# New insights in the function and regulation of the phosphorylated pathway for serine biosynthesis in the human brain

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L-serine (L-Ser) is the precursor of D-Ser, the dominant co-agonist of N-methyl-D-aspartate receptors in mammalian CNS. In the human brain the L-Ser pool is maintained by *de novo* biosynthesis through the “phosphorylated pathway” (PP). 3-Phosphoglycerate dehydrogenase (PHGDH), 3-phosphoserine aminotransferase (PSAT) and 3-phosphoserine phosphatase (PSP) catalyse the three steps of the pathway, and defects in any of the three enzymes cause a group of diseases known as “serine deficiency disorders”.

We have investigated the functional and structural properties of the three enzymes, both *in vitro* and in human astrocytes where they were found to form a metabolic assembly we named “serinosome”. Through the *in vitro* reconstruction of the PP we concluded that PSAT and PSP are the main players in shifting the flux towards L-Ser synthesis (previously published in: Rabattoni, V et al. (2023) FEBS J 290, 3877-3895). Several known pathogenic variants of PSAT and PSP were analysed, and the molecular basis of their defective function was assessed. In the case of PSP, two new variants were characterized: the N133S, found in two siblings with intellectual deficiency and spastic paraparesis, was proven to destabilize the protein and to affect serinosome assembly; the R27S/D32G, identified in Alzheimer’s disease patients, showed a deeply impaired catalytic efficiency because of a 20-fold reduction in $k_{cat}$. One interesting feature that emerged from the *in vitro* reconstructed PP is that PSP hypofunctional variants are less likely to affect the flux through the PP as compared to PHGDH or PSAT variants (previously published in: Marchesani, F et al. (2024) BBA Mol Basis Dis 1870, 167034; Marchesani, F et al. (2023) Biomolecules 13, 1219). Moreover, inhibition by L-Ser, albeit at play on the isolated PSP, only affects the flux through the PP when the enzyme activity is severely impaired by inactivating substitutions.

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