LARGE-SCALE PROFILING OF PHYSIOLOGICALLY RELEVANT NATURALLY OCCURRING RARE HUMAN GPCR VARIANTS USING THE BIOSENS-ALL® TECHNOLOGY

Frank Cao[1], Claire Normand[1], Hasna Mebrouk[1], Jenny Lau[1], Adeline Gadzinski[1], Meriem Semache[1], Arturo Mancini[1], Melissa R. Miller[2], Shunjie Guan[2], Dong Yan[2], Craig L. Hyde[2], Jean-Philippe Fortin[2], Eric B. Fauman[2], Xavier Leroy[1], and Laurent Sabbagh[1]

[1]Domain Therapeutics, Montreal, Quebec, Canada, and Strasbourg, France and [2] Worldwide Research, Development and Medical, Pfizer Global R&D, Cambridge, MA, USA

Abstract

G protein-coupled receptors (GPCRs) represent the largest class of membrane-bound proteins which regulate diverse physiological processes by engaging multiple signaling pathways. GPCR signaling can be differentially modulated by specific ligands to selectively promote the engagement of different subsets of signaling pathways, an outcome known as biased signaling. Although dysregulation of GPCR activity has been linked to pathological consequences, the involvement of their signaling pathways toward specific clinical repercussions remain unresolved. Thus, linking the role of GPCR signaling pathways to distinct physiological processes is not only important for understanding GPCR biology, but also essential for successfully targeting these receptors as potential therapeutic avenues. Here, to exploit the potential of naturally occurring GPCR variants emerging from large scale exome and genome sequencing efforts, we assessed the signaling profiles of rare variants across different GPCRs found in human populations with documented clinical phenotypes. Signaling profiles were assessed through an automated platform using our bioSens-All® technology, which consists of a panel of 16 pathway-selective bioluminescence resonance energy transfer (BRET)-based biosensors that monitor the activation of heterotrimeric G proteins (Gαs, Gαi1, Gαi2, GαoA, GαoB, Gαz, Gαq, Gα11, Gα14, Gα15/16, Gα12, Gα13) or the recruitment of β-arrestins (β-arrestin 1, and β-arrestin 2) to the plasma membrane upon agonist stimulation. To achieve the large-scale screen in a high throughput system, bioSens-All® assays were first miniaturized and adapted to a 384-well format. Subsequently, HEK293 cells were co-transfected with receptors and biosensors, and a scheduling software was used to coordinate the interaction between various instruments via a robotic microplate mover. Analysis was performed through an automated tool to generate and compile signaling profiles for each variant normalized to the wild-type receptor, revealing gain- or loss-of-function properties induced by the variants across different signaling pathways, thus also allowing us to identify genetic alterations that impart biased signaling profiles. By pairing the multiparametric signaling profiles of variants found in human populations generated through our automated bioSens-All® platform with information on their associated clinical phenotypes, we can uncover associations between altered receptor pharmacology and clinical outcomes to accelerate the discovery of disease-relevant targets and improve translation from bench to the clinic.

Objectives

PROJECT AIM

- Assess signaling profiles of naturally occurring rare variants across GPCRs found in human populations

GOAL

- Link GPCRs and their signaling pathways to distinct clinical phenotypes

STRATEGY

- Receptors + variants selected from biobank
  - Large-scale biomedical database containing genetic and health info of ~500,000 participants
- ~12 000 variants across 60 GPCRs were selected based on two criteria:
  1) Computationally predicted to be damaging
  2) Higher mutation frequency with genomic data paired with clinical phenotypes
- Characterize signaling profiles using our automated screening platform bioSensAll®

bioSens-All®

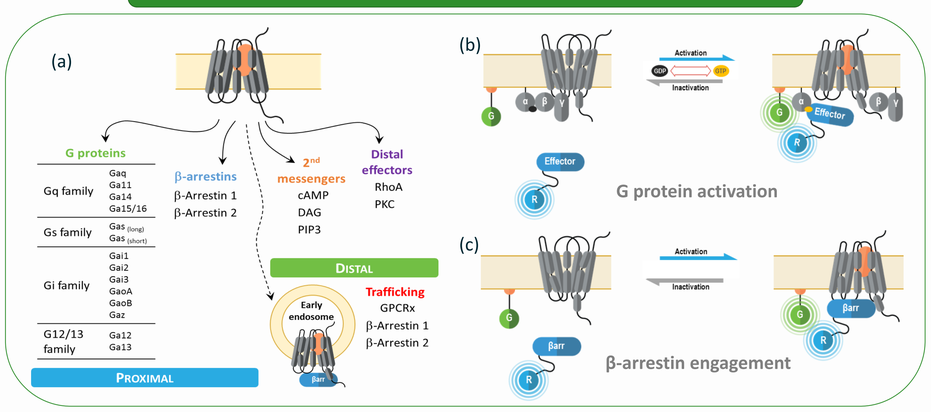
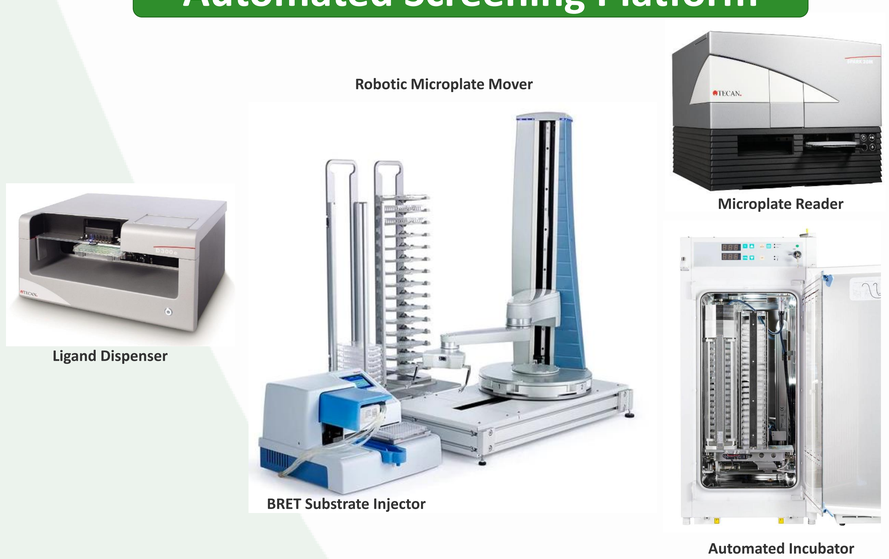


Fig. 1. BRET-based biosensors for G protein and βarrestin activation. (a) The bioSens-All® platform consists of 16 biosensors for monitoring the activation of specific Gα proteins and βarrestins downstream of GPCRs. These biosensors are designed to measure receptor proximal events that are engaged upon receptor activation, namely G protein activation and βarrestin recruitment. In addition to proximal biosensors, the technology includes sensors enabling detection of distal effector activity and second messenger generation. The latter assays can be used to complement and/or confirm events observed with proximal sensors. **(b)** Upon receptor activation, RlucII-tagged effector proteins (Effector-RlucII) translocate towards and interact with active Gα subunits from each G protein family, leading to increased BRET. **(c)** Upon receptor activation, RlucII-tagged βarrestins (βarrestin-RlucII) translocate to the plasma membrane, thus increasing BRET with membrane-anchored rGFP.

Automated Screening Platform



Results

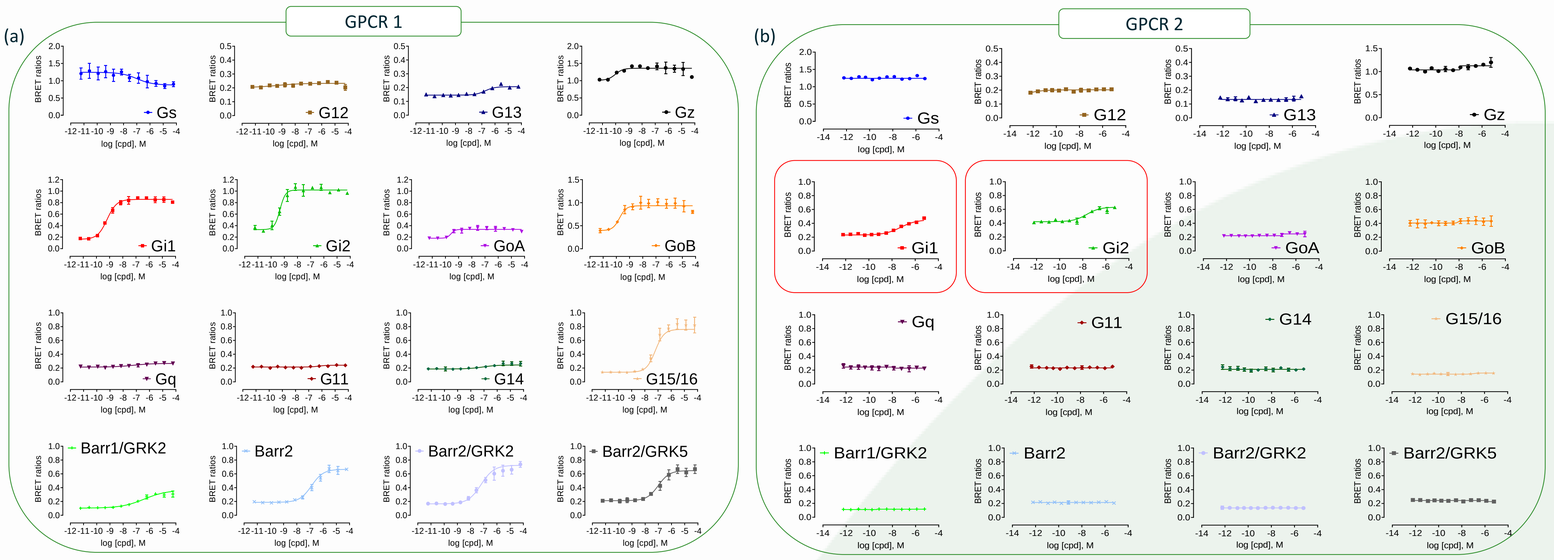


Fig. 2. Signaling profiles of wildtype human GPCRs. HEK293 cells were transfected with plasmids coding for a specific GPCR and one of 16 biosensors. Cells were treated with GPCR-specific ligand and the response recorded 30 mins later. **(a)** GPCR 1 demonstrated promiscuous signaling across many G protein and βarrestin pathways. **(b)** GPCR 2 demonstrated selective signaling on Gi1 and Gi2 pathways only.

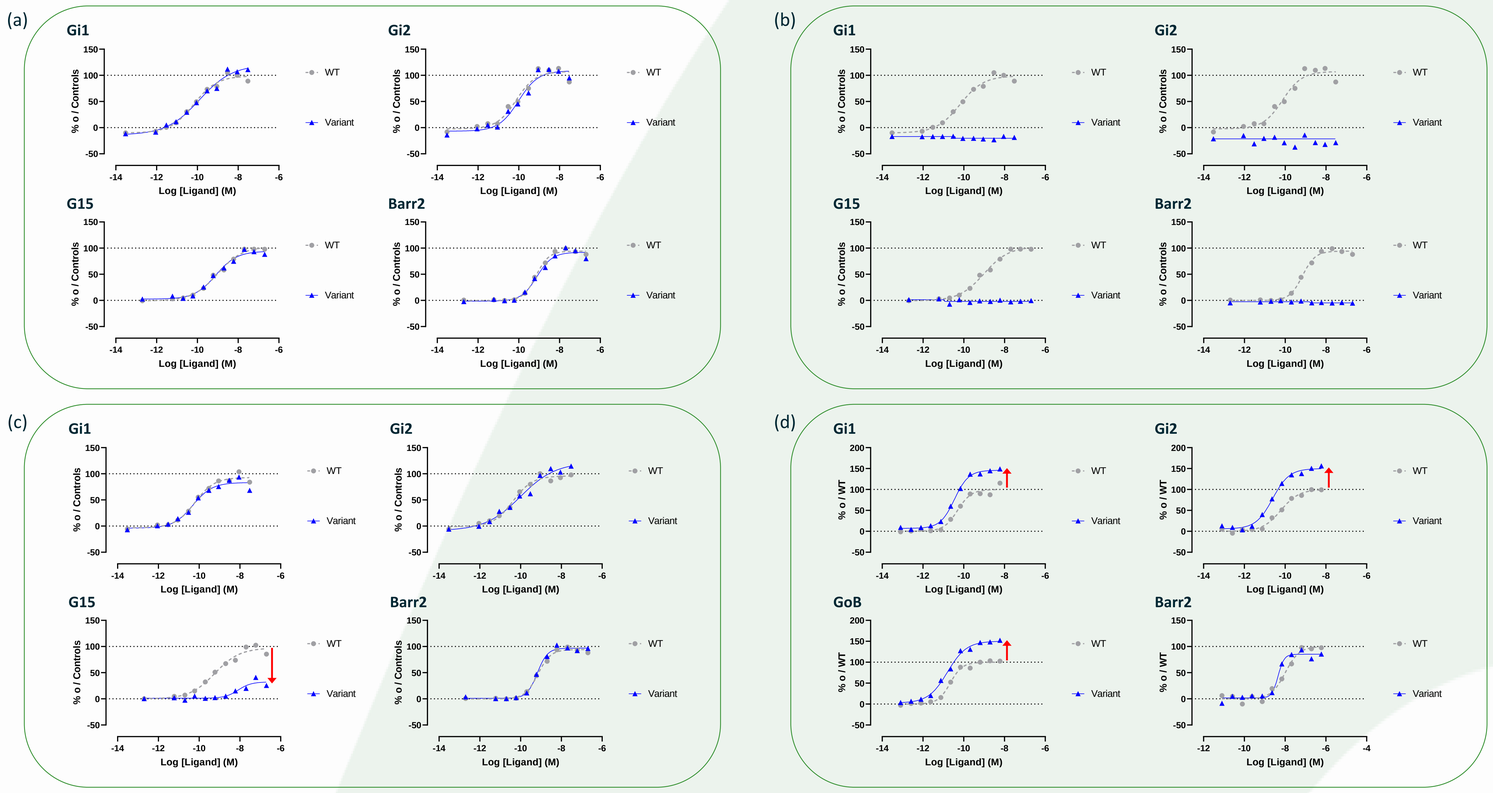
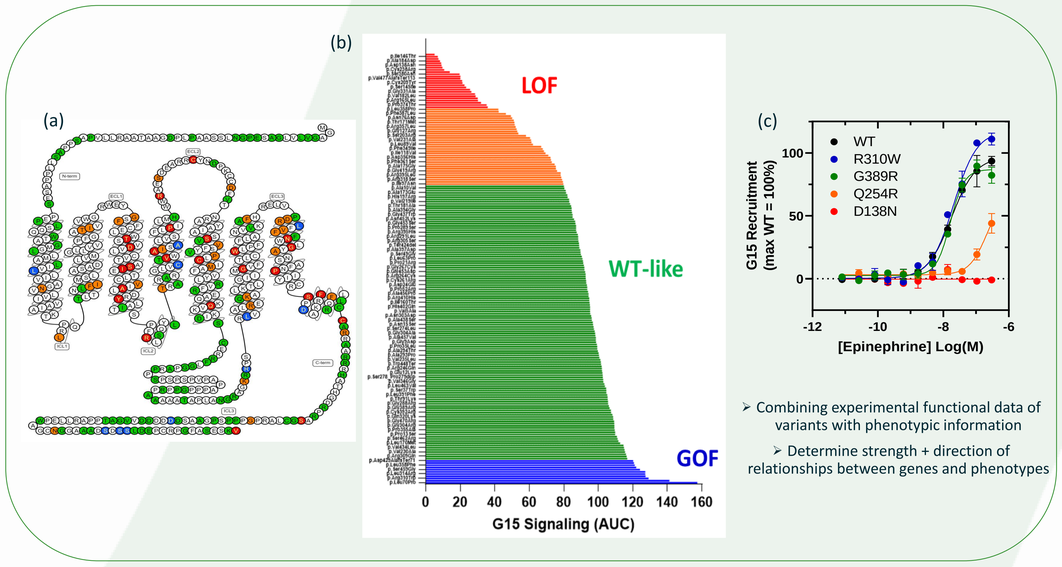


Fig. 3. Signaling profiles of variant receptors. HEK293 cells were transfected with plasmids coding for a specific GPCR and one of 16 biosensors. Cells were treated with GPCR-specific ligand and the response recorded 30 mins later. **(a)** Variant displays WT-like profile on all pathways selected. **(b)** Variant displays complete loss of function profile on all pathways selected. **(c)** variant displays loss of function on G15 and WT-like profile on Gi1, Gi2, and βarr2, thus yielding an overall biased profile. **(d)** Variant displays gain of function on Gi1, Gi2, and GoB and WT-like profile on βarr2, thus yielding an overall biased profile.



- Combining experimental functional data of variants with phenotypic information
- Determine strength + direction of relationships between genes and phenotypes

Fig. 4. Activity of 265 variants of ADRB1. (a) Location of variants on the serpentine map of ADRB1. **(b)** In vitro pharmacology of G15 area under the curve (AUC) for all profiled ADRB1 variants. **(c)** G15 signaling of four representative variants.

Conclusions

- bioSens-All® is a robust and flexible platform to assess GPCR signaling:
  - Uses unmodified receptors and G proteins (except for Gαs)
  - Permits pan-pathway analysis in parallel using one protocol
  - Automation of platform allows large scale GPCR variant profiling
- Pairing signaling data generated through bioSens-All® with clinical phenotypes, we begin to uncover associations between altered receptor pharmacology and disease states
- Analysis can be easily applied to receptors with strong human genetics data such as CASR, CCR5, etc.
- Accelerate the discovery of disease-relevant targets and improve translation from bench to the clinic