antibodies were detected in 73.8 and 52.5%, respectively. All patients with autoimmune diseases met the international classification criteria for their respective disease.[33–35]

The control group included 409 individuals without a history of chronic inflammatory autoimmune or infectious diseases; they were matched to patient groups by ethnicity and socioeconomic status, and were unrelated to patients. Their mean age was 45.84 ± 13.20 years and 93.4% were women.

All the study subjects were enrolled at the Cellular Biology and Immunogenetics Unit of the 'Corporación para Investigaciones Biológicas' (CIB), in Medellin, and were of Spanish ancestry, originating from the northwestern population of Colombia known as the Paisa community.[36] Anthropological and historical studies describe this population as the most clearly defined in Colombia because of the relative genetic isolation maintained until the late 19th century. Paisa ancestral ethnic component consists of 85% Caucasian and 15% Amerindian, whereas admixture between Paisa and

African populations has been documented as not significantly greater than zero.[36,37] Previous work from our group has shown the homogeneity of our cohorts and control group.[38,39] Exclusion criteria consisted of hematological disorders, active infection or breastfeeding. This research was conducted in compliance with resolution 008430 of 1993 of the Ministry of Health of Colombia, and was classified as minimal risk research. This study was also approved by the institutional review board at CIB and the University of Texas Health Science Center at San Antonio, USA.

Quantification of FCGR3B copy number

Genotyping for the copy number of *FCGR3B* was performed using real-time PCR with the ABI/PRISM 7700 or 7900 Sequence Detector System (PE, Applied Biosystems, Foster City, CA, USA). The system detected the emitted fluorescence as FAM (6-carboxyfluorescein, 6-FAM) from the probe detecting *FCGR3B* and VIC from the probe detecting the β-globin gene during amplification. *FCGR3B* primer sequences were as follows: sense primer 5′-CCCCTCCACCTTTTCTGGTAAG-3′; antisense primer 5′-TGGATCTGGGCTGGTCTGT-3′; probe 5′-FAM-CTGGAGCCCTGGATCC-MGB-3′. β-Globin primer sequences are as follows: sense primer 5′-TCGCTTT CTTGCTGTCCAATTTCTA-3′; antisense primer 5′-ATG CTCAAGGCCCTTCATAATATCC-3′; and probe 5′-VIC-CCTAAGTCCAACTACTAAACTG-MGB-3′ (synthesized by Applied Biosystems). The methods of genotyping *FCGR3B* copy number are similar to those used to quantify the copy number of *CCL3L1*, as described previously.[5]

Statistical analyses

We used the χ^2-test to determine the significance of the differences in the distribution of the copy number of *FCGR3B* between cases and controls. Next, we used unconditional multiple logistic regression analyses to assess the association between the copy number of *FCGR3B* and the risk of developing SLE, RA and primary SS in separate regression models. ORs were used as an estimate of the relative risk, and were determined along with their 95% CIs using unconditional logistic regression analyses. We also studied the association of copy number of *FCGR3B* with the risk of lupus nephritis and elevated autoantibody titers in the SLE cohort. To test whether the copy number of *FCGR3B* is independently associated with the risk of SLE, RA and primary SS, we included the copy number of *CCL3L1* in a single multivariate logistic regression model along with *FCGR3B*. In addition, we determined whether the association of the CNV in *FCGR3B* differed according to the genetic background of *CCL3L1* CNV. Next, we estimated the attributable fraction as $p(\mathrm{OR}-1)/(p(\mathrm{OR}-1)+1)$,[40] where p is the proportion of controls with *FCGR3B* copy number other than the average and OR represents the OR estimated from logistic regression analysis. Lastly, we formally tested whether the ORs reflecting association of *FCGR3B* CNV with the risk of SLE and SS were heterogeneous across the *CCL3L1* gene copy number using the Breslow–Day test of homogeneity. All the statistical analyses were conducted using the Stata 10.0 (College Station, TX, USA) statistical software.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

We thank Robert A Clark and George Crawford for their institutional support; Jill King and Adriana Benavides for their help with the bioinformatics for designing the primers and probe. We thank the reviewers of this study for their critical review that greatly helped in improving our paper. This work was supported by the Voelcker Fund Scholar Award to Dr Sunil Ahuja from the Max and Minnie Tomerlin Voelcker Fund. SKA is also a recipient of the Elizabeth Glaser Scientist Award, the Burroughs Wellcome Clinical Scientist Award in Translational Research and the Doris Duke Distinguished Clinical Scientist Award. This work was also supported in part by the Rosario University, Bogota, Colombia.

References

1 Bailey JA, Gu Z, Clark RA, Reinert K, Samonte RV, Schwartz S *et al.* Recent segmental duplications in the human genome. *Science* 2002; **297**: 1003–1007.
2 Conrad B, Antonarakis SE. Gene duplication: a drive for phenotypic diversity and cause of human disease. *Annu Rev Genomics Hum Genet* 2007; **8**: 17–35.
3 Nguyen DQ, Webber C, Ponting CP. Bias of selection on human copy-number variants. *PLoS Genet* 2006; **2**: e20.
4 Redon R, Ishikawa S, Fitch KR, Feuk L, Perry GH, Andrews TD *et al.* Global variation in copy number in the human genome. *Nature* 2006; **444**: 444–454.
5 Gonzalez E, Kulkarni H, Bolivar H, Mangano A, Sanchez R, Catano G *et al.* The influence of CCL3L1 gene-containing segmental duplications on HIV-1/AIDS susceptibility. *Science* 2005; **307**: 1434–1440.
6 Gornalusse G, Mummidi S, He W, Silvestri G, Bamshad M, Ahuja SK. CCL3L copy number variation and the co-evolution of primate and viral genomes. *PLoS Genet* 2009; **5**: e1000359.
7 Kuhn L, Schramm DB, Donninger S, Meddows-Taylor S, Coovadia AH, Sherman GG *et al.* African infants' CCL3 gene copies influence perinatal HIV transmission in the absence of maternal nevirapine. *AIDS* 2007; **21**: 1753–1761.
8 Meddows-Taylor S, Donninger SL, Paximadis M, Schramm DB, Anthony FS, Gray GE *et al.* Reduced ability of newborns to produce CCL3 is associated with increased susceptibility to perinatal human immunodeficiency virus 1 transmission. *J Gen Virol* 2006; **87** (Pt 7): 2055–2065.
9 Shostakovich-Koretskaya L, Catano G, Chykarenko ZA, He W, Gornalusse G, Mummidi S *et al.* Combinatorial content of CCL3L and CCL4L gene copy numbers influence HIV-AIDS susceptibility in Ukrainian children. *AIDS* 2009; **23**: 679–688.
10 Burns JC, Shimizu C, Gonzalez E, Kulkarni H, Patel S, Shike H *et al.* Genetic variations in the receptor-ligand pair CCR5 and CCL3L1 are important determinants of susceptibility to Kawasaki disease. *J Infect Dis* 2005; **192**: 344–349.
11 Mamtani M, Rovin B, Brey R, Camargo JF, Kulkarni H, Herrera M *et al.* CCL3L1 gene-containing segmental duplications and polymorphisms in CCR5 affect risk of systemic lupus erythaematosus. *Ann Rheum Dis* 2008; **67**: 1076–1083.
12 McKinney C, Merriman ME, Chapman PT, Gow PJ, Harrison AA, Highton J *et al.* Evidence for an influence of chemokine ligand 3-like 1 (CCL3L1) gene copy number on susceptibility to rheumatoid arthritis. *Ann Rheum Dis* 2008; **67**: 409–413.

13 Fanciulli M, Norsworthy PJ, Petretto E, Dong R, Harper L, Kamesh L *et al.* FCGR3B copy number variation is associated with susceptibility to systemic, but not organ-specific, autoimmunity. *Nat Genet* 2007; **39**: 721–723.
14 Breunis WB, van Mirre E, Bruin M, Geissler J, de Boer M, Peters M *et al.* Copy number variation of the activating FCGR2C gene predisposes to idiopathic thrombocytopenic purpura. *Blood* 2008; **111**: 1029–1038.
15 Aitman TJ, Dong R, Vyse TJ, Norsworthy PJ, Johnson MD, Smith J *et al.* Copy number polymorphism in Fcgr3 predisposes to glomerulonephritis in rats and humans. *Nature* 2006; **439**: 851–855.
16 Coxon A, Cullere X, Knight S, Sethi S, Wakelin MW, Stavrakis G *et al.* Fc gamma RIII mediates neutrophil recruitment to immune complexes. A mechanism for neutrophil accumulation in immune-mediated inflammation. *Immunity* 2001; **14**: 693–704.
17 Takai T. Roles of Fc receptors in autoimmunity. *Nat Rev Immunol* 2002; **2**: 580–592.
18 Anaya JM, Gomez L, Castiblanco J. Is there a common genetic basis for autoimmune diseases? *Clin Dev Immunol* 2006; **13**: 185–195.
19 Becker KG. The common variants/multiple disease hypothesis of common complex genetic disorders. *Med Hypotheses* 2004; **62**: 309–317.
20 Seldin MF, Amos CI. Shared susceptibility variations in autoimmune diseases: a brief perspective on common issues. *Genes Immun* 2009; **10**: 1–4.
21 Anaya JM, Mantilla RD, Correa PA. Immunogenetics of primary Sjogren's syndrome in Colombians. *Semin Arthritis Rheum* 2005; **34**: 735–743.
22 Castano-Rodriguez N, Diaz-Gallo LM, Pineda-Tamayo R, Rojas-Villarraga A, Anaya JM. Meta-analysis of HLA-DRB1 and HLA-DQB1 polymorphisms in Latin American patients with systemic lupus erythematosus. *Autoimmun Rev* 2008; **7**: 322–330.
23 Pan HF, Ye DQ, Wang Q, Li WX, Zhang N, Li XP *et al.* Clinical and laboratory profiles of systemic lupus erythematosus associated with Sjogren syndrome in China: a study of 542 patients. *Clin Rheumatol* 2008; **27**: 339–343.
24 Ramos-Casals M, Brito-Zeron P, Font J. The overlap of Sjogren's syndrome with other systemic autoimmune diseases. *Semin Arthritis Rheum* 2007; **36**: 246–255.
25 Isenberg DA. Systemic lupus erythematosus and Sjogren's syndrome: historical perspective and ongoing concerns. *Arthritis Rheum* 2004; **50**: 681–683.
26 Takai T, Li M, Sylvestre D, Clynes R, Ravetch JV. FcR gamma chain deletion results in pleiotrophic effector cell defects. *Cell* 1994; **76**: 519–529.
27 Willcocks LC, Lyons PA, Clatworthy MR, Robinson JI, Yang W, Newland SA *et al.* Copy number of FCGR3B, which is associated with systemic lupus erythematosus, correlates with protein expression and immune complex uptake. *J Exp Med* 2008; **205**: 1573–1582.
28 Fossati G, Moots RJ, Bucknall RC, Edwards SW. Differential role of neutrophil Fc gamma receptor IIIB (CD16) in phagocytosis, bacterial killing, and responses to immune complexes. *Arthritis Rheum* 2002; **46**: 1351–1361.
29 Ruth AJ, Kitching AR, Kwan RY, Odobasic D, Ooi JD, Timoshanko JR *et al.* Anti-neutrophil cytoplasmic antibodies and effector CD4+ cells play nonredundant roles in anti-myeloperoxidase crescentic glomerulonephritis. *J Am Soc Nephrol* 2006; **17**: 1940–1949.
30 Moore JH. The ubiquitous nature of epistasis in determining susceptibility to common human diseases. *Hum Hered* 2003; **56**: 73–82.
31 Williams SM, Haines JL, Moore JH. The use of animal models in the study of complex disease: all else is never equal or why do so many human studies fail to replicate animal findings? *Bioessays* 2004; **26**: 170–179.
32 Goh KI, Cusick ME, Valle D, Childs B, Vidal M, Barabasi AL. The human disease network. *Proc Natl Acad Sci USA* 2007; **104**: 8685–8690.
33 Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS *et al.* The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988; **31**: 315–324.
34 Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF *et al.* The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982; **25**: 1271–1277.
35 Vitali C, Bombardieri S, Jonsson R, Moutsopoulos HM, Alexander EL, Carsons SE *et al.* Classification criteria for Sjogren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. *Ann Rheum Dis* 2002; **61**: 554–558.
36 Bravo ML, Valenzuela CY, Arcos-Burgos OM. Polymorphisms and phyletic relationships of the Paisa community from Antioquia (Colombia). *Gene Geogr* 1996; **10**: 11–17.
37 Bedoya G, Montoya P, Garcia J, Soto I, Bourgeois S, Carvajal L *et al.* Admixture dynamics in Hispanics: a shift in the nuclear genetic ancestry of a South American population isolate. *Proc Natl Acad Sci USA* 2006; **103**: 7234–7239.
38 Correa PA, Gomez LM, Cadena J, Anaya JM. Autoimmunity and tuberculosis. Opposite association with TNF polymorphism. *J Rheumatol* 2005; **32**: 219–224.
39 Gomez LM, Anaya JM, Gonzalez CI, Pineda-Tamayo R, Otero W, Arango A *et al.* PTPN22 C1858T polymorphism in Colombian patients with autoimmune diseases. *Genes Immun* 2005; **6**: 628–631.
40 Hanley JA. A heuristic approach to the formulas for population attributable fraction. *J Epidemiol Community Health* 2001; **55**: 508–514.