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Mutations in G Protein–Coupled Receptors: Mechanisms, Pathophysiology and Potential Therapeutic Approaches^S

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| | |
|--|-----|
| Abstract | 90 |
| Significance Statement..... | 90 |
| I. Introduction | 90 |
| II. History | 92 |
| III. General Mechanisms of GPCR Pathologies | 93 |
| IV. Inactivating Mutations of GPCRs | 95 |
| A. Partially Inactivating Mutations—Loss of Basal Activity | 97 |
| B. Partially Inactivating Mutations—Alteration of Distinct Receptor Functions..... | 97 |
| C. The Special Case—Pseudogenization of GPCRs | 99 |
| V. Activating Mutations in GPCRs—GoF..... | 99 |
| A. Agonist-Independent GoF | 99 |
| B. Agonist-Dependent GoF | 100 |
| VI. Other Causes of GPCR Dysfunctions | 100 |
| A. Gene Dosage as Cause of GPCR-Related Pathologies..... | 100 |
| B. Messenger RNA Processing Events as Cause of GPCR-Related Diseases..... | 101 |
| C. Altered and Ectopic Expression of GPCRs | 102 |
| 1. Promoter Mutations | 102 |
| 2. Untranslated Region and Intronic Mutations | 102 |
| 3. Gene Fusion Events and Ectopic Expression..... | 103 |
| D. GPCR-Related Diseases Caused by Mutations in More than One Gene | 104 |
| E. GPCR-Directed Autoantibodies as Cause of Diseases..... | 105 |
| VII. Therapeutic Options and Approaches..... | 105 |
| A. Symptomatic Therapies of GPCR Pathologies..... | 105 |
| B. Direct Targeting of Malfunctional GPCRs..... | 107 |
| 1. Gene Replacement..... | 107 |
| 2. Chemical Chaperones and Pharmacoperones | 107 |
| 3. Receptor Fragment Replacement..... | 107 |
| 4. Suppression of Stop Codons..... | 108 |
| 5. Ligand-Based Functional Rescue | 108 |
| VIII. Identification of Disease-Relevant GPCR Using Population Genetic Data..... | 109 |
| IX. Future Perspectives..... | 111 |
| Acknowledgments..... | 111 |
| References..... | 112 |

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Abstract—There are approximately 800 annotated G protein-coupled receptor (GPCR) genes, making these membrane receptors members of the most abundant gene family in the human genome. Besides being involved in manifold physiologic functions and serving as important pharmacotherapeutic targets, mutations in 55 GPCR genes cause about 66 inherited monogenic diseases in humans. Alterations of nine GPCR genes are causatively involved in inherited digenic diseases. In addition to classic gain- and loss-of-function variants, other aspects, such as biased signaling, *trans*-signaling, ectopic expression, allele variants of GPCRs, pseudogenes, gene fusion, and gene dosage, contribute to the repertoire of GPCR dysfunctions. However, the spectrum of alterations and GPCR involvement is probably much larger because an additional 91 GPCR genes contain homozygous or hemizygous loss-of-function mutations in human individuals with currently unidentified

phenotypes. This review highlights the complexity of genomic alteration of GPCR genes as well as their functional consequences and discusses derived therapeutic approaches.

Significance Statement—With the advent of new transgenic and sequencing technologies, the number of monogenic diseases related to G protein-coupled receptor (GPCR) mutants has significantly increased, and our understanding of the functional impact of certain kinds of mutations has substantially improved. Besides the classical gain- and loss-of-function alterations, additional aspects, such as biased signaling, *trans*-signaling, ectopic expression, allele variants of GPCRs, uniparental disomy, pseudogenes, gene fusion, and gene dosage, need to be elaborated in light of GPCR dysfunctions and possible therapeutic strategies.

I. Introduction

Recent estimates suggest 19,000–20,000 protein-coding genes in the human genome (Ezkurdia et al., 2014). Although all these genes were required to maintain the organismal fitness, at least during a period of human evolution, only a subset of genes causes diseases when altered. Research over the past 25 years has resulted in the identification of genes responsible for ~50% of the estimated 7000 rare monogenic diseases, and it is predicted that most of the remaining disease-causing genes will be identified in the next decade (Boycott et al., 2013). There are over 800 annotated G protein-coupled receptor (GPCR) genes (4.1%–4.3% of all genes) (Lv et al., 2016; Alexander et al., 2019), making these membrane receptors the most abundant gene family in the human genome. Members of the GPCR superfamily are involved in almost every physiologic function, including the mediation of signal transduction of neurotransmitters, hormones, metabolites, odors, and ions. They are also involved in transducing mechanical forces (Petersen et al., 2015; Scholz et al., 2017; Erdogmus et al., 2019) and cell-cell and cell-matrix interactions (Hamann et al., 2015). Because of their ability to modulate such an immense range of physiologic signals, GPCRs are targeted by 34% of the pharmaceuticals used today

(Hauser et al., 2017). However, mutations in GPCR genes can severely alter their normal function, and mutant GPCR genes cause about 66 human monogenic diseases (Table 1). Notably, several individual GPCR genes can cause different monogenic diseases because of loss- and gain-of-function mutations. Considering the 66 monogenic diseases caused by mutations in GPCR genes and 363 annotated nonolfactory GPCR genes (~17.9%), the alteration of GPCR genes contributes proportionally to the 3500 currently defined monogenic human diseases compared with all human genes (17% to 18%).

With the advent of exome- and whole-genome sequencing, more than 180 genotype-phenotype relations were established for rare monogenic diseases between 2009 and 2013 (Boycott et al., 2013), but only two included mutations in GPCR genes—GPR179 for blindness and glutamate receptor (GRM) 1 for congenital cerebellar ataxia (Table 1). It seems possible that we have reached saturation in identifying the involvement of GPCRs in monogenic diseases, but genome-wide association studies (GWASs), phenotyping of gene-deficient animal models, and analyses of somatic mutations of GPCR genes will likely establish new links between diseases and GPCR mutations.

This review is motivated by the fact that the most recent comprehensive reviews on monogenic diseases

ABBREVIATIONS: ACTH, adrenocorticotrophic hormone; aGPCR, adhesion GPCR; AVPR2, V2 vasopressin receptor; BRS3, bombesin-like receptor type 3; CASR, Ca²⁺-sensing receptor; CCR5, C-C motif chemokine receptor 5; DI, digenic inheritance; EDNRB, endothelin type B receptor; eQTL, expression quantitative trait loci; FFA, free fatty acid; FFAR3, FFA receptor 3; FZD, frizzled class receptor; GABBR2, γ -aminobutyric acid type B receptor subtype 2; GEF, GTP exchange factor; GHSR, growth hormone secretagogue receptor; gnomAD, Genome Aggregation Database; GNRHR, gonadotropin-releasing hormone receptor; GoF, gain of function; GPCR, G protein-coupled receptor; GRM, glutamate receptor; GWAS, genome-wide association study; hCG, human chorionic gonadotropin; HIV, human immunodeficiency virus; IRES, internal ribosome entry site; KISS1R, kisspeptin receptor; LH, luteinizing hormone; LHCGR, LH/hCG receptor; LoF, loss of function; MC1R, melanocortin type 1 receptor; MC2R, melanocortin type 2 receptor; MC4R, melanocortin type 4 receptor; NDI, nephrogenic diabetes insipidus; OPRM1, μ -opioid receptor 1; ORF, open reading frame; PDE, phosphodiesterase; PROKR2, prokineticin receptor type 2; RHO, rhodopsin; SNP, single nucleotide polymorphism; 7TMD, seven-transmembrane helices domain; TACR3, tachykinin receptor 3; TSH, thyroid-stimulating hormone; TSHR, TSH receptor; UTR, untranslated region.

TABLE 1
Monogenic diseases caused by mutations in GPCRs

Currently known monogenic inherited human diseases caused by mutations in GPCRs [sources: MAlAcards (<https://www.malacards.org/>), Online Mendelian Inheritance in Man (<https://omim.org/>), Pubmed literature screen], and the TSHR mutation data base (<https://www.tsh-receptor-mutation-database.org/>) are listed together with the current number of causative missense, nonsense, splice-site, and large-deletion/rearrangement mutations (source: <http://www.hgmd.cf.ac.uk/ac/index.php>). Diseases written in bold are caused by activating mutations. Some GPCR genes were also identified in studies screening human genomes for LoF variants: *genes intolerant for LoF (Lek et al., 2016), #homozygous LoF genes in Pakistani adults (Saleheen et al., 2017), *orphan GPCR.

| GPCR Gene | Disease/Syndrome | Missense | Nonsense | In/ del | Splice | Large | Reference |
|-----------|--|----------|----------|------------|--------|-------|---|
| ADGRG1** | Neural tube defect, spina bifida | 28 | 1 | 3 | | | Robinson et al., 2012 |
| ADGRE2* | Vibratory urticaria | 1 | | | | | Boyden et al., 2016 |
| ADGRG1* | Bilateral frontoparietal polymicrogyria | 14 | 6 | 7 | 2 | 1 | Piao et al., 2004 |
| ADGRG2** | Congenital bilateral aplasia of the vas deferens | | | 3 | | | Patat et al., 2016 |
| ADRG6* | Arthrogryposis multiplex congenita, lethal congenital contracture syndrome-9 | 1 | 1 | 1 | | | Ravenscroft et al., 2015 |
| ADGRV1* | Usher syndrome type IIC | 69 | 21 | 41 | 7 | 4 | Weston et al., 2004 |
| AGTR1 | Renal tubular dysgenesis | 1 | 2 | 1 | | | Gribouval et al., 2005, 2012 |
| AVPR2 | X-linked NDI | 135 | 23 | 75 | 3 | 27 | Rosenthal et al., 1992 |
| AVPR2 | X-linked nephrogenic syndrome of inappropriate antidiuresis | 4 | | | | | Feldman et al., 2005 |
| CALCRL* | Autosomal recessive nonimmune hydrops fetalis with lymphatic dysplasia | | | 1 | | | Mackie et al., 2018 |
| CASR | Hypocalcemic hypercalcemia, neonatal hyperparathyroidism | 226 | 17 | 35 | 6 | 3 | Pollak et al., 1993 |
| CASR | Dominant and sporadic hypoparathyroidism | 62 | | 1 | | | Pollak et al., 1994 |
| CHRM3* | Prune belly syndrome, familial congenital bladder malformation, impaired pupillary constriction, dry mouth | | | 1 | | | Weber et al., 2011 |
| CXCR4 | WHIM syndrome | 1 | 5 | 4 | | 1 | Hernandez et al., 2003 |
| CXCR2 | Autosomal recessive severe congenital neutropenia due to CXCR2 deficiency | 3 | | 1 | | | Auer et al., 2014 |
| CYS1LTR2 | Uveal melanoma, blue nevi | 2 | | | | | Moore et al., 2016; Möller et al., 2017 |
| EDNRA* | Mandibulofacial dysostosis with alopecia | 2 | | | | | Gordon et al., 2015 |
| EDNRB | Susceptibility to Hirschsprung disease 2, Waardenburg syndrome type 4A, ABCD syndrome | 35 | 5 | 7 | 2 | 4 | Puffenberger et al., 1994; Verheij et al., 2002 |
| FPR1 | Juvenile periodontitis | 3 | | | | | Gwinn et al., 1999 |
| FSHR | Hypergonadotropic ovarian dysgenesis | 15 | | | | 1 | Aittomäki et al., 1995 |
| FSHR | Ovarian hyperstimulation syndrome | 8 | | | | | De Leener et al., 2008 |
| FZD2 | Autosomal dominant omodyspasia, Robinow syndrome | 1 | 2 | | | | Saal et al., 2015 |
| FZD4* | Dominant familial exudative vitreoretinopathy | 42 | 9 | 13 | | 2 | Robitaille et al., 2002 |
| FZD5 | Autosomal dominant coloboma | | | 1 | | | Liu et al., 2016 |
| FZD6 | Recessive isolated congenital nail dysplasia | 4 | 2 | | | | Frjörmark et al., 2011 |
| GCGR | Mahvash disease | 7 | 1 | 2 | | 1 | Zhou et al., 2009 |
| GHRHR | Growth hormone deficiency | 21 | 2 | 6 | 10 | 1 | Wajnrajch et al., 1996 |
| GHSR | Growth hormone deficiency and short stature | 7 | 1 | 1 | | | Pantel et al., 2006 |
| GNRHR | Hypogonadotropic hypogonadism | | | 4 | | 2 | de Roux et al., 1997 |
| GPR88* | Childhood-onset chorea with psychomotor retardation | 42 | 1 | 1 | | | Alkufri et al., 2016 |
| GPR101* | X-linked acrogiantism | 2 | | | | 8 | Kamenický et al., 2015 |
| GPR143 | Ocular albinism type I, congenital nystagmus, altered thickness of the iris | 44 | 7 | 29 | 17 | 29 | Bassi et al., 1995; Zhou et al., 2008b; Peng et al., 2009 |
| GPR179 | Congenital stationary night blindness | 5 | 1 | 5 | 1 | 1 | Audio et al., 2012 |
| GRM1* | Autosomal recessive spinocerebellar ataxia | | | 3 | 1 | | Guergueltcheva et al., 2012 |
| GRM1* | Autosomal dominant spinocerebellar ataxia | 2 | | | | | Watson et al., 2017 |
| GRM6# | Congenital stationary night blindness | 17 | 5 | 6 | 1 | | Dryja et al., 2005 |
| KISS1R | Hypogonadotropic hypogonadism | 19 | 4 | 3 | 2 | 1 | de Roux et al., 2003; Seminara et al., 2003 |
| KISS1R | Central precocious puberty | 1 | | | | | Teles et al., 2008 |
| LHCGR | Leydig cell hypoplasia, pseudohermaphroditism, primary amenorrhea | 18 | 6 | 5 | 2 | 5 | Kremer et al., 1995 |
| LHCGR | Male-limited precocious puberty, Leydig cell adenoma | 18 | | | | | Shenker et al., 1993 |
| LPAR6 | Hypotrichosis, wooly hair | 11 | 2 | 10 | | 2 | Pasternack et al., 2008; Shimomura et al., 2008 |
| MC1R | Hypopigmentation | 73 | 1 | 8 | | | Valverde et al., 1995 |
| MC2R | Glucocorticoid deficiency | 34 | 3 | 8 | | | Clark et al., 1993; Tsigos et al., 1993 |
| MC2R | ACTH-independent Cushing syndrome | 1 | | | | | Swords et al., 2002 |
| MC3R | Obesity | 25 | | | | | Lee et al., 2002 |
| MC4R | Obesity | 119 | 7 | 21 | | 1 | Vaisse et al., 1998; Yeo et al., 1998 |
| MTNR1B | Susceptibility to diabetes mellitus type 2 | 27 | | | | 1 | Bonnefond et al., 2012 |

(continued)

TABLE 1—Continued

| GPCR Gene | Disease/Syndrome | Missense | Nonsense | In/del | Splice | Large | Reference |
|--------------|--|----------|----------|--------|--------|-------|--|
| OGRI (GPR68) | Anelagenesis imperfecta | 1 | | 2 | | | Parry et al., 2016 |
| OPN1SW# | Tritanopia | 6 | | | | | Weitz et al., 1992 |
| OPN1MW | Deuteranomaly, cone dystrophy | 5 | 1 | 1 | | 6 | Winderickx et al., 1992 |
| OPN1LW* | Blue cone monochromacy | 12 | 3 | 1 | | 35 | Nathans et al., 1993 |
| P2RY12 | Bleeding disorder | 9 | | 2 | | | Hollopeter et al., 2001 |
| PROKR2 | Kallmann syndrome | 42 | 2 | 4 | | | Dodé et al., 2006 |
| PTHIR | Blomstrand chondrodysplasia, Eiken syndrome, primary failure of tooth eruption | 6 | 8 | 9 | | 9 | Jobert et al., 1998; Decker et al., 2008 |
| PTHIR | Murk Jansen type of metaphyseal chondrodysplasia | 6 | | | | | Schipani et al., 1995 |
| RGR | Retinitis pigmentosa | 4 | | 1 | 2 | | Morimura et al., 1999 |
| RHO | Congenital night blindness, autosomal dominant retinitis pigmentosa | 141 | 9 | 20 | 6 | 3 | Dryja et al., 1990 |
| SIPR2 | Autosomal dominant retinitis pigmentosa | 9 | | | | | Rao et al., 1994; Park, 2014 |
| SMO | Deafness | 2 | | | | | Santos-Cortez et al., 2016 |
| SMO | Basal cell carcinoma | 3 | | | | | Xie et al., 1998; Khamaysi et al., 2016 |
| SMO | Curry-Jones syndrome (Mosaicism) | 1 | | | | | Khamaysi et al., 2016 |
| TACR3 | Normosmic hypogonadotropic hypogonadism | 19 | 6 | 2 | 2 | | Topaloglu et al., 2009 |
| TBXA2R | Bleeding disorder | 4 | | 1 | | | Hirata et al., 1994 |
| TRHR | Hypothyroidism | 1 | 2 | 1 | | | Collu et al., 1997 |
| TSHR | Congenital hyperthyroidism, hyperfunctioning thyroid adenoma, and carcinoma | 80 | 10 | 12 | 4 | 4 | Sunthornthepvarakul et al., 1995 |
| TSHR | | 105 | | 3 | | | Parma et al., 1993 |

caused by GPCR mutations were published more than 10 years ago (Schöneberg et al., 2004; Spiegel and Weinstein, 2004; Lania et al., 2006; Insel et al., 2007; Tao, 2008; Vassart and Costagliola, 2011). In the meantime, the number of monogenic diseases and subphenotypes related to mutant GPCRs has increased, and our understanding of the functional impact of certain kinds of mutations has improved. This concise review will detail the current state of general mechanisms of disease-causing GPCR mutations, the resulting phenotypes, and potential therapeutic strategies to restore the altered GPCR function.

II. History

Understanding the contribution of GPCRs to the pathomechanisms of inherited human diseases is inseparably linked to efforts made in elucidating the function of rhodopsin (RHO), a prototypical GPCR. After the sequencing of the gene encoding human RHO of the retinal rods (Nathans and Hogness, 1984), RHO immediately became a candidate gene for a number of inherited forms of retinitis pigmentosa, a disease characterized by the degeneration of photoreceptors. Several groups identified mutations in the coding region of RHO that were strongly associated with this disease (Dryja et al., 1990; Inglehearn et al., 1991). To date, more than 180 RHO mutations have been identified that cause autosomal dominant forms of retinitis pigmentosa (Table 1).

After the first discovery of retinitis pigmentosa-causing mutations in RHO and the cloning of other GPCR cDNAs in the late 80s/early 90s, the number of mutant GPCRs directly linked to human diseases has increased continuously. Primarily, end-organ resistances to hormones were linked to mutations in GPCRs. Large pedigrees were screened to identify chromosomal regions associated with inherited diseases and linked to mutations in GPCRs (Lester et al., 1990; Bichet et al., 1992) (Fig. 1). For example, the V2 vasopressin receptor (AVPR2) cDNA was identified in 1992, and mutations in the AVPR2 gene were found in parallel to be the cause of X-linked nephrogenic diabetes insipidus (NDI) (Rosenthal et al., 1992). Similarly, the cloning of the receptors for thyroid-stimulating hormone (TSH) (Parmentier et al., 1989) and luteinizing hormone (LH) (McFarland et al., 1989) was closely followed by the identification of disease-causing mutations in these glycoprotein hormone receptors (Parma et al., 1993; Shenker et al., 1993).

The next “wave” in discovering human monogenic diseases caused by GPCR mutations was promoted by phenotyping GPCR gene-deficient mouse lines in a search for the phenotypic counterpart in humans (Fig. 1). For example, inactivating mutations in the melanocortin type 4 receptor (MC4R), which caused forms of inherited obesity in humans (Vaisse et al., 1998; Yeo et al., 1998), were discovered after description of obesity in MC4R-deficient mice (Huszar et al., 1997). Similarly, hyperglucagonemia,

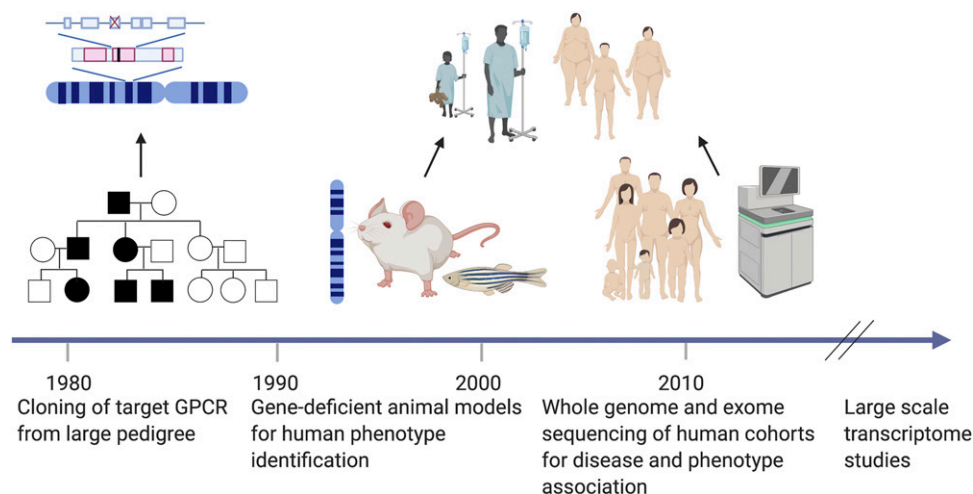


Fig. 1. Approaches to identify mutant GPCR as cause for inherited diseases in the course of time. After cloning and sequencing of the first GPCR genes in the mid-80s/90s, genomic loci information of large disease-affected families was matched with mutations in GPCR genes. In the 1990s, targeted GPCR gene disruption in animal models and subsequent phenotyping guided the identification of human phenotypes and diseases caused by mutations in orthologous human GPCR genes. Currently, large-scale exome and genome sequencing of disease and control cohorts allows for comparative analysis yielding rare GPCR variants that can be linked to human phenotypes. In the future, deep-sequenced transcriptomes of tissues and even defined cell populations will add information on the disease-relevant impact of mutations in the coding and noncoding regions with respect to promoter activity and splicing. Figure was created with BioRender.

hyperaminoacidemia, and glucagon cell hyperplasia were described in mice with a glucagon receptor deficiency (Gelling et al., 2003; Dean et al., 2017), and subsequently, the same phenotype was found in humans with a glucagon receptor defect (Zhou et al., 2009).

The current “wave” is still driven by mouse phenotypes but also by whole-genome and exome sequencing of patients with suspect phenotypes (Fig. 1). For example, whole-exome sequencing of patients with congenital stationary night blindness revealed recessive mutations in the orphan receptor GPR179 (Audo et al., 2012), and the disease-causing effect of loss of GPR179 function was demonstrated in mouse and zebrafish models (Peachey et al., 2012). Similarly, exome sequencing identified mutations in the γ -aminobutyric acid type B receptor subtype 2 (GABBR2) responsible for the Rett syndrome-like phenotype and epileptic encephalopathy (Yoo et al., 2017). Consistently, mice lacking GABBR2 have a complex neurologic phenotype that includes seizures (Gassmann et al., 2004). One can anticipate that whole-genome and transcriptome sequencing will expose additional GPCR genes and new pathomechanisms with alterations of genic components, such as promoters and intronic regions (Fig. 1).

III. General Mechanisms of GPCR Pathologies

A receptor molecule is defined by its ability to change its structure after specific binding of its agonist(s). This signal-driven conformational change is transduced into an effect, such as the recruitment of or loss of association with other proteins or biomolecules, modification of enzymatic activity, or change of ion permeability. GPCRs are so-called GTP exchange factors (GEFs) triggering the exchange of GDP to GTP in the α -subunit of heterotrimeric

G proteins. Mutations in GPCRs can lead to the inactivation of their GEF activity [loss of function (LoF)] (Fig. 2) or ligand-independent activation of this GEF activity [gain of function (GoF)] (Fig. 3). These two functional consequences are the most frequent alterations caused by mutations in GPCRs.

However, research of the last 25 years has extended this simple view on the GPCR being just an “on-off switch.” Coupling to more than one G-protein class (Gudermann et al., 1996), the existence of different active and inactive conformational states (Weis and Kobilka, 2018), biased signaling (Wootten et al., 2018), allosteric binding sites (May et al., 2004), receptor oligomerization (Milligan et al., 2019), and the modulation of the receptor activity by endogenous antagonists/inverse agonists (Adan and Kas, 2003) and other interacting proteins (van der Westhuizen et al., 2015) introduce a higher functional complexity into the GPCR superfamily. All these properties and functions can be individually or combinatorically altered by mutations (Stoy and Gurevich, 2015). A further level of complexity is added when mutations in GPCR genes change promoter activity, affect splicing, or lead to gene duplications or rearrangements (Fig. 4). Furthermore, GPCRs are also capable of G protein-independent signaling [e.g., by recruiting arrestins (Chen et al., 2018b)], scaffolding other intracellular proteins (Appert-Collin et al., 2006; Dunn and Ferguson, 2015; Knapp and Wolfrum, 2016; Garcia et al., 2018), or interacting with other receptors and serving as a signal by themselves (Schöneberg et al., 2016). The components organizing proper trafficking and signaling of GPCRs can be affected by mutation and may result in (partially) convergent phenotypes, as seen for inherited diseases caused by mutations in GPCRs. However, this review does not focus on inherited diseases

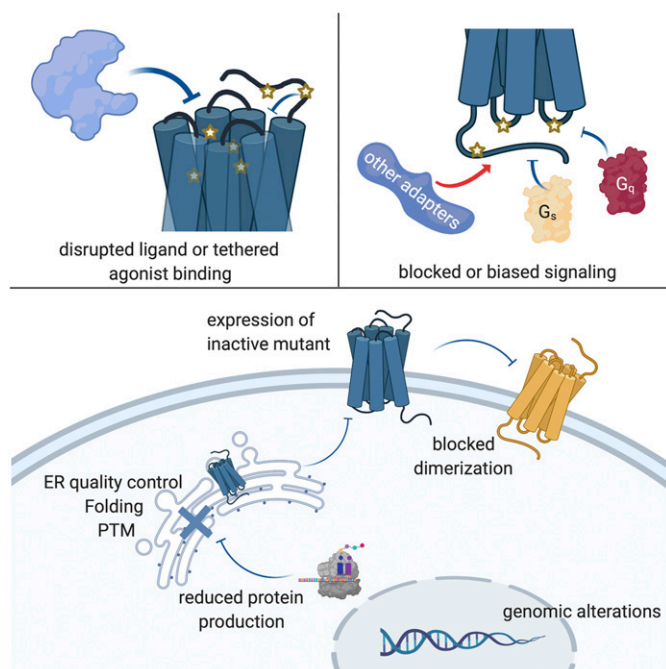


Fig. 2. General mechanisms of disease-relevant LoF mutations in GPCRs. The LoF of GPCRs can be caused by alterations at different levels of the receptor protein biosynthesis (genomic, transcriptomic, and/or translational level), protein folding, processes of peri- and post-transcriptional modifications, and trafficking (lower panel). In most of these cases, there is no or insufficient receptor protein at the cell surface to mediate signal transduction. The LoF of mutant GPCRs that are transferred to the plasma membrane can be the results of receptor oligomerization effects, mutations that interfere with proper binding of the agonist or tethered agonist (upper left panel), or mutations that interfere with interaction of G proteins or other adapter proteins (upper right panel). Figure was created with BioRender. ER, endoplasmic reticulum; PTM, post-transcriptional modification.

of components of GPCR signaling but refers to relevant reviews on this topic (Weinstein et al., 2006; Gurevich and Gurevich, 2019).

To date, over 2350 mutations in 55 GPCR genes have been causally linked to 66 human diseases. Approximately 14 disorders are caused by activating mutations in GPCRs (Table 1). Different diseases can result from a single GPCR gene, considering inactivating and activating mutations [e.g., hypothyroidism and hyperthyroidism caused by mutations in the TSH receptor (TSHR)]. Dominant traits do not always result from activating mutations, but, even in the heterozygous stage of an inactivating mutation, gene dose effects or dominant-negative effects of the misfolded protein on the remaining wild-type receptor (e.g., RHO, FZD2) may cause a disease (see below). Among these 55 GPCRs, there are seven (14.5%) orphan GPCRs (Hauser et al., 2020), which contribute only 9.4% of all disease-causing mutations in GPCRs. This is most probably due to the fact that the functional relevance of mutations found in orphan GPCRs is difficult to test because of missing agonists and unknown or uncertain signal transduction pathways. Therefore, the causal link between the disease and identified mutations is usually established from linkage data of phenotypically well-characterized

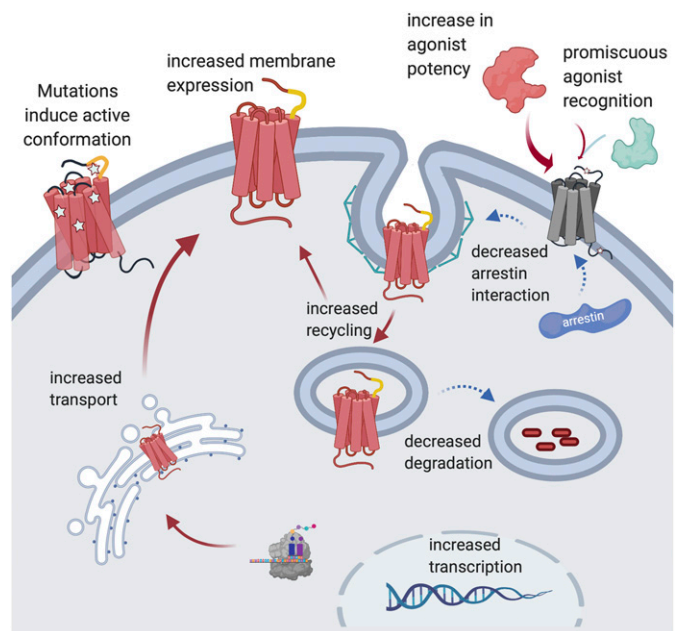


Fig. 3. General mechanisms of disease-relevant GoF mutations in GPCRs. There are two general forms of GPCR alterations that cause a gain of receptor function—agonist-independent and agonist-dependent causes. Mutations in transmembrane helices or, more rarely, in the extracellular loops promote an active conformation of this domain. Some GPCRs (see text) contain an internal agonist, and mutations within or surrounding the tethered sequence can shift this internal agonist into its active conformation, triggering the on-stage of the transmembrane domain. In rare cases, mutations can increase the potency of the physiologic agonist, which shifts the concentration-response significantly leftward and generates a quasi-constitutively active GPCR. Mutations can also change the agonist specificity, leading to a GPCR that now recognizes a related and more potent endogenous ligand. Decrease of receptor inactivation (e.g., due to lack of arrestin recruitment) or degradation and increased transport or recycling can also appear as an increased receptor activity. Figure was created with BioRender. uORF, upstream ORF.

cohorts or large consanguineous families and the occurrence of obviously inactivating mutations, such as frame-shift and premature stop mutations (Piao et al., 2004; Kamenický et al., 2015; Ravenscroft et al., 2015; Alkufri et al., 2016). Yet, GPCR-deficient mouse models often copy the human phenotype (Supplemental Table 1; Table 2) and are, therefore, useful to link human diseases to causative mutations even in orphan GPCRs. One example is a male fertility defect due to deficiency of the adhesion GPCR (aGPCR) ADGRG2, which was first found in a knockout mouse (Davies et al., 2004) and later found in patients with an X-linked infertility phenotype (Patat et al., 2016).

Most genomic alterations are missense mutations (68%), and this is followed by small inserts/deletions (16%), nonsense (stop) mutations (7%), gross deletions/rearrangements (6%), and splice-site mutations (3%) (Table 1). When compared with mutation entries of 11,320 human genes [Human Gene Mutation Database Professional release 2019.4; (Stenson et al., 2012)], there are some differences in the distribution of genomic alterations (48% missense mutations, 22% small inserts/deletions, 11% nonsense mutations, 10% gross deletions/rearrangements, 9% splice-site mutations).

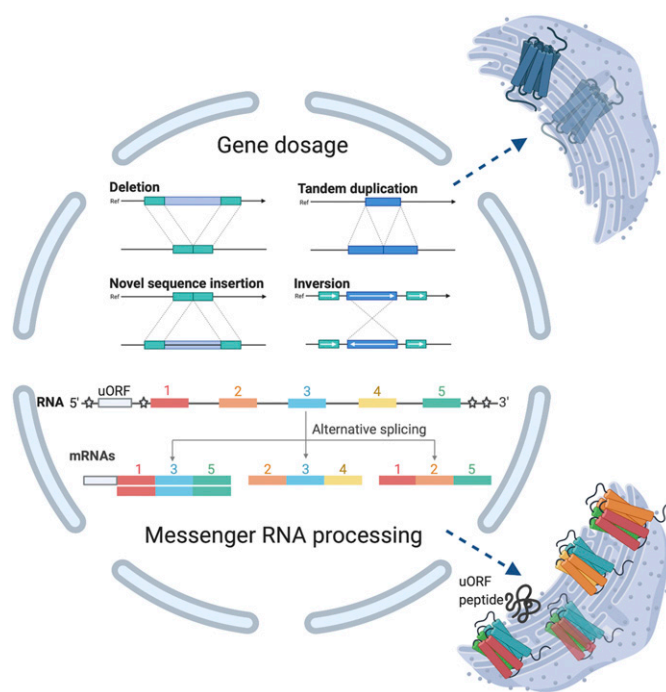


Fig. 4. Other mechanisms of disease-relevant GPCR pathologies. As in the case of many other inherited diseases, more complex genomic alterations can cause a loss but also a gain of GPCR function. Chromosomal mutations can have an impact of gene dosage (deletions, large insertions, rearrangements, duplications, gene fusions), which results in reduced, increased, or ectopic GPCR expression. Mutations may also have an impact on proper mRNA transcription and processing when promoter, UTR, or intronic regions are affected. For most of the known genomic alterations, there are also examples for GPCR genes (see text). Figure was created with BioRender.

However, one must consider that not all variants in the Human Gene Mutation Database are pathogenic and that, mechanistically, not all genes can be activated by missense mutations (e.g., structural proteins, such as collagens). The following sections will elaborate on these different genetic changes that influence GPCR functions.

IV. Inactivating Mutations of GPCRs

For GPCRs, “inactivation” means full or partial loss of responsiveness to the physiologic signal, leading to a full or partial loss of the signal transduction specifically mediated by the affected GPCR. Inactivating mutations include the full repertoire of mutations: single-nucleotide mutations, inserts/deletions, frameshifting mutations, and partial or complete deletion of the gene. Most inactivating mutations are *de novo* or recessive mutations transmitted only over a few generations. In some cases, heterozygosity can drift or even confer potential selective advantages to mutation carriers over a long period, as in the case of prokineticin receptor type 2 (PROKR2), in which the inactivating mutation L173R has persisted more than 9000 years (Avbelj Stefanija et al., 2012). A high frequency of inactivating mutations in populations may indeed have an advantage as

suggested for variants in the melanocortin type 1 receptor (MC1R) gene, a key regulator of melanogenesis (Martínez-Cadenas et al., 2013; Dib et al., 2017; Marano et al., 2017).

Most missense, insert/deletion, and nonsense mutations alter folding of the receptor proteins (about 80% of all inactivating mutations in GPCRs), which are retained intracellularly by the endoplasmic reticulum quality control system (Araki and Nagata, 2011) and, therefore, cause improper expression at the cell surface (Schöneberg et al., 2004; Conn et al., 2007) (Fig. 2). Misfolded GPCRs may even aggregate and accumulate, causing cell death (Park, 2019) and convergent phenotypes summarized as protein aggregate diseases (Aguzzi and O'Connor, 2010). One example is retinitis pigmentosa, in which rod photoreceptor cells degenerate because of deposits of misfolded mutant RHO (Miller et al., 2015).

Once a mutant GPCR has passed through the endoplasmic reticulum quality control machinery and reached the cell surface, the mutation can still interfere with agonist binding and/or G-protein coupling (Fig. 2). Functional assays that differentiate between the different molecular causes of receptor inactivation are well established, including second messenger, ligand-binding, and receptor expression assays. Such experimental setups have revealed numerous clinically relevant examples of GPCR mutations, showing that LoF of plasma membrane-expressed receptors is mainly caused by a reduced affinity for the physiologic agonist(s) (Biebrermann et al., 1997; Schöneberg et al., 1998; de Roux et al., 1999; Morello et al., 2001; Tarnow et al., 2003). In principle, alteration of the agonist binding pocket can occur either through direct or indirect mechanisms. The former case involves amino acid positions that directly participate in agonist interactions. For example, the Arg²⁵⁶Gln mutation in the ADP receptor P2RY12 causes an inherited bleeding disorder (Table 1) (Cattaneo et al., 2003). This mutation does not interfere with proper cell surface expression of P2RY12 but with agonist binding. The crystal structure of the human P2RY12 with a bound agonist revealed that the positively charged arginine residue (Arg²⁵⁶) directly interacts with the α phosphate moiety of the agonist 2-methylthioadenosine-5'-diphosphate-ADP (Zhang et al., 2014). Since Arg²⁵⁶ is an essential part of the agonist binding pocket, mutation of this position to Gln consequently leads to a loss of affinity. Indirect disturbance of the agonist binding site can occur if the mutated position is distantly involved in structuring and stabilizing the binding pocket. A clear assignment of direct and indirect effects on agonist binding is often difficult.

Isolated defects of receptor/G-protein coupling without effects on receptor trafficking and/or agonist binding are rare (Fig. 2) but do occur in Hirschsprung disease, which is caused by mutations in the endothelin type B receptor (EDNRB) gene (Table 1). Several mutant

TABLE 2
Monogenic diseases caused by mutations in GPCRs

| The top 10 autosomal diseases caused by mutations in GPCRs are listed together with the detailed human phenotype and the phenotype found in the respective gene-deficient mouse model (given reference). X-chromosomal diseases and their mouse phenotypes are listed in Supplemental Table 1. Mouse phenotypes are taken from the listed reference and the mouse phenotype data base (http://www.informatics.jax.org). | | |
|--|---|---|
| GPCR Gene | Human Phenotype/Main Symptoms | Mouse Phenotype/Main Symptoms |
| ADGRV1 | Usher syndrome type IIC; ciliopathy, sensorineural hearing deficiencies at birth and later development of progressive retinitis pigmentosa | Cochlear defects, progressive hearing impairment, deafness, audiogenic seizure |
| CASR | Neonatal hyperparathyroidism; hypocalciuric hypercalcemia elevated PTH, bone demineralization, failure to thrive, associated with parathyroid hyperplasia | Hypocalciuric hypercalcemia, decreased phosphate serum level, elevated parathyroid hormone level, parathyroid hyperplasia, bone abnormalities, retarded growth, premature death |
| EDNRB | Hirschsprung disease, aganglionic megacolon; congenital absence of intrinsic ganglion cells in the myenteric (Auerbach) and submucosal (Meissner) plexuses of the gastrointestinal tract | Required for neural crest-derived melanocyte and enteric neuron development, homozygous mice are predominantly white and die as juveniles from megacolon |
| FZD4 | Dominant familial exudative vitreoretinopathy due to heterozygous inactivation of FZD4; incomplete development of the retinal vasculature, clinical appearance varies from blindness during infancy to mildly affected patients | Homozygous animals develop cerebellar degeneration, severe ataxia, abnormal absence of a skeletal muscle sheath around the lower esophagus, progressive deafness, small kidney |
| GNRHR | Hypogonadotropic hypogonadism; absent or incomplete sexual maturation, low levels of circulating gonadotropins and testosterone | Small sexual organs, low levels of FSH, LH, and steroid hormones, failure of sexual maturation, infertility, inability to respond to exogenous GnRH |
| MC1R | Hypopigmentation; defect in eumelanin production, fair skin, red hair, increased risk of melanoma | Yellow coat color, reduced sensitivity to noxious stimuli, increased analgesic responsiveness |
| MC4R | Early-onset childhood obesity, hyperphagia due to alteration of hypothalamic appetite regulation, metabolic syndrome | Maturity-onset obesity syndrome, hyperphagia, hyperinsulinemia, hyperglycemia, nonalcoholic steatohepatitis, reduction in corpora lutea number |
| PROKR2 | Kallmann syndrome; anosmia related to defective olfactory bulb morphogenesis, absent or incomplete sexual maturation, low levels of circulating gonadotropins and testosterone | Hypoplasia in the olfactory bulb, small sexual organs, failure of sexual maturation, infertility |
| RHO | Autosomal dominant early-onset retinitis pigmentosa; progressive retinal rod cells degeneration, night blindness, peripheral visual field loss | Slow degeneration of the retina, decrease of light-evoked electroretinogram responses |
| TSHR | Congenital hypothyroidism, thyroid hypoplasia, bradycardia, oligophrenia, hypothermia, elevated TSH, reduced thyroid hormones | Very low to undetectable serum thyroxine, elevation of TSH, retarded growth, infertility, mild anemia, elevated serum cholesterol, delayed ossification, reduced cortical bone |

EDNRBs exhibit wild-type binding affinity but impaired coupling to $G_{q/11}$ proteins (Imamura et al., 2000).

The majority of disease-causing missense mutations impact receptor positions that have been highly conserved during evolution. For example, 62% of all missense mutations in AVPR2 that cause NDI (see Table 1) are at 100% conserved amino acid positions and 26% at positions that vary between only two amino acids in vertebrate AVPR2 orthologs (Schöneberg et al., 2004). Therefore, the evolutionary conservation of an individual position within a protein may serve as a predictive tool to evaluate the functional relevance of missense mutations. Indeed, there are a number of bioinformatics tools that use evolutionary information from automatically generated multiple-sequence alignments and a variety of features to describe protein function and structure (Ng and Henikoff, 2003; Adzhubei et al., 2010). The rationale of this approach comes from the fact that the sequence diversity and conservation of a given protein are the result of a long evolutionary process characterized by the continuous accumulation of mutations, which are subsequently sorted by natural selection. Deleterious mutations that inactivate a vital protein will be purged from the population (purifying selection), whereas functionally tolerated or favorable mutations may be swept up in frequency by drift and positive selection, respectively. We previously tested these methods on systematically generated experimental data on P2RY12 (Cöster et al., 2012) and naturally occurring mutations in monogenic diseases (Schöneberg et al., 2018) and found that evolutionary data allow for 92% correct prediction of the functional relevance of missense mutations (Schöneberg et al., 2018).

Mutational inactivation or even a loss of GPCR genes does not always result in severe pathology. Using large exome and genome-sequencing data bases, more than 90 human GPCR genes were identified harboring homozygous or hemizygous inactivating mutations (Supplemental Tables 1 and 2). These GPCRs have not been linked to any known human diseases, and the human individuals exhibiting the mutations were all adults without life-threatening pathologies. For example, GPR33 is mainly a pseudogene in European populations, but the intact allele has a significantly higher frequency in Southeast Asia (Römpler et al., 2005; Bohnkamp et al., 2010). A similar intact allele/pseudogene allele pattern in GPR33 was found in rats and gerbils, raising the hypothesis of a zoonotic pathogen triggering this synergistic allele distribution (Römpler et al., 2005; Bohnkamp et al., 2010). One should consider even a fitness advantage under given environmental circumstances due to a loss of distinct GPCR-mediated functions. For example, C-C motif chemokine receptor 5 (CCR5) inactivation provides some resistance to HIV and may have even caused the positive selection of this locus (Liu et al., 1996; Samson et al., 1996).

A. Partially Inactivating Mutations—Loss of Basal Activity

In the classic two-state model, GPCRs are considered to exist in an equilibrium between an active and an inactive conformation. Consequently, even in the agonist-unoccupied state, a subpopulation of receptors is always in an active state, which underlies some basal activity of many GPCRs. Extraordinarily high basal activity has been observed for several dozen wild-type receptors from all classes of GPCRs (Seifert and Wenzel-Seifert, 2002; Martin et al., 2015). Increased basal activity of the bombesin-like receptor type 3 (BRS3) was even found positively selected in placental mammals during vertebrate evolution (Tang et al., 2019). Therefore, the genuine property of a GPCR to display increased basal activity may significantly contribute to its specific physiologic role and, consequently, the loss of basal activity may have pathophysiological relevance.

The TSHR is the best-studied example presenting high basal activity in the G_s protein/adenylyl cyclase/cAMP pathway (Neumann et al., 2010b). However, the physiologic relevance of its constitutive activity is poorly studied. It very likely has biologic relevance on thyroid development and function because the congenital and complete inactivation of TSHR often leads to thyroid hypoplasia (Biebermann et al., 1997; Grasberger and Refetoff, 2017), whereas the central congenital hypothyroidism, because of the lack of the TSH β -subunit, presents with normally developed thyroid (Medeiros-Neto et al., 1997). One may speculate that the basal activity of TSHR is sufficient to promote embryonal thyroid development and growth and the very low thyroid hormone levels still detectable in patients with central hypothyroidism. There are a few other examples of the pathophysiological relevance of loss of basal receptor activity. Mutant MC4R and GHSR with reduced basal activity have been suggested as causes of inherited obesity (Srinivasan et al., 2004) and familial short stature syndrome (Pantel et al., 2006), respectively.

B. Partially Inactivating Mutations—Alteration of Distinct Receptor Functions

Studies of the past three decades have disproved the concept of “one agonist—one receptor—one signal transduction pathway” at each of the three levels. Many GPCRs have more than one natural (even endogenous) agonist. For example, early studies showed that structurally related endogenous ligands (adrenaline, nor-adrenaline, dopamine) can activate the β_1 adrenergic receptor (Weitl and Seifert, 2008). Diverse crossactivity between chemokines and chemokine receptors has been identified (de Munnik et al., 2015). Even chemically unrelated metabolites can be endogenous agonists at one GPCR (e.g., both UDP and prostaglandin E_2 glyceryl ester activate P2RY6), and ligand-specific positions in the binding pocket of P2RY6 were identified

(Brüser et al., 2017). It is therefore reasonable to assume that mutations can selectively affect binding of only one agonist, leading to partial LoF. Currently, there is no disease-relevant example.

Homo- and hetero-oligomerization of GPCRs, splice variants, and accessory proteins that modify specificity further contribute to the functional complexity of GPCRs. Coupling of a given GPCR to multiple G proteins after agonist activation is rather common (Gudermann et al., 1997). Although the C terminus and other determinants of the G protein α -subunit have been identified to contribute to specificity of the G protein/receptor interaction (Okashah et al., 2019), receptor sequence selectivity determinants remain elusive. To predict the functional relevance of variants found in GPCRs, it is of importance to determine common residues and/or structures within GPCRs, which provide G-protein contact sites and mediate G-protein specificity. Indeed, there is some convergence in the intramolecular activation pathways mediated by a conserved structural rearrangement of residue contacts that release G protein–contacting residues (Venkatakrishnan et al., 2016; Kayikci et al., 2018).

Furthermore, GPCR signaling can also recruit arrestin-dependent pathways (Reiter et al., 2012) and the ELMO/DOCK/Rac axis (Park et al., 2007; Weng et al., 2019) (Fig. 2). This complexity of GPCR functionalities can be described by different conformational states of the receptor protein. Biased agonists selectively stabilize only a subset of receptor conformations, thus preferentially activating certain signaling pathways. Theoretically, all the distinct properties contributing to the full functionality of a given GPCR can be selectively affected by mutations.

Investigation of GPCR mutations altering distinct receptor function and associating those alterations with human (sub)phenotypes has only recently begun, but there are already some examples demonstrating the pathophysiological relevance of such GPCR subfunctions (Fig. 2). For example, the TSHR is capable of stimulating members of all four G-protein families (Laugwitz et al., 1996). Mutagenesis studies have identified position Y⁶⁰¹ as a molecular switch for G-protein selectivity (Biebermann et al., 1998). Consistently, the TSHR mutation Y⁶⁰¹N found in a toxic adenoma (Table 1) caused constitutive activation of the G_s protein/cAMP pathway but was unable to couple to G_{q/11} proteins (Arseven et al., 2000). In transfected cells, the Ca²⁺-sensing receptor (CASR) preferentially increases inositol 1,4,5-trisphosphate and inhibits cAMP production. Several disease-causing CASR mutations have been shown to switch this preferential signaling by stabilizing receptor conformations that couple differentially to intracellular signaling pathways [e.g., arrestin-biased signaling (Gorvin et al., 2018)]. Furthermore, an inactivating NDI-causing mutation (R¹³⁷H) in the AVPR2 affecting the conserved DRY motif at the C terminus of

transmembrane helix 3 does not disturb G-protein coupling (Schöneberg et al., 1998) but desensitizes AVPR2 in the absence of agonist because of constitutive recruitment of arrestin, which is termed “constitutive desensitization” (Barak et al., 2001).

Mutational alteration of receptor dimerization and oligomerization is a rather rare cause of inherited GPCR diseases (Fig. 2). It has been shown that three retinitis pigmentosa–causing missense mutations in RHO behave as monomers, whereas wild-type RHO functionally reconstitutes into liposomes as dimers or multimers (Ploier et al., 2016). On the other hand, oligomerization of GPCRs can also be the cause of dominant-negative effects due to retention of the wild-type receptor by interacting with mutationally truncated receptors (Wise, 2012). First evidence of a dominant-negative effect came from splice variants truncating the gonadotropin-releasing hormone receptor (GNRHR) in the seven-transmembrane helices domain (7TMD) (Grosse et al., 1997). Therefore, it is reasonable to assume that truncated or even missense mutations may retain or influence the wild-type GPCR leading to dominant diseases, even if the mutation is inactivating. Such a condition has also been observed in metabotropic GRM1, which naturally functions as dimer, in which mutations can have a dominant-negative effect leading to early-onset cerebellar ataxia (Watson et al., 2017). A similar case of dominant variants in normally autosomal recessive diseases was described for a bleeding disorder caused by an inactivating mutation of P2RY12 (Mundell et al., 2018).

There are several GPCRs, such as the aGPCRs, that exhibit large N termini with multiple domains. These large N termini are required to integrate a multitude of extracellular signals, such as protein-ligand binding, cell-cell contacts, and even mechanical forces, into uniform intracellular signals via a tethered agonist (Monk et al., 2015; Schöneberg et al., 2015, 2016). Mutations, therefore, can alter distinct properties, such as prevention of the interaction of an N-terminal domain of GPR56 with collagen III, causing bilateral frontoparietal polymicrogyria (Luo et al., 2012). Adhesion GPCRs are not only capable of *cis*-signaling (e.g., G-protein activation) but also function as a signal for other receptors (*trans*-signaling) (Liebscher et al., 2013). It has been shown in *Caenorhabditis elegans* and mouse that phenotypes caused by complete aGPCR gene knockouts can be partially rescued only by a membrane-anchored N terminus (Prömel et al., 2012; Patra et al., 2013; Duman et al., 2019), indicating 7TMD-independent functions of the N terminus. Indeed, transcript analyses determined that the domain architecture of the N terminus of aGPCRs often differs, and N termini without or with an incomplete 7TMD anchor as well as separate 7TMD are frequently derived from a single aGPCR gene (Knierim et al., 2019). Therefore, it is reasonable to assume that the pathophysiological consequence of mutations in aGPCR genes and,

therefore, the disease phenotype will significantly differ depending on what function of an aGPCR is altered.

C. The Special Case—Pseudogenization of GPCRs

Pseudogenes are genomic elements with gene-like structures that do not have the function of the gene from which they originated. There are two major pseudogene classes: processed pseudogenes, which are derived from RNA intermediates, and unprocessed pseudogenes, which result from gene duplications during replication. Unitary pseudogenes belong to a special class of unprocessed pseudogenes that are formed without duplication when a single original gene is inactivated through mutation such that no functional copy of the gene remains (Cheetham et al., 2020). An inactivating mutation (missense, frameshifting, deletion, insertion, splice site, premature nonsense mutation) of a gene can be seen as the first step in a process leading to pseudogenization of one of the copies. Over time, this inactive variant will accumulate further alterations (loss of constraint) beyond recognition. Depending on the methods used to identify pseudogenes, 8000 to 14,700 potential pseudogenes are still detectable in the human genome (Pei et al., 2012; Cheetham et al., 2020). Among them, more than 3500 are unprocessed pseudogenes, which also contain many GPCR-derived sequences (e.g., 5-HT-7, Y6R, GNRHR2). About 25% of all unprocessed pseudogenes are still transcribed. As alleles of active genes, pseudogenes can also increase in frequency in a population until fixation because of drift, genetic bottlenecks, hitchhiking by neighbored positively selected genes, or positive selection of the inactivated gene itself.

Pseudogenes are usually not in the focus of inherited phenotypes or diseases. However, the repertoire of pseudogenes differs between individuals because of incomplete fixation of gene inactivation in some individuals and populations. Classic examples for such cases are the chemokine receptors Duffy antigen and CCR5. Lack of Duffy antigen expression or inactivating mutations in Duffy confer some degree of resistance against a malaria form (*Plasmodium vivax*) (Tournamille et al., 1995; Tamasauskas et al., 2001). The most frequent Duffy variant FY*0 is approximately 58,000 years old (McManus et al., 2017). Similarly, a protective role against infection with HIV and AIDS progression was found because of pseudogenization of CCR5 by the deletion of 32 amino acids (CCR5-Δ32) (Dean et al., 1996; Liu et al., 1996; Samson et al., 1996). The CCR5-Δ32 allele arose in frequency approximately 5000 years ago, and it is still under debate whether its emergence is consistent with a historic selective event (Stephens et al., 1998; Novembre et al., 2005; Hedrick and Verrelli, 2006; Bouwman et al., 2017). However, identification of persons who are HIV-1-infected and homozygous for CCR5-Δ32 supported evidence that the lack of functional CCR5 at the cell surface does not confer absolute protection against HIV-1 infection (Smoleń-Dzirba et al., 2017).

GPR33 is an orphan chemoattractant GPCR that was previously identified as a pseudogene in humans (Marchese et al., 1998). The analysis of human individuals revealed that the intact allele of GPR33 is still present in the human population (Römpler et al., 2005). Estimates of the age of the hominin alleles suggest inactivation within the past 1 million years. Large exome sequencing studies [collected and curated in Genome Aggregation Database (gnomAD), (Karczewski et al., 2020)] verified a higher frequency of the intact allele in Asian populations (3.5%–6%), even with homozygote individuals, and a significantly lower frequency in African (1.5%) and European (0.2%) populations. There are 14 additional inactivating variants in GPR33 with a frameshift variant (rs58865778) at higher frequency in the African population (2%) that is almost absent in Asian and European populations. Therefore, GPR33 belongs to a class of rare pseudogenes that have disabling mutations in the reference genome but are intact in some individuals (Cheetham et al., 2020). GPR33 seems to be involved in innate immunity, as supported by its expression in immune-relevant tissues and functional studies (Römpler et al., 2005; Bohnkamp et al., 2010; Morri et al., 2018). The coincidental inactivation and its fixation in several species of distantly related mammalian orders (Römpler et al., 2005) suggest a selective immune response-related pressure on this chemoattractant receptor gene.

Free fatty acid receptor 3 (FFAR3 or GPR41) and GPR42, previously assigned as a pseudogene, are two very closely related GPCRs, whereas FFAR3 is a receptor for short fatty acids (Brown et al., 2003). Mutagenesis studies have previously shown that amino acid position 174 is important for functional signaling since the conversion of R¹⁷⁴ (found in FFAR3) to W¹⁷⁴ (found in GPR42) silences the response to short-chain fatty acids, raising the possibility that GPR42 might be an inactive pseudogene. However, in GPR42 a Trp was found at position 174 only in 26% and 39% of the populations investigated (Liaw and Connolly, 2009; Puhl et al., 2015). Revisiting allele frequency in gnomAD (Karczewski et al., 2020), we found that the R¹⁷⁴ variant is dominant in European populations (89%) but less frequent in African (68%) and East Asian (33%) populations. Therefore, the R¹⁷⁴ variant of GPR42 should be reconsidered as a functional GPCR and the W¹⁷⁴ variant as a unitary pseudogene.

These examples demand thoughtful re-examinations of genes previously annotated as pseudogenes. In any case, pseudogenization of formerly intact GPCR genes is a tradeoff between disadvantageous and even advantageous phenotypes/functionalities related to these GPCR genes under given environmental situations.

V. Activating Mutations in GPCRs—GoF

A. Agonist-Independent GoF

The concept that mutations can activate GPCRs and cause diseases has been introduced by site-directed

mutagenesis work at the $\alpha 1$ adrenergic receptor in the early 90s (Allen et al., 1991). Soon thereafter, inherited diseases, such as hyperthyroidism (Parma et al., 1993), male precocious puberty (Shenker et al., 1993), and retinitis pigmentosa (Rao et al., 1994), caused by constitutive activation of GPCRs were described. Roughly 21% of all monogenic diseases related to mutations in GPCR genes are caused by activating mutations (Table 1). However, only 1% of the individual mutations in GPCRs listed in Table 1 are responsible for these inherited and acquired genetic diseases. Most are missense mutations (~95%), and only a few are deletions or rearrangements. Currently, GoF is explained by the release of the receptor from inactive state conformational constraints or the generation of new interactions that stabilize the active state (Parnot et al., 2002) (Fig. 3). As a consequence, receptor phosphorylation, internalization, and desensitization may also be activated by the changed receptor conformations. However, there is only a partial overlap of latter consequences and G-protein activation. Most activating mutations are found in the transmembrane helices of GPCRs directly triggering conformational changes of the 7TMD (Tao, 2008) (Fig. 3). Overall, there are no significant sites enriched for mutations within the 7TMD of GPCRs causing constitutive receptor activation. However, in glycoprotein hormone receptors, activating mutations are quite homogeneously distributed among the transmembrane helices with two exceptions: 1) no activating mutations have been found yet in transmembrane helix 4, and 2) most activating mutations accumulate over the entire transmembrane helix 6 (Kreuchwig et al., 2013). Most activating mutations are missense mutations, but small in-frame deletions in the third intracellular loop of TSHR can also cause ligand-independent receptor activation (Wonerow et al., 1998). Interestingly, several disease-causing mutations were identified in the extracellular loops and N termini of the TSHR (Grüters et al., 1998; Kleinau et al., 2007). It is assumed that such mutations mimic agonist binding at extracellular loops and expose or isomerize integral agonists as in case of glycoprotein hormone receptors (Brüser et al., 2016) (Fig. 3).

Following the concept of an equilibrium between active and inactive conformations, the total amount of a given receptor matters in respect to the resulting agonist-independent basal activity (see above). Consequently, mutations that increase receptor cell surface expression, promote receptor transport and recycling, or decrease receptor degradation would increase basal G-protein signaling. There are reports showing increased cell surface expression and, therefore, basal receptor signaling of naturally occurring GPCR variants [e.g., for GPR133 (Fischer et al., 2016)]. However, the disease- or phenotype-causing relevance of such mechanisms is poorly studied and probably needs more attention in evaluating genotype-phenotype associations.

B. Agonist-Dependent GoF

There are rare cases in which GoF is caused by different molecular mechanisms than agonist-independent receptor activation (Fig. 3). A special case of GoF was found in patients with hyperthyroidism in which mutations in the hormone-binding domain enabled the TSHR to bind the structurally related glycoprotein hormone human choriongonadotropin (hCG). It was shown that the mutant TSHR was thereafter sensitive to hCG concentrations during gestation (Rodien et al., 1998; Coulon et al., 2016). A mutation in the N terminus of the CASR with a significant left-shifted concentration-response curve of the agonist was found in an autosomal dominant hypocalcemia (Vargas-Poussou et al., 2002). Paradoxical GoF due to coexpression of the wild-type PROKR2 and an inactivating mutation in PROKR2 was described for a patient with precocious puberty (Fukami et al., 2017) and also shown in vitro (Sposini et al., 2015).

Phosphorylation of GPCRs upon activation followed by arrestin recruitment is one mechanism to shut off receptor signaling. There are several cases in which dominant retinitis pigmentosa is caused by mutations of the RHO C terminus (Apfelstedt-Sylla et al., 1993; Restagno et al., 1993). It was shown that these mutations led to defects in receptor phosphorylation and arrestin recruitment and result in excessive signaling (Song et al., 2009) (Fig. 3). Similarly, several mutations in the MC4R lead to an agonist-induced biased G_s -protein and β -arrestin signaling. Increased agonist-induced β -arrestin recruitment correlates with lower body mass index and incidence of associated diseases (Lotta et al., 2019).

However, a note of caution should be added here. Increased basal activity may not always result from mutations but might occur from present endogenous agonists. GPCR activated by metabolites (Tan et al., 2017) and orphan GPCRs in which the endogenous agonist is not yet known can appear with constitutive activity. A role for such endogenous agonists needs to be ruled out especially in native cell systems. For example, ATP/ADP P2Y receptors and their mutants can appear constitutively active because of released nucleotides. Thus, functional assays require proper controls, such as by applying apyrase, which degrades ATP to AMP (Schmidt et al., 2013).

VI. Other Causes of GPCR Dysfunctions

A. Gene Dosage as Cause of GPCR-Related Pathologies

In a diploid organism, the two alleles define the gene dosage required to maintain the physiologic function of the gene products. Alterations of the gene dosage can occur in both directions—reduction and increase (Fig. 4). Gene copy number variation can occur by recombination-based and replication-based mechanisms. In most cases,

the LoF of one allele does not cause a pathologic phenotype because the wild-type allele can compensate. However, there are cases in which an LoF of one allele results in a partial or even full disease phenotype called haploinsufficiency. Haploinsufficiency explains the dominance of a deleterious allele in a diploid organism. For example, mutations in *EDNRB* causing Waardenburg syndrome type II (Table 1) have been found in the heterozygous state (Issa et al., 2017). Haploinsufficiency has also been claimed for mutations in *TSHR*, causing hypothyroidism (Moia et al., 2013), microdeletion of *PROKR2* in a patient with congenital hypopituitarism and growth hormone deficiency (Parsons et al., 2017), deletion of *FZD4* in patients with exudative vitreoretinopathy (Li et al., 2006), and microdeletions of *MC4R* in patients who are obese (Cody et al., 1999). It has recently been shown that haploinsufficiency of the *MC4R* locus can be rescued, at least in mice, by increasing the gene dosage (Matharu et al., 2019).

One specific form of allelic reduction of the gene dosage is related to X-chromosomal diseases. Usually, only males are affected by inactivating mutations in X-chromosomal genes because of hemizyosity. In females, the X-chromosome inactivation usually occurs randomly, generating a 50:50 inactivation of one X chromosome. However, skewed X-chromosome inactivation occurs when the inactivation of one X chromosome is favored over the other, leading to an uneven number of cells with each chromosome inactivated. When the X chromosome carries the altered allele, even females can develop the disease. Indeed, there are cases in which female individuals show full symptoms of NDI caused by mutations in *AVPR2* (Nomura et al., 1997; Sangkuhl et al., 2004; Bösel et al., 2012). There is also a report of a skewed X-chromosome inactivation causing blue cone monochromacy (Table 1) by a heterozygous, long wavelength- (red) sensitive opsin (*OPN1LW*) LoF mutation (Frederiksen et al., 2013).

Genomic imprinting is the monoallelic and parent of origin-dependent expression of a subset of genes. Mutations in such imprinted genes can cause human diseases even in the heterozygous state, depending on the parental origin. For example, the Angelman syndrome is a disease caused by a functional loss of the maternally expressed gene ubiquitin protein ligase *E3A*. Approximately 100 genes have been identified as imprintable in humans (<http://www.geneimprint.com/>), but there are only two GPCRs among them: *GPR1* (paternal) and calcitonin receptor (maternal). For *HTR2A* (maternal), there are conflicting data about the relevance of imprinting at this locus. None of these genes have been linked to inherited diseases in humans.

Sexual dimorphism describes different characteristics between the two sexes of one species beyond the differences in their sexual organs. Imprinting and sex-biased transcriptomic expression account for sexual dimorphism. In adult humans, 24 GPCRs are significantly

sex-biased expressed (adjusted p -value < 0.05) (Shi et al., 2019), which include the GPCR disease-related kisspeptin receptor (*KISS1R*), *GHSR*, tachykinin receptor 3 (*TACR3*), and *ADGRG1*. It is therefore very likely that phenotypes caused by mutation of sex-biased expressed GPCRs may differ. The glycoprotein hormone receptor [LH/hCG receptor (*LHCGR*)] is, per se, sex-biased because of its expression in ovary Theca cells and testis Leydig cells regulating specific gonadal function. Interestingly, activating mutations in *LHCGR* are only dominant in males, causing male-limited precocious puberty, but have no phenotype in female carriers. Temperature sensitivity of some *LHCGR* mutants (Jaquette and Segaloff, 1997), low *LHCGR* expression in prepubertal girls, and the requirement for both LH and follicle-stimulating hormone to induce puberty have been offered as explanations (Themmen and Huhtaniemi, 2000). Interestingly, unlike women with the activating *LHCGR* mutation $D^{578}G$ who are normal, female mice carrying the corresponding activating mutation $D^{582}G$ are infertile because of irregular estrous cyclicity, anovulation, and precocious puberty, indicating species differences between mouse and human (Hai et al., 2015).

Almost all GPCRs have been found included in microscopic chromosomal deletion or duplication, with many of them causing syndromic diseases (see <https://www.ncbi.nlm.nih.gov/clinvar/>; <https://decipher.sanger.ac.uk/>) (Firth et al., 2009) (Fig. 4). GPCRs included in such gross chromosomal alterations may contribute to the clinical phenotype; however, their individual impact is hard to dissect among other gene deletions or duplications. Submicroscopic structural variations (1 kb to 5 Mb), including duplications, inversions, and more complex rearrangements, are widespread in normal human genomes and can increase gene dosage with no apparent effects. For example, low-copy repeat, duplication, and inversion at the vasoactive intestinal peptide receptor 2 gene are common in a healthy population (Berl et al., 2013). However, such copy number variations may impact the susceptibility to some conditions like schizophrenia (Jin et al., 2016) and autism (McLysaght et al., 2014; Firouzabadi et al., 2017), similar to how vasoactive intestinal peptide receptor 2 or copy number differences of glutamate receptors contribute to attention-deficit/hyperactivity disorder (Elia et al., 2011). There is currently only one example reproducibly showing that a locus duplication containing a GPCR leads to an inherited disease: the increase of *GPR101* copy number (locus duplication) causes X-linked acroigantism and acromegaly (Trivellin et al., 2014, 2018; Beckers et al., 2015; Rodd et al., 2016; Hou and Tao, 2019).

B. Messenger RNA Processing Events as Cause of GPCR-Related Diseases

High-throughput sequencing data of transcriptomes and genomes have revolutionized our understanding of

alternative splicing and promoter usage. By comparing transcript variants and genomes, many mRNA processing events can be determined, such as the transcriptional start, splicing, and polyadenylation. Alternative splicing includes exon skipping, intron retention, and removal of cryptic introns. Higher complexity is further achieved by alternative promoter usage and tissue-specific splicing. GPCR genes also undergo the spectrum of RNA processing events. More than half of all nonolfactory GPCR genes contain more than one protein-coding exon (Markovic and Challiss, 2009), including some with over 50 exons (Knierim et al., 2019), giving rise to multiple GPCR variants derived from one gene.

Although there is no significant difference in the nonsynonymous mutation rates (60.2 ± 18.1 versus 66.6 ± 23.5 nonsynonymous SNP/100 codons) between GPCRs without and with introns in their coding region, pathogenic splicing alterations caused by point mutations at splice sites are increasingly recognized as an essential mechanism through which gene mutations cause human diseases (Fig. 4). Thoughtful transcriptome profiling can expose such disease-causing variants in addition to simple gene variants in the coding region (exome profiling). In addition to mutations of the splice acceptor or donor sites, mutations in auxiliary *cis*-regulatory elements as well as intronic and exonic variants can affect proper splicing and generate mis-spliced mRNA to a relevant extent (Xiong et al., 2015). Therefore, pathogenic splicing mutations may escape identification or correct interpretation by sequencing because they may not be distinguishable from neutral polymorphisms. Mutations can also affect the delicate balance of transcript variants produced by alternatively spliced exons and consequently cause diseases. Indeed, in studies of neurofibromatosis type 1 and ataxia telangiectasia in which analysis was performed both on DNA and RNA, approximately 50% of the patients were found to have the disease due to mutations that resulted in aberrant splicing. Of these mutations, 11%–13% would have been misclassified only as frameshift, missense, or nonsense mutations if the analysis had been limited to genomic sequence alone, and their effect on splicing would have been overlooked (Baralle and Baralle, 2005).

In 18 GPCRs, altered splicing has been found to cause the respective GPCR-related disease (Table 1). Most mutations change the nucleotide sequences of the donor or acceptor splice sites flanking coding exons [e.g., in GPR143 (Mayeur et al., 2006)] (Table 1). As a consequence of such loss of splice sites, introns are not eliminated, or exons are skipped, leading to frameshifts, insertions, or loss of coding sequence (Baralle and Baralle, 2005). There are rare cases in which a gain of a splice site in an exon or intron is reported for GPCR-related diseases. For example, in RHO, the mutation c.620T>G generates a strong splice acceptor site, resulting in a 90-bp in-frame deletion and protein mislocalization in vitro and

in vivo (Riedmayr et al., 2020). In X-linked NDI, a novel c.276A>G mutation creates a novel splice acceptor site, leading to 5' truncation of AVPR2 exon 2 as shown by minigene analysis (Scherthaner-Reiter et al., 2016).

C. Altered and Ectopic Expression of GPCRs

1. Promoter Mutations. In addition to mutations in the GPCR coding regions, alterations of expression-regulating regions may cause GPCR-related pathologies (Fig. 4). One of the first examples was identified in patients with blue cone monochromacy in which the deletion of the upstream locus control region of the X-chromosomal red opsin (OPN1LW) and green opsin (OPN1MW) led to a loss of expression of both opsins (Wang et al., 1992; Smallwood et al., 2002). Variants in the promoter region of human GPCRs influencing their expression levels and, therefore, with possible pathophysiological relevance were described [e.g., for the human N-formyl peptide receptor 2 gene promoter (Simiele et al., 2012)]. Also, resistance to the malaria-causing parasite *Plasmodium vivax* can be mediated by inactivating mutations in a chemokine receptor (Duffy antigen) (Parasol et al., 1998) but also by mutations in the Duffy promoter leading to reduced receptor expression levels (Tournamille et al., 1995). Several mutations in the promoters of the dopamine receptors DRD4 and DRD5 have been associated with attention-deficit/hyperactivity disorder (Kustanovich et al., 2004). Experimentally confirmed mutations in the promoter region of GPCRs demonstrating the causality of inherited diseases are rather rare. It has been shown that decreased expression of the growth hormone–releasing hormone receptor gene due to a mutation in a Pit-1 transcription factor binding site was causative for growth hormone deficiency (Salvatori et al., 2002) (Table 1). An interesting case of a 15-bp deletion in the regulatory region of GPR56 (ADGRG1) led to local disruption of GPR56 expression, resulting in restricted polymicrogyria limited to the cortex surrounding the Sylvian fissure (Bae et al., 2014). Menstrual cycle-dependent febrile episodes seem to be caused by a serotonin receptor HTR1A promoter mutation altering receptor expression levels (Jiang et al., 2012).

2. Untranslated Region and Intronic Mutations. Regulatory elements that define mRNA expression levels and mRNA stability can be found in the promoter region but also within the so-called 5'- and 3'-untranslated regions (UTRs) of the mRNA and in intronic regions. Such *cis*-acting regulatory RNA elements are binding sites of long noncoding RNAs, G-quadruplex structures, short open reading frames (ORFs), and internal ribosome entry sites (IRESs) (Leppek et al., 2018). Mutations in such regulatory parts are not immediately obvious but may affect gene function. In contrast to the prokaryotic translation machinery, mammalian ribosomes generally do not translate the downstream cistron of a bicistronic mRNA. There are two exceptions to this general

rule. The first is when the first ORF is short (fewer than ~30 codons, “minicistron” or upstream ORF), and the translation machinery resumes scanning in a 5′ → 3′ direction and reinitiates translation at a downstream AUG codon. The second exception is an IRES allowing a cap-independent initiation of translation. In GPCR genes, an upstream ORF has been identified, such as in the $\beta 2$ adrenergic receptor and the corticotropin-releasing hormone receptor 1 encoding for small peptides, which function as inhibitors of receptor translation (Parola and Kobilka, 1994; Xu et al., 2001). Cap-independent translation via an IRES element has been experimentally verified for angiotensin II receptor type 1 (Martin et al., 2003). Recently, a data base has been launched (<http://reprod.njmu.edu.cn/cgi-bin/iresbase/index.php>) listing potential IRES in 27 human GPCR mRNAs (Zhao et al., 2020). Therefore, mutations in the 5′-UTR need to be considered; however, to our best knowledge there is no experimentally verified example for an inherited GPCR-related disease known yet. However, some GPCRs (CXCR5, GPR34, and several olfactory receptors) contain SNPs in their IRES sequence, and it needs to be determined whether they influence receptors’ ORF translation.

The region downstream of an ORF plays an important role in regulating mRNA 3′ end formation, nuclear export, subcellular localization, translation, and degradation (Mayr, 2017). The mean length of the 3′-UTR of a mature human mRNA is, with about 1200 nucleotides, six times greater than the average 5′-UTR (Mayr, 2016). Consequently, 3′-UTRs are rich in regulatory elements, which can be identified with web-based tools (Grillo et al., 2010; Dassi et al., 2014) (<http://aura.science.unitn.it>; <http://utrdb.ba.itb.cnr.it>). There are estimates that approximately 0.2% of all known disease-associated mutations are located in 3′ regulatory region (Chen et al., 2006). Although there are a number of SNPs in 3′-UTRs of GPCRs associated with diseases [e.g., schizophrenia and a 3′-UTR SNP in GPR85 (Matsumoto et al., 2008)], experimental proof of disease causalities or phenotype contributions is still missing.

3. Gene Fusion Events and Ectopic Expression. Gene fusions via chromosomal rearrangement initiated by DNA double-strand breakage can cause ectopic expression and/or altered gene expression. In the advent of high-throughput sequencing methods, gene fusion can be easily detected by genomic DNA and mRNA sequencing. Numerous gene fusions involving GPCR genes have been detected by such methods, most without direct clinical correlate (<https://ccsm.uth.edu/FusionGDB/>) (Kim and Zhou, 2019) but some in association with cancer (<https://fusionhub.persistent.co.in/>) (Panigrahi et al., 2018) (Fig. 4). For example, the receptor tyrosine kinase–fused gene was found to be fused genomically and at the mRNA level with ADGRG7 (GPR128) in healthy humans (Chase et al., 2010). Translocation of immunoglobulin heavy chain locus targeting the GPR34

gene at Xp11.4 [t(X;14)(p11.4;q32.33)] has been frequently associated with cancer. As a consequence of this fusion, upregulation of GPR34 mRNA and aberrant expression of GPR34 was found in mucosa-associated lymphoid tissue lymphoma (Ansell et al., 2012; Baens et al., 2012; Akasaka et al., 2017). Here, the G_i protein–coupled GPR34 most probably drives cell proliferation by activating the mitogen-activated protein kinase pathway because of immunoglobulin heavy chain locus-driven high expression and the receptors’ high basal activity (Schöneberg et al., 2018). Also, regulatory elements of GPCR genes can promote oncogenesis. High frequency of fusions involving a region 5′ of the orphan GPCR P2RY8 and the coding region of type I cytokine receptor cytokine receptor-like factor 2 (CRLF2) is found in B-progenitor acute lymphoblastic leukemia (Mullighan et al., 2009; Hertzberg et al., 2010; Nikolaev et al., 2014).

The ectopic expression of GPCRs has been frequently implicated in oncogenesis and metastasis (Marín and Chen, 2004; Qu et al., 2004; Boire et al., 2005; Tang et al., 2013; Zhao et al., 2019). A first link between cell transformation and GPCRs was described in 1986 with the identification of the MAS oncogene (Young et al., 1986). Pioneering work of the Gutkind group introduced the concept that chronic activation of GPCRs can lead to transformation of cells (Gutkind et al., 1991). Later, this concept has been verified by the identification of somatic activating mutations in several GPCRs causing adenomas and carcinoma (Table 1). Overexpression of GPCRs and significant relations between GPCR expression and survival rates have been found in numerous tumors (Sriram et al., 2019), making GPCRs potential targets in cancer therapy (Insel et al., 2018; Wu et al., 2019).

Besides genomic rearrangements fusing the coding exons of GPCRs to other promoters (see above) or regulation-relevant mutations in promoter regions (Chevalier et al., 2014), altered regulation of GPCR expression is probably the cause for its ectopic expression. For example, the aGPCR ADGRD1 (GPR133) is usually not (highly) expressed in glial cells. In the hypoxic regions of glioblastoma, ADGRD1 expression is significantly increased because of local hypoxia, drives tumor growth, and is a prognostic marker for malignancy (Bayin et al., 2016). In this case, the involvement of the hypoxia-inducible factor 1 α has been shown.

A special form of ectopic expression of GPCRs is caused by the introduction of active receptors encoded by viral genomes (Arvanitakis et al., 1997; Montaner et al., 2013). Several human herpesviruses carry GPCR genes in their genomes, showing the highest homology to human chemokine receptors. It has been shown that viral GPCRs hijack receptor-mediated signal transduction pathways of the host for survival, replication, and pathogenesis. Constitutive activity of many viral GPCRs significantly contribute to these processes

(Montaner et al., 2013; Vischer et al., 2014). For example, the constitutive, agonist-independent activity of the viral GPCR ORF74 encoded by the Kaposi sarcoma-associated herpesvirus significantly contributes to virus-driven malignancies (Arvanitakis et al., 1997). The constitutively active GPCR encoded by Epstein-Barr virus downregulates cell surface major histocompatibility complex class I expression as part of the immune evasion strategy of this virus (Zuo et al., 2009; Fares et al., 2019).

D. GPCR-Related Diseases Caused by Mutations in More than One Gene

In quantitative genetics, epistasis encompasses any nonadditive interaction among genes. In projection on inherited diseases, epistasis describes the interaction of two or more genetic loci, which can substantially modify disease severity (“genetic modifier”) or result in entirely new phenotypes. Whole-exome and genome sequencing have provided enormous amounts of information on human genetic variation, offering the chance to identify digenic and oligogenic inherited diseases. Digenic inheritance (DI) considers pathologies with the simplest form of multigenic etiology, implicating more than one gene. As suggested by Deltas (2018), several forms have to be distinguished. First, an important element of true DI is that the patient will only manifest the disease when two mutations on separate genes are coinherited. Both mutations are necessary and sufficient to cause the defined clinical symptoms. Second, DI is also encountered when pathogenic mutations responsible for two distinct diseases are coinherited, leading to a mixed phenotype. Here, the patient presents with a combination of symptoms found in the two individual monogenic diseases. Third, a pseudo-DI scenario is present when the clinical symptoms caused by a single mutation that is causal for the inherited disease are modified by a second variant in a different gene (“genetic modifier”).

In many genetic mouse models, loss of GPCR function in combination with other gene defects contributes to obvious pathologies. Even the combination of two different GPCR deficiencies can expose phenotypes. For example, although the loss of GPR116 or ADGRL4 alone in mice had no obvious effect on cardiovascular or kidney function, mice lacking both showed malformations of the aortic

arch arteries and the cardiac outflow tract (Lu et al., 2017). Similarly, triacylglycerol-induced increase in plasma GLP-1 was only significantly reduced in free fatty acid receptor FFAR1/FFAR4 double-deficient mice but not in the individual FFAR1- and FFAR4-deficient mice (Ekberg et al., 2016).

In contrast, examples for true DIs involving at least one GPCR gene are rare in humans (Table 3). Heterozygous mutations of FZD4 responsible for familial exudative vitreoretinopathy (Table 1) were found together with heterozygous mutations in other genes also known to cause inherited exudative vitreoretinopathy (Li et al., 2018). Similarly, a heterozygous LoF mutation in PROKR2 (Table 1) together with a mutation in WD repeat domain 11 protein, which alters its capacity to bind to its functional partner the homeobox protein empty spiracles homeobox 1, was found in a patient with pituitary stalk interruption syndrome (Kallmann-like phenotype) (McCormack et al., 2017). Also, patients heterozygous for a PROKR2 mutation were heterozygous for mutations in the KAL1 gene, suggesting possible DI (Dodé et al., 2006; Sarfati et al., 2013). Screening a DI data base (Gazzo et al., 2016), one can find heterozygous LoF mutations of ADGRV1 (GPR98) combined with inactivating mutations in MYO7A, MYO6, or PDZD7, all genes known to cause Usher syndrome subtypes.

There are numerous reports of GPCR variants being genetic modifiers of human diseases. Although such variants may not directly cause diseases, they appear to be risk factors for and modifiers of diseases and the response to environmental factors, stress, and drugs. For specific search of such GPCR variants and modified phenotypes, we recommend the PhenoModifier data base (<https://www.biosino.org/PhenoModifier/index>) (Sun et al., 2020), which contains several dozen entries related to GPCR variants. Besides GPCR variants, which may synergistically promote disease-relevant pathways, mutations in GPCRs may also counteract dysfunction of other genes and positively modify the severity of diseases. Indeed, P2RY2 polymorphisms may have an impact on Ca²⁺ influx in airway epithelia of patients with cystic fibrosis (Büscher et al., 2006). Similarly, a naturally occurring variant of the β2-adrenergic receptor (Gly¹⁶-Glu²⁷) is more frequent in patients with cystic fibrosis.

TABLE 3
Digenic inheritance of GPCR-caused diseases

GPCRs involved in currently known digenic inherited human diseases are listed together with the comutated gene. Data were taken from the digenic diseases data base DIDA (<http://dida.ibsquare.be>) and the indicated literature.

| GPCR Gene | Disease | DI Genes |
|----------------|---|----------------------------|
| ADGRV1 (GPR98) | Usher syndrome | MYO7A, DFNB37, PDZD7 |
| EDNRB | Hirschsprung disease | RET |
| FZD4 | Exudative vitreoretinopathy | LRP5, F5 |
| GNRHR | Hypogonadotropic hypogonadism | PROKR2, FGFR1 |
| KISS1R | Pituitary stalk interruption syndrome, Kallmann syndrome | PROKR2, FGFR1, IL17RD |
| OPN1LW | Blue cone monochromacy (Nathans et al., 1993; Katagiri et al., 2018) | OPN1MW |
| PROKR2 | Pituitary stalk interruption syndrome, Kallmann syndrome | FGFR1, GNRHR, KAL1, KISSR1 |
| TACR3 | Hypogonadotropic hypogonadism | NSMF |
| TSHR | Mild congenital hypothyroidism (Sato et al., 2015; Fu et al., 2016; Abe et al., 2018) | DUOX2, TG |

Here, enhanced receptor function and, therefore, an up-regulated cystic fibrosis transmembrane conductance regulator activity may be responsible for improved cystic fibrosis phenotype (Steagall et al., 2007).

Currently, there are only four examples of DI diseases in humans known wherein different GPCR genes carry LoF mutations (Table 3). For example, the combination of two heterozygous LoF mutations in *KISS1R* and *PROKR2*, respectively, seems to be responsible for a case of hypogonadotropic hypogonadism (Gazzo et al., 2016). However, it is likely a matter of time that additional true DI diseases caused by two different LoF mutation-harboring GPCR genes are identified because of the statistical chance and reflecting the many LoF variants, which have already been found by exome and genome sequencing (see Supplemental Tables 1 and 2).

It should also be noted that the DI involving inactivation of GPCR genes can have advantages. As mentioned above, impaired expression of chemokine receptor Duffy (pseudogenization) has some advantages against the malarial parasite *Plasmodium vivax*. A combination of the *P. falciparum*-resistant hemoglobin variant HbS, and the Duffy negativity is likely to be selected in areas in which *P. falciparum* and *P. vivax* infections are endemic and mixed infections frequently occur (Gelpi and King, 1976).

E. GPCR-Directed Autoantibodies as Cause of Diseases

Autoantibodies are immunoglobulins that bind to endogenous epitopes of the host with their paratopes. Several autoimmune diseases have been associated with the presence of autoantibodies, but there are also healthy individuals with autoantibodies who never develop an autoimmune disease (Plotz, 2003; Cabral-Marques and Riemekasten, 2017; Cabral-Marques et al., 2018). It is not surprising that in the process of developing autoantibodies against host structures, GPCRs may also be included in the endogenous epitope-directed immune response. However, in rare cases, such autoantibodies contribute to specific pathologies related to a functional consequence of antibody binding to a GPCR. In principle, antibodies can block or activate the function of a GPCR mainly in an allosteric mode of action (van der Westhuizen et al., 2015). Autoantibodies directed against the TSHR are well studied. Here, the antibodies are directed against the N terminus and can activate (Graves disease, hyperthyroidism) or block (Hashimoto's thyroiditis, hypothyroidism) the TSHR or be functionally neutral (Rees Smith et al., 1988). Recent experimental data suggest an activation mechanism in which upon extracellular autoantibody binding an intramolecular agonist induces structural changes in the 7TMD, triggering G-protein activation (Brüser et al., 2016). These results add glycoprotein hormone receptors to a group of GPCRs containing rhodopsin, protease-activated receptors, and aGPCRs, which have their agonists already covalently

bound or as an integral part of the receptor protein (Schöneberg et al., 2016). In the glycoprotein hormone receptors, protease-activated receptors, and adhesion GPCRs this tethered agonist is formed by a short peptide sequence directly N-terminal of the transmembrane helix 1 and interacts with the extracellular loops and the adjacent parts of the transmembrane helices domain.

Blocking autoantibodies against the CASR has been reported to cause acquired hypocalciuric hypercalcemia (Kifor et al., 2003). Disease-related autoantibodies that function as allosteric modulators and keep CASR in an active conformation with its agonist have been found in a patient with acquired hypocalciuric hypercalcemia (Makita et al., 2007). Furthermore, the melanin-concentrating hormone receptor 1 has been identified as a target of blocking autoantibodies, causing vitiligo (Kemp et al., 2002; Gottumukkala et al., 2003; Gavalas et al., 2009). Pathogenic autoantibodies against $\beta 1$ adrenergic receptors have been first identified in patients with Chagas' heart disease and associated with heart failure [reviewed in Boivin-Jahns and Jahns (2018)].

VII. Therapeutic Options and Approaches

There are at least two principle options to treat many of the pathologies caused by malfunctions in GPCRs. First, the treatment aims to cope with the symptoms of the GPCR-linked disease by pharmacological and/or surgical intervention. Second, approaches aim to directly target the mutant GPCR and rescue its function (Fig. 5).

A. Symptomatic Therapies of GPCR Pathologies

Many GPCR malfunctions cause endocrine diseases with end-organ resistance or autonomy, among them, for example, hypothyroidism or hyperthyroidism (TSHR) and glucocorticoid deficiency or ACTH-independent Cushing syndrome (MC2R) (Table 1). In case of endocrine end-organ resistance, such as hypothyroidism and glucocorticoid deficiency, simple hormone substitution of thyroxine and cortisol, respectively, can be the treatment of choice. End-organ autonomy, such as hyperthyroidism and ACTH-independent Cushing syndrome may require surgical removal of the hyperfunctional gland(s) followed by proper hormone substitution.

Many congenital GPCR malfunctions result in developmental and syndromic defects (Table 1), which can only be partially targeted by surgery or indirectly by drugs to reduce pathologic phenotypes. For example, the X-linked NDI (inactive AVPR2, Table 1) characterized by the production of large amounts of hypotonic urine, is commonly treated by water substitution, salt restriction, indomethacin, and, paradoxically, thiazide diuretics (Bockenhauer and Bichet, 2015). However, many GPCR-related diseases are so rare that the low number of patients (Table 1) is insufficient to provide an

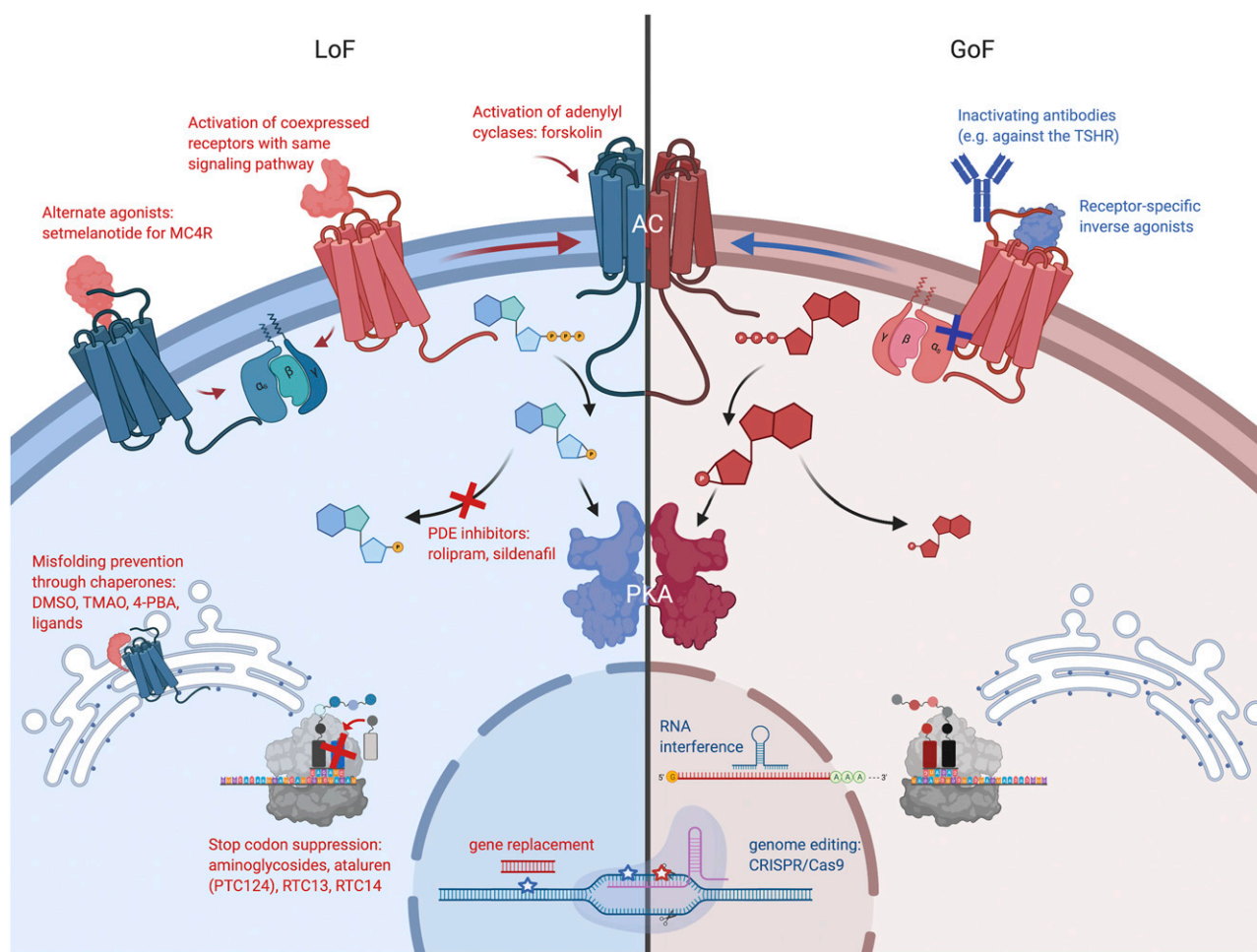


Fig. 5. Potential therapeutic strategies to influence altered GPCR functions. There are direct and indirect approaches to influence altered GPCR functions. Direct approaches target the affected receptor (ligands, pharmacoperones) or receptor gene or transcript (gene replacement, gene editing, premature stop-codon suppression) (for details see text). Indirect methods usually target the function of signaling pathway components downstream of the receptor (e.g., G proteins, adenylyl cyclases, PDE). It is also feasible to activate a different GPCR expressed in cells in which the disease-causing GPCR occurs, which should have a similar (in case of LoF) or an opposite (in case of GoF) signal transduction (for details, see text). AC, adenylyl cyclase; Cas9, CRISPR-associated protein 9; PKA, protein kinase A; TMAO, trimethylamine N-oxide; 4-PBA, 4-phenylbutyric acid.

evidence-based standard therapy. Although most GPCR-related diseases are rare, there are general strategies to restore cellular GPCR function. Most attempts are directed to activate the signal transduction pathway of the mutationally altered receptor. For example, the AVPR2 couples to the G_s protein/adenylyl cyclase pathway and increases intracellular cAMP levels, which promote insertion of aquaporin 2 into the apical membrane of collecting duct cells. This allows for water reabsorption from the urine and, therefore, urine concentration. Cyclic AMP and cGMP levels can also be increased by inhibition of phosphodiesterases (PDEs), which degrade cAMP and cGMP to AMP and GMP, respectively (Fig. 5). PDE inhibitors, such as rolipram and sildenafil, were found to be efficient in reducing the symptoms of NDI in mouse models of NDI (Coffey et al., 1991; Sohara et al., 2006) and in patients with congenital NDI (Bouley et al., 2005; Assadi and Sharbaf, 2015).

Single-cell RNA sequencing data revealed that almost every cell type investigated expresses dozens of

different GPCRs, with some sharing similar signaling properties (Kaur et al., 2017; Tischner et al., 2017). It is therefore reasonable to stimulate endogenously coexpressed GPCRs to restore signaling of the mutant receptor (Fig. 5). For example, receptors for prostaglandin E2 are expressed in renal tubule cells, and specific activation of prostaglandin E2 receptors could increase the urine concentrating ability in an NDI rat model (Olesen et al., 2011). In case of activating mutations in G_s protein-coupled receptors (e.g., TSHR, LHCGR), one can also consider targeting a coexpressed GPCR, which couples to the functionally opposite $G_{i/o}$ protein–signaling pathway.

Another example of indirectly restoring impaired signaling is the MC1R (Table 1), in which LoF mutations cause a pheomelanotic pigment phenotype in humans and, therefore, increase the UV-induced skin damage and the melanoma risk. The MC1R couples to the G_s protein/adenylyl cyclase pathway and increased intracellular cAMP levels promote eumelanin production.

Topical application of forskolin, a skin-permeable pharmacologic activator of adenylyl cyclases, can mimic MC1R signaling by increasing cAMP and epidermal eumelanin levels enhancing UV resistance in a Mc1r signaling defect mouse model (Bautista et al., 2020) (Fig. 5).

B. Direct Targeting of Malfunctional GPCRs

Defining the causality of GPCR dysfunction is a prerequisite to specifically rescue the receptor malfunction to therapeutically address the GPCR-related disease. All the above-mentioned approaches will help to prioritize and guide the still-required functional characterization of GPCR mutations in the different assays. That in-depth functional characterization of GPCR mutations is indeed of clinical relevance and has been shown in numerous successful attempts in rescuing functionally altered GPCRs by pharmacological chaperones (Conn et al., 2007; Beerepoot et al., 2017; Hou et al., 2018; Newton and Anderson, 2018), functional complementation (Schöneberg et al., 1997; Rivero-Müller et al., 2010), stop-codon suppression (Sanguhl et al., 2004), agonists, inverse agonists, and ligands binding at non-orthosteric sites (Gershengorn and Neumann, 2012) (Fig. 5). These different attempts are not applicable for all kinds of mutant GPCRs but are reserved for distinct structural or functional receptor defects. Pharmacological chaperones are mostly used for missense-mutated receptors, which show trafficking alterations due to improper protein folding, glycosylation, or post-translational modifications. Receptor truncations due to stop or frameshifting mutations can be rescued by fragment replacement (complementation) approaches. However, at least the first two to three transmembrane helices should remain in the mutant receptor for efficient rescue by the missing fragment (Ridge et al., 1995; Schöneberg et al., 1996). Some premature stop codons can be over-read by suppressing ribosomal fidelity leading to full-length receptor proteins. Ligand-based approaches require full-length receptors still able to bind drugs at their orthosteric or non-orthosteric binding sides. However, direct gene replacement or even the restoration of the normal base pair sequences by genome editing approaches (e.g., CRISPR/CRISPR-associated protein 9 method) are the most straightforward in treating inherited GPCR pathologies. However, the tissue-specific targeting of mutated GPCR genes has not been achieved in patients and awaits first successful application examples. Therefore, we briefly review current approaches for selected examples.

1. Gene Replacement. Gene replacement strategies in GPCR-deficient animal models are feasible and show promising results. For example, introduction of a 15–16-kb genomic rhodopsin DNA fragment, including endogenous promoters, all introns, and flanking regulatory sequences, into a rhodopsin knockout mouse using DNA nanoparticles resulted in high levels of physiologic transgene expression over a period of 5 months (Zheng et al., 2020). An interesting approach to cope with the

severe retinal degeneration due to dominant mutations in RHO includes both gene suppression and replacement gene therapy. Here, the mRNA encoding the mutant RHO, which would accumulate and degenerate the retina, is suppressed by mutant RHO-directed short hairpin or micro RNAs. The RHO replacement is achieved by a “codon-modified” rhodopsin transgene that is resistant to degradation by the interference RNA construct (O'Reilly et al., 2007; Greenwald et al., 2013; Cideciyan et al., 2018).

2. Chemical Chaperones and Pharmacoperones. Folding of GPCR polypeptides is a complex process often requiring chaperones. The failing of this assisted folding due to receptor mutations results in protein aggregation or degradation instead of formation of functional proteins (Tao and Conn, 2014). The cell surface expression level of most GPCRs is critical for their physiologic functions, and chemical chaperones and pharmacoperones can promote surface expression of the mutant receptor. Basically, there are two forms of compound-based chaperones that in principle can be applied in vivo: 1) chemical chaperones and 2) ligand-based chaperones (pharmacoperones). Chemical chaperones are low-molecular weight compounds that can support protein folding. Examples are glycerol, DMSO, trimethylamine-*N*-oxide (TMAO), and 4-phenylbutyric acid (Tao and Conn, 2018). Pharmacoperones are compounds that usually enter the cells, bind as ligand to a given GPCR and serve as a molecular scaffolding to support proper folding of the otherwise-misfolded mutant receptor protein, and route it correctly to the plasma membrane. Such ligands can function as agonists or antagonists and are identified in large-scale screening assays expressing misfolded GPCRs (Smith et al., 2016). Several misfolded GPCRs have been rescued by chemical and ligand-based folding support such as the GNRHR (Janovick et al., 2009), FZL4 (Generoso et al., 2015), and AVPR2 (Makita et al., 2016; Mouillac and Mendre, 2018). Most examples are GPCRs carrying a missense mutation. However, there are also some C-terminally truncated GPCRs, which can be rescued by pharmacoperones (Jean-Alphonse et al., 2009). For more details and mechanisms, we refer to excellent reviews (Tao and Conn, 2014, 2018).

3. Receptor Fragment Replacement. More than 20% of all inactivating mutations in GPCRs lead to receptor protein truncation because of premature stop codons or frameshift mutations (see above). Because GPCRs are composed of multiple folding units (Ridge et al., 1995; Schöneberg et al., 1995), it was demonstrated that mutant AVPR2 containing clinically relevant mutations in the carboxy-terminal third of the receptor protein can be functionally rescued by coexpression of a nonmutated carboxy-terminal AVPR2 fragment in vitro (Schöneberg et al., 1996). Generation of mice harboring a nonsense mutation (E242stop) in the AVPR2 gene resulted in an NDI phenotype (Yun et al., 2000).

However, mating these NDI mice with transgenic mice expressing the missing C-terminal AVPR2 fragment did not rescue the phenotype (unpublished data). In vivo complementation of truncated GPCRs with the missing folding unit seems to be too inefficient as therapeutic approach.

4. Suppression of Stop Codons. Translational readthrough of premature stop codons induced by pharmaceutical compounds is a promising way of restoring functional protein expression and reducing disease symptoms. Readthrough therapy is based on the discovery that small compounds, such as aminoglycosides, ataluren (PTC124), RTC13, and RTC14, modify the translation machinery to suppress a nonsense codon, elongate the nascent polypeptide, and consequently result in the synthesis of full-length protein (Nagel-Wolfrum et al., 2016) (Fig. 5). The efficiency of the readthrough depends on the nonsense codon sequence and the stop codon-surrounding sequence (context preference) (Dabrowski et al., 2015). This may explain the obviously low effect on the physiologic translation termination. Furthermore, there are usually more than one stop codon in the 3'-UTR and close proximity of the natural termination codon, increasing the chance of translation termination even in the presence of aminoglycosides. A number of clinically approved drugs, including the aminoglycosides gentamycin and tobramycin, have been identified to increase readthrough of premature stop codons (Mutyam et al., 2016). For example, the efficiency of gentamycin has not only been shown in cell culture and animal models but also in numerous clinical trials addressing disease-causing stop codons in patients with muscle dystrophies Duchenne and cystic fibrosis [for review see Dabrowski et al. (2018)]. However, aminoglycosides may exert strong ototoxic and nephrotoxic effects, which can be partially avoided when patients with the A1555G mutation in 12S ribosomal RNA gene of mitochondrial DNA, which is known to predispose to gentamicin-induced ototoxicity, are excluded (Malik et al., 2010). PTC124 (ataluren) has been selected as the most potent readthrough-promoting drug from over 800,000 compounds screened (Welch et al., 2007). Ataluren is an orally bioavailable compound that has been successfully tested in a cystic fibrosis mouse model (Du et al., 2008) but was inefficient in patients with nonsense-mutation cystic fibrosis (Kerem et al., 2014). The translational readthrough approaches have also been used to restore the function of stop codon-caused GPCR-related diseases. Treatment with aminoglycosides turned out to be more efficient than the complementation approach in rescuing AVPR2 nonsense mutations in vitro and in vivo (Schulz et al., 2002; Sangkuhl et al., 2004). Functional restoration of nonsense-truncated rhodopsin and MC4R was also demonstrated in rodent models of retinitis pigmentosa (Guerin et al., 2008) and obesity (Bolze et al., 2013), respectively.

5. Ligand-Based Functional Rescue. In case a missense mutation still allows for (partial) trafficking of the mutant GPCR to the plasma membrane, ligand-based approaches may be feasible. Specifically, activating mutations can be targeted by inverse agonists to reduce the constitutive activity. For example, small-molecule inverse agonists have been discovered for TSHR that may have therapeutic potential as orally active drugs to inhibit constitutive signaling of mutant TSHR in patients with thyroid cancer and in some patients with hyperthyroidism (Neumann et al., 2010a) (Fig. 5). In case of activating autoantibodies against the TSHR, peptides derived from the internal agonist or small compound are suitable to suppress autonomous receptor activity (Brüser et al., 2016; Marcinkowski et al., 2019). Besides small compounds, monoclonal antibodies that probably modulate the isomerization of the internal agonist of this receptor could also be suitable to suppress constitutive activity of the TSHR (Chen et al., 2018a). An inverse agonist approach has also been used to rescue the phenotype of mice expressing a constitutively active parathyroid hormone receptor (Noda et al., 2020). Viral genome-encoded GPCR can constitutively activate signaling cascades (see above). The inverse agonists GSK682753A and VUF2274 inhibit the constitutive activity of the Epstein-Barr virus-induced receptor 2 and the human cytomegalovirus GPCR US28, respectively, and may be of therapeutic use (Casarosa et al., 2003; Benned-Jensen et al., 2011). Moreover, VUF2274 inhibits US28-mediated HIV entry into cells (Casarosa et al., 2003).

In GPCRs, wherein inactivating mutations interfere with proper agonist binding, specifically selected agonists that still bind to the mutant receptor can be used for activation. For example, the small peptide and highly-selective MC4R agonist setmelanotide (formerly known as RM-493, BIM-22493) decreased food intake and reduced body weight in obese humans with deficiency in the proopiomelanocortin gene (Kühnen et al., 2016) or the leptin receptor gene (Clément et al., 2018). Later it was tested whether setmelanotide can still activate mutant MC4R, which causes obesity. It was shown that this high potent peptide drug can partially rescue the function of some of these receptor mutants in obese humans (Collet et al., 2017) (Fig. 5). The study also shows that a successful treatment requires an allele-specific in vitro testing because the rescue efficiency significantly differs between mutant MC4R.

Mutation-induced LoF of GPR126 (ADGRG6) causes myelination defects in zebrafish and mouse (Monk et al., 2009; Mogha et al., 2013). Small compound library screens using mutant zebrafish (hypomorph) identified drugs that were able to directly activate the function of GPR126 (Bradley et al., 2019; Diamantopoulou et al., 2019). These examples show that identification of small compounds targeting mutant receptors may be a suitable approach

toward individualized therapeutic strategies in GPCR pathologies.

VIII. Identification of Disease-Relevant GPCR Using Population Genetic Data

In the last 15 years, the number of GPCRs known to cause inherited human diseases almost doubled [(Schöneberg et al., 2004) vs. Table 1]. This increase was anticipated based on phenotype data of GPCR gene-deficient mouse models. Approximately 52% of GPCR-defective mouse strains showed a defined phenotype or embryonic or perinatal lethality, and about 41% had an obvious phenotype after challenge (e.g., by drugs or pathogens) (Schöneberg et al., 2004). Identification of genetic determinants contributing to disease susceptibility is currently performed by linking genetic markers (e.g., SNPs) or expression data with phenotypic traits in huge GWAS or expression quantitative trait loci (eQTL) studies, respectively. This review does not comprehensively focus on such variant-phenotype associations because there are countless GWAS and eQTL studies also extracting signals from GPCR loci [as reviewed in (Tang and Insel, 2005; Insel et al., 2007; Thompson et al., 2014; Kovacs and Schöneberg, 2016; Luo et al., 2019)]. Only some selected GPCR variant/disease associations are listed in Table 4 and may encourage further clinical and experimental work to

causally link those variants to human phenotypes. However, GWAS and eQTL studies only statistically link the determined genotype/expression-phenotype and usually lack causality. One critical future task will be the efficient filtering of candidate disease-causing variants in protein-coding genes by providing additional information and methodical input. Thus far, the prediction of LoF-intolerant genes based on extensive collections of exome data has already identified 3230 genes with near-complete depletion of predicted protein-truncating variants (Lek et al., 2016). Thirty GPCRs are in this list, with 22 of these genes having no currently established human disease phenotype and eight genes with known human phenotypes (Table 1, marked with *). Interestingly, 11 genes in this list encode for aGPCRs (ADGRA3, ADGRB1–3, ADGRC1–3, ADGRG2, ADGRL1–3) and six glutamate receptor genes (GRM1–5, GRM7) with an obvious underrepresentation of rhodopsin-like GPCRs. These GPCR genes are candidates of human pathologic phenotypes as suggested by gene-deficient mouse models [e.g., severe malformations in ADGRC-deficient mouse strains (Tissir et al., 2010; Shi et al., 2014)]. An exome-wide study of 10,503 Pakistani adult participants identified 1317 genes carrying homozygous LoF mutations (Saleheen et al., 2017). Besides numerous odorant and taste receptors, 21 nonodorant GPCRs were found homozygously inactivated (Supplemental Table 2

TABLE 4
Human phenotypes and diseases associated with GPCR dysfunction

A selection of GPCR genes in which variants show a significant association with human phenotypes is given. Some GPCR genes were also identified in studies screening human genomes for LoF variants: *genes intolerant for LoF (Lek et al., 2016).

| GPCR Gene | Human Phenotype | Reference |
|-----------------|--|--|
| ADGRB2* | Progressive spastic paraparesis | Purcell et al., 2017 |
| ADGRC2 | Idiopathic scoliosis | Einarsdottir et al., 2017 |
| ADGRG6 | Adolescent idiopathic scoliosis | Kou et al., 2013 |
| ADGRL2 | Microcephaly with severely reduced sulcation and rhombencephalosynapsis | Vezain et al., 2018 |
| ADORA1 | Early-onset parkinsonism and cognitive dysfunction | Jaberi et al., 2016 |
| ADRB2 | Nocturnal asthma | Turki et al., 1995; Contopoulos-Ioannidis et al., 2005 |
| ADRA2A | Atypical familial partial lipodystrophy | Garg et al., 2016 |
| ADRA2B | Familial adult myoclonic epilepsy/autosomal dominant cortical myoclonus and epilepsy | Guerrini et al., 2001; De Fusco et al., 2014 |
| CCKAR | Cholesterol gallstone disease and obesity | Miller et al., 1995 |
| CHRH2 | Bipolar disorder | Cruceanu et al., 2018 |
| CX3CR1 | Impaired human monocyte survival, developmental dysplasia of the hip | Feldman et al., 2013; Collar et al., 2018 |
| GABBR2 | Early infantile epileptic encephalopathy | EuroEPINOMICS-RES Consortium; Epilepsy Phenome/Genome Project; Epi4K Consortium, 2014; Hamdan et al., 2017 |
| GPR35 | Albright hereditary osteodystrophy/pseudohypoparathyroidism, and mild-to-moderate mental retardation | Shrimpton et al., 2004; Shim et al., 2014 |
| GPR180 | Congenital microcoria | Fares-Taie et al., 2015 |
| GPR161 | Spina bifida, pituitary stalk interruption syndrome, childhood medulloblastoma | Karaca et al., 2015; Kim et al., 2019; Begemann et al., 2020 |
| HTR2B | Severe impulsivity | Bevilacqua et al., 2010 |
| HTR7 | Autism spectrum disorder | Helsmoortel et al., 2016 |
| LGR4 (GPR48) | Low bone mineral density and osteoporotic fractures, aniridia-genitourinary anomalies-mental retardation syndrome (AGR syndrome) | Styrkarsdottir et al., 2013; Yi et al., 2014 |
| RXFP2 (LGR8) | Cryptorchidism | Gorlov et al., 2002 |
| TAS2R38 | Chronic rhinosinusitis | Lee et al., 2012 |

ADRB2, β_2 adrenergic receptor.

marked with #; Table 1). In a new analysis of the gnomAD data set, 3270 genes with LoF variants were identified, and after further filtering data, they defined a set of 1815 genes that are likely to be tolerant to biallelic inactivations (Karczewski et al., 2020), among them 32 GPCRs carrying homozygous inactivating stop or frameshifting mutations (Supplemental Table 2 marked with +).

Additional bioinformatical approaches may help to improve reliable predictions of gene variants, which can be linked to human phenotypes. Less than 3% of protein-coding genetic variants are predicted to result in obvious LoF through the introduction of a stop codon or frameshifting variants (Emdin et al., 2018). Such obvious LoF variants in a hemizygous or homozygous state are more clearly suitable for genotype-phenotype association studies (Supplemental Tables 1 and 2). For example, the variant Arg⁹⁵Ter (rs114285050) truncating GPR151 protects against obesity and type 2 diabetes (Emdin et al., 2018) (Supplemental Table 2). Inactivating stop and frameshifting mutations in GPR142 (Supplemental Table 2) have a high frequency in human populations (21% in the African population), leading to homozygous GPR142-deficient humans. GPR142 is a receptor for aromatic amino acids expressed in the pancreas and controls tryptophan-induced insulin and incretin secretion in mice (Lin et al., 2016). The synthetic GPR142 agonist C-22 strongly improved oral glucose tolerance in both lean and obese mice (Rudenko et al., 2019).

Based on the recent UniProt release (31-Jul-2019), there are 847 protein-coding genes at the X chromosome, among them 23 encoding nonodorant GPCRs (Supplemental Table 1). Currently, only six X-linked diseases caused by mutations in GPCRs are known, and mice deficient for these genes show very similar phenotypes. However, the number of X-linked GPCR diseases will likely increase in the future because mice carrying defects in 14 additional X-chromosomal GPCR genes present with distinct phenotypes (Supplemental Table 1). Indeed, a data base search of most X-chromosomal GPCR genes revealed human male individuals carrying clearly inactivating mutations (premature stop or frameshifting mutations) (Supplemental Table 1). Now, detailed clinical characterization is necessary to link these human GPCR “knock-outs” to defined phenotypes and compare these with data from pharmacological and GPCR-deficient mouse studies.

Recent population genetic approaches found roughly 100 predicted LoF mutations in protein-coding genes per genome and an estimated 0.13–0.29 recessive lethal mutations per haploid set of autosomes (MacArthur et al., 2012; Gao et al., 2015). However, the chance of homozygosity or compound heterozygosity for such LoF variants depends on the variant frequency and is still low (see Supplemental Tables 1 and 2). Usually, high rates of consanguineous marriages, bottlenecks, or specific

environmental niches can promote an increase in the frequency of a given LoF variant. In very rare cases, uniparental disomy can cause homozygosity. Here, two homologous chromosomes are inherited from the same parent. For example, in a 3-year-old patient with obesity, a homozygous premature stop codon in G protein-coupled bile acid receptor 1 was found in which the mother was heterozygous for this mutation but not the father (Yu et al., 2016). It has been shown in humans and mice that G protein-coupled bile acid receptor 1 induces energy expenditure (Watanabe et al., 2006).

Because GPCRs are a major target of therapeutic drugs, receptor variants may contribute to their therapeutic variability. A recent study extracted an average of four common and 128 rare variants for each GPCR from the exome aggregation consortium (Hauser et al., 2018). The exome aggregation consortium data set was later included into the gnomAD data, presenting now a data set of 125,748 exomes and 15,708 whole genomes (<https://gnomad.broadinstitute.org>) (Karczewski et al., 2020). Our own analysis of 144 nonodorant receptors of all GPCR classes (all GPCR genes listed in Supplemental Tables 1 and 2; Table 1) revealed 52,115 variants (quality filtered missense, nonsense, frameshifting, and splice site), giving an average of 362 rare and frequent variants per GPCR. In-depth analysis showed that there is an average of 64 nonsynonymous mutations per 100 codons in GPCR genes found in the currently sequenced populations (Supplemental Table 3). Surely, this number will rise further by sequencing more individuals.

Because natural selection eliminates deleterious variants from populations, methods to detect selection have modeled the reduction in variation (constraint) compared with an expectation (Samocha et al., 2014). The gene variation data base gnomAD has a constraint tool already implemented (Lek et al., 2016), and one can hypothesize that this may serve as predictor for the clinically relevant GPCR alteration. For example, if the observed and expected missense and LoF scores for a given GPCR are significantly lower than 1, one may expect a phenotype that reduces fitness. To test this hypothesis, we used two sets of GPCRs: 1) GPCR with inactivating mutations causing inherited diseases (Table 1, reduced fitness) and 2) GPCR with hemizygous or homozygous inactivating mutations occurring in obviously healthy human individuals (Supplemental Tables 1 and 2). On average, GPCR genes show an expected synonymous mutation rate, and there is no significant difference between GPCR genes related to known monogenic diseases and those genes that have not been already associated with monogenic phenotypes (Supplemental Table 3, $P = 0.19$). However, non-synonymous mutations show significantly lower ratios between the observed and expected mutation rates (missense $P = 0.008$; frameshifting/stop/splice $P = 0.001$) (Supplemental Table 3) in GPCR genes with

known disease relevance, indicating that, based on the constraint, one can predict candidate GPCR genes leading to severe functional defects upon inactivation. Candidates with very low observed/expected LoF mutation rates are BRS3, GPR173, and ADGRC3 (CELSR3) (Supplemental Tables 1 and 2). Indeed, mice deficient for BRS3 or GPR173 develop a metabolic syndrome-like or bone developmental phenotype, respectively (Supplemental Table 1). Constitutive and conditional *Adgrc3*-deficient mice revealed that this aGPCR is critical for the development of several major axonal bundles in the central nervous system (Tissir et al., 2005; Zhou et al., 2008a) and required to guide motor axons in the hindlimb (Chai et al., 2014). These analyses clearly indicate that the list of GPCRs being clinically relevant when mutated is much longer than currently estimated (Tables 1 and 5). Specifically, those GPCRs, which are not in the list of genes tolerant to LoF (Supplemental Table 2) but show low LoF observed/expected ratios, should be considered in the future focus of phenotype/genotype relations. Among them are, for example, the muscarinic acetylcholine receptors type 1 and type 4 (CHRM1, CHRM4), ADGRA1, ADGRC2, ADGRF5, and GPR61.

Regardless of their relevance for inherited phenotypes, analyzing and qualifying SNP data in drug-targeted GPCRs are of great importance with respect to possible changes in target function and undesired side effects (Hauser et al., 2018). Therefore, the frequency of functionally relevant SNPs in populations is relevant to test for and adjust pharmacotherapy if necessary. The μ -opioid receptor 1 (OPRM1) is a classic example of SNPs influencing the pharmacodynamic properties of analgesics. The OPRM1 N⁴⁰D variant (rs1799971) leads to the loss of a glycosylation site in the N terminus (Huang et al., 2012). It was reported that patients carrying the D⁴⁰ variant have lower pain thresholds (Fillingim et al., 2005) and require higher morphine doses to get an analgesic response (Klepstad et al., 2004). The D⁴⁰ allele frequency is higher in Asian populations (36%) than in European (19%) and African (2.4%) populations (rs1799971 in gnomAD data base). However, the relevance of N⁴⁰D for opioid treatment and addiction is still under debate (Walter and Lötsch, 2009; Taqi et al., 2019; Ho et al., 2020). Notably, there are also GoF variants in OPRM1. The very rare K²³⁵N variant displayed increased efficacy

and potency to buprenorphine relative to the wild type (Hauser et al., 2018).

IX. Future Perspectives

Methodical advances in mouse genetics and in the acquisition of population genetic data significantly increased the number of inherited diseases and phenotypes specifically assigned to functionally relevant mutations in GPCRs. Population genetic and evolutionary sources are mined with the help of bioinformatics to identify GPCR genes with high constraints (LoF-intolerant genes) that are likely to cause phenotypes when mutated. At present, such bioinformatic tools consider the entire coding sequence of a given gene and evaluate its constraint. However, these investigations could be restricted to distinct parts of a receptor molecule to identify local constraints and, therefore, increase the predictive value of such analyses. Currently, odorant and taste receptors are not well-characterized with respect to their disease relevance, although many of them are also highly expressed in nonsensory tissues, so-called ecomotopic odorant, and taste receptors (Di Pizio et al., 2019). This will surely change when their physiologic roles are determined in more detail. In the future, their participation in digenic and oligogenic diseases will also contribute to the significance of altered GPCR function in inherited disorders. Furthermore, the wealth of structural information—specifically how ligands bind, how signals are transduced through the receptor molecule, and how GPCRs interact with their intracellular partners (Venkatakrishnan et al., 2016; Hilger et al., 2018; Weis and Kobilka, 2018)—greatly improved our molecular understanding of pathologic mutations. Hopefully, this information can be used to more precisely predict the functional relevance of GPCR variants found in populations in order to explain phenotypes and to adjust pharmacotherapy. Again, cell and animal models carrying variants rather than constitutive or conditional whole-gene knockouts will be more easily accessible because of modern genome editing methods (Anzalone et al., 2019). This will also allow for studying the disease relevance of mutation in the 5'- and 3'-UTRs of GPCR transcripts and in the nontranscribed regulatory regions of GPCR genes. Finally, precise genome editing will rescue most of the disease-causing mutations found in GPCR genes. Until this is feasible with high efficiency in vivo, individualized and allele-specific pharmacological approaches are required to reverse GPCR malfunctions.

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Authorship Contributions

Wrote or contributed to the writing of the manuscript: Schöneberg, Liebscher.

TABLE 5
Main Points

- Extended data base mining to identify LoF-intolerant GPCRs
- Explore pathologic potential of variations in noncoding genic components of GPCR genes (promoter, introns, UTR)
- Explore pathologic potential of neglected GPCRs (e.g., ecomotopic odorant and taste receptors)
- Improve structural and evolutionary approaches to predict the functional relevance of GPCR variations
- Extended use of genome editing methods in research and therapy

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Supplements

Mutations in G protein-coupled receptors: Mechanisms, pathophysiology and potential therapeutic approaches

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Table S1 X-chromosomal GPCRs containing obvious loss-of-function (LoF) mutations

The gnomAD database (v2.1.1 <https://gnomad.broadinstitute.org>) was screened for stop and frameshifting mutations in X-chromosomal GPCR genes occurring in hemizygous (male) or homozygous (female) stage. Such individuals would be considered as deficient for the specific GPCR. The literature and the International Mouse Phenotyping Consortium (IMPC) website (<http://www.mousephenotype.org/>) were screened for the respective GPCR-deficient mouse line and their phenotypes. Light-gray rows contain GPCRs causing inherited human diseases. *LoF variants found in the C terminus, which may not interfere with function.

| Symbol | # of stop/fs variants (# individuals homo/hemizygous) | human phenotype/disease | mouse phenotype | Reference for mouse phenotype |
|---------|---|--|--|--|
| ADGRG2 | 1 (0/1) rs756053665* | congenital bilateral aplasia of the vas deferens | infertility | (Davies et al., 2004) |
| ADGRG4 | 44 (1/76) rs764463001* rs1310086936* rs146635325* | unknown | not determined yet | |
| AGTR2 | 8 (0/77) rs371470323* | unknown | altered drinking response, increased blood pressure | (Hein et al., 1995; Ichiki et al., 1995) |
| AVPR2 | 2 (0/3) rs37286283* Trp336Ter* | nephrogenic diabetes insipidus | nephrogenic diabetes insipidus | (Yun et al., 2000) |
| BRS3 | 1 (0/1) | unknown | metabolic defects and obesity | (Ohki-Hamazaki et al., 1997) |
| CXCR3 | 3 (0/56) | unknown | increased susceptibility to experimental autoimmune encephalomyelitis | (Liu et al., 2006) |
| CYSLTR1 | 6 (0/9) rs1310506059* | unknown | altered immune system | (Maekawa et al., 2002; Oyoshi et al., 2012) |
| GPR34 | 2 (0/2) | unknown | altered immune system | (Liebscher et al., 2011; Preissler et al., 2015) |
| GPR50 | 1 (0/1) rs756979771* | unknown | decreased percent body fat/body weight, increased food intake | (Ivanova et al., 2008) |
| GPR82 | 4 (0/4) rs1252758363* | unknown | reduced food intake and body weight | (Engel et al., 2011) |
| GPR101 | 1 (0/1) | acrogigantism | increased heart weight and serum calcium | IMPC |
| GPR119 | 8 (0/8) rs771380953* | unknown | abnormal skin morphology, decreased body weight and insulin levels | (Lan et al., 2009), IMPC |
| GPR143 | 3 (0/4) | ocular albinism type I, congenital nystagmus | hypopigmentation of the ocular fundus | (Incerti et al., 2000) |
| GPR173 | 0 (0/0) | unknown | decreased body mass, length, and bone density, abnormal coat coloration | IMPC |
| GPR174 | 2 (0/2) | unknown | abnormal T cell physiology, altered sexual dimorphism in B-cell physiology | (Barnes et al., 2015; Zhao et al., 2020) |

| | | | | |
|--------|---|-------------------------------|--|---|
| GRPR | 5 (0/5) rs1464753617* rs1418228152* rs760783114* | unknown | hyperactive, abnormal scratching behavior | (Sun and Chen, 2007; Wada et al., 1997) |
| HTR2C | 5 (0/7) rs781872799* | unknown | decreased food intake, weight, body length, increased insulin levels | (Kawahara et al., 2008) |
| LPAR4 | 3 (0/3) rs768426033* | unknown | abnormal embryonic blood and lymphatic vessel formation | (Sumida et al., 2010) |
| OPN1LW | 0 (0/0) | blue cone monochromacy | gene is missing | |
| OPN1MW | 0 (0/0) | deuteranomaly, cone dystrophy | abnormal vision | (Smallwood et al., 2003) |
| P2RY10 | 2 (0/2) | unknown | unknown | |
| P2RY4 | 6 (0/1055) rs1189582496* rs41310667* | unknown | abnormal digestive secretion | (Robaye et al., 2003) |
| P2RY8 | 0 (0/0) | unknown | gene is missing | |

Table S2 GPCR genes containing homozygous LoF variants in humans

The gnomAD database (v2.1.1 <https://gnomad.broadinstitute.org>) was screened for stop and frameshifting mutations (LoF) in all autosomal GPCR genes occurring in a homozygous stage. In total, 74 GPCR genes were identified where such individuals can be considered as deficient for the specific GPCR. The dbSNP database number is given when available. +genes tolerant for LoF (Karczewski et al., 2020). *LoF variants found in the C terminus, which may not interfere with function. #Some homozygous LoF GPCR gene variants were also identified in a study screening genomes of Pakistani adults for LoF variants (Saleheen et al., 2017).

| GPCR gene | # different homozygous inactivating mutation (# individuals) | dbSNP |
|-----------|--|--|
| ADGRA1 | 3 (12) | rs1049378588, rs532393541, rs145811832 |
| ADGRA2+ | 1 (1) | rs1442366259* |
| ADGRC1 | 2 (6) | rs749296817, rs149062226 |
| ADGRC3+ | 1 (1) | rs753268601* |
| ADGRD2+ | 2 (5) | rs542304419, rs141098727 |
| ADGRE2#+ | 5 (73) | rs548759776, rs534018804, rs375427337, rs143325445, rs1183101283 |
| ADGRF2+ | 3 (8) | rs76859817, rs768564109, rs147106931 |
| ADGRF3+ | 1 (4) | rs139522210 |
| ADGRF4# | 1 (1) | rs574873610 |
| ADGRG7#+ | 1 (1) | rs535088251 |
| ADGRL4#+ | 1 (1) | rs781215346 |
| ADRA1A | 1 (1) | rs778085459 |
| ADRA2B | 1 (1) | rs368204339 |
| ADRB3+ | 1 (1) | rs769733135 |
| BDKRB1+ | 1 (4) | rs145322761 |
| C5AR2# | 1 (1) | rs767175827 |
| CCR5# | 5 (1073) | rs777330502, rs1800560, rs938517991, rs775750898, rs146972949 |
| CRHR2+ | 2 (2) | rs8192492*, rs1414971420 |
| CXCR1+ | 1 (1) | rs532614335 |
| DRD4+ | 6 (73) | rs146562378, rs1327643360, rs1347977789, rs751359558, rs1290906588, rs34662058 |
| DRD5# | 2 (9) | rs570059380, rs145497708 |
| GPR1+ | 3 (4) | rs776449349, rs150274953, rs560796868 |
| GPR15 | 1 (2) | rs771269696 |
| GPR32 | 2 (8) | rs772552393, rs560406571 |
| ϕGPR33 | 2 (55) | rs58865778, rs17097921 |
| GPR35 | 1 (1) | rs758180346 |
| GPR39# | 2 (2) | rs371700670, rs773140509* |
| GPR42 | 1 (1) | rs770802036 |
| GPR84# | 1 (1) | rs777783623 |
| GPR87+ | 1 (1) | p.Val201GlyfsTer51 |
| GPR89B#+ | 2 (5) | rs143821798, rs782044418 |
| GPR135 | 1 (167) | rs143521018* |
| GPR142#+ | 3 (552) | rs59375334, rs569465136, rs777583208 |
| GPR151# | 4 (30) | rs756942272, rs558001563, rs140449635, rs114285050 |
| GPR152 | 1 (1) | rs745593287* |
| GPRC5A+ | 1 (3) | rs527915306 |
| GPRC5D+ | 1 (1) | rs576323196* |
| GPRC6A+ | 4 (5375) | rs550458778, rs371464745, rs144698290, rs6907580 |
| HCAR3 | 1 (1) | rs762492119 |
| HTR1F# | 1 (1) | Lys134Ter |

| | | |
|---------|----------|--|
| HTR2B | 1 (2) | rs79874540 |
| LTB4R2# | 1 (4) | rs374257326 |
| MAS1L | 1 (2) | rs559479676 |
| MLNR | 1 (1) | rs563947699 |
| MRGPRF | 1 (2) | rs756457218 |
| MRGPRG# | 4 (4) | rs749436617, rs1160877464, rs997734102, Trp184Ter |
| MRGPRX1 | 2 (2) | rs200974967, rs140371088* |
| MRGPRX3 | 2 (524) | rs188327405*, rs78408237* |
| MRGPRX4 | 1 (1) | rs528957025 |
| NMUR2+ | 1 (1) | rs145273801 |
| NPSR1+ | 2 (4474) | rs77892941, rs7809642* |
| NTSR2+ | 1 (1) | rs757448862 |
| OPN4+ | 1 (1) | rs571378526 |
| OPRD1 | 1 (1) | rs775451738 |
| OPRM1+ | 2 (89) | rs17174638, rs760566402 |
| OXER1 | 1 (1) | rs764276620 |
| OXGR1 | 1 (62) | rs565524916 |
| PRLHR | 1 (1) | rs765894801 |
| PTGDR | 2 (16) | rs41407349, rs41533946 |
| PTGDR2+ | 1 (1) | rs1485268895 |
| PTGIR | 1 (1) | rs771355379* |
| PTH2R+ | 1 (39) | rs61742329 |
| QRFPR+ | 2 (2) | rs1387943774, rs768925368 |
| RRH+ | 1 (1) | rs771791931 |
| RXFP1#+ | 2 (4) | rs756658609, rs756658609 |
| RXFP3 | 1 (1) | rs763917541 |
| SCTR+ | 1 (1) | rs200497817 |
| TAAR1 | 1 (1) | rs1178830423 |
| TAAR2# | 3 (550) | rs8192646, rs530446092, rs147900465 |
| TAAR8# | 1 (2) | rs200640516 |
| TAAR9 | 4 (31) | rs377459700, rs752622374, rs769937555, rs749840523 |
| UTS2R | 1 (1) | rs750153792 |
| XCR1#+ | 1 (1) | rs772431611* |

Table S3 Mutation rate in GPCR genes

The sequence data of 125,748 exomes and 15,708 whole genomes (gnomAD dataset v2.1.1, March 2020) (<https://gnomad.broadinstitute.org>) were taken to analyze the individual GPCRs listed in Tables 1, S1, S2 (except for ϕ GPR33). SNP: only quality-filtered non-synonymous mutations (missense, stop, frameshifting, splice site) were considered. The observed vs expected rates (o/e scores) were extracted for each GPCR gene using the gnomAD tool *Constraint* (for methodical details of the o/e score see (Lek et al., 2016)) and separated into synonymous, missense and obvious LoF mutations (stop, frameshifting, splice site): 1 is the expected value; ** $p < 0.01$

| | SNP | codons | SNP/100 codons | o/e score synonymous | o/e score missense | o/e score pLoF |
|---|-----------|-----------|-------------------|-------------------------|-----------------------|----------------------|
| All, N=145 mean (median) | 359 (272) | 580 (395) | 64.0 (64.9) | 1.03 (1.02) | 0.92 (0.93) | 0.68 (0.69) |
| unknown phenotype N=90, mean (median) | 329 (277) | 509 (387) | 66.5 (69.4) | 1.02 (1.01) | 0.95 (0.97) | 0.78 (0.79) |
| Disease N=55, mean (median) | 409 (262) | 695 (404) | 59.8 (61.4) | 1.06 (1.03) | 0.87 (0.89)** | 0.54 (0.49)** |

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