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REVIEW ARTICLE

Molecular mechanisms responsible for transglutaminase 2 involvement in the multiple sclerosis etiopathogenesis and its possible use as a neuroinflammation biomarker

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Abstract

Introduction: Transglutaminase 2 (TG2) is a ubiquitous enzyme involved in extracellular matrix remodeling, cell adhesion, signal transduction, and modulation of immune responses. While its role in celiac disease is well established, increasing evidence supports its involvement in the pathogenesis of multiple sclerosis (MS).

Objectives: The objectives of this review are to describe the molecular mechanisms responsible for TG2 involvement in the multiple sclerosis etiopathogenesis and to evaluate its potential as a neuroinflammation biomarker.

Methods: The literature on the involvement of TG2 in MS was reviewed, with particular attention to its molecular mechanisms, expression in central and peripheral immune cells, association with disease activity and progression, and potential role as a biomarker of neuroinflammation.

Results: TG2 has been detected in monocytes and macrophages within demyelinating lesions, is induced in astrocytes by pro-inflammatory mediators, and is expressed in neurons and endothelial cells under neuroinflammatory conditions. Its localization in astrocytes within MS plaques further supports a role in sustaining neuroinflammation. Modulation of TG2 activity influences immune cell trafficking across the blood–brain barrier and regulates microglial and macrophage polarization. In patients with MS, altered TG2 expression and splicing isoforms in peripheral blood mononuclear cells are associated with disease activity, progression, and specific monocytic functional profiles. Preliminary findings in individuals with radiologically isolated syndrome suggest that peripheral TG2 expression may serve as an indicator of subclinical neuroinflammation.

Conclusion: Overall, TG2 emerges as a promising biomarker in MS, potentially complementing established neuroaxonal markers and improving phenotypic stratification. Further validation of its diagnostic and prognostic value will require large prospective studies integrating multi-omics approaches and advanced neuroimaging within a precision medicine framework.

Keywords: Transglutaminase 2; Multiple sclerosis; Biomarkers; Neuroinflammation; Peripheral blood mononuclear cells; Gene expression

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1. Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) that primarily affects young adults and is one of the leading causes of non-traumatic disability. Its pathogenesis is multifactorial, involving interactions between genetic variants, most notably the *HLA-DRB1**15:01 allele, and environmental factors such as low vitamin D levels, obesity, smoking, and reduced UV exposure. Epstein–Barr virus infection plays a key role, being nearly ubiquitous among affected patients and associated with an increased risk of developing MS years after infectious mononucleosis. Importantly, a landmark longitudinal study on more than 10 million serum samples from United States military personnel demonstrated that seroconversion to Epstein–Barr virus precedes almost all MS cases and increases MS risk by approximately 32-fold, strongly supporting a causal relationship.[1] The disease exhibits high clinical heterogeneity, reflecting the diversity of perivenular inflammatory lesions that drive demyelination, oligodendroglial damage, and, in advanced stages, neurodegeneration. Diagnosis is challenging because clinical manifestations vary widely and often include nonspecific sensory, motor, visual, or cognitive symptoms. The 2024 McDonald criteria integrate magnetic resonance imaging (MRI), cerebrospinal fluid (CSF) analysis—including oligoclonal bands or κ-free light chains—and visual evoked potentials to demonstrate lesion dissemination in space and time.[2] However, MRI may not detect early or subclinical disease activity, and lumbar puncture remains an invasive procedure, limiting its utility for repeated monitoring.

The prolonged preclinical phase underscores the urgent need for sensitive, accessible peripheral biomarkers that can reflect neuroimmunological dynamics, thereby improving early diagnosis, disease monitoring, and prognostic stratification within a precision medicine framework. In this context, transglutaminase 2 (TG2) emerges as a molecule of interest. TG2, a multifunctional enzyme of the transglutaminase family (EC 2.3.2.13), catalyzes calcium-dependent cross-linking reactions between glutamine and lysine residues. It is ubiquitously expressed and participates in fundamental processes such as cell adhesion, apoptosis, extracellular matrix (ECM) remodeling, and signal transduction.[3,4] TG2 also interacts with key intracellular signaling pathways, particularly nuclear factor-κB (NF-κB), through mechanisms that include both canonical activation and TG2-mediated cross-linking of the NF-κB inhibitor IκBα. Sustained activation of the TG2/NF-κB axis promotes the expression of pro-inflammatory cytokines, chemokines, and adhesion molecules, thereby amplifying chronic inflammatory responses.

In the CNS, this signaling contributes to microglial and astrocyte activation, ECM remodeling, and neuronal dysfunction, suggesting a mechanistic role for the TG2/NF-κB axis in neurodegenerative disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and MS.[5] Increased TG2-mediated cross-linking activity has been associated with the formation of insoluble protein aggregates, a hallmark of many neurodegenerative conditions. For example, TG2 promotes amyloid-β oligomerization in AD, and similar mechanisms have been described in other disorders.[6] In MS, both TG2 expression and activity are elevated under conditions of chronic neuroinflammation and reactive fibrosis in the CNS, suggesting a role in amplifying immune responses and stabilizing protein aggregates.[7] Overall, TG2 involvement in neuroinflammation, protein cross-linking, and neurotoxicity positions it not only as a potential pathogenic mediator but also as a promising biomarker and therapeutic target in MS.

2. Transglutaminase 2: Structure, regulatory mechanisms, and multifunctional biochemistry

Transglutaminase 2 is the most ubiquitously expressed member of the transglutaminase family, present in nearly all cellular and extracellular compartments. This widespread distribution supports its role in integrating cellular stress signals, modulating proteins via post-translational modifications,[8] and influencing cell fate in inflammatory and degenerative conditions.[8,9] TG2 catalyzes calcium-dependent transamidation and deamidation reactions through a thioacyl-enzyme intermediate, leading to the formation of ε-(γ-glutamyl)-lysine and γ-glutamylpolyamine cross-links, which are effectively irreversible *in vivo*[3,4] (Figure 1).

In addition to its transamidation activity, TG2 exhibits calcium-independent functions, including guanosine triphosphate (GTP)/adenosine triphosphate (ATP) binding and hydrolysis,[10-13] protein disulfide isomerase activity,[14] and serine/threonine kinase activity.[15] These diverse activities allow TG2 to function not only as a catalytic enzyme in post-translational protein modifications, but also as a regulator of intracellular signaling through its G protein activity and its interactions with multiple signaling pathways and protein complexes. As a result, TG2 influences key cellular processes, including cell survival, differentiation, inflammatory responses, and ECM remodeling. This functional versatility underlies its involvement in both physiological tissue homeostasis and the pathogenesis of chronic inflammatory and neurodegenerative disorders, including MS (Figure 2).

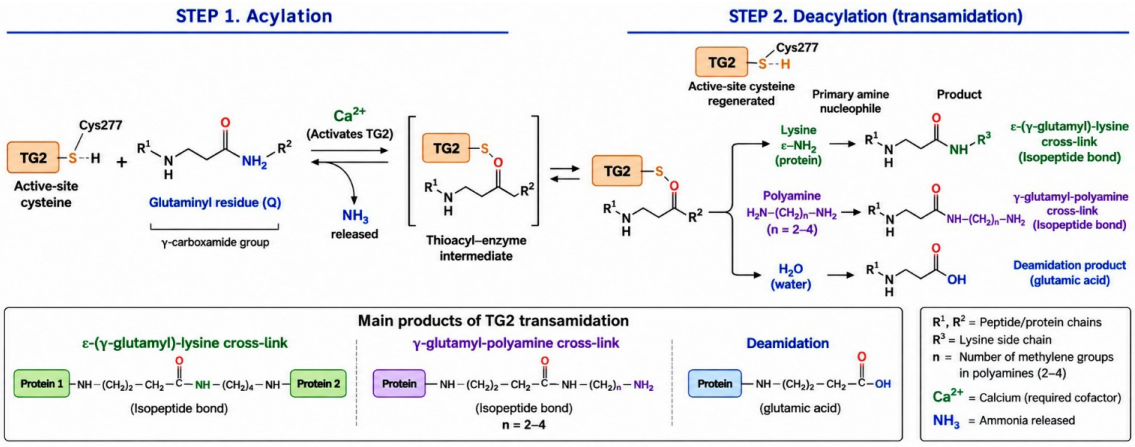


Figure 1. TG2 two-step reaction mechanism. In the first step, in the presence of Ca^{2+}, the active-site cysteine reacts with the γ-carboxamide group of a glutaminyl residue, forming a thioacyl–enzyme intermediate and releasing ammonia. In the second step, the thioacyl–enzyme intermediate reacts with an incoming nucleophilic primary amine, forming a covalent bond between the amine donor and the glutaminyl acceptor substrate, and regenerating the active-site cysteine of TG2.
Abbreviations: Cys277: Cysteine 277; TG2: Transglutaminase 2.

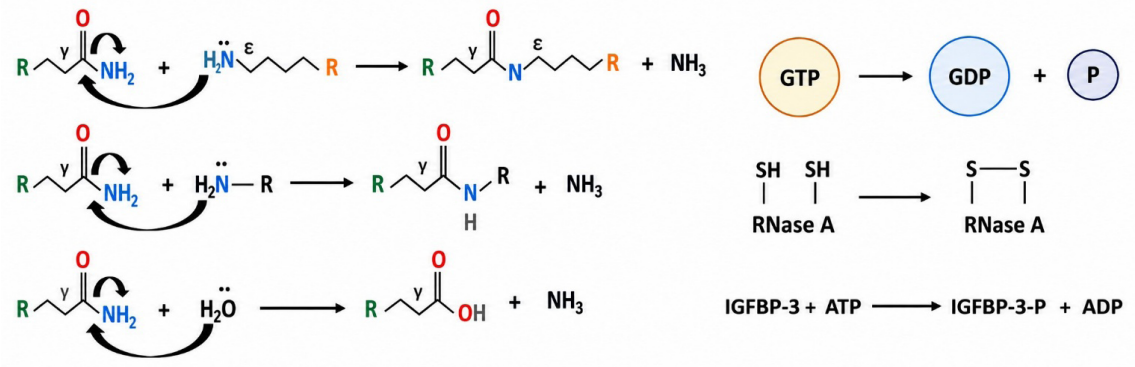


Figure 2. Reactions catalyzed by TG2. (I) Transamidation between the γ-carboxamide group of a glutaminyl residue and the ε-amino group of a lysyl residue; (II) transamidation between the γ-carboxamide group of a glutaminyl residue and a polyamine; (III) deamination; (IV) GTPase activity; (V) protein disulfide isomerase activity; (VI) kinase activity.
Abbreviations: ATP: Adenosine triphosphate; ADP: Adenosine diphosphate; GDP: Guanosine diphosphate; GTP: Guanosine triphosphate; GTPase: Guanosine triphosphatase; IGFBP-3: Insulin-like growth factor-binding protein-3; P: Phosphate; RNase A: Ribonuclease A; TG2: Transglutaminase 2.

However, its guanosine triphosphatase (GTPase) activity is markedly weaker than that of classical small GTPases. This suggests that nucleotide binding primarily acts as a conformational switch rather than a catalytic signaling mechanism.[16] Table 1 summarizes the relationship among TG2 conformational states, their regulatory factors, and the resulting biological functions. This distinction is particularly relevant to neuroinflammatory and neurodegenerative contexts. TG2 multifunctionality arises from its modular architecture and tightly regulated conformational states. The enzyme consists of an N-terminal β-sandwich domain, a central catalytic core containing the Cys277–His335–Asp358 catalytic triad,[17] and two C-terminal β-barrel domains involved in nucleotide binding and protein–protein interactions[18,19] (Figure 3A). TG2 dynamically shifts between a compact GTP-bound inactive conformation and an open calcium-bound active state[20,21] (Figure 3B). Intracellular conditions characterized by low calcium and high GTP favor the inactive form. Extracellular TG2—despite high calcium availability—is often restrained by redox-dependent disulfide bonds that can be reversed by thioredoxin. Lipids, ECM components, and phosphorylation events further modulate this conformational equilibrium,[22,23] allowing TG2 to respond selectively to inflammatory stimuli and tissue stress.

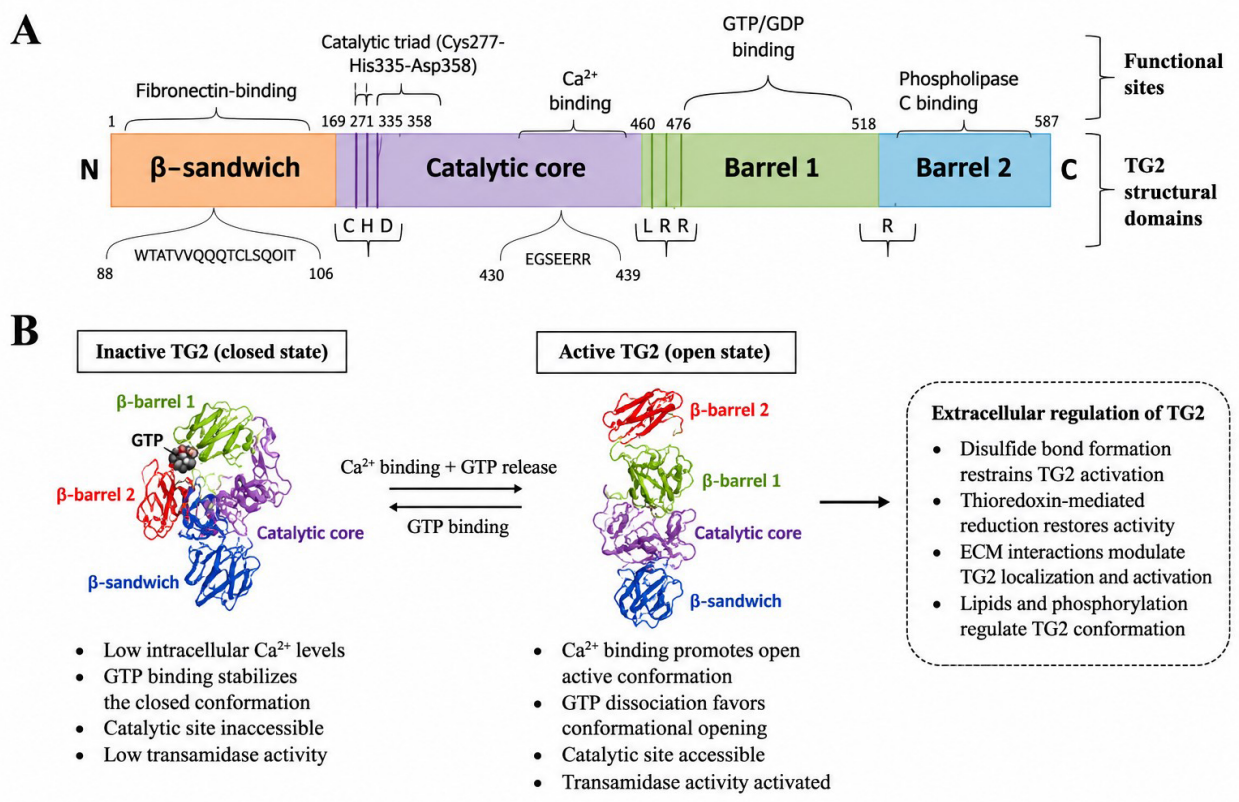


Figure 3. Schematic diagram of TG2 structure and functional domains. TG2 is composed of four domains. (A) The N-terminal β-sandwich mediates interactions with the ECM and contains binding sites for integrins and fibronectin. In its extended conformation, the catalytic core mediates protein polymerization and may contribute to apoptosis. (B) The β-barrel 1 domain contains the GTP/GDP-binding site and plays a key role in TG2-mediated signal transduction. The C-terminal β-barrel 2 domain, in the GTP-bound form, can recruit and activate phospholipase C and mediate pro-inflammatory functions.

Abbreviations: ECM: Extracellular matrix; GDP: Guanosine diphosphate; GTP: Guanosine triphosphate; GTPase: Guanosine triphosphatase; TG2: Transglutaminase 2.

Transglutaminase 2 expression is tightly regulated at multiple levels. Expression of the transglutaminase 2 gene (*TGM2*) is regulated by multiple stimuli, including hypoxia, inflammatory cytokines, and endoplasmic reticulum stress,[24-27] through interconnected signaling pathways such as hypoxia-inducible factor 1, NF-κB, and transforming growth factor β (TGF-β). These regulatory mechanisms, together with post-transcriptional and alternative splicing regulation, contribute to the modulation of TG2 expression and function (Table 2). TG2 also participates in feed-forward loops that amplify inflammatory signaling[5,28-30] (Figure 4). Epigenetic mechanisms and microRNAs provide additional levels of regulation of *TGM2* expression under pathological conditions. DNA methylation, histone modifications, and microRNA-mediated post-transcriptional mechanisms can influence *TGM2* transcription, mRNA stability, and protein expression, thereby modulating TG2 availability and function during inflammatory and stress-related responses.[31]

Finally, alternative splicing of *TGM2* generates multiple isoforms with distinct C-terminal regions. These splice variants may involve alterations or partial loss of the C-terminal β-barrel domains, which contribute to nucleotide binding and stabilization of the TG2 closed inactive conformation. Consequently, changes in the C-terminal region can affect protein folding, subcellular localization, and GTP-binding capacity, potentially modifying TG2 function in pathological contexts.[32] Figure 5 shows the main sequenced human TG2 splicing variants and putative isoforms.[33] While the full-length isoform is predominant, shorter variants such as TG2-S/TGH,[34,35] TGH2, and disease-associated transcripts (e.g., TGM2_v3 and TGM2_v4b) display distinct functional properties that depend on cellular context, subcellular localization, and disease-associated stimuli. These splice variants may differentially regulate TG2 intracellular trafficking,

protein–protein interactions, and enzymatic activity, thereby influencing inflammatory signaling, cellular stress responses, and neurodegenerative processes.[36-38] These isoforms add functional diversity, shaping TG2 involvement in pathological remodeling and chronic inflammation.

3. TG2 in cellular homeostasis, inflammation, and disease

Transglutaminase 2 is a ubiquitous multifunctional enzyme expressed across diverse cell types, particularly endothelial and smooth muscle cells. Under basal conditions, TG2 is

Table 1. Conformational states of TG2, their regulatory mechanisms, and associated biological functions

Conformational state	Regulatory condition	Structural conformation	Functional consequence	References
Inactive TG2 (closed)	High intracellular GTP/ATP, low Ca^{2+}	Compact closed conformation	Catalytic site becomes inaccessible; TG2 mainly functions as a scaffold/signaling protein through protein–protein interactions	10-13,16,20,21
Active TG2 (open)	High Ca^{2+} concentration	Open extended conformation	Catalytic site becomes exposed; transamidation and deamidation reactions promote protein cross-linking and post-translational modification	3,4,20,21
Extracellular TG2	ECM environment and redox regulation	Activity controlled by reversible disulfide bond formation	Regulation of cell adhesion, ECM remodeling, and cell migration	22,23
Inflammatory/stress conditions	Cytokines, hypoxia, oxidative stress	Increased expression and shift toward the active conformation	Amplification of inflammatory signaling, ECM remodeling, and fibrosis	5,22-30

Abbreviations: ATP: Adenosine triphosphate; ECM: Extracellular matrix; GTP: Guanosine triphosphate; TG2: Transglutaminase 2.

Table 2. Major regulators of TG2 expression and functional consequences

Regulator/stimulus	Signaling pathway or mechanism	Effect on TG2	Functional relevance	References
Hypoxia and cellular stress	HIF-1-associated signaling	↑ *TGM2* transcription	Adaptation to cellular stress and inflammatory responses	30
Pro-inflammatory cytokines (IL-1, IL-6)	NF-κB signaling	↑ TG2 expression and activity	Amplification of inflammation and immune cell activation	24,25,30
TGF-β	TGF-β signaling	↑ *TGM2* transcription	ECM remodeling, fibrosis, and tissue repair	28-30
Endoplasmic reticulum stress	PERK/ER stress pathway	↑ TG2 expression	Cellular stress adaptation and apoptosis regulation	26
miRNAs (e.g., miR-19)	Post-transcriptional regulation	Modulation of *TGM2* expression	Fine regulation of TG2 levels under pathological conditions	31
Alternative splicing	Generation of TG2 isoforms	Isoforms with distinct localization and functions	Functional diversification of TG2	32-37

Abbreviations: ECM: Extracellular matrix; HIF: Hypoxia-inducible factor; IL: Interleukin; miRNA: MicroRNA; NF-κB: Nuclear factor-κB; PERK/ER: Protein kinase R-like endoplasmic reticulum kinase/endoplasmic reticulum; TGF-β: Transforming growth factor β; TG2: Transglutaminase 2; *TGM2*: Transglutaminase 2 gene.

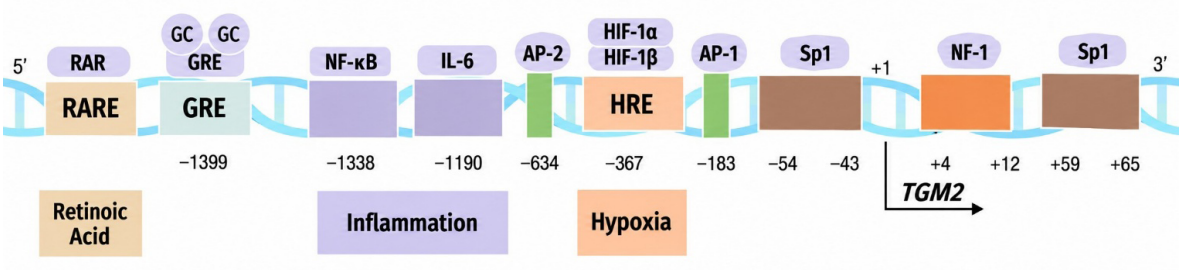


Figure 4. *TGM2* promoter and its response elements. Response elements for RAR/RXR (−1,731 bp and −1,720 bp), glucocorticoid, (GRE; −1,399 bp), NF-κB (−1,338 bp), IL-6 (−1,190 bp), TGF-β1 (−900 bp), estrogen (ERE; −656 bp), AP-2 (−634 bp), HIF (HRE; −367 bp), AP-1 (−183 bp), and NF-1 (+4 bp, +12 bp), as well as motif regions such as CAAT box (−96 bp), TATA box (−29 bp), and GC box (Sp1, −54 bp, −43 bp, +59 bp, +65 bp) have been identified within the *TGM2* promoter.
Abbreviations: AP-1: Activator protein-1; AP-2: Activator protein-2; ERE: Estrogen response element; GRE: Glucocorticoid response element; HIF: Hypoxia-inducible factor; HRE: Hypoxia response element; IL-6: Interleukin-6; NF-1: Nuclear factor-1; NF-κB: Nuclear factor-κB; RAR/RXR: Retinoic acid receptor/retinoid X receptor heterodimer; RARE: Retinoic acid response element; Sp1: Specificity protein 1; TGF-β1: Transforming growth factor β1; *TGM2*: Transglutaminase 2 gene.

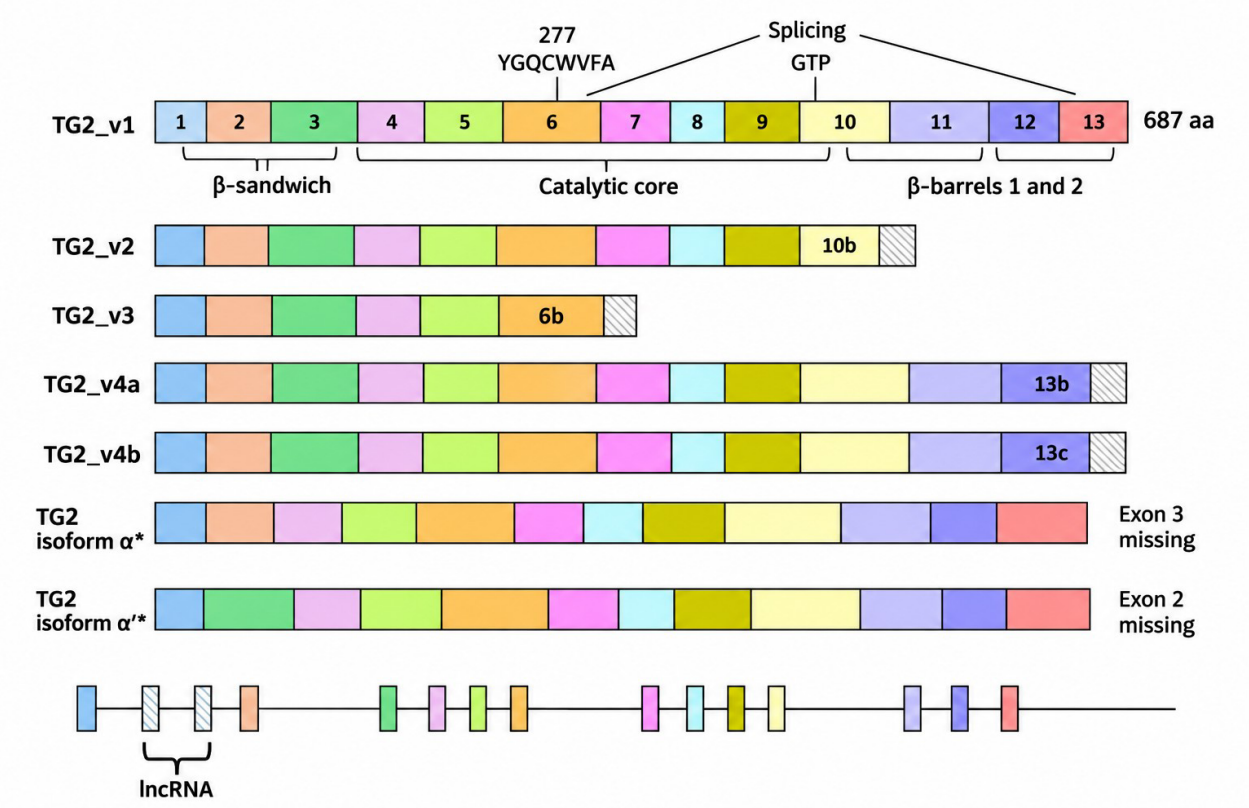


Figure 5. Sequenced human TG2 splicing variants. A long noncoding RNA (lncRNA) site is indicated at the level of the first intron. Two putative isoforms, isoform c* and isoform d*, are also included (redrawn based on Bianchi *et al.*[33]).
Abbreviations: GTP: Guanosine triphosphate; TG2: Transglutaminase 2.

predominantly localized within intracellular compartments, including the cytosol, nucleus, and mitochondria, where it participates in cellular homeostasis and stress responses. In response to inflammatory stimuli, tissue injury, or ECM remodeling, TG2 can be translocated or externalized to the extracellular space, where it contributes to cell adhesion, matrix stabilization, and signaling processes.[39,40]

Transglutaminase 2 regulates critical physiological

processes, including cell proliferation, differentiation, ECM organization,[41] and apoptosis.[42] Activation of TG2 during apoptosis promotes calcium-dependent cross-linking of cytoskeletal and structural proteins, including actin-associated proteins and other intracellular substrates, generating a more stable protein network that transiently preserves cytoplasmic integrity. This stabilization limits the leakage of intracellular components and facilitates immunologically silent clearance of apoptotic cells by phagocytes.[43]TG2 functions are highly context-dependent and are influenced by subcellular localization and cellular state. Extracellular TG2 promotes cell survival and ECM homeostasis by binding fibronectin and interacting with integrins (particularly β1, β3, and β5 integrins), thereby activating adhesion-dependent signaling pathways that regulate cell survival, migration, and tissue remodeling. In contrast, intracellular TG2 regulates cellular homeostasis through interactions with signaling molecules and transcriptional regulators, influencing apoptosis, differentiation, and responses to cellular stress in a context-dependent manner.[44,45] These functions are modulated by calcium levels, GTP binding, and redox conditions.[46,47] Mitochondrial TG2 regulates calcium homeostasis by modulating mitochondrial calcium handling and maintaining mitochondrial functional stability. In addition, TG2 interacts with members of the B cell lymphoma 2 protein family, including the pro-apoptotic protein BAX and the anti-apoptotic protein BCL-2, thereby influencing mitochondrial membrane integrity and apoptotic signaling pathways.[47,48] In the nucleus, TG2 regulates gene expression through interactions with nuclear proteins, transcriptional regulators, and stress-responsive pathways. Nuclear TG2 has been implicated in modulating NF-κB-dependent inflammatory signaling, apoptosis-related pathways, and cellular stress responses, thereby influencing inflammatory gene expression programs and cell survival mechanisms. Table 3 summarizes the main nuclear functions of TG2 and their contribution to inflammatory regulation.

Transglutaminase 2 is strongly implicated in inflammatory processes. Its expression and transamidation activity are upregulated by pro-inflammatory cytokines, including interleukin-6 (IL-6), interleukin-1β (IL-1β), and TGF-β, through NF-κB-dependent signaling. This occurs via canonical degradation of IκBα or through TG2-mediated cross-linking of IκBα (Figure 6). These mechanisms contribute to fibrosis, cell adhesion and migration, monocyte differentiation, and macrophage phagocytic activity. Extracellular TG2 contributes to

Table 3. Nuclear targets and signaling pathways regulated by TG2 during inflammatory and stress responses

Nuclear TG2 function	Molecular target/pathway	Effect of TG2	Impact on inflammation	References
Regulation of transcriptional activity	NF-κB signaling pathway	TG2 promotes NF-κB activation through non-canonical signaling mechanisms	Enhances transcription of pro-inflammatory genes and cytokine production	5,30
Interaction with transcription factors	Tumor protein p53	TG2 modulates p53-dependent transcriptional responses and apoptosis-related pathways	Influences inflammatory responses associated with cellular stress and damage	8,19
Regulation of hypoxia responses	HIF-1α	TG2 interacts with hypoxia-related signaling pathways, contributing to cellular adaptation	Modulates inflammatory responses during hypoxia and metabolic stress	6,8
Modification of nuclear proteins	Histones and nuclear substrates	TG2-mediated cross-linking alters nuclear protein organization and gene regulation	May affect expression of inflammatory and stress-response genes	9,19
Nuclear localization and cell fate regulation	Apoptosis-related pathways	Nuclear TG2 influences cell survival or apoptosis depending on cellular context	Modulates inflammatory outcomes by affecting damaged cell clearance	43,44
GTP-binding/signaling functions	Gh/G-protein activity	TG2 acts as a signaling molecule independent of its transamidase activity	Regulates intracellular pathways involved in inflammation and stress responses	12,13,44

Abbreviations: Gh/G: GTP-binding form/function of TG2; GTP: Guanosine triphosphate; HIF-1α: Hypoxia-inducible factor-1α; NF-κB: Nuclear factor-κB; TG2: Transglutaminase 2.

ECM stabilization and promotes cell adhesion through interactions with integrins (β1, β3, β5), heparan sulfate proteoglycans, and G protein-coupled receptors such as GPR56. These interactions coordinate signaling pathways involved in cell survival, proliferation, and migration.

Dysregulated TG2 has been associated with a wide spectrum of pathological conditions, including autoimmune disorders, tissue fibrosis, chronic inflammation, and neurodegenerative diseases, highlighting its central role as a modulator of cellular homeostasis, stress responses, and inflammation. TG2 also participates in transmembrane signaling via receptors such as oxytocin receptors, thromboxane A2 receptors, adrenergic receptors, and phospholipase C delta 1,[45] and modulates signaling pathways involving integrins, platelet-derived growth factor receptor, and vascular endothelial growth factor receptor, thereby promoting cell proliferation, survival, and migration.[49] These multifaceted roles underline TG2 as a pivotal factor in neurodegeneration, vascular inflammation, fibrosis, and autoimmunity.

4. Role of TG2 in neuroinflammation and neurodegenerative diseases

Neuroinflammation is a common feature of several neurodegenerative disorders and is characterized by the activation of resident CNS cells, particularly microglia and astrocytes, which release reactive oxygen species, nitric oxide, inducible nitric oxide synthase, and pro-inflammatory cytokines such as IL-6 and tumor necrosis factor-α (TNF-α).[50,51] TG2 has emerged as a relevant modulator of these inflammatory processes due to its ability to regulate immune cell activation, ECM remodeling, cellular stress responses, and protein aggregation. Table 4 summarizes the major inflammatory pathways, cellular

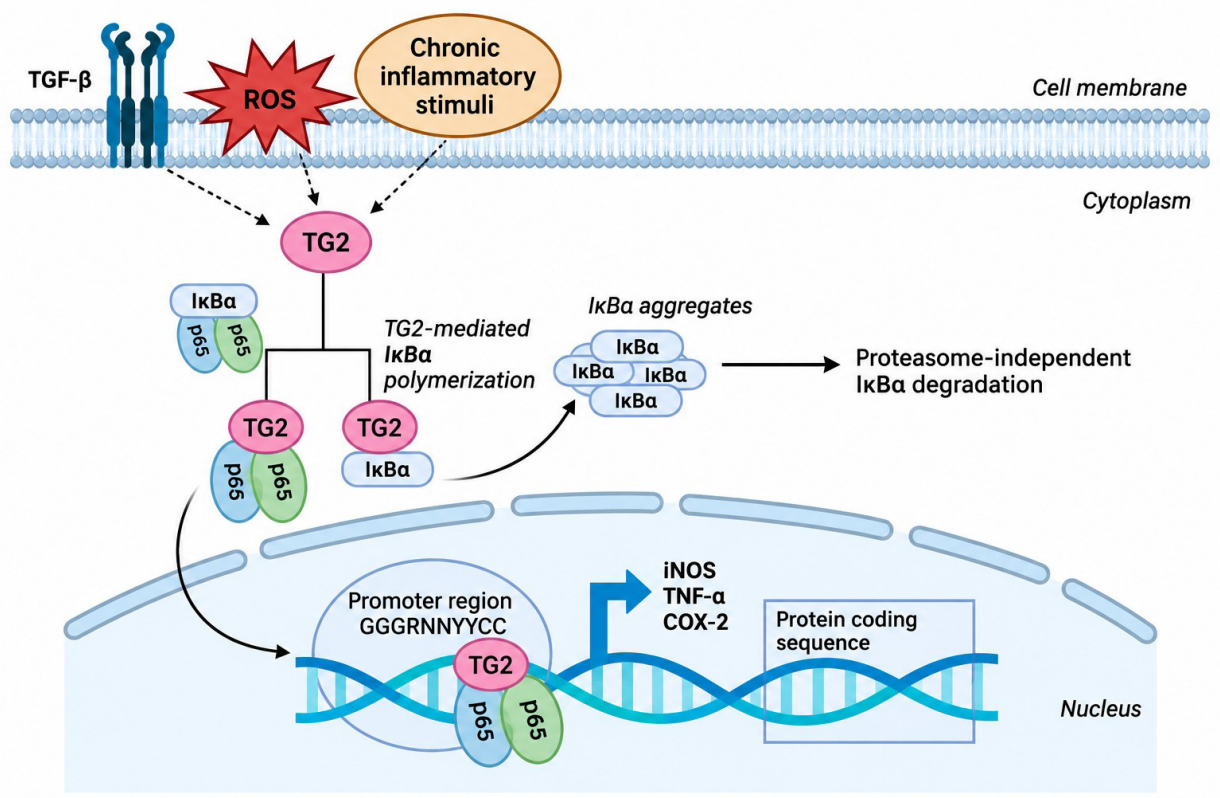


Figure 6. TG2/NF-κB axis in inflammation. Inflammatory stimuli induce TG2 activation and its interaction with the cytosolic inactive NF-κB complex, composed of the transcriptionally active p50 and p65 subunits, and the inhibitory IκBα subunit. Binding of TG2 to IκBα promotes its polymerization and dissociation from the NF-κB complex, followed by degradation of IκBα aggregates via a proteasome-independent pathway. The active p50/p65 heterodimer, in association with TG2, translocates to the nucleus, where it binds to the NF-κB consensus sequence (GGGRNNYYCC) in the promoter regions of genes involved in inflammation and tumorigenesis.

Abbreviations: COX-2: Cyclooxygenase-2; IκBα: NF-κB inhibitor; iNOS: Inducible nitric oxide synthase; NF-κB: Nuclear factor-κB; ROS: Reactive oxygen species; TG2: Transglutaminase 2; TGF-β: Transforming growth factor β; TNF-α: Tumor necrosis factor-α.

targets, and processes regulated by TG2. Its effects involve multiple cellular targets, including microglia, astrocytes, infiltrating macrophages, and neurons, and are dependent on its subcellular localization and disease context.

Evidence suggests that TG2 contributes to pathological mechanisms involved in HD,[52-54] AD,[55,56] PD,[57,58] MS,[38] and amyotrophic lateral sclerosis. Table 5 summarizes the disease-specific roles of TG2 in major neurodegenerative disorders. In these conditions, increased TG2 mRNA expression, protein levels, and enzymatic activity have frequently been reported.[59,60] In activated microglia and macrophages, TG2 may promote neuroinflammation by modulating the production of pro-inflammatory mediators and regulating cellular migration and phagocytic activity. In astrocytes, TG2 contributes to reactive gliosis and ECM remodeling through regulation of cell–matrix interactions and extracellular protein cross-linking, potentially sustaining a chronic inflammatory environment. In neurons, intracellular TG2 may influence cellular stress responses and apoptosis-related pathways through interactions with signaling proteins and mitochondrial regulators, thereby contributing to neuronal vulnerability during prolonged inflammation.

However, TG2 functions are highly context-dependent, and experimental models have also suggested protective roles, including supporting cell survival under metabolic stress and facilitating apoptotic cell clearance. Therefore, TG2 may exert either pathogenic or compensatory effects depending on the activated cell type, disease stage, subcellular localization, and relative contribution of its enzymatic and scaffolding functions. In addition to regulating inflammatory responses, TG2 may contribute to neurodegeneration through its ability to promote pathological protein aggregation. More than two decades ago, Selkoe *et al.*[61] proposed that transglutaminase activity might contribute to protein aggregation in AD. Supporting this hypothesis, tau protein has been identified as a strong transglutaminase substrate *in vitro*,[62,63] and ε-(γ-glutamyl)-lysine cross-links have been identified within neurofibrillary tangles and paired helical filaments in AD brain tissue.[64] Elevated bis-γ-glutamyl putrescine levels in the CSF of HD patients suggest that polyamine-mediated protein cross-linking occurs in the brain, particularly in HD.[65] Transglutaminase activity has also been shown to promote oligomerization and aggregation of amyloid-β under physiological conditions, potentially contributing to AD pathology.[66,67]

Transglutaminase 2 may further amplify neuroinflammatory responses associated with protein aggregation. For example, amyloid-β deposition promotes recruitment and activation of monocytes, macrophages, and microglia, leading to increased production of inflammatory cytokines and chemokines.[68-72] In this context, TG2 overexpression has been associated with amyloid-β-induced inflammatory responses, including increased IL-6 and IL-1β expression in THP-1 human monocytic cells[73] and BV2 murine microglia,[74] whereas TG2 silencing reduces TNF-α expression in these models.[75,76] Beyond AD, TG2 is implicated in protein aggregation in PD,[57,58] progressive supranuclear palsy,[60,77] and HD. Expanded polyglutamine domains, as observed in HD, are TG2 substrates,[78-80] suggesting that aberrant TG2 activity may contribute to CAG-repeat expansion disorders. *In vitro*, TG2 promotes the formation of soluble aggregates of α-synuclein and polyglutamine proteins,[81,82] indicating that TG2 may represent a common molecular link between protein aggregation and inflammation across multiple neurodegenerative conditions. Overall, TG2 represents a multifunctional regulator connecting immune activation, glial responses, ECM remodeling, protein aggregation, and neuronal dysfunction. These properties support its potential relevance as both a biomarker and therapeutic target in neurodegenerative diseases, although further studies are required to define its specific contribution in different cellular and pathological contexts.

5. Role of transglutaminase 2 in multiple sclerosis pathogenesis

Transglutaminase 2 is a multifunctional enzyme with GTPase, kinase, transamidation, and scaffolding activities that interacts with a wide range of intra- and extracellular substrates. Although these functions support macrophage survival and phagocytic activity during tissue repair,[83,84] they may also contribute to inflammatory processes and cell dysfunction under pathological conditions,[85] particularly in neuroinflammatory disorders such as MS.[86-89] TG2 may contribute to MS pathogenesis through multiple mechanisms involving immune cell trafficking, glial activation, ECM remodeling, and regulation of inflammatory responses. Several cell populations appear to be involved in TG2-mediated effects, including infiltrating monocytes/macrophages, resident microglia, reactive astrocytes, endothelial cells, and neurons.

A key mechanism through which TG2 may influence neuroinflammation is the regulation of immune cell adhesion and migration across the blood–brain barrier (BBB). Extracellular TG2 interacts with ECM components, including fibronectin and heparan sulfate proteoglycans, and binds cell surface receptors such as integrins (β1, β3, and β5), promoting cell–matrix interactions, migration, and tissue remodeling.[90-92] Through these mechanisms, TG2 may facilitate recruitment and accumulation of

inflammatory cells within the CNS, thereby contributing to lesion formation and maintenance of the inflammatory microenvironment characteristic of MS. Infiltrating monocytes and macrophages represent major cellular mediators of MS pathology by promoting myelin phagocytosis, antigen presentation, and release of inflammatory mediators. TG2 expression is increased in activated macrophages and microglia under inflammatory conditions, where it may regulate cellular migration, phagocytic activity, and inflammatory responses.[83-85] In addition, TG2 expressed by endothelial cells may contribute to vascular inflammatory processes and facilitate interactions between circulating immune cells and the BBB, further supporting leukocyte recruitment into the CNS.[90,91]

Astrocytes and neurons may also be affected by TG2 activity, particularly during chronic inflammatory conditions, where TG2 contributes to reactive gliosis, cellular stress responses, and tissue remodeling. Evidence from experimental autoimmune encephalomyelitis (EAE), an established animal model of MS, supports a pathogenic role for TG2 in neuroinflammation. TG2 knockout or pharmacological inhibition reduces leukocyte infiltration into the CNS, attenuates microglial activation, and decreases pro-inflammatory cytokine expression.[86,87] These findings suggest that TG2 contributes to early inflammatory phases of disease by promoting immune cell recruitment and activation.

However, TG2 may also exert phase-dependent effects, participating in chronic processes such as ECM remodeling, glial scar formation, and persistent inflammatory responses. Human pathological studies further support TG2 involvement in MS lesion development. Post-mortem analyses of MS brain tissue have demonstrated increased TG2 expression in macrophages and reactive astrocytes within active lesions, particularly in perivascular regions.[89] TG2 expression is associated with ECM alterations, including deposition of fibronectin and chondroitin sulfate proteoglycans, which may contribute to lesion persistence and impaired remyelination.[89,93]

Furthermore, interactions between TG2, cytosolic phospholipase A2, and cyclooxygenase-2 suggest a potential role for TG2 in inflammatory signaling pathways involved in demyelination and chronic lesion evolution.[94] Overall, TG2 represents a molecular link between immune cell trafficking, glial activation, ECM remodeling, and chronic neuroinflammation in MS. By regulating both acute inflammatory infiltration and long-term tissue remodeling processes, TG2 may contribute to different phases of disease progression, supporting its potential relevance as a biomarker and therapeutic target in MS. Although TG2 participates in pathogenic mechanisms shared with other neurodegenerative disorders, including neuroinflammation and protein remodeling, available evidence suggests that its contribution to MS is more closely associated with immune-mediated mechanisms, whereas TG2 has been primarily implicated in protein aggregation and aggregate stabilization in AD and PD.[55-58,61-82,86-94]

6. Transglutaminase 2 as an emerging biomarker in multiple sclerosis

Transglutaminase 2 has recently emerged as a promising biomarker in MS, supported by converging evidence from cellular, molecular, animal, and human studies. Its potential as a diagnostic and prognostic tool stems from correlations between TG2 expression, disease activity, lesion burden, and clinical severity.

6.1. Peripheral transglutaminase 2 expression and isoform-specific signatures as candidate biomarkers of disease activity in multiple sclerosis

Peripheral blood mononuclear cells (PBMCs) represent an accessible source of circulating immune cells, including monocytes and lymphocyte populations, which are involved in MS-associated inflammatory pathways. Therefore, analysis of TG2 expression in PBMCs has been explored as a minimally invasive approach to investigate peripheral immune signatures potentially associated with CNS inflammatory activity. TG2 can be assessed at different biological levels, including *TGM2* mRNA expression, protein abundance, and enzymatic transamidation activity. However, current studies investigating TG2 in MS have mainly focused on *TGM2* mRNA expression in PBMCs. Analyses of PBMCs have provided preliminary evidence that TG2 expression may represent a molecular indicator of disease activity in MS.

Transglutaminase 2 can be measured at different levels, including *TGM2* mRNA expression through quantitative polymerase chain reaction-based approaches, TG2 protein abundance through immunological assays, and enzymatic activity through evaluation of TG2-mediated transamidation reactions. Among these approaches, studies performed in MS have primarily investigated *TGM2* transcript levels in PBMCs, allowing assessment of peripheral immune cell activation and inflammatory signatures. In a cohort of 161 patients with MS, increased TG2 mRNA expression in PBMCs was associated with clinical severity and lesion burden, including higher Expanded Disability Status Scale scores and increased brain lesion volume, suggesting that peripheral TG2 expression may reflect systemic inflammatory activity associated with

CNS pathology.[95] Therefore, TG2 should be considered a candidate biomarker of disease activity and progression rather than an established diagnostic marker, as further validation in larger longitudinal cohorts is required.

Beyond total TG2 expression, alternative splicing of the *TGM2* gene generates multiple isoforms with distinct functional properties. Specific splice variants, including V4a and V4b, have been reported to be increased in PBMCs from patients with MS compared with healthy controls. These isoforms differ in their C-terminal regions, potentially affecting protein regulation, localization, and enzymatic activity under inflammatory conditions. Therefore, isoform-specific TG2 signatures may provide additional information regarding immune activation states and disease heterogeneity compared with total TG2 expression alone.[96]

Preliminary studies in individuals with radiologically isolated syndrome (RIS) further suggest that peripheral TG2 expression may have potential value in identifying individuals with subclinical inflammatory activity and increased risk of progression toward clinically definite MS.[97] However, prospective studies are required to determine whether TG2 measurements can reliably predict conversion, disease progression, or treatment response. The cohorts investigated in these studies were selected according to established diagnostic criteria for MS and RIS. The MS cohorts included patients diagnosed according to McDonald criteria, with clinical characterization including different MS phenotypes, whereas the RIS cohort consisted of individuals fulfilling RIS criteria without previous clinical manifestations of MS. Detailed inclusion and exclusion criteria, demographic characteristics, and clinical features of each cohort are reported in the original studies.[95-97]

6.2. Insights from animal models: Transglutaminase 2 in experimental autoimmune encephalomyelitis pathogenesis

Complementary evidence from EAE models underscores the pathogenic relevance of TG2. TG2 deficiency or pharmacological inhibition in EAE leads to reduced leukocyte infiltration into the CNS, attenuated neuroinflammation, and improved behavioral outcomes.[98] These findings suggest that increased TG2 expression may reflect activation of inflammatory pathways involved in disease progression rather than representing a disease-specific marker alone. Notably, TG2 expression increases in neurons adjacent to perivascular lesions, and its cytoplasmic localization correlates with apoptotic markers, suggesting a dual role in both immune cell-mediated inflammation and neuronal vulnerability. These findings indicate that TG2 actively participates in acute inflammatory phases, such as leukocyte migration across the BBB, as well as in chronic stages, including ECM remodeling and glial scar formation.

6.3. Histopathological evidence: Transglutaminase 2 in multiple sclerosis lesions

Histopathological studies of post-mortem MS brain tissues further support the relevance of TG2 as a lesion-associated biomarker. TG2 is highly expressed in macrophages and reactive astrocytes within active lesions and colocalizes with ECM components, such as fibronectin and chondroitin sulfate proteoglycans, which are known to inhibit remyelination.[7,94] Its expression in chronic lesions appears to contribute to glial fibrosis and persistent inflammatory microenvironments, potentially linking TG2 activity to impaired tissue repair and lesion chronicity.[89] These observations suggest that TG2 not only reflects ongoing pathology but may also participate directly in processes that limit recovery and sustain disease progression.

6.4 Overall role of transglutaminase 2 as a multi-level biomarker in multiple sclerosis

Taken together, these cellular, molecular, and histopathological data highlight TG2 as a multi-level biomarker candidate in MS. Measuring TG2 in PBMCs provides a minimally invasive means to monitor systemic inflammatory activity, while splice isoforms may serve as stable indicators of inflammatory subtype or disease phase. TG2's tissue expression also links molecular activity to lesion pathology. TG2's involvement in both leukocyte trafficking and ECM remodeling further underscores its dual role as a marker of inflammation and tissue damage. Collectively, these findings position TG2 as a central biomarker with potential applications in disease monitoring, patient stratification, and early intervention strategies, offering novel avenues for precision medicine approaches in MS.

7. Limitations of current biomarkers for multiple sclerosis and the potential added value of transglutaminase 2

7.1. Limitations of conventional and emerging biomarkers

The diagnosis of MS still relies primarily on identification of immunoglobulin G oligoclonal bands (OCBs) in CSF, detected through isoelectric focusing followed by immunofixation. These bands indicate intrathecal immunoglobulin synthesis, suggesting local immune activation within the CNS.[2,99,100] However, this technique has several limitations: It is labor-intensive, requires

Table 4. Molecular targets and cellular processes regulated by TG2 during neuroinflammation

TG2 target/process	Mechanism regulated by TG2	Effect on inflammation	References
Pro-inflammatory cytokines (IL-6, IL-1β, TNF-α)	TG2 modulates inflammatory signaling pathways, including NF-κB activation, influencing cytokine expression	Amplification and persistence of inflammatory responses	5,30,75-78
NF-κB signaling pathway	TG2 regulates NF-κB activity through interaction with signaling proteins and modification of IκBα	Increased transcription of inflammatory genes and cytokine production	5,19,30
Microglial activation	TG2 influences microglial phenotype and production of inflammatory mediators	Sustained neuroinflammation and contribution to neuronal damage	5,75,76,78
Macrophage/monocyte migration and activation	Extracellular TG2 interacts with ECM proteins and integrins, regulating adhesion, migration, and phagocytic activity	Increased immune cell recruitment and inflammatory cell accumulation	83,85,89,98
Astrocyte activation and reactive gliosis	TG2 regulates astrocyte responses and ECM remodeling through extracellular protein cross-linking and fibronectin organization	Maintenance of chronic inflammatory environment and altered tissue remodeling	7,87,94
ECM components (fibronectin, proteoglycans)	TG2 promotes ECM stabilization and remodeling through protein cross-linking and integrin-mediated interactions	Regulation of immune cell adhesion, migration, and inflammatory cell trafficking	8,28,29,41
Oxidative stress pathways (ROS, NO, iNOS)	TG2 participates in cellular stress responses and inflammatory signaling under oxidative conditions	Oxidative damage and amplification of inflammatory responses	22,23,27,30
Apoptosis-related pathways	Intracellular TG2 interacts with apoptosis regulators and mitochondrial proteins, influencing cellular stress responses	Modulation of neuronal survival or cell death depending on cellular context	42,43,46,47
Protein aggregation (amyloid-β, tau, α-synuclein, polyglutamine proteins)	TG2-mediated cross-linking promotes stabilization of pathological protein aggregates	Links protein aggregation with activation of inflammatory responses	54-66,75-84

Abbreviations: ECM: Extracellular matrix; IκBα: NF-κB inhibitor; iNOS: Inducible nitric oxide synthase; IL: Interleukin; NF-κB: Nuclear factor-κB; NO: Nitric oxide; ROS: Reactive oxygen species; TG2: Transglutaminase 2; TNF-α: Tumor necrosis factor-α.

Table 5. Disease-specific roles and mechanisms of TG2 involvement in neurodegenerative disorders

Disease	Evidence of TG2 involvement	Main effects/functions of TG2	References
HD	Increased TG2 expression and activity have been detected in HD brain tissue	TG2 catalyzes the cross-linking of expanded polyglutamine proteins, including huntingtin, thereby promoting protein aggregate formation; TG2 inhibition/ablation has been associated with reduced neuronal damage in experimental models	52,53,80-82
AD	Increased transglutaminase activity and TG2 expression have been reported in AD brain	TG2 promotes cross-linking of tau and amyloid-β, contributing to formation and stabilization of pathological aggregates; TG2 may also enhance amyloid-β-induced inflammatory responses in immune cells	55,56,60-67,75
PD	TG2 is implicated in α-synuclein aggregation and Lewy body-related pathology	TG2-mediated cross-linking promotes α-synuclein oligomerization and aggregation, potentially contributing to neuronal dysfunction	57,81
MS	Altered TG2 expression has been detected in peripheral immune cells and CNS cells in MS models and patients	TG2 regulates macrophage/monocyte migration, myelin phagocytosis, astrocyte responses, and extracellular matrix remodeling, influencing inflammation and demyelination	83,86-89,94,95,98
ALS	TG2 alterations have been associated with neurodegenerative mechanisms in ALS	TG2 may contribute to protein cross-linking, cellular stress responses, and neuronal vulnerability, although its specific role remains incompletely defined	6
PSP	Increased TG2 activity and expression have been detected in PSP brain	TG2-mediated tau cross-linking may contribute to abnormal tau aggregation and neurodegenerative pathology	59,77

Abbreviations: AD: Alzheimer's disease; ALS: Amyotrophic lateral sclerosis; CNS: Central nervous system; HD: Huntington's disease; MS: Multiple sclerosis; PD: Parkinson's disease; PSP: Progressive supranuclear palsy; TG2: Transglutaminase 2.

specialized technical skills, can take three to four hours per session, and demands strict parallel analysis of serum and CSF samples. Moreover, interpretative thresholds (two versus three bands as a cutoff) remain controversial, and assessment can be subjective.[101-103] To overcome these challenges, measurement of kappa free light chains (κFLCs) in CSF has emerged as a quantitative alternative or complement to OCB identification. Several studies have demonstrated that the κFLC index (calculated from CSF and serum levels, and normalized to the CSF/serum albumin ratio) achieves sensitivity and specificity comparable to OCBs.[104] In multicenter cohorts of MS and clinically isolated syndrome (CIS) patients, the κFLC index showed a sensitivity of approximately 88%, compared with approximately 85% for OCBs. κFLC levels also remain elevated in primary progressive MS, confirming its utility across disease subtypes.[105]

Nevertheless, the κFLC approach is not without limitations: No universally accepted cutoff exists, standardization across measurement methods is lacking, and κFLC levels can be influenced by BBB dysfunction.[106,107] Other emerging biomarkers in MS include glial fibrillary acidic protein (GFAP), a marker of astrocytic injury; chitinase-3-like protein 1 (CHI3L1/YKL-40), associated with glial activation and inflammation; C-X-C motif chemokine ligand 13 (CXCL13), reflecting B-cell chemotaxis; as well as microRNAs (miRNAs) and extracellular vesicles (exosomes), which provide information on transcriptional regulation and intercellular signaling.[108-111] While these markers offer important mechanistic insights and correlate with disease activity or progression, they face challenges such as variability across patients, lack of standardized assays, and uncertain specificity for MS versus other neuroinflammatory conditions.

7.2. Transglutaminase 2 as a complementary and pathogenic biomarker

In this context, TG2 offers several potential advantages as a complementary biomarker:

- Integration of pathogenic pathways: TG2 is directly involved in processes central to MS pathology, including leukocyte migration across the BBB, microglial and macrophage activation, ECM remodeling, and glial fibrosis.[90-92] These mechanisms are not captured by conventional markers such as OCBs or κFLCs.
- Peripheral accessibility and isoform specificity: TG2 expression can be measured in PBMCs, and specific splice variants (e.g., V4a, V4b) provide a stable molecular signature of inflammatory activity, potentially more informative than total protein levels.[96]
- Prognostic and risk stratification potential: TG2 levels in PBMCs correlate with clinical severity and lesion load, and early studies suggest its utility as a precursor biomarker in CIS or RIS for predicting conversion to MS.[95,97]
- Diagnostic complementarity: TG2 could enrich existing biomarkers by integrating functional and pathogenic dimensions, rather than only reflecting B-cell activity or general glial injury. Its measurement could therefore improve patient stratification, monitoring of disease progression, and evaluation of therapeutic response.[95]

In conclusion, while OCBs, κFLCs, GFAP, CHI3L1/YKL-40, CXCL13, and miRNAs/exosomes remain essential tools, they are limited by methodological constraints, inter-individual variability, or lack of specificity. TG2 emerges as a promising complementary biomarker that bridges molecular, cellular, and clinical information, potentially enabling a more precise and personalized approach to MS diagnosis and management.

8. Challenges and limitations of transglutaminase 2 as a biomarker in multiple sclerosis

Despite promising data, the potential use of TG2 as a biomarker in MS is constrained by several important limitations. First, there is a lack of standardization regarding what is measured: Some studies assess TG2 at the mRNA level in PBMCs, others at the protein level, or by evaluating enzymatic (transamidating) activity. This heterogeneity complicates comparisons across studies and makes it unclear which readout—mRNA, protein, or activity—better reflects disease activity or progression. For TG2 to become clinically applicable as a complementary biomarker within the diagnostic framework of MS, future studies will need to establish standardized assays and clinically validated cutoff values, and demonstrate independent diagnostic and prognostic performance beyond currently used biomarkers. For isoform-specific TG2 measurements, particularly splice variants such as V4a and V4b, additional technical harmonization will be required, including validated isoform-specific assays, appropriate normalization procedures, and assessment of inter-laboratory reproducibility.

Second, existing study cohorts are relatively small. For instance, the foundational study on PBMC-derived TG2 mRNA included 161 MS patients and 151 controls, providing significant results but still limited in population heterogeneity.[95] Population studies, such as those on

individuals with RIS, remain preliminary and are limited by small sample sizes, as demonstrated in a recent study comparing 10 RIS patients, 10 relapsing-remitting MS patients, and 10 healthy controls.[97]

Third, there is a lack of longitudinal studies following TG2 over time in relation to relapses, disability progression, treatment response, or conversion from RIS to clinically definite MS. Without these data, it is difficult to establish the temporal stability, intra-individual variability, and true predictive value of TG2 as a biomarker. To mitigate intra-individual variability of TG2 expression in PBMCs, future studies should adopt standardized sampling protocols, including controlled timing of blood collection, documentation of disease phase (relapse versus remission), treatment status, and concurrent inflammatory conditions. Longitudinal repeated measurements within the same patients, together with normalization strategies and standardized quantitative assays, will be essential to determine the biological stability of TG2 expression and improve its clinical reliability as a biomarker.

Fourth, the specificity of TG2 for MS remains unclear: TG2 is a ubiquitous enzyme involved in inflammation, cell migration, ECM remodeling, and fibrosis—mechanisms common to many neurological and inflammatory CNS diseases.[85] Therefore, the proposed biomarker model does not consider peripheral TG2 levels as an MS-specific marker, but rather as part of a multimodal signature integrating TG2 expression with clinical parameters, imaging findings, and established biomarkers. Future studies should evaluate TG2 in well-characterized cohorts while accounting for systemic inflammatory conditions and potential confounding factors to determine its disease-specific contribution. Although multi-omics and proteomic approaches are increasingly being applied to biomarker discovery in MS, specific validated pipelines integrating TG2 with established biomarkers such as GFAP and κFLCs are currently lacking. Integrative approaches combining proteomic, transcriptomic, and clinical data through computational strategies may help determine whether TG2 provides independent diagnostic or prognostic information beyond existing biomarkers. Therefore, increased TG2 levels (mRNA, protein, or activity) might reflect broader inflammatory or tissue responses rather than MS-specific pathology.

Finally, heterogeneity in cellular sources expressing TG2 (monocytes/macrophages, endothelial cells, astrocytes, neurons) and the variability in activation states (acute versus chronic phase, relapse versus remission) complicates interpretation of isolated measurements. Post-mortem immunohistochemical studies show TG2 expression in monocytes/macrophages but not in T or B lymphocytes, suggesting that TG2 reflects the activity of different cellular lineages rather than classical B-cell responses.[89]

9. Towards precision medicine: Transglutaminase 2 in multiple sclerosis monitoring and therapy

The emerging evidence of TG2's involvement in the pathogenesis and progression of MS opens several avenues for future research, both as a biomarker and as a potential therapeutic target. TG2 participates in key pathogenic processes, including leukocyte migration across the BBB, microglial activation, ECM remodeling, and macrophage polarization.[89,98] Understanding its role could enhance patient stratification, improve disease monitoring, and identify new therapeutic strategies.

9.1. Transglutaminase 2 as a biomarker: Proteomics and imaging integration

One promising direction involves unbiased proteomic studies to validate TG2 combined with other molecules in multi-marker panels. Such approaches could identify complementary biomarkers, enhance diagnostic specificity, and capture disease heterogeneity by integrating TG2 with established indicators, including OCBs, κFLCs, GFAP, YKL-40, CXCL13, miRNAs, and exosomal cargo. Proteomics could also improve biomarker robustness by clarifying which TG2 isoforms or post-translational modifications most accurately reflect disease activity. Another important avenue is integrating TG2 assessment with advanced imaging techniques. Combining TG2 measurements with MRI biomarkers, including paramagnetic susceptibility mapping for iron deposition or gadolinium enhancement for BBB leakage, could allow correlation between molecular activity and lesion characteristics. Similarly, coupling TG2 evaluation with optical coherence tomography may provide insights into retinal neurodegeneration and axonal loss, facilitating longitudinal monitoring of neuroinflammation and neurodegeneration. Such multimodal approaches could improve patient stratification, enable early intervention, and guide therapeutic decisions.

9.2. Transglutaminase 2 as a therapeutic target: Pharmacological and nutraceutical approaches

Transglutaminase 2 also represents a potential therapeutic target in MS. Given its involvement in leukocyte migration across the BBB, microglial activation, ECM remodeling, and macrophage polarization, pharmacological inhibition of TG2 may modulate neuroinflammation and tissue damage. Current strategies focus on blocking its transamidation activity, which is central to its pathogenic effects. Among TG2's multiple functions, transamidation activity currently

represents the most promising therapeutic target in MS, as it directly contributes to ECM remodeling, leukocyte infiltration, and chronic neuroinflammation. By contrast, although GTP binding regulates TG2 conformation and intracellular signaling, its therapeutic relevance in MS remains less well defined and requires further investigation. Competitive, reversible, and irreversible inhibitors have been developed, although challenges remain regarding specificity, BBB penetration, and off-target effects on other transglutaminase isoforms.[112-114] Innovative approaches, such as nanoparticle-based delivery systems, could enhance CNS targeting while reducing systemic side effects, potentially offering a disease-modifying strategy in MS, particularly in the early inflammatory phase of the disease.[115-117]

A further potential avenue involves using nutraceuticals to modulate neuroinflammatory and oxidative pathways relevant to TG2 regulation. Preclinical studies in microglia-based cellular models, including primary and immortalized microglial cells, have shown that natural bioactive compounds can reduce pro-inflammatory cytokine production, attenuate oxidative stress, and limit microglial activation.[118,119] Additional evidence from EAE and BBB dysfunction models indicates that nutraceuticals with antioxidant and vasoprotective properties can improve barrier integrity, decrease leukocyte infiltration, and promote a more balanced redox environment.[120] Given TG2's involvement in leukocyte migration and ECM remodeling, nutraceutical-based interventions may complement strategies targeting TG2 directly.

9.3. Future perspectives

Future research on TG2 in MS should aim to:

(i) standardize TG2 detection and activity assays across biological compartments;
(ii) incorporate TG2 into multi-marker panels validated by proteomics;
(iii) explore targeted TG2 inhibition using selective pharmacological or nanomedicine-based strategies;
(iv) integrate molecular TG2 data with imaging modalities (MRI, optical coherence tomography) to achieve comprehensive disease monitoring;
(v) evaluate nutraceutical-based approaches capable of modulating TG2-related inflammatory and oxidative pathways.

By combining these approaches, TG2 could evolve from a promising experimental biomarker into a clinically relevant tool for diagnosis, prognosis, and treatment monitoring in MS, ultimately bridging molecular pathology with personalized medicine.

10. Conclusion

In summary, TG2 is emerging as a mechanistically informative and potentially clinically relevant biomarker in MS. Its central involvement in neuroinflammatory cascades, BBB dysfunction, and neurodegenerative processes underscores its relevance for capturing both inflammatory and chronic-progressive disease components. Yet, despite promising experimental and early translational findings, its adoption in clinical practice requires further rigorous validation. Future efforts should prioritize harmonizing TG2 detection methods across biological matrices, characterizing isoform- and activity-specific signatures, and integrating TG2 measures within multimodal biomarker frameworks including proteomics, neuroimaging, and systems-level approaches. Large, longitudinal, phenotype-stratified cohorts will be essential to delineate TG2's diagnostic sensitivity, prognostic accuracy, and utility as a treatment-response indicator. Establishing these parameters will determine whether TG2 can evolve into a robust and clinically impactful biomarker, ultimately contributing to more precise biological profiling and improved therapeutic decision-making in neurodegenerative disorders such as MS.

Dedication

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