Association of a polymorphism of the Fcγ-receptor 2A (FCGR2A) gene with chronic periaortitis

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Abstract

Objective

Chronic periaortitis (CP) is an inflammatory disease associated in 20-60% of the cases with IgG4 related disease. Current evidence supports an autoimmune nature for CP. Fc gamma receptors (FcγRs) are involved in several immune system activities and are associated with autoimmunity in general. We explored the influence of genetic variants within this region on susceptibility to CP.

Methods

Genotyping of 4 candidate single nucleotide polymorphisms (SNPs) of the FCGR region was performed in CP patients and controls.

Results

One hundred and eighty-three cases and 181 controls were included. An association between the SNP rs1801274 of the FCGR2A and CP was detected (OR 1.6, 95%CI 1.18-2.16;corrected p-value, p_{corr}=0.0085). After stratification of the population according to clinical characteristics, the association was restricted to cases of idiopathic retroperitoneal fibrosis (OR 1.66, 95%CI 1.21-2.29;p_{corr}=0.028), without involvement of the thoracic aorta (OR 1.77, 95%CI 1.21-2.57;p_{corr}=0.043), with deep vein thrombosis at onset (OR 3.96, 95%CI 1.81-8.66;p_{corr}=0.0021) and with normal IgG4 levels (OR 2.67, 95%CI 1.39-5.12;p_{corr}=0.031).

Conclusion

In the largest candidate gene approach study performed so far in CP, we demonstrated an association for CP with a gene hallmark of autoimmunity. The association appears restricted to typical cases of CP without increase of IgG4 levels.

Key words

Fcγ-receptor 2A, chronic periaortitis, idiopathic retroperitoneal fibrosis, IgG4, gene polymorphisms

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Received on January 31, 2018; accepted in revised form on May 23, 2018.

Funding: this work was supported in part by the grant "A tailored approach to the immune-monitoring and clinical management of viral and autoimmune diseases" funded by the Regione Emilia-Romagna within the Programma di Ricerca Regione Università 2010–2012, in part by "Tailoring Rituximab treatment in ANCA-associated vasculitis; a genetic and an immunological approach" funded by the Regione Emilia-Romagna within the Programma di ricerca Regione-Università Giovani Ricercatori 'Alessandro Liberati' 2013, and by the "Fondazione Emma ed Ernesto Rulfo per la Genetica Medica"

Competing interests: none declared.

Introduction

Chronic periaortitis (CP) is a rare disease including idiopathic retroperitoneal fibrosis (IRF) and perianeurysmal retroperitoneal fibrosis (PRF) (1). The mainstays of therapy are glucocorticoids (2) although refractory/relapsing forms may need immunosuppressive medications (3). In 20–60% of cases CP is associated with IgG4-related disease (IgG4-RD). However, whether IgG4-related CP is a distinct subset is still unclear, since no clinically meaningful differences are evident between IgG4-related and -unrelated CP forms (2). So far, few genetic studies have been performed in CP, mainly in small cohorts; genetic associations were demonstrated with *HLA-DRB1*03* (4), a *CC chemokine receptor 5* gene single nucleotide polymorphism (SNP) (5) as well as a *CCL11* gene haplotype (6). Although genetic studies are difficult to perform in rare diseases, their impact may be significant as they allow the identification of variants associated with disease risk, provide useful pathogenic insights, improve classification of the diseases or their subgroups, and help identify potential therapeutic targets.

Fc gamma receptors (FcγR) are a group of cell surface proteins that bind the Fc portion of IgGs, thereby mediating the activation of both innate and adaptive immune responses. Several studies have shown how SNPs of their genes may have a role in influencing infection risk (7) and development of autoimmunity (8). FcγRs are encoded on chromosome 1 in a locus difficult to genotype mainly for the presence of copy number variations; inclusive genotyping approaches (such as genome-wide association studies) are not able to provide reliable information on this area with the exception of the only gene located outside the highly variable area, the *FCGR2A*. A candidate gene approach remains therefore the gold standard for exploring this region (9, 10). In a relatively large cohort of patients affected by CP, we investigated whether genetic determinants of the *FCGR* region may confer susceptibility to the disease.

Patients and methods

Patients with a diagnosis of CP followed at Parma University Hospital were included in the study. Secondary forms of retroperitoneal fibrosis were excluded through a standard screening process aimed at the identification of forms related to drugs, malignancies, genetically determined aortic diseases, infectious aortitis, Erdheim-Chester disease or other forms of autoimmune aortitis (*e.g.* giant cell arteritis [GCA], or Takayasu's arteritis [TAK]). Patients with IRF and PRF were included since both are idiopathic forms of CP (1). Age- and sex-matched healthy subjects served as controls. All the study subjects were Caucasian. The main clinical characteristics of the CP patients were collected; the definitions regarding the type of CP (IRF *vs.* PRF), the type of localisation (typical *vs.* atypical) and of the presence of thoracic aorta involvement are reported in the Appendix (11). Serum IgG4 levels were defined as high when >135 mg/dl.

Four candidate SNPs of the *FCGR* loci tagging *FCGR2A* (rs1801274), *FCGR3A* (rs396991), *FCGR2B* (rs1050501) and *FCGR3B* (NA1-NA2) were analysed. Genomic DNA was extracted from EDTA-treated peripheral blood samples using the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA) and stored at -20°C until use. Genetic analysis of rs1801274 and rs396991 SNPs was performed using TaqMan® SNP Genotyping Assay (Life Technologies™). TaqMan PCR and fluorescence measurements were performed using a real-time PCR system (ABI PRISM 7700, Life Technologies™). Genotyping of *FCGR2B* SNP was performed by Sanger sequencing (CEQ 2000XL Sequence Analysis, Beckman Coulter, CA, USA), using the specific sense and anti-sense primer pair 5'-TGGGACAAGGAGAGTACTGCCTGT-3' and 3'-CCAAGACCTTCTCCAACTGCC-5'. *FCGR3B* NA1-NA2 SNP genotyping was performed by multiplex PCR employing allele-specific sense and anti-sense primers. The primers NA-1 forward (5′-CAGTGGTTTCACAATGAGAA-3′), NA-2 forward (5′-CAATGGTACAGCGTGCTT-3′) and NA-1/2 reverse (5'-ATGGACTTCTAGCTGCAC-3') were used to amplify the fragment of *FCGR3B*

containing the polymorphic sites with an endpoint PCR. In order to confirm *FCGR2A*, *FCGR3A*, *FCGR3B* genotyping, one-third of the samples were sequenced by Sanger sequencing.

Statistical analyses was performed using the software R *(http://www.r-project.org)* and the packages coin (12) and SNPassoc (13). Association of the SNPs was tested using a log additive model of the likelihood ratio test; for the allelic analyses, the chi square test was used. Results are expressed as n(%) for categorical variables and median and interquartile range (IQR) for continuous variables. The association between proportion of the risk allele of the *FCGR2A* and number of disease characteristics associated to its SNP was explored using a log-additive model of the Cochrane Armitage test. Bonferroni correction for multiple testing was applied, with corrected p (p_{corr}) values <0.05 considered statistically significant.

This study complies with the Declaration of Helsinki; the Ethics Committee of Parma Hospital approved the study and all the participants signed a written informed consent.

Results

One hundred and eighty-three cases and 181 controls were included in the study. Patients' characteristics at the time of diagnosis are reported in Table I. All the genotyped SNPs were in Hardy Weinberg Equilibrium (Table II).

An association between the SNP rs1801274 (*FCGR2A*) and CP was detected (OR 1.6, 95%CI 1.18–2.16; p=0.0021, p_{corr}=0.0085) (Table III); the association was also confirmed after the allelic analyses with T being the risk allele (OR 1.58, 95%CI 1.18–2.13; p=0.0023, p_{corr}=0.012) (Table IV).

After stratification of the population according to clinical characteristics, this SNP remained associated in the following subgroups: patients with IRF (OR 1.66, 95%CI 1.21-2.29; p=0.0015, p_{corr}=0.028), without involvement of the thoracic aorta (OR 1.77, 95%CI 1.21–2.57; p=0.0024, p_{corr}=0.043), with deep vein thrombosis (DVT) at diagnosis (OR 3.9,6 95%CI 1.81–8.66; p=0.00012, p_{corr}=0.0021), and with normal IgG4 levels (OR 2.67, 95%CI 1.39–5.12; p=0.0017, p_{corr}=0.031) (Table V).

The proportion of the risk allele of the SNP rs1801274 (*FCGR2A*) increased significantly across the overall CP population with the increase of the number of the aforementioned disease characteristics associated with the SNP

Table I. Main demographic and clinical characteristics of the chronic periaoritis patients included in the study.

Characteristics		n=183
Gender	Male	123 (67%)
	Female	60 (33%)
Age at diagnosis- years		58.5 (52-65)
Serum creatinine at diagnosis (mg/dl)		1.4 (0.9-3.05)
ESR at diagnosis (mm/h)		61 (40-85)
CRP at diagnosis (mg/l)		20.2 (6.0-39.2)
Established atherosclerotic disease	Yes	37 (21%)
	No	140 (79%)
Type or retroperitoneal fibrosis	IRF	147 (82%)
	PRF	33 (18%)
Retroperitoneal fibrosis localisation	Typical	159 (88%)
	Atypical	21 (12%)
Thoracic aorta involvement	Yes	34 (27%)
	No	93 (73%)
Ureteral obstruction	Yes	134 (75%)
	No	45 (25%)
Deep vein thrombosis	Yes	23 (13%)
	No	152 (87%)
Increased IgG4	Yes	8 (22%)
	No	28 (78%)

Data are reported as n(%) for categorical variables and median (interquartile range) for continuous variables.
CP: chronic periaortitis; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; IRF: idiopathic retroperitoneal fibrosis; PRF: perianeurysmal retroperitoneal fibrosis.

Table II. Single nucleotide polymorphisms (SNPs) tested, minor allele frequency observed (MAF) and test for deviation from Hardy-Weinberg Equilibrium (HWE) in our overall population of 364 samples (183 cases + 181 controls).

SNP	Alleles	Gene	MAF	HWE - p
rs1801274	C/T	*FCGR2A*	42.7	1
rs396991	G/T	*FCGR3A*	46.8	0.207
rs1050501	C/T	*FCGR2B*	14	0.122
NA1-NA2	NA1/NA2	*FCGR3B*	29.4	0.256

Table III. Association of 4 candidate SNPs of the *FCGR* region with chronic periaortitis.

SNP	Gene	OR (95% CI)	p-value	p_{corr}
rs1801274	*FCGR2A*	1.6 (1.18-2.16)	0.0021	0.0085
rs396991	*FCGR3A*	0.97 (0.73-1.28)	0.82	1
rs1050501	*FCGR2B*	0.72 (0.48-1.08)	0.11	0.45
NA1-NA2	*FCGR3B*	1.21 (0.89-1.65)	0.23	0.92

Table IV. Distribution of the 2 alleles of the SNP rs1801274 of the gene *FCGR2A* in cases of chronic periaortitis and controls.

Allele	Cases (%)	Controls (%)
T	230 (63%)	187 (52%)
C	136 (37%)	175 (48%)

Table V. Association of the SNP rs1801274 of the gene *FCGR2A* with chronic periaortitis subgroups based on specific disease characteristics.

Characteristics		OR (95% CI)	*p*-value	p_{corr}
Established atherosclerotic disease	Yes	1.54 (0.92-2.60)	0.096	1
	No	1.56 (1.12-2.16)	0.007	0.13
Type or retroperitoneal fibrosis	IRF	1.66 (1.21-2.29)	0.0015	0.028
	PRF	1.21 (0.70-2.07)	0.5	1
Retroperitoneal fibrosis localisation	Typical	1.53 (1.12-2.10)	0.0065	0.12
	Atypical	1.91 (0.96-3.81)	0.058	1
Thoracic aorta involvement	Yes	1.71 (1-2.93)	0.047	0.85
	No	1.77 (1.21-2.57)	0.0024	0.043
Ureteral obstruction	Yes	1.59 (1.14-2.21)	0.0052	0.094
	No	1.48 (0.92-2.39)	0.103	1
Deep vein thrombosis	Yes	3.96 (1.81-8.66)	$1.2x10^{-04}$	0.0021
	No	1.41 (1.03-1.93)	0.032	0.57
Increased IgG4	Yes	0.93 (0.33-2.60)	0.89	1
	No	2.67 (1.39-5.12)	0.0017	0.031

IRF: Idiopathic retroperitoneal fibrosis; PRF: perianeurysmal retroperitoneal fibrosis.

Table VI. Changes of proportion of the risk allele T across different groups of patients affected by chronic periaortitis according to the number of disease characteristics with which the SNP rs1801274 of the gene *FCGR2A* remained significantly associated.

Number of disease characteristics*	T allele prevalence (%)
0	21/38 (55%)
1	80/136 (59%)
2	86/136 (63%)
3	37/50 (74%)
4	6/6 (100%)

*The disease characteristics included in this model are the ones for which the SNP rs1801274 of the gene *FCGR2A* remains as significantly associated to the disease after stratification.
The increase of proportion of the T allele with the increase of the number of the disease characteristics is statistically significant (Cochrane Armitage test – log additive model p=0.011).

(Cochrane Armitage test – log additive model p=0.011) (Table VI).

None of the other tested SNPs showed statistically significant associations with CP (Table II).

Discussion

In the largest genetic association study performed so far in CP, we identified an association of the *FCGR2A* SNP rs1801274 with the risk of developing the disease; interestingly the risk allele was T, the same found to be associated to Kawasaki disease (KD), a medium-vessel vasculitis which sometimes also causes large vessel involvement (10).

After subgroup analyses the association remained significant only for cases of IRF, without thoracic aorta involvement, showing DVT at disease onset and without increase of serum IgG4; altogether, these features are consistent with the most typical clinical presentation of CP as a disease mainly involving the peri-aortoiliac space, causing local complications such as DVT, without IgG4 increase and without extra-retroperitoneal lesions such as thoracic periaortitis or other IgG4-related complications (11). Of note, a trend for a progressive increase of the proportion of the T allele was detected with the increase of the number of these disease characteristics.

We detected a highly significant association of this SNP with the subgroup of patients presenting with DVT at disease onset. Notably, the A allele of this SNP has been linked to risk of thrombosis in patients affected by heparin-induced thrombocytopenia (HIT) although this mechanism appeared to be disease-specific (14). In our cohort the allele associated to the risk of DVT was the opposite, suggesting that the pathogenic mechanism in CP is probably different.

The association of the *FCGR2A* with autoimmune diseases is not restricted to KD (10) but includes other forms (15); for instance, it has also been described to be associated with TAK (16) and GCA (17). These findings point toward a central role of FcγR2A not only to autoimmunity susceptibility in general but interestingly also in several immune-mediated diseases causing blood vessel inflammation.

The tested *FCGR2A* SNP is located within one exonic region of this gene encoding for a variant of the protein characterised by higher affinity for the binding of the Fc portion of IgG2 but to some extent also of IgG1; FcγR2A is mainly expressed on the surface of myeloid cells and granulocytes and is activatory in nature (18). Its role in modulating susceptibility to autoimmune diseases characterised by the presence of autoantibodies appears therefore intuitive with the FcγR2A probably playing a role in autoantibody generation and clearance (15). Despite that, the impact of this protein on the immune system activity seems to be broader as shown by a clear association of this SNP with diseases characterised by the lack of a clear role for autoantibodies in their pathogenesis (10, 16, 17). Of interest, some reports also suggest a possible activity of this SNP in modulating autoimmune disease severity and a potential for predicting response to treatment (19, 20).

Several findings already support the autoimmune nature of CP (11); our study provides a further clue. Moreover, it may also have an implication from a classification point of view, suggesting that the genetic background of the typical IRF is somehow different from the forms with atypical features and extra-retroperitoneal lesions.

An overlapping genetic background between CP and KD, TAK and GCA also provides the rationale for a shared therapeutic approach across these diseases: methotrexate is the cornerstone of the immunosuppressive treatment in TAK and GCA and indeed effectiveness in CP has been shown (3); moreover the anti-IL6 receptor antibody to-

cilizumab has proven efficacy in both GCA and CP (21, 22). Intravenous immunoglobulins are key in the treatment of KD although so far no studies exploring their effectiveness in CP have been published.

Our report has limitations. First, the sample size that, although relatively large considering the rarity of the disease, remains small for a genetic association study; this is even more relevant for the subgroup analyses in general and for the one regarding the IgG4 levels in particular, suggesting caution in interpreting these results. Second, no information regarding the histology of the CP is available in our study: on the one hand, this does not allow us to explore associations of genetic variants with histological characteristics and more importantly it cannot allow us to rule out that a subgroup of patients with IgG4 related disease with normal circulating IgG4 levels in the peripheral blood may have been included. Third, the study design: although a candidate gene approach allows us to perform studies on cohorts that would be far too small for more inclusive genetic studies, it lacks the exploratory purposes of the latter. Fourth, the lack of a replication cohort is an additional weakness, although at the time of the study, a cohort of similar size would have not been available.

Conclusion

We have identified an association of the SNP rs1801274 of the gene *FCGR2A* with the risk of developing CP. The observation that the same genetic variant is associated with medium- and large-vessel vasculitides further supports the hypothesis that CP is an autoimmune condition primarily causing blood vessel inflammation.

Appendix

Study definitions

- Chronic periaortitis (CP): chronic inflammatory process characterised by the spreading of inflammatory tissue from the aorta and iliac arteries to the surrounding area.
- Idiopathic retroperitoneal fibrosis (IRF): idiopathic form of chronic periaortitis not associated with aneurysms of the aorta.
- Perianeurysmal retroperitoneal fibrosis (PRF): chronic periaortitis associated with abdominal aortic aneurysm.
- Typical localisation of retroperitoneal fibrosis: defined as
  - homogeneous plaque surrounding the anterolateral sides of the abdominal aorta and encircling the common iliac arteries (1)
  - medial ureteral deviation and/or obstruction and inferior vena cava encasement (1).
- Atypical localisation of retroperitoneal fibrosis: retroperitoneal fibrosis not fulfilling the above criteria.
- Thoracic aorta involvement: patients were considered to be screened for this localisation in case of presence of a contrast enhanced chest CT or MRI and whole body ^{18}F-FDG PET-CT.

References

1. VAGLIO A, BUZIO C: Chronic periaortitis: a spectrum of diseases. *Curr Opin Rheumatol* 2005; 17: 34-40.
2. VAGLIO A, PALMISANO A, ALBERICI F *et al*.: Prednisone versus tamoxifen in patients with idiopathic retroperitoneal fibrosis: an open-label randomised controlled trial. *Lancet* 2011; 378: 338-46.
3. ALBERICI F, PALMISANO A, URBAN ML *et al*.: Methotrexate plus prednisone in patients with relapsing idiopathic retroperitoneal fibrosis. *Ann Rheum Dis* 2013; 72: 1584-86.
4. MARTORANA D, VAGLIO A, GRECO P *et al*.: Chronic periaortitis and HLA-DRB1*03: another clue to an autoimmune origin. *Arthritis Rheum* 2006; 55: 126-30.
5. BOIARDI L, VAGLIO A, NICOLI D *et al*.: CC chemokine receptor 5 polymorphism in chronic periaortitis. *Rheumatology* (Oxford) 2011; 50: 1025-32.
6. MANGIERI D, CORRADI D, MARTORANA D *et al*.: Eotaxin/CCL11 in idiopathic retroperitoneal fibrosis. *Nephrol Dial Transplant* 2012; 27: 3875-84.
7. CASTRO-DOPICO T, CLATWORTHY MR: Fcgamma receptors in solid organ transplantation. *Curr Transplant Rep* 2016; 3: 284-93.
8. MARTORANA D, BONATTI F, ALBERICI F *et al*.: Fcgamma-receptor 3B (FCGR3B) copy number variations in patients with eosinophilic granulomatosis with polyangiitis. *J Allergy Clin Immunol* 2016; 137: 1597-99.
9. ERBE AK, WANG W, GALLENBERGER M, HANK JA, SONDEL PM: Genotyping single nucleotide polymorphisms and copy number variability of the FCGRs expressed on NK cells. *Methods Mol Biol* 2016; 1441: 43-56.
10. KHOR CC, DAVILA S, BREUNIS WB *et al*.: Genome-wide association study identifies FCGR2A as a susceptibility locus for Kawasaki disease. *Nat Genet* 2011; 43: 1241-46.
11. VAGLIO A, MARITATI F: Idiopathic retroperitoneal fibrosis. *J Am Soc Nephrol* 2016; 27: 1880-89.
12. HOTHORN T, HORNIK K, VAN DE WIEL MA, ZEILEIS A: A Lego System for Conditional Inference. *The American Statistician* 2006; 60: 257-63.
13. SNPassoc: SNPs-based whole genome association studies. R package version 1.9-2. [http://CRAN.R-project.org/package=SNPassoc]
14. ROLLIN J, POUPLARD C, SUNG HC *et al*.: Increased risk of thrombosis in FcgammaRIIA 131RR patients with HIT due to defective control of platelet activation by plasma IgG2. *Blood* 2015; 125: 2397-2404.
15. ZHU XW, WANG Y, WEI YH *et al*.: Comprehensive assessment of the association between FCGRs polymorphisms and the risk of systemic lupus erythematosus: evidence from a meta-analysis. *Sci Rep* 2016; 6: 31617.
16. SARUHAN-DIRESKENELI G, HUGHES T, AKSU K *et al*.: Identification of multiple genetic susceptibility loci in Takayasu arteritis. *Am J Hum Genet* 2013; 93: 298-305.
17. MORGAN AW, ROBINSON JI, BARRETT JH *et al*.: Association of FCGR2A and FCGR2A-FCGR3A haplotypes with susceptibility to giant cell arteritis. *Arthritis Res Ther* 2006; 8: R109.
18. SMITH KG, CLATWORTHY MR: Fcgamma-RIIB in autoimmunity and infection: evolutionary and therapeutic implications. *Nat Rev Immunol* 2010, 10(5):328-343.
19. CARTIN-CEBA R, INDRAKANTI D, SPECKS U *et al*.: The pharmacogenomic association of fcgamma receptors and cytochrome p450 enzymes with response to rituximab or cyclophosphamide treatment in antineutrophil cytoplasmic antibody-associated vasculitis. *Arthritis Rheumatol* 2017; 69: 169-75.
20. ALBERICI F, SMITH RM, FONSECA M *et al*.: Association of a TNFSF13B (BAFF) regulatory region single nucleotide polymorphism with response to rituximab in antineutrophil cytoplasmic antibody-associated vasculitis. *J Allergy Clin Immunol* 2017; 139: 1684-87.
21. VILLIGER PM, ADLER S, KUCHEN S *et al*.: Tocilizumab for induction and maintenance of remission in giant cell arteritis: a phase 2, randomised, double-blind, placebo-controlled trial. *Lancet* 2016; 387: 1921-27.
22. VAGLIO A, CATANOSO MG, SPAGGIARI L *et al*.: Interleukin-6 as an inflammatory mediator and target of therapy in chronic periaortitis. *Arthritis Rheum* 2013; 65: 2469-75.