Interleukin 1 receptor type 1 gene variants associate with disease susceptibility to IgG4-related periaortitis/periarteritis in IgG4-related disease

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Research Article

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Abstract

Background

IgG4-related disease (IgG4-RD) is an immune-mediated disorder characterized by high serum IgG4 concentration and IgG4-bearing plasma cell infiltration in affected organs. IgG4-related periaortitis/periarteritis is a recently identified disease entity in IgG4-RD that affects the cardiovascular system. Since the genetic factors related to disease onset are unclear, we examined the genetic associations with IgG4-related periaortitis/periarteritis susceptibility.

Methods

A recent genome-wide association study identified *interleukin 1 receptor type 1* (*IL1R1*) gene variants in 75 patients with IgG4-RD. Accordingly, 8 single nucleotide polymorphisms (SNPs) in the *IL1R1* gene were selected and genotyped in 124 patients with IgG4-RD (43 with IgG4-related periaortitis/periarteritis) and 344 healthy subjects. Serum IL-1β levels were also determined in 102 IgG4-RD patients.

Results

No remarkable SNP associations were detected between IgG4-RD patients and controls. However, the minor allele frequencies of 6 SNPs (rs2287049, rs3917273, rs2160227, rs951192, rs3917318, and rs7582198) were significantly increased in IgG4-related periaortitis/periarteritis patients (corrected $P < 0.05$). Serum IL-1β levels were significantly lower in patients with IgG4-related periaortitis/periarteritis ($P = 0.011$) but were not associated with any SNP.

Conclusions

This study indicated that *IL1R1* SNPs contributed to IgG4-related periaortitis/periarteritis, but not IgG4-RD, onset. Our findings raise the possibility of certain genetic factors influencing the risk of specific IgG4-RD manifestations.

Background

IgG4-related disease (IgG4-RD) is an immune-mediated disorder characterized by high serum IgG4 concentration and IgG4-bearing plasma cell infiltration in affected organs in association with lymphoplasmacytic infiltration, storiform fibrosis, and obliterative phlebitis [1]. In 2001, these clinical and pathological features were first identified in patients with autoimmune pancreatitis (AIP) [2], now referred to as type 1 AIP. Since then, simultaneous or metachronous lesions with distinguishing common pathological features have been found in nearly every organ system.

Recently, IgG4-RD has been recognized to also affect the cardiovascular system as retroperitoneal fibrosis, inflammatory aneurysms, lymphoplasmacytic aortitis, and aortic dissection, which has been newly termed IgG4-related periaortitis/periarteritis [3–5]. Although the clinical features of IgG4-related periaortitis/periarteritis are now being identified, the contributing factors to disease onset remain unclear. IgG4-related periaortitis/periarteritis tends to occur in patients with an older IgG4-RD onset age and in those with a highly active disease state displaying elevated serum IgG, IgG4, circulating immune complexes, and soluble interleukin (IL)2 receptor [3, 4]. The elevation of serum IgG4 is considered to be under the control of cytokine signals, including IL-4, IL-5, IL-10, IL13, IL12, transforming growth factor (TGF)-β, and B-cell activating factor [6]. In turn, serum IgG4 concentration is correlated with the number of pathologic T follicular helper type 2 cells. Therefore, high concentrations of both serum IgG4 and circulating T follicular helper type 2 cells may be involved in the pathogenesis of IgG4-related periaortitis/periarteritis.

Significant associations for HLA haplotype, Fc receptor-like protein 3, cytotoxic T-lymphocyte antigen 4, and other genetic factors with type 1 AIP have been reported [7–9]. A recent genome-wide association study (GWAS) showed that HLA-DRB1 and the Fc fragment of IgG receptor IIb regions were possible susceptibility loci for IgG4-RD [10], although no genetic studies of patients with IgG4-related periaortitis/periarteritis exist to date. We therefore sought to determine the susceptibility genes to IgG4-related periaortitis/periarteritis using GWAS-based, fine-tuned mapping of candidate genes in a Japanese population.

Methods

Subjects.

Between 2002 and 2015, a total of 223 Japanese patients were diagnosed as having IgG4-RD at Shinshu University Hospital or its affiliated institutions. Among them, 99 patients were excluded for the reason of no stored DNA samples (n = 55) or (2) no contrast-enhanced computed tomography (CT) analysis due to renal impairment or allergy (n = 44). The remaining 124 patients with IgG4-RD (median age: 67 years, 75% male) were included in this retrospective study along with 344 previously reported volunteer healthy subjects [11]. IgG4-RD was diagnosed based on the Japanese Comprehensive Diagnostic Criteria for IgG4-RD [12]. Contrast-enhanced CT findings were reviewed by an experienced radiologist (Y.F.). Periaortitis and periarteritis were defined as vessel wall thickness and wall enhancement either partially or circumferentially on CT images, as reported previously [4]. The protocol of this study was reviewed and approved by the Institutional Review Board of Shinshu University School of Medicine in Matsumoto, Japan (approval number: 571). Written informed consent was obtained from all participating patients. The investigation was conducted according to the principals of the Declaration of Helsinki.

GWAS and SNP genotyping.

Genomic DNA was isolated from whole-blood samples using Quick Gene-800 assays (Kurabo, Osaka, Japan). Genotyping was done with the GeneChip Human Mapping 500K Array Set (Affymetrix, CA, USA) according to the manufacturer's protocol in the first stage of this analysis. Samples with a < 93% genotype call rate were excluded from the study, as were SNPs with a call rate of < 95% or a minor allele frequency of < 5% overall. From a prior genome-wide association analysis between patients with IgG4-related periaortitis/periarteritis (n = 28) and those without (n = 47), we extracted 3 candidate genes (interleukin 1 receptor type 1 [IL1R1]; 2q11.2-q12.1, calcium/calmodulin-dependent protein kinase II alpha [CAMK2A]; 5q32, and vacuolar protein sorting 13 homolog B [VPS13B]; 8q22.2) containing highly significant SNPs (i.e., $P < 10^{-4}$). SNPs in the *IL1R1* gene were examined to identify possible susceptibility loci to IgG4-related periaortitis/periarteritis since this gene has been established as an important mediator involved in many cytokine-induced immune and inflammatory responses [13]. Three SNPs that showed a significant association with IgG4-related periaortitis/periarteritis by a GWAS were selected along with 5 additional SNPs. In total, 8 SNPs (rs3917225, rs2287049, rs2287048, rs3917273, rs2160227, rs951192, rs3917318, and rs7582198) having minor allele frequencies of > 5% in the *ILR1* gene were evaluated. The genotyping of all SNPs was performed using the ABI TaqMan allelic discrimination kit and the StepOnePlus™ Real-Time PCR System (Thermo Fisher Scientific, MA, USA) following the manufacturer's instructions.

Measurement of serum IL-1β levels.

Serum samples were obtained and stored below − 20 °C until measurements. Serum IL-1β levels were examined by a commercially available ELISA kit (Proteintech, IL, USA) following the manufacturer's instructions.

Statistical analysis.

We assessed the significance of allele distribution among patients using the chi-square test by means of 2 × 2 comparisons (df = 1). Differences between groups were examined by Mann–Whitney U-test. *P*-values were subjected to Bonferroni's correction by multiplication by the number of different alleles observed in each locus (*Pc*). Association strength was estimated by calculating the odds ratio (OR) and 95% confidence interval (CI). A $P < 0.05$ was considered statistically significant. Statistical analyses were performed using IBM SPSS statistics software version 24 (IBM, IL, USA). Genetic power was calculated using G*Power version 3.1.9.6 [14].

Results

Eight SNPs in the *IL1R1* gene (rs3917225, rs2287049, rs2287048, rs3917273, rs2160227, rs951192, rs3917318, and rs7582198) were genotyped in 124 IgG4-RD patients and 344 healthy subjects. The genotype frequencies of the tested SNPs were in Hardy–Weinberg equilibrium in all subject groups. Although no remarkable associations were seen between IgG4-RD patients and controls (Table 1), the minor allele frequency was significantly different (i.e., $Pc < 0.05$) for 6 SNPs (rs2287049, rs3917273, rs2160227, rs951192, rs3917318, and rs7582198) from 43 patients with IgG4-related periaortitis/periarteritis (Table 2). These genotype frequencies also exhibited statistical differences between IgG4-RD patients with IgG4-related periaortitis/periarteritis and those without (Table 3).

Table 1
Allelic association of 8 SNPs in the *IL1R1* gene in IgG4-RD patients and healthy subjects

dbSNP	Position (bp)	Gene location	Alleles (1 > 2)	Minor allele frequency, %		*P*	OR (95% CI)
				IgG4-RD (n = 124)	Healthy Subjects (n = 344)		
rs3917225	102769302	Intron	A > G	38.3	36.3	0.582	0.92 (0.68 to 1.24)
rs2287049	102770738	Intron	G > A	49.2	46.8	0.518	1.10 (0.82 to 1.47)
rs2287048	102773999	Intron	C > T	38.3	37.9	0.918	0.98 (0.73 to 1.33)
rs3917273	102779719	Intron	T > A	42.3	41.4	0.802	1.04 (0.77 to 1.39)
rs2160227	102783355	Intron	T > G	42.3	41.7	0.864	1.03 (0.76 to 1.38)
rs951192	102785854	Intron	C > A	41.5	41.9	0.928	0.99 (0.73 to 1.32)
rs3917318	102792760	Intron	G > A	40.7	41.4	0.848	0.97 (0.72 to 1.31)
rs7582198	102799834	3'UTR	G > A	41.5	41.6	0.992	1.00 (0.74 to 1.34)

1, major allele; 2, minor allele; OR, odds ratio; CI, confidence interval.

Table 2

Allelic association of 8 SNPs in the *IL1R1* gene in IgG4-RD patients with and without IgG4-related periaortitis/periarteritis

dbSNP	Position (bp)	Gene location	Alleles (1 > 2)	Minor allele frequency, %		*Pc*	OR (95% CI)
				Periaortitis/periarteritis (+) (n = 43)	Periaortitis/periarteritis (-) (n = 81)		
rs3917225	102769302	Intron	A > G	48.8	32.7	0.103	1.96 (1.15 to 3.35)
rs2287049	102770738	Intron	G > A	62.8	42.0	0.014	2.33 (1.36 to 3.99)
rs2287048	102773999	Intron	C > T	30.2	42.6	0.454	0.58 (0.34 to 1.02)
rs3917273	102779719	Intron	T > A	54.7	35.8	0.034	2.16 (1.27 to 3.68)
rs2160227	102783355	Intron	T > G	54.7	35.8	0.034	2.16 (1.27 to 3.68)
rs951192	102785854	Intron	C > A	54.7	34.6	0.018	2.28 (1.34 to 3.89)
rs3917318	102792760	Intron	G > A	54.7	33.3	0.009	2.41 (1.41 to 4.12)
rs7582198	102799834	3'UTR	G > A	54.7	34.6	0.018	2.28 (1.34 to 3.89)

1, major allele; 2, minor allele; OR, odds ratio; CI, confidence interval.

Table 3
Genotype distribution of *IL1R1* SNPs in IgG-RD patients with and without IgG4-related periaortitis/periarteritis

dbSNP	Alleles (1 > 2)	Genotype	Genotype frequency, %		Model*	*P*	OR (95%CI)
			Periaortitis/periarteritis (+) (n = 43)	Periaortitis/periarteritis (-) (n = 81)			
rs3917225	A > G	AA/AG/GG	9/24/10	36/37/8	Additive	0.0115	1.96 (1.15 to 3.35)
rs2287049	G > A	AA/AG/GG	17/20/6	12/44/25	Additive	0.0016	2.33 (1.36 to 3.99)
rs2287048	C > T	CC/CT/TT	19/22/2	25/43/13	Additive	0.044	0.58 (0.34 to 1.02)
rs3917273	T > A	AA/AT/TT	11/25/7	11/36/34	Dominant (AT + AA vs. TT)	0.0038	3.72 (1.48 to 9.35)
rs2160227	T > G	GG/GT/TT	11/25/7	11/36/34	Dominant (GG + GT vs. TT)	0.0038	3.72 (1.48 to 9.35)
rs951192	C > A	AA/AC/CC	11/25/7	10/36/35	Additive	0.0022	2.28 (1.34 to 3.89)
rs3917318	G > A	AA/AG/GG	11/25/7	9/36/36	Additive	0.0011	2.41 (1.41 to 4.12)
rs7582198	G > A	AA/AG/GG	11/25/7	10/36/35	Additive	0.0022	2.38 (1.34 to 3.89)

1, major allele; 2, minor allele; OR, odds ratio; CI, confidence interval. * The additive genetic model was tested using Armitage's test.

Median serum IL-1β level was significantly lower in IgG-RD patients with IgG4-related periaortitis/periarteritis than in those without (11.8 vs. 4.7 pg/mL, $P = 0.011$). No remarkable differences were observed for serum IL-1β levels and SNP genotypes in the *IL1R1* gene.

Discussion

IgG4-related periaortitis/periarteritis is a recently identified new disease entity candidate in IgG4-RD. As investigations on the clinical features of this disease have only just commenced, the exact pathogenesis of IgG4-related periaortitis/periarteritis is unknown. This study is the first to identify genetic factors related to IgG4-related periaortitis/periarteritis. While *IL1R1* gene polymorphisms were not linked to susceptibility in IgG4-RD involving other organs in a prior GWAS and this study [10], our data uncovered intriguing associations for 6 *IL1R1* SNPs and IgG4-related periaortitis/periarteritis. IL1R1 is a member of the IL-1 receptor family. This membrane receptor binds to IL-1 ligands (IL-1α and IL-1β cytokines) involved in the initiation of IL-1-mediated immune and inflammatory responses [13]. In spite of their identical activities, IL-1α is biologically active as a precursor molecule, whereas IL-1β is secreted and circulates systemically following inflammasome activation. Therefore, we also investigated whether relationships between serum IL-1β levels and *IL1R1* gene polymorphisms affected the development of IgG4-related periaortitis/periarteritis.

Several reports have shown that $CD4^+$ cytotoxic T cells (CTLs) are clonally expanded both in the peripheral blood and in inflammatory tissues to secrete IL-1β, TGF-β, and interferon-γ [15]. Moreover, CTLs were decreased after successful therapy, suggesting that IL-1β-, interferon-γ-, and TGF-β-secreting CTLs were related to IgG4-RD and other fibrotic inflammatory disorders. However, our study showed that serum IL-1β levels were lower in the periaortitis/periarteritis positive group than in the negative group among patients with IgG4-RD. This may have been due to increased levels of soluble sIL-1R1 receptors in the vasculitis positive group. Soluble sIL-1R1 and sIL-1R2 receptors were found to be significantly increased in IgG4-RD sera as compared with healthy controls [16]. Elevated sIL-1R1 binds serum IL-1β and may inhibit excess IL-1β activity to protect ongoing IL-1-mediated inflammation. However, no significant correlations were observed between serum IL-1β levels and *IL1R1* genotypes.

The limitations of this study are lack of disease controls, validation cohort, limited sample size, and no pathological specimens; thus, the possibility of type I error cannot be excluded. Additional series are needed to validate our newly discovered associations in a larger number of IgG4-related periaortitis/periarteritis subjects of other ethnicities. However, power calculations based on the study subjects of 43 patients with IgG4-related periaortitis/periarteritis and 81 IgG4-RD patients without IgG4-related periaortitis/periarteritis and an effect size of 0.44 at rs3917318 showed sufficient detection power (0.97) at the 0.05 level of significance.

In summary, the preset study revealed significant associations of *IL1R1* SNPs with the onset of IgG4-related periaortitis/periarteritis, but not with IgG4-RD onset, in a Japanese population. Our findings raise the possibility of certain genetic factors influencing the risk of specific IgG4-RD manifestations and require further study.

Abbreviations

AIP: autoimmune pancreatitis; CAMK2A: Calcium/calmodulin-dependent protein kinase II alpha; CI: confidence interval; CT: Computed tomography; CTLs: cytotoxic T cells; GWAS: genome-wide association study; IgG4: Immunoglobulin G4; IgG4-RD: Immunoglobulin G4-related disease; IL: interleukin; IL1R1: interleukin 1 receptor type 1; OR: odds ratio; Pc: corrected P; SNPs: single nucleotide polymorphisms; TGF-β: transforming growth factor-β; VPS13B: vacuolar protein sorting 13 homolog B; sIL-2R: Soluble interleukin-2 receptor

Declarations

Ethics approval and consent to participate

The protocol of this study was reviewed and approved by the Institutional Review Board of Shinshu University School of Medicine in Matsumoto, Japan (approval number: 571). Written informed consent was obtained from all participating patients. The investigation was conducted according to the principals of the Declaration of Helsinki.

Consent for publication

Not applicable

Availability of data and materials

The dataset analyzed in this paper is available from the corresponding author on reasonable request, and with appropriate additional ethical approvals, where necessary.

Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

T.U., and M.Ota designed the study, performed the data analysis, and wrote the manuscript. N.O., and A.M. conducted experiments and data analysis. Y.F. performed data collection, data analysis, and data interpretation. M.Ozawa, Y.K., T.W., and H.H. participated in the case and data collection. S.K. designed the study, and performed data interpretation. The authors read and approved the final manuscript.

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