Porf-2 = Arhgap39 = Vilse: A Pivotal Role in Neurodevelopment, Learning and Memory

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**Porf-2 = Arhgap39 = Vilse: A Pivotal Role in Neurodevelopment, Learning and Memory**

**ABSTRACT**

Small GTP converting enzymes, GTPases, are essential for the efficient completion of many physiological and developmental processes. They are regulated by GTPase activating proteins (GAPs) and guanine nucleotide exchange factors (GEFs). *Arhgap39*, also known as preoptic regulatory factor-2 (Porf-2) or Vilse, a member of the Rho GAP group, was first identified in 1990 in the rat CNS. It has since been shown to regulate apoptosis, cell migration, neurogenesis and cerebral and hippocampal dendritic spine morphology. It plays a pivotal role in neurodevelopment and learning and memory. Homologous or orthologous genes are found in over 280 vertebrate and invertebrate species suggesting preservation through evolution. Not surprisingly, loss of the *Arhgap39/Porf-2* gene in mice manifests as an embryonic lethal condition. Although *Arhgap39/Porf-2* is highly expressed in the brain, it is also widely distributed throughout the body, with potential additional roles in oncogenesis and morphogenesis. This review summarizes, for the first time, the known information about this gene under its various names, in addition to considering its transcripts and proteins. The majority of findings described have been made in rats, mice, humans and fruit flies. This work surveys the known functions, functional mediators, variables modifying expression and upstream regulators of expression, and potential physiological and pathological roles of *Arhgap39/Porf-2* in health and disease.

**Key Words:** Porf-2, Arhgap39, RhoGAP, Vilse, Arhgap93B, apoptosis, cell migration, dendritic spines, neurodevelopment, learning and memory, neurogenesis, conserved gene
SIGNIFICANCE STATEMENT

This review comprehensively includes what is currently known about *Arhgap39/Porf-2* under its multiple names. Arhgap39 is a critically required molecule for neurodevelopment, learning and memory that is expressed throughout the lifespan. It also has definitive roles in stem cell fate and cell migration, apoptosis and proliferation, in both neural and non-neural sites. Homologous or orthologous genes have been conserved for millennia through evolution with a wide phylogenetic distribution from invertebrates to mammals. Its expression is exquisitely regulated by age, sex, location and hormones. The consequences of dysregulation of *Arhgap39* are currently under investigation.

I. INTRODUCTION

GTP Converting Enzymes and their Modifying Proteins

Small GTP converting enzymes, GTPases, ranging in size from 20-40 KDa, are essential for the efficient completion of many physiological and developmental processes (De Filippis et al., 2014, Jiang and Ramachandran, 2006, Moon and Zheng, 2003, Peck et al., 2002, Tcherkezian and Lamarche-Vane, 2007, Ghosh et al., 2017). The GTPases switch between an active GTP-bound state and an inactive GDP-bound form as illustrated in Figure 1. These switches are mediated by GTPase activating (or accelerating) proteins (GAPs) and guanine nucleotide exchange factors (GEFs). GEFs promote the binding of GTP, thus favoring the active state, while GAPs enhance the intrinsic activity of the GTPase, leading to GTP hydrolysis and returning the GTPase to an inactive state. Thus, GAPs can also be considered as co-effectors with their GTPase partners. The inactive state is further promoted by guanine nucleotide dissociation inhibitors (GDIs) which sequester and stabilize the GDP-associated form.
There are five GTPase subfamilies: Ras (rat sarcoma), Rho (Ras homolog), Rab (rat brain), Arf/Sar (ADP-ribosylation factor/secreton-associated Ras-related protein), and Ran (Ras-related nuclear) in eukaryotes. These five subfamilies have been extensively studied in yeast, Arabidopsis, rice, Drosophila and selected vertebrates, especially rodents and humans (De Filippis, Romano, 2014, Jiang and Ramachandran, 2006, Yorimitsu et al., 2014). They regulate a diverse array of cellular functions, such as cytoskeletal reorganization, cell motility and polarity, vesicular trafficking, cell fate and differentiation. Each GTPase subfamily has an associated GAP subfamily. Several groups of Rho GTPases exist, including Rho, Rac, Cdc42, and Rnd. An individual RhoGAP can interact with several Rho GTPases, depending on the cellular and developmental context.

**A Brief History of Arhgap39/Porf-2 Discovery (for more detail see Section V below)**

The first paper on *Arhgap39/Porf-2* reported the partial sequence and tissue distribution of the messenger RNA in the rat (Nowak, 1990). Subsequently, the investigation of the physiology and regulation of *Arhgap39/Porf-2* in the rat led to the publication of 11 peer-reviewed manuscripts, including a summary book chapter on the discovery and characterization of Porf-2 (Nowak, 2014). It was found that Arhgap39/Porf-2 proteins are encoded by multiple RNA transcripts with tissue-specific expression. *Arhgap39/Porf-2* is highly expressed in mammalian brain, including hypothalamus (regulation of metabolism and reproduction) and hippocampus (essential for learning and memory) (Hu and Nowak, 1994, Nowak, 1990). The pattern of hypothalamic expression in the preoptic area-anterior hypothalamus (POA-AH) during a critical period of prenatal development is sex-specific, with levels peaking earlier in males (E18-19) than in females (P0) (Nowak and Gore, 1999). It was found that the hormones, estradiol (E2) and progesterone (P4), regulate its expression in the female rat brain (Nowak et al., 1999). In males,
gonadotropic pituitary factors decrease Arhgap39/Perf-2 mRNA in the POA and in the testes (Nowak et al., 1997) and testicular factors decrease Arhgap39/Perf-2 mRNA in the POA, but not in the medial basal hypothalamus (MBH) or cerebral cortex. Through this work it was also learned that Arhgap39/Perf-2 is highly expressed in a subset of immature germ cells in testes (Nowak, Torres, 1997). In sum, these results suggest that precise regulation of Arhgap39/Perf-2 expression has an impact on both CNS development and reproductive health. Finally it was discovered, using Southern blot analysis, that Arhgap39/Perf-2-like genes was present in nine widely ranging groups of vertebrates including primates, ungulates, rodents, birds and fishes (Schmerr et al., 2002), but the function(s) of this gene remained elusive.

*Arhgap39/Perf-2—From EST to ORF*

Arhgap39/Perf-2, also known as Vilse, KIAA1688, D15Ws1169e, CrossGAP and Rho GTPase activating protein39, is a RhoGAP protein. Its ortholog is known as Arhgap93B in *Drosophila.* Evidence that this gene contains a functional open reading frame emerged slowly, initially based on a partial sequence (Nowak, 1990) from a rat brain cDNA library. Its existence was hinted at as a member of several EST collections (Ko et al., 1998, Miki et al., 2001, Okazaki et al., 2002), including pre-implantation mouse embryo (Ko et al., 2000), and orphan gene libraries (Bonaldo et al., 1996, Carninci et al., 2000). *Arhgap39/Perf-2* has been identified in over 20 large scale comprehensive enumerations of genes expressed as RNA transcripts and proteins. The complete mouse coding sequence was predicted in 2001 (Kawai et al., 2001) and verified by Okazaki and colleagues (Okazaki et al., 2004). The Arhgap39/Perf-2 story has emerged slowly from a series of studies that demonstrate regional, temporal and physiological variations in expression, tissue and location, some of which likely reflect species-specific functional roles for this gene. These will be discussed in more detail below.
II. GENE, RNA and PROTEIN (Table 1)

Phylogenetic Distribution of Arhgap39/Porf-2

In 2002, the first phylogenetic distribution analysis was reported for Arhgap39/Porf-2 (Schmerr, Schleter, 2002). Southern blot analysis using a rat probe revealed homologs in human, mouse, pig, sheep, cow, chicken (two bands) and zebra fish. It was shown that, in the rat, Arhgap39/Porf-2 is a single copy gene (Nowak, Torres, 1997) and this has been confirmed in human and mouse with the sequencing of their complete genomes. In Drosophila there is a single ortholog. However, the possibility remains that duplications have occurred in other species, including chickens.

In 2002, the Mammalian Gene Collection Program team identified murine Arhgap39/Porf-2 as a candidate full-ORF clone (Strausberg et al., 2002). Subsequently, in 2004, the Mammalian Genome Project reported the full-ORF cDNA sequences in mouse and human (Gerhard et al., 2004). In 2011 the mouse gene location was determined by Jackson Laboratories (Bar Harbor, ME) to be on chromosome 15 (Diez-Roux et al., 2011). The gene is positioned on chromosome 7 in rats and on chromosome 8 in humans.

To date, 180 orthologs, identified by gene and exome sequencing, have been reported in a variety of vertebrate species, from platypuses (NCBI103) to equids (Hestand et al., 2015) and penguins (NCBI101). Moreover, the homologous mouse and human genes are located in a large conserved syntenic region corresponding to human chromosome 8 and mouse chromosome 15, supporting derivation from a common ancestor that existed at minimum 75 Myr ago (Mouse Genome Sequencing Consortium et al., 2002). A related gene (Arhgap93B) in Drosophila and other invertebrates has now been identified in > 100 additional species. As the common ancestor of
vertebrates and insects existed at minimum ~530 million years ago, this raises the possibility that

*Arhgap39/Porf-2* is even older, but this idea remains to be rigorously evaluated.

**DNA and RNA Structure and Modifications**

The mouse *Arhgap39/Porf-2* gene is ~94,000 base pairs in length and contains 13 exons. The

*Rattus norvegicus* gene is slightly shorter at ~92,000 bp, also with 13 exons. The *Homo sapiens*

genome is ~171,000 bp with 16 exons. The arrangement and spacing of the 12 most-3’ exons are

similar in these three species; the increased length of the human gene appears to reflect

extensions of the introns upstream of the fourth rat/mouse exon. Whereas the *Drosophila*

counterpart is only 14,785 bp, it has 14 exons and three domains that are highly conserved within

the vertebrate genes noted above; these are WW, myosin tail homology 4 (MyTH4) and GAP

domains described below.

Through *in silico* and Northern blot analyses, as well as sequencing of full-length cDNAs in

human, rat and mouse, it has been clearly demonstrated that the *Arhgap39/Porf-2* gene can give

rise to multiple RNA transcripts (Nowak, 1990, 1997, Rietveld et al., 2014). Although it was

briefly reported that there was a second related gene in the mouse (Lundstrom et al., 2004), it

was subsequently shown to represent an alternative splice form of Arhgap39/Porf-2 mRNA.

However, there is evidence that two of the major transcripts give rise to slightly different

proteins, due to alternative splicing of the in-frame exon 7 (Figure 2). The mouse protein

isoforms 1 and 2 differ by elimination of exon 7 in isoform 2 which results in a 31 amino acid

deletion in the MyTH4 domain. Human isoforms 1 and 2 are similar to mouse isoform 2 and the

single verified rat isoform in that they all encode a protein that lacks exon 7. Human isoforms 1

and 2 differ in their 5’ untranslated regions. In addition to the verified transcripts, there are a

number of predicted transcripts. To a large extent, the resulting protein or other products of these
transcripts have not been characterized. Alternative splicing occurs in 41% of mouse transcriptome and, in transcripts which contain a coding sequence, 79% of splice variations alter the protein products, and potentially, their subcellular localization and function (Okazaki, Furuno, 2002). It remains to be seen whether there are different forms of Arhgap39/Porf-2 protein in different subcellular compartments or with alternative posttranslational modifications that allow for it to have several separate functions.

Protein Domains and Modifications

The RhoGAP family members are notable for having multiple functional domains in addition to the GAP domain, including many that have been implicated in protein-protein interactions. This organization may facilitate interactions with more than one signaling pathway (Moon and Zheng, 2003, Tcherkezian and Lamarche-Vane, 2007). In the mouse, as well as in Drosophila melanogaster, Arhgap39/Porf-2 protein has been shown to have three potentially functional domain regions, as shown in Figure 2.

Two WW Domains

The first hypothesized functional region is represented by a pair of WW domains at the N-terminus of the predicted Drosophila melanogaster protein (Lundstrom, Gallio, 2004) and mouse isoform 1 (Lim et al., 2014). WW domains are approximately 35-45 amino acids in length and are characterized by the presence of hydrophobic amino acids with cyclic side chains, including tryptophan, tyrosine, phenylalanine and proline, but do not usually otherwise exhibit significant homology between different genes. However, the two WW domains of human Arhgap39/Porf-2 exhibit 84% and 77%, respectively, with the related drosophila Arhgap93B (Lundstrom, Gallio,
WW domains recognize and interact with proline rich segments of other proteins to facilitate protein-protein interactions (Otte et al., 2003).

**MyTH4 Domain**

The second region is a MyTH4 domain, an approximately 150 amino acid module. MyTH4 domains have been identified as a conserved sequence in the tail domains of several different unconventional myosins and a plant kinesin-like microtubule protein. There is a 44% protein sequence similarity between the *Homo sapiens* Arhgap39/Porf-2 and *Drosophila melanogaster* Arhgap93B MyTH4 domains (Lundstrom, Gallio, 2004). Although the function of MyTH4 domains is not yet fully understood, there is evidence that the MyTH4 domain of Myosin-X (Myo10) binds to microtubules (Kerber and Cheney, 2011). The MyTH4 domain has also been found in several non-motor proteins. Interestingly, the results of Huang et al. (Huang et al., 2002) suggest a possible role for one of these (MAX-1) in netrin-induced axon repulsion in *Caenorhabditis elegans* by modulating the UNC-5 receptor signaling pathway. MAX-1 homologues are also found in drosophila, mice and human (Kikuno et al. 2000, Huang et al. 2002). An emerging theme is that many, and perhaps most, guidance cues are bifunctional. Arhgap39/Porf-2 and MAX-1 are both MyTH4 domain-containing proteins that display this attraction-repulsion dichotomy.

**RhoGAP Domain**

The third functional region is a RhoGAP domain close to the carboxy-terminus. RhoGAP domains are approximately 140 amino acids, sharing 20-40% amino acid identity (Lamarche and Hall, 1994). The actual GTPase(s) with which they interact cannot be predicted by the sequence, but must be experimentally determined. The human Arhgap39/Porf-2 GAP domain shares 86.5%
homology with the rat, 88.8% with the mouse (personal calculation) and 61% with drosophila (Lundstrom, Gallio, 2004). The rat also has transcripts that include the RhoGAP domain; however, it also has at least two variants, X4 and X5, that lack this domain. Instead, each contains a distinct carboxy terminal sequence.

Additional Motifs and Modifications

In addition to the WW, MyTH4 and GAP domains, the predicted protein isoforms 1 and 2 have 19 potential motif sites, including 8 serine/threonine phosphorylation sites, two SH2, and a single SH3 motif (Obenauer et al., 2003). Serine or tyrosine phosphorylation of regulatory proteins including GAPs is one mechanism by which activation and subcellular location can be controlled. One example is CdGAP, a RhoGAP whose activity is regulated through phosphorylation by ERK (Tcherkezian et al., 2005). Another example is the phosphorylation of the RacGAP protein, FilGAP, at Ser-402 (Morishita et al., 2015). Phosphorylation increases its RacGAP activity in vivo and promotes diffuse distribution in the cytoplasm which stimulates cell spreading on fibronectin. The total number and active sites of phosphorylation in the Arhgap39/Porf-2 protein have not been determined. One of the predicted phosphorylation sites in Arhgap39/Porf-2 at serine 169, within a casein kinase I motif, was confirmed by a screen of mouse synaptosomal proteins isolated from 2-5 month cortex (Munton et al., 2007). In addition, phospho-serine 589 was identified in a large screening of the developing mouse brain (E16.5) for phosphorylated peptides by Ballif and colleagues (Ballif et al., 2004); interestingly, this was not one of the phosphorylation sites previously predicted.

SH2 and SH3 are homology domains found in a family of oncogenic non-receptor tyrosine kinases. An SH2 domain, which was first identified in the oncoproteins Src and Fujiyami poultry sarcoma, is about 100 amino-acid residues long. It functions as a regulatory module of
intracellular signaling cascades by interacting with high affinity to phosphotyrosine-containing target peptides in a sequence-specific and strictly phosphorylation-dependent manner. SH3 domains are small protein domains of about 60 amino acid residues. SH3 domains are found in proteins of signaling pathways regulating the cytoskeleton, the Ras protein, the Src kinase and many others. SH3 domains interact with other proteins and mediate assembly of specific protein complexes, typically via binding to proline-rich peptides in their respective binding partner. The presence of these motifs that accelerate protein localization and interactions in Arhgap39/Porf-2 are consistent with its role as a RhoGAP that is involved in several complex signaling pathways.

III. TISSUE DISTRIBUTION of ARHGAP39/PORF-2 RNAs and PROTEINS (Table 2)

The first partial Arhgap39 (Porf-2) sequence was reported in a cDNA library constructed with RNA isolated from adult rat hypothalamus (Nowak, 1990). This group detected multiple polyA-enriched RNA transcripts expressed widely in the adult rat, including cerebral cortex, hippocampus, hypothalamus, cerebellum, amygdala, anterior pituitary, adrenal, testis, liver and kidney (Nowak, 1990, 1997, 2014). ESTs and alternative splicing sequence enriched tags (ASSETs) (Watahiki et al., 2004) were also found in 7.5 day mouse embryo (E7.5) placental growth cone (Ko, Threat, 1998) and E17.5 day mouse skin and testes (Miki, Kadota, 2001). In 2001 human KIAA1688 cDNA was sequenced and human tissue distribution of Arhgap39/Porf-2 RNA was quantified by PCR (Kikuno et al., 2004). Although RNA levels were highest in brain and spinal cord, it was also found in testis, kidney, liver, pancreas, lung and in other tissues at lower levels.
RNA transcripts have also been detected in human hypothalamus, adrenal, prostate, and placenta (Nowak, 2014) and in several cultured cell types, including human melanocytes (Watahiki, Waki, 2004), human vascular endothelial cells (Kaur et al., 2008), mouse cerebellar stem cells (C17.2) (Ma and Nowak, 2011), Fisher rat thyroid-like (FRTL-5) cells, mouse hypothalamic neurons (GT-1) (Nowak, 2014), and human pancreatic beta cells (Wang J., 2012, personal communication.). Recently, several extensive studies of gene expression in human (Fagerberg et al., 2014), mouse (Yue et al., 2014) and rat (Yu et al., 2014) have expanded the molecular distribution of Arhgap39/Porf-2 RNA to include intestine, spleen, heart, thymus, uterus, lung, stomach, ovary, urinary bladder, mammary gland, prostate, salivary gland, thyroid gland and adipose tissue.

In 2004 Ballif et al. identified an Arhgap39/Porf-2 phosphopeptide in a large proteomic analysis of E16.5 mouse brain (Ballif, Villen, 2004). Diez-Roux et al. found moderate protein expression of Arhgap39/Porf-2 (D15Wsu169e) in E14.5 mouse embryo brain and spinal cord and peripheral nervous system, and low expression in multiple other peripheral locations, including renal, hematopoetic, gastrointestinal and smooth and striated muscle (Diez-Roux, Banfi, 2011). Arhgap39/Porf-2 protein expression was also reported in mouse cerebellar stem cells (Ma and Nowak, 2011). Recently, several Arhgap39/Porf-2 proteins were detected in human male liver (unpublished observations, Liu, Zhang, Guo and Nowak).

In the brain, Arhgap39/Porf-2 RNA was first localized to neurons (Nowak, Torres, 1999), in the 60 day old female rat hypothalamus by in situ hybridization analysis. It was later detected as a phosphoprotein by LC-MS/MS in synaptic terminals isolated from 2-5 month C57Bl/6 mouse cortex (Munton, Tweedie-Cullen, 2007). Arhgap39/Porf-2 is also detected in neurospheres derived from mouse hippocampus (Huang et al., 2016).
IV. FUNCTIONAL ROLES and DOWNSTREAM EFFECTORS (Table 3)

Arhgap39/Porf-2 has been shown to function through regulation of two Rho GTPases, Rac1 and Cdc42.

Several investigators have shown that Arhgap39/Porf-2 is a regulator of Rho GTPases and as such plays a role in endothelial cell migration (Kaur et al., 2008), ganglion and axon tracking (Hu et al., 2005, Lundstrom et al., 2004), neural stem cell fate (Ma and Nowak, 2011, Huang et al., 2017) and dendritic spine formation (Lee et al., 2017, Lim et al., 2014). These studies further link Arhgap39/Porf-2 to angiogenesis, tracheal innervation, and CNS development.

Axon Directional Migration

Lündstrom and colleagues (Lundstrom, Gallio, 2004) were the first to show a functional role for Arhgap93B, the Drosophila melanogaster ortholog of Arhgap39/Porf-2. They demonstrated that the WW domains of Vilse, as they termed it, bind to the CC2 domain of the Slit receptor, Roundabout (Robo), and inactivate RacGTP to mediate Slit-directed midline repulsion of CNS axons in tracheal cells. They further demonstrated that both drosophila Arhgap93B and human Arhgap39/Porf-2 GAP domains effectively stimulated GTP hydrolysis of Rac1. Soon thereafter, Hu et al. (Hu, Li, 2005) identified Vilse, which they called CrossGAP, as a regulator of midline axonal repulsive guidance in Drosophila. They confirmed the direct interaction between Vilse and Robo and the inactivation of Rac1 by Vilse. Interestingly, either too little or too much Vilse resulted in defective Robo-mediated, Slit-directed axon repulsion at the midline.

Endothelial Cell Migration

In human vascular endothelial cells, Arhgap39/Porf-2/Vilse is sequestered by Robo1 and Robo4, resulting in an increase in activated Cdc42. Cdc42-GTP then activates insulin receptor protein 53
(IRSp53) by binding to its CRIB domain. This exposes an SH3 domain that binds to Mena, an
IRSp53 effector and mediates actin nucleation, resulting in filopodia formation and endothelial
cell directional migration (Kaur et al., 2008).

**Neural Stem Cell Fate**

In 2011, the roles of Arhgap39/Porf-2 in the mouse neural stem cell (NSC) line, C17.2, were
discovered, including its negative effects on cell cycle and proliferation and potentiating effects
on apoptosis. The key cell signaling components involved in mediating these effects were
identified (Ma and Nowak, 2011). These include increased levels of p21 leading to cell cycle
arrest and decreased cell proliferation, and enhanced drug-induced apoptosis through both p53-
dependent and -independent pathways by upregulating Bcl-2 associated X protein (BAX). These
findings are consistent with those of others who have shown that BAX and p53 are regulators of
programmed cell death in mouse cerebellum (Geng et al., 2010) as well as with the finding that
upregulation of BAX and p53 can suppress the growth of tumors, including gliomas (Zhen et al.,
2017).

This work also demonstrated that Arhgap39/Porf-2 had no effect on NSC differentiation induced
by serum starvation (Ma and Nowak, 2011). Although this study did not identify the GTPase
substrate involved, Ras, Rho and Cdc42 have all been shown to regulate cell cycle entry and cell
proliferation (Olson et al., 1995, Olson et al., 1998, Welsh et al., 2001) and to determine the
fate of NSCs by influencing the balance of stem cell maintenance with proliferation and
apoptosis (Joseph and Hermanson, 2010). Other RhoGAPs including DLC2 (Ching et al., 2003)
and tGAP1 (Modarressi et al., 2004) have been shown to decrease cell proliferation by slowing
down the cell cycle. tGAP1 also induces apoptosis when overexpressed in somatic cells.
In 2016, Huang et al. showed that the effects of Arhgap39/Porf-2 on cell proliferation of NSCs were mediated through its GAP domain. They extended previous in vitro findings in C17 cells that Arhgap39/Porf-2 is anti-proliferative (Ma and Nowak 2011) and demonstrated that Arhgap39/Porf-2 decreases proliferation in neurospheres from newborn mouse hippocampus as well as in hippocampal sections from 6 week old mice (Huang, Yang, 2016). Transfection with a full length Arhgap39/Porf-2 construct, but not one lacking the GAP domain, resulted in decreased levels of intra-nuclear β-catenin. The authors postulated that the observed effects on cell proliferation are mediated through the GAP domain causing decreased translocation of β-catenin into the nucleus.

Dendritic Spine Morphology

In 2014, Lim at al. (Lim et al., 2014) demonstrated that Arhgap39/Porf-2 is required for normal dendritic spine formation in the rat dentate gyrus, a portion of the hippocampal formation, and in mouse neuroblastoma x rat glioma hybrid cells. Interaction of a proline motif in the neuron scaffold protein connector enhancer of kinase suppressor of ras2 (CNK2) with the WW domain of Vilse regulates Arhgap39/Porf-2 localization. This critical interaction maintains the RacGDP/GTP balance required for dendritic spine formation. It is of note that CNK2 has been implicated in X-linked cognitive impairment in a human subject (Houge et al., 2012). Additionally, constitutive lack of the RhoGAP, oligophrenin 1, in mice results in decreased density and increased length of dendritic spines in the amygdala (Khelfaoui et al., 2014) and failure of hippocampal CA1 dendritic spines to mature during development (Khelfaoui et al., 2007). These mice also display deficits in learning and memory as measured with Y-maze, O-maze, Morris water maze and conditioned fear extinction, as well as altered hippocampal LTP. Deficits in mice of additional Rho GTPase regulators, including SRGAP3, BCR and ABR, ARG,
ABL, Integrin 3α, KALRN, and ARGEF6, have also been associated with cognitive and anxiety-related behavioral alterations, such as decreases in novel object recognition and Y-maze alternation and social interactions, altered synaptic plasticity, and abnormal dendritic spine morphology (De Filippis, Romano, 2014). Finally, exome sequencing of individuals from a family with late-onset Parkinson’s Disease identified a mutation (p.Arg667Gln) in Arhgap39/Porf-2 as a rare variant possibly associated with the disease (Schulte et al 2014), indicating that dysregulation of this gene may also play a role in neurodegeneration.

**Learning and Memory**

In 2014, Arhgap39/Porf-2 was among the genes identified to be associated with cognitive performance in humans (Rietveld, et al., 2014). This may reflect variation in expression of mRNA variants 1 and 2, an interesting observation since they both encode the same protein isoform, but differ in the 5’ UTR. Quite recently, Lee et al. (2017) described the effects in hippocampus of a widespread Arhgap39/Porf-2 knockout in mouse forebrain under the direction of the CamKII promoter. These mice show behavioral deficits in learning and memory as measured by Morris water maze and Y-maze performance. They also exhibit abnormal hippocampal signaling and dendritic spine morphology. Not surprisingly, given its high level of expression in placental growth cone and developing nervous system, a global constitutive knockout resulted in embryonic lethality, with incomplete embryo development.

Taken together, these findings indicate that Arhgap39/Porf-2 is an integral part of the signaling machinery that regulates hippocampal structure and function and more broadly neuronal function, through its effects on neuronal cell proliferation and apoptosis, its regulation of axonal migration and its role in dendritic spine morphology, synaptic plasticity, and cognitive performance.
More recently, several bioinformatics analyses have uncovered associations of Arhgap39/Porf-2 mutations or variations in copy number or expression level with several types of cancer (URL 1, URL 2). These include tumors of the CNS, skin, prostate, and gastrointestinal tract; one conserved variant has been shown to correlate with prostate tumors and sarcomas in a family lineage (Jones 2017). Given its roles in apoptosis and proliferation and interactions with p53 and BAX, a mutation that decreased expression of Arhgap39/Porf-2 could result in increased cell proliferation, leading to tumorigenesis.

V. VARIABLES MODIFYING EXPRESSION and UPSTREAM REGULATORS of ARHGAP39/PORF-2 (Table 4)

Whereas it is important to know the downstream effects of Arhgap39/Porf-2 action, it is also critical to understand the physiological factors that regulate Arhgap39/Porf-2 expression. It has been known for some time that Arhgap39/Porf-2 mRNA in the rat brain is localized mainly to neurons (Nowak, Torres, 1999) and that in the rat age, sex differences, brain region, gonadal steroids and developmental stage regulate/influence the expression of Arhgap39/Porf-2 mRNAs in hippocampus, hypothalamus and cerebral cortex (Hu and Nowak, 1994, 1995, Nowak, 1997, Nowak and Gore, 1999, Nowak, Torres, 1999).

Developmental and Age-Related Changes in CNS Expression of Arhgap39/Porf-2

As measured by nuclease protection assays of cytoplasmic RNA, Arhgap39/Porf-2 RNA averages 40 μg/g total RNA from 15 days to 2 months of age in the male rat hippocampus. The levels increase over 2-fold at 6 and 12 months, then decline with age. Why Arhgap39/Porf-2 is
Arhgap39/Porf-2 RNA levels in the POA are high at E18-19, then fall gradually until P15. This is followed by a plateau through 2 months of age when postnatal levels are highest (19.9 μg/g) followed by a progressive decrease with age (Hu and Nowak, 1994). Postnatal age-related changes in expression have also been identified in the MBH, and cerebral cortex (Hu and Nowak, 1995) where Arhgap39/Porf-2 RNA is several fold higher in both male and female rats at 15 days compared to 30 and 60 days, followed by a long plateau through 24 months of age. Arhgap39/Porf-2 RNA in the MBH also declines rapidly by 2-fold from E18-19 to P15 in both sexes (Nowak and Gore, 1999). Its regionally distinctive, developmentally modified neuronal expression suggested early on that Arhgap39/Porf-2 has a critical role in prenatal and postnatal CNS development and function.

Sex Differences in the Developing CNS

There are also sex differences in Arhgap39/Porf-2 cytoplasmic RNA levels during development in the rat, being higher in male than in female hippocampus and MBH at P15 (Hu and Nowak, 1995). In the POA, late embryonic levels are higher in males at E18-19, then decline rapidly resulting in lower levels in males at P0 and P15 (Nowak and Gore, 1999). Thus there is a sex difference in timing for peak levels of Arhgap39/Porf-2 in the developing rat fetal POA, a brain region that is critical for normal feedback regulation of gonadotropin releasing and inhibiting hormones, and reproductive function more generally.

Gonadal Hormones

In the adult rat, Arhgap39/Porf-2 expression is responsive to extrinsic variations in circulating gonadal hormones in both females and males. In females ovariectomized at 44 days, treatment...
with E2 alone results in an increased level of Arhgap39/Porf-2 RNA in the POA and
hippocampus compared with placebo treated controls (Nowak, Torres, 1999). Replacement of
only P4 upregulates Arhgap39/Porf-2 in POA and cerebral cortex. Treatment with E2 plus P4
also increases Arhgap39/Porf-2 in the hippocampus but this combination decreases the RNA in
POA and cortex. No changes are observed in the MBH. Thus the response of the female rat brain
to gonadal steroids is highly region-dependent. Based on Northern blot analysis of
Arhgap39/Porf-2 mRNA in male rats subjected to hypophysectomy or castration, both pituitary
and testicular factors directly or indirectly affect alternative splicing of Arhgap39/Porf-2 mRNA
transcripts in the POA, MBH and cerebral cortex (Nowak, 1997). At the present time, neither
how this specifically impacts sex-related gene function, nor how Arhgap39/Porf-2 mediates the
effects of reproductive axis hormones in the brain is known.

**Pituitary Factors**

Expression of Arhgap39/Porf-2 RNA in rat testes is predominantly localized to spermatogonia
and primary spermatocytes and is regulated by age and by pituitary gonadotrophic hormones
(Nowak, Torres, 1997). Arhgap39/Porf-2 testicular expression declines with age in the rat
(Nowak, Torres, 1997, Yu, Fuscoe, 2014). At 60 days of age, Arhgap39/Porf-2 RNA has
deprecated to 40% of the level at 15 days; there is a further 50% decline between 60 days and 6-12
months. By 24 months the level is 8-28% of that at 15 days. Hypophysectomy of young adult
male rats results in a significant increase in testicular Arhgap39/Porf-2 to levels normally seen at
15 days. Although the developmental pattern of expression, in addition to the germ cell location
and regulation by hormones of reproduction, suggests a function for Arhgap39/Porf-2 proteins in
mammalian testes, that specific function is currently unknown. Another RhoGAP, tGAP1 that is
mainly found in testes, has been shown to be expressed later, in round spermatids, where it slows down the cell cycle and may induce apoptosis (Modarressi, Cheng, 2004).

**Epigenetic Mechanisms**

Recently, epigenetic mechanisms have been implicated in the regulation of Arhgap39/Porf-2 expression (URL 3). miRTargetLink currently lists 23 miRNAs with weaker experimental evidence that may target Arhgap39/Porf-2.

**Metabolism, Insulin and IGF-1**

More recently it was discovered that Arhgap39/Porf-2 expression is also modified by two important regulators of metabolism and growth, insulin and IGF-1 (Wang, 2011). Both insulin and IGF-1 decrease Arhgap39/Porf-2 expression in FRTL-5 cells in a dose and time-dependent manner. That this downregulation is signaled through the PI-3-kinase/AKT and Raf kinase/MEK pathways was shown by use of specific chemical inhibitors, including wortmannin and Ly29402 for PI-3-K, Akt inhibitor-4, Raf kinase inhibitor, and high-dose wortmannin and PD98059 for MEK/Erk phosphorylation. Additionally, knockdown of IGF-1 receptor (IGF-1R) and possibly insulin receptor results in a partial reversal of the downregulation. An additional study of interest (Bentov et al., 2014) showed an association of IGF-1R deficiency in aged dermal fibroblasts with decreased Erk phosphorylation and decreased cell proliferation. Although Arhgap39/Porf-2 is expressed in skin and is a potential mediator of this effect, neither it nor other potential downstream regulators were measured in this study.

It makes physiological sense that stimulators of growth and metabolism would act counter to Arhgap39/Porf-2, a regulator that promotes apoptosis and inhibits cell proliferation. Recent findings have shown that neurogenesis is impaired and apoptosis increases in hippocampi of...
subjects with diabetes (Ho et al., 2013). A logical question that follows is—what happens to
endogenous levels of Arhgap39/Porf-2 when insulin or IGF-1 is deficient as in Type 1 diabetes
or growth hormone deficiency, either congenital (Laron syndrome) or age-related (Gong et al.,
2014), or if the response to insulin or IGF-1 is blocked, as seen in obesity with insulin resistance,
Type 2 diabetes, or aging (Bentov, Damodarasamy, 2014)?

**Insulin Resistance and Obesity**

Two recent findings suggest a link between insulin resistance and *Arhgap39/Porf-2*. In a Zucker
rat model of obesity-related diabetes, treatment with an antioxidant-fortified diet resulted in
partial preservation of glomerular filtration rate in 20 week old diabetic females (Slyvka et al.,
2009). This group also found decreased expression of Arhgap39/Porf-2 RNA in the kidney
compared to diabetic females on a control diet. Obese humans and rodents also have been shown
to have decreased hepatic expression of Arhgap39/Porf-2 and carcinoembryonic antigen related
cell adhesion molecule 1 (Heinrich et al., 2017). Decreased carcinoembryonic antigen related
cell adhesion molecule 1 leads to a decrease in insulin clearance, exacerbating hyperinsulinemia.
Thus the decrease in Arhgap39/Porf-2 could be a result of increased suppression by insulin in the
pre-diabetic state, although later, as insulin resistance with hyperinsulinemia advances to frank
diabetes, Arhgap39/Porf-2 levels can be anticipated to rise. Both insulin and IGF-1 deficiencies
have been linked to neurodegenerative (Biessels et al., 2014, Ekblad et al., 2017) and
cardiovascular disease (Higashi et al., 2012). This, in conjunction with the above findings, raises
the question of whether increased *Arhgap39/Porf-2* expression factors into the pathophysiology
of diabetic complications more generally.

**Potential Physiological and Pathophysiological Roles of Arhgap39/Porf-2**
Tables 5 and 6 summarize the potential physiological and pathophysiological roles of 

*Arhgap39/Porf-2*, based upon the findings to date. Its wide phylogenetic and tissue distribution, 
its complex physiological regulation, and its pivotal role in basic cell functions, support the 
equation that there is much more to discover about this particular RhoGAP.

**VI. SUMMARY**

*Arhgap39/Porf-2* (Vilse, CrossGAP) is a Rho GTPase activating protein that interacts with Rac1 
and Cdc42. As is the case with many RhoGAPs, it has multiple functions. Among these are 

inhibition of cell proliferation, promotion of apoptosis, regulation of axon guidance and 
endothelial cell migration, and development of normal dendritic spine morphology in the 

hippocampus which may underlie support of normal learning and memory. Various 

physiological and hormonal cues direct region-specific expression in the brain, suggesting that 

day-to-day expression is carefully monitored and maintained. Finally, the finding that deletion of 

*Arhgap39/Porf-2* is fatal and the fact that this gene has been conserved through evolution for 

millennia highlight the crucial importance of this gene.


Qualitative and quantitative analyses of protein phosphorylation in naive and stimulated mouse synaptosomal preparations. Mol Cell Proteomics 6:283-293.


coding sequences of mouse homologues of KIAA gene: IV. The complete nucleotide sequences of 500 mouse KIAA-homologous cDNAs identified by screening of terminal sequences of cDNA clones randomly sampled from size-fractionated libraries. DNA Res 11:205-218.


URL 2. http://useast.ensembl.org/Homo_sapiens/Gene/Variation_Gene/Table?db=core;g=ENSG00000147799;r=8:144529179-144605816 (oncogenesis)


Figure Legends

Figure 1. Schematic of Rac/Rho Functional Conversion, Activation and Inactivation. GTPase: guanine nucleotide triphosphate hydrolase; GAP: GTPase activating protein; GEF: guanine nucleotide exchange factor; GDI: guanine nucleotide dissociation inhibitor.

Figure 2. Sequences of Two Isoforms of Arhgap39/Porf-2 Protein in Mouse. The protein sequence numbers correspond to those in the NCBI database. Adapted from www.ncbi.nlm.nih.gov/gene/22366 (mouse). The functional domains include two WW, and one each of MyTH4 and GAP. Protein isoform 2 differs from isoform 1 in the deletion of 31 amino acids, encoded by exon 7, from the MyTH4 domain.
<table>
<thead>
<tr>
<th>Table 1. Arhgap39/Porf-2 Gene Structure, RNA transcripts and Proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gene Structure</strong></td>
</tr>
<tr>
<td>• The human gene is over 171,000 base pairs in length and contains 16 exons</td>
</tr>
<tr>
<td>• The mouse homolog is 94,000 base pairs and has 13 exons</td>
</tr>
<tr>
<td>• The rat homolog is 92,000 base pairs long and has 13 exons</td>
</tr>
<tr>
<td>• Homologues/orthologs identified in &gt;190 vertebrate species</td>
</tr>
<tr>
<td>• &gt; 100 additional orthologs identified in non-vertebrate animal species</td>
</tr>
<tr>
<td>• Chromosome locations are on 15 in mouse, 8 in human and 7 in rat</td>
</tr>
<tr>
<td><strong>mRNA transcripts</strong></td>
</tr>
<tr>
<td>• 3 verified and 5 predicted in human (<em>Homo sapiens</em>)</td>
</tr>
<tr>
<td>• 2 verified and 1 predicted in mouse (<em>Mus musculus</em>)</td>
</tr>
<tr>
<td>• 1 verified and 5 predicted in rat (<em>Rattus norvegicus</em>)</td>
</tr>
<tr>
<td>• 1 verified in Drosophila</td>
</tr>
<tr>
<td><strong>Proteins, Domains and Motifs</strong></td>
</tr>
<tr>
<td>• Proteins range in size from 16.5-133 kDa</td>
</tr>
<tr>
<td>• Domains include WW, MyTH4 and RhoGAP</td>
</tr>
<tr>
<td>• Additional predicted motifs include serine and threonine phosphorylation, SH3 and SH2.</td>
</tr>
</tbody>
</table>
Table 2. Tissue and cell type distribution of Arhgap39/Porf-2 Expression in Human, Mouse, Rat and Fruit fly.

<table>
<thead>
<tr>
<th>Type of Tissue or Cell</th>
<th>Location (Species)</th>
<th>Method of Detection</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Brain</strong></td>
<td>•Hippocampus (R, M, H)</td>
<td>•N, NPA, RNAi, IHC</td>
</tr>
<tr>
<td></td>
<td>•Hypothalamus (R)</td>
<td>•N, NPA, ISH</td>
</tr>
<tr>
<td></td>
<td>•Cerebellum (R) (M)</td>
<td>•N, NPA, IHC; RNA Seq (Yue)</td>
</tr>
<tr>
<td><strong>Peripheral Nervous System</strong></td>
<td>•Amygdala (R)</td>
<td>•N, RNA Seq (Yue)</td>
</tr>
<tr>
<td></td>
<td>•Ventral neuropile (D)</td>
<td>•ISH</td>
</tr>
<tr>
<td><strong>Endocrine organs</strong></td>
<td>•Tracheal ganglia (D)</td>
<td>•ISH (Löndstrom)</td>
</tr>
<tr>
<td></td>
<td>•Testes (R, H, M)</td>
<td>•RNAi</td>
</tr>
<tr>
<td></td>
<td>•Anterior pituitary (R)</td>
<td>•N</td>
</tr>
<tr>
<td></td>
<td>•Adrenal (H) (M) (R)</td>
<td>•N, RNA Seq (Yue, Yu)</td>
</tr>
<tr>
<td></td>
<td>•Placenta (H, R, M)</td>
<td>•N, EST (Ko) RNA Seq (Yu,Yue)</td>
</tr>
<tr>
<td><strong>Other organs</strong></td>
<td>•Ovary (M) (H)</td>
<td>•RNA Seq (Yu)</td>
</tr>
<tr>
<td></td>
<td>•Adipose tissue (M) (H)</td>
<td>•RNA Seq (Pas)</td>
</tr>
<tr>
<td></td>
<td>•Mammary gland (M)</td>
<td>•RNA Seq (Pas)</td>
</tr>
<tr>
<td></td>
<td>•Thyroid (H)</td>
<td>•RNA Seq (Pas)</td>
</tr>
<tr>
<td></td>
<td>•Pancreas (H)</td>
<td>•RNA Seq (Pas)</td>
</tr>
<tr>
<td><strong>Cells</strong></td>
<td>•Prostate (H) (R)</td>
<td>•N, RNA Seq</td>
</tr>
<tr>
<td></td>
<td>•Liver (R, H) (M)</td>
<td>•N, PCR, WB; RNA Seq (Yue)</td>
</tr>
<tr>
<td></td>
<td>•Skin (M) (H)</td>
<td>•EST (Kensh); RNA Seq (Fag)</td>
</tr>
<tr>
<td></td>
<td>•Uterus (R) (H)</td>
<td>•RNA Seq (Yu); (Fag)</td>
</tr>
<tr>
<td></td>
<td>•Lung (R) (H)</td>
<td>•RNA Seq (Yu); (Fag)</td>
</tr>
<tr>
<td></td>
<td>•Spleen (R) (H)</td>
<td>•RNA Seq (Yu); RNA Seq (Fag)</td>
</tr>
<tr>
<td></td>
<td>•Heart (R) (M)</td>
<td>•RNA Seq (Yu) (Yue)</td>
</tr>
<tr>
<td></td>
<td>•Thymus (R) (M)</td>
<td>•RNA Seq (Yu) (Yue)</td>
</tr>
<tr>
<td></td>
<td>•Skeletal Muscle (R) (M)</td>
<td>•RNA Seq (Yu) (Yue)</td>
</tr>
<tr>
<td></td>
<td>•G1 tract (M) (H)</td>
<td>•RNA Seq (Yu) (Yue)</td>
</tr>
<tr>
<td></td>
<td>•Bladder (M)</td>
<td>•RNA Seq (Yu) (Yue)</td>
</tr>
<tr>
<td></td>
<td>•Kidney (R) (H)</td>
<td>•PCR; RNA Seq (Pas)</td>
</tr>
<tr>
<td></td>
<td>•FRTL-5 (R thyroid-like)</td>
<td>•WB, PCR</td>
</tr>
<tr>
<td></td>
<td>•Pancreatic beta cells (H)</td>
<td>•PCR (Wang)</td>
</tr>
<tr>
<td></td>
<td>•cos7 cells (nHP kidney)</td>
<td>•PAGE</td>
</tr>
<tr>
<td></td>
<td>•C17.2 (M cerebellar stem)</td>
<td>•PCR, WB</td>
</tr>
<tr>
<td></td>
<td>•GT-1 (M hypothalamic neuron)</td>
<td>•WB</td>
</tr>
<tr>
<td></td>
<td>•Vascular endothelial (H)</td>
<td>•IP (Kaur)</td>
</tr>
<tr>
<td></td>
<td>•Melanocytes (H)</td>
<td>•ASSET (Watahiki)</td>
</tr>
</tbody>
</table>
Table 3. Functions and Mediators of Arhgap39/Porf-2

<table>
<thead>
<tr>
<th>Functions</th>
<th>Mediators of Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Mediates axonal guidance and midline repulsion in developing <em>Drosophila</em>.</td>
<td>1. Robo, RacGTP, Slit (Lundström et al., 2004; Hu et al., 2005)</td>
</tr>
<tr>
<td>2. Regulates filopodia formation and directional migration of human vascular endothelial cells.</td>
<td>2. Robo1 and Robo4, Cdc42, IRSp53, MENA, actin nucleation (Kaur et al., 2008)</td>
</tr>
<tr>
<td>3. Delays cell cycle and decreases proliferation of mouse neural stem cell line, C17.2.</td>
<td>3. p21, decreased progression from G1 to S (Ma and Nowak, 2011)</td>
</tr>
<tr>
<td>4. Promotes apoptosis in C17.2 cells.</td>
<td>4. BAX, p53 (Ma and Nowak, 2011)</td>
</tr>
<tr>
<td>5. Regulates dendritic spine morphology in embryonic rat hippocampal neurons.</td>
<td>5. CNK2, Rac1 (Lim et al., 2014)</td>
</tr>
<tr>
<td>6. Decreases neurogenesis in mouse hippocampus.</td>
<td>6. Rac1, β-catenin (Huang et al., 2016)</td>
</tr>
<tr>
<td>7. Plays a role in learning and memory.</td>
<td>7. Hippocampal synaptic signaling (Lee et al., 2017)</td>
</tr>
</tbody>
</table>
Table 4. Variables Modifying Expression and Upstream Regulators of \textit{Arhgap39/Porf-2} 

- Developmental Stage (rat brain) 
- Age (rat brain and testes) 
- Sex (rat brain) 
- Brain region (rat) 
- Gonadal hormones, including estradiol and progesterone (rat brain) 
- Pituitary factors (rat brain and testes) 
- Epigenetic mechanisms such as miRNAs 
- Insulin and IGF-1 (FRTL-5 cells) 
- Type 2 diabetes (Zucker rat kidney) 
- Obesity (human liver) 

Table 5. Potential Physiological Roles of \textit{Arhgap39/Porf-2} 

- Sexual dimorphism of hypothalamus 
- Axon guidance and directional migration 
- Regulation of early sperm development 
- Endothelial cell migration 
- Regulation of cell cycle in neural stem cells 
- Development of normal dendritic spine morphology 
- Cognitive performance 
- Development and function of extraembryonic structures 

Table 6. Potential Pathophysiological Roles of \textit{Arhgap39/Porf-2} 

- Congenital cognitive insufficiency 
- Age-related cognitive decline/ Neurodegeneration 
- Diabetic nephropathy 
- Diabetes-related cognitive dysfunction 
- Obesity-related hepatic dysfunction 
- Cognitive decline related to IGF-1 deficiency 
- Oncogenesis
Figure 1. The Rho GTPase Cycle

- GDP-bound: inactive conformation
- GTP-bound: active state

- GDIs
- GEFs
- GAPs (Arhgap39)
- Pi

- Effectors
  - cell cycle
  - cell proliferation
  - apoptosis
Figure 2. Arhgap39 Mouse Protein Isoforms

- Isoform 1: exon 7 starts at 1109
- Isoform 2: exon 7 starts at 1078

- WW domains
- myTH4 domain
- RhoGAP domain