ZOOLOGICAL RESEARCH

Biological implications and limitations of a cynomolgus monkey with naturally occurring Parkinson's disease

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We recently identified a cynomolgus monkey with naturally occurring Parkinson's disease (PD), indicating that PD may not be a uniquely human disease (Li et al., 2020). In our previous study, four lines of evidence, including typical PD clinical symptoms, pharmacological responses, pathological hallmarks, and genetic mutations, strongly supported the identification of a monkey with spontaneous PD (Figure 1). To the best of our knowledge, this is the first reported case of naturally developed PD in animals. This suggests that PD is not a disease restricted to humans, with its existence in a non-human primate providing a novel evolutionary angle for understanding PD. As a close relative to humans (Buffalo et al., 2019; Phillips et al., 2014; Yan et al., 2011), this rare case of PD in another primate species provides solid evidence that monkeys are ideal candidates for the development of a genuine "animal version of PD", with conserved etiology and pathogenesis (Li et al., 2020). Furthermore, it allows us to compare similarities and differences in PD development between species and to understand PD pathogenesis from an evolutionary point of view.

Many intriguing questions arise from our study (Li et al., 2020). The first is why only one spontaneous PD monkey was identified in the screening of more than 1 500 monkeys in our animal center. Does this mimic the incidence rate of PD in human populations? In our study, the identified PD monkey was an adult male. The incidence of PD in the human male population is around 61.21 per 100 000 person-years (Hirsch et al., 2016). Assuming a similar incidence rate among our male monkeys, the probability of discovering a spontaneous PD case in our colony is around 0.06%. The male monkey population in our primate center stands at about 1 000, therefore the probability of discovering a monkey with spontaneous PD is: 1 000×61.21/100 000=0.61. Adjustment of the age factor is difficult as the monkeys screened in our study ranged from 7 to 30 years old, corresponding to an age range of 20 to 90 years in humans (Roth et al., 2004; Tigges et al., 1988). A 10-year-old monkey corresponds to a 30–40-year-old human (Roth et al., 2004; Tigges et al., 1988), and the incidence of PD in this age range in the human male population is as low as 3.59 per 100 000 person-years (Hirsch et al., 2016). Thus, the probability of discovering a 10-year-old PD case in our population is about 20 times lower than 0.61. Therefore, we were fortunate to find one PD case in our monkey colony. This rough estimation demonstrates that naturally occurring PD is likely a rare event in monkey colonies within an animal center, and estimation may be biased because of potential inbreeding within the colony. Thus, assessments based on our monkey colony may not

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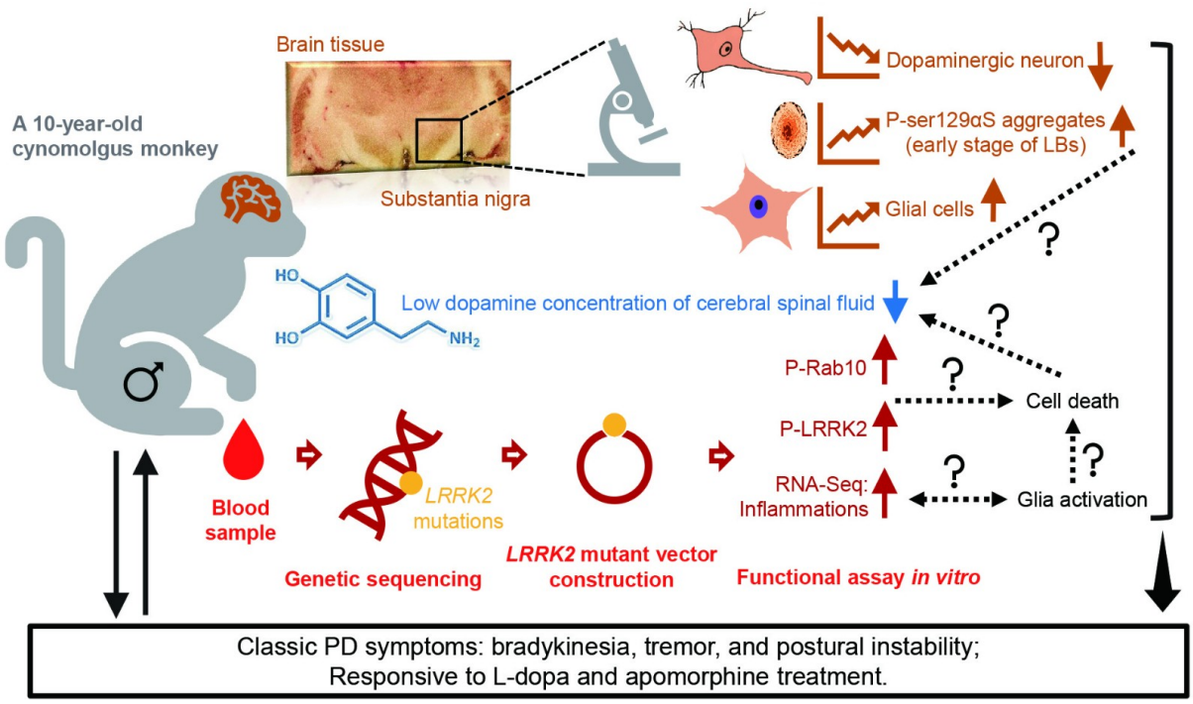

Figure 1 Spontaneous PD monkey displayed typical Parkinsonian symptoms and pathological hallmarks

Potential pathogenetic mechanisms are illustrated.

accurately reflect the incidence rate of PD in a realistic breeding scenario across wild monkey populations. Moreover, due to greater survival challenges and the fact that PD is a motor deficit disease, we speculate that inflicted animals in the wild may not reach an adequate age to display PD phenotypes. Nonetheless, the occurrence of this disease suggests the possibility that PD-like behaviors may appear in natural wild populations and should be monitored during field observations.

The second question to arise is to what extent the identified monkey PD mimics human PD. Human PD has several clinical types, but key phenotypes usually include motor symptoms such as bradykinesia, resting tremor, postural instability, and rigidity, and classic pathological changes such as severe nigral dopaminergic neuronal loss and Lewy pathology (Poewe et al., 2017). The spontaneous PD monkey showed classic clinical symptoms, similar responses to levodopa and apomorphine treatments as PD patients, and all typical PD pathological hallmarks (Li et al., 2020). In other words, the monkey PD mimicked nearly all important phenotypes of human PD. However, our previous study (Li et al., 2020) has several limitations in regard to the characterization of pathogenesis in the spontaneous PD monkey. Firstly, due to the monkey's sudden death, we were unable to collect fresh tissue samples for biochemical analyses to obtain direct evidence for the functional consequences of the LRRK2 mutations p.I835L, p.N1506Y, and p.E2381G. Secondly, the *in vitro* cell culture data only provided indirect evidence for the possible pathogenicity of the LRRK2 mutations causing the PD phenotypes in the monkey. We found that the levels of serine 1292-phosphorylated LRRK2 (p-Ser1292 LRRK2) and threonine 73-phosphorylated Rab10 (p-Thr73 Rab10) were increased, which may have triggered PD pathogenesis, as seen in previous studies in humans (Fan et al., 2018; Zimprich et al., 2004). Moreover, the RNA-sequencing data indicated that immunological reactions and inflammation may also have contributed to the PD pathogenesis in this monkey, consistent with the glial cell activation observed in pathological staining (Li et al., 2020). To demonstrate a causal relationship between the LRRK2 mutations and PD phenotypes, *in vivo* data from transgenic monkeys bearing these mutations are required. This could be accomplished in monkeys using recently popularized gene-editing techniques (Chen et al., 2017; Luo et al., 2016; Niu et al., 2014; Zhao et al., 2019).

The third question to arise is how our understanding of this spontaneous model will help in the future construction of genetically modified PD monkeys. As the naturally occurring PD monkey identified in our study exhibited a sensitive response to classic PD treatment drugs, e.g., levodopa and apomorphine (Li et al., 2020), we predict that genetically modified monkeys with LRRK2 mutations may be feasible models. Furthermore, models with LRRK2 as the target could be used for screening and verifying novel drugs in PD preclinical studies in the future.

In summary, we identified a rare PD monkey case in captivity (Li et al., 2020), which suggests that PD is not a uniquely human disease and LRRK2 may be a promising target for creating genetically modified PD monkeys.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

H.L., Y.G.Y., and X.T.H. conceived and prepared the draft. All authors read and approved the final version of the manuscript.

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