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Targeting GFAT for diabetes management: a therapeutic approach to alleviate hexosamine pathway-induced complications

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ABSTRACT

Diabetes mellitus (DM) is one of the leading non-communicable metabolic disorders. Over time it may lead to the development of serious complications. Glutamine fructose-6-phosphate amidotransferase (GFAT), is the first and rate-limiting enzyme that plays an important role in regulating the hexosamine biosynthesis pathway (HBP). During hyperglycemia, excess glucose that enters the cell gets diverted into the HBP by this GFAT enzyme. Recent studies have suggested that the overexpression of GFAT is associated with insulin resistance and diabetic complications and it is mainly seen in patients with diabetes.

Using various sources, an extensive literature survey was conducted to determine the complex role of GFAT enzyme and their involvement in the modification of various proteins and transcription factors, contributing to the development of diabetes complications.

The overexpression of GFAT during hyperglycemia increases the flux through the HBP, resulting in insulin resistance, and various vascular complications such as nephropathy, neuropathy, retinopathy, delayed wound healing, and cardiovascular complications.

Inhibiting GFAT emerges as a potential therapeutic strategy to counteract hexosamine pathway-induced insulin resistance and alleviate vascular complications in diabetes. The multifaceted role of GFAT in diabetic complications underscores its significance as a therapeutic target for future advancements in diabetes management.

Keywords: GFAT, Diabetes, Hexosamine pathway, Insulin resistance, Diabetic complications

1. INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by elevated plasma glucose levels [1], resulting from defective insulin secretion (T1DM) or resistance to insulin action (T2DM) [2]. Globally, diabetes stands as the 7th leading cause of mortality. "The estimated global prevalence was 463 million people cases in 2019, a number projected to rise to 578 million cases by 2030 and 700 million cases by 2045, presenting a substantial global health challenge" [3]. Chronic hyperglycemia is the major factor responsible for the development of microvascular and macrovascular complications [4]. Several cellular and molecular mechanisms are involved. Among them, the hexosamine biosynthesis pathway (HBP) recently emerged as one of the important mechanisms through which hyperglycemia exerts its effect [5,6].

The hexosamine biosynthesis pathway is a relatively minor branch of glycolysis and it is regulated by the enzyme glutamine fructose-6-phosphate amidotransferase (GFAT), a first and rate-limiting enzyme responsible for directing incoming fructose-6-phosphate into the hexosamine pathway [7].

Several studies have highlighted the crucial role of GFAT enzyme in the involvement of insulin resistance and diabetic complications. For instance, during hyperglycemia, the flux through the hexosamine pathway increases as a result of increased GFAT activity, which leads to glucose-induced insulin resistance and vascular complications[8,9]. Since the HBP depends on the GFAT activity, inhibiting this enzyme could be beneficial in treating the hexosamine pathway-induced insulin resistance and vascular complications in diabetes. This comprehensive review overviews the multifaceted role of GFAT in diabetic complications. Additionally, it also summarizes the strategies for targeting the GFAT, offering insight into future prospects to develop effective therapies, paving the way for future advancements in treatment strategies for type 2 diabetes complications.

GFAT enzyme and hexosamine pathway

The hexosamine biosynthesis pathway constitutes a relatively minor branch of glycolysis, utilizing approximately 2-3% of

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total glucose [7]. HBP is catalyzed by the enzyme GFAT, which is the primary and rate-limiting enzyme. As shown in Figure 1, the GFAT enzyme plays a main role in converting fructose-6-phosphate (F-6-P) and glutamine to glucosamine-6-phosphate (GlcN-6-P) and glutamate. Further consequent steps lead to the metabolism of GlcN-6-P into UDP-N-acetyl glucosamine (UDP-GlcNAc). UDP-GlcNAc has three major actions 1) as a major end product of HBP, 2) as an allosteric feedback inhibitor of the enzyme GFAT, 3) as an obligatory substrate of O-GlcNAc transferase (OGT). 'OGT is a cytosolic and nuclear enzyme, facilitating a reversible post-translational modification wherein N-acetyl glucosamine (GlcNAc) is transferred in O-linkage to specific serine/threonine residues of numerous proteins' and transcription factors [10] include, IRS-1, IRS-2 [11], Sp1[12–16], CREB [17], PDX-1[18], c-myc [19], Stat 5 [20].

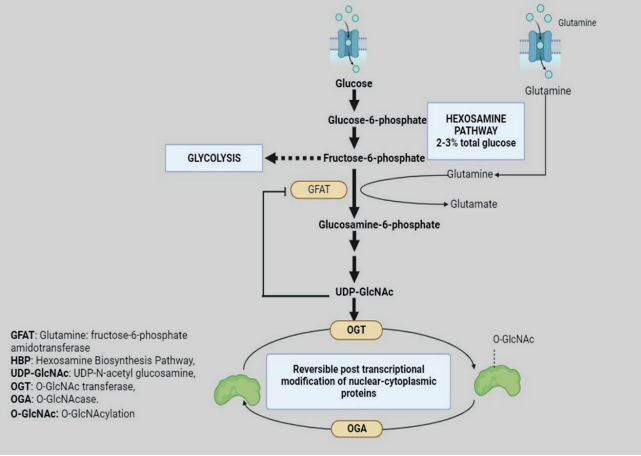

Figure 1. *GFAT Enzyme and Hexosamine Biosynthesis Pathway*

GFAT isoforms and tissue-specific expressions

Three distinct isoforms of GFAT have been identified in humans: hGFAT1, hGFAT2, and hGFAT1Alt (also known as GFAT1-L) [21]. hGFAT1 is expressed all over the body, and high expression was seen in the placenta, testis, pancreas, skeletal muscle, and heart. hGFAT2 is highly expressed in the central nervous system (CNS), heart, liver, and pancreas [22]**.** Various cell types within a tissue may tend to express hGFAT1 or hGFAT2. For instance, hGFAT1 is the primary isoform in cardiac myocytes and GFAT2 in cardiac fibroblasts [23]. hGFAT1 Alt represents an alternatively spliced variant of hGFAT1, is highly expressed in striated muscle [24].

Mechanism of GFAT enzyme regulation and substrate binding

In the initial step, as shown in Figure 2(a), GFAT catalysis the synthesis of d-glucosamine-6-phosphate (GlcN6P) from two substrates: l-glutamine (l-Gln) and Fructose-6-phosphate (Frc6p). This reaction results in the release of l-Glutamate (l-Glu) as a byproduct [25].

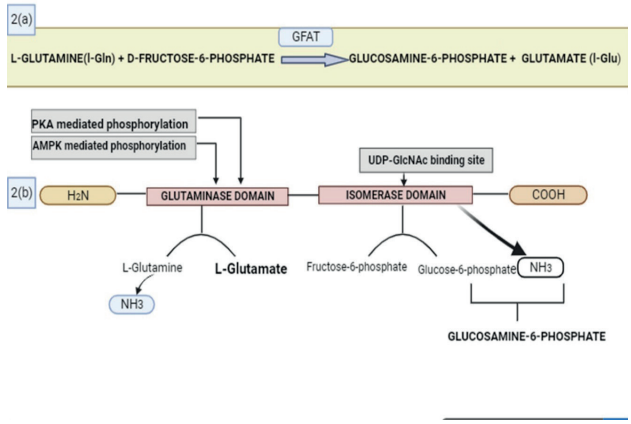

Figure 2. *Mechanism of GFAT enzyme regulation and substrate binding. 2(a) Reaction catalyzed by GFAT enzyme. 2(b) GFAT structural domains and its regulations.*

Glutamine fructose-6-phosphate amidotransferase comprises two distinct domains: the glutaminase domain, responsible for hydrolyzing l-Gln to l-Glu by releasing ammonia, and the isomerase domain, which has a dual function. The isomerase domain not only converts Fructose-6-Phosphate to d-glucose-6-phosphate (Glc6P) but also facilitates the ammonia transfer to Glc6P, leading to the production of GlcN6P [26], as shown in Figure 2(b).

Importantly, GFAT interacts with both of its substrates, l-Gln and Frc6P, to activate the ammonia channel that connects the active region of the two domains [27]. The sequence of amino acids and biochemical function of GFAT enzyme are preserved from bacteria to humans, however, the only significant difference is that the UDP-GlcNAc exclusively inhibits only eukaryotic GFAT by binding to the isomerase domain [28]. This binding event disrupts inter-domain communications, which not only impacts the isomerase domain but also inhibits glutaminase activity. Furthermore, as shown in Figure 2(b), eukaryotic GFAT may be controlled by phosphorylation by AMP-activated protein kinase (AMPK) and cAMP-dependent protein kinase (PKA) [29,30]. However, these effects are controversial, and further studies are needed to fully understand the mechanisms.

Difference in GFAT activity between Type 1 and Type 2 diabetes

Insulin resistance is a key feature of type 2 diabetes, and GFAT enzyme has been shown to play a significant role in this condition [8]. In contrast, the role of GFAT enzyme in type 1 diabetes remains less understood. Type 1 diabetes is primarily characterized by autoimmune – mediated destruction of pancreatic beta cells, leading to absolute insulin deficiency. Unlike type 2 diabetes, where insulin resistance is a central feature, type 1 diabetes involves a different pathophysiological mechanism. As a result, the involvement of GFAT in type 1 and type 2 diabetes might differ.

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Factors influencing the potential increase in GFAT activity

The potential increase in the GFAT activity could result from various factors, including the loss of negative feedback inhibition, phosphorylation of serine 205mediated by PKA, hyperglycemia, and oxidative stress.

Gain-of-function mutant G451E of GFAT enzyme

Under normal conditions, UDP-GlcNAc inhibits the eukaryotic GFAT enzyme through allosteric feedback inhibition. UDP-GlcNAc binds to the isomerase domain of GFAT, disturbing inter-domain communication. However, the sensitivity to this inhibition can be diminished by the gain-of-function mutation G451E of the GFAT enzyme. This decreased sensitivity to UDP-GlcNAc causes the potential to increase enzyme activity, consequently resulting in an elevated flux through the hexosamine pathway [31].

R203H Variant of GFAT enzyme

The GFAT R203H variant is less likely to be phosphorylated by PKA at position 205 (a modification that typically reduces the enzyme activity). Additionally, R203H gain-of-mutation is not affected by UDP-GlcNAc feedback inhibition, thereby increasing the enzyme activity [32].

Hyperglycemia-induced mitochondrial superoxide production:

As shown in Figure 3, Hyperglycemia is the primary factor for upregulating GFAT activity by increasing superoxide production or directly entering into the cells. During hyperglycemia, there will be elevated ROS production within the mitochondria. These ROS subsequently induce strand breakage of strands in nuclear DNA, causing the activation of poly (ADP-ribose) polymerase (PARP). PARP, in turn, modifies glyceraldehyde-3 phosphate dehydrogenase (GADPH), resulting in the inhibition of its activity. This inhibition causes an increase in the number of glycolytic products that are upstream of GADPH. Moving further upstream, there is an elevation in the level of glycolytic metabolite fructose-6-phosphate. Since fructose-6-phosphate is a substrate of the GFAT enzyme, its increased level enhances the GFAT enzyme activity [33].

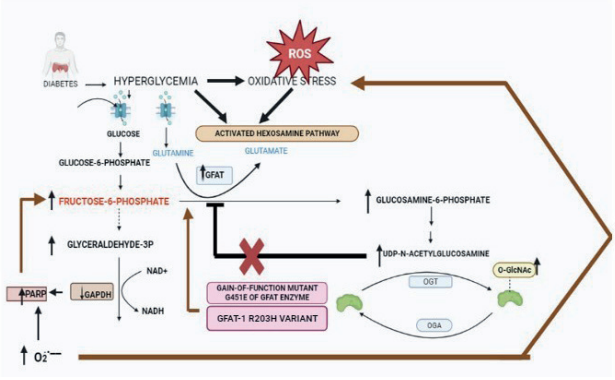

Figure 3. *Various factors influencing the potential increase in GFAT activity*

GFAT enzyme and oxidative stress

Oxidative stress arises from an imbalance between the production of reactive oxygen species (ROS) and body's antioxidant defences, leading to damage in the cells and tissues [34]. Increased levels of oxidative stress biomarkers, such as TBARS (lipid-related thiobiobarbituric acid reactive substances, MDA (melondialdehydes) and isoprostanes; protein related protein carbonyls and nitrosylated proteins, AGEs (carbohydrate-related advanced glycation end-product and 8-OHdG (DNA-related 8-hydroxy-deoxyguinine) observed in various in-vitro and in-vivo experimental models of diabetes [35].

The relationship between the GFAT enzyme and oxidative stress is complex. In the diabetic individuals, the GFAT activity has been positively associated with increased levels of TBARS, and PCO (protein carbonyl contents, which are the markers of lipid peroxidation and oxidative stress in proteins, respectively [36]. This suggests that GFAT may influence oxidative stress through its effects on cellular glycosylation patterns, which can modulate the functions of various cellular proteins, involved in oxidative stress responses. But the exact mechanism is not fully understood.

Role of GFAT enzyme in the development of insulin resistance and vascular complications

Glutamine fructose-6-phosphate amidotransferase enzyme plays a crucial role in the hexosamine biosynthesis pathway and regulates glucose metabolism and insulin signaling. As shown in Figure 4, the Dysregulation of the GFAT enzyme has been implicated in the development of insulin resistance and beta cell dysfunction, which is a common cause of type 2 diabetes. Moreover, the GFAT enzyme has been linked to the development of diabetic complications, such as diabetic nephropathy, delayed wound healing, cardiac hypertrophy, and heart failure.

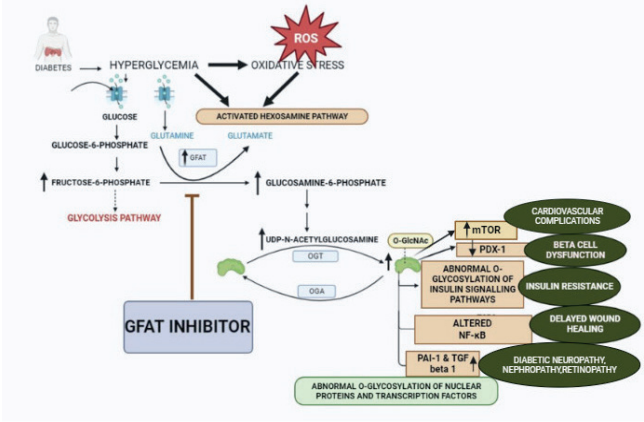

Figure 4. *Dysregulation of GFAT enzyme and associated complications.*

a. Insulin resistance

Insulin resistance is characterized by a diminished biological response to insulin stimulation in target tissues, predominately

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affecting the liver, muscle, and adipose tissues. This impairment hinders the effective disposal of glucose, leading to a compensatory elevation in beta-cell insulin production and the development of hyperinsulinemia [37].

Recent studies have suggested that GFAT overexpression in muscle, fat [38], and liver[39] results in glucose intolerance and insulin resistance in mice. Furthermore, modest GFAT overexpression in the pancreas may result in either hyperinsulinemia, obesity, and insulin resistance in transgenic mice [40] or impaired glucose-stimulated insulin secretion in isolated rat islets.

The GFAT enzyme overexpression impairs glucose transporters by causing aberrant

O-GlcNAcylation of proteins at serine/threonine residues that could potentially influence the protein activity, transcription factors and signaling transduction pathways. Specifically O-GlcNAc modifies the IRS-1/2, PI3K, PDK1, Akt, and GLUT 4 which are the components of the cell signaling pathway[11,41–43]. Additionally, in diabetes, FOXO, ChREBP1, CRTC2, PGC1α, LXR a key transcriptional factor regulating gluconeogenesis and lipogenesis genes in liver, was subjected to GFAT-induced O-GlcNAcylation [44-46].

Furthermore, insulin resistance was observed due to the of impaired GLUT4 translocation in transgenic mice overexpressing GFAT enzymes in their muscles and adipose tissues [47].

b. Beta-cell dysfunction

The overexpression of GFAT causes beta-cell deterioration, resembling the dysfunction observed in people with diabetes. This is due to the suppression of glucose-stimulated secretion of insulin and decreased mRNA levels of many genes specific to beta cells, including, insulin, GLUT2, and glucokinase. Additionally, GFAT overexpression increases O-linked glycosylation and subsequent decrease in the binding ability of PDX-1 to DNA. PDX-1 is a crucial transcription factor that controls all three genes [48].

c. Vascular complications

The risk of developing vascular complications in patients with (T2DM) is related to the GFAT activity. Hyperglycemia-induced overexpression of the GFAT causes a four-fold increase in O-GlcNAcylation of the transcription factor Sp1[12–16], which activates PAI-1 promoter, TGF-alpha, and TGF-β1 in smooth muscles, endothelial cells, and mesangial cells resulting in vascular complications like diabetic nephropathy, retinopathy and neuropathy [9,12,35,49-53].

d. Delayed wound healing

Thrombospondin 2(TSP 2) is a matricellular protein with a demonstrated role in response to injury and was associated with delayed healing[54]. During increased flux, the GFAT enzyme activity increases, which in turn NF-κB response element present in the promoter region of Thrombospondin 2 (TSP 2) gets activated, through increased O-glycosylation. This activation ultimately results in the overexpression of TSP2 protein and is implicated in delayed wound healing [55].

e. Cardiovascular complications

The upregulation of GFAT1 in cardio myocytes is associated with pathological cardiac remodeling, including cardiac hypertrophy and heart failure. This is due to the direct stimulation of the mTOR pathway by GFAT1 through O-GlcNAcylation. However, inhibiting GFAT1 has been found to suppress mTOR and cell growth [56].

Strategies for targeting GFAT enzyme

The significant role of GFAT enzyme in diabetic complications has been increasingly emphasized by recent evidence. Therefore, developing a potential GFAT inhibitor can emerge as a promising target for the treatment of diabetes. The GFAT enzyme can be targeted by developing glutamine analogs such as small molecule inhibitors, identifying potential biomarkers, exploring natural compounds, targeting particular isoforms and tissue-specific expressions of GFAT, feedback inhibition, and by PKA phosphorylation.

Glutamine analogs and small molecule inhibitors: The mechanism of glutamine analogs in inhibiting GFAT enzyme involves interaction with the active sites, thereby interfering with the binding of the natural substrate, glutamine. This inhibition could be competitive or non-competitive. Small molecule inhibitors such as 6-diazo-5-oxo-norleucine (DON) and O-diazo acetyl-L-serine (azaserine) are reactive electrophiles that mimic glutamine and shown to inhibit GFAT activity in cancer cells[57], but their gastrointestinal (GI) toxicity limits its use[58]. Therefore, developing prodrugs with reduced gastrointestinal toxicity may be beneficial for advancing its clinical utility.

GFAT and HbA1c in diabetes management: Hemoglobin A1c (HbA1c) is a widely recognized and clinically valuable biomarker for diabetes management. Higher HbA1c levels indicate poor glycemic control. The study reported a positive correlation between GFAT activity and HbA1c levels in type 2 diabetic patients, indicating that higher GFAT activity is associated with increased HbA1c [36]. Therefore, integrating GFAT activity into diabetes monitoring could enhance the ability to predict and manage glycemic control more effectively.

Isoforms and tissue – specific targeting: Understanding the tissue-specific expression and functional differences of GFAT isoforms can provide insight into potential targets against diabetes. Recently researchers have also developed an interest in developing GFAT inhibitors for diabetic complications, with GFAT 1 being the most promising target due to its high expression in liver and fat. A reversible GFAT inhibitor, RO0509347, has demonstrated fewer GFAT inhibition in vitro, and efforts to enhance its efficacy or identify alternative scaffolds as potential GFAT inhibitors are ongoing[59].

Natural compounds: Natural compounds hold significance because of their lower toxicity as compared to synthetic drugs. The inhibitory potential of natural compounds, such

as anacardium occidentale [60] and anthocyanins [61] against GFAT activity, has been identified in recent in-*silico* studies, warranting further *in vitro* and *in vivo* experiments to confirm their inhibitory capacities.

Feedback inhibitors: UDP-GlcNAc is the feedback inhibitor of the GFAT enzyme. Research has shown that the GFAT1 activity can be modulated by UDP-GlcNAc. Developing inhibitors that mimic the UDP-GlcNAc feedback inhibition effect, can potentially be used to regulate GFAT-1 activity [62].

PKA phosphorylation: Furthermore, GFAT-1 activity is regulated by phosphorylation by PKA. Targeting the PKA signaling pathway to modulate GFAT phosphorylation could be a potential approach to regulate GFAT-1 activity [62].

Lifestyle modifications: Lifestyle factors such as diet, exercise, and other lifestyle modifications play a crucial role in regulating GFAT activity. Diets rich in antioxidants and low in refined sugars can help lower glucose levels and oxidative stress, thereby potentially mitigating glucose-induced GFAT activity.

Future prospectives

GFAT as a marker for diabetes treatment response: Studies indicates that the GFAT activity is elevated in individuals with type 2 diabetes compared to non – diabetic controls and is positively associated with key markers of diabetes severity such as HbA1c, insulin resistance (measured by HOMA-IR), postprandial plasma glucose levels, and oxidative stress indicators like protein carbonyls and lipid peroxidation. Given these association, future research should explore the potential of GFAT as a biomarker for monitoring diabetes treatment responses and effectiveness of therapeutic interventions, potentially leading to more precise management of glycemic control and overall disease progression.

Predictive marker for complications: Research should focus on exploring GFAT as a predictive marker for the risk of diabetic complications due to its role in mediating the expression of several genes associated with vascular complications, including TGF-β1 and PAL-1, NF-κβ, which are involved in fibrosis and inflammation.

Research gap: Further research in this area should address the knowledge gap and to fully understand the molecular mechanisms and potential side effects of GFAT inhibitors, as well as optimal dosing and combination strategies for maximizing their efficacy in the clinic.

Investigating combination therapies involving GFAT inhibitors with other conventional anti-diabetic drugs may lead to effective and personalized treatment strategies for diabetes management.

While GFAT is recognized for its role in type 2 diabetes, there is a significant gap in understanding its specific function in type 1 diabetes. Therefore, further research is needed, with a particular focus on its role in type 1 diabetes.

Well-designed clinical trials are essential for evaluating the safety and efficacy of GFAT inhibitors for optimal therapeutic regimens.

Research should aim to address the challenges associated with developing interventions targeting GFAT in diabetic complications, such as off-target effects, and potential impact on normal cellular functions.

Conclusion

In conclusion, L-Glutamine D-fructose-6-phosphate amidotransferase (GFAT), a first and rate-limiting enzyme in the hexosamine biosynthesis pathway (HBP). The dysregulation of GFAT has been associated with insulin resistance, beta cell dysfunction, and the development of vascular complications. Several factors, including gain-of mutation, phosphorylation, hyperglycemia, and oxidative stress, can influence GFAT activity, further exacerbating these complications. Several approaches such as small molecule inhibitors and natural compounds

offer a potential strategy for targeting GFAT. Also targeting particular isoforms and tissue-specific expressions of GFAT, feedback inhibition, and PKA phosphorylation offer additional dimensions to therapeutic strategies. However, despite the abundance of research on GFAT dysregulation in diabetes, there is a limited number of available GFAT inhibitors, and none have advanced to clinical trials. Therefore further research and clinical investigations are needed to develop potential therapeutic strategies for inhibiting GFAT. Future research should prioritize addressing knowledge gaps, investigating new biomarkers, investigating thorough clinical trials, investigating combination therapies, its role in type 1 diabetes and overcoming challenges linked to interventions targeting GFAT. Progress in these areas will not only just enhance the understanding of the clinical implications of GFAT enzyme but will also create opportunities for formulating effective treatment approaches for individuals with diabetes.

Compliance with ethical standards

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Authors contributions: IO :Conceptualization, writing original draft, writing-review and editing, data curation, VR:Formal analysis, supervision, investigation, HM: Data curation, validation and software. All authors approved the final version of the manuscript.

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