ABHD6: its place in endocannabinoid signaling and beyond.

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Abstract

The endocannabinoid (eCB) signaling system modulates neurotransmission and inflammation, amongst other physiological functions. Its newest member, α/β-hydrolase domain containing 6 (ABHD6), has emerged as a promising therapeutic target to treat several devastating diseases, including epilepsy. We review the molecular mechanisms that mediate and control eCB signaling and within it, the specific role of ABHD6. We also discuss how ABHD6 controls the abundance of additional lipids and the trafficking of ionotropic receptors to plasma membranes. We finish with several unexplored questions regarding this novel enzyme. Our current understanding of the molecular mechanism and biological function of ABHD6 provides a strong foundation for the development of small molecule therapeutics to treat devastating diseases.

Keywords

ABHD6; endocannabinoids; enzymes; neurons; neuroinflammation; epilepsy

The canonical endocannabinoid signaling system

The existence of an endocannabinoid (eCB) signaling system was first inferred from studies on the bioactivity of Δ^9-tetrahydrocannabinol (THC), the psychoactive compound produced by the cannabis plant [1]. This signaling system consists of two cannabinoid (CB) receptors (CB$_1$R and CB$_2$R), two main signaling lipids (anandamide, AEA, and 2-arachidonoylglycerol, 2-AG) that activate these receptors, and the enzymes that produce and inactivate eCBs. The lipid nature of eCBs provides both autocrine and paracrine modalities to regulate the physiology and function of cells in the brain and periphery. Accordingly, eCBs are 1) produced in an activity-dependent manner by independent lipases located at the plasma membrane, 2) exhibit distinct pharmacology at CB receptors and 3) are inactivated by independent enzymes [1]. Thus, the eCB signaling system encompasses parallel signaling lipids that independently regulate membrane receptors and fundamental physiological functions throughout the body.

The CB$_1$R is a G protein-coupled receptor expressed by many cell types and most abundantly expressed in neuron terminals [2]. In neurons, through the activation of G proteins, CB$_1$R regulates the activity of second messengers and effector proteins, including adenylyl cyclase and extracellular signal–regulated kinases (ERK) kinases activities, the opening probability of L-, N- and P/Q-type calcium channels and of potassium hyperpolarization-activated cyclic nucleotide–gated (HCN) channels, the combination of which controls action potential-dependent vesicular neurotransmitter release [3–7]. Under certain conditions, CB$_1$Rs activate phospholipase C and PI3K pathways through G protein β/γ subunits also reduce presynaptic neurotransmitter release [8–11]. The prolonged, repeated activation of CB$_1$Rs regulates gene expression and synaptic arborization [12–14]. Thus, activation of CB$_1$Rs expressed by neurons regulates neurotransmitter release and is involved in eCB-dependent synaptic plasticity [15].

The CB$_2$R also couples to G proteins, has 44% protein homology to CB$_1$R and is primarily expressed by hematopoietic cells [16, 17]. eCBs also modulate CB$_2$R activity, but with a different pharmacological action than at CB$_1$R, which is likely to explain a portion of the select modulatory control of immune cell function by CB$_2$R [2]. Activation of CB$_2$Rs reduces inflammation by lowering the production of immune mediators, reducing macrophage's ability to process antigens and prime helper T-cells, and by eliminating immune cells by apoptosis [18]. Small molecules that target CB$_2$Rs also regulate immune cell proliferation and migration [19]. Accumulating evidence show that CB$_2$Rs are also expressed in neurons and recent work uncovered cell-type specific roles of CB$_2$Rs on contextual fear, spatial memory and anxiety, illustrating an increasing appreciation for CB$_2$Rs in neurons [20]. There are several additional receptors that are also modulated by phytocannabinoids (phytoCB), synthetic CB ligands and eCBs, including GPR55, several transient receptor potential (TRP) channels and glycine receptors [1]. These are all likely to play roles in the bioactivity of phytoCBs that are independent of what is often referred to as the canonical eCB signaling pathway that encompasses CB$_1$R, CB$_2$R, AEA, and 2-AG [21–23].

This first eCB, AEA, is a partial agonist of CB$_1$R [24]. The production and release of AEA is tonically low and is increased through the stimulation of a multi-step enzymatic pathway that starts by generating its precursor, N-arachidonoylphosphatidylethanolamide (NAPE), which is converted to NAPE-specific phospholipase D (PLD) into AEA and phosphatidic acid [25–29]. The second eCB, 2-AG, is 200 times more abundant than AEA in mouse brain, and its production is transiently increased by rises in intracellular calcium that activates diacylglycerol lipase (DGL) to cleave DAG and produce 2-AG [30–35] (Figure 1, Key Figure). 2-AG then acts at several targets, including as a paracrine full agonist at both CB$_1$R and CB$_2$R and an autocrine positive allosteric modulator of gamma-aminobutyric acid receptors A (GABA$_A$ receptors).

The potency and efficacy of eCBs to activate CB receptors is regulated by at least six mechanisms: 1) the amount of eCB produced, 2) the assisted travelling of eCBs from their site of synthesis to CB receptors (presynaptic, postsynaptic and on neighboring cells), which is most likely mediated by fatty acid binding proteins [36], 3) the number of CB receptors expressed and their functional coupling, which are controlled by agonist-induced receptor

recycling and desensitization, 4) CB receptor biased signaling to different second messengers, 5) clearance of eCBs by uptake through the plasma membranes, and 6) eCB inactivation by intracellular enzymatic hydrolysis, which primarily involves fatty acid amide hydrolase (FAAH) for AEA and monoacyglycerol lipase (MGL) and α/β-hydrolase domain containing 6 (ABHD6) for 2-AG (Figure 1).

Significant effort dedicated to understanding the molecular mechanism by which eCBs are hydrolyzed has revealed clear similarities and cardinal differences in the enzymatic mechanisms that hydrolyze eCBs. While the majority of AEA hydrolysis in the brain is carried by FAAH, 2-AG hydrolysis involves multiple enzymes, including 85% by MGL, 4% by ABHD6 and 9% by α/β-serine hydrolase domain 12 (ABHD12), at least when measured in mouse brain homogenates [37]. Importantly, recent evidence shows that the overall activity of 2-AG hydrolyzing enzymes in brain homogenates do not fully explain the relative ability of each enzyme to control the levels and efficacy of 2-AG at CB receptors, and that their subcellular expression pattern and the proximity of 2-AG production to its specific target better reflects their contribution to the regulation of signaling. Here we will review the exciting new evidence that established ABHD6's place in eCB signaling, as well as several emerging evidences of its role in controlling other eCB-independent signaling pathways, and how better understanding these molecular interactions will help develop novel therapeutics.

Initial evidence for new 2-AG-hydrolyzing activities and identification of ABHD6

The identification of a novel eCB-hydrolyzing activity and the demonstration of its ability to control eCB signaling requires addressing several fundamental criteria. This often starts with the pharmacological and genetic demonstration that blocking the candidate enzyme halts eCB hydrolysis in both tissue homogenates and intact cells, leads to an increase in the levels of the eCB, and that this activity controls the ability of eCB to activate its target receptors. The first evidence that suggested the existence of another enzyme besides FAAH and MGL that hydrolyzes 2-AG came from Muccioli et al, which found that the mouse microglial cell line, BV-2, does not express MGL yet actively hydrolyzes 2-AG [38]. Concomitantly, three novel enzyme candidates that hydrolyze 2-AG (ABHD6, ABHD12 and neuropathy target esterase) were identified by a multi-dimensional protein identification platform, known as **activity-based protein profiling (ABPP)** (see Glossary) [37, 39]. The demonstration that ABHD6 is responsible for hydrolyzing 2-AG in BV-2 cells that do not express MGL was established by using the first generation ABHD6 inhibitor, WWL70, and by short interfering RNA (siRNA) knockdown, both of which reduced 2-AG hydrolysis in BV-2 homogenates and intact BV-2 cells [40]. ABHD6 expression was detected in several cells, including neurons, and its pharmacological and genetic inhibition enhanced the magnitude of 2-AG activation of CB_1Rs and CB_2R [40]. Together, these studies established that ABHD6 regulates 2-AG signaling through CB_1Rs in neurons and CB_2Rs in immune cells and provided initial evidence that ABHD6 might represent a new member of the eCB signaling system.

ABHD6: A member of the serine hydrolase family

ABHD6 is a 30kDa integral membrane protein that belongs to the serine hydrolase family, one of the largest family of enzymes. Although all members of this family contain the canonical hydrolase fold of an 8-stranded parallel α/β structure with a nucleophile-his-acid catalytic triad, each member is often different in sequence, substrate specificity and catalytic activity [41]. For example, ABHD6 and ABHD12 both hydrolyze 2-AG to similar extents when their activities are measured in mouse brain homogenates despite only sharing less than 20% sequence homology [37]. ABHD6 includes a catalytic triad determined by amino acids S148-D278-H306 that is responsible for 2-AG hydrolysis [41,42]

Expression profile of ABHD6

The expression profile of ABHD6 RNA in tissue and cells suggests high expression in the immune system, particularly the spleen, and the small intestine, then followed by the brain (particularly the cerebral cortex, pituitary gland and hippocampus)[i]. Single cell RNA-seq analyses of mouse brain shows that ABHD6 mRNA expression is different in young and adult brain. In young brains, ABHD6 mRNA is found in progenitor cells and astrocytes, while in adults, it is abundant in select types of neurons (such as GABAergic) and astrocytes [43][ii]. ABHD6 mRNA expression is regulated by hormones such as estrogen, suggesting sex-dependent differences in its expression [44]. Together, these results suggest that ABHD6 expression in select cell types is likely to change as a function of development and sex.

Further, the expression profile of ABHD6 protein in brain tissue appears to be differentially regulated in distinct brain areas and neural cell subtypes. ABHD6 enzymatic activity measured using ABPP in mouse brain has shown that the highest enzyme activity is in the frontal cortex, hippocampus, striatum, and cerebellum, where it is higher than ABHD12 and even MGL [45]. ABHD6 is primarily expressed on the cytoplasmic side by principal glutamatergic neurons, with some expression on GABA interneurons and astrocytes [40]. In murine neurons in culture, ABHD6 expression is prominently localized to dendrites and colocalized with the post-synaptic protein microtubule associated protein 2 (MAP2) and not with presynaptic CB_1R, suggesting a post-synaptic expression pattern that complements the presynaptic expression profile of MGL (Figure 1) [40, 46]. It is likely that the distinct subcellular localization of ABHD6 and MGL (post- and presynaptic, respectively) allows for differential spatial and temporal control of 2-AG levels.

Activity of ABHD6:

Studies in mouse brain homogenates have shown that there are several lipid substrates that can be hydrolyzed by ABHD6 including monoacylglycerols (MAG) with saturated acyl chains varying from 8-18 carbons [42]. Thus, ABHD6 hydrolyzes select lipids with distinct activities 1-arachidonoyl-glycerol (1-AG) > 2-AG > 2-lauroyl-glycerol (2-LG) [42]. Independent of eCB signaling, ABHD6 hydrolyzes 90% of multichain lipid constituents bis(monoacylglycero)phosphate (BMP) and lysobisphosphatidic acid in late endosomes and lysosomes in the liver [47]. Together, these studies suggest that ABHD6 will regulate the levels of several classes of lipids, including signaling, metabolic and structural lipids.

ABHD6 inhibitors

Several ABHD6 inhibitors with distinct chemical scaffolds have been developed, including carbamate-based inhibitors (WWL70, WWL123, KT-182, KT-195 and MJN193) that block its hydrolyzing activity through a covalent modification of the catalytic S148 [37, 48, 49] and fatty acid-based inhibitors (UCM710, CP7B, JAC88, JAC93) that block substrate hydrolysis by competition [50] (Figure 2). It is postulated that with carbamate-based inhibitors, the hydrogen bond between the inhibitor's carbonyl group and the main chain of P80 of ABHD6 is weakened and a new hydrogen bond is formed with the side chain of S148, facilitating a covalent bond [51]. However, the selectivity of early generation carbamate-based and fatty acid-based ABHD6 inhibitors has been questioned by studies reporting their ability to inhibit other enzymes. For example, WWL70 also modulates cyclooxygenase 2 (COX-2) and prostaglandin E1/2 (PGE-1/2) activities in microglia [52]. The carbamate-based inhibitor MJN110 inhibits MGL and ABHD6 activities, although ABHD6 to a lesser extent [53]. Many fatty acid-based inhibitors reduce ABHD6, MGL and FAAH activities with similar IC_{50}s within the micromolar (μM) range and thus cannot pharmacologically differentiate these activities. For example, UCM710 inhibits ABHD6 and FAAH with IC_{50}s of 2.4 and 4.0 μM, respectively, and MGL of 30 μM[50].

However, the next generation of carbamate-based ABHD6 inhibitors have reached greater potency, selectivity and *in vivo* activity, including the brain-penetrant compound KT-182, the peripherally-restricted compound KT-203, and the orally-available compound KT-185 [49]. In addition, triazole urea featuring chiral hydroxylated 2-benzylpiperidines have recently been synthesized to act as dual inhibitors of both DGL and ABHD6, citing the chirality of the carbon at the C2 substituent and position of the C5 hydroxyl to dictate inhibitory activity on the enzymes in mouse brain extracts [54]. Finally, the recent chemical biology tools based on fluorescence activity-based probes that are being developed to try and differentiate the enzymatic activity of these enzymes will certainly help develop more select inhibitors [55]. Thus, much effort has been dedicated to developing selective inhibitors of ABHD6 to unravel the role of this enzyme in regulating cell functions and explore the possibility of targeting this enzyme for therapeutic purposes.

eCB-dependent and eCB-independent roles of ABHD6 in modulating neuronal functions

Evidence suggests that ABHD6 activity controls eCB-dependent neuronal functions through remarkably distinct mechanisms that impact long-term, but not short-term, synaptic plasticity. An involvement of ABHD6 in **long-term synaptic depression (LTD)** was first suggested when it was observed that addition of ABHD6 inhibitor, WWL70, reduced the threshold to LTD at glutamatergic synapses which persisted for at least 40 min after induction [40]. By sharp contrast, WWL70 did not affect short-term plasticity, which is exemplified by **depolarization-induced suppression of inhibition (DSI)** and **depolarization-induced suppression of excitation (DSE)** [56–58]. Specifically, these two forms of short-term CB_1R-mediated synaptic plasticity that regulate GABAergic and glutamatergic transmission, respectively, and are mediated by retrograde 2-AG signaling [59]. Since the durations of DSE and DSI are determined largely by enzymatic degradation

of 2-AG, one might predict that blocking 2-AG hydrolysis would extend the time course of DSE/DSI [46, 60]. However, *in vitro* recordings of neurons revealed that it is mediated by MGL and another enzyme known to modify 2-AG, COX-2 and remains unaffected by ABHD6 inhibition [46, 60, 61]. These studies provide an important differentiator between the biological roles plays by ABHD6, MGL and COX-2 by emphasizing their involvement in short-term versus long-term synaptic plasticity mechanisms.

The initial clue for the existence of an eCB-independent function of ABHD6 in regulating neuronal function came from unbiased high-resolution proteomics on brain tissue unveiling that ABHD6 is part of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor macromolecular complex and reducing its expression results in decreasing AMPA receptor surface expression and ensuing glutamatergic transmission independently of cannabinoid receptors [62, 63] (Figure 1). The reduction in surface expression of the AMPA receptor happens via reduction in surface expression of one of the subunits of AMPA, GluA1. It was found that the C-terminal tail of GluA1 is required for binding between ABHD6 and AMPA subunits GluA1, GluA2 and GluA3 [64]. Importantly, mutation of the S148 site on ABHD6, which is critical to its serine hydrolase activity, did not affect the ability of ABHD6 to regulate AMPA expression, showing that this function of ABHD6 is independent of the enzyme's ability to hydrolyze eCBs [62]. It is interesting to note that this reduction in glutamatergic signaling by ABHD6's regulation of functional AMPA receptor expression contrasts with its eCB-dependent role of enhancing the sensitivity of LTD induction [62]. Therefore, ABHD6's impact on long-term synaptic plasticity at excitatory synapses results from the net effect of two competing mechanisms involving eCB-dependent and eCB-independent mechanisms.

It is important to point out that the findings with ABHD6 inhibitors or genetic deletion of ABHD6 in mice on obesity and diabetes are also independent of eCB signaling as indicated by the results showing the liver, adipose, pancreas eCB signaling were unaltered when ABHD6 is blocked down [65–67]. Thus, ABHD6 is likely to play both eCB-dependent and eCB-independent roles in both central and peripheral tissues.

Pathological consequences of impaired ABHD6 activity

Sever reports show that ABHD6 expression and activity changes in select tissues and as a function of specific pathological processes. ABHD6 expression is increased in select cancer subtypes such as bone, prostate, leukemia and Burkitt's lymphoma, as well as in systemic lupus erythematosus [41, 68, 69]. The gene is also known to be targeted by the Epstein-Barr virus (EBV), a DNA tumor virus associated with Hodgkin's lymphoma and post-transplant lymphoma [70]. Decreased ABHD6 expression may also be involved in select pathological processes, as shown in inflammatory-associated pathologies where infection of macrophages by *Salmonella* typhimurium downregulates hydrolysis activity of ABHD6 and FAAH, which increases 2-AG and AEA levels and enhances phagocytic function in host cells [71]. Thus, changes in ABHD6 expression and functionality may be involved in select pathological processes, including cancer, which provides a framework to test the therapeutic value of targeting ABHD6 for the treatment of select maladies.

Targeting ABHD6 for therapeutic benefit

An initial clue to maladies that might benefit from ABHD6 inhibitors came from discovering that ABHD6 blockade regulates activity-dependent production of 2-AG and ensuing activation of CB_1R, a characteristic of certain epilepsies [40]. Accordingly, ABHD6 inhibition by WWL123 significantly decreases seizure frequency in mice models of chemically-induced and genetically-induced seizures [72]. However, it is interesting to note that ABHD6 inhibition reduced these seizures by increasing $GABA_A$ receptor activity and not by activating CB_1Rs or CB_2Rs [72, 73] (Figure 1). This suggests that ABHD6 inhibitors dampen excessive excitatory transmission in seizures by two mechanisms that provide therapeutic advantages: 1) increasing the level of an endogenous ligand, 2-AG, instead of directly targeting the receptor (here $GABA_A$ receptors) and 2) allosterically increasing $GABA_A$ receptor signaling instead of targeting the orthostatic site of the receptors, a combination of assets that will likely reduce desensitization and therapeutic tolerance [74].

Additional diseases that might benefit for ABHD6 inhibitors are fragile × mental retardation, and traumatic brain injury (TBI). Specifically, in the mouse model of fragile × mental retardation, ABHD6 inhibitors rescue the impaired eCB-dependent LTD [75]. In this model, the transgenic fragile × mental retardation 1 protein directly disrupts the expression of DGL mRNA, reduces 2-AG signaling and increased mGluR5 function, the combination of which impairs neurotransmission in this pathology [75]. In TBI, the ensuing secondary injury mediated by excitotoxicity, neuroinflammation and oxidative stress propagates because of insufficient increase in 2-AG levels to counteract these pathological processes [76]. In a TBI mouse model, post-injury treatment with the ABHD6 inhibitor WWL70 improved motor coordination and working memory performance but did not affect spatial learning or memory impairments [77]. Remarkably, WWL70 attenuated several TBI-induced neurodegenerative processes through both CB_1R and CB_2R, including reduced lesion volume and neuronal loss, suppressed expression of intercellular adhesion molecule 1 (ICAM-1), a marker of inflammation and blood-brain-barrier (BBB) dysfunction, and attenuated nitric oxide and prostaglandin E2 production, two mediators of proinflammatory responses [77]. It should be mentioned that the effect of ABHD6 blockade in fragile × mental retardation and in TBI were carried out using WWL70 and thus will require further validation using genetic tools and more selective ABHD6 inhibitors.

ABHD6 inhibitors have also been tested in experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis (MS) [78]. Here chronic treatment with WWL70 ameliorated clinical symptoms and accordingly reduced both mRNA levels of proinflammatory mediators tumor necrotic factor (TNF-α), inducible nitric oxide synthase (iNOS) and interleukin (IL)-iβ, and the number of CD4+ T-cell infiltrates and TNF-α+ cells [78]. This therapeutic effect was blocked by the CB_2R antagonist, AM630, and lost in the CB_2R-knockout (KO) mice, confirming a CB_2R-dependent mechanism [78]. As mentioned above, the lack of selectivity of WWL70 required further validations [52]. Accordingly, recent work revisited the therapeutic value of ABHD6 inhibitors in MS with the next generation ABHD6 inhibitor, KT-182, and showed that treatment did ameliorate neurological symptoms of EAE but had a small overall effect in disease progression [79]. In another model of MS, the cuprizone model of non-immune dependent demyelination, which

allows to study a different aspect of MS pathogenesis, KT-182 only partially attenuated myelin damage, astrogliosis and microglia/macrophage reactivity without affecting oligodendrocytes [80]. Taken together, these results indicate limited efficacy in therapeutic efficacy of ABHD6 inhibitors in autoimmune-mediated diseases.

When considering systemic diseases, ABHD6 inhibitors might help for the treatment of obesity as indicated by its role in the control of lipid metabolism [42]. Specifically, downregulation of ABHD6 expression in the liver protects against body weight and fat mass gain, increases energy expenditure, and improved glucose and insulin tolerance in mice fed a high-fat diet [65]. β cell-specific ABHD6-KO mice show similar results suggesting that ABHD6 negatively regulates glucose-stimulated insulin secretion [66, 67]. Both functions of ABHD6 are independent of eCB signaling [65–67]. Remarkably, mice lacking ABHD6 in the ventromedial hypothalamus exhibit CB_1R-mediated blunting effects on fasting-induced feeding and consume less food overall, expend less energy and have reduced adaptive thermogenesis in response to a metabolic challenge, suggesting a role for ABHD6 in the counter-regulatory responses associated with metabolic shifts [81]. Together, these studies identify central and peripheral maladies that might benefit from blocking ABHD6 activity.

Conclusion and future perspectives.

Our understanding of the molecular mechanism and biological function of ABHD6, as well as the therapeutic potential of ABHD6 inhibitors, has rapidly increased during the last decade. We've learned that ABHD6 finetunes multiple signaling pathways and that it functions as a molecular hub that controls several biological functions. The studies published on the molecular mechanisms and biological function of ABHD6 during the last decade provided the foundation to establish this enzyme both as a *bona fide* member of the eCB signaling system and revealed a versatile molecular machinery that regulates additional signaling systems, including $GABA_A$ and AMPA receptors through distinct mechanisms (Figure 3A). However, important questions remain unanswered regarding ABHD6's multifunctionality (see Outstanding Questions). While ABHD6 is highly expressed in the brain, it is only responsible for a very small portion of 2-AG hydrolysis when its activity is measured in brain homogenates. What are the major functional lipids metabolized by ABHD6? While it is known that the serine-hydrolase activity of ABHD6 cleaves several lipid substrates, an unbiased global metabolomics analysis of the changes triggered by the acute and prolonged blockade of ABHD6 activity has yet to be reported and will likely identify novel lipid substrates and role in lipid membrane turn-over (Figure 3B). Furthermore, the remarkable ability of ABHD6 to directly interact with AMPA receptors independently of its serine-hydrolase activity and chaperone their trafficking to the plasma membrane raises fundamental questions about the specific ABHD6-AMPA receptors amino acid interaction sites, the type of molecular motor that controls this trafficking and whether additional receptors use this platform to traffic to the plasma membrane (Figure 3C). The range of signaling lipids and interacting receptors that are modulated by ABHD6 suggests a multifunctional role that positions this protein as a critical molecular hub to modulate multiple signaling systems

We also have a limited understanding of the cell-specific expression pattern of ABHD6 and how it changes during development and aging. ABHD6 expression is upregulated by estrogen, indicating that its expression will be tightly controlled by hormone levels [44]. The remarkably high expression of ABHD6 in astrocytes tallies well with the recent studies establishing a dominant role for eCB signaling in this glial subtype and raises the question whether other glial cell types might express this enzyme and whether ABHD6 expression changes as a function of reactive gliosis associated with select neurological diseases [82]. The tools that were recently developed to follow ABHD6 expression and activity, which include specific antibodies that were validated using genetic controls and fluorescence activity-based probes that differentiate serine hydrolase enzymatic activities, will certainly help answer these questions [55, 83].

Further, what are the next therapeutic indications that might benefit from targeting ABHD6? The activity-dependent regulation of 2-AG levels by ABHD6 suggest that its inhibitors might show benefit in rapid onset neuronal dysfunctions and diseases, such as stroke and pain, and more limited efficacy in slow-progressing neuronal dysfunctions and diseases. Is there a role for ABHD6 in brain development? Probably "yes," as suggested by the changes in ABHD6 expression in select cell types as a function of brain development and sex, and that this enzyme regulates eCB signaling and AMPA receptor expression at the plasma membrane, two key molecular mechanisms involved in neuronal differentiation.

Selective manipulation of ABHD6 activities represents an exciting opportunity to develop novel therapeutic modalities. However, the utility of targeting of ABHD6 for therapeutic benefit should be systematically validated using genetic approaches, such as knockout and knockdown strategies. Furthermore, there remains a clear need for developing highly selective inhibitors that target the different activities carried by ABHD6 and lack off-target activity before moving towards the clinic. The escalating interest to study ABHD6's role in eCB signaling and beyond promises to continue on this steep trajectory, a venture will help promptly move ABHD6 inhibitors towards the clinic for the treatment of several devastating diseases.

Acknowledgments:

Glossary

ABPP:	activity-based protein profiling; used in functional proteomic technology utilizing specific chemical probes to target and identify mechanistically related classes of enzymes
DSE:	depolarization-induced suppression of excitation; following neuronal depolarization, glutamate-mediated neurotransmission is reduced
DSI:	depolarization-induced suppression of inhibition; following neuronal depolarization, GABA-mediated neurotransmission is reduced

LTD: long-term synaptic depression; long-lasting decreases in synaptic strength following prolonged activation

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Highlights

- ABHD6 is a *bona fide* member of the endocannabinoid signaling system, enzymatically controlling levels of the most abundant endocannabinoid 2-AG, controlling activation of CB_1 and CB_2 receptors, and thereby modulating cell function.

- ABHD6 modulates neuronal activity in an endocannabinoid-independent manner, through AMPA receptor trafficking and allosteric modulation of GABA receptor signaling. This illustrates a valuable multifunctionality of this enzyme with multiple therapeutic possibilities.

- Novel inhibitors targeting ABHD6 have been developed with increasing selectivity and sensitivity that led to elucidation of its biological function.

- Inhibition of ABHD6 has emerged as a promising therapeutic treatment of select neurological diseases.

Outstanding Questions

- What are the global metabolomic and phenotypic changes caused by either acute or prolonged blockade of ABHD6 activity?

- What are the specific amino acids sites on ABHD6 involved in its multifunctional activity?

- What is the cell-specific expression pattern of ABHD6, and does this change during development, aging and select disease processes?

- Are there different therapeutic benefits of ABHD6 inhibition in rapid versus slow-progressing neuronal dysfunctions and diseases?

Resources:

i. http://mouse.brain-map.org/search/show?
page_num=0&page_size=20&no_paging=false&exact_match=false&search_t
erm=ABHD6&search_type=gene.
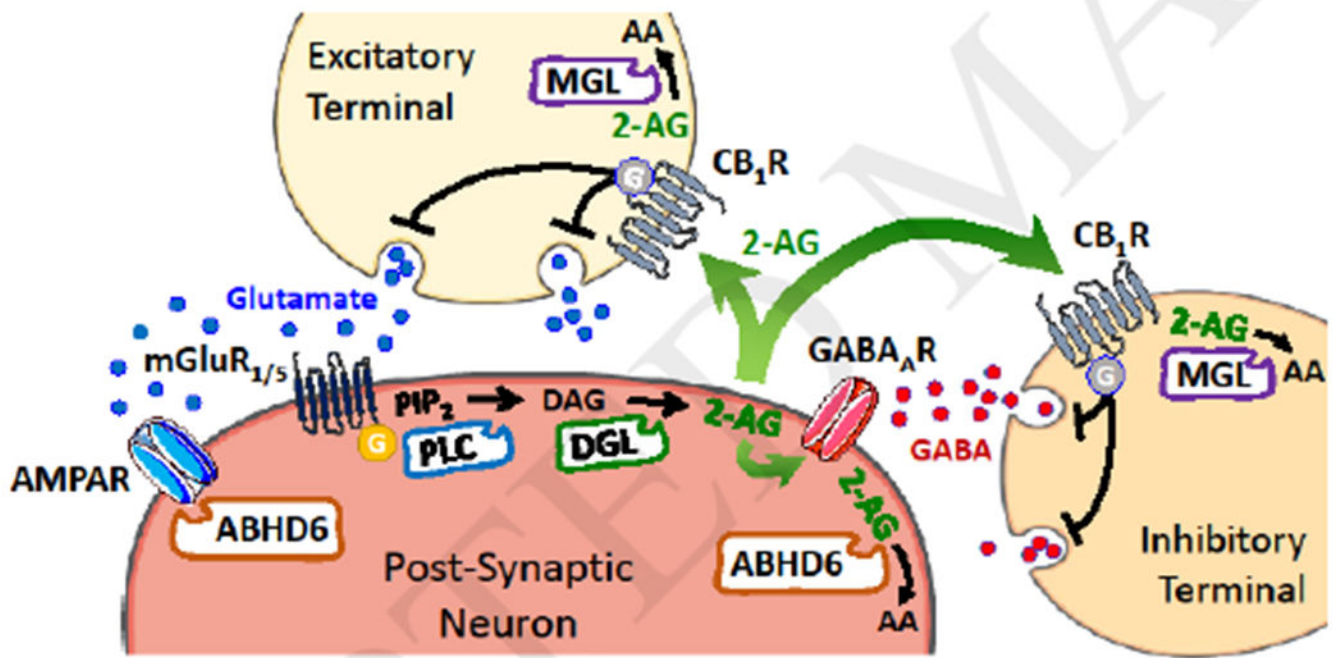
ii. http://www.brainrnaseq.org/

Figure 1, Key Figure. Molecular components involved in the regulation of 2-AG signaling by neurons.

Stimulation of excitatory neurons releases glutamate (blue spheres), activating metabotropic glutamate receptors 1/5 (mGluR$_{1/5}$) on the postsynaptic neuron, which couple to G proteins (orange sphere), activate phospholipase C (PLC) and lead to the production of diacylglycerol (DAG) from phosphoinositol bisphosphate (PIP$_2$). DAG is cleaved by DAG lipase (DGL), which produces 2-AG that acts as 1) paracrine agonist at CB$_1$ receptors (CB$_1$R) expressed by excitatory terminals, 2) paracrine agonist at CB$_1$R expressed by inhibitory terminals that release GABA (red spheres) to activate GABA$_A$ receptors on the post-synaptic neurons and 3) autocrine positive allosteric modulator of GABA$_A$ receptors on the post-synaptic neurons. Excess 2-AG is hydrolyzed to arachidonic acid (AA) and glycerol (not shown) by monoacylglycerol lipase (MGL) in presynaptic neurons or by ABHD6 in postsynaptic neurons. ABHD6 interacts with α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and controls their trafficking to the membrane of post-synaptic compartments.
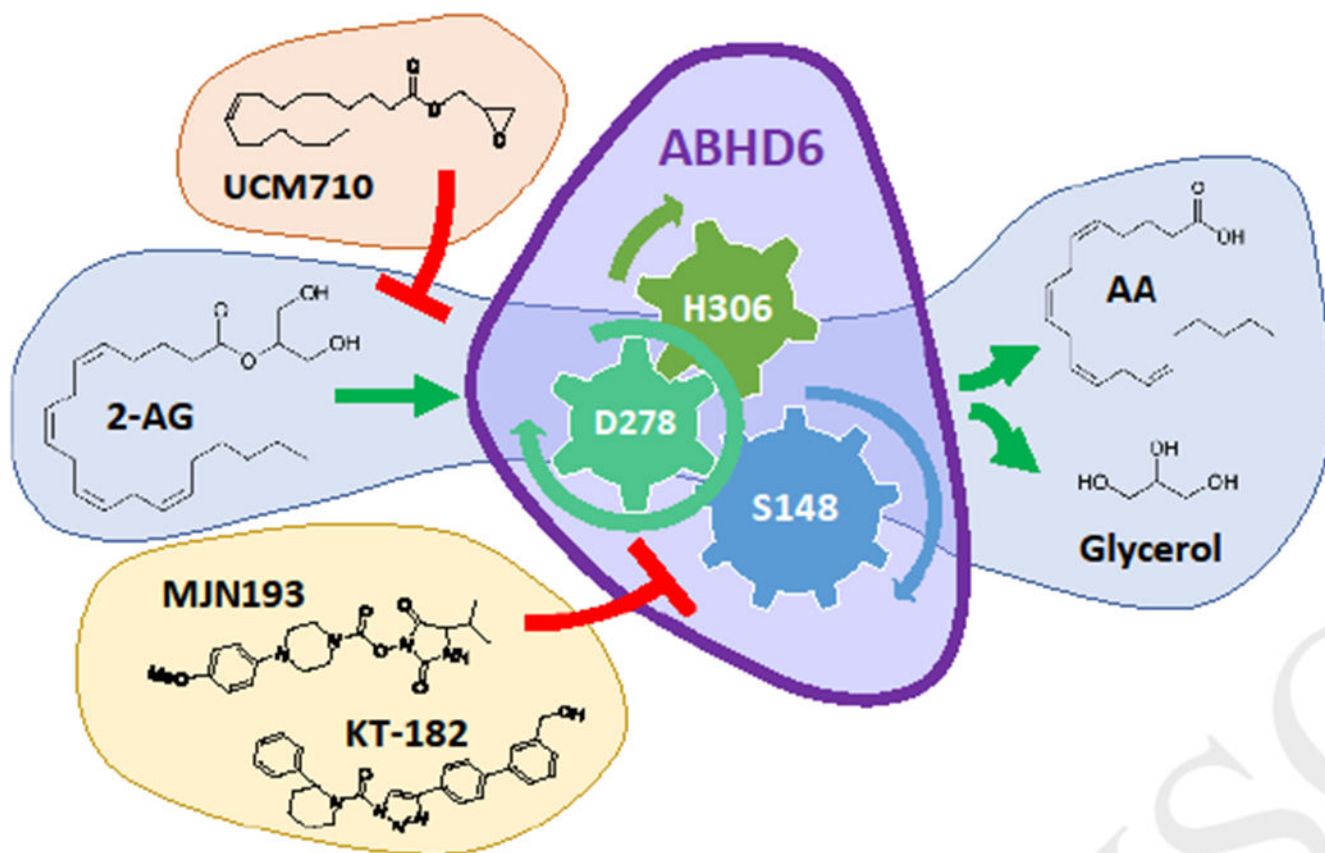
Figure 2. ABHD6 activity and inhibition.

The lipid substrate (e.g. 2-AG) is hydrolyzed by ABHD6 (purple shape) containing a catalytic triad made by amino acids S148, D278 and H306 and leads to the release of arachidonic acid (AA) and glycerol. Fatty-acid based inhibitors (e.g. UCM710) compete with the lipid substrate whereas irreversible carbamates inhibitors (KT-182) and reversible inhibitors (MJN193) interact with catalytic triad.
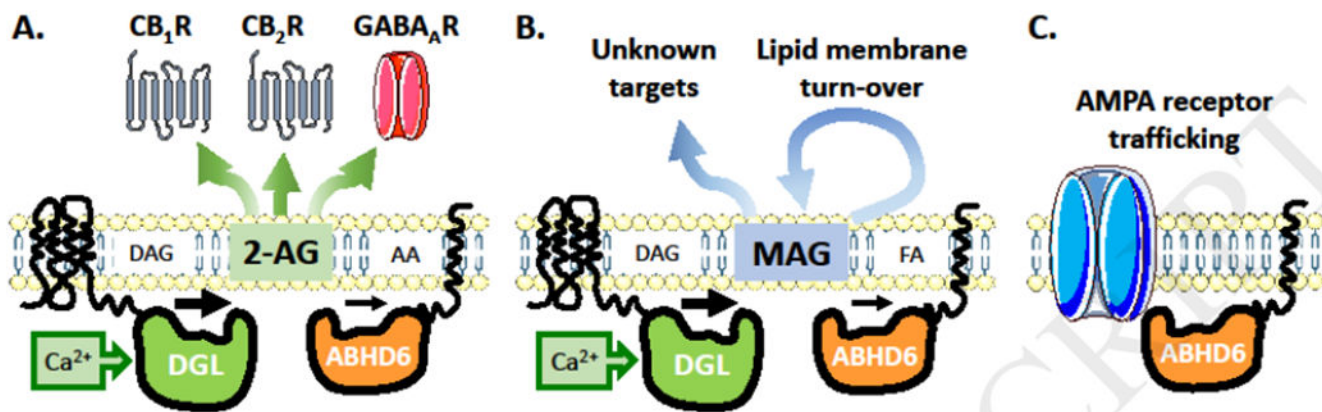
Figure 3. Multifunctional activity of ABHD6.
A. Rise in intracellular calcium concentration activates diacylglycerol lipase (DGL) that cleaves diacylglycerol (DAG) into 2-AG. ABHD6 hydrolyzes 2-AG into arachidonic acid (AA) and glycerol (not shown). Thus, ABHD6 controls the activity-dependent 2-AG accumulation and eventual activation of cannabinoid receptors CB_1R and CB_2R and $GABA_A$ receptors. **B.** ABHD6 controls the hydrolysis of monoacylglycerols (MAG) into fatty acids (FA). MAG accumulation might module the activity of unknown receptors targets and/or modify lipid membrane composition. **C.** ABHD6 acts as a chaperone to traffic AMPA receptors to the plasma membrane.