The fragile site WWOX gene and the developing brain

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Abstract

WWOX was cloned as a tumor suppressor gene mapping to chromosomal fragile site FRA16D. Loss of WWOX is closely related to tumorigenesis, cancer progression, and therapy resistance. Recent studies demonstrate the growing role of WWOX gene in other human pathologies such as metabolic and nervous system-related conditions. The neurologic phenotype of WWOX mutation includes seizures, ataxia, developmental delay, and spasticity of variable severity. WWOX is a ubiquitous protein with high expression in many tissues including brain, cerebellum, brain stem, and spinal cord. WWOX is highly expressed in different brain regions during murine fetal development and remained unchanged in the cortex and the corpus callosum in adult mice. The mechanism or the putative role of WWOX in the nervous system is still unclear but may include abnormal signaling protein, disruption of neuronal pathways, neuronal differentiation, mitochondrial dysfunction, or apoptosis. Homozygous mutations affecting WWOX in humans are likely to be more described in the future using exome sequencing. The described findings highlight that WWOX plays a critical role in normal central nervous system development and disease.

The aim of this review is to summarize the roles of WWOX in the developing brain.

Keywords: WWOX gene, seizures, ataxia, spasticity, brain

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Introduction

WWOX was cloned as a putative tumor suppressor gene mapping to chromosomal fragile site FRA16D. The WWOX gene spans a genomic locus of 1–2 Mbp encoding an open reading frame of 1.2 kb and consists of nine coding exons in a region of approximately one million base pairs. The full-length WWOX, which encodes a 414 amino acid protein, possesses two typical N-terminal WW domains, a C-terminal short-chain dehydrogenase reductase domain, and nuclear localization sequence.^{1–4}

Numerous studies have shown that WWOX is an important tumor suppressor and that deletions affecting WWOX accompanied by loss of expression are frequent in various epithelial cancers.⁵ Translocations and deletions affecting WWOX are also common in multiple myelomas and are associated with a worse prognosis.⁶ In addition, recent studies demonstrate a growing important role of WWOX gene in other human pathologies such as metabolic syndrome,⁷ and more recently central nervous system (CNS)-related pathologic conditions.^{8,9}

The aim of this review is to summarize the roles of WWOX in the developing brain.

Expression of WWOX expression in tissues including the developing nervous system

Immunohistochemical staining corroborated by RNASeq analysis from multiple human tissues has shown that the WWOX is a ubiquitous protein with high expression in many tissues. Thyroid, brain, testis, ovary, and prostate demonstrated to be the tissues with the highest expression levels.^{10–12}

Chen et al.¹⁰ studied the expression and distribution of WOX1 in developing and adult murine nervous system. They showed that WWOX gene is differentially expressed during various stages of brain development in mice. The immunoreactivity is seen in the derivatives of ectodermal layers during embryonic development and includes cells of the CNS, peripheral nervous system, and sense organ and mesenchymal cells of the head and neck. The general pattern is that WWOX expression in the developing brain is low during the early embryonic stage; however, moderate-to-high WWOX immunoreactivity is detected in the brain and spinal cord during the middle and late fetal stages. In the adult's brain, high levels WOX1 immunoreactivity are seen in the epithelial cells of the choroid plexus and

ependymal cells, while the expression is low to moderate within white matter tracts, mainly axonal profiles of the corpus callosum, striatum, optic tract, and cerebral peduncles.

Experimental studies: Mutation of the WWOX gene and audiogenic seizures in rats with epilepsy and lethal dwarfism

Suzuki et al.¹³ analyzed a spontaneous rat mutant (Ide/Ide) and reported a phenotype characterized by dwarfism, hypogonadism, epilepsy, and postnatal lethality. Brain pathology showed extracellular vacuoles in the hippocampus and amygdala. Sound stimulation of Ide/Ide rats induced epileptic seizures in 95% of cases, with staring, sometimes progressing to tonic-clonic convulsions. Electroencephalogram showed interictal spikes, fast waves during wild running, and burst of spikes during clonic phases. The authors showed that the Ide mutation is a 13-bp deletion in exon 9 of WWOX leading to a frame-shift of the last 44 codons of WWOX and replacement with a novel open reading frame of 54 codons. This mutation causes a frame shift, resulting in aberrant amino acid sequences at the C-terminal. Western blot analysis detected WWOX proteins of 47 and 42 kD in normal hippocampi, whereas both products were undetectable in the hippocampi of homozygous mutant rats. The authors concluded that WWOX protein is expressed in the CNS, and the abnormal neuronal excitability in Ide/Ide rats may be because of a lack of WWOX function.

The WWOX gene and human CNS-related pathologic conditions

1. Germline loss-of-function WWOX mutations lead to epilepsy, ataxia, and mental retardation

Recently, Mallaret et al.⁸ showed by exome sequencing that WWOX is homozygously mutated in four children from a consanguineous Saudi family with autosomal recessive spinocerebellar ataxia-12 (SCAR12; MIM#614322). These children presented with cerebellar ataxia, generalized epilepsy, and mental retardation. MRI brain of one patient revealed posterior white matter hyperintensities, whereas muscle biopsy showed vacuolization of the sarco-tubular system. The mutation Pro47Thr affects an extremely conserved proline residue that is a critical component of the first WW domain consensus sequence of WWOX. Analysis of patient fibroblasts showed normal amounts of the mutant P47T protein, but functional studies showed that the mutant protein was unable to bind a PPPY-containing oligopeptide suggesting that the mutation causes a conformational change that alters its ability to interact with normal protein motifs. A second consanguineous family was identified; the phenotype of the two affected siblings in this family also included generalized tonic-clonic seizures, mental retardation, and ataxia, but also prominent upper motor neuron disease. This family is carrying a highly conserved homozygous mutation Gly372Arg of the WWOX protein. The mutation affects a glycine found at the

C-terminus in the oxidoreductase domain of WWOX, and its functional consequences are less clear.

In this study, Mallaret et al.⁸ also demonstrated that WWOX full knockout mice developed spontaneous and audiogenic seizures and balance disturbances, phenotype reminiscent of the clinical presentation of all affected patients with WWOX mutations.

From this study, the authors concluded that germline mutations in WWOX cause a recessive form of early onset neurodegenerative disease including epilepsy, mental retardation, cerebellar ataxia, and prominent spasticity.

2. Mutation in WWOX gene cause microcephaly, growth retardation, epilepsy, retinal degeneration, and early death

More recently, Abdel-Salam et al.⁹ identified by whole-exome sequencing a homozygous nonsense mutation in the WWOX gene in an Egyptian family. The two siblings from this consanguineous family presented with severe failure to thrive, early intractable epilepsy, progressive microcephaly, severe psychomotor retardation, retinopathy, and early death at 3 and 16 months of age. MRI brain showed supratentorial atrophy with thin cerebral cortex, hippocampal dysplasia, hypoplasia of corpus callosum, hypotrophic temporal lobes, and gyral pattern anomaly. In this case, the homozygous WWOX germline alteration is a nonsense mutation at arginine 54 (pArg54*) leading to a virtual WWOX human full knockout case.

We also observed three siblings from a consanguineous Saudi family (unpublished data) with onset in the first month of life of refractory seizures, spasticity, microcephaly, failure to thrive, and early death. MRI brain showed hypomyelination and global brain atrophy. Exome sequencing revealed homozygous mutation (Chr16_78458766G > A) in the WWOX gene. This mutation was confirmed by Sangar sequencing.

These two reports confirm the observation of Mallaret et al.,⁸ but in more severe phenotype, and are highly similar to the abnormalities reported in Ide/Ide rats with a spontaneous functional null mutation of WWOX, expanding the nervous system clinical presentation of WWOX gene mutation.

Conclusions and future directions

WWOX is a ubiquitous protein with high expression in different brain regions during murine fetal development including cerebrum, cerebellum, brain stem and spinal cord, and remained unchanged in the cortex and the corpus callosum in adult mice. The neurologic phenotype of WWOX gene mutation includes seizures, most of the time refractory, ataxia, psychomotor retardation, retinal degeneration, and spasticity. The neurological phenotype associated with WWOX gene mutation is likely to be expanding with the use of whole exome sequencing.

The common feature describing these patients in the appearance of epilepsy early in life; in many cases, the epilepsy becomes refractory to treatment. There are several factors that may contribute to the seizures observed in patients with WWOX mutations: (1) WWOX is expressed

in the brain region, such as the hippocampus and cortical neurons, involved in seizure generation. (2) WWOX induces neuropathological changes (extracellular vacuoles in the hippocampus) in mutant rats and brain malformation in humans. (3) Other mechanisms of the putative role of WWOX in the nervous system may include abnormal signaling proteins, disruption of neuronal pathways, neuronal differentiation, or apoptosis probably via the mitochondrial pathway. In fact, WOX1 significantly downregulate the apoptotic inhibitors Bcl-2 and Bcl-xL, and upregulates p53 expression and tumor necrosis factor cytotoxicity.^{14,15} The WWOX is also implicated in the regulation of neuronal differentiation mainly by negative regulation of glycogen synthetase kinase 3 β .¹⁶ In rats, it was found that WOX1 plays an essential role in the 1-methyl-4-phenyl-pyridinium-induced neuronal death and is present in the condensed nuclei and damaged mitochondria of degenerative neurons in the striatum and cortex, ipsilaterally to intoxication.¹⁷

The described findings highlight that WWOX is associated with multiprotein networks and plays a critical role in normal CNS development and disease. Further research is needed to understand the signaling network to shed light upon the role of WWOX in the developing brain.

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