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ORIGINAL ARTICLE

Association of NFKB1 −94ins/del ATTG promoter polymorphism with susceptibility to and phenotype of Graves' disease

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Recently, a functional polymorphism in the NFKB1 gene promoter (−94ins/del ATTG) has been identified and associated with chronic inflammatory diseases. The aim of this study was to analyze the association of NFKB1 polymorphism with susceptibility to and phenotype of Graves' disease (GD). The initial case–control association study, performed in a Polish–Warsaw cohort (388 GD patients and 688 controls), was followed by the two replication studies performed in Polish–Gliwice and Japanese–Kurume cohorts (198 GD patients and 194 controls, and 424 GD patients and 222 controls, respectively). The frequency of the −94del ATTG (D) allele was increased in GD compared to controls in Warsaw cohort. This finding was replicated in Gliwice cohort. Combining both Polish–Caucasian cohorts showed that the NFKB1 polymorphism was significantly associated with susceptibility to GD with a codominant mode of inheritance (P = 0.00005; OR = 1.37 (1.18–1.60)). No association with GD was found in Japanese cohort. However, subgroup analysis in Japanese GD patients revealed a correlation between the NFKB1genotype and the development of ophthalmopathy (P = 0.009; OR = 1.49 (1.10–2.01)), and the age of disease onset (P = 0.009; OR = 1.45 (1.09–1.91)). Our results suggest that NFKB1 −94ins/del ATTG polymorphism may be associated with susceptibility to and/or phenotype of GD.
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Keywords: *NFKB1; genetic polymorphism; Graves' disease; ophthalmopathy*

Introduction

The nuclear factor-κB (NF-κB) signaling pathway regulates the expression of hundreds of genes, many of which play important roles in the immune system, such as genes encoding cytokines (IL-2, IL-6, IL-12, TNF-α, IFN-γ), cytokine receptors (IL-2 receptor), cell adhesion molecules (VCAM-1, ICAM-1) and chemokines (IL-8).[1,2] The NF-κB family of transcription factors has five members: p50/p105 (encoded by the *NFKB1* gene, 4q24), p52/p100 (*NFKB2*, 10q24.32), RelA (*RELA*, 11q13.3), RelB (*RELB*, 19q32.2) and c-Rel (*REL*, 2p16.1). All these proteins share a highly conserved DNA-binding/dimerization domain called the Rel homology domain. The p50 and p52 transcription factors, which are derived from precursor proteins, p105 and p100 respectively, lack transcriptional activation domains, and their homodimers are thought to act as repressors. In contrast, RelA, RelB and c-Rel carry transcriptional activation domains. Although the p50/RelA heterodimer is the major and most active NF-κB dimer in many cells, the balance between different NF-κB homo- and heterodimers will control the level of transcriptional activity. Moreover, the activity of NF-κB signaling is tightly regulated by interaction with several inhibitory IκB proteins and by IκB kinases (IKK).

Clearly, any change in the delicate balance of the NF-κB signaling pathway can lead to an alteration in immune response.[3] Thus, genes involved in the NF-κB pathway are important candidate genes for autoimmune diseases. So far, two genes, *SUMO4* and *MAP3K7IP2* (6q25), have been studied in autoimmune thyroid diseases (AITD). *SUMO4* encodes a protein involved in the ubiquitination of IκBα, and a single nucleotide polymorphism (SNP) causing methionine to valine substitution (rs237025, A163G, M55V) results in greater NF-κB transcriptional activity.[4] This polymorphism has been associated with susceptibility to AITD, type 1 diabetes (T1D) and rheumatoid arthritis in Asian populations.[5,6] However, the association of M55V with Graves' disease (GD) and T1D could not be replicated in Caucasian populations, suggesting heterogeneity in the genetic effect of the *SUMO4* locus among diverse ethnic groups.[7–10] *MAP3K7IP2* (also known as *TAB2)* encodes a different inhibitor of the NF-κB signaling pathway and a

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SNP (001Msp) has been shown to be associated with AITD.[4,9]

Recently, a functional *NFKB1* promoter polymorphism (−94ins/del ATTG) has been identified and associated with the development of ulcerative colitis.[11] In this study, we analyzed the association of this polymorphism with GD. First, we performed a population-based, case–control study in a Polish–Warsaw cohort. Next, replication studies have been performed in a second set of Polish–Caucasian (from Gliwice) and Japanese subjects. Finally, we analyzed the interactions between *NFKB1* genotypes and other established GD susceptibility markers (HLA-DRB1*03 and CTLA-4 A49G) and the association of *NFKB1* polymorphism with the clinical phenotype of GD (age of onset, sex, presence of ophthalmopathy, family history of AITD).

Results

Association of NFKB1 −94ins/del ATTG polymorphism with susceptibility to GD

The initial case–control study in the Polish–Warsaw cohort had a power >0.80 to detect an association similar to the one originally reported by Karban *et al*.[11] at α 0.05, with one-tailed χ^2-test. We found that the frequency of the D allele (−94del ATTG) was significantly greater in GD compared to controls (46.4 vs 38.8%; $P=0.0006$; odds ratio (OR) = 1.36 (1.14–1.63)). There were also significant differences in genotype distributions, suggesting a codominant or recessive effect (Table 1). For a replication study, we analyzed a second set of unrelated GD patients and control subjects from the Polish–Gliwice cohort. The study confirmed a significant increase of the D allele in GD (46.2 vs 38.9%; $P=0.039$; OR = 1.35 (1.01–1.79)), whereas the genotype distribution suggested a dominant or codominant effect. No evidence of significant heterogeneity between Warsaw and Gliwice studies has been detected. Combining all Polish GD samples and controls showed that the *NFKB1* −94ins/del ATTG polymorphism was significantly ($P=0.00005$) associated with susceptibility to GD with a codominant mode of inheritance, producing an OR of 1.37 (1.18–1.60). Based on the genotype distribution in all Polish samples, the dominant and recessive model could be rejected ($P=0.013$ and $P=0.023$, respectively).

In addition, we analyzed the association of *NFKB1* polymorphism with GD in an ethnically different group of Japanese subjects. The frequencies of D allele and DD homozygote were similar in Polish–Caucasian and Japanese control groups. No statistically significant differences in D allele (37.4 vs 39.6%; $P=0.428$; OR = 0.91 (0.72–1.15)) or genotype frequencies were observed among cases and controls in the Japanese cohort.

Association of the NFKB1 −94ins/del ATTG polymorphism with clinical and genetic characteristics of GD

The distribution of *NFKB1* genotypes was compared in subgroups of GD patients stratified by clinical and genetics parameters. In Polish–Caucasian patients (the pooled Warsaw and Gliwice cohorts), there was a tendency towards an increase of D allele in the subgroup with clinically evident ophthalmopathy (49.8 vs 43.9%; $P=0.058$; OR = 1.26 (0.99–1.61)) (Table 2). The distribution of *NFKB1* genotypes in GD with or without eye disease suggested a codominant ($P=0.056$; OR = 1.27 (0.99–1.63)) or recessive model ($P=0.035$; OR = 1.56 (1.03–2.36)). The *NFKB1* genotypes were equally distributed among *HLA-DRB1*03* and *CTLA-4* 49G alleles carriers. Moreover, no significant interaction between *CTLA-4* CT60, *PTPN22* C1858T and *NFKB1* polymorphism was detected in the Polish–Warsaw cohort (data not shown).

In Japanese GD patients we observed a significant association of the *NFKB1* polymorphism with the development of clinically evident ophthalmopathy (Table 3). The most significant genotype effect was found under a codominant model, producing an OR of 1.49 (1.10–2.01). There was no correlation between the frequencies of D allele and the severity of eye disease: NOSPECS class 0–II ($N=301$)—34.5%, class III ($N=94$)—44.1% and class IV–VI ($N=29$)—44.8%. The

Table 1 The association of *NFKB1* −94ins/del ATTG promoter polymorphism with susceptibility to Graves' disease

Population	*Group*	*N*	*NFKB1 genotype frequencies*			P-*value** *OR (95%CI)*		
			W/W	*W/D*	*D/D*	*Dominant model*	*Codominant model*	*Recessive model*
Poland–Warsaw	GD	388	116 (29.9%)	184 (47.4%)	88 (22.7%)	0.022**	0.001	0.001
	Control subjects	688	253 (36.8%)	336 (48.8%)	99 (14.4%)	1.36 (1.04–1.78)	1.36 (1.14–1.63)	1.75 (1.27–2.40)
Poland–Gliwice	GD	198	51 (25.8%)	111 (56.1%)	36 (18.2%)	0.027	0.030	0.25**
	Control subjects	194	70 (36.1%)	97 (50.0%)	27 (13.9%)	1.63 (1.06–2.51)	1.39 (1.03–1.88)	1.37 (0.80–2.37)
Poland–Pooled	GD	586	167 (28.5%)	295 (50.3%)	124 (21.2%)	0.001**	0.00005	0.001**
	Control subjects	882	323 (36.6%)	433 (49.1%)	126 (14.3%)	1.45 (1.16–1.82)	1.37 (1.18–1.60)	1.61 (1.22–2.12)
Japan–Kurume	GD	424	170 (40.1%)	191 (45.0%)	63 (14.9%)	0.178	0.428	0.759
	Control subjects	222	77 (34.7%)	114 (51.4%)	31 (14.0%)	0.79 (0.57–1.11)	0.91 (0.72–1.15)	1.08 (0.68–1.71)

Abbreviations: CI, confidence interval; GD, Graves' disease; OR, odds ratio.
W = −94ins ATTD allele; *D* = −94del ATTG allele.
**P*-values for the comparison of genotype distribution between GD and controls calculated with the use of χ^2-test. The most likely model (dominant, recessive, codominant) for the genotype distributions was analyzed by χ^2-test using Web-Assotest (www.ekstroem.com).
**Indicates a *P*-value for model fit $P<0.05$ suggesting the exclusion of this model of genotype effect. Homogeneity of OR: Warsaw vs Gliwice: dominant model $P=0.50$, codominant model $P=0.9$, recessive model $P=0.46$; Poland Pooled vs Japan: dominant model $P=0.004$, codominant model $P=0.004$, recessive model $P=0.14$.

Table 2 Distribution of *NFKB1* −94ins/del ATTG promoter genotypes in subgroups of patients with GD stratified by clinical and genetic parameters in Polish–Caucasian cohort

Parameter	*Subgroup*	N	*NFKB1 genotypes frequencies*			P	*OR (95%CI)*
			W/W	*W/D*	*D/D*		
Gender	Males	108	34 (31.5%)	51 (47.2%)	23 (21.3%)	0.640	0.93 (0.69–1.26)
	Females	478	133 (27.8%)	244 (51.0%)	101 (21.1%)		
Ophthalmopathy	NOSPECS ⩾III	204	53 (26.0%)	99 (48.5%)	52 (25.5%)	0.056	1.27 (0.99–1.63)
	NOSPECS ⩽II	362	109 (30.1%)	188 (51.9%)	65 (18.0%)		
Disease onset	Onset <40 years	267	67 (25.1%)	149 (55.8%)	51 (19.1%)	0.557	1.08 (0.84–1.37)
	Onset ⩾40 years	274	85 (31.0%)	130 (47.4%)	59 (21.5%)		
Family history	Positive	168	50 (29.8%)	80 (47.6%)	38 (22.6%)	0.933	1.01 (0.78–1.32)
	Negative	351	97 (27.6%)	184 (52.4%)	70 (19.9%)		
HLA DRB1*03	Present	187	58 (31.0%)	92 (49.2%)	37 (19.8%)	0.385	0.88 (0.67–1.17)
	Absent	241	62 (25.7%)	130 (53.9%)	49 (20.3%)		
CTLA4 49G	Present	364	108 (29.7%)	179 (49.2%)	77 (21.2%)	0.958	0.99 (0.75–1.32)
	Absent	135	36 (26.7%)	74 (54.8%)	25 (18.5%)		

Abbreviation: CI, confidence interval; GD, Graves' disease; OR, odds ratio.
P-values and odds ratios (OR) were calculated for codominant model.

Table 3 Distribution of *NFKB1* −94ins/del ATTG promoter genotypes in subgroups of patients with GD stratified by clinical and genetic parameters in Japan cohort

Parameter	*Subgroup*	N	*NFKB1 genotypes frequencies*			P	*OR (95%CI)*
			W/W	*W/D*	*D/D*		
Gender	Males	92	43 (46.7%)	36 (39.1%)	13 (14.1%)	0.249	0.82 (0.59–1.15)
	Females	332	127 (38.3%)	155 (46.7%)	50 (15.1%)		
Ophthalmopathy	NOSPECS ⩾III	123	39 (31.7%)	59 (48.0%)	25 (20.3%)	0.009	1.49 (1.10–2.01)
	NOSPECS ⩽II	301	131 (43.5%)	132 (43.9%)	38 (12.6%)		
Disease onset	Onset <40 years	232	81 (34.9%)	110 (47.4%)	41 (17.7%)	0.009	1.45 (1.09–1.91)
	Onset ⩾40 years	192	89 (46.4%)	81 (42.2%)	22 (11.5%)		
Family history	Positive	116	39 (33.6%)	60 (51.7%)	17 (14.7%)	0.336	1.16 (0.86–1.58)
	Negative	296	124 (41.9%)	126 (42.6%)	46 (15.5%)		
CTLA4 49G	Present	283	115 (40.6%)	123 (43.5%)	45 (15.9%)	0.881	1.04 (0.59–1.84)
	Absent	26	12 (46.2%)	9 (34.6%)	5 (19.2%)		

Abbreviations: CI, confidence interval; GD, Graves' disease; OR, odds ratio.
P-values and odds ratios (OR) were calculated for codominant model.

NFKB1 polymorphism was associated also with an early (<40 years) onset of GD, producing an OR of 1.45 (1.09–1.91). Patients carrying the D allele had a significantly younger age of disease onset (median 32 years (upper and lower quartiles 24–46)) compared to W/W homozygotes (median 40 (28–50)), as analyzed by Mann–Whitney *U*-test ($P = 0.006$).

Next, we applied multiple logistic regression analysis to adjust for effects of other factors on the development of ophthalmopathy in Polish–Caucasian and Japanese populations (Table 4). The independent variables included in the model were: *NFKB1* genotypes, disease onset, tobacco smoking and male sex. In Caucasians population, we found a statistically significant effect of the *NFKB1* genotype under the recessive model and a trend for an effect assuming a codominant model. Analysis of other variables showed a strong effect of cigarette smoking and older age of onset. In the Japanese cohort, only the *NFKB1* polymorphism had a statistically significant effect on the development of ophthalmopathy under all three models considered although the data suggested that the codominant model may be most appropriate.

Discussion

The *NFKB1* promoter −94ins/del ATTG polymorphism was first reported by Karban *et al*.[11] to be linked to and associated with susceptibility to ulcerative colitis (UC) in a non-Jewish, North American–Caucasian population. Although the authors suggested a recessive effect, we found that the most significant genotype effect was actually present under a codominant model ($P = 0.004$, OR = 1.30 (1.09–1.56)). Additionally, *in vitro* functional assays suggested that the D allele may result in reduced gene expression and hence decreased p50/p105 NF-κB protein production. The authors hypothesized that (i) a decrease in p50 homodimers may result in a relative overexpression of Rel homo- and heterodimers, or (ii) low levels of p50 lead to a poor innate immune response.[11]

Table 4 Analysis by multiple logistic regression of an association between *NFKB1* −94ins/del ATTG promoter genotypes and Graves' ophthalmopathy after adjustment for age at onset, smoking status and sex

Population	*Variable*	P-*value OR (95%CI)*		
		Dominant model	*Codominant model*	*Recessive model*
Poland–pooled	*NFKB1*	0.25 1.27 (0.84–1.93)	0.054 1.30 (0.99–1.70)	0.043 1.49 (1.02–2.50)
	Disease onset[a]	0.0004 1.26 (1.11–1.44)	0.0004 1.26 (1.11–1.44)	0.0004 1.26 (1.11–1.44)
	Tobacco smoking	0.001 1.91 (1.31–2.77)	0.001 1.90 (1.30–2.76)	0.001 1.90 (1.30–2.74)
	Male sex	0.36 0.80 (0.49–1.30)	0.3 0.79 (0.49–1.29)	0.3 0.79 (0.49-1.28)
Japan	*NFKB1*	0.03 1.65 (1.04–2.61)	0.009 1.52 (1.11–2.08)	0.03 1.88 (1.06–3.32)
	Disease onset[a]	0.12 0.89 (0.77–1.03)	0.14 0.90 (0.78–1.04)	0.09 0.88 (0.77–1.02)
	Tobacco smoking	0.52 1.18 (0.71–1.95)	0.49 1.20 (0.70–1.99)	0.65 1.13 (0.68–1.86)
	Male sex	0.31 1.34 (0.76–2.38)	0.3 1.35 (0.76–2.40)	0.39 1.28 (0.73–2.26)

Abbreviations: CI, confidence interval; OR, odds ratio.
[a]OR shows risk increment per 10 years increase in age at onset.

The association of the *NFKB1* polymorphism with UC has been replicated in an independent study in a Dutch population.[12] Moreover, a significant genotype–phenotype correlation was identified—mean age of disease onset was significantly lower in patients homozygous for the D allele.[12] However, the association of −94ins/del ATTG polymorphism with susceptibility to or phenotype of UC could not be replicated in German, UK and Spanish populations.[13–15] The discrepancies between these studies may be explained by differences in environmental factors for UC in different populations and/or differences in the haplotypic context of the −94 del ATTG variant among the studied populations.

The *NFKB1* −94ins/del ATTG polymorphism was not studied in detail in other autoimmune diseases, although there are single reports in which no associations with rheumatoid arthritis, systemic lupus erythematosus, celiac disease or T1D were found.[16–18] However, one study reported an epistatic interaction between *NFKB1* and Fc receptor-like 3 (*FCRL3*) gene in Spanish–Caucasian patients with rheumatoid arthritis.[19] Interestingly, *FCRL3* has been recently associated with susceptibility to GD.[9,20] A different (CA) repeat polymorphism located in the 3′ region of *NFKB1* has been reported to be associated with T1D but subsequent studies were unable to confirm this finding.[21–23]

In this study, the frequency of the D allele in control populations were similar to those observed in other Caucasian populations, as reported by Karban *et al.* (D allele frequency—38%), Borm *et al.* (37%) and Glas *et al.* (39%).[11–13] First in the Warsaw cohort, we observed an increased frequency of the D allele in GD patients compared to controls. This observation was replicated in a second, independent set of Polish GD cases and healthy controls. The results from both Polish cohorts suggest a codominant effect. However, the association with susceptibility to GD was not confirmed in a different ethnic population—Japanese. Although a potential functional variant could be expected to be associated with a disease independent of ethnicity, this is not always so, even for established associations such as *SUMO4*.[7]

Search for genotype–phenotype correlations revealed a trend tendency towards an increase of D allele in Polish–Caucasian patients with Graves' ophthalmopathy. Consistent with this finding, in Japanese GD patients the *NFKB1* polymorphism was significantly associated with clinically evident eye disease. Among Japanese we also found a correlation between the *NFKB1*-94del ATTG allele and younger age of onset. These results are interesting given the established role of NF-κB in pathogenesis of ophthalmopathy. Several proinflammatory cytokines (IL-1, TNF-α and IFN-γ), which are associated with the NF-κB signaling pathway, are known to play a central role in the autoimmune inflammation in orbital tissues.[24,25] Apart from leukocyte activation, NF-κB plays an important role in glycosaminoglycans production by orbital fibroblast.[26] Therefore, the treatment of Graves' ophthalmopathy with glucocorticoids is predominantly directed against the NF-κB signaling pathway.[27,28] To analyze further the effect of *NFKB1* vs other environmental and demographic factors on the development of eye symptoms, we applied multiple logistic regression analysis. In both cohorts the *NFKB1* polymorphism increased the risk of developing eye disease. In addition, we confirmed the strong association of cigarette smoking and older age of onset of GD with ophthalmopathy in Polish–Caucasian patients.[29] Apart from *NFKB1* genotypes, no other variables had a significant effect on the development of ophthalmopathy in Japanese GD patients, confirming our previous results.[30] Thus, the predisposing factors to ophthalmopathy in Japanese population remain to be established.

Finally, some limitations of our study need to be pointed out: (i) before accepting as proven the association of *NFKB1* −94ins/del ATTG with GD should be further replicated in other Caucasian populations; (ii) −94ins/del ATTG may be linked to other functional

polymorphisms within the *NFKB1* locus and defining 'haplotype blocks' by analyzing more markers in the region may be necessary to delineate the primary association and explain differences between populations;[31] (iii) genotype–phenotype correlation results should be regarded as preliminary since the analysis was underpowered, which may result in both, false-negative and -positive results—thus the association with clinically apparent ophthalmopathy also requires replication.

In conclusion, our results indicate that the *NFKB1* polymorphism may be associated with susceptibility to and/or phenotype of GD.

Patients and methods

Subjects

The following cohorts of patients and controls were studied: (i) Poland–Warsaw, comprising of 388 GD patients and 688 healthy subjects; (ii) Poland–Gliwice, comprising of 198 GD patients and 194 healthy subjects and (iii) Japan–Kurume, comprising of 424 GD patients and 222 healthy subjects.

Patients with GD were consecutive series of unrelated cases from the Department of Endocrinology, Medical University of Warsaw; Department of Nuclear Medicine and Endocrine Oncology, Institute of Oncology in Gliwice and Department of Endocrinology, Kurume University School of Medicine. The clinical characteristics of the studied groups are shown in Table 5. The diagnosis of GD was based on clinical and biochemical symptoms of hyperthyroidism and was confirmed by the presence of diffuse goiter, detectable TSH receptor auto antibodies (TRAK Lumitest, BRAHMS Diagnostica GmbH, Germany) and/or increased radioiodine uptake. The severity of ophtalmopathy was assessed according to the NOSPECS classification. Patients with proptosis (NOSPECS class III), extraocular muscle dysfunction (class IV), exposure keratitis (class V) and optic neuropathy (class VI) were considered clinically evident. The control group comprised of ethnically matched healthy subjects, who had no family history of autoimmune diseases. The research program was approved by the Local Ethical Committees, and written informed consent was obtained from all of the participants.

Genotyping

Genotyping of the *NFKB1* −94ATTG ins/del promoter polymorphism (GeneBank accession AF213884, gi 7012904) was determined by PCR amplification (primers: 5′-TTTAATCTGTGAAGAGATGTGAATG-3′ and 5′-CTCTGGCTTCCTAGCAGGG-3′), followed by digestion with the restriction enzyme *Van*91I (Fermentas International Inc., Ontario, Canada), as described by Karban *et al*.[11] The obtained restriction fragments were visualized on a 2% agarose gel. To confirm the accuracy of the method employed, all heterozygotes were analyzed twice by RFLP and randomly selected subjects were analyzed by direct sequencing. Replication studies in Gliwice and Kurume have been performed blinded to initial results obtained in Warsaw. Analysis of *CTLA-4* and *HLA-DRB1* polymorphisms were reported previously.[32,33]

Statistical analysis

Allele frequencies were compared between groups by χ^2 test on a 2×2 contingency table using Statistica software package (StatSoft Inc., Tulsa, OK, USA). The genotype distribution was analyzed assuming three models of inheritance: dominant, codominant or recessive. Under each model the OR with 95% confidence interval and the *P*-value for an association was calculated. Also, the fit of the model was assessed by a χ^2-test. These calculations were performed using Web-Assotest program (available at: http://www.ekstroem.com/assotest/assotest.html). *P*-values less than 0.05 were considered significant. The conservative Bonferoni's correction for multiple testing was not applied.[34] Homogeneity of OR across the population strata was assessed with Breslow–Day test (dominant or recessive model) or multiple logistic regression (codominant model) using SPSS software package (SPSS Inc. Chicago, IL, USA). In the analysis by logistic regression, the *NFKB1* genotype was encoded

Table 5 Clinical characteristics of patients with Graves' disease (GD)

Characteristics	*Studied groups*					
	N[a]	*Poland–Warsaw*	N[a]	*Poland–Gliwice*	N[a]	*Japan–Kurume*
Male	388	19.6%	198	16.2%	424	21.7%
Age of onset of GD (year)[b]	357	41.8±15.4	184	40.1±13.5	424	37.0±15.1
Disease duration (year)[b]	357	4.2±6.1	173	5.7±4.6	424	4.8±7.7
Ophthalmopathy (NOSPECS class III and higher)	369	37.1%	197	34.0%	424	29.0%
Cigarette smokers	334	44.0%	181	45.3%	417	34.3%
Family history of AITD	330	34.2%	189	29.1%	412	27.4%
Therapy for hyperthyroidism						
Antithyroid drugs	366	36.9%	193	1.5%	424	81.7%
Radioactive iodine		45.1%		88.1%		0%
Surgery		18.0%		10.4%		8.3%

Abbreviations: AITD, autoimmune thyroid disease; GD, Graves' disease.
Values are percentages of the group.
[a]*N*-number of patients available for analysis.
[b]Age of onset and disease duration are presented as mean±s.d.

as 0, 1, or 2 according to the number of alleles with the −94ATTG deletion and the homogeneity of OR was assessed by analyzing the statistical significance of an interaction between population and genotype by the likelihood ratio test.[35] Multiple logistic regression was also used to analyze the association between the *NFKB1* genotypes and Graves' ophthalmopathy after adjustment for age at onset, smoking status and sex.

Differences in distribution of age of disease onset were analyzed by a non-parametric Mann–Whitney test. All the cases and controls in studied cohorts were in Hardy–Weinberg equilibrium.

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Disclosure summary

None.

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