Individuals with IgG4-related Disease Do Not Have an Increased Frequency of the K409 Variant of IgG4 that Compromises Fab-arm Exchange

To the Editor:

Immunoglobulin (Ig)G4 is considered a noninflammatory antibody because of its relative inability to fix complement and its poor binding to activating Fc receptors[1,2]. This antibody is also unique in its ability to exchange "half-antibodies" comprised of 1 heavy chain and 1 light chain by a process called "Fab-arm exchange"[3]. Fab-arm exchange is thought to render IgG4 functionally monovalent, interfering with its ability to crosslink target antigens and limiting its ability to form large immune complexes. Apart from the limited ability of IgG4 to bind to activating Fc receptors and its inability to fix complement, Fab-arm exchange of IgG4 may also contribute to the putative immunoregulatory properties of this isotype[3]. Fab-arm exchange is facilitated by residues in the hinge region, as well as in the CH3 domain of IgG4, which are unique to this subclass[4]. An arginine residue (R409) in the CH3 domain of IgG4, unique to the IgG4 subclass, is necessary for Fab-arm exchange[4]. Analysis of the crystal structure of the CH3 domain of IgG4 suggests that this arginine residue (R409) facilitates Fab-arm exchange by preventing the proper formation of an interchain hydrogen bond network that is generated in other IgG isotypes, which all have a lysine in position 409[5].

A single-nucleotide polymorphism (SNP) in the CH3 exon of IgG4 results in a nonsynonymous change in codon 409 (AGG > AAG) and alters the R409 residue, which is critical for Fab-arm exchange. This SNP was previously recognized as an isoallotypic variant of IgG4, in which a lysine (K409) is present in place of arginine (R409) in the CH3 domain of IgG4[6].

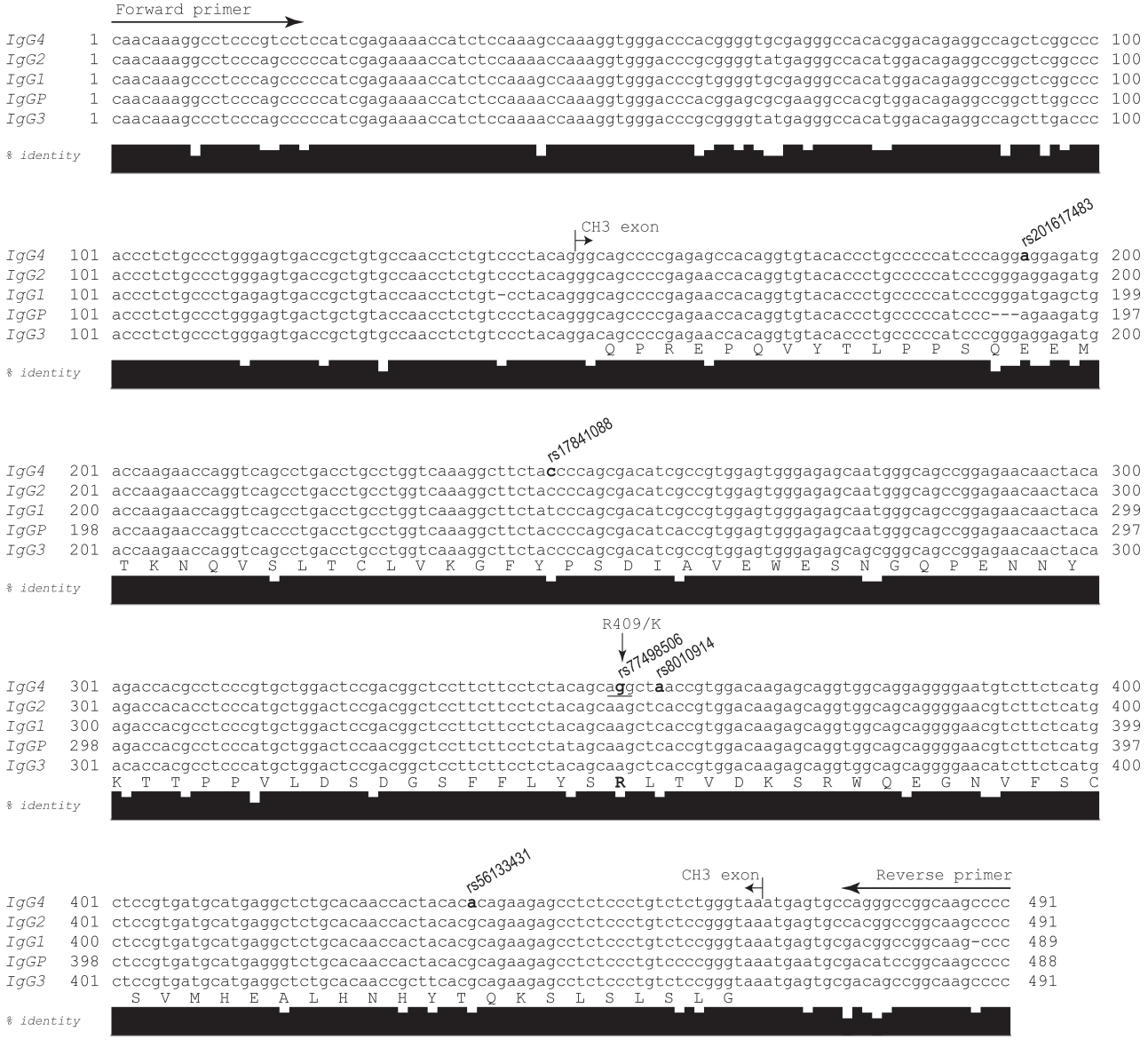

Figure 1. Single-nucleotide polymorphisms (SNP) in the CH3 exon of immunoglobulin (Ig)G4. SNP (shown in boldface) in the CH3 exon of IgG4 are depicted in an alignment of the CH3 exons of IgG1, IgG2, IgG3, IgG4, and IgGP. The amino acid translation of the IgG4 CH3 exon is shown below the alignment. The positions of the primers used for specific amplification of the CH3 exon of IgG4 are indicated by arrows. The minor allele of rs77498506 is identical to the K409 variant of IgG4 and results in a nonsynonymous substitution from arginine to lysine (AGG > AAG). The codon encoding arginine (R409) is underlined.

The K409 variant of IgG4 resembles IgG1, IgG2, and IgG3, which also encode a lysine at this position (Figure 1), and do not undergo Fab-arm exchange[5]. Serum concentrations of IgG4 correlate with certain IgG allotypes, some of which are genetically linked to the K409 variant of IgG4[7]. It has been speculated that the K409 variant of IgG4 might be enriched in subjects with IgG4-related disease (IgG4-RD) and that this polymorphic variant could contribute to the pathogenesis of IgG4-RD, a fibroinflammatory disorder of possible autoimmune etiology, characterized by elevations in circulating IgG4 levels as well as an expansion of IgG4+ plasma cells in the affected tissues[8,9]. Although extensive genetic studies in IgG4-RD have not yet been reported, it remains likely that IgG4-RD is caused by environmental triggers in a genetically susceptible background.

We have evaluated the occurrence of the K409 variant of IgG4 in a cohort of 25 subjects with IgG4-RD who presented to the rheumatology clinic at the Massachusetts General Hospital. All patients signed written informed consent for the investigations described. All had biopsy-proven IgG4-RD affecting 1 or more of the following organs: pancreas, lacrimal gland, submandibular gland, parotid gland, biliary tree, retroperitoneum, kidney (tubulointerstitial nephritis), lymph node, lung, mediastinum, aorta, common carotid artery, palate, pharynx, larynx, lymph node, and skin. Nineteen patients self-identified as white, 3 as Asian, and 2 as black. One patient declined to provide information about race.

Because of the high degree of nucleotide sequence conservation among the constant regions encoding IgG subclasses and the IgGP pseudogene, the K409 variant of IgG4 is not included in most high-throughput genotyping panels (Figure 1). Therefore, we designed primers for the specific amplification of the CH3 exon of IgG4 (5'-CAA CAA AGG CCT CCC GTC CT-3' and 5'-GGG GCT TGC CGG CCC TG-3'). PCR was performed for 35 cycles at 67°C (KAPA2G HotStart ReadyMix; KapaBiosystems) using 50–100 ng of genomic DNA as a template. The PCR products were sequenced using the Sanger method. The resulting sequences included several bases that were specific to IgG4, which were used to confirm that the amplified sequences were indeed IgG4. The amplified region includes 5 SNP: rs56133431, rs8010914, rs77498506, rs17841088, and rs201617483 (Figure 1). The K409 variant of IgG4 corresponds to the minor allele (T) of the rs77498506 SNP. The rest of the SNP encode synonymous substitutions.

The SNP frequencies in the CH3 exon of IgG4 observed in the subjects with IgG4-RD are summarized in Table 1. The SNP frequencies in control populations from the dbSNP database are also listed for comparison. We did not identify any subject with the K409 variant of IgG4 in our study cohort. These data suggest that this variant is not a major contributor to disease susceptibility in IgG4-RD. Statistical analyses have limitations when the frequency of an allele is zero in a population, and it is reasonable to assume that the frequency of the K409 polymorphism is in the same range as the control population of healthy subjects of European descent. Based on the modified Wald method of CI determination and assuming random sampling of IgG4-RD subjects, we estimate with > 95% CI that the K409 variant of IgG4, which compromises Fab-arm exchange, is absent in at least 88.4% of subjects with IgG4-RD. Although IgG4 Fab-arm exchange has not been directly examined in individuals with disease at the protein level, we believe that dysregulation of IgG4 Fab-arm exchange is unlikely to be a major contributor to the pathogenesis of this disease. Rather, the pathology in IgG4-RD appears to be driven by T cells[10]. However, immunological evaluation of individuals bearing the K409 variant of IgG4 may help in a broader understanding of the pathophysiological implications of IgG4 Fab-arm exchange.

MAIMUNA AHMAD, Undergraduate Research Fellow; VINAY S. MAHAJAN, MD, PhD, Research Fellow; HAMID MATTOO, PhD, Research Fellow, Center for Cancer Research, Massachusetts General Hospital; JOHN H. STONE, MD, Professor of Medicine, Harvard Medical School, Director, Clinical Rheumatology, Massachusetts General Hospital; SHIV PILLAI, MD, PhD, Professor of Medicine and Health Sciences and Technology, Harvard Medical School, Center for Cancer Research, Massachusetts General Hospital, Boston, Massachusetts, USA. Address all correspondence to Dr. S. Pillai, Massachusetts General Hospital, 149 13th Street, Boston, Massachusetts, 02129 USA.
E-mail: pillai@helix.mgh.harvard.edu
Supported by grants AI 076505 and AI064930 to SP from the US National Institutes of Health.

REFERENCES

1. Bindon CI, Hale G, Bruggemann M, Waldmann H. Human monoclonal IgG isotypes differ in complement activating function at the level of C4 as well as C1q. J Exp Med 1988;168:127-42.
2. Bruhns P, Iannascoli B, England P, Mancardi DA, Fernandez N, Jorieux S, et al. Specificity and affinity of human Fcgamma receptors and their polymorphic variants for human IgG subclasses. Blood 2009;113:3716-25.
3. Aalberse RC, Stapel SO, Schuurman J, Rispens T. Immunoglobulin G4: an odd antibody. Clin Exp Allergy 2009;39:469-77.
4. Labrijn AF, Rispens T, Meesters J, Rose RJ, den Bleker TH, Loverix S, et al. Species-specific determinants in the IgG CH3 domain enable Fab-arm exchange by affecting the noncovalent CH3-CH3 interaction strength. J Immunol 2011;187:3238-46.
5. Davies AM, Rispens T, den Bleker TH, McDonnell JM, Gould HJ, Aalberse RC, et al. Crystal structure of the human IgG4 C(H)3 dimer reveals the role of Arg409 in the mechanism of Fab-arm exchange. Mol Immunol 2013;54:1-7.
6. Brusco A, Saviozzi S, Cinque F, DeMarchi M, Boccazzi C, de Lange G, et al. Molecular characterization of immunoglobulin G4 gene isoallotypes. Eur J Immunogenet 1998;25:349-55.
7. Steinberg AG, Morell A, Skvaril F, van Loghem E. The effect of Gm(23) on the concentration of IgG2 and IgG4 in normal human serum. J Immunol 1973;110:1642-5.

Table 1. Frequency of single-nucleotide polymorphisms (SNP) in the CH3 exon of IgG4.

	Data from dbSNP				Patients with IgG4-RD, n = 25
SNP	Source	Population Details	REFSNP Alleles	MAF	MAF
rs56133431	1000 genomes	1094 worldwide individuals from 1000 genomes project	C/T	C = 0.253	C = 0.36 G = 0.08
rs8010914	1000 genomes	1094 worldwide individuals from 1000 genomes project	G/T	G = 0.122	G = 0.04
rs17841088	CEPH	92 individuals from CEPH pedigrees [UTAH (93%), French (4%), and Venezuelan (3%)]	C/T	T = 0.210	T = 0.00*
rs201617483	CS Agilent	662 participants of European descent	C/G/T	G = 0.028	G = 0.04
rs77498506	CS Agilent	662 participants of European descent	C/T	T = 0.011	T = 0.00*

* 100% of the IgG4-RD subjects in this study were of the C/C genotype. MAF: minor allele frequency; dbSNP: single-nucleotide polymorphism database; REFSNP: Reference SNP; IgG4-RD: immunoglobulin G4-related disease.

8. Pandey JP. Genetic markers of immunoglobulin G as potential risk factors for IgG4-related disease. J Rheumatol 2012;39:2048.
9. Stone JH, Zen Y, Deshpande V. IgG4-related disease. N Engl J Med 2012;366:539-51.
10. Mahajan VS, Mattoo H, Deshpande V, Pillai S, Stone JH. IgG4-Related Disease. Annu Rev Pathol 2013 Oct 2 (E-pub ahead of print).

J Rheumatol 2013; 41:1; doi:10.3899/jrheum.131017