Overexpressing PLA2G6 mutations cause symptoms of young–onset dystonia–parkinsonism type 14

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Introduction

Parkinson's disease (PD) is the most common neurodegenerative motor disorder, affects over 10 million people worldwide. It is caused by the progressive degeneration of the ventral midbrain dopaminergic neurons, which project to the dorsal striatum, thus disrupting the neural circuitry responsible for regulating voluntary movement. In advanced stages of the disease, non-motor features such as emotional and cognitive deficits also appear. Despite decades of research, PD remains incurable. Current treatments are symptomatic and center on dopamine-replacement strategies, which are effective for a limited time; however, the neurodegeneration continues unabated (Hegarty et al., 2014).

Typically, PD is considered as a degenerative neurological disorder that typically manifests symptoms in late adulthood. However, compelling evidence from animal models showed that a range of irregular molecular mechanisms or environmental factors occurring during the prenatal period can either directly cause a reduction in the number of dopamine neurons or enhance the susceptibility to accelerated dopaminergic cell death during aging (Barlow et al., 2007). Indeed, studies have indicated that genetic mutations in specific genes (PARKs) could be the primary risk factors for familial PD, which comprises about 10% of all PD cases (Reed et al., 2019). Many of these genetic mutations can cause neurological defects in addition to parkinsonism.

In 2009, Paisan-Ruiz et al. (2009) described three individuals from two unrelated families of young-adult onset of a rapidly progressive neurodegenerative disorder characterized by parkinsonism, dystonia, and severe cognitive decline (Paisan-Ruiz et al., 2009); more cases were identified since then. This disease has been named adult-onset dystonia-parkinsonism, also known as Parkinson disease-14 (PARK14), which is caused by a homozygous mutation in the *PLA2G6* gene on chromosome 22q13. In addition to PARK14, mutations in the *PLA2G6* gene can also cause early-onset forms of neurodegeneration with brain iron accumulation (NBIA) (Morgan et al., 2006) and infantile neuroaxonal dystrophy (INAD) (Khateeb et al., 2006). Particularly, the symptoms of PARK14 develop during early adulthood, which leads to the hypothesis that neurodevelopmental defects during fetus or infantile stages may be responsible for the progression of PARK14.

Phospholipase A2, group VI (PLA2G6) is a calcium-independent phospholipase that

is involved in the metabolism of glycerophospholipids, phospholipid remodeling, arachidonic acid release, synthesis of prostaglandins and leukotrienes, and apoptosis. PLA2G6 plays an important role in the inner mitochondrial membrane homeostasis, in particular in maintaining normal functioning of cardiolipin, which tethers electron transfer chain molecules to the inner mitochondrial membrane. PLA2G6 also plays a role in capacitive Ca^{2+} entry, a mechanism that is important in intracellular Ca^{2+} homeostasis (Karkheiran et al., 2015). *PLA2G6* is highly expressed in the brain (Yang et al., 1999b, Yang et al., 1999a). In addition, it catalyzes the hydrolysis of phospholipids at sn-2 position, thereby producing lipid metabolites that might mediate the downstream signaling pathways. As a consequence, these lipid signals regulate multiple physiological and pathophysiological processes in the nervous system, hence suggesting that altered lipid signaling might contribute to the pathology of the PLA2G6 mutation (Yung et al., 2015, Choi and Chun, 2013). However, the molecular mechanism of PLA2G6 mutations, especially in PARK14, is still unknown. The presence of demyelination and axonal swellings in both the central and peripheral nervous systems have been observed in patients with INAD and in *PLA2G6* knockout mice (Khateeb et al., 2006, Beck et al., 2011). In addition, the catalytic activity of PLA2G6 is impaired in some patients with PARK14, NBIA, and INAD (Engel et al., 2010, Gui et al., 2013). These findings highlighted a role of phospholipase function and lipid regulation in PLAN pathology.

Many PLA2G6 mutations that have been identified are spread across different locations on the entire PLA2G6 coding sequence. However, most of the PARK14-associated *PLA2G6* mutations are not located in the critical catalytic patatin domain. Therefore, the role of PLA2G6 and PLA2G6 mutations in PARK14 and parkinsonism-like phenotype is currently unclear. The clinical genetic study showed that PLA2G6 D331Y mutation is associated with increased risk for early-onset PD in a Taiwanese cohort of PD patients (Lu et al., 2012). In addition, an increasing number of PLA2G6 mutations have been identified to date; hence, a model to examine the neurological effect of different PLA2G6 mutations efficiently is desperately required.

In this study, we aimed to investigate the pathological role of *PLA2G6* mutations in PARK14 using the zebrafish model system. To this end, we first overexpressed six human *PLA2G6* mutations in zebrafish. We demonstrated that three of the six *PLA2G6* mutations caused locomotion defects and resulted in a decrease in the number of dopaminergic neurons in zebrafish. In addition, two of the mutations, including D331Y and T572I, were found to cause defective phospholipase activity, which further lead to a reduction in the DHA level.

Material and Methods

Statistical Analysis

For spontaneous contraction, touch response and axon counts, statistical analysis was performed using the unpaired Student's *t* test in Microsoft Excel 2007, and results with $P < 0.05$ are described as statistically significant. All graphs show the mean and SD and are represented as the mean ± SD in the text.

Results

Discussion

Although previous studies have provided insights into the significant impact of genetic factors on PD, the molecular mechanism underlying PD remains largely unclear. A comprehensive analysis focusing on the biological function and interactions of PD-related genes may provide valuable information to understand the pathogenesis of PD.

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