

ENZYMATIC LANDSCAPE OF ANXIETY DISORDERS: INTEGRATING GAD, MAO-A, HPA-AXIS ENZYMES, EPIGENETIC MODULATORS, CYP450 PATHWAYS AS DIAGNOSTIC BIOMARKERS AND THERAPEUTIC TARGETS

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ABSTRACT

According to current trends in Anxiety research anxiety is recognised as multi enzymatic pathological condition of upstream biosynthetic and regulatory dysfunctions rather than classical receptor-centric GABAergic models. This review proposes and elaborates an “Enzymatic Theory” of anxiety which describes the upstream enzymatic dysregulation as a primary mechanistic pathophysiology of anxiety. We examine and explain the role of Glutamic Acid Decarboxylase isoforms (GAD65/GAD67) in main inhibitory neurotransmitter GABA-Aminobutyric Acid synthesis, Monoamine Oxidase-A (MAO-A) in serotonergic homeostasis, and 11 β -Hydroxysteroid Dehydrogenase (11 β -HSD1/HSD2) in hypothalamic-pituitary-adrenal axis modulation as core anxiogenic and anxiolytic determinants and Epigenetic enzymes which including HDACs, DNMTs, and HATs, are reviewed as master chromatin regulators or DNA methylation modulators that encode stress-induced transcriptional silencing within fear circuits in brain which are responsible for anxiety. Furthermore, cytochrome P450 (CYP3A4, CYP2C19) and UDP-glucuronosyltransferase (UGT) systems are analysed as main enzymes responsible for anxiolytic drug pharmacokinetics and pharmacogenomic polymorphisms. Diagnostic enzyme panels include COMT Val158Met, MAO-A uVNTR, and CYP genotyping are evaluated as translational biomarkers for precision psychiatry. The integration of enzyme-responsive drug delivery, AAV-mediated GAD gene therapy, and epigenome-editing strategies will be the best move from symptom-based treatment to mechanistically biomarker-guided precise anxiolytic therapy.

KEYWORDS: Anxiety disorders, Enzymatic theory, Epigenetic enzymes, Anxiolytic drug metabolism, Precision psychiatry

1. INTRODUCTION

The Latin word "anxieta," indicating "trouble of thoughts about an uncertain occurrence," and the Greek word "anxo" that indicates "to squeeze, grip, or press tight," are the sources of the English word "anxiety." The term "anxiety" describes the emotions that ordinary people experience when they are in danger or under stress. Anxious people often feel tight, irritated, and uncomfortable. Everyone worries in response to certain circumstances, but those who suffer from anxiety disorders encounter excessive and illogical feelings that disrupt their social lives, relationships, job and educational activities, as well as complimentary time [1].

1.1 Enzymatic theory of Anxiety

The “Enzymatic theory” of anxiety is evolving because due to the limitations of a completely receptor-centric GABA model, current research has focused more on upstream enzymatic mechanisms that regulate the production and synaptic availability of GABA, giving rise to the "Enzymatic theory" of anxiety. The rate-limiting enzyme, glutamic acid decarboxylase, (GAD), which catalyses the transformation of the excitable amino acid L-Glutamate into GABA, an inhibitory neurotransmitter, is the foundation of this concept. The enzyme GAD controls the rate at which GABA is produced by catalyzing the conversion of glutamic acid to GABA. These two isoforms play complementary and functionally different roles in the regulation of inhibitory neurotransmission: GAD65 is primarily localized to synaptic terminals and plays a specific role in the activity-dependent, fine-tuning regulation of inhibitory neurotransmission during times of increased neuronal activity, such as stress, while GAD67 is constitutively active, distributed throughout the neuron, and responsible for synthesizing over 90% of basal GABA levels in the brain [2]. According to the enzymatic theory, a fundamental and upstream pathogenic mechanism in anxiety disorders may be a main deficit or dysregulation in the enzymatic activity of GAD, as opposed to merely a downstream decrease in GABA receptor sensitivity. From the standpoint of translational pharmacology, this finding is especially important because it implies that the anxious phenotype and the clinically noted decreased therapeutic response to benzodiazepines in a subset of patients may be caused by decreased enzymatic synthesis of GABA at the synapse rather than just receptor dysfunction. Genetic variations in the glutamic acid

decarboxylase gene have also been linked to anxiety phenotypes in both human and animal models [3]. When taken as a whole, these convergent data indicate that studying the enzymatic regulation of the GABA biosynthesis pathway or other pathways more especially, the expression, activity, and modulation of GAD65 and GAD67—may reveal new, upstream, and mechanistically accurate therapeutic targets for the treatment of anxiety disorders that go far beyond the traditional receptor-level pharmacology of benzodiazepines and SSRIs.

2. ENZYMATIC REGULATION IN NEUROTRANSMITTER HOMEOSTASIS

2.1 Monoamine Oxidase-A (MAO-A) and Serotonergic Turnover

At the top of a regulated enzyme cascade, monoamine oxidase type A (MAO-A) (EC 1.4.3.4) effectively regulates the precise availability of serotonin (5-hydroxytryptamine, or 5-HT) around the brain's central nervous system. The mitochondria include a flavoprotein called monoamine oxidase. (MAO), and these catalyses the deamination process caused of dietary and minor amines including β -phenylethylamine and a variety of monoamine neurotransmitter compounds like dopamine, serotonin, and norepinephrine. MAO essentially comes in two isoforms: MAO-A as well as MAO-B, which vary in their preferred substrates, inhibitor sensitivity, and regional distribution within the nervous system. Both isoforms are well encoded by different genes on the X chromosome and share roughly 70% amino acid sequence identity [4]. In the isoforms of MAO, MAO-A is primarily responsible for maintaining serotonergic homeostasis because MAO-B selectively oxidises phenylethylamine whereas MAO-A selectively oxidises serotonin and norepinephrine. Dopamine in the central nervous system may also be oxidised by both isoforms [5]. The catalytic system of MAO-A depends on a covalently bound FAD, which stands for flavin cofactor at the molecular level. The 8-methylene molecule of the isoalloxazine. ring connects the covalently attached flavin, adenine dinucleotide component to the enzyme cysteine in both isoforms of MAO. In monoamine turnover the final metabolic endpoint is not aldehyde intermediate compound oxidised by the initial oxidative deamination. In monoamine turnover MAO-A transforms 5-HT into 5-hydroxy-3-indolacetaldehyde (5-HIAL), which is then further processed by the NAD⁺-dependent enzyme aldehyde dehydrogenase (ALDH) into 5-hydroxy-3-indolacetic acid (5-HIAA) [6]. So, the ratio of 5-HIAA to 5-HT in brain tissue and cerebrospinal fluid serves as a direct biochemical indicator or biomarker of MAO-A enzymatic activity and serotonergic turnover rate. Genetic knockout studies have confirmed this relationship, demonstrating that MAO-A-deficient mice have significantly lower 5-hydroxy-3-indolacetic acid and higher 5-HT in all major brain regions, with the 5-HT oxidative deamination index dropping to 41–45% of control values [7]. The production of reactive oxygen species (ROS) as enzymatic byproducts is an equally significant outcome of MAO-A catalytic activity. Aldehydes, ammonia, and hydrogen peroxide are byproducts of the oxidative deamination mechanism, and the production of hydrogen peroxide and other reactive oxygen species during this process has the potential to be neurotoxic. Isoforms of MAO will be able to produce reactive oxygen species so, MAO-A is considered not just as a catabolic enzyme but also as a possible factor in oxidative stress-mediated neurodegeneration, especially when substrate availability is increased or enzyme expression is enhanced. The cytoprotective result of this enzymatic loop is that MAO's main function in physiological settings is to maintain neurotransmitter homeostasis by preventing monoamine neurotransmitters from acting in peripheral and central tissues, keeping cytosolic monoamine concentrations low, and maintaining neurotransmitter reuptake, so MAO will be considered a candidate biomarker for precision therapy of anxiety disorders [4].

2.2 GAD(GAD65/67): The Anxiolytic Synthesis

Neurotransmitter dysfunction includes GABA/glutamate imbalances, where nervousness is associated with low GABA and high Glu levels in the brain, disrupting the essential equilibrium between stimulated and inhibition. Specifically, loss of neuronal GABAergic neurones can cause excessive stimulation within the basolateral amygdala and elevated anxiety [8]. Glutamate Decarboxylase (GAD) uses pyridoxal 5-phosphate (PLP) as cofactor to stimulate the decarboxylation reaction in serotonin to GABA, the main inhibitory neurotransmitter. The vesicular GABA the carrier (VGAT) then carries it into neurones. GADs are broadly dispersed inside the cell, with large concentrations seen in presynaptic clusters and on the Golgi complex. GABA levels and the ensuing inhibiting neurotransmission at synapses are strongly associated between GAD transcription and activation [9,10]. The key physiological barrier that regulates the excitatory- inhibitory (E/I) equilibrium throughout a central nervous system is the transformation of L-glutamate to the gamma-aminobutyric acid via a GAD enzyme; anxiety-related conditions inherently disturb this E/I balance. GAD is a “isoenzyme” with two distinct isoforms, GAD67 nor GAD65, that are found in neurones in both humans and rabbits. This is not a consistently structured enzyme [11]. GABAergic neurones in the brain manufacture GAD67 and GAD65, which have molecular weights that are normal of 65 and 64 kDa, respectively. These are the recognised enzymes that produce GABA [12]. At the molecular level, GAD uses the other factor the pyridoxal-5-phosphate (PLP), an active form of vitamin B6, to assist the irreversibly α -decarboxylation of glutamate [13]. The biochemical pathway of the conversion of Glutamate to GABA initiates when glutamate binds to the GAD-PLP holoenzyme complex, leading to the release of carbon dioxide (CO₂) and the formation of GABA [14]. While both isoforms of GAD perform the same chemical reaction (Glutamate = GAD+PLP/GABA + CO₂), they occupy different functional roles like GAD67 is constitutively active and distributed throughout the neuron to maintain “basal” GABA levels necessary for general network stability, whereas GAD65 is localized to synaptic terminals and is triggered by “phasic” demands, such as acute stress or high-frequency neuronal firing in case of Panic attacks [15]. This triggering mechanism for

GAD65 involves a cycle of palmitoylation and depalmitoylation that delivers the enzyme to synaptic vesicle membranes, allowing it to rapidly synthesize GABA for immediate vesicular packaging during periods of intense synaptic activity [16]. In the context of diagnostic biomarker for precision anxiety therapy, the GAD enzyme is targeted not just as a metabolic byproduct but as a dynamic “rheostat” for emotion regulation [17]. Diagnostic and therapeutic strategies have converted from simple receptor modulation to direct enzymatic enhancement, aiming to increase GAD expression or activity via several pathways. In the category of precision medicine by gene editing gene therapy uses Adeno-associated viral (AAV) vectors to deliver functional GAD genes into hyperactive circuits like the basolateral amygdala (BLA), effectively “turning on” a local GABA factory to suppress anxiety-driving through glutamatergic signals [18]. Furthermore, emerging research into the Gut-Brain Axis suggests that certain probiotics mentioned in Table 1. can trigger endogenous GAD activity within the enteric nervous system, providing a peripheral anxiolytic signal that reaches the brain via the vagus nerve [19]. Precision medicine pathway followed by GAD as a diagnostic biomarker for anxiety disorders provide a more sustainable and physiologically “tuned” relief from anxiety [20], avoiding the sedation and dependence typically associated with traditional benzodiazepines basically acting on postsynaptic GABA receptors. Polymorphisms in the GAD1 gene, which encodes for the GABA-synthesizing enzyme GAD67, have been associated with anxiety-relevant phenotypes. Genetic variation may also affect epigenetic regulation of GAD1. In panic disorder, the overall methylation of GAD1 DNA was decreased compared to controls and the environmental stressor of negative life events (NLE) led to further decreases in GAD1 methylation. GAD1 rs3762555 was also shown to be associated with GAD1 methylation at selected CpG sites within GAD1 and is likely to be an example of how genetic background, environmental stress and epigenetic modifications converge to regulate GAD67-dependent GABA levels and the excitatory/inhibitory balance of neural circuits involved in anxiety [21].

Table 1: GAD targeted drugs with their mechanism and therapeutic status

Drug	Target Mechanism	Therapeutic Status	Reference
AAV-GAD (NLX-P101)	Viral vector-mediated overexpression of GAD65/67.	Clinical Trials	[22]
Valproate	Increases GAD activity & inhibits GABA-transaminase.	FDA Approved	[23]
Pyridoxine (B6)	Increases GAD-PLP saturation; activates GAD65.	Supplement	[24]
L-Theanine	Upregulates mRNA expression of GAD1 (GAD67).	Nutraceutical	[25]
Phenelzine	Increases GAD expression via chronic modulation.	FDA Approved	[26]
Probiotic (L. rhamnosus)	Triggering of GAD activity via the vagus nerve.	Experimental	[19]
Apo-GAD Activators	Conversion of inactive apo-GAD65 to active holo-enzyme.	Pre-clinical	[15]

3. HPA-AXIS ENZYMES: 11 β -HYDROXYSTEROID DEHYDROGENASE (11 β -HSD)

The enzymatic modulation of glucocorticoids which plays a major role in anxiety pathophysiology regulates by the Hypothalamic-Pituitary-Adrenal (HPA) axis. The mechanistic flow of corticosterone in rodents and cortisol in humans is regulated by the **11 β -Hydroxysteroid Dehydrogenase** enzyme system. 11 β -HSDs metabolize glucocorticoids, so by this controlling the intracellular concentrations of hormones that can activate corticosteroid receptors (Figure 1). 11 β -HSD basically have two types of isoenzymes type 1 and type 2 that drive the enzyme reaction in opposing directions in the majority of tissues and circumstances [27].

11 β -HSD2(dehydrogenase)

Active Corticosterone $\xleftarrow{11\beta\text{-HSD1(reductase)}} \xrightarrow{11\beta\text{-HSD2(dehydrogenase)}} \text{Inactive Corticosterone}$

3.1 11 β -HSD1: Context dependent effects on anxiety

11 β -HSD1 basically acts as a reductase in the conversion of inactive to active corticosterone and converting inactive cortisone into active cortisol within the hippocampus and prefrontal cortex. Chronic stress has been shown responsible for the upregulation 11 β -HSD1, leading to localized “hypercortisolemia” even when systemic cortisol levels appear normal [28]. The enzymatic dysregulation lead by 11 β -HSD1 responsible for the neurotoxic effects of glucocorticoids and increase of intracellular 11 β -HSD1 regulate glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) signaling in stress-sensitive brain areas such as the hippocampus, amygdala, and prefrontal cortex by converting inactive Corticosterone into active Corticosterone [29]. The endoplasmic

reticulum's hexose-6-phosphate dehydrogenase produces NADPH, which is essential for the central nervous system's reductase function and raises local cortisol levels independent of blood levels. It is the enzyme which mostly expressed in neurons and glia. Chronic 11 β -HSD1 overactivity causes dendritic atrophy in CA3 pyramidal cells, lowers hippocampus neurogenesis, disturbs HPA axis homeostasis, and impairs neuroplasticity so when these all dysregulated simultaneously increasing anxiety [30]. Mechanistically over production of cortisol saturates GRs, while inhibiting serotonin signaling, BDNF-TrkB pathways, and affecting pro-anxiety GABA-A receptor subunits. In microglia, neuroinflammatory stimuli including IL-1 β and IL-6 further upregulate 11 β -HSD1 thus by causing neuroinflammation and severe anxiety. Reduced fear conditioning and restored amygdala-hippocampal synaptic plasticity are confirmed by preclinical knockout and pharmacological inhibition investigations employing drugs such as BVT.2733 [29,30]. Nevertheless, anxiety was strangely reported as a side effect in clinical trials of inhibitors like ABT-384 and AZD4017, indicating either compensatory ACTH rebound or disruption of adaptive glucocorticoid oscillations. All things considered, 11 β -HSD1 is still a promising candidate diagnostic biomarker of anxiety and personalized therapy necessitating brain-penetrant, isoform-selective inhibitors for successful anxiety treatment [31].

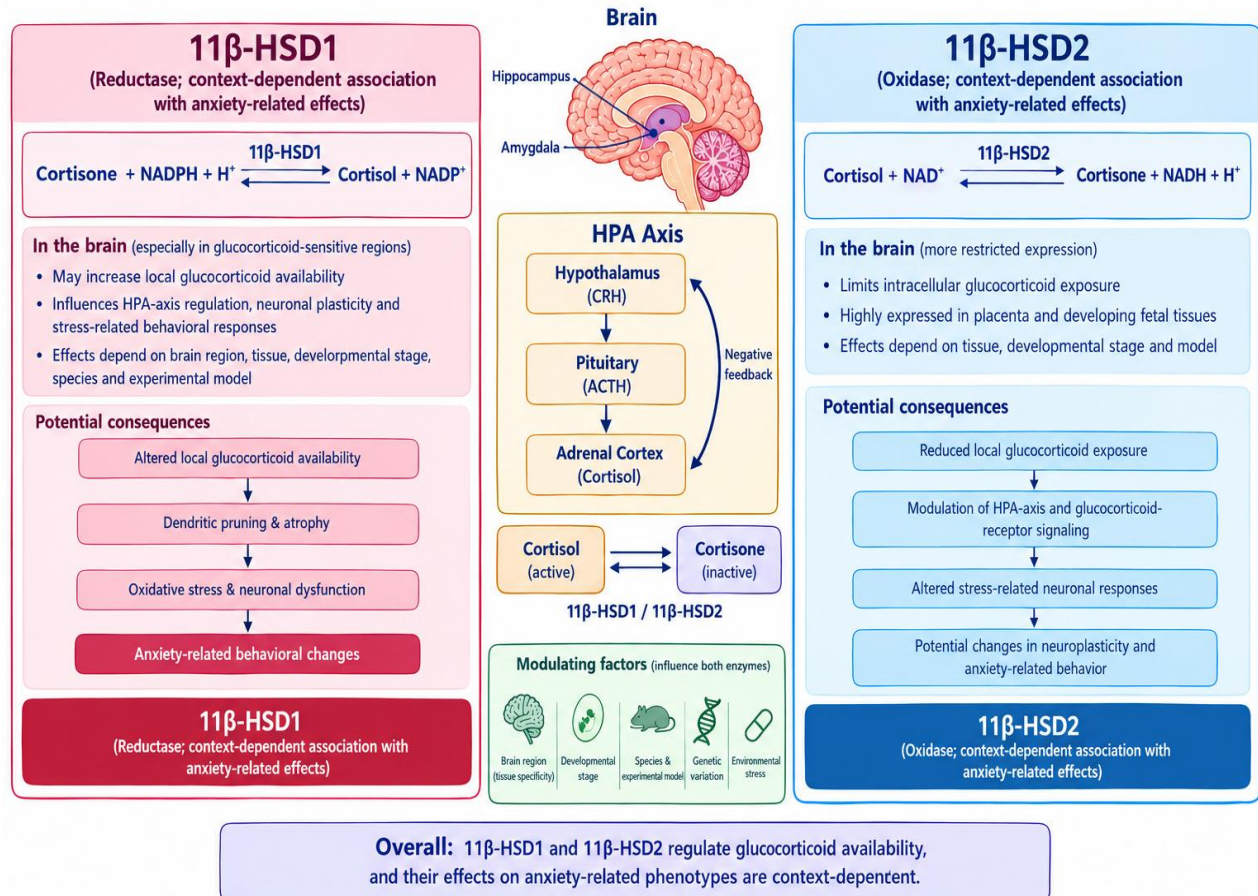


Figure 1. Context-dependent roles of 11 β -HSD1 and 11 β -HSD2 in glucocorticoid regulation and anxiety-related phenotypes.

3.2 11 β -HSD2: Context dependent effects on anxiety

Acting as an oxidase, this enzyme inactivates cortisol. Its high expression in specific brain regions protects neurons from the “stress-scars” of maternal or environmental trauma. Recent research indicates that enhancing 11 β -HSD2 activity may provide a novel therapeutic pathway to buffer the brain against stress-induced anxiety [28]. By converting cortisol into cortisone through its NAD⁺ dependent oxidase activity, 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD2) preserves brain region's mineralocorticoid receptors (MR) and glucocorticoid receptors (GR) from glucocorticoid overload, especially during development [29,30]. 11 β -HSD2, is highly expressed in the placenta, fetal brain, and distinct adult CNS nuclei such as the nucleus tractus solitarius, keeps active glucocorticoid levels low, preventing cortisol from inappropriately activating MR and promoting the balanced HPA axis tone necessary for anxiolytic resilience [32,33]. 11 β -HSD2 prevents premature neuronal maturation, dendritic remodelling deficits, and HPA hyper-reactivity in the developing brain by limiting maternal glucocorticoid transfer across the placenta and foetal blood-brain barrier; brain-specific conditional knockouts confirm no overt adult anxiety alterations, suggesting placental/foetal peripheral dominance, but global deficiency programs persistent emotional dysregulation [34]. Stress-induced downregulation may exacerbate anxiogenic glucocorticoid surges in limbic circuits such as the hippocampus and amygdala. Adult expression, however

decreased, regulates salt intake, blood pressure via NTS MR protection, and stress responses [32,35]. 11 β -HSD2 is downregulated in microglia by inflammatory cytokines like IL-6, which prolongs glucocorticoid excess and anxiety comorbidities in conditions like PTSD; on the other hand, its overexpression through peroxisome proliferator-activated receptors imparts neuroprotection. Current zebrafish models demonstrate dynamic 11 β -HSD2 modulation under acute and chronic stress, reducing the inhibition of forebrain regeneration caused by cortisol and encouraging anxiolytic behavioral adaption [36]. Although isoform specificity and blood-brain barrier penetration are obstacles, while 11 β -HSD2 agonists or gene treatments show promise for glucocorticoid-hypersensitive anxiety subtypes in precision psychiatry. 11 β -HSD2's anxiolytic function is demonstrated by glucocorticoid inactivation protecting limbic homeostasis, preventing acute and planned anxiogenesis, and establishing it as a translational target in contrast to 11 β -HSD1's amplification [29,32].

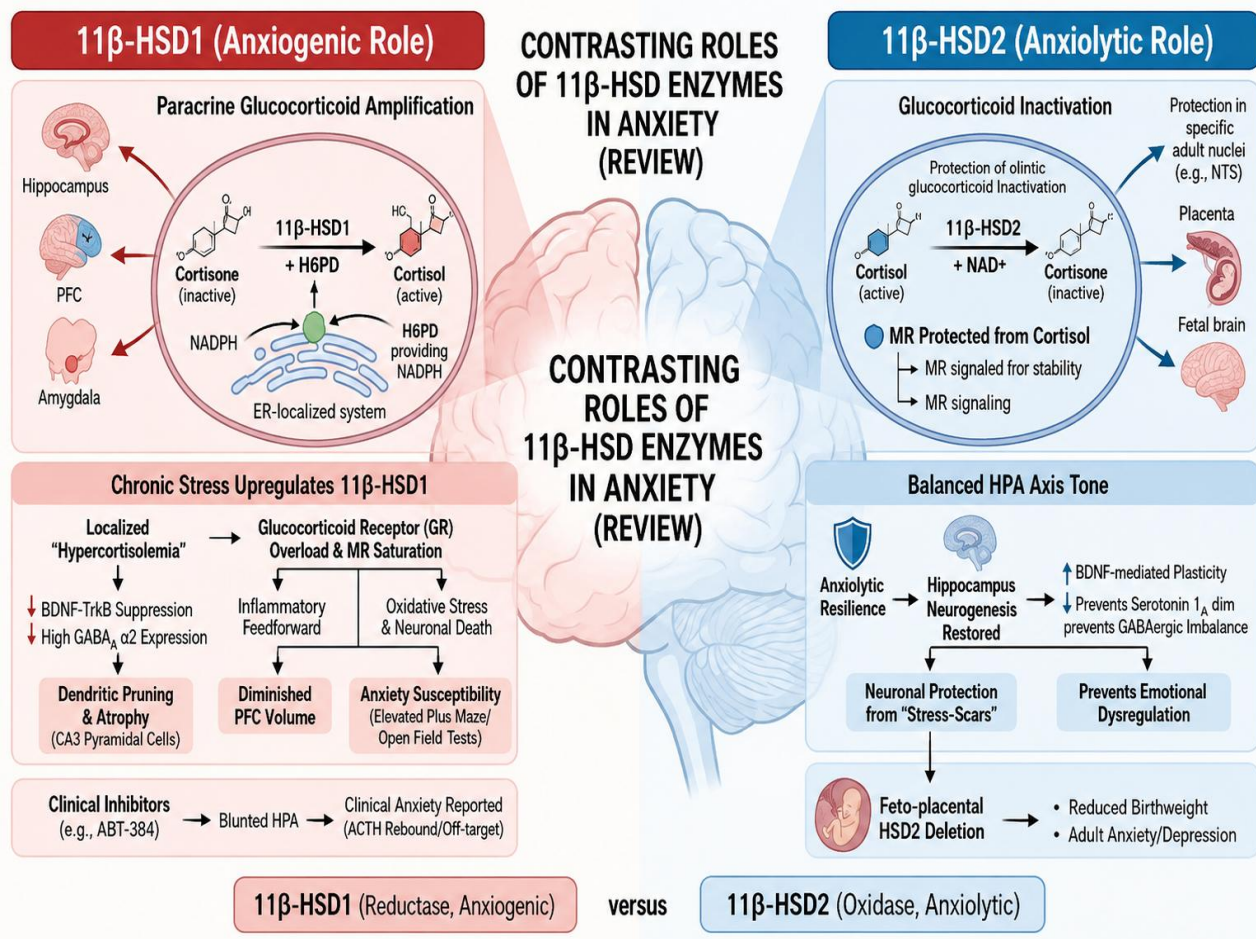


Figure 2: Roles of 11 β - HSD1 & 11 β - HSD2 in anxiety as anxiogenic and anxiolytic [28,30]

4. EPIGENETIC ENZYMES: THE MASTER MODULATORS OF GENE EXPRESSION IN ANXIETY

The developing study of neuro epigenetics has completely changed our knowledge of chronic anxiety. The pathophysiology of anxiety is not only governed by chemical imbalances means decreasing serotonin or increasing glutamate, but also anxiety is directly regulated by the structural lookup of chromatin. epigenetic enzymes, such as DNA Methyltransferases (DNMTs), Ten-Eleven Translocation (TET) dioxygenases, Histone Acetyltransferases (HATs), Histone Deacetylases (HDACs), and Histone Methyltransferases (HMTs), co-ordinately regulate dynamic chromatin remodelling and non-coding RNA regulation by Without changing the underlying DNA sequence [37,38]. DNMTs, HATs, and HDACs are the three interdependent enzyme systems which mean s their regulation depends simultaneously to each other and these enzymes working together as molecular gatekeepers for the determination of whether genes associated with stress resistance, emotional regulation, and neuroplasticity are permanently silenced or stay in active state. They all functions within the coordinated multiprotein complexes with transcriptional, metabolic, and signaling inputs. The expression of HDAC and DNMT further also controlled by non-coding RNAs including miR-132/212, miR-101a, and lncRNA Gomafu. Stress-induced dysregulations in these networks in RNA or DNA sequences persist over generations, altering HPA feedback and serotonin/glutamate balance. Neuroinflammatory mediators such IL-1 β and IL-6 also boosts DNMT3B and HDAC activity in microglia, sustaining feedforward loops that re-enforced the pathophysiology of anxiety [39].

4.1 Histone Deacetylases (HDACs): The Anxiogenic Silencers

In the category of epigenetic enzymes HDACs enzymes mostly studied in relation with the anxiety. The HDACs Enzyme family also known as zinc dependent enzymes, which are further divided into various classes like Class I which contains HDAC1, 2, 3, 8, and class IIa contains HDAC4, 5, 7, 9, and class IIb contains HDAC6, 10, and

IV contains HDAC11. They are known as “anxiogenic silencers” because of the deacetylation means removal of acetyl groups from ϵ -N-acetyl lysine residues on histone tails encourages chromatin condensation into a densely packed heterochromatin state, which is further physically blocking transcriptional machinery from accessing DNA [40]. Class I HDACs, especially HDAC1 and HDAC2, are significantly increased in the prefrontal cortex, hippocampus, and basolateral amygdala in anxiety disorders such as PTSD, GAD, and specific phobias. Long-term psychological stress is the main reason for this upregulation of Class I HDACs by activating NF- κ B through glucocorticoid receptors. This leads to hypoacetylation at important histone sites like H3K9/14 and H4K12/5/8/16, which then inhibits the transcription of BDNF exon IV, this gene basically essential to emotional resilience and synaptic plasticity [41]. The main function of HDAC2 in the fear circuitry is suppressing Nr4a1 and Nr4a2, which are immediate early genes essential for fear extinction, HDAC2 overexpression in BLA neurons improves fear training [42]. HDAC6 uses non-histone processes to add another level of anxiogenic intricacy, instead of just condensing chromatin, HDAC6 increases Corticosterone Releasing Hormone expression in paraventricular nucleus (PVN) neurons and facilitates GR nuclear translocation by stabilizing the glucocorticoid receptor chaperone Hsp90, this process directly increases hyperactivity of the HPA axis [43]. Along with above HDACs, HDAC10 also a candidate biomarker for diagnostic and therapeutic purpose because HDAC10 promotes neuroinflammation by activating the NLRP3 inflammasome in microglia [44].

4.2 DNA Methyltransferases (DNMTs): Coding the Stress Response

DNA methyltransferases are the epigenetic enzymes which have the power to create more permanent, heritable molecular structure of genes by depositing 5-methylcytosine (5mC) marks at CpG islands in gene promoters, whereas HDACs temporarily silence genes through chromatin compaction. DNMTs include DNMT1 which maintains methylation of DNA, DNMT3A and DNMT3B also involved in de novo methylation and DNMT3L acts as a catalytic cofactor in DNA methylation of genes that control emotional homeostasis and produce chronic anxiety vulnerability [45]. Anxiety disorders sometimes convert into chronic anxiety after traumatic events because when glucocorticoid receptor phosphorylation binds DNMT3A to limbic promoters under chronic stress, which then anchoring permanent epigenetic alterations that survive the initial stressor [46].

4.2.1 Enzymatic Reversal and Therapeutic Strategies

Therapeutic strategy of anxiety will be changed from symptom-based treatment to true epigenetic remodelling by correcting disrupted DNA methylation process and this process can lead by epigenetic enzymes. In the brain TET dioxygenases mediated demethylation maintains the transcriptional flexibility which is necessary for neuroplasticity and behavioral changes adaptation by reacting dynamically to learning and memory consolidation, neuronal activity, and stress exposure [47]. Nucleoside DNMT inhibitors like decitabine produce irreversible covalent binding effects which uses proteasomal mechanism to capture and destroy DNMT1 action in DNA methylation. The non-nucleoside inhibitor RG108 directly inhibits the DNMT active site without covalent enzyme binding, which then resulting in targeted demethylation and reactivation of silenced genes without the broad hypomethylation seen with nucleoside analogues. When RG108 injected directly into brain target areas, then it abolished long-term contextual fear memory and showed anxiolytic and antidepressant-like effects in animal models [48].

4.3 Histone Acetyltransferases (HATs): The Natural Counterbalance

Histone Acetyltransferases (HATs) are known as the natural biochemical key if HDACs are the chromatin “lock” in the anxiety pathophysiology. In order to neutralize the positive charge of histone tails, relax DNA-histone interactions, and create an open chromatin (euchromatin) configuration that allows transcriptional machinery to access resilience genes, HATs transfer acetyl groups from acetyl-CoA to ϵ -amino groups of histone lysine residues [49]. This open chromatin state permits the expression of genes mediating fear extinction, affective modulation, and synaptic plasticity in anxiety-relevant areas such as the prefrontal cortex, hippocampus, and amygdala. HATs are categorized structurally into three major nuclear families: MYST, the CBP/p300 family, and GNAT (Gcn5-related N-acetyltransferases). Type A HATs are nuclear transcriptional regulators, while type B HATs work cytoplasmically to prepare freshly synthesized histones for nuclear incorporation. HATs extend their regulatory effect over the entire spectrum of molecular cascades related to anxiety by acetylating non-histone substrates such as transcription factors, nuclear receptors, and signaling enzymes in addition to histones. Research on fear and anxiety has focused largely on the p300/CBP family. HAT activity is directly linked to the pathophysiology of phobias, PTSD, and GAD. Pharmacological inhibition of p300/CBP activity using C646 delivered locally into the lateral nucleus of the amygdala (LA) significantly impairs fear memory consolidation and reconsolidation, confirming that p300/CBP-mediated H3 acetylation is essential for the molecular encoding of fear in this structure [50]. This also applies to the prefrontal cortex: p300/CBP inhibition given within an hour of memory reactivation in the prelimbic cortex prevents the H3 acetylation elevation that is normally seen following retrieval in control animals, disrupting fear memory reconsolidation. Histone acetylation at H3K9ac, H3K14ac, and H4K5ac is dynamically regulated at the chromatin mark level in fear conditioning, retrieval, and extinction in a brain-region-, task-, and time-dependent manner, especially at the BDNF gene locus. This shows that HAT activity is a dynamically regulated determinant of anxiety circuit function rather than a static background process [51].

5. ENZYMES IN ANXIOLYTIC DRUG METABOLISM: CYP450 AND UGT SYSTEMS

Two main enzymatic routes control the pharmacokinetics of anxiolytic drugs: Phase I (oxidative) metabolism, which is mediated by cytochrome P450 (CYP450) enzymes, and Phase II (conjugative) metabolism, which is mediated by uridine 5'-diphosphate glucuronosyltransferases (UGTs). The pharmacokinetics, therapeutic efficacy, and safety profiles of medications used in clinical anxiety therapy are all determined by these enzymatic systems taken together [52]. Alprazolam is a triazole-benzodiazepine that is frequently used to treat anxiety and panic disorders. It is oxidized by CYP3A4 and CYP3A5 to produce 4-hydroxyalprazolam and alpha-hydroxy alprazolam, which are then glucuronidated [53]. The main active metabolite of diazepam is desmethyldiazepam, which is produced by N-demethylation by CYP2C19 and CYP3A4. Temazepam and oxazepam are produced in trace quantities by CYP3A4. CYP3A4 and CYP3A5 oxidize midazolam, which has a quick onset and a fast plasma clearance, to produce alpha-hydroxy midazolam and 4-hydroxymidazolam. While both metabolites quickly conjugate with glucuronic acid to create pharmacologically inactive products, the former plays a major role in the pharmacological effects of midazolam at high drug concentrations. The CYP2C19 genetic polymorphism is a major cause of inter-individual variability. While extensive metabolizers (EMs) may need dosage escalation to achieve therapeutic effectiveness, poor metabolizers (PMs) accumulate greater plasma concentrations of diazepam and citalopram, raising risks of respiratory depression and cognitive impairment [52,53]. Rebound anxiety and subtherapeutic drug levels are the outcomes of co-administration of CYP3A4 inducers like carbamazepine. Additionally susceptible to CYP3A4-mediated oxidation, which produces inactive metabolites, are non-benzodiazepine anxiolytics such as buspirone, a 5-HT_{1A} partial agonist. Therapeutic drug monitoring (TDM) is becoming more and more important in precision psychiatry since genetic variations like CYP2D6*4 and medication interactions like fluoxetine inhibition highlight the dominance of CYP450 in determining an individual's response to anxiolytics. The main metabolic pathway for the "LOT" trio lorazepam, oxazepam, and temazepam is Phase II conjugation via UGT enzymes, avoiding significant Phase I dependence. This makes them especially appropriate for older people who are prone to CYP downregulation or patients with hepatic impairment. While UGT2B7 and UGT1A9 are primarily responsible for oxazepam metabolism, UGT1A4 manages lorazepam glucuronidation with limited CYP450 involvement. However, by reducing drug exposure, rifampin-induced acceleration of UGT activity may jeopardize anxiolytic coverage. While pregabalin and gabapentin completely escape CYP/UGT routes through renal excretion, newer drugs like zuranolone (a GABA_A modulator) undergo dual CYP3A4/UGT1A9 metabolism, where enzyme inhibition risks neurotoxic BZD-type overshooting [53].

Table 2: Anxiolytic Drugs, Mechanisms, and Metabolizing Enzymes

Anxiolytic Drug	Scientific Mechanism of Action	Metabolizing Enzymes & Organ	Reference
Benzodiazepines			
Diazepam	Positive Allosteric Modulator (PAM) of GABA-A receptors; increases Cl ⁻ channel opening frequency.	CYP3A4, CYP2C19 (Liver)	[54]
Alprazolam	High-affinity PAM of GABA-A; binds to the alpha-gamma subunit interface to enhance inhibitory neurotransmission.	CYP3A4 (Liver)	[54]
Lorazepam	Direct GABA-A modulation; possesses high relative potency for omega-1 and omega-2 receptor subtypes.	UGT2B15, UGT1A4 (Liver)	[55]
Oxazepam	3-hydroxy metabolite; acts as a GABA-A PAM with direct glucuronidation (phase II metabolism).	UGT2B7, UGT1A9 (Liver)	[55]
Clonazepam	Nitro-benzodiazepine; enhances GABAergic inhibition with high affinity for central BZD receptors.	CYP3A4 (Liver)	[54]
Chlordiazepoxide	Pro-drug converted to active metabolites (demoxepam); facilitates GABA-mediated pre-synaptic inhibition.	CYP3A4, CYP2C19 (Liver/Gut)	[55]
Midazolam	Short-acting GABA-A PAM; high lipophilicity at physiological pH allows rapid CNS penetration.	CYP3A4 (Liver/Intestine)	[54]
Triazolam	Ultra-short-acting BZD; binds non-selectively to alpha-1, alpha-2, alpha-3, and alpha-5 subunit-containing GABA-A receptors.	CYP3A4 (Liver)	[55]
Temazepam	GABA-A receptor modulator; bypasses Phase I oxidation, primarily undergoing direct conjugation.	UGT2B7 (Liver)	[55]
Non-Benzodiazepines			

Propranolol	Non-selective beta-adrenergic antagonist; prevents autonomic symptoms by blocking beta-1 and beta-2 receptors.	CYP2D6 (Liver)	[56]
SSRIs / SNRIs			
Escitalopram	S-enantiomer of citalopram; highly selective Serotonin Transporter (SERT) inhibitor.	CYP2C19, CYP3A4 (Liver)	[56]
Sertraline	Selective inhibitor of SERT; also exhibits weak Dopamine Transporter (DAT) inhibition.	CYP2C19, CYP2B6 (Liver)	[56]
Paroxetine	Potent SERT inhibitor; also possesses significant muscarinic (mACh) receptor antagonism.	CYP2D6 (Liver)	[56]
Fluoxetine	SERT inhibitor with 5-HT-2C antagonism; has a very long-lived active metabolite (norfluoxetine).	CYP2D6, CYP2C19 (Liver)	[56]
(Newer Agents) Zolpidem	Imidazopyridine; selective agonist at the $\alpha 1$ subunit-containing GABA-A receptors.	CYP3A4, CYP1A2 (Liver)	[54]

5.1 Cytochrome P450 (CYP) System: CYP3A4 and CYP2C19

The most common hepatic and intestinal isoform is CYP3A4 which is responsible for about half of total CYP450 enzyme mediated drug clearance. In order to control onset of action, duration of action, and accumulation risk, it mainly increases the N-demethylation and 4-hydroxylation of benzodiazepines, converting diazepam to active nordiazepam and temazepam. It also metabolizes triazolam, alprazolam, and midazolam via α -hydroxylation to inactive forms [57]. Benzodiazepines are especially susceptible to drug-drug interactions because CYP3A4 is expressed in the endoplasmic reticulum of hepatocytes and enterocytes. While inhibitors like ketoconazole, erythromycin, and ritonavir increases plasma BZD levels 5 to 15 times and prolong sedation and respiratory depression, inducers like rifampin accelerate clearance and cause breakthrough of anxiety. Clock gene modification also contributes to diurnal oscillations in CYP3A4 activity; DBP downregulation in hepatocytes exposed to BZD inhibits CYP3A4 production. Together with CYP3A4, CYP2C19 synergistically N-demethylates diazepam and clobazam to active desmethyldiazepam, contributing 30–50% of metabolic flux. Due to their half-lives being two to four times longer than average, patients which belongs to the poor metabolizers category are more likely to experience excessive sedation [58]. Additionally, CYP2C19 controls the demethylation of citalopram and escitalopram, which increases the risk of QT prolongation in Poor Metabolizer patients by 1.5-2 times drug exposure [57]. In order to avoid oversedation during alcohol withdrawal or status epilepticus, CPIC guidelines advise 50% dosage reductions of diazepam in PMs category patients of CYP2C19. Management is further complicated by enzyme interactions, omeprazole competitively decreases PM clearance, whereas fluvoxamine, a dual CYP2C19/1A2 inhibitor, raises diazepam Area Under Curve by 2.5 times [57,58].

5.2 Uridine 5-Diphospho-Glucuronosyltransferases (UGT)

UGT enzymes improve water solubility, encourage renal and biliary excretion by catalyzing the transfer of glucuronic acid to hydroxylated substrates, and build-up of less Phase I metabolites or pharmacologically active parent chemicals. The 3-hydroxy moieties of the LOT trio- lorazepam, oxazepam, and temazepam are directly glucuronidated, resulting in inert O-glucuronides that are eliminated 80–90% unchanged in urine. They are therefore beneficial for individuals with immature or compromised CYP systems, such as newborns, the elderly, and people with liver illness. The most prevalent isoform, UGT2B7 which glucuronidates around 35% of frequently given drugs, such as lamotrigine and oxazepam. UGT1A9 and UGT1A4 process lorazepam and temazepam, respectively. Therapeutic windows in the treatment of generalized anxiety disorder (GAD) are impacted by polymorphic UGT variations, which cause 2-5-fold inter-individual clearance variability. According to preclinical data, UGT2B7 mutant mice mimic human poor metabolizer phenotypes by showing three-fold increases in oxazepam exposure with potentiated hypnosis. Clinically, DDI care is necessary since phenytoin induction reduces short-acting anxiolysis and atazanavir inhibits UGT1A1, prolonging exposure to BZD metabolites. An emerging technique in precision psychiatry is UGT phenotyping employing probe substrates like acetaminophen for UGT1A6, especially for individuals with renal or hepatic comorbidities [59].

6. PHARMACOGENOMICS AND ENZYME-BASED DIAGNOSTICS: THE PATH TO PERSONALIZED ANXIOLYTIC THERAPY

The lack of reliable biological testing through biomarkers that can confirm diagnosis, stratify severity, or predict treatment response makes anxiety disorders one of the most underdiagnosed or poorly diagnosed and poorly treated mental illnesses in the world [1]. The intersection of enzyme biology and pharmacogenomics for diagnostic biomarkers has revolutionary potential for moving forward from past trial-and-error prescription to biomarker-guided precision therapy.

6.1 Clinical Significance of CYP450 Polymorphisms

In psychiatric pharmacology, the most completely verified family of enzyme-based pharmacokinetic indicators are the cytochrome P450 isoenzymes CYP2D6 and CYP2C19. Along with CYP2B6, genetic diversity in these enzymes directly impacts the metabolism of SSRIs and SNRIs, affecting individual plasma drug concentrations,

therapeutic effectiveness, and tolerance. Each of the four phenotypic categories - poor metabolizers (PMs), intermediate metabolizers (IMs), normal metabolizers (NMs), and ultra-rapid metabolizers (UMs)—has unique therapeutic consequences. Treatment non-response and adverse drug responses from psychiatric drugs are clearly more common in people with genetic variations that result in decreased CYP2D6 or CYP2C19 enzyme activity. While acknowledging the need for additional research to clarify their impact on therapeutic response beyond pharmacokinetics, a large-scale meta-analysis integrating genetic and clinical data from 13 studies across European and East Asian ancestry populations confirmed CYP2C19 and CYP2D6 as significant predictors of antidepressant metabolite levels. Actionable dose recommendations for CYP2D6, CYP2C19, CYP2B6, SLC6A4, and HTR2A genotypes in regard to serotonin reuptake inhibitor antidepressants have been defined by the Clinical Pharmacogenetics Implementation Consortium (CPIC). Pharmacogenomics (PGx)-guided prescription for psychiatric reasons was being used by at least 70 institutions globally as of 2023. Monoamine oxidase A (MAOA), the mitochondrial enzyme that catalyzes the oxidative deamination of serotonin, norepinephrine, and dopamine, has become a consistently implicated enzymatic biomarker across anxiety subtypes, going beyond CYP pharmacokinetics. Among the genetic polymorphisms most commonly linked to the pathophysiology of panic disorder are long alleles of the MAOA upstream variable number tandem repeat (uVNTR). Further implicating enzyme-related indicators in the diagnosis and prognosis of anxiety disorder trajectories is epigenetic dysregulation, which includes modified DNA methylation patterns at the MAOA gene and neuroendocrine loci such as OXTR, BDNF, NET, GAD1, CRHR1, and NR3C1. The primary enzyme that deactivates dopamine and norepinephrine at synaptic terminals, catechol-O-methyltransferase (COMT), has also been found to be a pharmacogenomic and diagnostic biomarker [58][59].

6.2 The Rise of Diagnostic Enzyme Panels: The COMT Val158Met Assay

A major focus of precision therapy in psychiatry is the incorporation of pharmacogenetic biomarkers-based testing into clinical treatment. There are number of enzyme-encoding genes present for modern diagnostic panels, such as MAO-A, COMT, and CYP variations which allowing for genotype-guided or biomarker guided prescription decisions [60]. The genotyping of the COMT Val158Met polymorphism (rs4680) is known to be a single nucleotide variation on chromosome number 22q11.2 where a G-to-A substitution causes a valine-to-methionine alteration at 158th amino acid of the COMT enzyme. Val/Val homozygotes had much lower dopamine levels in the prefrontal cortex than Met/Met homozygotes because the alleles are co-dominant. There are some important therapeutic modifications to this functional difference like Patients with Met/Met exhibit better improvement in positive symptoms and are more susceptible to antipsychotics, especially when using atypical drugs while according to meta-analyses of more than 4,600 people in the field of pain treatment Rs4680 has small but substantial benefits on pain thresholds in patients with fibromyalgia and headaches [61]. TaqMan SNP genotyping with real-time PCR is now often used for the Val158Met test, providing effective allelic discrimination from genomic DNA extracted from blood samples. Diagnostically, this assay guides clinicians in determining whether patients are more likely to benefit from dopaminergic versus serotonergic anxiolytics or not [62][63].

7. CONCLUSION AND FUTURE PERSPECTIVES: ENZYME-TARGETED DRUG DELIVERY

Anxiety is a multi-enzymatic illness that includes abnormalities in xenobiotic metabolism, neurotransmitter production, neuroendocrine control, and epigenetic chromatin remodelling. In comparison between traditional receptor-centric GABAergic theory and the “Enzymatic theory” of anxiety, which is based on upstream deficits in Glutamic Acid Decarboxylase (GAD65/GAD67) activity and MAO alterations and genetic remodelling by epigenetic enzymes, “Enzymatic theory” of anxiety provides a more mechanistically comprehensive framework for the biomarker-based precision therapy of anxiety disorders. Inhibitory neurotransmission is hampered by decreased GAD65 activity in limbic areas including the basolateral amygdala, which results in chronic hyperarousal and decreased sensitivity to conventional benzodiazepines. The pharmacokinetic disposition and safety of almost all approved anxiolytic medications are regulated by the CYP450 superfamily, especially CYP3A4 and CYP2C19, and UGT conjugative enzymes. CPIC-concordant genotyping panels are gradually transitioning from experimental to standard-of-care practice. The integration of multi-enzyme pharmacogenomic panels that combine CYP2D6, CYP2C19, MAO-A, and COMT genotyping into regular pre-prescription screening is the most pressing translational goal going forward. This will replace the existing trial-and-error paradigm with a physiologically informed framework. The clinical-stage NLX-P101 program is an example of how gene therapy approaches, such as AAV-mediated delivery of functional GAD65/GAD67 constructs to hyperactive amygdala circuits, represent a potentially disease-modifying strategy that targets the upstream enzymatic deficiency rather than downstream receptor manipulation. Non-nucleoside DNA methyltransferase inhibitors such as RG108, isoform-selective HDAC inhibitors, and locus-specific epigenome editing with dCas9-TET1 fusion constructs show promise for long-term transcriptional reprogramming of fear circuits, transforming temporary symptom relief into enduring neurobiological resilience. The problems of blood-brain barrier penetration and systemic side effects are also targeted by enzyme-responsive nanoparticle drug delivery systems that release anxiolytics load inside brain areas which have the strong COX-2 or HDAC enzymatic activity. Enzymatic alterations are not the only reliable diagnostic or prognostic biomarkers for anxiety disorders but with the consideration of enzymatic biomarkers along with receptor-based biomarkers the anxiety disorders will be treated more precisely and by consideration of enzymatic biomarkers the precised therapy for anxiety disorders will be achieved. All things considered, the next ten years of anxiety disorder research and clinical practice must be defined by a deliberate

shift from receptor-centric pharmacological treatment to enzymatic biomarkers-based precision medicine, where each patient who have different enzymatic signature serves as both the diagnostic cornerstone and the completely precised therapeutic roadmap.

8. ABBREVIATIONS

1. GABA- Gamma-Aminobutyric Acid
2. MAO- Monoamine Oxidase
3. GAD- Glutamic Acid Decarboxylase
4. CNS- Central Nervous System
5. 5-HT- 5-Hydroxytryptamine (Serotonin)
6. PLP- Pyridoxal 5-phosphate
7. 11 β -HSD- 11 β - Hydroxysteroid Dehydrogenase
8. ACTH- Adrenocorticotrophic Hormone
9. DNMTs- DNA Methyltransferases
10. HDACs- Histone Deacetylases
11. PTSD- Post-Traumatic Stress Disorder.
12. HATs- Histone Acetyltransferases
13. CYP450- Cytochrome P450
14. UGT- Uridine 5-diphospho-glucuronosyltransferase
15. PAM- Positive Allosteric Modulator
16. PM- Poor Metabolizer
17. BZD- Benzodiazepine
18. COMT- Catechol-O-methyltransferase

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12. CONFLICTS OF INTEREST

There are no conflicts of interest.

13. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

14. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

15. USE OF ARTIFICIAL INTELLIGENCE (AI) ASSISTED TECHNOLOGY

The authors declare that they have not used artificial intelligence (AI) tools for writing and editing of the manuscript, and no images were manipulated using AI.

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