

Review

Swiprosin-1/ EFhd2: from Immune Regulator to Personality and Brain Disorders

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Key Words

Swiprosin-1 • EFhd2 • Immunity • Personality • Neurogenesis • Dopamine • Addiction • Neurodegenerative disorders

Abstract

Background/Aims: Swiprosin-1/ EF-hand domain 2 (EFhd2) is a Ca²⁺ sensor protein that plays an important role in the immune system. Its abundant expression in the brain, however, suggested also a role in neuronal circuits and behavior. **Methods:** Here we review recent discoveries on the structure and molecular function, its role in immunity and its function in the brain regarding behavioral control and pathologies. **Results:** While EFhd2 did not emerge as a vital protein for brain development, changes in its expression may nevertheless shape the adult behavioral repertoire significantly and contribute to adult personality traits. A defective function of EFhd2 may also render individuals more prone to the development of psychiatric disorders. Most prominently, EFhd2 proved to be a resilience factor protecting from fast establishment of drug addiction. Moreover, EFhd2 is critical for adult neurogenesis and as a modulator of monoaminergic systems. **Conclusion:** Dysregulated activity of EFhd2 is increasingly considered as a contributing factor for the development of numerous neurodegenerative disorders. Whether EFhd2 can be used as biomarker or in therapeutic approaches has to be addressed in future research.

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Introduction

Swiprosin-1 (EFhd2) was originally named in reference to the Swiss-Prot database used for the tandem mass spectrometry data analysis [1]. It is a 240 amino acid Ca²⁺ binding, small adaptor protein consisting of two EF-hand domains (EF1, EF2) with predicted and

apparent molecular weights of 27 kDa and 33 kDa, respectively (<http://elm.eu.org>) [2-4]. The EFhd2 coding gene D4Wsu27e consists of a conserved structure that contains 4 exons, with a long intron between exon 1 and 2, and a long 3'-UTR in exon 4, in both mice and humans. The proximal promoter region from -100/+41 to -70/+41 seems to play a pivotal role in activating the D4Wsu27e gene in Jurkat T cells. ADR1 and Sp1 were shown to bind to 3 conserved noncoding sequences located between the exon 1 and exon 2 of the D4Wsu27e gene within its promoter *in vitro* and *in vivo*. They were identified as transactivators of the D4Wsu27e gene suggesting that they may serve as an important transcription factors for EFhd2 [5]. In mice, D4Wsu27e locates on chromosome 4 and in humans it locates on chromosome 1. In humans, there were three protein-coding splice variants predicted so far [6]. A structural analysis study revealed that the EFhd2 protein is thermostable [7]. EFhd2 is highly conserved among species, especially among mouse, rat, human and Drosophila [8, 9]. It appears also conserved among other homologous EF-hand containing proteins. As such, EFhd2 and its fraternal twin Swiprosin-2/EFhd1 have a similar predicted structure with a 69.7% homology [6]. It is the region between amino acids 20-80, based on the numbering of EFhd2 that is likely to specify their distinct functions [6]. A comparison of amino acid sequences between different species revealed that mouse EFhd2 (Swiss-Prot Q9D8Y0) is 91% identical to human and 98% to rat orthologues [8]. It is also 52% identical to DEFhd2 [10]. Rat and human EFhd2 are 93% identical [8]. Another characteristic that has been described is the ability of EFhd2 to form oligomers and filamentous structures, such as amyloid structures [11-13]. Although the physiological function of EFhd2 in its entirety is still unknown, it has been documented to have a role in apoptosis, cell motility, invasion, metastasis, calcium signaling, actin cytoskeleton, synapse formation, micro-morphology of neuronal cells, neurogenesis, cortical development, neurodegenerative diseases, addiction-prone personality traits, and behavioral plasticity. Its differential expression indicates that EFhd2 expression is regulated in response to many pathological processes, suggesting that it also has a role in many disease processes.

Methods

Here we review the occurrence and physiological function of EFhd2, thereby following its original discovery in the immune system up to more recent links with brain function and the control of normal and pathological behavior.

Structure and function of EFhd2

A blot-overlay assay with radioactive Ca^{2+} revealed that both EF-hand domains of EFhd2 have an intrinsic capacity to bind Ca^{2+} [14]. Equilibrium dialysis with recombinant GST-EFhd2 showed that EFhd2 binds Ca^{2+} with a rather low K_d of 110 μM at physiological ionic strength [12]. Point mutations revealed that the conserved residues E116 and E152 of the EF1 and EF2 domains, respectively, are essential for qualitative binding of Ca^{2+} in blot overlay assays [12]. In contrast to the data obtained by Hagen et al. [14], isothermal titration calorimetry (ITC) experiments using full-length EFhd2^{EF1} and EFhd2^{EF2} unraveled that each EF-hand domain binds Ca^{2+} with high affinity (EF1, $K_d = 96 \pm 15$ nM; EF2, $K_d = 70 \pm 1$ nM) [15]. The authors noted furthermore that there was a decrease in entropy upon Ca^{2+} binding, indicating that the flexible conformation of the Ca^{2+} binding site in the absence of Ca^{2+} changes to a rigid conformation [15]. The K_d s (70–100 nM) for the EF hand domains of EFhd2 could only be measured at low ionic strength (50 mM Tris-HCl, pH 8.5, 20 mM NaCl) [15]. In accordance with the data obtained by Hagen et al. [14], using the full length protein under physiological ionic strength, the authors hypothesize that the actual affinity of EFhd2's EF-hands for Ca^{2+} is much lower than 100 nM under physiologic ionic strength, and that both EF hands would not be occupied by Ca^{2+} at resting Ca^{2+} levels in live cells [15]. Taken together,

these data suggest that EFhd2 can respond to physiologic intracellular changes of the Ca^{2+} concentration, thereby, modulating cytoskeleton dynamics related to cell migration or dendritic spine morphology [15, 16].

Ca^{2+} binding to the EF1 and EF2 domains and also to the N-, but not to the C-terminal was shown to control the intracellular Ca^{2+} concentration in response to BCR stimulation in WEHI231 cells, suggesting that EFhd2 regulates the BCR-induced Ca^{2+} flux through a Ca^{2+} -dependent positive feedback loop. The underlying mechanism still remains to be determined. It should be noted here that EFhd2 did not control the BCR-elicited Ca^{2+} flux in naïve primary splenic B cells [17]. It may still be possible that EFhd2 controls BCR-induced Ca^{2+} signals further downstream upon Ca^{2+} binding, or in proliferating B cells, such as germinal center B cells, where EFhd2 is most prominently expressed within the B cell lineage (<http://www.immgen.org>) [18].

A hydrophobicity plot of murine EFhd2 according to Kyte and Doolittle revealed that the EF1 domain of EFhd2 is more hydrophilic than the EF2 domain [14]. A structural analysis study revealed that EFhd2 is predominantly composed of alpha helix and random coil structures. Thereby, EFhd2's thermo-stability depends on its N-terminus and the Ca^{2+} binding [7]. Mutations of a conserved aspartate on either EF1 or EF2 domain disrupted the Ca^{2+} binding activity, indicating that these domains work in pair as a functional Ca^{2+} binding domain. A conserved phenylalanine at site 89 seems to be important for the EFhd2 Ca^{2+} binding activity [7]. *In vitro* kinase assays demonstrated that Cdk5, a hyper-activated kinase in tauopathies, but not GSK3b, directly phosphorylates EFhd2 and regulates its physiological and pathological function. Mass spectrometry and mutagenesis analyses indicated that Cdk5 mono-phosphorylates EFhd2 at S74, but not the adjacent S76, and affects its Ca^{2+} binding activity [19].

Primary and secondary structure analysis has shown that EFhd2 is an actin binding protein (ABP) that contains three F-actin-binding sites, ABS 1-3, in its core domain (residues 70–184) and modulates actin bundling [15, 20]. The crystal structures of the inactive apo form of EFhd2 could not yet be determined due to structural instability during protein purification [15]. The crystal structure of the EFhd2 core domain, which is mutant defective for one Ca^{2+} -binding site (E116A for EF1; E152A for EF2) was determined [15]. The core domain of the crystal structure of the Ca^{2+} -bound EFhd2 has been shown to adopt a compact and globular fold [15]. The EFhd2 domain architecture is composed of a N-terminal disordered region, forming a moiety of low complexity (LC) with an alanine stretch and an SH3 binding domain [8, 10], followed by a proline-rich region (PR, residues 80–90, ABS1), two EF-hand domains (EF1 and EF2, residues 91–163, ABS2) a connecting short α -helix, which resembles the ligand helix of EF-hand proteins (ligand mimic-LM helix) [21], and a C-terminal coiled-coil (CC) domain (residues 164–184, ABS3) [8, 15, 20].

EFhd2 binds to F-actin in a direct Ca^{2+} independent manner and displays F-actin bundling activity in a Ca^{2+} dependent manner, directly through its EF-hand domains and mainly through its CC domain [15, 22]. Thereby, EFhd2 dimerizes in a parallel manner [15], opposed to what has been anticipated before [20]. Particularly, structures of mutants defective for Ca^{2+} -binding revealed that the F-actin bundling activity depends on the structural rigidity of F-actin binding sites conferred by the binding of the EF-hands to Ca^{2+} . In the presence of Ca^{2+} , two Ca^{2+} ions bind to the EF-hand domains and maintain a structural rigidity of the EFhd2 homodimer through the parallel CC interaction. This subsequently enhances the F-actin-bundling ability of EFhd2 [15]. However, in the absence of Ca^{2+} , the EFhd2 core region displays local conformational flexibility around the Ca^{2+} -binding loop of the EF-hand and C-terminal linker, leading to the re-organization of actin binding sites of EFhd2 which retains F-actin binding, but not F-actin bundling activity [5, 15].

The F-actin-bundling ability of EFhd2 is regulated by a phosphorylation-dependent mechanism. Epidermal growth factor (EGF)-induced phosphorylation at Ser183 of EFhd2 mediates the transition of the C terminal linker region from an ordered to a disordered structure [23]. This results in an increased thermo-stability and in an increased electrostatic repulsion between the ABSs of the core domains of EFhd2 in the homodimer [23]. This re-

organization of the ABSs does not change the affinity of EFhd2 for F-actin, but reduced the F-actin-bundling activity [23].

N-terminal, PR region and actin binding site 1

The LC of the flexible N-terminus contributes to EFhd2's thermo-stability [7] and its functional-binding ability [10, 14]. Particularly the PR region of EFhd2 was identified as part of the ABS1 [15], as well as a site important for the binding of EFhd2 to recombinant SH3-domains of Lyn, Fgr, and PLC γ [10]. Moreover, the PxxP motif in this region may mediate phosphoinositide binding with its hydrophobic residues [24]. This is in accord with its requirement for proper association of EFhd2 with lipid rafts at the B-cell membrane [10, 15].

Deletion of the LC and PR regions might hamper the EF1 folding resulting in a reduced Ca²⁺ binding to the EF-hand domains of EFhd2 [14]. Deletion of EF1 could influence the N-terminal part of EFhd2 in such a way that it loses its function, comparable to its deletion [14].

Deletion of either the N- or C-terminus may render the EF-hand domains more accessible and increases the Ca²⁺ binding ability of EFhd2 protein [7]. Thus, the N- and C-terminus may preclude Ca²⁺ binding activity or deletion of these domains induces a conformational change that generates a more efficient Ca²⁺ binding protein [7]. While the deletion of the N-terminus generates a more globular protein, maintaining the EF-hand domains almost at the same distance as the full-length protein, the distance between the two EF-hand domains was reduced in the C-terminus deletion mutant [7].

EF-hand domains and actin binding site 2

EFhd2 consists of 2 EF-hand domains, the EF1 and the EF2, that show structural stabilization in the presence of Ca²⁺ [15]. They both have the capability to bind Ca²⁺ [7] with high affinity and in a cooperative manner [14, 15]. The binding of Ca²⁺ to EFhd2 is a requirement that equips the protein with the ability to enhance the B cell receptor (BCR)-elicited Ca²⁺ flux [14]. Although both EF-hand domains of EFhd2 are functional [7, 14], EF1 which is more hydrophilic than EF2, contributes to the BCR-induced Ca²⁺ flux more than EF2. That is possibly because EF1 might influence either the folding or the cooperative Ca²⁺ binding of EF2 [14] or since the Ca²⁺-binding loop of EF1 adopts a more flexible structure than EF2 in the absence of Ca²⁺, resulting in large conformational fluctuations to EF1 [15].

EF-hand-domains represent robustly folding super secondary structures. Their single conserved polar residues, such as F89, D105, D109, E116 for EF1, and D141, D143, D145, E152 for EF2, are predicted to be important for the Ca²⁺ binding at these domains [7, 14, 15]. It is important to note that E116 and E152 are the conserved Glu residues that are located at the last position of the loop of EF1 and EF2, respectively. The D105 and D141 represent amino acids at the first position within each loop of EF1 and EF2, respectively [7, 14]. The Phenylalanine residue in EF1 represents a conserved amino acid affected by the identified single nucleotide polymorphism (SNP) rs12131549 [7]. Phosphorylation of the tyrosine residues of EF1, Y83 and Y104, was found to be irrelevant for the actin assembly/disassembly though [25].

EF-hand domains interact tightly via its helix 1 and 4 with the PR region (ABS1). They are also associated with the LM-helix (ABS3) through intramolecular interactions such as those found at the Ca²⁺/calmodulin (CaM) complex [15]. The influence of EF1 on the N-terminal part of EFhd2 pointed to a Ca²⁺-myristoyl switch in EFhd2 [14].

CC domain and actin binding site 3

The CC domain of EFhd2 regulates its dimerization in a Ca²⁺-dependent manner [11, 22]. It facilitates the formation of filaments ranging from 50 to 500 nm lengths [11], possibly enhances its avidity for Ca²⁺ binding [14], and regulates the actin bundling activity through its lysine-rich stretch [22]. Its dimerization or interactions with other partners mediated through its CC domain, which assembles into a parallel dimer, regulates the *in vitro* and *in vivo* function of EFhd2 [14, 15, 22]. EFhd2 has been shown to interact through its CC domain

with a tau binding domain of the mutant tauP301L [11]. Swiprosin-2 contains a very similar CC domain [6], but there is no reported evidence for hetero-dimerization of EFhd2 and Swiprosin-2/EFhd1 yet [26].

In *Drosophila* S2 cells, the CC domain of DEFhd2 is responsible for its localization to the plasma membrane since its deletion, similarly to the deletion of both EF-hand domains and the PR region, led to solely localization in the cytoplasm [9]. Interestingly, in embryos both the EF-hand domains and the CC domain were required to recruit DEFhd2 in the fusion component myoblasts (FCMs) during myoblast fusion [9].

Even when EFhd2 is able to mediate actin-bundling through both its EF-hand domains and its CC domain in the presence of the Ca^{2+} , Kwon et al. [22] suggested that the CC domain is more essential than the EF1 and EF2 domains for the actin bundling activity. Moreover, it was reported that a EFhd2 variant with a mutation in the CC region lost its activity to enhance cell spreading and lamellipodium formation [22].

The ability of EFhd2 to bind Ca^{2+} requires its EF-hand domains [14] and is related to the protein stability. Thereby, Ca^{2+} is essential to maintain a stable form [15]. This ability is controlled by phosphorylation [19]. The dynamic exchange of EFhd2 phosphorylation and dephosphorylation is a molecular mechanism that regulates actin dynamics by modulating the accessibility of cofilin to F-actin [27]. EFhd2 is the only protein among its orthologues that contains a highly conserved S183 residue, which is known to be phosphorylated. It has been shown that EGF-induced phosphorylation at S183 inhibits F-actin bundling, but not the binding activity of EFhd2 and can be blocked by inhibiting PKC. This suggests that PKC may be an upstream kinase catalyzing EFhd2 phosphorylation [27]. EGF-induced phosphorylation at Ser183 of EFhd2 and, likewise, the phosphorylation-mimicking mutant S183E permit cofilin access to the F-actin. This results in actin de-polymerization and induction of loose and thin actin filaments [23, 27]. On the other hand, the unphosphorylated form of EFhd2 and the phosphorylation-deficient mutant S183A induced dense and thick actin filaments [27]. Other studies reported that EFhd2 may be phosphorylated at the sites S11, S74, S76, and Y83, Y104, S204, and at other residues [4, 15, 27, 28] (<http://www.phosphosite.org>).

EFhd2 expression

Among species, physiological systems and organs

EFhd2 is highly conserved among species, especially among mouse, rat, human, frog, chicken, insects, and *Drosophila* [6, 8, 9] and among other homologous EF-hand containing proteins, such as Swiprosin-2/EFhd1 or AIF-1 [6, 8, 15].

Although EFhd2 is a highly conserved protein expressed across many species, it appears to be absent in primitive species like yeast [2, 6, 11]. EFhd2 has been shown to be expressed during the development of the mesoderm [6]. In species such as worm, zebrafish, and higher vertebrates it was also expressed in endodermal and ectodermal tissues [6]. In the adult mice brain, EFhd2 mRNA and protein have been identified in many different regions [13, 29]. Purohit et al. [29] showed that areas with high density of neurons show more intense staining for EFhd2, whereas regions with low density of neurons show less EFhd2 expression [29]. Thus, EFhd2 is not detected in the molecular layer I, the external granular layer II and in brain regions that mostly consist of white matter, such as e.g. the corpus callosum [29]. Conversely, EFhd2 expression is strong in the grey matter, in the neuropil throughout the CNS, the deeper pyramidal III, IV, V and the multiform VI layer of the cortex, the dentate gyrus, and the CA1 and CA2 areas of the hippocampus [29]. Upon a closer look, within the CNS, EFhd2 is expressed in the brain stem, cerebellum, amygdala, striatum, cortex, prefrontal cortex (PFC), as well as the hippocampus [13, 30, 31], in the thalamus and in the olfactory bulb [29]. Particularly, in the cerebellum EFhd2 is expressed in the Crus1 and Crus2 of the ansiform lobule and the flocculonodular lobe, the most primitive division of the cerebellum that can affect the locomotion disequilibrium, vertigo, and gait ataxia. Apart from its high abundance in the ectodermal tissue, EFhd2 has been detected in other systems including

the immune system and several tissues such as spinal cord, lung, heart, liver, spleen, skeletal muscle [8, 13] bone marrow [2], the lenses and fetal eye [32], the mouse renal glomerulus [33] and with lower abundance in kidney and thymus [8]. Particularly, the EFhd2 orthologue in *C. elegans* was found to be expressed in pharynx, body wall muscle, the nervous system and the ventral nerve cord. In zebrafish, EFhd2 associated expressed sequence tags (ESTs) were expressed in brain, eye, genitourinary tissue, gills, muscle, and olfactory rosettes [6].

EFhd2 was also found to be expressed in other cell types such as epithelial cells and endothelial cells [34]. Cells which originate from the ectoderm, expressed also EFhd2 on mRNA and protein level, such as the primary immortalized human keratinocytes [35] and adult rat ventricular cardiomyocytes (ARVC) [36]. In *Drosophila*, the DSwiprosin-1 was transiently expressed during myoblast fusion and endogenously accumulated at the ends of the muscles of the pharynx [9]. It should be noted that the term “expression” used here refers to both RNA and protein data that were not normalized amongst each other- “Expression” is therefore a qualitative statement.

Expression during embryonic development

In the embryonic mice brain, EFhd2 is expressed in the ectodermal tissue, such as the rostral foregut and the caudal hindgut, and in the telencephalon at E8.5 (embryonic stage 5, 5-6) and E14.5 (embryonic stage 22), respectively [37]. In addition, EFhd2 was found to be expressed in the developing cortex, hippocampus, and thalamus of wild-type embryonic mice at days E16 and E18 (embryonic stage 24 and 26) and in the adult brain at postnatal day 150 (P150) [29]. EFhd2 is abundant in the human forebrain [38] where it is expressed throughout adult neuronal development, as well as in mature neurons [29, 39, 40]. It should be noted that in adult versus embryonic brain tissue the expression of EFhd2 is increased roughly two-fold [29]. EFhd2 is expressed in all forebrain areas at embryonic and adult stages [29]. It is already present in murine embryonic stem cells and at birth. However, EFhd2 knock out (KO) and wild type (WT) littermates are indistinguishable at gross brain morphology level, as well as at tissue/organ level. This may suggest that EFhd2 expression may not be necessary for survival during embryonic development, but is required for full capacity development.

In *Drosophila*, DEFhd2 is expressed during muscle development at the E10 (embryonic stage 16). Apart from the embryo, it is expressed in the larvae and in pupae from stage 9 on in the ventral head mesoderm and at stage 10 in hemocytes of the head mesoderm [10]. During germ-band retraction, its expression is detectable in the head region of hemocytes, as well as in foci in the somatic mesoderm [10, 41]. At late fusion stages, it is visible in a few loci within the somatic mesoderm [10, 41].

Immunity related function of EFhd2

EFhd2 in cells of the immune system

EFhd2 is expressed across species in many cell types of the innate and adaptive immune system [6]. Although EFhd2 was initially identified in human lymphocytes, especially in T cells [2, 25] and has been thought to play a role in lymphocyte physiology [42], it is now understood that EFhd2 is not exclusively expressed in lymphoid tissue [8], but in most of the immune cells [34]. It has been identified to be preferentially expressed in the human CD8+ cytotoxic T lymphocytes when compared to CD4+ T helper cells and CD19+ B lymphocytes [2, 43]. A recent exciting report showed that EFhd2 binds to the cytoplasmic domain of PD-1 [44]. PD-1 is an inhibitory molecule on T cells that has caught attracted attention as a target of checkpoint inhibitors, monoclonal antibodies that disrupt the interactions of PD-1 with its ligands, PD-L1 and PD-L2, to support anti-tumour T cell responses [45]. Compelling evidence suggests that the PD-1/PD-L axis has also important functions in the brain [46]. EFhd2 was required for PD-1 to suppress cytokine secretion, proliferation and adhesion of human T cells [44]. However, EFhd2 was also required for human T cell mediated cytotoxicity and for anti-

tumour response in a mouse model [44]. Along this line, it was also found to be expressed in natural killers cells [47], antigen-presenting cells (APCs), such as human and murine B cells [3, 6, 8] and infected macrophages [48]. During murine B-cell development, EFhd2 is highly expressed in immature bone marrow B cells. It is also expressed in resting and activated splenic B cells. Its level did not change during B-cell activation with anti-gM /IL-4, LPS, or with anti-CD40/IL-4 stimulation [8]. However, its expression in Jurkat T cells was up-regulated during T cell activation [11] and during differentiation in a murine RAW264 macrophage cell line after treatment with RANK-L and receptor activator of NF-KB ligand [49], as well as in osteoclasts [50]. EFhd2 is also expressed in human peripheral blood mononuclear cells (PBMCs) [51] where its proteolytic cleavage is associated with rheumatoid arthritis [51]. EFhd2 shows a 5-fold higher expression in monocytes when compared to B cells [26, 50]. Ramesh et al. [34] reported that EFhd2 is expressed in human and primary murine mast cells and modulates their activation. In *Drosophila*, that does not possess an adaptive immune system, DEFhd2 is known to be expressed in innate immune cells, such as macrophage-like hemocytes (phagocytes), during myoblast fusion in the embryo [6, 9].

EFhd2 in B cells

EFhd2 was initially identified by a high-resolution 2-DE and mass spectrometry analysis [1, 3]. On protein level, EFhd2's predominant presence in CD8 lymphocytes when compared to CD19 and CD4 lymphocytes suggested that it may be involved in functions that are important for cytotoxic lymphocytes [3], a hypothesis that has been tested positively recently [44]. Amongst B cells, however, EFhd2 exhibited the highest expression in immature B cells of the bone marrow, but was also expressed in resting and activated splenic B cells. Using ectopic expression and short hairpin RNA (shRNA)-mediated downregulation, it was shown that EFhd2 may regulate the lifespan and BCR signaling thresholds in immature B cells. It was suggested that it is part of the NF-KB-activating branch of the BCR pathway [8]. Moreover, murine EFhd2 was found enriched in the lipid rafts of WEHI231 and NYC31.1 cells, suggesting that it is involved in lipid raft-regulated transduction of BCR signals [3]. Kroczek et al. [10] showed that EFhd2 binds to the rSH3 domains of the Src kinases Lyn and Fgr, as well as to that of PLC γ . It enhances the constitutive interaction of Syk and PLC γ 2 with Lyn. Additionally, the authors showed that EFhd2 stabilized the association of BCR with tyrosine-phosphorylated proteins, specifically Syk and PLC γ 2, in membrane rafts. Thus, it was suggested that EFhd2 is a positive regulator of the BCR-induced Ca²⁺ flux since it provides a membrane scaffold that is required for Syk-, SLP-65-, and PLC γ 2-dependent BCR-induced Ca²⁺ flux [10]. Another study showed that during murine B-cell development, EFhd2 is expressed 5-fold higher in monocytes compared to the B-cells of healthy donors, suggesting to be related to monocyte migration under inflammatory conditions [26, 52]. EFhd2 also showed significantly increased expression in the memory B and T cells compared to naive cells [43].

In EFhd2 KO mice it was shown that T cell-dependent immunization with sheep red blood cells and infection with the helminth *Nippostrongylus brasiliensis* (N.b) increased the production of antibodies of multiple isotypes and the germinal center formation. The serum IgE levels and numbers of IgE+ plasma cells were strongly increased in EFhd2 KO N.b.-infected mice in a B cell intrinsic manner. Accordingly, the authors suggested that EFhd2 may serve as a negative regulator of germinal center-dependent humoral type 2 immunity [17]. Along this line, EFhd2 is a target gene of STAT6, a transcription factor that is mandatory for type 2 humoral immunity [53, 54]. This suggests that STAT6, by up-regulation of EFhd2, creates its own negative feedback loop.

EFhd2 and signaling complexes and actin dynamics

A proteomics approach that was used to identify proteins in caspase-9 protein complexes in extracts derived from untreated and cytochrome c/dATP stimulated lysates of

NSCLC cells revealed that under non-apoptotic conditions, caspase-9 associated with EFhd2. It was assumed that among a large number of cytoskeletal proteins associated with inactive caspase-9, EFhd2 might bind to caspase-9 via ERM proteins indicating a scaffold function and/or that it might regulate Ca^{2+} -dependent activation of caspase-9 during apoptosis [55]. Another study showed that over-expression of EFhd2 promoted the interaction between EFhd2 and caspase-9 and increased the formation of apoptosomes [56]. Kim et al. [25] showed that EFhd2 expression is up-regulated in T cells by the PKC- θ . It is down-regulated by treatment with NF- κ B inhibitors, but not by NF-AT inhibitor, suggesting that EFhd2 expression is PKC- θ -inducible that may modulate the late phase of T cell activation after antigen challenge [25].

In a diabetic mice model, it was shown that EFhd2 was up-regulated by PKC β in the early stage of diabetic neuropathy (DN), and that PKC β facilitated glomerular endothelial cell (GEC) apoptosis through the mitochondrial-dependent pathway [56]. Moreover, EFhd2 expression was also found to be up-regulated in streptozotocin (STZ)-treated mice and a conditionally immortalized mouse podocyte cell line (MPC-5) treated with high levels of glucose. This study using an EFhd2 KO diabetic mouse model and the cell line MPC-5 concluded that EFhd2 expression in glomerular podocytes plays a critical role in early-stage DN. A lack of EFhd2 in early DN attenuates mitochondria-dependent podocyte apoptosis induced by hyperglycemia or high glucose via p38 MAPK signaling pathway [57].

It is known that membrane protrusions and cell movement depend on actin dynamics, which are regulated by a variety of actin-binding proteins working cooperatively to reorganize actin filaments. The expression of EFhd2 was correlated with that of the epidermal growth factor receptor (EGFR) and induced by EGF [58]. Particularly, EFhd2 was at least 1.5-fold down-regulated upon EGF stimulation, indicating a potential role in growth factor-dependent actin remodeling [4]. A study by Huh et al. [58] showed that while EFhd2 overexpression enhanced lamellipodia formation in B16F10 melanoma cells, EFhd2 knockdown inhibited EGF-induced lamellipodia formation, and led to a loss of actin stress fibers at the leading edges of cells, but not at the cell soma [58]. Similar to previous findings suggesting that EFhd2 is an actin bundling protein that regulates actin dynamics in general and cell spreading and migration in particular [22], the authors here showed that EFhd2 modulated lamellipodia formation and actin dynamics by regulating the accessibility of F-actin to cofilin. They showed that EGF-induced phosphorylation of EFhd2 at Ser183 allowed cofilin access to F-actin and led to actin depolymerization. This suggested that the dynamic alteration between EFhd2 phosphorylation and dephosphorylation regulates actin dynamics by modulating the pattern of cofilin activity at the leading edges of the cell [27]. Another study documented that ectopic expression of EFhd2 enhanced lamellipodia and membrane ruffles. These findings showed that at the lamellipodia and membrane ruffles, EFhd2 controlled the direction of cell protrusion and enhanced migration velocity and that its level was up-regulated at highly invasive stages of malignant melanoma [58]. We found that knock-out of EFhd2 accelerated dephosphorylation of Cofilin (corresponding to its activation) after BCR stimulation of primary B cells [17].

EFhd2, tumors and heart disease

In a mouse metastasis model, EFhd2 overexpression induced pulmonary metastasis, whereas EFhd2 knockdown led to marked inhibition of metastasis of highly invasive melanoma cells. The authors concluded that EFhd2 might stimulate cancer invasion and metastasis through activation of the Rho family of small GTPases, including Rac1, Cdc42, and RhoA. Thereby, EFhd2 may serve as a therapeutic target to prevent cancer invasion and metastasis [58]. It was shown that EFhd2 expression increased the formation of protrusive invadopodia structures and associated with increased metastasis, promoted epithelial-to mesenchymal transition partly through inhibition of caveolin-1. Therefore, EFhd2 can serve as an independent marker to predict postsurgical recurrence of patients

with stage I lung adenocarcinoma [59]. A study searching for novel protein biomarkers for acute myeloid leukemia (AML) revealed that monocyte differentiation (FAB M4–M5) correlated with enrichment of EFhd2 [36]. Another study revealed that the expression levels of EFhd2 were up-regulated in human keratinocytes immortalized by human papilloma virus type 16 E6 and E7 oncogenes [35]. It was suggested that EFhd2 may play a key role in cardiac remodeling. Particularly, its expression was increased when adult rat ventricular cardiomyocytes (ARVC) started to spread. This suggested that EFhd2 is required for ARVC to adapt to culture conditions [60]. EFhd2 was also identified as one of the key markers in the process of de- and re-differentiation of cultivated ARVC [61]. Furthermore, in ARVC, silencing of EFhd2 was associated with a down regulation of G protein-coupled receptor kinase 2 (GRK2) and caused a sensitization of β -adrenergic receptors suggesting that EFhd2 is involved in the desensitization of β -adrenergic receptors [60]. Whole blood RNA-seq in an attempt to identify disease-related gene expression in unclassified rare-disease patients, an allelic imbalance towards the deleterious allele was found in the *efhd2* gene. The carrier of this event was diagnosed with idiopathic cardiomyopathy and had accompanying symptoms, namely elevated inflammatory markers, Raynaud's disease and alopecia, indicative of auto-immune issues [62].

EFhd2 in monocytic cells, macrophages and mast cells

EFhd2 becomes up-regulated after stimulation with LPS in macrophages, translocates to the membrane and gets phosphorylated on tyrosine [63]. Furthermore, EFhd2 regulates LPS-induced macrophage recruitment via enhancing actin polymerization and cell migration [63], through up-regulation of Rac1/Cdc42, N-WASP/WAVE2 and Arp2/3 [63]. It was suggested that EFhd2 plays an important role in the macrophage immune response to LPS-induced or cecal ligation and puncture-induced sepsis in mice. Genetic deletion of EFhd2 led to higher mortality, more severe organ dysfunction, restrained macrophage recruitment in the lung and kidney, and attenuated inflammatory cytokine production (including IL-1 β , IL-6, TNF- α , IL-10, and IFN- γ) [64]. Moreover, there was decreased HLA-DR expression in EFhd2-deficient macrophages [64]. Mechanistically, EFhd2 controlled the JAK2/STAT1/STAT3 pathway through the expression level of IFN- γ receptors in macrophages [64]. In corroboration, Xu et al. [65] revealed that extrinsic E-selectin engagement induced phosphorylation of the cytoplasmic IFN- γ R2 by Bruton's tyrosine kinase, thereby, facilitating EFhd2 binding [65]. This event promoted IFN- γ R2 trafficking from the Golgi to cell membrane and was found to be essential for the macrophage response against intracellular bacterial infection [65]. Whereas mRNA expression profiles from a septic mouse model revealed that EFhd2 was also differentially regulated in megakaryocytes between 0-hour control mice and septic mice at 24- and 48-hour status after cecal ligation and puncture [66], EFhd2 had no function in platelets [67]. However, upon thrombin stimulation, the expression level of EFhd2 in rat and human platelets was found to be up-regulated by 1.5-fold [68]. Finally, EFhd2 was up-regulated in the rBCG-AN-E-AC-infected macrophages compared with rBCG-A-E-infected macrophages [48].

Molecular and pharmacological methods investigating the potential mechanism of EFhd2 in the modulation of mast cell activation suggested that EFhd2 regulates cytokine expression of the human mast cell line HMC-1 through actin remodeling [34]. Thylur et al. [42] demonstrated that EFhd2 is expressed in mast cells and is up-regulated in both, *in vitro* cultured mast cells by phorbol ester and *in vivo* model tissues of passive cutaneous anaphylaxis and atopic dermatitis, through the protein kinase C β 1/ η pathway. The authors of this study suggested that PKC-C β 1/ η involves in the expression of EFhd2 in the human mast cell line HMC-1 and that EFhd2 modulates mast cell activation through a NF-KB-dependent pathway [42].

Moreover, EFhd2 was found 3-fold down-regulated in peripheral blood mononuclear cells (PBMCs) of rheumatoid arthritis patients compared to the controls. This suggested a

diagnostic value of protein expression patterns [51]. Furthermore, EFhd2 was identified to be differentially expressed in osteoclast-like cells, suggesting its involvement in bone remodeling and Ca^{2+} homeostasis [50]. Similarly, EFhd2 at mRNA level showed differential expression in human mesenchymal stem cells suggesting that it may play an important role in osteoblast differentiation and bone related diseases [49]. Another study suggested an important role of EFhd2 during bone mesenchymal stem cell differentiation [69].

The role of EFhd2 in normal behavior

EFhd2 in cells of the nervous system

Northern Blot analysis has revealed that EFhd2 is predominantly expressed in the brain [6, 8]. In microglia cells, EFhd2 was found up-regulated and secreted in response to stimulation with nitrated α -synuclein [70]. In cortical neurons EFhd2 is present in neurites [29]. Its synaptic presence was confirmed by biochemical analysis of isolated synaptosomes [29, 71]. Thereby, EFhd2 might play a role in the number of synapses that could be formed, as 1) a decrease in EFhd2 expression led to the production of more synapses [72] and more presynaptic structures [38], 2) both overexpression as well as silencing increased dendritic spine branching in primary murine cortical neurons [16], 3) freshly integrated newborn EFhd2 KO neurons in the hippocampus exhibited less dendritic branching as determined by the Scholl-index [40]. In kinesin-mediated microtubule gliding, an increase in the transport velocity and the number of moving particles was observed in EFhd2 KO neurons [29], suggesting that EFhd2 is involved in intracellular trafficking. This suggestion is in accord with the notions that EFhd2 controls IFN- γ R2 trafficking from the Golgi to the membrane in macrophages [65]. In line, using anti-EFhd2 monoclonal antibodies, two EFhd2 pools in B cells were detected. One was found at the plasma membrane and one as a cytoplasmic pool [26]. EFhd2 was enriched in lipid rafts - membrane microdomains that originally were proposed to be sorting stations and that serve as platforms for BCR signal transduction - of B-cell lines that undergo BCR-induced apoptosis [3, 8]. EFhd2 was also present in the murine spinal cord lipid rafts, but only when mice over-express a G93A mutant (mutant toxic gain of function form of superoxide dismutase 1, SOD1) [71]. Associated with cell bodies, but apparently not nuclear [29], EFhd2 was found at high levels in the cytosol and proximal to the membrane in neurons of most brain regions [29]. EFhd2 was also found specifically in the laminae of the granular and pyramidal cells of the hippocampus [29]. In neurites marked by tau and MAP2, EFhd2 was found in proximity to pre- and post-synaptic markers [29]. EFhd2 localizes in axons, dendrites, and synaptic complexes [29, 38]. It associated closely with PSD-95, confirming its dendritic localization [29]. It was also present in neuronal growth cones and nascent presynaptic structures in developing neurons [38]. Furthermore, EFhd2 was detected in high abundance in the cytosolic fraction and the synaptic plasma membrane compartments of biochemically isolated synaptosomes [29], like amphiphysin which is linked to synaptic vesicle endocytosis [72].

Altogether, its localization in the brain and the increasingly recognized effects of immune factors on behavior [73, 74], suggests a prominent role of EFhd2 in the organization of behavior.

Motor functions

Although EFhd2 is mainly expressed in CNS regions that associate with motor function, such as motor cortex and cerebellum [13], there were no motor coordination or balance impairments detected in EFhd2 mutant mice, neither in hemizygous nor in null mutant animals. However, a study by Wang et al. [75] revealed that EFhd2 is strongly expressed in the vestibular nuclei (VN) of the medulla oblongata. Decreased EFhd2 expression levels in the VN temporally associated with motion sickness [75]. Furthermore, the recovery of EFhd2 expression coincided with motor coordination recovery [75]. This may suggest a role of EFhd2 in motor pathologies.

Feeding behavior

Food intake and drinking behavior was not altered in mice lacking EFhd2 (EFhd2 KO). Neither was body weight affected [16].

Emotional behavior

The emotional behavior of EFhd2 KO mice was characterized by enhanced sensation-seeking behavior in the open field test and reduced anxiety in the elevated plus maze test. Depression-related behavior was reduced in the forced swim test and in the novelty suppressed feeding test. However, the consumption of a hedonic stimulus was not altered, suggesting a preserved hedonic tone [16]. These findings suggest that a lack of EFhd2 results in a phenotype characterized by high sensation seeking and reduced anxiety, which is often associated with generally enhanced risk taking behavior and drug abuse in animals and humans [76, 77]. There is a strong relationship between sensation seeking and arousal [78, 79]. In this study, EFhd2 KO mice not only showed an enhanced exploratory response to a novel environment, but also enhanced tissue noradrenaline (NA), but not serotonin (5-HT) levels in the nucleus accumbens (Nac) and prefrontal cortex (PFC). This suggests a higher baseline arousal level in EFhd2 KO mice [16]. These findings were paralleled in a human sample of healthy adults, which also showed an association of the EFhd2 coding gene D4Wsu27e SNP rs112146896 with anxiety traits [16].

EFhd2 in behavioral pathologies

Drug addiction

Drug addiction is a very common psychiatric disorder with severe health consequences for the individual and detrimental effects for social environment and society [80]. The molecular mechanisms that lead from controlled drug use to addiction and those that prevent chronic consumers from such a transition are not sufficiently understood. Although symptoms of drug addiction are very similar among affected individuals and well classified in current diagnostic manuals like DSM-5 of the American Psychiatric Association (2013) [81], there are different pathways which lead from a controlled consumption, which is an integral part of Western society culture [82], to addiction. Interestingly, only a minority of chronic drug consumers of up to 30%, depending on the drug used, develop an addiction. Others control their consumption and instrumentalization of the drug over life time [82-84]. Thus, there appear resilience factors that protect individuals from becoming addicted even after they established regular drug consumption. These resilience factors and their neuronal mediators are increasingly considered as predictors and targets for prevention strategies and possibly also during addiction treatment [85].

A study by MacLaren and Sikela [86] compared the gene expression profiles and sensitivity to the sedative effects of alcohol of inbred short sleep (ISS) vs. inbred long sleep (ILS) mice. EFhd2 expression was higher in the cerebellum of ILS compared to ISS mice. ISS mice were also more sensitive to the sedating effects of alcohol. This was the first evidence suggesting a role of EFhd2 as potential resilience factor for alcohol effects. They were confirmed and expanded in other mouse models. It was shown that EFhd2 KO mice drink more alcohol than controls and spontaneously escalate their consumption. This coincided with a sensation-seeking and low anxiety phenotype [16]. A reversal of the behavioral phenotype with β -carboline, an anxiogenic inverse benzodiazepine receptor agonist, normalized alcohol preference in EFhd2 KO mice, suggesting a EFhd2-driven relationship between personality traits and alcohol preference [16]. These findings were confirmed in a human sample of healthy adolescents where a positive association of the D4Wsu27e SNP rs112146896 was found with lifetime drinking, and a negative association with anxiety [16]. In the mouse model, a lack of EFhd2 reduced the extracellular DA levels in the Nac, but enhanced responses to alcohol in this structure. Thereby, the EFhd2 effect was region

specific, as it did not affect basal DA levels or alcohol-induced responses the PFC [16]. A gene expression analysis revealed reduced tyrosine hydroxylase expression and the regulation of genes involved in cortex development, Eomes and Pax6, in EFhd2 KO mice. These findings were corroborated in *Xenopus* tadpoles by EFhd2 knock-down. Magnetic resonance imaging (MRI) in mice showed that a lack of EFhd2 during development reduced cortical, but not subcortical region volumes at an adult age [16]. Moreover, human MRI confirmed the negative association between lifetime alcohol drinking and superior frontal gyrus volume. The EFhd2 KO did not affect the sedative effects of alcohol, conditioned rewarding effects of alcohol, or alcohol bioavailability in mice [16]. From these results the authors concluded that EFhd2 may work as a conserved resilience factor against alcohol consumption and its escalation, mediated by developmental effects on Pax6/Eomes controlled cortical development and its influence on the reward circuitries of the brain. A naturally occurring reduced EFhd2 function may induce personality traits of sensation seeking/ low anxiety which may explain the higher risk of later enhanced susceptibility for alcohol consumption and addiction [16].

A subsequent study investigated whether the resilience role of EFhd2 also applies to other drugs of abuse, like the psychostimulants cocaine and methamphetamine. This study confirmed that EFhd2 has no role in the establishment of the conditioned rewarding effects of both drugs. EFhd2 deficiency led to hyperactivity in a novel environment, but a reduced methamphetamine-induced hyperlocomotor response. Furthermore, methamphetamine partially normalized the low anxiety trait in the EFhd2 KO mice [85]. EFhd2 also controls the psychostimulant drug-induced DA- and 5-HT increases in the brain in a resilience-like way. Thereby, EFhd2 appears to significantly limit DA and 5-HT responses in the Nac, but less so in the PFC. A more complex bi-directional modulation was found for the NA response [87].

Electrophysiological slice recordings showed that the lack of EFhd2 enhanced the excitability of dopaminergic neurons in the ventral tegmental area (VTA). EFhd2 KO dopaminergic neurons showed significantly higher rates of spontaneous firing [87], possibly resulting from an imbalance between excitation and inhibition [88]. There was also an enhanced firing rate after stimulation of VTA dopaminergic neurons lacking EFhd2. The latter is in accordance with the enhanced extracellular DA responses after a challenge with a psychostimulant drug. Further mechanistic investigation showed that VTA dopaminergic EFhd2 KO neurons displayed a stronger hyperpolarizing response to the DA D2 receptor agonist quinpirole. This suggests that the EFhd2 KO neurons are actually more sensitive to DA D2 auto-inhibitory control [87]. This altered dopaminergic feedback control may, however, not suffice to explain the observed effects on dopaminergic basal activity. Thus, other systems may be implicated in the modulation of the somatodendritic DA release. For instance, the glutamate input can modulate somatodendritic DA release [89], as it was also observed in EFhd2 deficient neurons in the vestibular nuclei that show increased glutamate-induced excitation [56].

Altogether, accumulating evidence points towards a specific role of EFhd2 in drug abuse and addiction. Thereby, EFhd2 is required to provide natural resilience to drug consumption, which is mediated by EFhd2 effects in the brain's reward system.

Suicide

Among other cytoskeletal proteins that have a functional connection to glutamate, GABA, and serotonin receptors, EFhd2 exhibited altered expression in the PFC and amygdala of suicide victims [90].

Schizophrenia

A proteomic analysis of post-mortem medio-dorsal thalamus samples from schizophrenia (SCZ) patients by using quantitative shotgun mass spectrometry and 2DE-gel electrophoresis revealed that EFhd2 was 2.5-fold upregulated in SCZ patients compared to healthy individuals [91].

EFhd2 and neurodegenerative diseases

An early finding connecting EFhd2 with neurodegenerative disease was its identification with a mutant tau protein (tau P301L) that elicits frontotemporal dementia associated with Parkinson's disease on chromosome 17 (FTDP-17) [13]. Subsequently, Ferrer-Acosta et al. [11] demonstrated the *in vitro* ability of EFhd2 to form amyloid structures which was reduced by the presence of Ca²⁺ ions. Very recently, Vega et al. [92, 93] suggested that EFhd2 modulates the structural dynamics of tau proteins based on two experiments. First, incubation of mutant (K19)-tau with substoichiometric amounts of EFhd2 promoted the formation of amyloid structures *in vitro*. Second, co-incubation of EFhd2 and tau in the absence of Ca²⁺ lead to the formation of solid-like structures containing both proteins. However, in the presence of Ca²⁺, EFhd2 and tau separated together into liquid droplets. EFhd2's coiled-coil domain was necessary to alter tau's liquid phase separation. A future task will be to integrate Ca²⁺-dependent structural alterations of EFhd2 with its apparent ability to alter tau structure. The same group also showed that EFhd2 associated with pathological tau proteins in the brain of patients with Alzheimer's disease (AD), suggesting that EFhd2 may play an important role in the pathobiology of tau-mediated neurodegeneration [11]. An RNA-Seq analysis found alternative splicing of EFhd2 at a statistically significant level in the frontal lobe between normal and AD post-mortem tissue [94]. Whether this alternative splicing is identical to the deleterious allelic event observed in an unclassified patient with autoimmune syndroms [62] remains to be determined. Moreover, the EFhd2 level could be significantly increased by magnetic field (MF) stimulation in AD patients, suggesting that EFhd2 may be a promising molecule for further investigating the mechanisms of MF exposure on the development of AD [95]. Another study reported a profound increase of TAU and p-TAU in the hippocampus of adult EFhd2 KO mice, suggesting that the loss of EFhd2 leads to hippocampal tauopathy, which in turn impairs the integration of adult newborn neurons [40]. Additionally, microarray analyses of the prefrontal cortex of EFhd2 KO mice revealed a dysregulation of genes involved in axonal guidance, glutamatergic synapse formation, chemokine receptor signaling, focal adhesion formation and extracellular matrix receptor interaction, including guidance cues such as Semaphorin-3C and Ephrin-A3. Interestingly, the positive regulation of dendrite growth by Semaphorin-3C is mediated by Cdk5 and both TAU and EFhd2 can be phosphorylated by Cdk5. Therefore, dysregulation of environmental factors regulating axon and synapse formation may mediate the cell extrinsic effect of EFhd2 deletion on adult hippocampal neurogenesis [40]. An *in vitro* study showed that absence of EFhd2 did not alter the synaptic endocytosis and exocytosis, but enhanced the transport of synaptophysin-GFP containing vesicles. The presence of EFhd2 notably inhibited the kinesin mediated transport [29]. The demonstrated involvement of EFhd2 in the control of synapse development and maintenance, but not in neurite development, provided a missing link between EFhd2 and the onset and progression of human dementia, a disorder characterized by the loss of synapses [38]. In addition, adult newborn neurons in EFhd2 KO mice showed reduced survival starting at the early neuroblast stage, spine formation and dendrite growth, thus linking EFhd2 to impaired synaptic plasticity. It may also suggest a role of EFhd2 in neuronal survival and synaptic integration in the adult hippocampus [39].

A study using a Huntington's disease (HD) mouse model identified changes in the EFhd2 protein expression that occurred at very early stages and preceded the phenotype of HD onset [96]. EFhd2 showed higher co-expression increases than α -synuclein in a study of Parkinson's disease (PD) brains [97]. A QUICK screen in NIH3T3 cells identified EFhd2 as an interaction partner of the leucine-rich repeat kinase (Lrrk2) which has a role in PD cases and is involved in actin cytoskeleton dynamics [33]. Another study suggested a EFhd2 abundance in the supernatants of aggregated nitrated α -synuclein stimulated microglia [69]. Protein expression patterns in the striatum, substantia nigra and cerebral cortex of the PINK1-KO mice, a PD mouse model, showed that EFhd2 expression was altered, with an expression ratio 0.66 in PINK-1 KO versus the WT mice [98].

A role of EFhd2 in amyotrophic lateral sclerosis (ALS) was suggested after a proteomic analysis using the G93A mutant SOD1 ALS mouse model found differences in spinal cord lipid

raft proteomes. EFhd2 was among the proteins uniquely identified in the lipid rafts isolated from G93A mouse spinal cord, but not from the WT [71]. In a previous study, however, it was shown that the expression levels of EFhd2 protein were not significantly changed in the hippocampus of a transgenic mouse model overexpressing human SOD1 as compared to non-transgenic mice [30]. Another study using a label-free quantitative proteomic analysis in order to identify co-aggregating proteins in transduced primary cortical neurons with a lentivirus expressing GA149-GFP or GFP alone revealed that EFhd2 was strongly enriched in C9orf72 patients, the most common pathogenic mutation in human ALS cases [99]. An involvement of EFhd2 in experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis, was reported as well. This study revealed an increase in EFhd2 expression in the spinal cord, thus suggesting that it may be the consequence of infiltration of inflammatory cells or glial activation [100].

A cerebral stroke mouse model found EFhd2 to be regulated early following a permanent occlusion of the middle cerebral artery (pMCAO) [101].

Conclusion

EFhd2 is a Ca^{2+} sensor protein that plays an important role in immune regulation. It is abundantly expressed in the brain, however, suggested also a role in behavioral organization. Recent research has now identified specific functions of EFhd2 in brain development. While EFhd2 does not appear to be a vital protein for development, changes in expression may shape adult behavioral repertoire and contribute to personality traits. Those naturally emerging differences in EFhd2 activity may also render individuals prone to the development of psychiatric disorders. Most prominently, EFhd2 emerged as a resilience factor protecting from fast establishment of drug addiction. EFhd2 is critical for neurogenesis in the brain and as a modulator of the mesolimbic dopamine system.

Dysregulated activity of EFhd2 is increasingly considered as a contributing factor in the development of various neurodegenerative disorders. Whether this can be used as biomarker or in therapeutic approaches has to be addressed in future research.

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Disclosure Statement

The authors declare no conflicts of interest.

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