Take it personal! Genetic differences in G protein-coupled receptors as studied with label-free technology

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Summary

The traditional medical treatment paradigm focuses on prescribing one drug to treat all patients with a specific disease or condition, so called 'one-size-fits-all'. However, it has been shown increasingly that differences between persons, such as in lifestyle or genes, can change both the course of a disease and effect of a drug. In order to adapt medical treatment and drug development to that, a concept know as precision medicine, it is essential to study which and how genetic differences, i.e. polymorphisms, affect drug response. In this thesis I studied of the influences of genetic variation on a specific class of drug targets, the G protein-coupled receptors (GPCRs), by using a combination of personal cellular models and novel label-free assay technology.

Chapter 1 introduces the main subjects and concepts around precision medicine, GPCRs and genetic variation discussed in this thesis. Chapter 2 continues with discussing the concept of using patient-derived cell lines as model systems and highlights the advantages of label-free technology assays to investigate these. To better understand drug action and pathological processes in the human individual, physiologically more appropriate model systems are needed. For this, patient-derived cells can offer specific advantages. Traditional GPCR assays are often label-based, which has disadvantages when aiming to represent the physiological situation as closely as possible. Novel label-free cellular assays enable the study of complex biological processes in their native environment. Examples and advantages of the combination of these two are discussed in chapter 2.

Chapter 3 describes the optimization and application of an impedance-based label-free assay methodology, the xCELLigence, to a type of personal cell line, the lymphoblastoid cell lines (LCLs) from individuals of the Netherlands Twin Registry (NTR), to allow direct measurement of cellular effects of GPCR signaling. Generally, this label-free assay technology was deemed only compatible with adherent cell lines, while LCLs are suspension cells. Therefore, the methodology was optimized and applied to study cellular properties and GPCR signaling in LCLs. A prototypical GPCR present on LCLs, the cannabinoid receptor 2 (CB_2R), was selected for proof-or-principle. Effects of several compound types were studied and proved comparable between LCLs of two unrelated individuals with the same genotype, providing the first evidence that the technology and model system were well suited to evaluate genetic influences on GPCR-mediated drug responses.

Chapter 4 presents the case of another GPCR, the adenosine A_{2A} receptor ($A_{2A}R$). The $A_{2A}R$ is a potential drug target for a variety of respiratory and inflammatory conditions, including Parkinson's disease, as well as the receptor for caffeine. After identifying which adenosine receptor subtypes were present on LCLs, the cellular effects of various types of compounds targeting the $A_{2A}R$ were compared between LCLs derived from a family of four individuals, consisting of parents and their identical twin children. In the presence of a specific type of genetic variation, an intron Single nucleotide polymorphism (SNP) that is potentially linked to caffeine-induced sleep disorders, different cellular effects were found for a specific type of compound, a partial agonist, but not for other compounds such as full agonists or antagonists. Although this does not provide causal evidence that response differences are directly related to this genetic variation, it does show that the chosen methodology is capable of picking up individual differences in GPCR signaling.

After this demonstration, genetic differences in other GPCRs were studied. The CB_2R is a GPCR investigated intensively as therapeutic target due to its important role in the immune system. In chapter 5, responses to agonists, partial agonists and antagonists of various chemical classes were characterized in LCLs from individuals with varying CB_2R genotypes. One of the interesting findings was that endogenous cannabinoids such as 2-AG induced cellular effects vastly different from all synthetic cannabinoids, especially in their time-profile. More importantly, it was also found that compounds with different chemical scaffolds showed different sensitivity to a highly common amino-acid altering polymorphism in the CB_2R, the Q63R variant. In a similar manner it may be possible to identify compounds prone to personal differences, so for precision medicine, or more suited as drugs for the general population early on in drug development.

Genetic differences may however not only influence drug effects, but can alter a person's susceptibility to disease or alter disease progression. Chapter 6 presents the case of the Glucose-Dependent Insulinotropic Polypeptide Receptor (GIPR), in which an amino-acid altering SNP that has often been associated with diseases changed the cellular effects of the endogenous ligand. The GIPR plays an important role in whole-body metabolism, and its amino-acid altering SNP E354Q has been associated with several diseases including diabetes. When studying this receptor in a panel of LCLs of individuals with different genotypes for E354Q, responses to the endogenous agonist GIP showed enhanced potency in Q354 homozygous individuals. This study hereby provides more insight into how GPCR polymorphisms could change physiology in the human individual.

In summary, a novel cellular approach for studying genetic effects on GPCRs has been explored and detailed throughout this thesis. Several GPCRs and different types of genetic

variations were investigated, and the findings highlight that various kinds of genetic differences in GPCRs, can profoundly influence drug response. These include differing effects depending on compound type or chemical scaffold, as well as on endogenous signaling. The overall conclusion from the results described in this thesis and forthcoming opportunities for drug discovery and treatment are discussed in detail in chapter 7. In concert, the findings in this thesis may contribute to the progress of applying precision medicine concepts to the GPCR class of drug targets and hence the development of clinically more effective and more tailored drugs.