Cryo-EM structures of two clinically-relevant glucagon-like peptide 1 missense variants

Kieran Deane-Alder*, Matthew Belousoff**, Denise Wootten**, Patrick Sexton**

*Umeå University, Department of Medical Biochemistry & Biophysics
**Monash University, Drug Discovery Biology & ARC Centre for Cryo-EM of Membrane Proteins

G protein-coupled receptors (GPCRs) are transmembrane proteins that convey extracellular signals through intracellular transducers, especially the eponymous guanine nucleotide-binding (G) proteins. GPCRs are involved in almost all physiological processes in humans, and therefore are major drug targets.

The glucagon-like peptide 1 receptor (GLP-1R) has emerged as a striking success story for drug discovery. GLP-1R is a metabolic class B1 GPCR that is activated by GLP-1, a peptide hormone released postprandially, to trigger insulin secretion. It is a major regulator of blood glucose, and consequently has become the premier target for new blockbuster drugs to treat type 2 diabetes mellitus (T2DM), a disease driven by loss of insulin secretion and sensitivity.

A large body of research has established that a missense variant of GLP-1R, alanine-316-to-threonine (A316T), substantially reduces the risk of developing T2DM. A316T carriers, which are around 1% of the population, have lowered blood sugar in the fasting state (1). However, functional studies into this variant have struggled to reconcile its signalling profile with clinical observations.

Loss-of-function GLP-1R variants have also been observed clinically: an individual with a methionine 149 missense variant (T149M) had particularly severe T2DM (2). Pharmacological studies indicate that any non-polar substitution at this site, including T149A, dramatically impairs canonical signalling by GLP-1R - though this can be rescued by allosteric modulation (3). However, it is unclear how a mutation outside the orthosteric site or G protein interface could have such a large effect.

Recent advances in cryogenic electron microscopy (cryo-EM) have facilitated near-routine structure determination of many GPCRs (4). We extended these techniques to obtain 3 - 4 Å structures of both the A316T and T149A variants of GLP-1R, in complex with agonists and Gs proteins. These structures of gain- and loss-of-function GLP-1R variants explain aspects of their physiology and underscore the functional importance of dynamic motion between the peptide and receptor transmembrane domains.

To our knowledge, this is the first time that structures of a GPCR missense variant which occurs in humans have been determined. In addition to providing new insights into agonist-transducer coupling by GLP-1R, these results also demonstrate that structural biologists can now probe structure-function relationships of naturally-occuring GPCR variants.

1. Lagou, *et al*. Nat Genet. 2023 Sep;55(9):1448–61.
2. Tokuyama, *et al*. Diabetes Res Clin Pract. 2004 Oct;66(1):63–9.
3. Koole, *et al*. J Pharmacol Exp Ther. 2015 Apr 1;353(1):52–63.
4. Danev, *et al*. Nat Commun. 2021 Jul 15;12(1):4333.