Lack of association of Graves' disease with the A2 allele of the interleukin-1 receptor antagonist gene in a white European population

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Abstract

Objective: To assess whether the A2-type IL-1RA polymorphism is associated with Graves' disease and Graves' ophthalmopathy. Several reports have described a genetic association between the A2 allele of the interleukin-1 receptor antagonist (IL-1RA) gene and certain inflammatory and autoimmune diseases, suggesting that certain loci within the IL-1-related genes may modulate the autoimmune inflammatory response. Recently, we demonstrated marked differences in the expression and regulation of IL-1RA gene and protein between orbital fibroblasts derived from patients with active Graves' ophthalmopathy and healthy individuals.

Design: A total of 144 white European patients with Graves' disease were genotyped to compare their IL-1RA A2 allele frequency with that of 174 healthy controls.

Methods: The polymerase chain reaction was used to amplify the pentallelic variable-number tandem-repeat locus in intron 2 of the IL-1RA gene.

Results: We found no significant differences in IL-1RA A2 allele frequencies (0.20 and 0.26 respectively) and IL-1RA A2 carriage rates (31% and 40% respectively) between patients with Graves' disease and the control group. Moreover, presence or absence of Graves' ophthalmopathy in patients with Graves' disease was not related to significant differences in IL-1RA A2 allele frequencies and IL-1RA A2 carriage rates.

Conclusions: Our data do not support an association between the IL-1RA A2 allele and Graves' disease or Graves' ophthalmopathy in our study population. Thus the A2-type IL-1RA gene polymorphism does not appear to indicate an increased susceptibility to develop Graves' disease and Graves' ophthalmopathy. Mechanisms unrelated to the IL-1RA A2 allele may be responsible for altered IL-1RA production within the orbital tissues in Graves' ophthalmopathy.

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Introduction

The importance of genetic predisposition in the pathogenesis of Graves' disease is clearly established. Multiple genes, including both human leucocyte antigen (HLA)- and non-HLA alleles, are likely to be involved in the susceptibility to this autoimmune disorder (1–8). The presence of interleukin (IL)-1 in thyroid and retro-orbital tissue of patients with Graves' disease (9–11), together with numerous immunological and proinflammatory properties of this cytokine, emphasizes the prominent role of IL-1 in the pathogenesis of this autoimmune thyroid disease (12–14). The important role of IL-1 in the orbital immune process in Graves' ophthalmopathy is highlighted by its capacity to stimulate retro-orbital fibroblast proliferation, glycosaminoglycan synthesis, and various immunomodulatory

molecules expressed by retro-orbital fibroblasts (15–19). The IL-1-dependent biological activities require binding of IL-1 to specific cell-surface receptors and normally are balanced by the naturally occurring IL-1 receptor antagonist (IL-1RA), which acts competitively to inhibit binding of IL-1 to its receptors (20, 21). Recently, it has been shown that exogenous addition of recombinant IL-1RA can inhibit IL-1-induced stimulation of glycosaminoglycan synthesis in cultured human retro-orbital fibroblasts, suggesting that certain IL-1-mediated effects in Graves' ophthalmopathy may be counteracted by IL-1RA *in vitro* (22). More recently, we have demonstrated marked differences in the expression and regulation of IL-1RA gene and protein between retro-orbital fibroblasts derived from patients with active Graves' ophthalmopathy and healthy individuals (23, 24).

A variable-number tandem-repeat polymorphism of the gene encoding the IL-1RA has been identified (25). Several reports have described a genetic association of the A2 allele of the IL-1RA gene and certain inflammatory and autoimmune diseases such as systemic lupus erythematosus, ulcerative colitis, alopecia areata and lichen sclerosus, and with certain forms of nephropathy (26–31). Moreover, two studies have yielded contradictory results concerning the association of the A2-type IL-1RA polymorphism and Graves' disease (32, 33). Blakemore *et al.* (33) have reported a significantly greater frequency of the IL-1RA A2 allele polymorphism in patients with Graves' disease compared with healthy controls, whereas Cuddihy & Bahn (32) failed to confirm such a difference. In the present study, we assessed whether the A2-type IL-1RA polymorphism is associated with non-HLA allele susceptibility in Graves' disease, and whether presence of this genetic locus may predispose individuals with established Graves' disease to develop Graves' ophthalmopathy.

Study participants and methods

Study participants

Investigations adhered to the tenets of the Declaration of Helsinki. Informed consent was obtained from the participants after the nature and possible consequences of the study had been explained to them. The research programme was approved by the Institutional Human Experimentation Committees.

A total of 144 white German patients (27 men and 117 women) with Graves' disease, all native residents of Saxony, were genotyped for the IL-1RA polymorphism and compared with 174 healthy control individuals (52 men and 122 women) who were natives of the same geographic region. Graves' disease was diagnosed by standard criteria (clinical examination, thyroid function tests, ultrasonography, thyroid-stimulating hormone receptor and thyroid peroxidase antibody titres, quantitative technetium scintigraphy). Patients with Graves' disease were divided into two subpopulations: those with clinically apparent Graves' ophthalmopathy and those without clinically apparent ophthalmopathy (periorbital swelling, proptosis, conjunctivitis, chemosis, diplopia). Patients with Graves' ophthalmopathy were followed according to the American Thyroid Association recommendations for classification. All healthy

control participants had documented normal thyroid function and had no evidence of thyroid autoimmune disease as determined by a careful personal and family history, clinical examination, thyroid function and thyroid autoantibody testing.

Polymorphism typing

Genomic DNA was extracted from EDTA-anticoagulated peripheral blood. The polymorphic region within the second intron of the IL-1RA gene was amplified by polymerase chain reaction (PCR) using specific primers, as described previously (25). Amplifications were conducted on an automated thermocycler (Thermodux, Wertheim, Germany) with 1 min denaturation at 96 °C followed by 30 cycles of 94 °C for 1 min, 60 °C for 1 min, 70 °C for 2 min and a final 10-min extension at 72 °C. PCR products of 410 bp (allele 1 = 4 repeats of the 86-bp region), 240 bp (allele 2 = 2 repeats), 500 bp (allele 3 = 5 repeats), 325 bp (allele 4 = 3 repeats) and 595 bp (allele 5 = 6 repeats) were analysed by electrophoresis on a 2% agarose gel stained with ethidium bromide. Negative controls without DNA template were included in each experiment.

Statistical analysis

Allelic frequencies (number of copies of a specific allele divided by the total number of alleles in the group) and carriage rates (number of individuals with at least one copy of a specific allele divided by the total number of individuals in the group) were calculated for the study groups. Because the two- and four-repeat alleles are common and the three-, five- and six-repeat alleles make up less than 5%, the frequencies and carriage rates of the A2 allele under study were compared with the non-A2 allele group consisting of A1, A3 and A4 alleles. Statistical significance was determined using χ^2 test (2×2 contingency tables). $P < 0.05$ was considered to indicate a statistically significant difference.

Results

The distribution of IL-1RA genotypes is shown in Table 1. The vast majority of individuals in our study population carried either the A1/A1 or A1/A2 haplotype. None of the individuals studied revealed the rare A5 allele.

Table 1 IL-1RA allele composition of the 318 individuals in the study population.

Sample group	IL-1RA genotype					
	A1/A1	A1/A2	A2/A2	A1/A3	A2/A3	A1/A4
Graves' patients ($n = 144$)	93	32	12	5	1	1
Normal controls ($n = 174$)	100	47	21	4	2	0

Table 2 Allele frequencies and carriage rates of the interleukin-1 receptor antagonist allele A2 and the combined non-A2 alleles in patients with Graves' disease (GD) compared with normal individuals, and in subgroups of patients with Graves' disease.

Sample group	A2 frequency	Non-A2 frequency	A2 carriage rate (%)	Non-A2 carriage rate (%)
Graves' patients (<i>n</i> = 144)	0.20 ^a	0.80	31 ^a	69
Normal controls (<i>n</i> = 174)	0.26	0.74	40	60
GD alone (<i>n</i> = 100)	0.18 ^a	0.82	28 ^a	72
GD and GO (<i>n</i> = 44)	0.23 ^{a,b}	0.77	39 ^{a,b}	61

n, number of individuals; GO, Graves' ophthalmopathy. ^a*P* > 0.05; ^b*P* > 0.05 compared with GD alone.

The allelic frequency of the IL-1RA A2 allele was 0.21 in the Graves' disease group and 0.26 in the control group. There were no significant differences between these groups (*P* > 0.05; Table 2). Comparison of the carriage rates of the A2 allele and grouped non-A2 alleles also failed to reveal a significant difference (*P* > 0.05; Table 2).

The A2-allelic frequencies and carriage rates of the subgroups of patients with Graves' disease alone and those with both Graves' disease and Graves' ophthalmopathy were compared with those of normal individuals and with one another. Presence or absence of clinically apparent Graves' ophthalmopathy in patients with Graves' disease was not related to any significant differences in IL-1RA A2 allele frequencies (0.23 and 0.19 respectively) and IL-1RA A2 carriage rates (40% and 32% respectively) (each *P* > 0.05; Table 2).

Discussion

IL-1 is a potent proinflammatory mediator that is responsible for a variety of immunological and metabolic effects involved in the evolution and propagation of Graves' disease and Graves' ophthalmopathy (12–19). There is increasing evidence that an imbalance between IL-1 and IL-1RA may play an important part in the pathogenesis of various inflammatory disease (34–37). We have recently demonstrated that cultured retro-orbital fibroblasts derived from patients with Graves' ophthalmopathy express and release significantly lower quantities of intra-cellular IL-1RA and soluble (s)IL-1RA compared with normal retro-orbital fibroblasts (23, 24). A biological significance for the IL-1RA in Graves' disease and Graves' ophthalmopathy has been implicated in studies by Rasmussen *et al.* (38) using cultured human thyroid cells and those by Tan *et al.* (22) using cultured human retro-orbital fibroblasts (22, 38).

Tarlow *et al.* (25) have previously identified a polymorphism in intron 2 of the IL-1RA gene that is caused by the variable copy number of an 86-bp sequence. Several reports have described a genetic association between increased frequencies of the A2 allele (two copies of the 86-bp unit) and certain chronic inflammatory diseases of epithelial tissue (26–31, 39, 40); however, few of these studies have been confirmed

(41, 42). Furthermore, lack of an association between the IL-1RA A2 allele and certain immune-mediated diseases such as rheumatoid arthritis, juvenile chronic arthritis, Crohn's disease and multiple sclerosis has been reported (39, 43, 44).

Two previous studies have examined whether the A2 allele of the IL-1RA gene is associated with an increased susceptibility to develop Graves' disease (32, 33). Blakemore *et al.* (33) have reported a significantly greater frequency of the IL-1RA A2 allele polymorphism in patients with Graves' disease than in healthy controls. In contrast to this report, and in agreement with the findings of Cuddihy and Bahn (32), our data do not support the IL-1RA A2 allele as a novel genetic susceptibility marker for Graves' disease. The reasons for this discrepancy remain unclear. Differences in the genetic pools studied are not a satisfactory explanation, because patients were drawn from a similar genetic background, and because a marked similarity of allelic frequencies of the IL-1RA A2 allele in all control groups does not indicate genetic heterogeneity. Further, our comparison of A2 allelic frequencies and carriage rates in patients with Graves' disease with and without Graves' ophthalmopathy have failed to reveal a statistically significant difference, confirming similar previous observations (32, 33). Thus possession of the IL-1RA A2 allele does not appear to indicate an increased risk for patients with Graves' disease to develop Graves' ophthalmopathy.

The majority of studies reporting an association of the IL-1RA A2 allele with certain chronic inflammatory disorders have suggested a correlation with disease severity or chronicity, supporting the notion that the A2 allele may represent a marker of disease severity rather than one of disease susceptibility (26–30). In contrast, Blakemore *et al.* (33) failed to reveal a correlation between the A2 allele and severity of Graves' disease, as assessed by thyroid autoantibody titres, serum concentrations of thyroxine, and outcome 1 year after antithyroid drug treatment. Of note, however, Cuddihy and Bahn (32) reported a trend towards a greater prevalence of the A2-type IL-1RA gene polymorphism in a small subgroup of patients with two or more extrathyroidal manifestations of Graves' disease. In common with those authors, we were unable to

examine this issue satisfactorily, because of the small number of patients with pretibial dermatopathy and acropachy who were available for IL-1RA polymorphism genotyping. A collaborative effort is now in progress to clarify this issue.

Polymorphisms in regions of cytokine genes that affect transcription may account for some of the interindividual variation in cytokine production (43, 45, 46). We have previously reported that smokers with active Graves' ophthalmopathy have lower sIL-1RA serum concentrations than non-smokers with Graves' ophthalmopathy, and that low sIL-1RA serum concentrations before and after orbital radiotherapy may be related to a poor response to therapy (23). Because impaired production of sIL-1RA may enhance the consequences of orbital inflammation and contribute to a prolonged course of active disease, experiments are now in progress to assess whether the A2-type IL-1RA gene polymorphism may contribute to differences in IL-1RA serum concentrations between smokers and non-smokers with Graves' ophthalmopathy (23). In addition, it remains to be determined whether this genetic marker indeed alters IL-1RA gene function, thereby influencing basal and cytokine-stimulated IL-1RA production in smokers with Graves' ophthalmopathy.

In conclusion, our current data do not support an association between the IL-1RA A2 allele and Graves' disease or Graves' ophthalmopathy in our study population. Thus the A2-type IL-1RA gene polymorphism does not appear to indicate an increased susceptibility to develop Graves' disease and Graves' ophthalmopathy. Altered IL-1RA production within the orbital tissue of patients with Graves' ophthalmopathy may involve mechanisms unrelated to the IL-1RA A2 allele.

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