Expression and Regulation of *INPP5D* in Alzheimer's Disease

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Alzheimer's disease (AD) is the most common form of dementia, accounting for approximately 38.5 million cases of all-cause dementia. Microglial cells, the innate immune cells of the central nervous system (CNS), have long been established as guardians of the brain by providing neuroprotection and maintaining cellular homeostasis. A protein with a myriad of effects on various important signaling pathways that is expressed in microglia is the Src Homology 2 (SH2) domain-containing Inositol 5′ Phosphatase 1 (SHIP1) protein. Encoded by the *INPP5D* (Inositol Polyphosphate-5-Phosphatase D) gene, SHIP1 has diminutive effects on most microglia signaling processes. Polymorphisms of the *INPP5D* gene have been found to be associated with a significantly increased risk of AD.

Alzheimer's disease | CNS | microglia | *INPP5D* | phosphatase domain | expression

1. Introduction

Alzheimer's disease (AD) is a common progressive neurodegenerative disease typically affecting individuals over the age of 65. It accounts for approximately 70% of all-cause dementia. Recent statistics have revealed that approximately 55 million individuals are living with dementia worldwide [1]. Cognitive decline and neuropsychiatric symptoms cause deterioration in quality of life and the need for 24-hour care, culminating in serious personal impact on patients and caregivers. Dementia, therefore, is an enormous global health burden, and the development of effective therapies is critical to effectively managing AD.

Genetic predisposition is an important AD risk determinant, with heritability estimates of 60–80% [2]. Microglia are the predominant immune cells in the brain, and genes exclusively or highly expressed in microglia, such as *TREM2*, *CD33*, *PLCG2*, *ABI3* [3] and *INPP5D* [4], have been found to exert various influences on the development of AD.

Central nervous system (CNS)-resident microglia provide innate immune defense against infection and trauma and maintain tissue homeostasis by performing synaptic pruning and clearing apoptotic cells and cellular debris. The blood–brain barrier prevents the adaptive immune system from playing a significant role in the CNS by excluding peripheral adaptive immune cells from the brain, although new evidence suggests that antigens and antigen-presenting cells originating from the CNS have been found in the peripheral lymphatic system and may stimulate adaptive and humoral immune reactions [5]. Once activated from their homeostatic or quiescent state, microglia assume a ramified morphology and efficiently clear various cellular and acellular material that has been marked for disposal. Such materials are generally produced by a variety of insults such as acute damage caused by trauma,

or ongoing damage, such as that characteristic of chronic neurodegenerative diseases, including AD. In AD, these damage-related materials and signals arise from plaques, fibrils and insoluble aggregates of amyloid-β (Aβ), damaged myelin, extracellular matrix, and stressed as well as degenerating neurons [6].

As AD develops, microglia lose their innate function and form clusters surrounding amyloid plaques and other damage signals [7]. Either through local proliferation or migration, microglia alter their morphology to become more amoeboid as they transform into a reactive state and prime their protein-synthesizing machinery to express various proteins needed to mount an immune response and, importantly, promote phagocytosis [7].

In 1994, Saxton and colleagues [8] reported that a 145 kDa protein became phosphorylated and associated with the adaptor protein Shc1 following B-cell or cytokine receptor stimulation. Shortly thereafter, the 145 kDa protein was identified by a number of research groups as Src Homology 2 (SH2) domain-containing Inositol 5′ Phosphatase (SHIP) [9][10][11], a protein encoded by the gene *INPP5D* (a member of the inositol polyphosphate-5-phosphatase) at the locus 2q37.1. Polymorphisms of *INPP5D* have been associated with higher risk of late-onset AD (LOAD) [4]. The single-nucleotide polymorphisms (SNPs) rs35349669 and rs10933431 in the *INPP5D* gene were significantly associated with increased AD risk [12]. *INPP5D* is associated with another major AD risk gene—*TREM2* (Triggering Receptor Expressed on Myeloid cells 2). It inhibits TREM2 signaling through its interactions with DAP12 (DNAX-activating protein of 12 kDa), an adaptor protein that is important for TREM2 signaling and function [13]. Thus, SHIP1 potentially plays important roles in the pathophysiology associated with Alzheimer's disease, and therefore, numerous small-molecule inhibitors of SHIP1 protein are currently being studied as possible immune-based therapies for AD.

2. Role of *INPP5D* in AD

INPP5D is a gene that is advancing to the forefront of genetics research in AD as a result of one of its SNPs, rs35349669, being associated as a common variant of the disease, accounting for 3.8% of all genetic risk for AD [14].

INPP5D, expressed in the brain, mostly in microglia, typically includes a 27-exon gene that encodes several isoforms. The full-length 27-exon isoform encodes an amino-terminal SH2 domain followed by a phosphatase domain, while the transcription of truncated isoforms lacking the SH2 domain originates from internal transcription start sites (TSS) [12].

The minor intron 10 allele of rs35349669 is associated with increased risk of AD, while the minor allele of rs10933431 located in intron 2 is associated with decreased AD risk [4][15]. According to Zajac et al. [12], isoforms of *INPP5D* associated with TSSs located in exon 1 and intron 14 are markedly increased in individuals with high AD neuropathology. In addition, expression of a novel variant (referred to as the D47 variant) that lacks a 47 bp segment from exon 12 was also increased in AD brains, resulting in ~13% of total *INPP5D* expression, and was found to undergo nonsense-mediated decay [12]. The missing 47 base pairs results in a frameshift and a

consequent introduction of a premature termination codon (PTC), with the resulting protein being devoid of a phosphatase domain. The rs35349669 SNP correlates with an allele-specific expression of full-length *INPP5D* [12].

The identified TSS of *INPP5D* includes TSS-A in the SH2 domain, and TSS-B and TSS-C in the phosphatase domain, all of which result in the production of different SHIP1 isoforms. TSS-A is located in exon 1 and generates isoforms 201, 204 and 205, whilst TSS-B originates in exon 11, producing isoform 202, and TSS-C is located in intron 14 and generates isoform 213. They also showed that isoforms originating from TSS-A and TSS-C are upregulated in the AD brain compared to the isoform produced by TSS-B. They concluded that individuals with AD may have an altered transcription factor profile or open chromatin sites that influence access to the TSS of the *INPP5D* gene, which in turn promotes transcription of various SHIP1 isoforms, leading to the propagation of AD pathology [12]. The Ikaros family of genes encodes for five zinc-finger proteins—Ikaros (*IKZF1*), Helios (IKZF2), Aiolos (*IKZF3*), Eos (*IKZF4*) and Pegasus (*IKZF5*)—that act as transcription factors, exerting critical regulatory roles during the development and function of various immune cells [16][17]. Ikaros exerts a crucial regulatory role in B-cell receptor (BCR) signaling, as studies have found that disruption of Ikaros in the chicken B cell line DT40 results in a diminution of BCR signaling, with reduced PLCγ2 phosphorylation and impaired intracellular calcium mobilization and signaling [18][19]. Other studies have revealed that Helios exerts a directly opposite effect as its knockout resulted in elevated BCR signaling [20]. Both Ikaros and Helios bind similar DNA sequences and chromatin remodeling complexes in the upstream regulatory region of the *INPP5D* gene to exert their effects as transcription factors [21][22].

Studies by Tsai and colleagues [23] revealed significantly elevated *INPP5D* expression in human AD microglial cells, and demonstrated a direct correlation between increased *INPP5D* expression and amyloid deposition in specific brain regions, including the inferior frontal, parahippocampal and superior temporal gyri, as well as in the frontal pole, although they did not delve into mechanisms driving specific *INPP5D* gene expression. There have been attempts to explain the increased expression of *INPP5D* in AD, where it has been proposed to be related to neuroinflammation and amyloid-plaque-associated increase in the expression of the transcription factor PU.1, which has been strongly associated in AD genetics. PU1 has been shown to bind to and upregulate *INPP5D* expression as it does other AD-related genes including *TYROBP*, *MS4As*, *TREM2*, and *CD33* [24]. However, more studies are required to characterize the mechanisms that underpin the increase in *INPP5D* expression in AD, how this might be mitigated, and if such manipulations may be of any tenable therapeutic benefits.

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