

Current views on the etiopathogenesis of thyroid ophthalmopathy

KATARZYNA ŁĄCKA¹, JOANNA SZARZYŃSKA², JAN K. ŁĄCKI^{3,4}

¹Department of Endocrinology, Metabolism and Internal Disease, Poznan University of Medical Sciences, Poznan, Poland; ²Unit of Internal Medicine, City Hospital of Poznan, Poland; ³Department of Rheumatology, Institute of Rheumatology, Warsaw, Poland; ⁴Department of Rheumatology and Clinical Immunology, Poznan University of Medical Sciences, Poznan, Poland

Abstract

Thyroid associated ophthalmopathy (TAO) is an autoimmune disorder often coexisting with Graves' disease, where clinical symptoms result from the inflammatory reactions occurring in retroorbital tissue. The mechanisms responsible for disease evolution have not been precisely recognized. Infectious, environmental, immune and genetic factors are likely to take part in the development of the disease. Recently, intensive research of cytokines influence on thyroid cells and retroorbital tissue have been undertaken. The cytokines group which takes part in TAO pathogenesis is very large, but the role of each individual cytokine is not yet fully known. The gene polymorphisms of these cytokines are also being investigated. The results of the studies obtained so far are not explicit. However, it can be expected that in the near future a cytokines genes polymorphism estimation in patients with thyroid associated ophthalmopathy and Graves' disease will be a helpful means to choose the treatment method and to establish the prognosis.

Key words: thyroid associated ophthalmopathy, inflammatory process, cytokines, gene polymorphism

(Centr Eur J Immunol 2004; 29 (3-4): 94-99)

Introduction

Graves' disease (GD) is a heterogeneous autoimmune disorder affecting the thyroid, eyes and skin. Based on epidemiological data from the Mayo Clinic, USA, annually in 45/100000 people, a new incident is confirmed. In this group 37 were women. This disease concerns women more frequently (women: men relation was 5:1); the mean age at which the disease was diagnosed was 20–40 years. In Poland the morbidity of Graves – Basedow disease is estimated as 1/25 000 persons/year. In children it is twenty times less frequent.

While some ocular symptoms may be shown in almost all patients with GD, clinically ophthalmopathy occurs in only 10% of patients. The eye changes of thyroid associated ophthalmopathy (TAO) range from mild to very severe and can include sight loss or persistent diplopia. Over 90% occurs in GD, about 5% in Hashimoto disease. The isolated form appears in 2-5% of cases.

TAO (or TED – thyroid eye disease, TAO – thyroid associated ophthalmopathy or thyroid associated orbitopathy) is an inflammatory disease of the orbital tissues. The effects of inflammation, mediated through cytokine release, include the proliferation of fibroblasts, an increased deposition of the extracellular matrix, and adipocyte differentiation and proliferation. As a result, edema, an enlargement of the extraocular muscles and an increased volume of the orbital soft tissues occur with exophthalmus. About 5% of patients with TAO have a compression of the optic nerve. These patients may present the following symptoms: damage of a central vision, defect of colour distinction, loss in a field of vision, damage of a papillary reflex.

Oedema, inflammation and late fibrosis account for the decreased function of the extraocular muscles, despite relative by intact fibers.

Correspondence: Ass. Prof. Katarzyna Łącka, MD, Department of Endocrinology, Metabolism and Internal Medicine, Poznan University of Medical Sciences, Przybyszewskiego 49, 60-355 Poznan, e-mail: K_Lacka@wp.pl

Clinical symptoms and epidemiology

Most patients with Graves' disease have ocular symptoms, but only a small percent develop an active form. The changes may concern one or both eye sockets. Ophthalmopathy features are present in 10% of patients with Graves' disease [1]. Research performed in Minnesota showed a higher incidence of the disease in females; for females it was 16/100,000/year, whereas for males, 2.9/100,000/year. Two age peaks of incidence were observed: in the female population the ages of 40-44 years and 60-64 years, and five years later in the male population. A correlation between the risk of severe ophthalmopathy and age and sex was also defined. Males of an advanced age present more intensive symptoms [2].

The occurrence and intensity of eye involvement is different among patients. The most frequent clinical symptom is eyelid retraction. The full symptomatic form of Graves' ophthalmopathy occurs relatively rarely. In the early development phase only functional changes occur: eyelid retraction, exophthalmos, Graefe, Kocher, Dalrymple, Joffroy, Stellwag' symptoms. In the subsequent period a dysfunction of the optic organ sets in: an aggravation of the extraocular muscle function (Moebius' symptom), diplopia, corneal damage and vision loss. An enlargement of eye muscle volume and optic nerve compression are responsible for most of the above-mentioned symptoms.

To estimate the changes in orbital tissues various classifications were implemented, taking into account the severity of clinical signs inflammatory processes. One of the first was Werner's scale, established in 1969, and in use up till the 1980's, which let us evaluate the progression of eye lesions [3]. NO SPECS classification includes the intensity of clinical symptoms from no symptoms, through eyelid retraction, exophthalmos, soft tissue infiltration and eye muscle involvement, to vision loss due to optic nerve compression. At present, it is the most often used scale elaborated by the American Thyroid Association [4, 5]. It offers 6 progression classes of eye changes. The first class is eyelid retraction. The second class includes eyelid and conjunctival oedema and lacrimation. The third class includes exophthalmos. The fourth class involves extraocular muscle volume changes and their results: movement reduction, diplopia. The fifth class involves corneal damage. The sixth class is optic nerve destruction and visual disturbances.

Besides clinical classifications, examinations allowing for the visualization of orbits contents is invaluable in the identification of changes progression. Particularly helpful are: B-mode ultrasonography, computed tomography and magnetic resonance [6-8]. They provide an estimation of the progress of the eye muscle and retroorbital tissue infiltration, particularly their volume measurement and optic nerve compression.

Pathogenesis

So far, the mechanisms leading to ophthalmopathy extension, have not been precisely recognized. Environmental, immune and genetic factors are being investigated. Some papers report the influence of infectious factors (congenital rubella, retroviruses, *Yersinia enterocolitica* [YE]), which reveal an antigen similarity with the thyroid-stimulating hormone-receptor (TSH-receptor). Infection can induce cross reactions of antibodies with self-antigens, lymphocytes T activation and influence MHC-antigens expression on thyroid cells [9]. The relationship between thyroid receptor antibodies (TRAb) and antibodies against different serotypes *Yersinia Enterocolitica*, O:3, O:5, O:8, O:9 in patients with AITD (Autoimmune Thyroid Diseases) was investigated. The levels of bacterial antibodies and TRAb were higher in patients with Graves' disease and Hashimoto' disease. Therefore, an infection can play a role in the etiology of these diseases [10]. It was also discovered that cross reactions are induced through antibodies anti epitopes with YE membrane and plasmids proteins (YOPs), which react with the outer membrane domain of the TSH-receptor (TSHr), because it shows homologies with YE antigens [11].

Another modifiable factor which has an influence on the occurrence and progress of the disease is cigarette smoking. There is a higher prevalence and an ophthalmopathy intensity in smokers. Much research has been done to prove that cigarette smoking is a risk factor of Graves' disease. There is no such correlation among patients who used to smoke in the past. The ophthalmopathy risk is much higher in smokers and also involves the patients who had been smoking in the past [12]. Smoking increases cellular hypoxia and the progression of pathological changes, at the same time decreasing the effectiveness of both orbital radiotherapy and glucocorticoid treatment [13, 14].

Environmental factors affect T-suppressor lymphocytes impairment that is related to the lack of control of activated Th2 lymphocytes, reactive with organism cells. Once the autoimmune process is initiated, it is never interrupted, with reactions between antigen presenting cells and thyrocytes inducing antibody production against thyroid cells [15]. It is known that in autoimmune thyroid diseases antibodies against several elements of the thyroid cells, TSH-receptor (antibodies enhanced and inhibited receptor activity), thyroid peroxidase (TPO) and thyroglobulin (TG), are present in the organism.

The orbital symptoms mentioned earlier were thought to be related to a depressed interaction between T cells, matrix' proteins and the inflammatory process. Lymphocytes circulating in blood, which recognize specified antigens in thyroid tissue, also recognize epitops on cells retroorbital tissue – fibroblasts and preadipocytes (cross reaction). Some so far unknown factors may stimulate immature cells to differentiation into adipocytes, which demonstrates an accelerated expression for the TSH-receptor [16]. Cytokines produced by lymphocytes may induce fibroblasts

Research mainly concerned the expression of these compounds in blood, the thyroid gland and retroorbital tissue. IL-1 together with TNF and IFN gamma moderate the thyrocyte function. IL-1 induced NO and cGMP are released from thyroid cells, at the same time inhibiting the release of TG and cAMP [19]. Receptors for TNF alpha were found on thyroid follicular cells; together with TNF alpha they participated in cytotoxic processes, characteristic for gland destruction in autoimmune diseases. In Graves' disease and the auto- and non-autoimmune hypothyreosis, the concentration of TNF alpha and sTNFR-1 was elevated. The normalization of the thyroid function through L-thyroxine therapy in hypothyreosis is related to a decrease in TNF alpha and sTNFR-1 levels; no such influence is visible on the concentration of these parameters in hypothyreosis [22]. The influence of IL-2 on T suppressor cells activation in AITD was also investigated. In the case of IL-2 absent, T suppressor lymphocytes showed lower activity when HLA-DR expression was analyzed. The activation of lymphocytes by IL-2 was reduced, too [30]. It was suggested that the thyroid gland may itself participate in the inflammatory process in autoimmune diseases through IL-1, IL-6, IL-8 release. mRNA expression of these factors in thyroid follicular cells was investigated. A stimulation of these cells by IL-1 gave rise to an increase in IL-1, IL-6, IL-8 mRNAs levels. IFN gamma did not have such an influence, but its elevated concentration caused IL-6 mRNA rise. TSH increased IL-1 mRNA concentration [31]. A tendency of IL-6 to increase was also observed in patients with ophthalmopathy, the higher, the longer the disease persisted. The association between IL-6 level and the severity of the inflammatory process in retroorbital tissues is visible. Euthyreosis and thyroid inflammation shows no influence on IL-6 level in serum. Pharmacological treatment or radiotherapy does not decrease IL-6 concentration [29].

IL-10 is likely to be an important mediator in stimulating antithyroid antibodies production; moreover, it may contribute to inhibiting thyroid cells destruction in Graves' disease [32]. Not only does IL-10 repress cellular immunity, it also stimulates the humoral response. Higher levels of IL-10 in autoimmune diseases were found. Simultaneously, B, Th1 and Th2 lymphocytes number as well as MHC class II expression on thyrocytes was higher. IL-10 is able to influence B lymphocytes stimulation and antibodies production [27].

IL-1, IFN gamma, TNF alpha affect adhesion molecules (ICAM-1) and LFA-1 expression in retroorbital tissue. The adhesion molecules presence is one of the characteristic features of active ophthalmopathy. An elevated expression of ICAM-1, ELAM-1, VCAM-1 correlates with the early disease phase and decreases with time [33]. sICAM-1 concentration falls with treatment, together with an improvement of orbital changes and reaching euthyreosis [34]. A LFA-3 and ICAM-1 expression increase was found in fibroblasts, connective tissue surrounding extraorbital

muscles and blood-vessels of retrobulbar tissue in ophthalmopathy. It is characteristic for the healthy population that adhesion molecules are in a very low concentration or are undetectable [35].

Genetic factors

Research of the HLA, CTLA4, TNF alpha and TSHR genes revealed a poor relationship of HLA and CTLA4 genes with ophthalmopathy, which is a relative risk for thyroid associated ophthalmopathy in patients with Graves' disease. However, research indicates environmental factors to be more important than genes in ophthalmopathy development [36].

The HLA region has for long been the main concern for investigators. Research of the HLA role in AITD in the Caucasian population suggests the HLA region not to be the main gene in AITD expression, even in HLA-DR3 positive families. It may, however, be a modulating gene. A higher susceptibility to GD in patients of Caucasian origin possessing HLA-DR3 antigen, was detected [37].

Research of genes polymorphisms, IL-1 alpha, IL-1 beta, IL-1R antagonist, IL-1R, IL-4, IL-4R, IL-6, IL-10 and TGF beta in patients with AITD, revealed a reduced frequency of T allele variant in IL-4 promotor in this group of patients that is reflected in the rarer presence of the heterozygotic genotype. Significant differences in polymorphisms frequency of the remaining investigated cytokines were not observed. The results may suggest a protective effect of this IL-4 variant in autoimmune thyroid diseases, particularly in GD [38].

Numerous studies performed by many centers report that CTLA4 gene polymorphism and G allele presence is significantly more frequent in GD sufferers [36, 39-51].

A TNF alpha gene polymorphism may constitute one of the factors leading to ophthalmopathy in Graves' disease. In the Japanese population TNF alpha polymorphism is more frequent in AITD, but the difference is not statistically significant. The rate of the polymorphism -1031C association is increased with the severity of TAO. The phenotype frequency of DRB1*0901(-)/TNF alpha -1031C(+) significantly increases in patients with GD and ophthalmopathy. -1031C and -863A alleles predispose Japanese patients with Graves' disease to thyroid associated ophthalmopathy [52]. The opinion prevails that single genes and their polymorphism show a modulating effect in TAO, and they are more likely to predispose people to GD together with other genes than if acting alone. The research findings suggest the presence of different loci predisposing to AITD in different ethnic groups.

Conclusion

Though the identification of genes which are responsible for thyroid diseases has not been successful, current intensive

research of such genes and cytokines gene polymorphisms may facilitate the choice of the most optimal therapy. Such research, having been performed for a few years all over the world, has just started in Poland. It seems possible that the estimation of cytokines gene polymorphism in Polish patients with Graves' disease and thyroid associated ophthalmopathy will be helpful in finding a response to the question whether the occurrence of possible polymorphisms affects treatment results and patients' susceptibility to pharmacological, including steroid, therapy. By establishing a correlation between polymorphism and environmental factors, it might be possible to judge if such research could influence the choice of treatment. If a relation was found between cytokine polymorphism and the form and course of the disease, the application of directed anticytokine treatment could be possible in individual cases, unresponsive to other forms of therapy, and the treatment choice would be made likely thanks to gene polymorphism investigations.

References

- Bartley GB, Fatourehchi V, Kadrmaz EF, et al. (1995): The incidence of Graves' ophthalmopathy in Olmsted County, Minnesota. *Am J Ophthalmol* 120: 511-517.
- Perros P, Crombie AL, Matthews JNS, Kendall-Taylor P (1993): Age and gender influence the severity of thyroid-associated ophthalmopathy: a study of 101 patients attending a combined thyroid-eye clinic. *Clin Endocrinol (Oxf)* 38 (4): 367-372.
- Werner SC (1969): Classification of the eye changes of Grave's disease. *J Clin Endocrinol Metab* 29: 982-984.
- Werner SC (1977): Modification of the classification of the eye changes of Graves' disease: recommendations of the Ad Hoc Committee of the American Thyroid Association. *J Clin Endocrinol Metab* 44: 203-204.
- Classification of eye changes of Graves' disease (1992): *Thyroid* 2 (3): 235-236.
- Hallin ES, Feldon SE (1988): Graves' ophthalmopathy: I. Simple CT estimates of extraocular muscle volume. *Br J Ophthalmol* 72 (9): 674-677.
- Yamamoto K, Itoh K, Yoshida S, et al. (1979): A quantitative analysis of orbital soft tissue in Graves' disease based on B-mode ultrasonography. *Endocrinol Jpn* 26 (2): 255-261.
- Wing SD, Hunsaker JN, Anderson RE, et al. (1979): Direct sagittal computed tomography in Graves' ophthalmopathy. *J Comput Assist Tomogr* 3 (6): 820-824.
- Tomer Y, Davies TF (1993): Infection, thyroid disease, and autoimmunity. *Endocr Rev* 14 (1): 107-120.
- Corapcioglu D, Tonyukuk V, Kiyani M, Yilmaz AE, Emral R, Kamel N, Erdogan G (2002): Relationship between thyroid autoimmunity and Yersinia enterocolitica antibodies. *Thyroid* 12 (7): 613-617.
- Wenzel BE, Peters A, Zubashev I (1996): Bacterial virulence antigens and the pathogenesis of autoimmune thyroid diseases (AITD). *Exp Clin Endocrinol Diabetes* 104 Suppl 4: 75-78.
- Vestergaard P (2002): Smoking and thyroid disorders – a meta-analysis. *Eur J Endocrinol* 146 (2): 153-161.
- Bartalena L, Marcocci C, Tanda ML, et al. (1998): Cigarette smoking and treatment outcomes in Graves ophthalmopathy. *Ann Intern Med* 129 (8): 632-635.
- Bartalena L, Martino E, Marcocci C, et al. (1989): More on smoking habits and Graves' ophthalmopathy. *J Endocrinol Invest* 12 (10): 733-737.
- Mann K, Gieseler R, Hormann R (1999): Aetiopathogenese des Morbus Basedow. *Z Arztl Fortbild Qualitatssich* 93 (Suppl 1): 29-34.
- Heufelder AE (2000): Pathogenesis of ophthalmopathy in autoimmune thyroid disease. *Rev Endocr Metab Disord* 1 (1-2): 87-95.
- Bednarczuk T, Kiljanski J, Mrowiec T, et al. (1998): T cell interactions with extracellular matrix proteins in patients with thyroid-associated ophthalmopathy. *Autoimmunity* 27 (4): 221-230.
- Hiromatsu Y, Kaku H, Miyake I, et al. (2002): Role of cytokines in the pathogenesis of thyroid-associated ophthalmopathy. *Thyroid* 12 (3): 217-221.
- Rasmussen AK (2000): Cytokine actions on the thyroid gland. *Dan Med Bull* 47 (2): 94-114.
- Wakelkamp IM, Gerding MN, Van Der Meer JW, et al. (2000): Both Th1- and Th2-derived cytokines in serum are elevated in Graves' ophthalmopathy. *Clin Exp Immunol* 121 (3): 453-457.
- Ajjan RA, Watson PF, Weetman AP (1996): Cytokines and thyroid function. *Adv Neuroimmunol* 6 (4): 359-386.
- Diez JJ, Hernanz A, Ma S, et al. (2002): Serum concentrations of tumour necrosis factor-alpha (TNF-alpha) and soluble TNF-alpha receptor p55 in patients with hypothyroidism and hyperthyroidism before and after normalization of thyroid function. *Clin Endocrinol (Oxf)* 57 (4): 515-521.
- Valyasevi RW, Jyonouchi SC, Dutton CM, et al. (2001): Effect of tumor necrosis factor-alpha, interferon-gamma, and transforming growth factor-beta on adipogenesis and expression of thyrotropin receptor in human orbital preadipocyte fibroblasts. *J Clin Endocrinol Metab* 86 (2): 903-908.
- Ban Y, Taniyama M, Tozaki T, et al. (2001): SEL1L microsatellite polymorphism in Japanese patients with autoimmune thyroid diseases. *Thyroid* 11 (4): 335-338.
- Takiyama Y, Miyokawa N, Tokusashi Y, et al. (2002): Thyroid-stimulating hormone induces interleukin-18 gene expression in FRTL-5 cells: immunohistochemical detection of interleukin-18 in autoimmune thyroid disease. *Thyroid* 12 (11): 935-943.
- de la Vega JR, Vilaplana JC, Biro A, et al. (1998): IL-10 expression in thyroid glands: protective or harmful role against thyroid autoimmunity? *Clin Exp Immunol* 113 (1): 126-135.
- Ajjan RA, Watson PF, Weetman AP (1997): Detection of IL-12, IL-13, and IL-15 messenger ribonucleic acid in the thyroid of patients with autoimmune thyroid disease. *J Clin Endocrinol Metab* 82 (2): 666-669.
- Molnar I, Balazs C (1997): High circulating IL-6 level in Graves' ophthalmopathy. *Autoimmunity* 25 (2): 91-96.
- Paschke R, Schuppert F, Taton M, Velu T (1994): Intrathyroidal cytokine gene expression profiles in autoimmune thyroiditis. *J Endocrinol* 141 (2): 309-315.
- Yoshikawa N, Morita T, Arreaza G, et al. (1995): The effect of interleukin-2 on suppressor T lymphocytes in autoimmune thyroid disease. *Clin Invest Med* 18 (2): 91-98.
- Watson PF, Pickerill AP, Davies R, Weetman AP (1995): Semi-quantitative analysis of interleukin-1 alpha, interleukin-6 and interleukin-8 mRNA expression by human thyrocytes. *J Mol Endocrinol* 15 (1): 11-21.
- Watson PF, Pickerill AP, Davies R, Weetman AP (1994): Analysis of cytokine gene expression in Graves' disease and multinodular goiter. *J Clin Endocrinol Metab* 79 (2): 355-360.

33. Pappa A, Calder V, Fells P, Lightman S (1997): Adhesion molecule expression in vivo on extraocular muscles (EOM) in thyroid-associated ophthalmopathy (TAO). *Clin Exp Immunol* 108 (2): 309-313.
34. Kulig G, Pilarska K, Kulig J, et al. (2002): Usefulness of soluble ICAM-1 measurements for the evaluation of the disease activity and efficiency of therapy in patients with infiltrative Graves' ophthalmopathy. *Pol Arch Med Wewn* 108 (6): 1161-1169.
35. Heufelder AE, Bahn RS (1993): Elevated expression in situ of selectin and immunoglobulin superfamily type adhesion molecules in retroocular connective tissues from patients with Graves' ophthalmopathy. *Clin Exp Immunol* 91 (3): 381-389.
36. Villanueva R, Inzerillo AM, Tomer Y, et al. (2000): Limited genetic susceptibility to severe Graves' ophthalmopathy: no role for CTLA-4 but evidence for an environmental etiology. *Thyroid* 10 (9): 791-798.
37. Ban Y, Davies TF, Greenberg DA, et al. (2002): The influence of human leucocyte antigen (HLA) genes on autoimmune thyroid disease (AITD): results of studies in HLA-DR3 positive AITD families. *Clin Endocrinol (Oxf)* 57 (1): 81-88.
38. Hunt PJ, Marshall SE, Weetman AP, et al. (2000): Cytokine gene polymorphisms in autoimmune thyroid disease. *J Clin Endocrinol Metab* 85 (5): 1984-1988.
39. Chistyakov DA, Savost'anov KV, Turakulov RI, et al. (2000): Complex association analysis of Graves' disease using a set of polymorphic markers. *Mol Genet Metab* 70 (3): 214-218.
40. Kouki T, Sawai Y, Gardine CA, et al. (2000): CTLA-4 gene polymorphism at position 49 in exon 1 reduces the inhibitory function of CTLA-4 and contributes to the pathogenesis of Graves' disease. *J Immunol* 165 (11): 6606-6611.
41. Vaidya B, Imrie H, Perros P, et al. (1999): The cytotoxic T lymphocyte antigen-4 is a major Graves' disease locus. *Hum Mol Genet* 8 (7): 1195-1199.
42. Vaidya B, Imrie H, Perros P, et al. (1999): Cytotoxic T lymphocyte antigen-4 (CTLA-4) gene polymorphism confers susceptibility to thyroid associated orbitopathy. *Lancet* 354 (9180): 743-744.
43. Kotsa K, Watson PF, Weetman AP (1997): A CTLA-4 gene polymorphism is associated with both Graves disease and autoimmune hypothyroidism. *Clin Endocrinol (Oxf)* 46 (5): 551-554.
44. Yanagawa T, Taniyama M, Enomoto S, et al. (1997): CTLA4 gene polymorphism confers susceptibility to Graves' disease in Japanese. *Thyroid* 7 (6): 843-846.
45. Badenhoop K, Donner H, Braun J, et al. (1996): Genetic markers in diagnosis and prediction of relapse in Graves' disease. *Exp Clin Endocrinol Diabetes* 104 (Suppl 4): 98-100.
46. Yanagawa T, Hidaka Y, Guimaraes V, et al. (1995): CTLA-4 gene polymorphism associated with Graves' disease in a Caucasian population. *J Clin Endocrinol Metab* 80 (1): 41-45.
47. Zaletel K, Krhin B, Gaberscek S, Pirnat E, Hojker S (2002): The influence of the exon 1 polymorphism of the cytotoxic T lymphocyte antigen 4 gene on thyroid antibody production in patients with newly diagnosed Graves' disease. *Thyroid* 12 (5): 373-376.
48. Kouki T, Gardine CA, Yanagawa T, Degroot LJ (2002): Relation of three polymorphisms of the CTLA-4 gene in patients with Graves' disease. *J Endocrinol Invest* 25 (3): 208-213.
49. Mochizuki M, Amemiya S, Kobayashi K, et al. (2003): Association of the CTLA-4 gene 49 A/G polymorphism with type 1 diabetes and autoimmune thyroid disease in Japanese children. *Diabetes Care* 26 (3): 843-847.
50. Einarsdottir E, Soderstrom I, Lofgren-Burstrom A, et al. (2003): The CTLA4 region as a general autoimmunity factor: an extended pedigree provides evidence for synergy with the HLA locus in the etiology of type 1 diabetes mellitus, Hashimoto's thyroiditis and Graves' disease. *Eur J Hum Genet* 11 (1): 81-84.
51. Akamizu T, Sale MM, Rich SS, et al. (2000): Association of autoimmune thyroid disease with microsatellite markers for the thyrotropin receptor gene and CTLA-4 in Japanese patients. *Thyroid* 10 (10): 851-858.
52. Kamizono S, Hiromatsu Y, Seki N, et al. (2000): A polymorphism of the 5' flanking region of tumour necrosis factor alpha gene is associated with thyroid-associated ophthalmopathy in Japanese. *Clin Endocrinol (Oxf)* 52 (6): 759-764.