Perspective

Is serine racemase (SRR) a second hit target for LRRK2-G2019S induced Parkinson's disease?

Sarah Louise Nickels, Jens Christian Schwamborn[*]

To date, at least 7 million people are suffering from Parkinson's disease (PD) worldwide, which is the second most prevalent, age-associated, and progressive neurodegenerative disorder (Tysnes and Storstein, 2017). Given the accelerated global pace of aging, it becomes of fundamental importance that we start understanding the origins of neurodegeneration in order to develop effective disease modifying treatments. Most PD patients suffer from a combination of motor and non-motor disabilities. The motor symptoms typically manifest in bradykinesia, tremor and rigidity (Hoehn and Yahr, 2001). The physical decline is directly linked to the loss of dopaminergic neurons in the *substantia nigra pars compacta* of the midbrain, which disrupts dopamine signalling responsible for movement and mobility (Lanciego et al., 2012). The non-motor symptoms are more diverse and include depression, anxiety, loss of smell, constipation, sleep disorders, and dementia (Chaudhuri and Schapira, 2009). Some of them precede the motor-ones by decades, raising the possibility that PD might have a long-term-compensated neurodevelopmental origin, which only manifests at older age and in presence of other contributing factors (Le Grand et al., 2014).

Both, the origin of neurodegeneration and the disease itself, remain elusive. In regard to the multiple-hit hypothesis, a multitude of different environmental risk factors, as well as genetic mutations most likely contribute to the onset and progression of PD (Sulzer, 2007). As the major, idiopathic form of PD, representing 85% of all cases (Klein and Schlossmacher, 2006), has no known genetic origin, its study remains a major challenge. Therefore, research often focuses on its underrepresented, familial counterpart that possesses a known underlying mono-genetic cause (Klein and Schlossmacher, 2006). PD, however, is far from being a single risk factor disorder (Lesage and Brice, 2009).

Interestingly, the most common mutation underlying PD, LRRK2-G2019S, phenotypically strongly resembles idiopathic cases (Lesage and Brice, 2009). Additionally, the age of onset of PD within these patients varies considerably and the penetrance of LRRK2-G2019S in the general population is incomplete, meaning that not all its carriers develop PD (Golub et al., 2009; Lesage and Brice, 2009). This observation highlights that PD is a complex multi-factorial disease. Consequently, other either inherent factors within the patient's genetic background or extrinsic, through lifestyle acquired factors seem to contribute to LRRK2-G2019S-induced PD. The microbiome-gut-brain axis for instance is involved in PD and LRRK2 plays a role in intestine inflammation (Liu and Lenardo, 2012; Benakis et al., 2020). As such, by studying LRRK2-G2019S induced PD, we can build a bridge between the known and unknown origins of PD.

Within this conceptual context, we were able to confirm that PD is a multi-variant polygenic disease (Nickels et al., 2019). We discovered the enzyme serine racemase (SRR) as a possible developmental genetic modifier that might predispose for PD in LRRK2-G2019S carriers. We demonstrated that neural epithelial stem cells (NESC) from these patients display impaired self-renewal and viability. Interestingly, LRRK2-G2019S was only sufficient but not necessary to cause these phenotypes, meaning that one part of their manifestation was LRRK2-G2019S independent. We could reveal that in our PD disease model the genetic background plays a decisive role. This was demonstrated by gene-correcting or inhibiting LRRK2-G2019S within patients, which was not rescuing the phenotypes. Meaning that the phenotypes within the patient cells- increased cell death and decreased proliferation were partly due to the patient genetic background and not the mutation itself. In our attempts to reveal potential contributing factors from the patient genetic background, we identified *SRR* as a possible susceptibility factor. We could show that serine metabolism was deregulated in LRRK2-G2019S carriers and we rescued NESC phenotypes by serine complementation. NESCs derived from patients who carry LRRK2-G2019S exhibited a severe downregulation in SRR and an intracellular accumulation of L-serine, which is in accordance with reduced SRR activity. The phenotypes within the patient NESCs were rescued by administrating D-serine, the product of SRR, suggesting that SRR represents the driving force behind the phenotypes. These results were further validated in human blood plasma of LRRK2-

G2019S carriers, which revealed increased serine levels, compared to idiopathic PD cases and healthy individuals. We identified SRR as being responsible for the observed defects and proposed that it acted as a potential novel enzymatic or genetic modifier within the patient genetic background and complements LRRK2-G2019S pathogenicity (**Figure 1**).

Interestingly, LRRK2-G2019S alone is also able to increase serine independently of the genetic background and SRR deregulations (**Figure 1**). Serine levels were increased, independently of *SRR* transcript levels when inserting the LRRK2-G2019S mutation into cells derived from individuals having a healthy background as well as *in vivo* in the mouse striatum of specifically LRRK2-G2019S carrying mice. This led us to the conclusion that both factors together might be responsible for disease manifestation (**Figure 1**).

Overall, our study highlighted the importance of selecting the appropriate model and isogenic controls to study PD and demonstrates the influence of the highly variable patient genetic background on PD pathogenesis. Nevertheless, some questions remain to be answered.

Taking into consideration the incomplete penetrance of LRRK2-G2019S we hypothesise that impaired SRR represents a metabolic second hit for developing PD. We speculate that this deregulation of SRR affects in particular the LRRK2-G2019S carriers. These patients might have an increased susceptibility towards SRR deregulations because their serine metabolism is already impaired by LRRK2-G2019S. In healthy or unaffected individuals, the serine impairments caused by LRRK2-G2019S might be compensated either by regulating LRRK2 or by upregulating serine levels. However, in PD patients having- additionally to the LRRK2-G2019S mutation and the upregulated serine levels- a SRR impairment, the compensatory strategy might fail and the disease manifests (**Figure 1**). Interestingly, it was shown that increased serine levels correlate with PD progression and that PD patients have different metabolite profiles that clearly separate from those of LRRK2-G2019S patients (Lewitt et al., 2017). Moreover, D-serine metabolism and SRR activity, as well as SNPs within that gene are involved in several other neurodegenerative diseases such as schizophrenia, Alzheimer's, and amyotrophic lateral sclerosis, which share certain features with PD (Labrie et al., 2009; Billard, 2018). Therefore, LRRK2-G2019S patients could be stratified for SNP's involved in serine metabolism, the *SRR* locus or its upstream regulators.
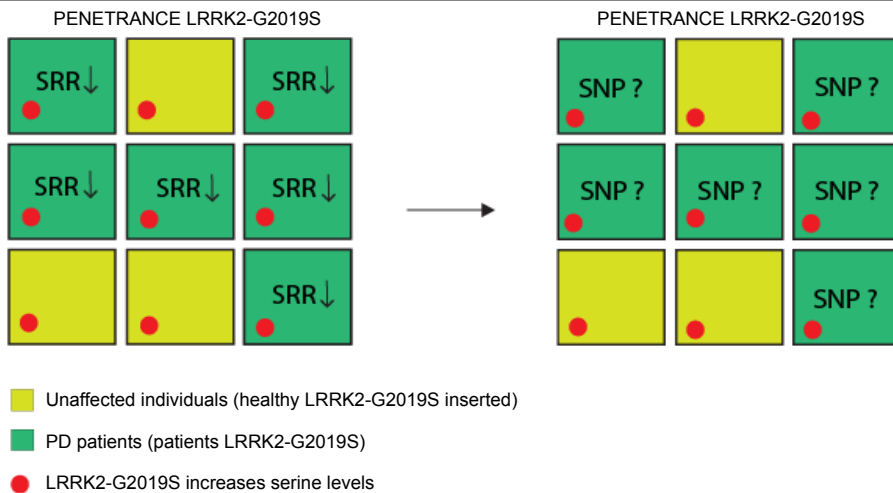
Figure 1 │ **A SNP in SRR acts as a second hit for developing LRRK2-G2019S induced PD.**
SRR downregulation, in the patient's genetic background, happens independent of LRRK2-G2019S and increased serine levels. A SNP for instance could be the underlying cause of the decreased SRR expression. PD: Parkinson's disease; SNP: single nucleotide polymorphism; SRR: serine racemase.

In addition, the underlying mechanism of serine metabolism and its interaction with LRRK2-G2019S are not yet identified. Regardless of the presence of a SNP, there is a clear need to clarify how LRRK2-G2019S can increase serine levels. There might be a either a direct interaction leading to a point of no return or some epigenetic mechanisms at work, which should be elucidated. Serine deficiency for instance increases inflammation and oxidative stress through the gut-brain axis in aging mice and D-serine production in the intestine, was able to rescue sleep phenotypes in SRR-KO drosophila (Dai et al., 2019; Wang et al., 2020). Moreover, some evidence suggests that the impact of serine and SRR might extend towards PD in general. Serine levels have been significantly elevated in PD cohorts (Lewitt et al., 2017). Therefore, the correlations between serine metabolism, SRR, and PD in general should be investigated. The underlying mechanism could be linked to N-methyl-D-aspartate receptor signaling, synaptic plasticity and the increased vulnerability of dopaminergic neurons towards the molecular mechanisms of PD (Sulzer, 2007). Moreover, treatment strategies based on serine complementation should be considered. Dopaminergic neurons are regulated by N-methyl-D-aspartate receptor signaling, and in PD, hyperactive glutamatergic transmission could cause and worsen motor neuron degeneration (Gelfin et al., 2012). Moreover, within the Parkinson-diseased brain, deregulated N-methyl-D-aspartate receptor signaling and the associated motor dysfunctions can be alleviated by D-serine treatment (Gelfin et al., 2012).

Based on our previous work, we here propose that further stratifying the origins of PD, especially for LRRK2-G2019S carriers and discovering the underlying mechanisms, such as impaired serine metabolism and SRR functionality would enable patient-specific, disease-modifying treatments, prevention strategies, and would lead the way towards precision medicine.

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