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Pathogenesis of thyroid eye disease — does autoimmunity against the TSH receptor explain all cases?

Patogeneza orbitopatii tarczycowej — czy reakcja autoimmunologiczna przeciwko receptorowi TSH tłumaczy wszystko?

Jack R. Wall, Hooshang Lahooti

Department of Medicine, the University of Sydney, Australia

Abstract

Thyroid associated ophthalmopathy, or thyroid eye disease (TED), is a complex inflammatory disorder of the eye that, as its name implies, is usually associated with thyroid disease. Clinical observation supports the existence of three main TED subtypes, namely ocular myopathy, congestive myopathy, and mixed congestive and myopathic ophthalmopathy. Although the precise pathophysiology of TED remains unclear, it is likely to reflect an autoimmune reaction involving sensitised T lymphocytes and autoantibodies directed against a specific orbital or thyroid-and-orbital shared antigen(s). One well-studied candidate in this immune reaction is the thyroid-stimulating hormone receptor (TSHR), which is also expressed in the orbital fibroblast and preadipocyte. Most patients with ophthalmopathy have associated Graves' disease, 10% have Hashimoto's thyroiditis in which the eye changes are often mild and expressed mainly as upper eyelid retraction (UER), and 10% have no apparent associated thyroid disease — so-called "euthyroid Graves' disease". Ophthalmopathy can also occur in some patients with transient thyroiditis, thyroid cancer, and Graves' disease many years after treatment of the hyperthyroidism — situations where TSHR antibodies are not expected to be present, suggesting that the relationship between TSHR antibodies and the eye disorder has not been established for all cases. In our studies of TED we have investigated the nature and significance of antibodies targeting other eye muscle and orbital connective tissue (OCT) antigens, in particular the calcium binding protein calsequestrin (CASQ1) and the orbital fibroblast membrane antigen collagen XIII. Our working hypotheses for the pathogenesis of TED are: i) the initial reaction in the orbit is antibody and T lymphocyte targeting of the TSHR in the OCT compartment, and ii) the associated extra ocular and upper eyelid muscle inflammation reflects either autoimmunity against primary skeletal muscle antigens such as CASQ1 or a secondary, non specific effect of the OCT reactions as proposed by the main proponents of the "TSHR hypothesis". Here, we review the evidence that autoimmunity against the TSHR expressed in the orbit can be implicated in the development of all cases of TED. Although there is a close general correlation between ophthalmopathy and TSHR antibodies there are many exceptions, suggesting that the continued study of the possible role of autoimmunity against calsequestrin and collagen XIII is justified. **(Pol J Endocrinol 2010; 61 (2): 222–227)**

Key words: *ophthalmopathy, TSH-receptor, Graves' disease, extra ocular muscle, Hashimoto's thyroiditis, euthyroid Graves' disease, autoimmunity*

Streszczenie

Orbitopatia tarczycowa lub tarczycowa choroba oczu (TED, *thyroid eye disease*) jest złożoną chorobą zapalną oka i jak nazwa wskazuje zwykle towarzyszy chorobom tarczycy. Obserwacje kliniczne potwierdzają istnienie trzech głównych podtypów TED: miopatia oczna, miopatia zastoinowa i mieszana orbitopatia zastoinowa i miopatyczna. Chociaż dokładna patofizjologia TED pozostaje niejasna, prawdopodobnie jest ona wyrazem reakcji autoimmunologicznej z udziałem uczulonych limfocytów T i przeciwciał skierowanych przeciwko swoistym antygenom oczodołu i wspólnemu antygenowi nabłonka pęcherzykowego tarczycy i tkanek oczodołu. Jednym dobrze poznanym powodem tej reakcji jest receptor hormonu stymulującego tarczycę (TSHR, *thyroid-stimulating hormone receptor*), którego ekspresję stwierdzono także na fibroblastach i preadipocytach pobranych z oczodołu osób chorych. Większość pacjentów z orbitopatią choruje także na chorobę Gravesa, 10% na zapalenie tarczycy Hashimoto z łagodnymi zmianami ocznymi wyrażonymi głównie pod postacią retrakcji powieki górnej i 10% nie jest związana z chorobami tarczycy — tak zwana choroba Gravesa z eutyreozą. Orbitopatia może wystąpić także u pacjentów z przejściowym zapaleniem tarczycy, rakiem tarczycy i chorobą Gravesa wiele lat po leczeniu nadczynności — sytuacja, w której nie spodziewa się obecności przeciwciał przeciw TSHR sugeruje, że we wszystkich przypadkach związek między przeciwciałami TSHR i chorobą oczu został wnikliwie zbadany. W niniejszym badaniu nad TED badano znaczenie przeciwciał przeciwko innym antygenom mięśni oka i tkanki łącznej oczodołu (OCT, *orbital connective tissue*), a szczególnie kalsekwestrynie (CASQ1, *calcium binding protein calsequestrin*), białku wiążącemu wapń i antygenowi błony oczodołowych fibroblastów, kolagenowi XIII. Powstały następujące robocze hipotezy patogenezy TED: a) reakcją inicjującą w oczodole jest połączenie przeciwciała i limfocytu T z TSHR w obszarze tkanki łącznej oczodołu; b) towarzyszące zapalenie mięśni zewnątrzoczodołowych i powieki wskazuje na reakcję autoimmunologiczną przeciw, po pierwsze, antygenom mięśni szkieletowych, takim jak CASQ1, lub na nieswoistą reakcję tkanki łącznej oczodołu jak proponuje większość zwolenników „hipotezy TSHR". W prezentowanej pracy przedstawiono dowody na to, że reakcja autoimmunologiczna przeciwko TSHR obserwowana w obrębie oczodołu może być stwierdzona w rozwoju wszystkich przypadków TED. Chociaż istnieje ogólnie bliska korelacja między orbitopatią i przeciwciałami TSHR jest wiele wyjątków sugerujących, że dalsze badania nad potencjalną rolą autoimmunizacji przeciwko kalsekwerynie i kolagenowi XIII są uzasadnione. **(Endokrynol Pol 2010; 61 (2): 222–227)**

Słowa kluczowe: *orbitopatia, receptor TSH, choroba Gravesa, mięśnie zewnątrzoczodołowe, zapalenie tarczycy Hashimoto, choroba Gravesa z eutyrozą, autoimmunizacja*

Jack R. Wall M.D., University of Sydney, Nepean Clinical School, Nepean Hospital, PO Box 63, Penrith NSW 2751, tel.: +61 0 4734 26 13, fax: +61 02 4734 26 14, e-mail: jackw@med.usyd.edu.au

Introduction

Ophthalmopathy is a common manifestation of Graves' disease. The association between "poppy eyes" and goitre was described as early as AD 1000 by the Persian physician and philosopher Avicenna [1]. Ophthalmopathy is most commonly seen in patients with Graves' hyperthyroidism where it is called "Graves' ophthalmopathy" [2, 3]. However, ophthalmopathy also occurs in a small proportion of patients with transient (subacute and silent) thyroiditis [4, 5] and in about a third of patients with progressive (Hashimoto's) thyroiditis [6]. Two other situations where ophthalmopathy is found in the apparent absence of TSHR antibodies are thyroid cancer [7] and Graves' disease many years after treatment of the hyperthyroidism (Wall et al., unpublished observations). In about 10% of cases, the ophthalmopathy occurs in the apparent absence of thyroid autoimmunity, where it is called "euthyroid Graves' disease (EGD)" [2]. Here, we use the generic description of "thyroid eye disease (TED)" for all of these situations. We review the TSHR hypothesis, its strengths and weaknesses, and attempt to provide a unified hypothesis that may explain all the features of TED in all patients.

Autoimmune nature of thyroid eye disease

Although the precise pathophysiology of TED remains unclear it is likely to reflect an autoimmune reaction involving sensitised T lymphocytes and autoantibodies directed against specific orbital or "thyroid and orbital tissue shared antigen(s)". What makes TED a controversial disorder is 1) the identity of the primary antigen(s) is unresolved, 2) it is not clear whether the eye muscles or OCT is/are the primary target tissue in the orbit or whether both tissues are always involved, and 3) one must explain how the ophthalmopathy is linked to thyroid autoimmunity. While most workers believe that the eye disorder is due to cross reactivity against the TSHR in the orbital tissues [8], there is growing evidence that autoimmunity against eye muscle antigens, especially the calcium binding protein calsequestrin (CASQ1), may also play a role in the evolution of the disease once the eye muscle fibre has been breached (since CASQ1 is mainly an intra cellular protein). Another possibility is that the initial reaction is against the TSHR, which leads to orbital fibroblast stimulation and proliferation, and that the eye muscle damage is secondary to this.

TED has been described by one of us (JW) as a "limited multi system autoimmune disorder" involving antigens in the OCT, eye muscle fibre, the lacrimal gland, human Harderian gland equivalent, and the thyroid gland [9]. Apart from the TSHR and CASQ1, another putative orbital antigen is collagen XIII, a connective tissue antigen expressed in the orbital fibroblast cell membranes. It seems likely that the tissue reactions reflect multiple autoimmune targeting of these and other antigens, possibly including nuclear proteins.

Classification of thyroid eye disease

There are three main subtypes of TED, namely: congestive ophthalmopathy, ocular myopathy, and mixed congestive and myopathic ophthalmopathy — which is the most common. The main features and candidate auto-antigens for each subtype are listed in Table I. Congestive ophthalmopathy is characterised by inflammation of the OCT with relative sparing of the extra ocular muscles, presenting as eye swelling, conjunctival injection, chemosis, watery or gritty eyes, and exophthalmos. In contrast, ocular myopathy is characterised by inflammation and swelling of the extra ocular

Table I. *Thyroid eye disease subtypes, clinical features, and candidate autoantibodies*
Tabela I. *Podtypy orbitopatii tarczycowej, cechy kliniczne i autoprzeciwciała występujące u pacjentów*

TED subtype	Main clinical features	Candidate auto-antigens
Ocular myopathy	Diplopia	Calsequestrin
	EOM dysfunction	G2s*
	Exophthalmos	Flavoprotein
Congestive ophthalmopathy	Watery, gritty eyes	TSHR
	Periorbital oedema	Collagen XIII
	Conjunctival injection/chemosis	
	Exophthalmos	
Mixed congestive and myopathic ophthalmopathy	Congestive and myopathic signs/symptoms	All of the above

*G2s is a fragment of the FOX-P1 transcription factor; EOM — extra ocular muscle; TSHR — thyroid-stimulating hormone receptor

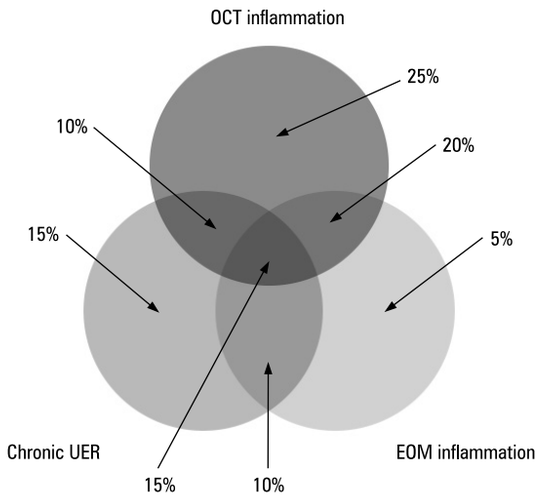

Figure 1. *Proposed relationships between the three main components of "thyroid eye disease" (extra ocular muscle (EOM) dysfunction, orbital connective tissue (OCT), and fat inflammation and upper eyelid retraction [UER]) and estimated frequencies (%) of their associations*

Rycina 1. *Propoonowany związek między trzema głównymi składowymi orbitopatii tarczycowej (dysfunkcja mięśnia zewnętrznego oka (EOM), tkanka łączna oczodołu (OCT) i stan zapalny tkanki tłuszczowej oraz opadanie powieki górnej (UER) oraz szacunkowa częstoliwość współwystępowania (%)*

muscles and manifests as eye muscle dysfunction, diplopia, and, occasionally, painful eye movement.

Although congestive and myopathic features can occur in isolation, the most common presentation of TED is mixed congestive and myopathic ophthalmopathy, half of which have UER as well, occurring in about 40% of patients with "Graves' ophthalmopathy" [10]. In patients with Hashimoto's thyroiditis, UER and lag are often the only features of an ophthalmopathy, except for mild proptosis ([6], and may represent a separate subtype. The estimated prevalence of the three components of TED alone and in association with one, or the other, or both other subtypes, are summarised in Figure 1. Overall, approximately 70% of patients with TED (*i.e.* Graves' disease or Hashimoto's thyroiditis plus ophthalmopathy, or EGD) have OCT inflammation, 50% have EOM involvement, and 50% have chronic UER, which are found as isolated abnormalities in 25%, 5% and 15%, respectively of cases.

TSH-receptor hypothesis

The generally held theory for the pathogenesis of TED is that the primary reaction involves antibodies targeting the TSHR expressed in the OCT, which leads to orbital inflammation manifesting as orbital fibroblast stimulation, collagen and glycosaminoglycans (GAGS) production, and signs and symptoms of peri orbital and conjunctival inflammation and congestion [11]. According to this theory, ocular myopathy is due to ischaemic damage to the eye muscles following a primary OCT inflammatory reaction, *i.e.* it is secondary. Certainly, the TSHR is a logical candidate antigen as it is expressed in the orbital preadipocytes and fibroblasts (as well as the thyroid gland) [9, 11]. While its expression in other tissues such as testis, systemic fat, and skin connective tissue [12, 13] could explain the development of pretibial myxedema and thyroid acropachy as local manifestations of a generalise connective tissue disorder in Graves' disease, it could also be argued that the tissue non specificity of the reactions makes a primary eye muscle reaction more likely. Certainly, the eye muscle fibre always seemed to us a more likely candidate autoantigen than the loose, fatty connective tissue in and around the eye muscles (hence our long-standing interest in the eye and eyelid muscles).

Although there is strong overall support for a key role of the TSHR in the pathogenesis of TED, whether or not TSHR antibodies are linked to ophthalmopathy in all cases of TED remains unclear. For example, it seems unlikely that TSHR antibodies can explain the development of ophthalmopathy in patients in whom the eye changes occur many years after the development of Graves' hyperthyroidism or in patients with Hashimoto's thyroiditis in whom eye changes occur in the frequent absence of TSHR antibody production. Furthermore, newborns with neonatal thyrotoxicosis do not have ophthalmopathy even when the mother has eye signs, and Amato et al. [14] reported the case of a Caucasian male who developed euthyroid Graves' disease after proven sub-acute thyroiditis in the absence of TSHR antibodies but with detectable eye muscle antibodies. Finally, as discussed above, the TSHR is also expressed in fibroblasts and adipocytes at sites such as the abdominal wall, which are presumed to be unaffected in Graves' disease patients [15]. However, one cannot exclude the possibility that TSHR antibodies were present at the onset of their eye disease in all patients with TED, including those with Hashimoto's thyroiditis and EGD, but negative at the time of presentation or initial testing.

Overall, these findings do suggest that a specific link between thyroid autoimmunity, the TSHR, and ophthalmopathy has not yet been proven in all situations. In order to test the notion that autoimmunity against TSHR can explain all cases of TED carried out a PubMed search for the period 2005 to the present, examining all relevant papers published during this period that address the relationship between ophthalmopathy and TSHR antibodies. While it is certain that some studies have been missed, the 10 papers reviewed seem repre-

Table II. *Relationship between TSH-receptor antibodies and ophthalmopathy in patients with autoimmune thyroid disease*
Tabela II. *Zależność między przeciwciałami przeciwko receptorowi TSH i orbitopatią u pacjentów z autoimmunologiczną chorobą tarczycy*

Group	Main findings	Comment	Reference
Hypothyroid GO (n = 11) Euthyroid GO (n = 28)	Mean TBII 2.2 iu/L for the two groups *v.* 8.6 in hyperthyroid patients with ophthalmopathy (p = 0.02)	TSHR Ab levels were "very low" in both groups of patients. TSI was not measured	Eckstein et al [16]
One case of EGD	TSHR Ab negative	TSI was not measured	Cakir [17]
Graves' disease before treatment	Patients with GO had greater TSHR Ab levels than patients with no ophthalmopathy	TSI was not measured	Massart et al. [18]
One patient with differentiated thyroid cancer who developed ophthalmopathy	There was a close correlation between eye signs and TSHR Ab positivity and titres	TSI was not measured	Antonelli et al. [7]
GO	65% of patients had positive TSI, 69% of whom developed ophthalmopathy *v.* 24% of those with negative or borderline positive tests	Overall close positive correlation but many exceptions	Acuna et al. [19]
GO and isolated ocular myopathy (n = 7), 6 of whom had increased EOM volumes	5 out of 7 patients had positive TBII, 1 had positive TPO Abs but negative TBII	"Same TSHR Ab profile as other patients with GO", except in one patient	Gerlach, Febert [20]
Recurrent GO post total thyroidectomy	Mean TRAb 33.8 iu/L *v.* 3.4 iu/L before and after total thyroidectomy	Close overall correlation between ophthalmopathy and TSHR Abs	Nart et al. [21]
Graves' disease (n = 210)	92% TBII positive but titres did not correlate with severity of ophthalmopathy	Retrospective study	Lin et al. [22]
EGD (n = 35; A), GO (n = 9; B)	TBII pos. in 28.6% of A and TSAb pos. in 83% of B; in B, both pos 100%	TSI is more closely related to EGD than TBII	Kazuo et al. [23]
GO (n = 482)	"Thyroid dysfunction is associated with a more severe ophthalmopathy compared to euthyroid state"	A retrospective study	Kim et al. [24]

GO — Graves' ophthalmopathy; TBII — TSH binding inhibiting immunoglobulin; Ab — antibody; EGD — euthyroid Graves' disease; TSI — thyroid stimulating immunoglobulin; EOM — extra ocular muscle; TPO — thyroid peroxidase.

sentative of the total experience of workers over the past 20 years. The results are summarised in table II. While a close general relationship between serum TSHR antibodies — especially those that stimulate the TSHR, so-called thyroid-stimulating immunoglobulin (TSI) — and ophthalmopathy in patients with thyroid autoimmunity is confirmed, this is less close for patients with EGD, and even in Graves' disease there are exceptions.

Euthyroid Graves' disease

In order to further address the relationship between ophthalmopathy and TSHR antibodies we have studied 10 patients with EGD, all of whom have been followed for several years (range 3–7 years, median duration 4 years) with always normal TSH and fT_4 levels and negative thyroid antibodies (Wall et al., in preparation). TSHR antibodies were not detected in any of the 4 patients tested, CASQ1 antibodies were positive in 4 of 8 patients tested, and collagen XIII antibodies were detected in 2 of the 8 patients. The eye features were quantified according to standard classification and nomenclature systems [25–27] at the first visit to the thyroid clinics at Nepean Hospital. Clinical activity score (CAS) ranged from 1–7 (median score 5) indicating that the disease was active, whereas 4 patients had eye muscle involvement (NOSPECS class 4, Nunery type 2) of whom 3 had increased eye muscle volumes on orbital CT imaging and 2 of the 8 patients had significant UER (Margin-reflex distance > 5 mm). Two patients were being treated with low doses of prednisolone at the time of study. Interestingly, three patients had thyroid ultrasound abnormalities, namely follicular adenoma (proven at surgery), goitre, and nodules, but there was

no evidence for thyroid autoimmunity except for "mild thyroiditis" in one patient (Wall et al., in preparation).

Role of autoimmunity against calsequestrin

In our earlier studies, using a crude preparation of calsequestrin prepared from rabbit heart muscle, and by measuring corresponding calsequestrin antibodies by western blot analysis, we found a modest relationship between anti-calsequestrin antibodies and ophthalmopathy. Anti-calsequestrin antibodies were detected in 40% of patients with clinically active TAO but only in 5% of normal subjects [28]. When Porter et al. [29] showed that calsequestrin was expressed 4.8 times more in eye muscle compared to other skeletal muscle, thus offering an explanation for the orbital specificity of skeletal muscle inflammation in Graves' disease, we decided to re-address its possible role in the pathogenesis of TED. Calsequestrin seemed a good candidate as it was shown to be distributed throughout the cell during the myotube stage of differentiation, where it could be seen by antibodies and T-cells. In our initial studies we determined the prevalence of anti-calsequestrin antibodies in a large group of patients with thyroid autoimmunity, with and without ophthalmopathy, using enzyme-linked immunosorbent assay (ELISA) incorporating highly purified rabbit skeletal muscle calsequestrin. We demonstrated that calsequestrin antibodies were good markers of ophthalmopathy, in particular of the ocular myopathy subtype of "Graves' ophthalmopathy" [10, 30, 31].

Next, we set out to identify differentially expressed genes within the thyroid of "Graves' ophthalmopathy" (GO) and Graves' hyperthyroidism (without ophthalmopathy) (GH) patients as a possible explanation for a thyroid-initiated orbital autoimmunity. RNA was extracted from thyroid glands of patients with Graves' disease. RNA samples were arrayed on Illumina® Human Ref-8 Expression BeadChips™ representing 20,5193 genes, the results of selected genes were validated by quantitative PCR (qPCR), and levels of protein translation were measured by Western Blot analysis. Two hundred and ninety-five genes were differentially expressed between GO and GH patients. Of these, the cardiac calsequestrin gene (CASQ2) was the most highly expressed gene in GO (2.2-fold increase) [32]. The succinate dehydrogenase flavoprotein subunit gene (sdha) was also significantly up-regulated in GO (1.4-fold) while genes encoding the thyroid antigens thyroglobulin, thyroid peroxidase, and TSHR were not differentially expressed between GH and GO [32]. The skeletal and cardiac calsequestrin proteins share 68.4% amino acid homology [33]. Previous work has shown that RNA levels of skeletal muscle calsequestrin are 4.7 times higher in extraocular muscle than in masticatory skeletal muscle [29], and that cardiac calsequestrin is expressed 2.7 times more in extra ocular muscle [34]. From these surprising findings we postulated that up-regulation of the CASQ2 gene in the thyroid of patients with Graves' disease may lead to the production of autoantibodies and sensitised T lymphocytes, which cross-react with calsequestrin in the extra ocular muscle of patients who develop ophthalmopathy.

Ophthalmopathy in patients with thyroiditis

Subacute thyroiditis (SAT) and silent thyroiditis (ST) are the most common causes of transient thyrotoxicosis. There have been reports of the development of TSHR positive Graves' hyperthyroidism and ophthalmopathy following SAT [35, 36]. We studied the prevalence of eye and eyelid signs and positive eye muscle and collagen XIII antibody tests in 11 patients with transient thyroiditis, 5 with SAT, and 6 with ST, and in age and sex-matched healthy controls. Five patients with transient thyroiditis developed ophthalmopathy at the first visit or on follow-up. TSHR antibodies were found in only one of these patients, a 20-year-old woman who developed Graves' hyperthyroidism following an episode of ST, but one or more eye muscle or collagen XIII antibodies were detected in 7 of the patients and antibody levels correlated generally with eye signs. Calsequestrin and Fp antibodies were the most frequently detected, and collagen XIII antibodies were detected in two patients with ST [5].

More recently, we studied the prevalence and phenotype of ophthalmopathy in patients with Hashimoto's thyroiditis, correlating eye signs with calsequestrin and collagen XIII antibodies [6]. We showed that mild eye changes are common in patients with Hashimoto's thyroiditis, and the overall prevalence of "ophthalmopathy" is much greater than previously thought, being present in about a third of the patients. However, the relationship between eye signs and calsequestrin antibodies was only modest in this study, and some patients with positive tests did not have eye signs, which could have reflected the long natural history of Hashimoto's thyroiditis.

Risk factors for ophthalmopathy

The phenotypic variation of eye signs in patients with TED suggests that complex interactions between endogenous (genetic factors, increasing age, male sex) and exogenous (cigarette smoking, hyperthyroidism and hypothyroidism, radioiodine treatment) factors may influence the development and severity of ophthalmopathy [37]. Smoking appears to be the greatest risk factor for ophthalmopathy [38, 39], while the role of ge-

netic factors in its development remains unclear. Recent work has focused on identifying genetic alterations associated with GO through small scale and inconclusive case-controlled association studies with candidate genes. The relationship between the autoimmune reactions against the TSHR and calsequestrin and putative genetic factors are unknown but can be addressed using traditional population studies, for example determining if there is any linkage between ophthalmopathy and polymorphisms of the CASQ1, TSHR, and collagen XIII genes — studies which are in progress in our laboratory.

Conclusions

Most workers and readers accept the TSHR hypothesis as being fact. However, the role of autoimmunity against calsequestrin also deserves to be taken seriously. While there is a strong overall association between TSHR Abs and ophthalmopathy, this varies according to the test used and patient population studied. We postulate that while antibodies directed against TSHR may be the initiating event that leads to orbital inflammation, antibody and T lymphocyte reactivity against calsequestrin, or some other eye muscle cell membrane antigen, may separately and independently lead to eye and eyelid muscle inflammation and damage, manifest as diplopia and UER. While it is less likely that autoimmunity against the TSHR in the OCT leads to secondary eye muscle damage, particularly as ocular myopathy can occur as an isolated abnormality in patients with no OCT inflammation (or in the case of EGD, thyroid inflammation), this has not been excluded. Larger prospective studies of well-characterised patients with thyroid autoimmunity with and without ophthalmopathy and of patients with EGD long term and control patients with other muscle and eye disorders should be carried out.

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