Review Article

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Experimental Neurobiology

A featured article of the special issue on Parkinsonian Syndrome

Hypothesis: Somatic Mosaicism and Parkinson Disease

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Mutations causing genetic disorders can occur during mitotic cell division after fertilization, which is called somatic mutations. This leads to somatic mosaicism, where two or more genetically distinct cells are present in one individual. Somatic mutations are the most well studied in cancer where it plays an important role and also have been associated with some neurodegenerative disorders. The study of somatic mosaicism in Parkinson disease (PD) is only in its infancy, and a case with somatic mutation has not yet been described. However, we can speculate that a somatic mutation affecting cells in the central nervous system including substantia nigra dopaminergic neurons could lead to the development of PD through the same pathomechanisms of genetic PD even in the absence of a germ-line mutation. Theoretically, a number of genes could be candidates for genetic analysis for the presence of somatic mosaicism. Among them, *SNCA* and *PARK2* could be the best candidates to analyze. Because analyzing brain tissues in living patients is impossible, alternative tissues could be used to indicate the genetic status of the brain. Performance of the technology is another factor to consider when analyzing the tissues.

Key words: somatic mutation, somatic mosaicism, genetics, Parkinson disease

INTRODUCTION: GENETICS OF PARKINSON DISEASE

Parkinson's disease (PD) is characterized by the degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc) in association with α–synuclein immunoreactive intracytoplasmic inclusions (Lewy bodies). However, studies over the last decade have shown that these pathologic changes are not limited to the SNpc and that many other brainstem nuclei and cortical areas are also involved [1]. Although the exact pathomechanisms of PD still remain unclear, several genes causing PD when mutated have been identified (Table1), and approximately 10% of patients with PD are familial, which suggests a genetic etiology even in sporadic cases [2]. However, the mechanism by which these mutations lead to PD is not fully understood, and the link between these mutations and α–synuclein pathology still remains elusive [3].

SOMATIC MOSAICISM

Until recently, genetic disorders were believed to be caused by inherited DNA variation. Except in mitochondrial disorders, mutation(s) is present in one of the parents (or both parents, in the case of autosomal recessive conditions) and found in all organs and tissues of the affected individual. The reason why some organs or tissues are affected and others are not could be related to the role of the mutated gene in each organ or tissue [4]. However, in

Table 1. Genetic etiology of Parkinson disease

Locus	Chromosome	Gene	Inheritance
PARK1	4q	*SNCA* (point mutation)	AD
PARK2	6q	*PARK2* (parkin)	AR
PARK3	2p	?	AD
PARK4	4q	*SNCA* (multiplication)	AD
PARK5	4p	*UCHL1*	?
PARK6	1p	*PINK1*	AR
PARK7	1p	*DJ-1*	AR
PARK8	12p-q	*LRRK2*	AD
PARK9	1p	*ATP13A2*	AR
PARK10	1p	?	?
PARK11	2q	*GIGYF2*	?
PARK12	Xq	?	X-linked
PARK13	2p	*HTRA2*	?
PARK14	22q	*PLA2G6*	AR
PARK15	22q	*FBXO7*	AR
PARK16	1q	?	?
PARK17	16q	*VPS35*	AD
PARK18	3q	*EIF4G1*	AD
PARK19	1p	*DNAJC6*	AR
PARK20	21q	*SYNJ1*	AR
	1q	*GBA*	AD
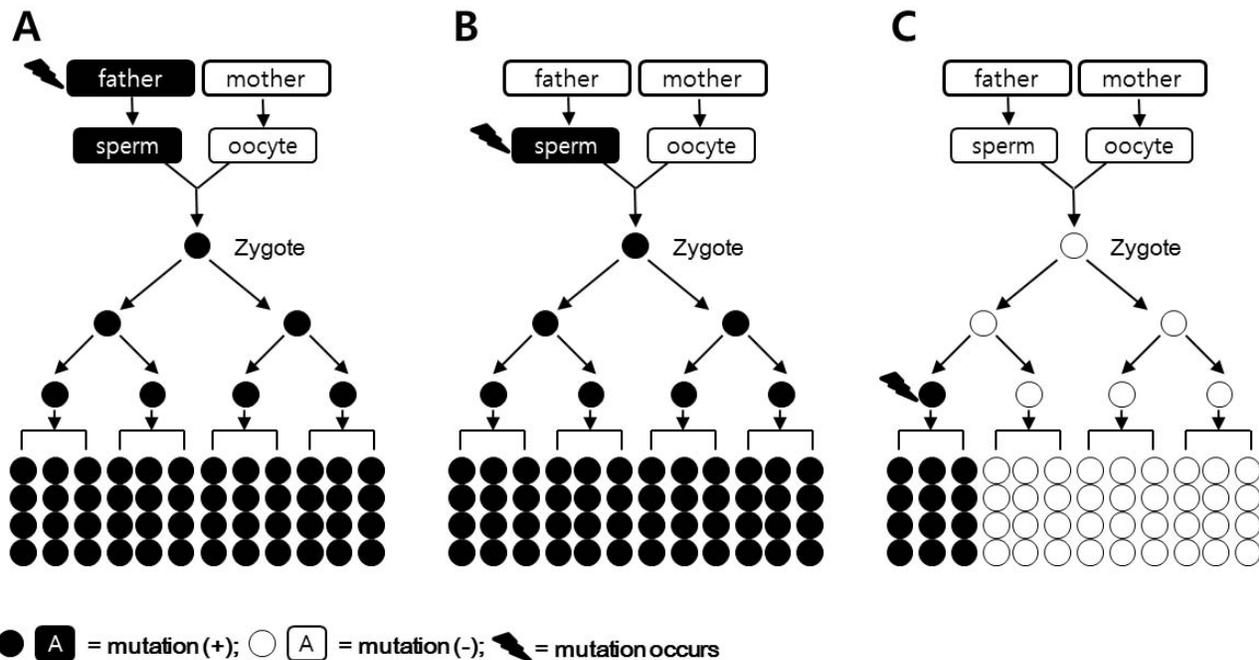
Fig. 1. Schematic figures depicting (A) inherited, (B) de novo, and (C) somatic mutations.
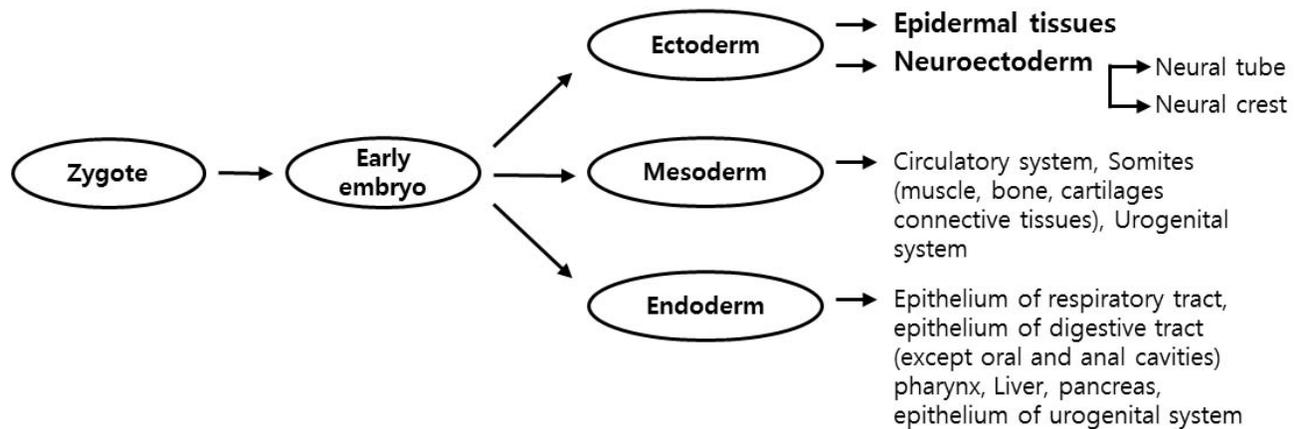
Fig. 2. Derivatives of primary germ layers.

some patients with genetic disorders, a mutation is not detected in the parents. This kind of mutation is called a 'de novo' mutation (Fig. 1). Usually, a de novo mutation is the result of a mutation in the egg or sperm of one parent thus undetectable in the parents by blood testing. Indeed, many patients with genetic disorders, especially those with earlier onset or with severe symptoms, have 'de novo' mutations rather than 'inherited' mutations because individuals with such genetic disorders are more likely to be selected against and thus are less likely to pass down the mutated genes.

Mutations causing genetic disorders can also occur during mitotic cell division after fertilization, which is called somatic mutation. This leads to somatic mosaicism, where two or more genetically distinct cells are present in one individual. It is not well studied yet how many cells in a human body carry somatic mutations. However, given that a human body has approximately 10^{14} cells which were derived from a single fertilized egg through roughly 30 cellular generations and that the mutation rate for each gene per cell division is 10^{-6}, one can speculate that a substantial number of cells in a human body will carry somatic mutations [5]. In fact, human bodies should be mosaics of genetic mutations [6]. The extent of mosaicism will be different between individuals and even between different tissues in a single individual. Somatic mutations are the most well studied in cancer where it plays an important role [7]. However, recent studies indicate that they can give rise to other diseases including neurodegenerative disorders [4, 8, 9].

PD AND SOMATIC MOSAICISM

The study of somatic mosaicism in PD is only in its infancy. In genetic PD, inherited mutations cause neuronal dysfunction and death preferentially in SNpc dopaminergic cells and lead to the development of PD. We can speculate that a somatic mutation affecting cells in the central nervous system including the SNpc dopaminergic neurons can lead to the development of PD through the same pathomechanisms of genetic PD even in the absence of germ-line mutations. This is a hypothetical condition not expected in a large number of patients with 'sporadic' PD but probably includes some cases of PD.

Somatic mosaicism in cancer tissues or organs with dividing cells can develop after birth and even later in life. However, neurons are non-dividing cells, and their close and distant interconnections have already been established at birth. Thus, somatic mutations leading to mosaicism in brain neuronal cells, if present, should have occurred during embryogenesis or fetal development. During embryogenesis, the neural plate, which later forms the neural tube, is formed from the ectoderm (Fig. 2), which also gives rise to the epidermis and the lining of the oral cavity. The neural tube is then subdivided into distinct regions: the prosencephalon, the mesencephalon, the rhombencephalon, and the spinal cord. The prosencephalon develops into the telencephalon (the forebrain) and the diencephalon (thalamus and hypothalamus). The mesencephalon develops into the midbrain. The rhombencephalon develops into the metencephalon (the pons and cerebellum) and myelencephalon (the medulla oblongata). Although it is not clear when the cells destined to be SNpc dopaminergic neurons complete their final mitosis, tyrosine hydroxylase-immunoreactive cells, which finally occupy the SN, are the first detected at the ventricular zone along the aqueduct of the Sylvius at around E11. They then migrate along the radial glia to the ventral surface of the mesencephalon to form the SN [10, 11]. A somatic mutation occurring at any stage of development

of SNpc dopaminergic neurons before the final mitosis takes place will result in an individual with somatic mosaicism in the SNpc. Two important considerations regarding the role of somatic mosaicism for the development of PD in this individual are as follows: (1) how many cells in the final SNpc are harboring mutations, and (2) how deleterious is the mutation. The onset of PD would be late, and the symptoms would be mild even if the proportion of dopaminergic cells with a mutation is high when the mutation has only a mild effect on neuronal function and survival. A deleterious mutation in only a tiny proportion of dopaminergic neurons will also result in mild disease. Another important point is the gene in which mutation occurs. If the mutation occurs in a gene that is essential for cell survival regardless of the cell type, it should be limited to the SNpc dopaminergic neurons to cause PD. Otherwise, the patient will present with a wide range of neurological dysfunctions or multi-organ dysfunctions rather than PD. On the contrary, if the mutation occurs in a gene which specifically affects the SNpc dopaminergic cells when mutated, the patient will develop PD even when the mutation is present beyond the SN. In summary, the clinical features and neuropathology in a patient with PD from a somatic mutation could be determined by the extent of the somatic mosaicism, the proportion of SNpc dopaminergic cells with the mutation, and the effect of the mutation on neurons.

Somatic mosaicism has already been associated with some adult-onset neurodegenerative disorders. In Alzheimer's disease, a patient with somatic mosaicism for a mutation in the presenilin-1 gene has been described. Degrees of mosaicism were 8% in peripheral lymphocytes and 14 % in cerebral cortex in this patient [12]. A case of hereditary spastic paraplegia associated with somatic mosaicism for mutation in SPG4/SPAST has been reported [13]. Recently, a patient with sporadic Creutzfeldt-Jakob disease caused by somatic mutation in codon 178 of the prion protein gene was described. In this patient, the proportion of mutated cells in peripheral blood cells and brain tissue was similar at approximately 97%, suggesting an early event of somatic mutation [14]. In Rett syndrome, somatic mosaicism for a mutation in the MECP2 gene has repeatedly been reported [15-17]. Somatic mosaicism in trinucleotide repeat expansion disorders including Huntington's disease, Friedreich's ataxia, fragile X syndrome, and dentatorubropallidoluysian atrophy has well been described [18-20].

In PD, a case with somatic mutation has not yet been described. Theoretically, a number of genes can be candidates for genetic analysis for the presence of somatic mosaicism. Among them, SNCA and PARK2 could be the best candidates to analyze because they are located in the chromosomal fragile sites which make them susceptible to copy number variation (CNV) generation [21],

and CNVs in these genes actually constitute an important cause of genetic PD [22, 23]. Furthermore, it has been reported that mosaic CNV is abundant in human neurons [24]. Mutation in SNCA leads to PD with autosomal dominant inheritance. Both point mutations and CNVs cause disease, and it has been shown that both types of mutations facilitate the aggregation of α–synuclein, which is the core neuropathology of PD [1]. According to a recent α–synuclein propagation hypothesis [24], even a very small number of cells with a SNCA mutation can give rise to PD by serving as a starting point of α–synuclein aggregation which then are transmitted to neighboring neurons without mutation and seed aggregation there. In this situation, the connection of neurons with a SNCA mutation as well as the location of those neurons will be an important factor in determining the extent of the final pathology. Still, study on somatic mosaicism for CNV in SNCA is lacking, and a recent study using postmortem brain tissues failed to detect somatic mosaicism for point mutation in SNCA [26]. Mutation in PARK2 is the most important genetic cause of young-onset PD with autosomal recessive inheritance. Interestingly, heterozygous carriers of the PARK2 mutation are at increased risk of developing PD [27, 28], which leads to the hypothesis that these patients may have a homozygous or compound heterozygous PARK2 mutation in the brain through a somatic mutation which has occurred after the formation of the ectoderm. LRRK2 and GBA also can be targets for analysis given that de novo mutations have been reported [29, 30].

TESTING THE HYPOTHESIS

The most convincing way to investigate the contribution of somatic mosaicism in PD is to analyze the genetic status of SNpc dopaminergic neurons in patients. However, this method is applicable only to postmortem brain tissues and impossible in clinical practice for obvious reasons. Even when a postmortem brain is available, the test may yield a negative result if the number of neurons with a somatic mutation was small, and they already had disappeared long before the patient's death, which is possible given the greater vulnerability of the mutated neurons. In living patients, tests should be done using alternative tissues which could indicate the genetic status of the brain (more specifically, the SNpc). Skin tissue or the epithelium of the oral mucosa can be considered because they are derived from the ectoderm. Although more invasive, salivary gland tissues will make better candidate because the connective tissue of the salivary glands is derived from the neural crest and has a greater chance of sharing somatic mosaicism with the brain tissue [31-33].

The performance of technology is another factor to consider

when analyzing the tissues. Conventional Sanger sequencing is not suitable because of the low resolution especially when low-level mosaicism is expected. A recent study using human brain tissue adopted high-resolution melting analysis which can detect a 5%-10% proportion of mutant DNA [26]. This technique has been reported to detect a mutation proportion as low as 0.5% [34]. With further technological advances, new techniques such as single-cell sequencing and high depth sequencing will become more affordable and readily available [4].

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