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REVIEW

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# Advances in thyroid peroxidase (TPO) and thyroid stimulating hormone receptor (TSHR) biomarkers for autoimmune thyroid diseases

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**ABSTRACT**

The development of immunological methods in the 1970s and 1980s led to the identification of thyroid peroxidase (TPO) and thyroid stimulating hormone receptor (TSHR) as key players in the onset of autoimmune thyroid diseases (AITD). TSHR is primarily associated with hyperthyroidism, while TPO is linked to hypothyroidism. Advances in understanding TPO and TSHR as biomarkers over recent decades have enhanced their clinical application. This review explores the molecular basis of TPO and TSHR as biomarkers, highlighting their importance in diagnosing AITD and their potential to guide effective therapeutic strategies. Additionally, it discusses the development of lateral flow assay (LFA) kits developed by the authors, which show promise as rapid and reliable diagnostic tools for AITD.

**Keywords:** advanced biomarkers, AITD, detection, TFO, TSHR

## Introduction

Autoimmune thyroid diseases (AITD) are complex disorders arising from the interplay of genetic predispositions, environmental triggers, and immune system dysregulation, ultimately leading to autoimmune attacks on the thyroid gland [1]. In 2023, Ban et al. documented the first recognized case of AITD, providing robust epidemiological evidence of genetic susceptibility. Their study highlighted key factors such as familial clustering, an elevated sibling risk ratio, and the presence of thyroid autoantibodies in relatives of AITD patients [2].

Diagnosing AITD can be challenging, as its symptoms often resemble those of other disorders, complicating clinical recognition. Hashimoto's thyroiditis and Graves' disease (GD) are the two most prevalent forms of AITD, both of which require medical intervention. AITD primarily results from a breakdown in immune tolerance to thyroid-specific autoantigens, leading to the production of autoantibodies that target the thyroid gland [3]. A critical aspect of AITD pathogenesis is the generation of autoantibodies that either mimic or block the action of thyroid-stimulating hormone (TSH). In particular, autoantibodies targeting the thyroid-stimulating hormone receptor (TSHR) are implicated in thyroid hyperactivity, as seen in Graves' disease [4].

The TSHR, located on the surface of thyroid follicular cells, regulates thyroid growth, hormone synthesis, and hormone release through its interactions with TSH [5–7]. In humans, the TSHR protein consists of 764 amino acids and function as a G protein-coupled receptor (GPCR). Conversely, Hashimoto's thyroiditis is characterized by autoantibodies against thyroid peroxidase (TPO), resulting in thyroid gland damage and hypothyroidism [8]. TPO is a 993-amino-acid enzyme with multiple glycosylation sites and cysteine residues in its primary sequence.

These biomarkers, particularly anti-TPO antibodies, are key indicators of disease prevalence, progression, and thyroid dysfunction, and are frequently used in diagnosing and monitoring AITD [9–11]. The prognostic significance of these biomarkers is critical in clinical settings, where timely intervention may prevent or mitigate the long-term consequences of thyroid dysfunction. Elevated levels of TPO and TSHR are often associated with insufficient regulatory T cells (Tregs), which play a vital role in maintaining immune homeostasis. Tregs prevent autoimmune reactions by ensuring tolerance to self-antigens, and their deficiency is linked to AITD development [12].

Recent studies have shown that interactions between the immune and neuroendocrine systems further complicate the AITD pathophysiology. Hormonal regulation, immune responses, and neurodegenerative processes observed in autoimmune thyroiditis patients underscore the complexity of these interactions [13]. The systemic nature of AITD is further evidenced by its association with various extrathyroidal conditions such as recurrent miscarriages, mitral valve prolapse, cerebellar ataxia, and thrombocytopenia [14–16]. These associations suggest that AITD is not solely a thyroid disorder but may contribute to a broader spectrum of systemic diseases.

This review explores the intricate relationships among TPO/TSHR, immune dysregulation, environmental factors, and neuroendocrine pathways in AITD. It emphasizes the significance of TPO and TSHR as biomarkers for early detection and diagnosis. Additionally, it highlights the development of a novel lateral flow assay (LFA) kit, designed by the authors, designed to simultaneously detect TPO and TSHR antibodies, showcasing the practical application of biomarker research in diagnosing and managing AITD.

## Thyrotropin receptor (TSHR) as a biomarker

The thyrotropin receptor (TSHR) plays a critical role in thyroid function and is central to the pathology of AITD such as Graves' disease and Hashimoto's thyroiditis [6]. Structurally, TSHR is a G protein-coupled receptor (GPCR) characterized by seven transmembrane domains and a large extracellular region responsible for binding throid-stimulating hormone (TSH) [17]. The extracellular domain contains a leucine-rich repeat (LRR) region, forming a horseshoe-shaped structure that is essential for TSH recognition and binding.

The interaction between TSHR and autoantibodies involves the formation of disulfide bonds (-S-S-), with cysteine playing a pivotal role in creating these bonds. These disulfide bonds are crucial for the proper assembly and functionality of complex proteins such as TSHR [18]. In Graves' disease, hyperthyroidism occurs when autoantibodies bind to TSHR, mimicking TSH activity and overstimulating the thyroid [19]. Conversely, autoantibodies that block TSH from binding to TSHR can lead to hypothyroidism [17].

A deeper understanding of the molecular interactions between autoantibodies and TSHR is essential for elucidating the pathophysiology of AITD. Studies have shown that autoantibodies directed against TSHR can disrupt normal TSH signaling by binding directly to the receptor [17]. Structural studies have also provided insights into the specific requirements for autoantibody binding to TSHR, revealing the complexity of this interaction [20]. These findings underscore the intricate nature of the receptor-autoantibody relationship, which continues to be a focus of ongoing research.

Clinically, TSHR has become a valuable biomarker for diagnosing AITD, particularly in confirming Graves' disease. The detection of thyrotropin receptor antibodies (TRAb) in serum is highly specific for diagnosing Graves' disease [21]. A specific subtype of TRAb, known as TSAb, has proven useful in assessing treatment efficacy and predicting the recurrence of Graves' disease after oral anti-thyroid therapy [22].

The predictive value of TSHR-based biomarkers is significant in the clinical management of AITD. Research demonstrates that the TSHR autoantibody levels correlate with the disease severity and

can serve as prognostic indicators of thyroid dysfunction [23]. In AITD patients, monitoring TSHR autoantibody dynamics helps predict relapse risks and informs therapeutic decisions [21]. Additionally, insights into the structural interactions between TSHR and autoantibodies offer potential targets for developing novel therapeutic approaches aimed at modulating immune responses in AITD [24].

## Thyroid peroxidase (TPO) as a biomarker

Thyroid peroxidase (TPO) is an crucial enzyme in the biosynthesis of thyroid hormones, playing a key role in the production of thyroxine (T4) and triiodothyronine (T3) [25]. Structurally, TPO is a transmembrane protein located on the apical membrane of thyroid follicular cells, where it catalyzes key reactions for thyroid hormone synthesis [26]. The human TPO protein consists of approximately 900 amino acid residues, with its sequence dictating the correct folding and formation of its active structure, necessary for its enzymatic function.

TPO's interaction with TPO antibodies (anti-TPO) involves various biochemical properties, including hydrophobic/hydrophilic interactions, charge, and polarity. TPO hydrophilic nature arises from amino acids such as glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine [18]. For TPO to carry out its catalytic function, hydrogen peroxide ($H_2O_2$) is required as a cofactor to oxidize iodide, enabling the iodination of tyrosine residues on thyroglobulin, which ultimately leads to the synthesis of thyroid hormones T4 and T3 [25].

Elevated levels of TPO are frequently observed in AITD [27]. Recent in silico study of nine human TPO mRNA variants have revealed three non-polymorphic conserved regions (1-273, 447-520, and 590-934), highlighting areas rich in antigenic sites [28]. This extensive antigenicity makes TPO a promising and cost-effective biomarker for detecting thyroid diseases.

In Hashimoto's thyroiditis, the presence of anti-TPO antibodies is a hallmark feature, with research indicating that up to 90% of AITD patients exhibit elevated serum levels of these antibodies [29]. Anti-TPO antibodies target TPO, triggering immune-mediated destruction of thyroid tissue, which ultimately leads to hypothyroidism [29]. The detection of anti-TPO antibodies in blood samples is not only linked to thyroid diseases but also to several non-thyroid conditions, underscoring their diagnostic relevance [30].

In clinical practice, TPO has become an invaluable tool for the early detection of thyroid disorders. Measurement of anti-TPO antibodies has proven effective not only in diagnosing hypothyroidism in humans but also in veterinary medicine, where it is used to identify hypothyroidism in dogs [31]. Furthermore, the presence of anti-TPO antibodies has been associated with suboptimal ovarian stimulation in women undergoing in vitro fertilization, suggesting broader clinical relevance of TPO in reproductive health [32]. This connection emphasizes the importance of considering thyroid autoimmunity in fertility planning and treatment.

The predictive value of TPO in AITD is underscored by its strong association with disease severity and progression. Anti-TPO antibodies are recognized as early indicators of thyroid disease, often detectable before the onset of thyroid hormone dysfunction [33]. Additionally, analysis of genetic loci associated with TPO antibody production provides additional insights into the clinical significance of TPO in predicting thyroid autoimmunity [29].

## Molecular mechanisms underlying TSHR and TPO

The molecular mechanisms underlying the autoimmune targeting of TSHR and TPO involve a complex interplay of genetic predisposition, environmental factors, and epigenetic changes. Specific genetic variants, such as amino acid substitutions in the HLA-DR peptide-binding region, have been identified as key contributors to the development of AITD [34]. These genetic alterations disrupt the immune system's ability to distinguish self-antigens like TSHR and TPO, triggering autoimmune responses [35]. This underscores the importance of molecular research in developing reliable biomarkers for AITD, particularly

TSHR and TPO, to improve diagnostic accuracy and prevent failures.

Our experimental work demonstrated high-affinity interactions between the partial structure of TPO and the Fab fragment of a high-affinity anti-TPO antibody (1VGE; TR1.9, a human IgG1 kappa autoantibody) using molecular docking studies (Figure 1).

Molecular docking revealed that hydrophobic interactions, which operate over short distances, help align the complementary surfaces of TPO and the antibody. Simultaneously, electrostatic interactions between charged side chains and hydrogen bonds between oxygen and nitrogen atoms further stabilize the interaction by accommodating specific structural or reactive groups, thereby enhancing the overall binding strength.

**Figure 1.** Three-dimensional structure modeling results for docking of partial TPO in the TPO antibody Fab complex.

Our findings support previous research indicating that the autoimmune interaction with TPO involves the dissociation of the TPO dimer into monomers. Le et al. explain that this dissociation exposes specific epitopes, rendering the thyroid more susceptible to autoimmune attack. Furthermore, the results suggest that conformational changes occur only when antibodies bind to immunodominant region B (IDR-B) of TPO, but not to immunodominant region A (IDR-A) [36]. This distinction is crrucial for understanding TPO's role in hypothyroidism and underscores the need for further exploration of these molecular mechanisms.

Regarding TSHR, understanding its molecular mechanisms is equally important, especially in hyperthyroid conditions such as Graves' disease. Zulkarnain et al. [37] conducted simulations of TSHR-antibody interactions using the HADDOCK web server (Figure 2).

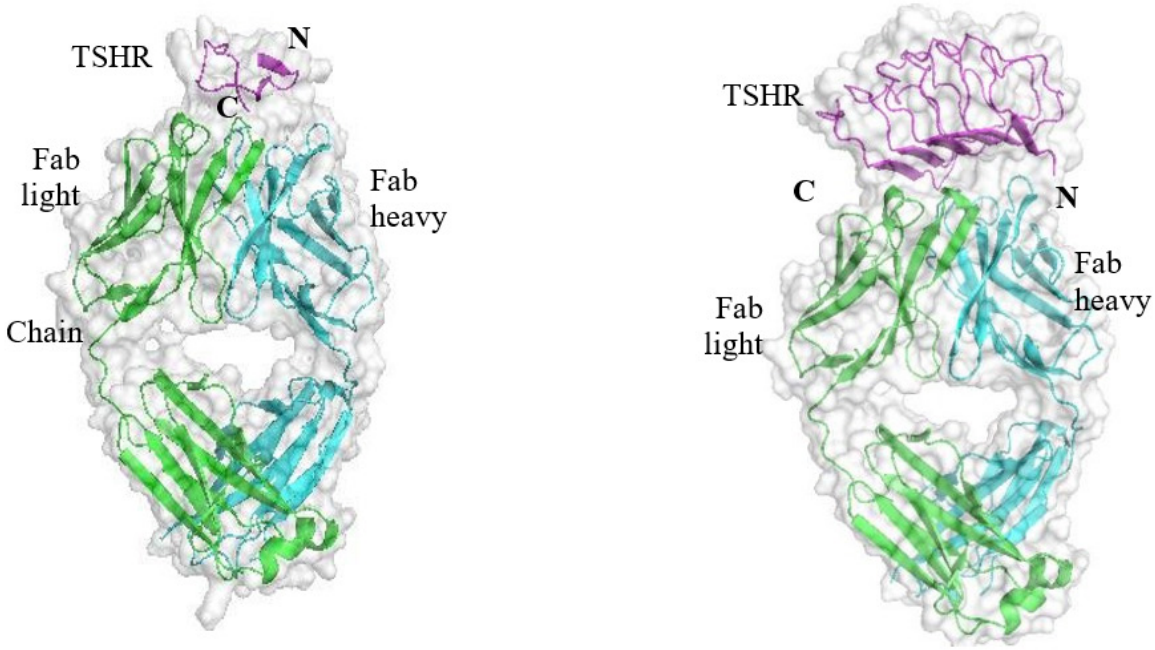


**Figure 2.** Three-dimensional structural modeling results for docking of the TSHR56 (left) and TSHR169 (right) peptides in the M22 Fab complex [37].

As shown in Figure 2, the TSHR169 variant displays a higher affinity for the TSAb-M22 Fab. The M22 antibody binds to TSHR through weak, noncovalent interactions, including electrostatic forces, hydrogen bonds, and hydrophobic interactions. Hydrophobic interactions, which function over short distances, facilitate surface complementarity, while electrostatic interactions and hydrogen bonds further stabilize the binding [37]. These interactions are key to understanding how antibodies can mimic or block TSH action, leading to thyroid dysfunction.

Further research has demonstrated that TSHR autoantibodies target specific regions of TSHR, particularly the N-terminal region, in a conformation-dependent manner [38]. Autoantibodies often bind through conformational epitopes, while neutral antibodies may interact with linear peptide sequences [39].

Various assays, such as those measuring ligand-binding inhibition and cAMP stimulation in cells, have been employed to assess TSHR autoantibody activity [40,41]. Structural studies of the TSHR260-JMG55 complex show that the TSHR260 structure closely resembles those of TSHR260-M22 and TSHR260-K1-70 complexes, suggesting that mutations and autoantibody binding do not significantly alter the leucine-rich repeat domain (LRD) of TSHR [42]. This structural stability is critical for understanding how TSHR autoantibodies can cause thyroid dysfunction without significantly changing the receptor's conformation.

Recent computational simulations by Mezei et al. [43] revealed two key findings: (a) a strong affinity between the leucine-rich (LR) region of TSHR and the TSH ligand, and (b) binding of the LR region to the TSH ligand reduces structural fluctuations within the LR region. These findings are critical for understanding the molecular basis of TSHR-mediated thyroid autoimmunity and pave the ways for potential targeted therapies.

## Predictive value and diagnostic accuracy of TSHR and TPO

A critical aspect of biomarker development is assessing their predictive value and diagnostic accuracy, particularly for TSHR and TPO. Recent studies demonstrate that TSAb are strongly correlated with Graves' disease in pregnant women, establishing them as crucial biomarkers for definitive diagnosis [22]. Research by Rapoport et al. [44] further confirmed the pathogenic role of TSAb by showing how their interaction with the thyrotropin receptor leads to hyperthyroidism, a hallmark of Graves' disease.

TSAb testing is particularly valuable for evaluating treatment responses and predicting the likelihood of Graves' disease relapse following oral anti-thyroid therapy. Additionally, elevated levels of TPO antibody have been observed in Graves' disease patients, underscoring their diagnostic potential. The strong correlation between TSAb and Graves' disease supports the development of rapid diagnostic tests based on TSHR and TPO, which could help prevent Graves' disease-related complications in pregnant women, such as prematurity, low birth weight (LBW), and neonatal thyrotoxicosis [22].

Anti-TSHR antibodies have proven effective for the early detection of AITD, providing a method for identifying these conditions in their earlier stages [45]. Studies have also explored the relationship between microRNA levels in serum and thyroid tissue and their association with TRAb in Graves' disease, suggesting a potential diagnostic link between these factors [46]. These findings highlight the potential of using TSHR antibodies as predictive and diagnostic tools for AITD.

Moreover, biased signaling by TSHR-specific antibodies, which can influence thyrocyte survival in autoimmune conditions, provides insights into the mechanisms underlying AITD [47]. Identifying unique signaling patterns within the thyroid gland could prove critical for improving diagnosis and prognosis.

TPO also serves as a key biomarker for predicting autoimmune diseases, particularly AITD. Anti-TPO antibodies are almost universally present in AITD, recognizing specific conformational epitopes on the TPO molecule and causing direct thyrocyte damage through complement system activation [48]. Detecting anti-TPO antibodies is a hallmark

of AITD, strongly suggesting the presence of underlying thyroid disorders that require further clinical investigation [49]. These antibodies target immunodominant regions on TPO, reinforcing their role as predictive markers for AITD [50], and making them invaluable for early diagnosis and disease management.

Anti-TPO antibodies are also predictive of AITD before clinical thyroid dysfunction becomes evident. For example, in patients with type 1 diabetes, the presence of anti-TPO antibodies indicates a higher risk of developing thyroid complications, highlighting the importance of monitoring TPO antibody levels in this population [51]. This predictive value supports proactive management of both thyroid and diabetic conditions.

Studies have also demonstrated that lifestyle modifications, such as weight loss, can reduce TPO antibody levels, suggesting a link between metabolic factors and the prediction of AITD [52]. Clinically, TPO antibodies are essential for diagnosing AITD, detecting subclinical and overt thyroid dysfunction, and forecasting disease progression by tracking antibody levels over time [53].

## Therapeutic implications of TSHR and TPO biomarkers

TSHR biomarkers hold significant therapeutic potential in managing thyroid disorders. Recent studies have explored TSHR as a therapeutic target in conditions like Graves' disease and Graves' orbitopathy. Small-molecule ligands targeting TSHR have shown promise as potential treatments for hyperthyroidism and Graves' disease [54]. Administration of TSHR-derived cyclic peptides has also demonstrated improvements in thyroid function and tissue characteristics in Graves' disease and Graves' orbitopathy, offering a novel therapeutic approach [55].

Additionally, Gq-biased activation of TSHR has been proposed as a strategy to modulate thyroid growth [56]. Forskolin, which activates downstream TSHR signaling pathways, has been found to enhance skeletal muscle stem cell regeneration, suggesting therapeutic implications for muscle-related conditions, such as muscular dystrophy [57].

Targeting TSHR with small-molecule ligands and antibodies has emerged as a promising strategy for treating autoimmune hyperthyroidism, including Graves' disease, where antigen-specific antibodies and T cells play critical roles in disease development [58]. Genetic and epigenetic dysregulation of TSHR expression in the thymus has been linked to thyroid autoimmunity, suggesting that modulating TSHR expression could be an effective approach for preventing or treating autoimmune responses [59]. The relatively lower concentrations of TSHR autoantibodies, compared to TPO antibodies, underscore the need for therapies that specifically target immune mechanisms associated with TSHR in AITD [23].

Insights from animal models have further clarified the mechanisms of AITD. Inducing Graves-like disease in animals through immunization with fibroblasts transfected with TSHR and class II molecules has provided valuable information on potential therapeutic interventions [60]. Understanding the role of TSHR autoantibodies, particularly their selective recognition of specific TSHR components in thyroid eye disease, may guide the development of targeted therapies aimed at reducing disease progression and alleviating associated symptoms [61].

TPO also plays a critical role in AITD, such as Hashimoto's thyroiditis and Graves' disease [36, 62, 63]. Research suggests that anti-TPO antibody can impair thyroid function by inhibiting TPO activity, leading to reduced thyroid hormone production and elevated TSH levels [64]. The presence of TPO antibodies has been linked to subclinical hypothyroidism, hypertension, and thyroid cysts, highlighting the broader clinical implications of TPO autoimmunity [64].

Further studies have investigated the relationship between anti-TPO antibody levels and thyroid dysfunction, indicating that TPO autoantibodies may represent secondary responses to thyroid injury [65]. Additionally, research has explored the correlation between TPO antibody levels and thyroid gland enlargement, emphasizing the

clinical relevance of assessing TPO antibodies for diagnosing thyroid diseases [66]. The detection of TPO antibodies in cerebrospinal fluid has also been associated with conditions like Hashimoto's encephalopathy, underscoring the systemic effects of TPO autoimmunity [67].

## Emerging research and future directions

As the landscape of AITD continues to evolve, the identification and utilization of biomarkers have become increasingly vital for early detection and accurate diagnosis. Among these, TSHR and TPO have emerged as critical biomarkers, providing valuable insights into the pathogenesis and progression of these disorders.

High titers of thyroid autoantibodies, particularly against TPO, are found in most patients with AITD, underscoring the importance of early detection. However, the high cost of diagnostic tests remains a challenge. To address this, the development of recombinant proteins derived from the TPO gene has been proposed as a solution to improve the sensitivity, specificity, and cost-effectiveness of AITD diagnostics [27].

To effectively develop TPO and TSHR biomarkers, it is crucial to investigate the cellular and molecular mechanisms underlying thyroid autoimmunity. Given that AITD represents the most common autoimmune disorder in humans [6], understanding the prevalence and impact of these conditions is essential. Recent studies have identified microRNAs (miRNAs) as important biomarkers for diagnosing and treating autoimmune diseases. Specific miRNA signatures associated with TPO and TSHR in autoimmune conditions are gaining attention and may lead to targeted therapeutic interventions [68].

Innovative chimeric TSHR bioassays have also been developed to detect thyroid-stimulating immunoglobulins, highlighting the importance of functional biomarkers in identifying autoimmune diseases involving TSHR autoantibodies [69]. Future research could focus on refining these bioassays and assessing their clinical utility across various autoimmune conditions.

## Conclusion

The exploration of TPO and TSHR as biomarkers has greatly enhanced the understanding and management of AITD. These biomarkers are essential for the early detection and diagnosis of conditions such as Graves' disease and Hashimoto's thyroiditis, offering insights into the molecular mechanisms driving these disorders. Their predictive value in identifying patients at risk of thyroid dysfunction underscores their importance in clinical practice, enabling earlier interventions that can prevent disease progression and long-term complications.

The complex interplay of immune dysregulation, genetic predisposition, and environmental factors in AITD highlights the need for a comprehensive approach to diagnosis and treatment. As research continues to uncover the specific roles of TSHR and TPO in thyroid autoimmunity, there is significant potential for these biomarkers to guide individualized therapy, improving patient outcomes and quality of life. Furthermore, incorporating these biomarkers into innovative therapeutic strategies, such as immunomodulation and precision medicine, holds promise for more effective treatments. Future investigations should continue to explore the potential of TSHR, TPO, and other emerging biomarkers to enhance the diagnostic accuracy and treatment of AITD.

## Author contributions

Conceptualization, AA; Writing – Original Draft, ABW; Writing – Review & Editing, AA, AR and ABW.

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## Declaration of competing interest

The authors declare that they have no known conflicting financial interests or personal ties that could have compromised the credibility of this review article.

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