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Splicing variants of porcine synphilin-1☆

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ABSTRACT

Parkinson's disease (PD), idiopathic and familial, is characterized by degradation of dopaminergic neurons and the presence of Lewy bodies (LB) in the substantia nigra. LBs contain aggregated proteins of which α-synuclein is the major component. The protein synphilin-1 interacts and colocalizes with α-synuclein in LBs. The aim of this study was to isolate and characterize porcine synphilin-1 and isoforms hereof with the future perspective to use the pig as a model for Parkinson's disease. The porcine *SNCAIP* cDNA was cloned by reverse transcriptase PCR. The spatial expression of *SNCAIP* mRNA was investigated by RNAseq. The presented work reports the molecular cloning and characterization of the porcine (*Sus scrofa*) synphilin-1 cDNA (*SNCAIP*) and three splice variants hereof. The porcine *SNCAIP* cDNA codes for a protein (synphilin-1) of 919 amino acids which shows a high similarity to human (90%) and to mouse (84%) synphilin-1. Three shorter transcript variants of the synphilin-1 gene were identified, all lacking one or more exons. *SNCAIP* transcripts were detected in most examined organs and tissues and the highest expression was found in brain tissues and lung. Conserved splicing variants and a novel splice form of synhilin-1 were found in this study. All synphilin-1 isoforms encoded by the identified transcript variants lack functional domains important for protein degradation.

Abbreviations: ORF, open reading frame; LB, Lewy body; SNCAIP, α-synuclein interacting protein

☆ The sequence of the porcine *SNCAIP* cDNA, encoding the synphilin-1 protein and the three splice variants of synphilin-1, synphilin-1tv1, synphilin-1tv2, and synphilin-1tv3 have been submitted to GenBank under the accession numbers, GenBank ID: NM_001105053, GenBank ID: NM_001105054, GenBank ID: EF154192, and GenBank ID: NM_001098601, respectively. The genomic sequences representing the porcine *SNCAIP* were submitted under the accession numbers GenBank ID: JQ916898 and GenBank ID: JQ941708, respectively.

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Introduction

Parkinson's disease (PD) is a common progressive neurodegenerative movement disorder. Clinically, PD is characterized by resting tremor, rigidity and bradykinesia along with characteristics as postural instability and marked response to dopaminergic therapy (Hardy et al., 2006). In the past 15 years, genetic studies have revealed that different gene mutations and copy number variants cause familial forms of PD (Crosiers et al., 2011). The pathological hallmark of idiopathic and most monogenic (familial) forms of PD comprises loss of nigrostriatal dopaminergic neurons in the substantia nigra pars compacta and α-synuclein containing Lewy bodies (LB) in the surviving neurons (Dickson et al., 2009). The mechanisms of α-synuclein aggregation in LB and the influence on neurodegeneration are still unresolved.

Synphilin-1 (α-synuclein-interacting protein) is a cytoplasmic protein which was identified by its interaction with α-synuclein in a yeast two-hybrid analysis (Engelender et al., 1999). Also, in vivo interaction between synphilin-1 and α-synuclein has been demonstrated (Engelender et al., 1999; Kawamata et al., 2001; Lee et al., 2004; Marx et al., 2003). Synphilin-1 localizes close to synaptic vesicles (Wheeler et al., 2002) and as the protein constitutes an intrinsic component of LB and it is likely involved in the pathogenesis of PD (Wakabayashi et al., 2000). The coding sequence of the human *SNCAIP* gene spans 10 exons resulting in a 919-amino acid protein. The human *SNCAIP* gene has been mapped to chromosome 5q23.1–23.3 (Engelender et al., 2000).

The human synphilin-1 protein contains several functional domains such as four ankyrin-like repeats (tandemly repeated modules of about 33 amino acids), a coiled-coil domain and an ATP/GTP-binding motif (Fig. 1) (Engelender et al., 1999). All these domains, being highly conserved in the human, mouse (O'Farrell et al., 2001) and porcine (this report) sequence, have been identified in proteins involved in or mediating protein-protein interactions. Although the physiological function of synphilin-1 is still unknown, the presence of the protein domains delivers circumstantial evidence for a critical function. Synphilin-1 interacts with proteins such as α-synuclein (Neystat et al., 2002), parkin (Chung et al., 2001), dorfin, and SIAH-1 and SIAH-2 (Liani et al., 2004; Nagano et al., 2003). These proteins are all E3 ubiquitin ligases and bind in the central part of the synphilin-1 polypeptide sequence. To date only one mutation in the *SNCAIP* gene has been described; an arginine to cysteine substitution in position 621 of the encoded amino acid sequence (Marx et al., 2003). The R621C mutation is located in the fifth ankyrin-like repeat. *SNCAIP* is constitutively expressed in several tissues and at a particularly high level in brain, heart and placenta (Engelender et al., 2000). The expression profiles for human and mouse synphilin-1 are very similar (Engelender et al., 2000; O'Farrell et al., 2001). The synphilin-1 protein is, like α-synuclein, predominantly expressed in neurons and is enriched in presynaptic nerve terminals during development (Ribeiro et al., 2002).

Synphilin-1 has an important function in protein degradation mediated by autophagic clearance of aggresome-like inclusions (Wong et al., 2012). Autophagy is a process that facilitates degradation of intracellular components through the sequestration of portions of the cytosol inside double membrane vesicles that then fuse with lysosomes. Small synphilin-1 aggregates and large aggresomes are differentially targeted by constitutive and inducible autophagy (Wong et al., 2012). Specific regions in synphilin-1 are necessary for its own basal and inducible aggrepaghy and for degradation of other pro-aggregating proteins (Wong et al., 2012).

Synphilin-1 displays trophic and neuroprotective effects (Li et al., 2010). Overexpression of synphilin-1 in mouse neuroblastoma cells leads to promoted neurite outgrowth and to protection against Rotenone-induced toxicity (Li et al., 2010). This suggests that synphilin-1 may have a protective role in PD pathogenesis.

Synphilin-1 is also involved in the control of energy balance. Overexpression of human synphilin-1 in transgenic mice resulted in hyperphagia and obesity (Li et al., 2012, 2014). These mice also displayed hyperinsulinemia, hyperleptinemia and impaired glucose tolerance (Li et al., 2012). Similarly, synphilin-1 overexpression in *Drosophila* positively regulates energy homeostasis (Liu et al., 2012). Synphilin-1 expression in fruitfly neurons induces obesity-like phenotypes including body weight, body fat and food intake (Liu et al., 2012).

To date, a minimum of eight different isoforms of human synphilin-1 have been reported (Humbert et al., 2007). They all arise from the *SNCAIP* gene by alternative splicing. Similarly, four different isoforms of mouse synphilin-1 can be retrieved from sequence databases. Alternatively spliced transcript variants encoding different isoforms of human synphilin-1 have been described, but their full-length nature remains to be determined. A synphilin-1 isoform, named synphilin-1A, lacking exons three and four and containing an insertion in exon nine has been described (Eyal et al., 2006). The synphilin-1A isoform has enhanced aggregatory properties and causes neurotoxocity (Eyal et al., 2006).