

## CLINICAL STUDY

# Variation in phenotypic appearance of Graves' disease: effect of genetic anticipation and duration of complaints

Xander G Vos<sup>1</sup>, Natalie Smit<sup>1</sup>, Erik Endert<sup>2</sup>, Jan G P Tijssen<sup>3</sup> and Wilmar M Wiersinga<sup>1</sup>

<sup>1</sup>Department of Endocrinology and Metabolism, <sup>2</sup>Laboratory of Endocrinology, Department of Clinical Chemistry and <sup>3</sup>Department of Cardiology, F5-161, Academic Medical Center, University of Amsterdam, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands

(Correspondence should be addressed to X G Vos; Email: x.g.vos@amc.nl)

## Abstract

**Objective:** Both genetic and environmental factors contribute to susceptibility of Graves' disease. In this study, we evaluated whether the duration of symptoms or a positive family history of autoimmune thyroid disease (AITD) are related to specific phenotypes in patients with a first episode of Graves' hyperthyroidism (GH).

**Design:** Cross-sectional multicentre observational study.

**Patients:** Two hundred and sixty-three consecutive untreated patients (mean age ( $\pm$  s.d.)  $42.6 \pm 12.4$  years; range 16–79 years) with a first episode of GH were included. Biochemical and clinical severity of GH was evaluated. Participants were asked to complete questionnaires about environmental factors (smoking behavior, use of estrogens, stress etc.), the duration of symptoms (interval between start of symptoms and date of referral) and family history for AITD. We ascertained the autoimmune nature of thyroid disease in affected relatives. Family history scores (FHS; high score indicating a close genetic relationship and/or a large number of affected relatives) were calculated for patients with a positive family history for AITD.

**Results:** The peak incidence for the diagnosis of GH was 2–3 months after onset of symptoms (32% of patients). Duration of symptoms was negatively associated with age ( $P$  for trend = 0.04). A positive family history for AITD was present in 42.6% of patients. Patients with the highest FHS were more often male ( $P=0.01$ ) while age at onset was lower ( $P=0.02$ ) compared to patients with a lower FHS. Among patient groups with different FHS, no differences were found in exposure to environmental factors, nor in clinical or biochemical severity of hyperthyroidism.

**Conclusion:** Our study does not support the hypothesis that a short duration of thyrotoxic symptoms until diagnosis is related to more severe hyperthyroidism in Graves' disease. We have found supporting evidence for the existence of genetic anticipation in Graves' disease by means of a lower age of onset in the group with the highest FHS.

*European Journal of Endocrinology* 161 113–118

## Introduction

The etiology of Graves' disease is multifactorial. Twin studies support that 79% of the susceptibility to develop Graves' hyperthyroidism (GH) can be attributed to genetic factors, leaving 21% for environmental factors (1). As a consequence of the multifactorial etiology, there is marked variation in phenotypic appearance of Graves disease: duration of symptoms before diagnosis, severity of hyperthyroidism, and the extent of thyroid enlargement vary greatly, and Graves' orbitopathy and pretibial myxedema may or may not be present. Little is known about the determinants of different phenotypes, apart from the effect of age (older age being associated with less severe hyperthyroidism) (2–4) and smoking behavior (smoking being associated with greater risk on Graves' orbitopathy) (5).

In this study, we evaluated whether duration of symptoms or a family history of autoimmune thyroid disease (AITD) are related to specific phenotypes in patients with a first episode of GH. With regard to duration of hyperthyroid symptoms, we have seen some patients over the last few years that presented themselves after a short period of symptoms with very severe GH and responded remarkably fast to antithyroid drugs. It reminded us of a recently described novel subtype of type 1 diabetes mellitus, characterized by a rapid onset, more severe metabolic derangement and absence of diabetes-related antibodies (6). We thus hypothesized that a short duration of thyrotoxic symptoms until diagnosis is related to more severe hyperthyroidism in Graves' disease patients.

It is well known that AITD clusters in families, and about 50% of patients with GH have a positive family

history for AITDs (4, 7). In view of genetic anticipation, one may presume that GH develops at a younger age in patients with a positive family history for AITD than in patients with a negative family history, and this was our second hypothesis.

Both hypotheses were tested in a cross-sectional observational study of consecutive patients with newly diagnosed untreated GH. Phenotypic appearance of Graves' disease was carefully determined, together with exposure to a number of environmental factors involved in the pathogenesis of Graves' disease.

## Subjects and methods

### Study design

We included 263 consecutive untreated patients (69 males and 194 females, age 16–79 years at study entrance) with a first episode of GH in a cross-sectional, multicenter, and observational study. Patients were included from nine participating centers in The Netherlands from July 2002 until September 2005. Inclusion criteria were: biochemical hyperthyroidism (thyrotrophin-stimulating hormone (TSH) < 0.4 mU/l, free thyroxine (fT<sub>4</sub>) > 23 pmol/l and/or triiodothyronine (T<sub>3</sub>) > 2.7 nmol/l) and a diffuse homogeneous uptake on thyroid scintigraphy (<sup>99m</sup>Tc-pertechnetate). Exclusion criteria were: relapse of Graves' disease; no written informed consent; no understanding of the Dutch language, and serious alcohol or i.v. drugs abuse. Clinical parameters at baseline like sex, age, goiter size (classification by means of WHO 1960 criteria) (8), clinical thyrotoxic score (HSS-score) and the existence of pretibial myxedema and Graves' orbitopathy, both diagnosed by inspection were recorded. Participants filled in the hyperthyroid symptom scale questionnaire (HSS-score) (9) which quantitatively measures clinical severity of hyperthyroidism through 10 items on 0- to 4-point subscales, including sweating, heat intolerance, nervousness, hyperactivity, tremor, weakness, hyperdynamic precordium, diarrhea, appetite, and degree of incapacitation. Venous blood samples were taken for thyroid hormone and antibody measurements before treatment was started. The participants were asked about the duration of symptoms (interval between start of symptoms and date of referral) and family history for AITDs. Furthermore, participants were asked to complete questionnaires asking about environmental factors (i.e. use of estrogens, smoking habits, exposure to iodine excess, pregnancies, and stress at time of diagnosis as published recently) (10). We ascertained the autoimmune nature of thyroid disease in affected relatives after having obtained their written consent via our patients. Family history scores (FHS) were made for all patients with a positive family history for AITD. Patients were scored according to the number and degree of affected relatives. Identical twins scored 1.0,

first degree relatives scored 0.5, second degree relatives scored 0.25, third degree relatives scored 0.125, and fourth degree relatives scored 0.0625. Patients with more than one affected relative received the cumulative score of all relatives. Of the 263 subjects who were included in this study, ten noticed that they had no information whatsoever about diseases in their family and two subjects did not complete the questionnaires. This left 251 subjects who were eligible for the present investigation.

The study was approved by the Ethics Committee of the Academic Medical Center of Amsterdam and the eight other participating centers. All patients gave written informed consent.

### Laboratory measurements

Non-fasting venous blood samples were taken for thyroid hormone and autoantibody measurements and were stored at –20 °C until assay. Serum T<sub>3</sub> and T<sub>4</sub> were measured with in-house RIA (11). T<sub>3</sub>-uptake was determined by a no-extraction, solid-phase <sup>125</sup>I RIA (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA, USA). FT<sub>4</sub>-index and fT<sub>3</sub>-index were calculated by multiplying T<sub>3</sub>-uptake with T<sub>4</sub> and T<sub>3</sub> respectively (reference ranges 70–140; 1.0–3.0 respectively). Serum TSH was determined with a fluoroimmuno-metric assay (Delfia hTSH, Perkin Elmer, Turku, Finland; detection limit 0.01 mU/l, reference range 0.4–4.0 mU/l). Serum thyrotropin-binding inhibitory Ig (TBII) was determined by a second generation luminescence receptor assay (TRAK human LIA, BRAHMS, Berlin, Germany; detection limit 1.0 IU/l, reference range < 2.0 IU/l). Autoantibodies against thyroid peroxidase (TPO-Ab) were analyzed by anti-TPO LIA (BRAHMS; detection limit 30 U/l, reference range < 60 kU/l). All measurements were performed at the laboratory of the Academic Medical Center of Amsterdam.

### Statistical analysis

To analyze the association between the duration of symptoms and phenotypic appearance of Graves' disease, patients were subdivided into five groups: ≤ 2 months (n = 80), 3–4 months (n = 57), 5–6 months (n = 50), 7–11 months (n = 28), and ≥ 12 months (n = 36). To analyze the influence of family history, patients were subdivided into five groups according to their FHS; 0.00 (no positive family history; n = 144), 0.06–0.49 (n = 19), 0.50–0.99 (n = 36), 1.00 (n = 32), and > 1.00 (n = 18). The one-way ANOVA and Kruskal–Wallis tests were used for comparison between means and medians of different subgroups respectively. Regression analyses were performed between the five subgroups and serum fT<sub>3</sub>-index, fT<sub>4</sub>-index, and HSS-score. Trend analyses between the five subgroups for duration of symptoms and age, serum TSH, serum TBII,

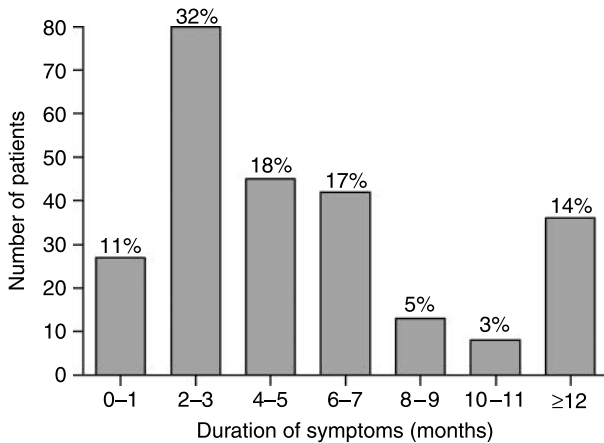
serum TPO-Ab, smoking pack years, and stress scores were performed using the Jonckheere–Terpstra test. The association between different subgroups and sex, goiter size, prevalence of Graves' orbitopathy, and pretibial myxedema, smoking behavior, iodine excess, estrogen use, and number of pregnancies was determined using  $\chi^2$  tests.

P values <0.05 indicated statistical significance. All statistical analyses were carried out using the SPSS statistical package for Windows, version 15.0. (SPSS Inc., Chicago, IL, USA).

**Results**

Figure 1 shows the frequency histogram of the duration of thyrotoxic symptoms before referral in 251 patients with newly diagnosed GH. The majority of patients (61%) had symptoms for <6 months. The median duration of symptoms until diagnosis upon referral was 4 months. The peak incidence for the diagnosis of GH is at 2–3 months after onset of symptoms (32% of patients). In 14% of patients, symptoms lasted for  $\geq 12$  months until diagnosis was made.

Table 1 shows the duration of symptoms as determinant for phenotypic appearance of Graves' disease at time of diagnosis. Duration of symptoms was negatively associated with age (the shorter the duration, the older the age; P for trend=0.04), and positively with TPO antibodies and goiter size (the longer the duration, the higher the TPO-Ab concentration and the greater the goiter size; P for trend=0.04 and 0.05 respectively). No associations were observed with sex, severity of hyperthyroidism, and extrathyroidal manifestations of Graves' disease (Table 1), nor with any of the environmental factors (i.e. estrogen use, pregnancies, smoking habits, exposure to iodine excess, and stress scores; data not shown).



**Figure 1** Frequency histogram for the duration of thyrotoxic symptoms before referral in 251 newly diagnosed patients with Graves' hyperthyroidism.

**Table 1** Duration of symptoms as determinant for phenotypic appearance of Graves' disease in 251 newly diagnosed patients with Graves' hyperthyroidism.

	0-2 months (n=80)	3-4 months (n=57)	5-6 months (n=50)	7-11 months (n=28)	$\geq 12$ months (n=36)	P value (trend)
Male gender	30.0 (%)	19.3 (%)	26.0 (%)	17.9 (%)	25.0 (%)	0.38
Age (years)	45.5 (39.0-52.0)	43.0 (30.5-50.0)	37.5 (29.0-51.5)	36.0 (29.0-45.5)	41.5 (39.0-52.0)	0.04
Positive family history	45 (%)	44 (%)	44 (%)	48 (%)	31 (%)	0.30
HSS-score	14.3 (6.7)	16.0 (7.3)	15.3 (7.1)	14.2 (6.0)	14.9 (6.7)	0.89
TSH (mU/l)	<0.01 (<0.01-<0.01)	<0.01 (<0.01-<0.01)	<0.01 (<0.01-<0.01)	<0.01 (<0.01-<0.01)	<0.01 (<0.01-<0.01)	0.45
Free T <sub>3</sub> -index	7.1 (4.4)	6.7 (2.9)	7.7 (3.1)	5.9 (2.6)	7.4 (4.4)	0.89
Free T <sub>4</sub> -index	334 (122)	320 (107)	368 (124)	300 (108)	365 (122)	0.34
TBII (IU/l)	8.7 (4.2-15.7)	8.6 (4.2-19.2)	12.7 (5.8-26.4)	9.2 (5.5-12.7)	8.2 (4.2-15.7)	0.39
TPO-Ab (kU/l)	205 (40-1622)	520 (40-2630)	590 (40->3000)	730 (50->3000)	910 (40-1622)	0.04
Goitersize						0.05
0	69 (%)	67 (%)	55 (%)	68 (%)	50 (%)	
I	19 (%)	16 (%)	22 (%)	11 (%)	25 (%)	
II	8 (%)	18 (%)	16 (%)	21 (%)	17 (%)	
III	5 (%)	0 (%)	6 (%)	0 (%)	8 (%)	
Graves' orbitopathy	19 (%)	19 (%)	12 (%)	21 (%)	28 (%)	0.40
Pretibial myxedema	5 (%)	4 (%)	6 (%)	0 (%)	8 (%)	0.74

Values for age, TSH, TBII and TPO-Ab are in median + interquartile range (range between the 25th and the 75th percentiles), values for HSS-score, free T<sub>3</sub>-index and free T<sub>4</sub>-index are given in mean + s.d.

**Table 2** Family history score as determinant for the phenotypic appearance in 249 newly diagnosed patients with Graves' hyperthyroidism.

Family history score (FHS):	0.00 (n=144)	0.06–0.49 (n=19)	0.50–0.99 (n=36)	1.00 (n=32)	> 1.00 (n=18)	P value*	P value <sup>†</sup> vs > 1.00 <sup>†</sup>
Male gender	29.9 (%)	10.5 (%)	16.7 (%)	12.5 (%)	38.9 (%)	0.04	0.15
Age (years)	43.0 (34.0–50.0)	48.0 (36.0–54.0)	43.0 (29.0–52.8)	46.0 (35.2–55.0)	35.0 (39.0–52.0)	0.06	<0.02
Duration of symptoms (months)	4.0 (2.0–7.8)	3.0 (2.0–4.0)	5.0 (3.0–8.0)	4.5 (2.0–7.2)	3.5 (%)	0.30	0.81
HSS-score	13.9 (6.8)	14.2 (5.1)	16.6 (7.5)	15.8 (7.4)	15.7 (6.7)	0.21	0.54
TSH (mU/l)	<0.01 (<0.01–<0.01)	<0.01 (<0.01–<0.01)	<0.01 (<0.01–<0.01)	<0.01 (<0.01–<0.01)	<0.01 (<0.01–<0.01)	0.99	0.77
Free T <sub>3</sub> -index	6.9 (4.0)	5.8 (1.8)	6.7 (2.3)	8.1 (4.4)	7.1 (4.4)	0.33	0.83
Free T <sub>4</sub> -index	336 (132)	329 (83)	315 (93)	367 (152)	325 (122)	0.61	0.70
TBII (IU/l)	8.9 (4.5–18.8)	7.2 (3.9–14.4)	11.3 (6.9–21.4)	8.2 (6.5–19.0)	8.4 (4.2–15.7)	0.35	0.56
TPOAb (kU/l)	395 (40–2798)	240 (40–540)	630 (102–2940)	940 (82–>3000)	940 (40–1622)	0.36	0.51
Goitersize						0.64	0.95
0	64 (%)	74 (%)	56 (%)	62 (%)	61 (%)		
I	18 (%)	21 (%)	28 (%)	12 (%)	17 (%)		
II	15 (%)	5 (%)	11 (%)	16 (%)	17 (%)		
III	2 (%)	0 (%)	6 (%)	0 (%)	6 (%)		
Graves' orbitopathy	22 (%)	5 (%)	19 (%)	19 (%)	6 (%)	0.28	0.14
Pretibial myxedema	4 (%)	0 (%)	11 (%)	0 (%)	11 (%)	0.08	0.15

Family history score: score according to the number and degree of AITD affected relatives. Identical twins scored 1.00, first degree relatives scored 0.50, second degree relatives scored 0.25, third degree relatives scored 0.125, and fourth degree relatives scored 0.0625. Patients with more than one affected relative received the cumulative score of all relatives. Values for age, TSH, TBII and TPO-Ab are in median + interquartile range (range between the 25th and the 75th percentiles), values for HSS-score, free T<sub>3</sub>-index and free T<sub>4</sub>-index are given in mean ± s.d. \* P values for analyses between all groups; † P values for analyses between FHS > 1.00 and ≤ 1.00.

A positive family history for AITD was present in 107 (42.6%) of the 251 patients. Two out of the 107 patients with a positive family history did not report the degree (first, second, third or fourth) of affected relatives. The remaining 105 patients were subdivided into four groups according their FHS (Table 2). Analyses of the different subgroups for FHS showed a difference in gender ratio ( $P=0.04$ ). Furthermore, we found a non-significant difference in age at onset between the different subgroups ( $P=0.06$ ). Interestingly, patients in the group with the highest FHS ( $>1.00$ ), were significantly younger ( $P=0.02$ ) compared with all patients with lower FHS (Table 2). The five groups did not differ from each other in exposure to environmental factors. No significant trends were found between FHS and thyroid function tests or thyroid antibody concentrations.

## Discussion

The first part of our study dealt with the duration of thyrotoxic symptoms until GH was diagnosed at referral. Quantitative data on this subject are scarce in the literature, but the general clinical experience is that Graves' disease develops over months rather than years. In line with this notion is the almost complete absence of TBII (the immediate cause of Graves' disease) in serum of healthy people, even in high-risk populations such as healthy relatives of AITD patients (12), and the occurrence of TBII in serum when Graves' disease manifests itself clinically. Our results substantiate these clinical notions: median duration of symptoms until diagnosis is 4 months. Graves' disease thus indeed develops rather rapidly over months, in contrast to Hashimoto's disease that develops slowly over years.

An unexpected finding was the inverse relationship between duration of thyrotoxic symptoms and age; the shorter the duration, the older the patients were. In view of the less severe hyperthyroidism in elderly patients with Graves' disease (2–4), we would have expected the opposite. The finding cannot be explained by earlier recognition of symptoms as being caused by hyperthyroidism (which might be the case in recurrent GH), because only patients with a first episode of Graves' disease had been included. It might be that advancing age is associated with greater concern on the significance of symptoms and on health in general, leading to earlier consultation. Longer duration of symptoms until diagnosis was associated with greater goiter size, which can be understood from the longer exposure time to TBII in view of the thyroid growth promoting effect of TBII (13, 14). Longer duration of symptoms was also related to higher TPO-Ab concentrations, tentatively explained from epitope spreading after the initial immune reaction against the TSH receptor (15). Consequently, we had to reject our initial hypothesis that a very short duration of symptoms might be related

to very severe hyperthyroidism. Clinical and biochemical severity of GH were not associated with duration of symptoms.

The second part of our study dealt with the significance of a positive family history of AITD in patients with GH. We observed a positive family history in 42.6% of our patients, a figure in good agreement with a frequency of around 50% reported in literature (4, 7). It is intriguing why this figure is not higher because twin studies have shown that around 79% of the susceptibility to develop Graves' disease can be attributed to genetic factors (1). Does it mean that genetic susceptibility for Graves' disease is lower in patients with a negative family history? To answer this question, one should compare the frequency of polymorphisms in susceptible genes (like *HLA-DR*, *CTLA4*, *PTPN22*, *CD40*, and *TSH-R*) between patients with and without a positive family history for AITD, but such a study has not been done. Could it be that environmental factors play a greater role in the development of Graves' disease in patients without a family history of AITD? Our data do not support this proposition, because exposure to environmental factors (like smoking, estrogen use, pregnancy, iodine excess, and stress) were not different between patients with a high or low FHS (data not shown).

We found some supporting evidence for our hypothesis that genetic anticipation might occur in familial cases of Graves' disease. When taking into account the number of affected relatives and the degree of kinship, the age at diagnosis was much lower in the group with the highest FHS. The difference in median age at onset between the group with the highest FHS and the lower ones varied between 8 and 13 years (Table 2). This interesting observation is in agreement with the scarce literature about genetic anticipation in Graves' disease. Brix *et al.* investigated age at diagnosis in 33 same-gender parent–offspring pairs with Graves' disease from multiple affected families and found that mean age at diagnosis in children was 12.5 years lower than in their parents (16). Manji *et al.* found that reporting of a relative with thyroid dysfunction was associated with a lower median age at diagnosis of Graves' disease (in women, 38 vs 43 years in patients with no family history, and in men 42 vs 47 respectively) (4). A lower median age in view of genetic anticipation is biologically plausible. Manji *et al.* also observed that the greater the number of affected relatives, the greater the effect (4). A limitation of our data is that we did not correct for family size and that there might be ignorance about the existence of family members with AITD, especially patients who do not have contact with their relatives (i.e. moved abroad, deceased etc).

Genetic anticipation in our study was not associated with more severe GH or greater exposure to environmental insults.

In summary, our study demonstrates that a short duration of thyrotoxic symptoms until diagnosis is not related to biochemically more severe hyperthyroidism in Graves' disease. We have furthermore obtained evidence for genetic anticipation in Graves' disease by means of a lower age of onset in the group with the highest FHS.

### Declaration of interest

All authors declare that they do not have any financial or other potential conflict of interest, and that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

### Funding

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

### Acknowledgements

We gratefully thank the participating physicians for their collaboration in this study: Dr A B Arntzenius (Spaarne Ziekenhuis, Hoofddorp), Dr C B Brouwer (Onze Lieve Vrouwe Gasthuis, Amsterdam), Dr J Derksen (Medisch Spectrum Twente, Enschede), Dr M J M Diekman and Dr M N Gerding (Ziekenhuis Deventer, Deventer), Dr V E A Gerdes (Slotervaartziekenhuis, Amsterdam), Dr W E de Graaff (Tergooiziekenhuizen, Blaricum), Dr R Heijligenberg (Ziekenhuis Gelderse Vallei, Ede), and Dr A F Muller (Diakonessenhuis, Utrecht). We would like to thank Ms E M Johannesma for all laboratory measurements.

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Received 5 April 2009

Accepted 19 April 2009