Development of new GBA1 substrates for the discovery of novel glycosylated metabolites

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Abstract for oral presentation

Gaucher disease (GD) is a lysosomal storage disorder (LSD) resulting from inherited glucocerebrosidase (GBA1) deficiency. GD diagnosis relies on GBA1 activity assays, typically employing 4-methylumbelliferyl-β-D-glucopyranoside (4MU-β-Glc) as fluorogenic substrate. However, these assays suffer from background 4MU release by the non-lysosomal GBA2 and cytosolic GBA3 enzymes. We developed GBA1-selective fluorogenic substrates by synthesizing a series of 6-*O*-acyl-4MU-β-Glc substrates with diverse fatty acid tails. Because of chemical and enzymatic instability of the ester bonds, analogues of 6-*O*-palmitoyl-4MU-β-Glc (**3**) with different chemical linkages were synthesized. 6-*O*-alkyl-4MU-β-Glc **9**, featuring an ether linkage, emerged as the most optimal GBA1 substrate, exhibiting both a low K_m and compared to substrate **3** a high V_{max}. Importantly, substrate **9** is not hydrolyzed by GBA2 and GBA3, and therefore acts as superior substrate for GD diagnosis (Figure 1).[1] Plants contain glycosyl phytosterols (campesterol, β-sitosterol and sigmasterol) that may also be acylated at C-6. LC-MS/MS analysis revealed that 6-*O*-acylated and regular glycosylcholesterol (HexChol) tend to be increased in GD patient spleens. Moreover, significant increases in 6-*O*-acyl-glycosyl-phytosterols were detected in GD spleens. Our findings suggest uptake of (6-*O*-acyl)-glycosyl-phytosterols from plant food and subsequent lysosomal processing by GBA1, and comprise the first example of accumulation of an exogenous class of glycolipids in GD. Excessive exposure of rodents to glycosylated phytosterols has been reported to induce manifestations of Parkinson's disease (PD). Further investigation is warranted to determine whether (6-*O*-acyl)-glycosyl-phytosterols could contribute to the enigmatic link between inherited defects in GBA1 and the risk for PD. Furthermore, a derivative of substrate **9** has been synthesized with a high ionizing handle which could help with the identification of novel metabolites in the trans glucosylation reaction catalyzed by GBA1 using LC-MS/MS strategies.

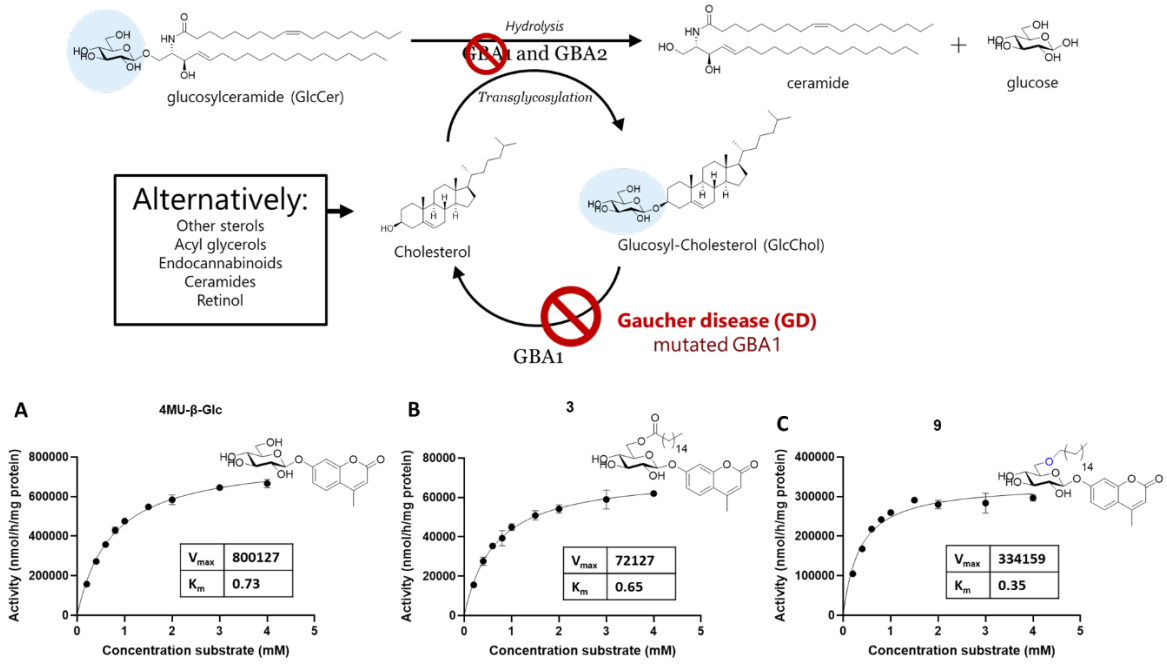

Figure. Maximum rate of hydrolysis (Vmax) and the Michaelis constant (Km) for fluorogenic substrates 4MU-β-Glc (A), 6-*O*-acyl-4MU-β-Glc **3** (B) and 6-*O*-alkyl-4MU-β-Glc **9** (B)

References

1. S. Bannink, et al., *6-O-Alkyl 4-methylumbelliferyl-β-D-glucosides as selective substrates for GBA1 in the discovery of glycosylated sterols.* Journal of lipid research, **2024**, *under minor revision.*