**Stabilisation Methods for structural determination of ligand free parathyroid receptor 1 in complex with Gs protein**

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The parathyroid hormone receptor 1 (PTH1R) is a class B1 G protein-coupled receptor (GPCR) that signals primarily through the stimulatory G protein, $G\alpha_s$ [1]. Naturally occurring polymorphisms in PTH1R that induce constitutive activity (spontaneous activation of receptor in the absence of an agonist) have been linked to Jansen's metaphyseal chondrodysplasia, a form of short limbed dwarfism; these polymorphisms include $H223^{2.50}R$, $H223^{2.50}K$ and $T410^{6.42}P$ [2]. These mutations disrupt known polar networks within class B1 GPCRs that are responsible for maintaining the receptor in an inactive confirmation [3]. Cell surface expression and constitutive $G\alpha_s$-dependent cAMP signalling of these 3 PTH1R mutants relative to the wildtype (WT) PTH1R was assessed using flow cytometry and cAMP accumulation assays. This revealed a range of constitutive activity, with all three mutants exhibiting constative activity relative to the WT; H223K induced a moderate increase in basal signalling, followed by T410P and H223R as the most constitutively active.

To investigate the mechanism behind the constitutive activity of these polymorphisms, biochemical samples of wildtype and three mutant receptors coupled to Gs protein, with and without a truncated version of the endogenous peptide, PTH (1-34), were prepared. Purified complex samples were sufficient for structural studies of WT and all three mutants in the presence of peptide agonist, thus high resolution cryo-EM structures were determined of these complexes. However, only the most constitutively active mutant receptors, T410P and H223R, were able to be purified in a sufficient yield for structural determination in the absence of the PTH (1-34) agonist. Here we present the structure of the three constitutively active mutants in complex with peptide and G protein, as well as two mutants in complex with G protein in the absence of peptide. This work provides insights into the link between structure and constitutive activity of these clinically relevant mutations.

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