1521-0081/73/1/89–119$35.00
PHARMACOLOGICAL REVIEWS
https://doi.org/10.1124/pharmrev.120.000011
Pharmacol Rev 73:89–119, January 2021

ASSOCIATE EDITOR: PAUL INSEL

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

Torsten Schöneberg and Ines Liebscher

Rudolf Schönheimer Institute of Biochemistry, Molecular Biochemistry, Medical Faculty, Leipzig, Germany

Address correspondence to: Dr. Torsten Schöneberg, Rudolf Schönheimer Institute of Biochemistry, Molecular Biochemistry, Medical Faculty, Johannisallee 30, 04103 Leipzig, Germany. E-mail: schoberg@medizin.uni-leipzig.de

Over the last 15 years, our research was mainly supported by the German Research Foundation (DFG) in FOR749 (T.S.), FOR2149 (T.S. and I.L.), CRC1052 project number 209933838 (T.S. and I.L.), CRC1423 project number 421152132 (T.S. and I.L.), the Integrated Research and Treatment Center (IFB) AdiposityDiseases (BMBF) (T.S. and I.L.), and intramural funding of the State of Saxony, Germany (T.S. and I.L.). We acknowledge support from Leipzig University for Open Access publishing.

Financial disclosure statement: no author has an actual or perceived conflict of interest with the contents of this article.

[S]This article has supplemental material available at pharmrev.aspetjournals.org.

https://doi.org/10.1124/pharmrev.120.000011.

***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, adrenocorticotropic hormone; aGPCR, adhesion GPCR; AVPR2, V2 vasopressin receptor; BRS3, bombesin-like receptor type 3; CASR, Ca^{2+}-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 choriogonadotropin; 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/)(Rappaport et al., 2017), 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
ADGRC1*°	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
ADGRG6°	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	Hypocalciuric 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
CYSLTR2	***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 omodysplasia, 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				Fröjmark 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	42	1	4	1	2	de Roux et al., 1997
GPR88°	Childhood-onset chorea with psychomotor retardation		1				Alkufri et al., 2016
GPR101°	***X-linked acrogigantism***	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	Audo 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				1	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					Bonnefond et al., 2012

(*continued*)

TABLE 1—*Continued*

GPCR Gene	Disease/Syndrome	Missense	Nonsense	In/del	Splice	Large	Reference
OGR1 (GPR68)	Amelogenesis 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
PTH1R	Blomstrand chondrodysplasia, Eiken syndrome, primary failure of tooth eruption	6	8	9	9		Jobert et al., 1998; Decker et al., 2008
PTH1R	***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
RHO	***Autosomal dominant retinitis pigmentosa***	9					Rao et al., 1994; Park, 2014
S1PR2	Deafness	2					Santos-Cortez et al., 2016
SMO	***Basal cell carcinoma***	3					Xie et al., 1998; Khamaysi et al., 2016
SMO	Curry-Jones syndrome (Mosaizism)	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	Hypothyroidism	80	10	12	4	4	Sunthornthepvarakul et al., 1995
TSHR	***Congenital hyperthyroidism, hyperfunctioning thyroid adenoma, and carcinoma***	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,

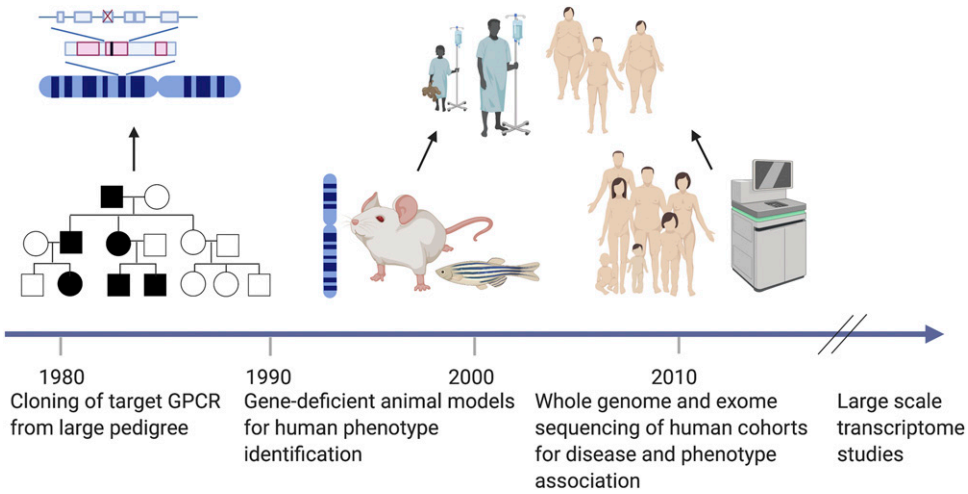

Fig. 1. Approaches to identify mutant GPCR as cause for inherited diseases in the course of time. After cloning and sequencing of the first GPCR genes in the mid-80s/90s, genomic loci information of large disease-affected families was matched with mutations in GPCR genes. In the 1990s, targeted GPCR 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

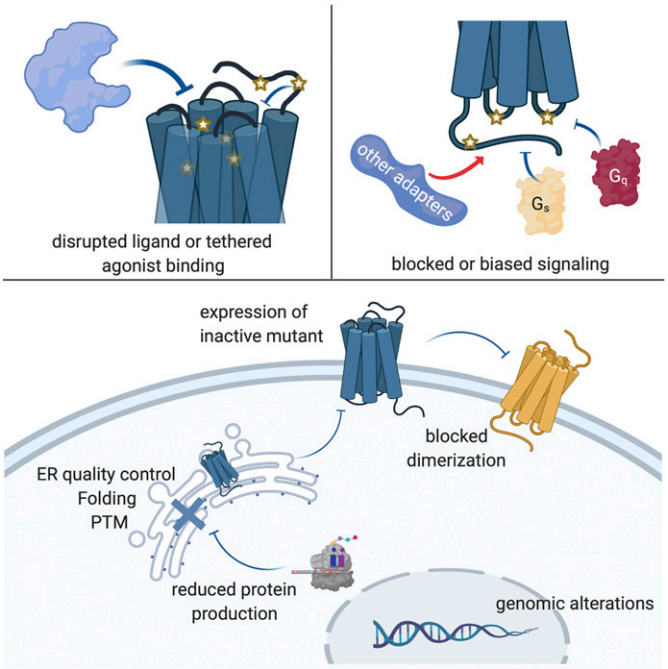

Fig. 2. General mechanisms of disease-relevant LoF mutations in GPCRs. The LoF of GPCRs can be caused by alterations at different levels of the receptor protein biosynthesis (genomic, transcriptomic, and/or translational level), protein folding, processes of peri- and post-transcriptional modifications, 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.

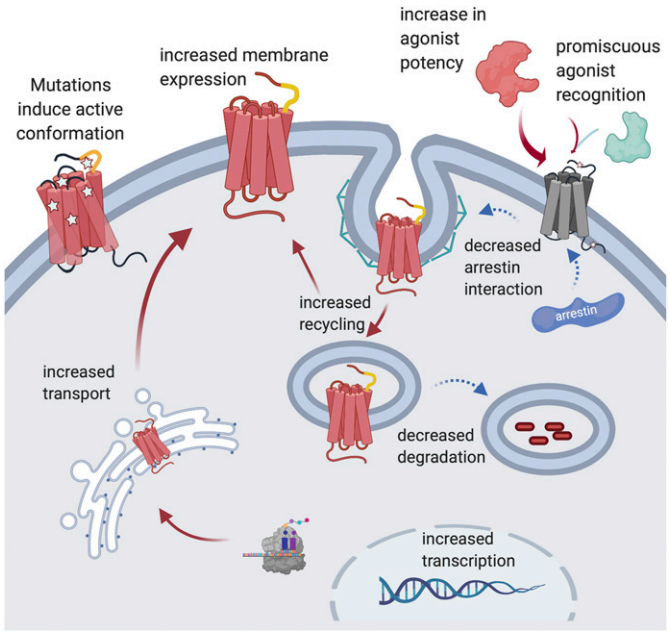

Fig. 3. General mechanisms of disease-relevant GoF mutations in GPCRs. There are two general forms of GPCR alterations that cause a gain of receptor function—agonist-independent and agonist-dependent causes. Mutations in transmembrane helices or, more rarely, in the exctracellular loops promote an active 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.

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 cohorts or large consanguineous families and the occurrence of obviously inactivating mutations, such as frameshift 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).

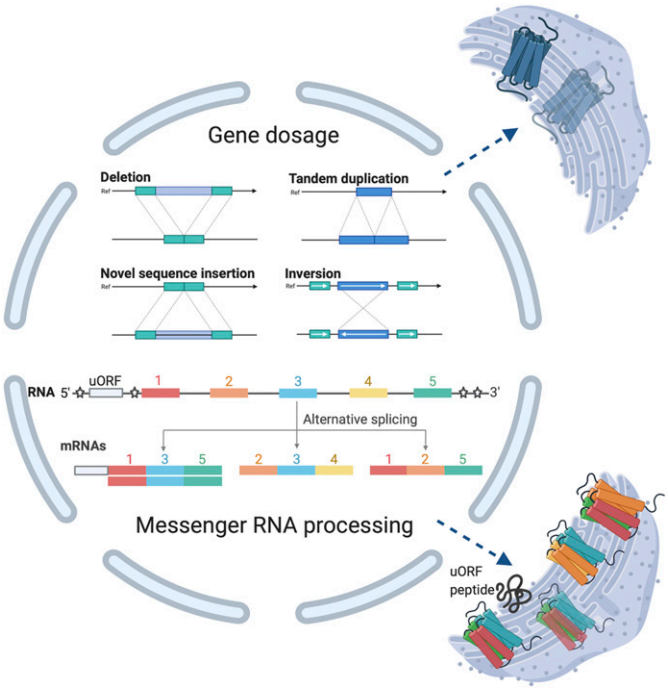

Fig. 4. Other mechanisms of disease-relevant GPCR pathologies. As in the case of many other inherited diseases, more complex genomic alterations can cause a loss but also a gain of GPCR function. Chromosomal mutations can have an impact of gene dosage (deletions, large insertions, rearrangements, duplications, 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) (Biebermann 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 $Arg^{256}Gln$ 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 (Arg^{256}) directly interacts with the α phosphate moiety of the agonist 2-methylthioadenosine-5′-diphosphate-ADP (Zhang et al., 2014). Since Arg^{256} 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	**References**
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	McGee et al., 2006; Yagi et al., 2007
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	Ho et al., 1995
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	Shin et al., 1997
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	Wang et al., 2001; Xu et al., 2004
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	Wu et al., 2010
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	Robbins et al., 1993; Mogil et al., 2005; Wada et al., 2005; D'Orazio et al., 2006
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	Huszar et al., 1997; Sandrock et al., 2009; Lede et al., 2017
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	Matsumoto et al., 2006
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	Naash et al., 1993
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	Beamer et al., 1981; Abe et al., 2003; Bassett et al., 2008

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; Bohnekamp 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; Bohnekamp 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, noradrenaline, dopamine) can activate the β1 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 Y^{601} as a molecular switch for G-protein selectivity (Biebermann et al., 1998). Consistently, the TSHR mutation $Y^{601}N$ 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 Ca^{2+}-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 ($R^{137}H$) 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; Bohnekamp 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 R^{174} (found in FFAR3) to W^{174} (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 R^{174} variant is dominant in European populations (89%) but less frequent in African (68%) and East Asian (33%) populations. Therefore, the R^{174} variant of GPR42 should be reconsidered as a functional GPCR and the W^{174} 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 α1 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 homogenously 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 choriogonadotropin (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 hemizygosity. 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; Böselt 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 (Beri 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 acrogigantism 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 (Schernthaner-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 β2 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 1α 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 Ca^{2+} influx in airway epithelia of patients with cystic fibrosis (Büscher et al., 2006). Similarly, a naturally occurring variant of the β2-adrenergic receptor (Gly^{16}-Glu^{27}) 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 (Satoh et al., 2015; Fu et al., 2016; Abe et al., 2018)	DUOX2, TG

Here, enhanced receptor function and, therefore, an upregulated 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 β1 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 thyroxin 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

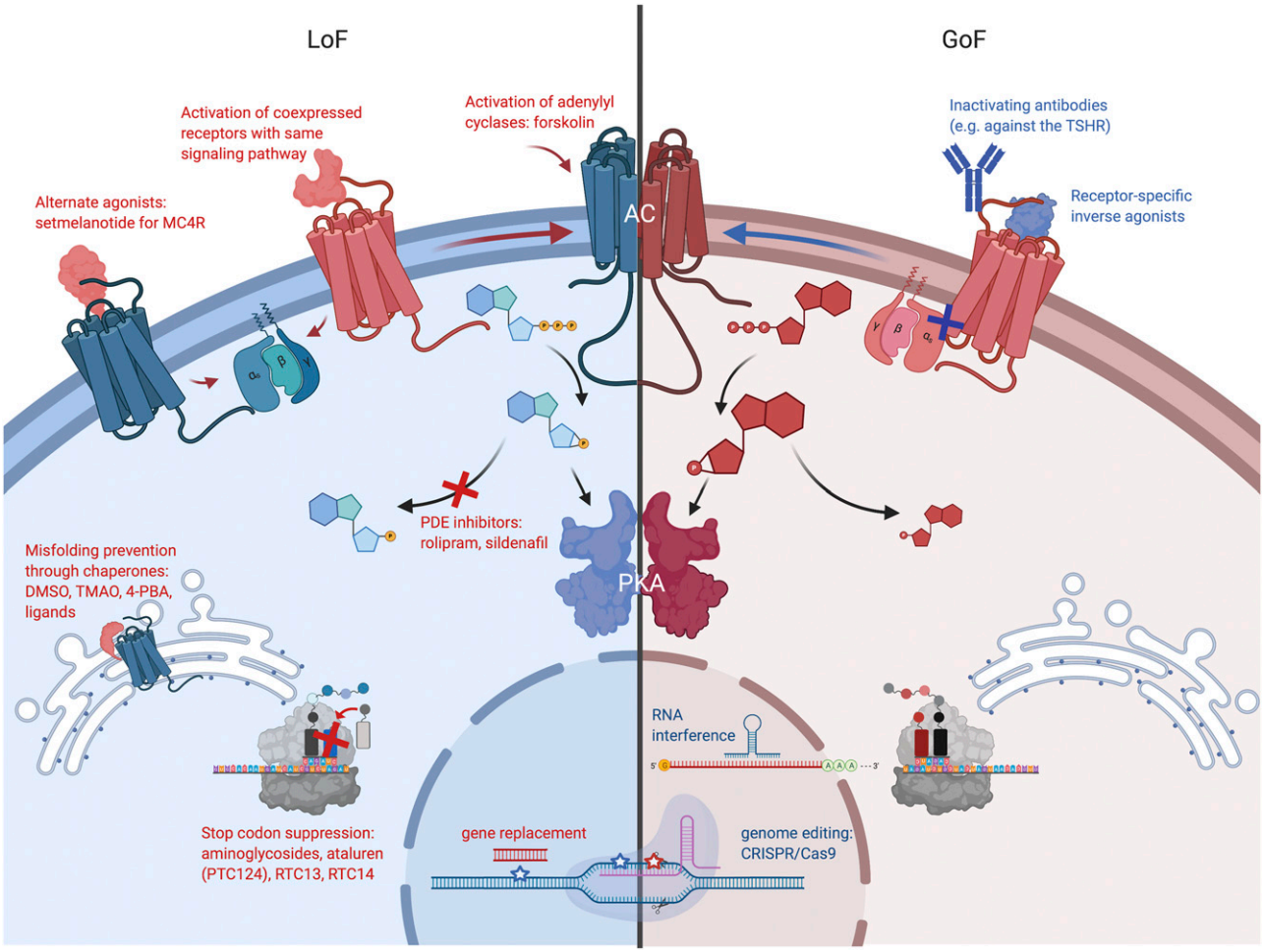

Fig. 5. Potential therapeutic strategies to influence altered GPCR functions. There are direct and indirect approaches to influence altered GPCR functions. Direct approaches target the affected receptor (ligands, pharmacoperones) or receptor gene or transcript (gene replacement, gene editing, premature 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 (Sangkuhl et al., 2004), agonists, inverse agonists, and ligands binding at nonorthosteric 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 helixes 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 nonorthosteric 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 dystrophia 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, β2 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 $Arg^{95}Ter$ (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, nonsynonymous 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 $N^{40}D$ 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 D^{40} variant have lower pain thresholds (Fillingim et al., 2005) and require higher morphine doses to get an analgesic response (Klepstad et al., 2004). The D^{40} 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 $N^{40}D$ 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 $K^{235}N$ variant displayed increased efficacy and potency to buprenorphine relative to the wild type (Hauser et al., 2018).

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., ecnomotopic odorant and taste receptors)
- Improve structural and evolutional approaches to predict the functional relevance of GPCR variations
- Extended use of genome editing methods in research and therapy

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 nonsensoric tissues, so-called ecnomotopic 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.

Acknowledgments

We are very thankful to the anonymous reviewers for their very helpful suggestions and input.

Authorship Contributions

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

References

Abe E, Marians RC, Yu W, Wu XB, Ando T, Li Y, Iqbal J, Eldeiry L, Rajendren G, Blair HC, et al. (2003) TSH is a negative regulator of skeletal remodeling. *Cell* **115**: 151–162.

Abe K, Narumi S, Suwanai AS, Adachi M, Muroya K, Asakura Y, Nagasaki K, Abe T, and Hasegawa T (2018) Association between monoallelic *TSHR* mutations and congenital hypothyroidism: a statistical approach. *Eur J Endocrinol* **178**:137–144.

Adan RA and Kas MJ (2003) Inverse agonism gains weight. *Trends Pharmacol Sci* **24**: 315–321.

Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS, and Sunyaev SR (2010) A method and server for predicting damaging missense mutations. *Nat Methods* **7**:248–249.

Aguzzi A and O'Connor T (2010) Protein aggregation diseases: pathogenicity and therapeutic perspectives. *Nat Rev Drug Discov* **9**:237–248.

Aittomäki K, Lucena JL, Pakarinen P, Sistonen P, Tapanainen J, Gromoll J, Kaskikari R, Sankila EM, Lehväslaiho H, Engel AR, et al. (1995) Mutation in the follicle-stimulating hormone receptor gene causes hereditary hypergonadotropic ovarian failure. *Cell* **82**: 959–968.

Akasaka T, Lee YF, Novak AJ, Honjo G, Takeoka K, Maekawa F, Fukutsuka K, Hayashida M, and Ohno H (2017) Clinical, histopathological, and molecular features of mucosa-associated lymphoid tissue (MALT) lymphoma carrying the t(X;14) (p11; q32)/GPR34-immunoglobulin heavy chain gene. *Leuk Lymphoma* **58**:1–4.

Alexander SPH, Christopoulos A, Davenport AP, Kelly E, Mathie A, Peters JA, Veale EL, Armstrong JF, Faccenda E, Harding SD, et al.; CGTP Collaborators (2019) The Concise Guide to Pharmacology 2019/20: G protein-coupled receptors. *Br J Pharmacol* **176** (Suppl 1):S21–S141.

Alkufri F, Shaag A, Abu-Libdeh B, and Elpeleg O (2016) Deleterious mutation in GPR88 is associated with chorea, speech delay, and learning disabilities. *Neurol Genet* **2**:e64.

Allen LF, Lefkowitz RJ, Caron MG, and Cotecchia S (1991) G-protein-coupled receptor genes as protooncogenes: constitutively activating mutation of the alpha 1B-adrenergic receptor enhances mitogenesis and tumorigenicity. *Proc Natl Acad Sci USA* **88**:11354–11358.

Ansell SM, Akasaka T, McPhail E, Manske M, Braggio E, Price-Troska T, Ziesmer S, Secreto F, Fonseca R, Gupta M, et al. (2012) t(X;14)(p11;q32) in MALT lymphoma involving GPR34 reveals a role for GPR34 in tumor cell growth. *Blood* **120**: 3949–3957.

Anzalone AV, Randolph PB, Davis JR, Sousa AA, Koblan LW, Levy JM, Chen PJ, Wilson C, Newby GA, Raguram A, et al. (2019) Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature* **576**:149–157.

Apfelstedt-Sylla E, Kunisch M, Horn M, Rüther K, Gerding H, Gal A, and Zrenner E (1993) Ocular findings in a family with autosomal dominant retinitis pigmentosa and a frameshift mutation altering the carboxyl terminal sequence of rhodopsin. *Br J Ophthalmol* **77**:495–501.

Appert-Collin A, Baisamy L, and Diviani D (2006) Regulation of g protein-coupled receptor signaling by a-kinase anchoring proteins. *J Recept Signal Transduct Res* **26**:631–646.

Araki K and Nagata K (2011) Protein folding and quality control in the ER. *Cold Spring Harb Perspect Biol* **3**:a007526.

Arseven OK, Wilkes WP, Jameson JL, and Kopp P (2000) Substitutions of tyrosine 601 in the human thyrotropin receptor result in increase or loss of basal activation of the cyclic adenosine monophosphate pathway and disrupt coupling to Gq/11. *Thyroid* **10**:3–10.

Arvanitakis L, Geras-Raaka E, Varma A, Gershengorn MC, and Cesarman E (1997) Human herpesvirus KSHV encodes a constitutively active G-protein-coupled receptor linked to cell proliferation. *Nature* **385**:347–350.

Assadi F and Sharbaf FG (2015) Sildenafil for the treatment of congenital nephrogenic diabetes insipidus. *Am J Nephrol* **42**:65–69.

Audo I, Bujakowska K, Orhan E, Poloschek CM, Defoort-Dhellemmes S, Drumare I, Kohl S, Luu TD, Lecompte O, Zrenner E, et al. (2012) Whole-exome sequencing identifies mutations in GPR179 leading to autosomal-recessive complete congenital stationary night blindness. *Am J Hum Genet* **90**:321–330.

Auer PL, Teumer A, Schick U, O'Shaughnessy A, Lo KS, Chami N, Carlson C, de Denus S, Dubé MP, Haessler J, et al. (2014) Rare and low-frequency coding variants in CXCR2 and other genes are associated with hematological traits. *Nat Genet* **46**: 629–634.

Avbelj Stefanija M, Jeanpierre M, Sykiotis GP, Young J, Quinton R, Abreu AP, Plummer L, Au MG, Balasubramanian R, Dwyer AA, et al. (2012) An ancient founder mutation in PROKR2 impairs human reproduction. *Hum Mol Genet* **21**: 4314–4324.

Bae BI, Tietjen I, Atabay KD, Evrony GD, Johnson MB, Asare E, Wang PP, Murayama AY, Im K, Lisgo SN, et al. (2014) Evolutionarily dynamic alternative splicing of GPR56 regulates regional cerebral cortical patterning. *Science* **343**:764–768.

Baens M, Finalet Ferreiro J, Tousseyn T, Urbankova H, Michaux L, de Leval L, Dierickx D, Wolter P, Sagaert X, Vandenberghe P, et al. (2012) t(X;14)(p11.4;q32.33) is recurrent in marginal zone lymphoma and up-regulates GPR34. *Haematologica* **97**:184–188.

Barak LS, Oakley RH, Laporte SA, and Caron MG (2001) Constitutive arrestin-mediated desensitization of a human vasopressin receptor mutant associated with nephrogenic diabetes insipidus. *Proc Natl Acad Sci USA* **98**:93–98.

Baralle D and Baralle M (2005) Splicing in action: assessing disease causing sequence changes. *J Med Genet* **42**:737–748.

Bassett JH, Williams AJ, Murphy E, Boyde A, Howell PG, Swinhoe R, Archanco M, Flamant F, Samarut J, Costagliola S, et al. (2008) A lack of thyroid hormones rather than excess thyrotropin causes abnormal skeletal development in hypothyroidism. *Mol Endocrinol* **22**:501–512.

Bassi MT, Schiaffino MV, Renieri A, De Nigris F, Galli L, Bruttini M, Gebbia M, Bergen AA, Lewis RA, and Ballabio A (1995) Cloning of the gene for ocular albinism type 1 from the distal short arm of the X chromosome. *Nat Genet* **10**: 13–19.

Bautista RM, Carter KM, Jarrett SG, Napier D, Wakamatsu K, Ito S, and D'Orazio JA (2020) Cutaneous pharmacologic cAMP induction induces melanization of the skin and improves recovery from ultraviolet injury in melanocortin 1 receptor-intact or heterozygous skin. *Pigment Cell Melanoma Res* **33**:30–40.

Bayin NS, Frenster JD, Kane JR, Rubenstein J, Modrek AS, Baitalmal R, Dolgalev I, Rudzenski K, Scarabottolo L, Crespi D, et al. (2016) GPR133 (ADGRD1), an adhesion G-protein-coupled receptor, is necessary for glioblastoma growth. *Oncogenesis* **5**:e263.

Beamer WJ, Eicher EM, Maltais LJ, and Southard JL (1981) Inherited primary hypothyroidism in mice. *Science* **212**:61–63.

Beckers A, Lodish MB, Trivellin G, Rostomyan L, Lee M, Faucz FR, Yuan B, Choong CS, Caberg JH, Verrua E, et al. (2015) X-linked acrogigantism syndrome: clinical profile and therapeutic responses. *Endocr Relat Cancer* **22**:353–367.

Beerepoot P, Nazari R, and Salahpour A (2017) Pharmacological chaperone approaches for rescuing GPCR mutants: current state, challenges, and screening strategies. *Pharmacol Res* **117**:242–251.

Begemann M, Waszak SM, Robinson GW, Jäger N, Sharma T, Knopp C, Kraft F, Moser O, Mynarek M, Guerrini-Rousseau L, et al. (2020) Germline *GPR161* mutations predispose to pediatric medulloblastoma. *J Clin Oncol* **38**:43–50.

Benned-Jensen T, Smethurst C, Holst PJ, Page KR, Sauls H, Sivertsen B, Schwartz TW, Blanchard A, Jepras R, and Rosenkilde MM (2011) Ligand modulation of the Epstein-Barr virus-induced seven-transmembrane receptor EBI2: identification of a potent and efficacious inverse agonist. *J Biol Chem* **286**:29292–29302.

Beri S, Bonaglia MC, and Giorda R (2013) Low-copy repeats at the human VIPR2 gene predispose to recurrent and nonrecurrent rearrangements. *Eur J Hum Genet* **21**: 757–761.

Bevilacqua L, Doly S, Kaprio J, Yuan Q, Tikkanen R, Paunio T, Zhou Z, Wedenoja J, Maroteaux L, Diaz S, et al. (2010) A population-specific HTR2B stop codon predisposes to severe impulsivity. *Nature* **468**:1061–1066.

Bichet DG, Hendy GN, Lonergan M, Arthus MF, Ligier S, Pausova Z, Kluge R, Zingg H, Saenger P, Oppenheimer E, et al. (1992) X-linked nephrogenic diabetes insipidus: from the ship Hopewell to RFLP studies. *Am J Hum Genet* **51**:1089–1102.

Biebermann H, Grüters A, Schöneberg T, and Gudermann T (1997) Congenital hypothyroidism caused by mutations in the thyrotropin-receptor gene. *N Engl J Med* **336**:1390–1391.

Biebermann H, Schöneberg T, Schulz A, Krause G, Grüters A, Schultz G, and Gudermann T (1998) A conserved tyrosine residue (Y601) in transmembrane domain 5 of the human thyrotropin receptor serves as a molecular switch to determine G-protein coupling. *FASEB J* **12**:1461–1471.

Bockenhauer D and Bichet DG (2015) Pathophysiology, diagnosis and management of nephrogenic diabetes insipidus. *Nat Rev Nephrol* **11**:576–588.

Bohnekamp J, Böselt I, Saalbach A, Tönjes A, Kovacs P, Biebermann H, Manvelyan HM, Polte T, Gasperikova D, Lkhagvasuren S, et al. (2010) Involvement of the chemokine-like receptor GPR33 in innate immunity. *Biochem Biophys Res Commun* **396**:272–277.

Boire A, Covic L, Agarwal A, Jacques S, Sherifi S, and Kuliopulos A (2005) PAR1 is a matrix metalloprotease-1 receptor that promotes invasion and tumorigenesis of breast cancer cells. *Cell* **120**:303–313.

Boivin-Jahns V and Jahns R (2018) GPCR-autoantibodies in chronic heart failure. *Front Biosci* **23**:2065–2081.

Bolze F, Rink N, Brumm H, Kühn R, Mocek S, Schwarz AE, Kless C, Biebermann H, Wurst W, Rozman J, et al. (2013) Characterization of the melanocortin-4-receptor nonsense mutation W16X in vitro and in vivo. *Pharmacogenomics J* **13**:80–93.

Bonnefond A, Clément N, Fawcett K, Yengo L, Vaillant E, Guillaume J-L, Dechaume A, Payne F, Roussel R, Czernichow S, et al.; Meta-Analysis of Glucose and Insulin-Related Traits Consortium (MAGIC) (2012) Rare MTNR1B variants impairing melatonin receptor 1B function contribute to type 2 diabetes. *Nat Genet* **44**:297–301.

Böselt I, Tramma D, Kalamitsou S, Niemeyer T, Nykänen P, Gräf KJ, Krude H, Marenzi KS, Di Candia S, Schöneberg T, et al. (2012) Functional characterization of novel loss-of-function mutations in the vasopressin type 2 receptor gene causing nephrogenic diabetes insipidus. *Nephrol Dial Transplant* **27**:1521–1528.

Bouley R, Pastor-Soler N, Cohen O, McLaughlin M, Breton S, and Brown D (2005) Stimulation of AQP2 membrane insertion in renal epithelial cells in vitro and in vivo by the cGMP phosphodiesterase inhibitor sildenafil citrate (Viagra). *Am J Physiol Renal Physiol* **288**:F1103–F1112.

Bouwman A, Shved N, Akgül G, Rühli F, and Warinner C (2017) Ancient DNA investigation of a medieval German cemetery confirms long-term stability of CCR5-Δ32 allele frequencies in Central Europe. *Hum Biol* **89**:119–124.

Boycott KM, Vanstone MR, Bulman DE, and MacKenzie AE (2013) Rare-disease genetics in the era of next-generation sequencing: discovery to translation. *Nat Rev Genet* **14**:681–691.

Boyden SE, Desai A, Cruse G, Young ML, Bolan HC, Scott LM, Eisch AR, Long RD, Lee CC, Satorius CL, et al. (2016) Vibratory urticaria associated with a missense variant in ADGRE2. *N Engl J Med* **374**:656–663.

Bradley EC, Cunningham RL, Wilde C, Morgan RK, Klug EA, Letcher SM, Schöneberg T, Monk KR, Liebscher I, and Petersen SC (2019) In vivo identification of small molecules mediating Gpr126/Adgrg6 signaling during Schwann cell development. *Ann N Y Acad Sci* **1456**:44–63.

Brown AJ, Goldsworthy SM, Barnes AA, Eilert MM, Tcheang L, Daniels D, Muir AI, Wigglesworth MJ, Kinghorn I, Fraser NJ, et al. (2003) The Orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. *J Biol Chem* **278**:11312–11319.

Brüser A, Schulz A, Rothemund S, Ricken A, Calebiro D, Kleinau G, and Schöneberg T (2016) The activation mechanism of glycoprotein hormone receptors with implications in the cause and therapy of endocrine diseases. *J Biol Chem* **291**: 508–520.

Brüser A, Zimmermann A, Crews BC, Sliwoski G, Meiler J, König GM, Kostenis E, Lede V, Marnett LJ, and Schöneberg T (2017) Prostaglandin E_2 glyceryl ester is an endogenous agonist of the nucleotide receptor $P2Y_6$. *Sci Rep* **7**:2380.

Büscher R, Hoerning A, Patel HH, Zhang S, Arthur DB, Grasemann H, Ratjen F, and Insel PA (2006) P2Y2 receptor polymorphisms and haplotypes in cystic fibrosis and their impact on Ca2+ influx. *Pharmacogenet Genomics* **16**:199–205.

Cabral-Marques O, Marques A, Giil LM, De Vito R, Rademacher J, Günther J, Lange T, Humrich JY, Klapa S, Schinke S, et al. (2018) GPCR-specific autoantibody signatures are associated with physiological and pathological immune homeostasis. *Nat Commun* **9**:5224.
Cabral-Marques O and Riemekasten G (2017) Functional autoantibodies targeting G protein-coupled receptors in rheumatic diseases. *Nat Rev Rheumatol* **13**:648–656.
Casarosa P, Menge WM, Minisini R, Otto C, van Heteren J, Jongejan A, Timmerman H, Moepps B, Kirchhoff F, Mertens T, et al. (2003) Identification of the first non-peptidergic inverse agonist for a constitutively active viral-encoded G protein-coupled receptor. *J Biol Chem* **278**:5172–5178.
Cattaneo M, Zighetti ML, Lombardi R, Martinez C, Lecchi A, Conley PB, Ware J, and Ruggeri ZM (2003) Molecular bases of defective signal transduction in the platelet P2Y12 receptor of a patient with congenital bleeding. *Proc Natl Acad Sci USA* **100**:1978–1983.
Chai G, Zhou L, Manto M, Helmbacher F, Clotman F, Goffinet AM, and Tissir F (2014) Celsr3 is required in motor neurons to steer their axons in the hindlimb. *Nat Neurosci* **17**:1171–1179.
Chase A, Ernst T, Fiebig A, Collins A, Grand F, Erben P, Reiter A, Schreiber S, and Cross NC (2010) TFG, a target of chromosome translocations in lymphoma and soft tissue tumors, fuses to GPR128 in healthy individuals. *Haematologica* **95**:20–26.
Cheetham SW, Faulkner GJ, and Dinger ME (2020) Overcoming challenges and dogmas to understand the functions of pseudogenes. *Nat Rev Genet* **21**:191–201.
Chen CR, McLachlan SM, Hubbard PA, McNally R, Murali R, and Rapoport B (2018a) Structure of a thyrotropin receptor monoclonal antibody variable region provides insight into potential mechanisms for its inverse agonist activity. *Thyroid* **28**: 933–940.
Chen JM, Férec C, and Cooper DN (2006) A systematic analysis of disease-associated variants in the 3′ regulatory regions of human protein-coding genes I: general principles and overview. *Hum Genet* **120**:1–21.
Chen Q, Iverson TM, and Gurevich VV (2018b) Structural basis of arrestin-dependent signal transduction. *Trends Biochem Sci* **43**:412–423.
Chevalier N, Paul-Bellon R, Camparo P, Michiels JF, Chevallier D, and Fénichel P (2014) Genetic variants of GPER/GPR30, a novel estrogen-related G protein receptor, are associated with human seminoma. *Int J Mol Sci* **15**:1574–1589.
Cideciyan AV, Sudharsan R, Dufour VL, Massengill MT, Iwabe S, Swider M, Lisi B, Sumaroka A, Marinho LF, Appelbaum T, et al. (2018) Mutation-independent rhodopsin gene therapy by knockdown and replacement with a single AAV vector. *Proc Natl Acad Sci USA* **115**:E8547–E8556.
Clark AJ, McLoughlin L, and Grossman A (1993) Familial glucocorticoid deficiency associated with point mutation in the adrenocorticotropin receptor. *Lancet* **341**: 461–462.
Clément K, Biebermann H, Farooqi IS, Van der Ploeg L, Wolters B, Poitou C, Puder L, Fiedorek F, Gottesdiener K, Kleinau G, et al. (2018) MC4R agonism promotes durable weight loss in patients with leptin receptor deficiency. *Nat Med* **24**:551–555.
Cody JD, Reveles XT, Hale DE, Lehman D, Coon H, and Leach RJ (1999) Haplosufficiency of the melancortin-4 receptor gene in individuals with deletions of 18q. *Hum Genet* **105**:424–427.
Coffey AK, O'Sullivan DJ, Homma S, Dousa TP, and Valtin H (1991) Induction of intramembranous particle clusters in mice with nephrogenic diabetes insipidus. *Am J Physiol* **261**:F640–F646.
Collar AL, Swamydas M, O'Hayre M, Sajib MS, Hoffman KW, Singh SP, Mourad A, Johnson MD, Ferre EM, Farber JM, et al. (2018) The homozygous CX3CR1-M280 mutation impairs human monocyte survival. *JCI Insight* **3**:e95417.
Collet TH, Dubern B, Mokrosinski J, Connors H, Keogh JM, Mendes de Oliveira E, Henning E, Poitou-Bernert C, Oppert JM, Tounian P, et al. (2017) Evaluation of a melanocortin-4 receptor (MC4R) agonist (Setmelanotide) in MC4R deficiency. *Mol Metab* **6**:1321–1329.
Collu R, Tang J, Castagné J, Lagacé G, Masson N, Huot C, Deal C, Delvin E, Faccenda E, Eidne KA, et al. (1997) A novel mechanism for isolated central hypothyroidism: inactivating mutations in the thyrotropin-releasing hormone receptor gene. *J Clin Endocrinol Metab* **82**:1561–1565.
Conn PM, Ulloa-Aguirre A, Ito J, and Janovick JA (2007) G protein-coupled receptor trafficking in health and disease: lessons learned to prepare for therapeutic mutant rescue in vivo. *Pharmacol Rev* **59**:225–250.
Contopoulos-Ioannidis DG, Manoli EN, and Ioannidis JP (2005) Meta-analysis of the association of beta2-adrenergic receptor polymorphisms with asthma phenotypes. *J Allergy Clin Immunol* **115**:963–972.
Cöster M, Wittkopf D, Kreuchwig A, Kleinau G, Thor D, Krause G, and Schöneberg T (2012) Using ortholog sequence data to predict the functional relevance of mutations in G-protein-coupled receptors. *FASEB J* **26**:3273–3281.
Coulon AL, Savagner F, Briet C, Vernin M, Munier M, Chabre O, and Rodien P (2016) Prolonged and severe gestational thyrotoxicosis due to enhanced hCG sensitivity of a mutant thyrotropin receptor. *J Clin Endocrinol Metab* **101**:10–11.
Cruceanu C, Schmouth JF, Torres-Platas SG, Lopez JP, Ambalavanan A, Darcq E, Gross F, Breton B, Spiegelman D, Rochefort D, et al. (2018) Rare susceptibility variants for bipolar disorder suggest a role for G protein-coupled receptors. *Mol Psychiatry* **23**:2050–2056.
Dabrowski M, Bukowy-Bieryllo Z, and Zietkiewicz E (2015) Translational readthrough potential of natural termination codons in eucaryotes--The impact of RNA sequence. *RNA Biol* **12**:950–958.
Dabrowski M, Bukowy-Bieryllo Z, and Zietkiewicz E (2018) Advances in therapeutic use of a drug-stimulated translational readthrough of premature termination codons. *Mol Med* **24**:25.
Dassi E, Re A, Leo S, Tebaldi T, Pasini L, Peroni D, and Quattrone A (2014) AURA 2: empowering discovery of post-transcriptional networks. *Translation (Austin)* **2**: e27738.
Davies B, Baumann C, Kirchhoff C, Ivell R, Nubbemeyer R, Habenicht UF, Theuring F, and Gottwald U (2004) Targeted deletion of the epididymal receptor HE6 results in fluid dysregulation and male infertility. *Mol Cell Biol* **24**:8642–8648.
Dean ED, Li M, Prasad N, Wisniewski SN, Von Deylen A, Spaeth J, Maddison L, Botros A, Sedgeman LR, Bozadjieva N, et al. (2017) Interrupted glucagon signaling reveals hepatic α cell axis and role for L-glutamine in α cell proliferation. *Cell Metab* **25**:1362–1373.e5.
Dean M, Carrington M, Winkler C, Huttley GA, Smith MW, Allikmets R, Goedert JJ, Buchbinder SP, Vittinghoff E, Gomperts E, et al. (1996) Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CKR5 structural gene. Hemophilia Growth and Development Study, Multicenter AIDS Cohort Study, Multicenter Hemophilia Cohort Study, San Francisco City Cohort, ALIVE Study. *Science* **273**:1856–1862.
Decker E, Stellzig-Eisenhauer A, Fiebig BS, Rau C, Kress W, Saar K, Rüschendorf F, Hubner N, Grimm T, and Weber BH (2008) PTHR1 loss-of-function mutations in familial, nonsyndromic primary failure of tooth eruption. *Am J Hum Genet* **83**: 781–786.
De Fusco M, Vago R, Striano P, Di Bonaventura C, Zara F, Mei D, Kim MS, Muallem S, Chen Y, Wang Q, et al. (2014) The α2B-adrenergic receptor is mutant in cortical myoclonus and epilepsy. *Ann Neurol* **75**:77–87.
De Leener A, Caltabiano G, Erkan S, Idil M, Vassart G, Pardo L, and Costagliola S (2008) Identification of the first germline mutation in the extracellular domain of the follitropin receptor responsible for spontaneous ovarian hyperstimulation syndrome. *Hum Mutat* **29**:91–98.
Deltas C (2018) Digenic inheritance and genetic modifiers. *Clin Genet* **93**:429–438.
de Munnik SM, Smit MJ, Leurs R, and Vischer HF (2015) Modulation of cellular signaling by herpesvirus-encoded G protein-coupled receptors. *Front Pharmacol* **6**:40.
de Roux N, Genin E, Carel JC, Matsuda F, Chaussain JL, and Milgrom E (2003) Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. *Proc Natl Acad Sci USA* **100**:10972–10976.
de Roux N, Young J, Brailly-Tabard S, Misrahi M, Milgrom E, and Schaison G (1999) The same molecular defects of the gonadotropin-releasing hormone receptor determine a variable degree of hypogonadism in affected kindred. *J Clin Endocrinol Metab* **84**:567–572.
de Roux N, Young J, Misrahi M, Genet R, Chanson P, Schaison G, and Milgrom E (1997) A family with hypogonadotropic hypogonadism and mutations in the gonadotropin-releasing hormone receptor. *N Engl J Med* **337**:1597–1602.
Diamantopoulou E, Baxendale S, de la Vega de León A, Asad A, Holdsworth CJ, Abbas L, Gillet VJ, Wiggin GR, and Whitfield TT (2019) Identification of compounds that rescue otic and myelination defects in the zebrafish *adgrg6* (*gpr126*) mutant. *eLife* **8**:e44889.
Dib L, San-Jose LM, Ducrest A-L, Salamin N, and Roulin A (2017) Selection on the major color gene melanocortin-1-receptor shaped the evolution of the melanocortin system genes. *Int J Mol Sci* **18**:2618.
Di Pizio A, Behrens M, and Krautwurst D (2019) Beyond the flavour: the potential druggability of chemosensory G protein-coupled receptors. *Int J Mol Sci* **20**:1402.
Dodé C, Teixeira L, Levilliers J, Fouveaut C, Bouchard P, Kottler ML, Lespinasse J, Lienhardt-Roussie A, Mathieu M, Moerman A, et al. (2006) Kallmann syndrome: mutations in the genes encoding prokineticin-2 and prokineticin receptor-2. *PLoS Genet* **2**:e175.
D'Orazio JA, Nobuhisa T, Cui R, Arya M, Spry M, Wakamatsu K, Igras V, Kunisada T, Granter SR, Nishimura EK, et al. (2006) Topical drug rescue strategy and skin protection based on the role of Mc1r in UV-induced tanning. *Nature* **443**: 340–344.
Dryja TP, McGee TL, Berson EL, Fishman GA, Sandberg MA, Alexander KR, Derlacki DJ, and Rajagopalan AS (2005) Night blindness and abnormal cone electroretinogram ON responses in patients with mutations in the GRM6 gene encoding mGluR6. *Proc Natl Acad Sci USA* **102**:4884–4889.
Dryja TP, McGee TL, Reichel E, Hahn LB, Cowley GS, Yandell DW, Sandberg MA, and Berson EL (1990) A point mutation of the rhodopsin gene in one form of retinitis pigmentosa. *Nature* **343**:364–366.
Du M, Liu X, Welch EM, Hirawat S, Peltz SW, and Bedwell DM (2008) PTC124 is an orally bioavailable compound that promotes suppression of the human CFTR-G542X nonsense allele in a CF mouse model. *Proc Natl Acad Sci USA* **105**: 2064–2069.
Duman JG, Mulherkar S, Tu YK, Erikson KC, Tzeng CP, Mavratsas VC, Ho TS, and Tolias KF (2019) The adhesion-GPCR BAI1 shapes dendritic arbors via Bcr-mediated RhoA activation causing late growth arrest. *eLife* **8**:e47566.
Dunn HA and Ferguson SS (2015) PDZ protein regulation of G protein-coupled receptor trafficking and signaling pathways. *Mol Pharmacol* **88**:624–639.
Einarsdottir E, Grauers A, Wang J, Jiao H, Escher SA, Danielsson A, Simony A, Andersen M, Christensen SB, Åkesson K, et al. (2017) CELSR2 is a candidate susceptibility gene in idiopathic scoliosis. *PLoS One* **12**:e0189591.
Ekberg JH, Hauge M, Kristensen LV, Madsen AN, Engelstoft MS, Husted AS, Sichlau R, Egerod KL, Timshel P, Kowalski TJ, et al. (2016) GPR119, a major enteroendocrine sensor of dietary triglyceride metabolites coacting in synergy with FFA1 (GPR40). *Endocrinology* **157**:4561–4569.
Elia J, Glessner JT, Wang K, Takahashi N, Shtir CJ, Hadley D, Sleiman PM, Zhang H, Kim CE, Robison R, et al. (2011) Genome-wide copy number variation study associates metabotropic glutamate receptor gene networks with attention deficit hyperactivity disorder. *Nat Genet* **44**:78–84.
Emdin CA, Khera AV, Chaffin M, Klarin D, Natarajan P, Aragam K, Haas M, Bick A, Zekavat SM, Nomura A, et al. (2018) Analysis of predicted loss-of-function variants in UK Biobank identifies variants protective for disease. *Nat Commun* **9**:1613.
Erdogmus S, Storch U, Danner L, Becker J, Winter M, Ziegler N, Wirth A, Offermanns S, Hoffmann C, Gudermann T, et al. (2019) Helix 8 is the essential structural motif of mechanosensitive GPCRs. *Nat Commun* **10**:5784.
EuroEPINOMICS-RES Consortium; Epilepsy Phenome/Genome Project; Epi4K Consortium (2014) De novo mutations in synaptic transmission genes including DNM1 cause epileptic encephalopathies [published correction appears in *Am J Hum Genet* (2017) 100:179]. *Am J Hum Genet* **95**:360–370.
Ezkurdia I, Juan D, Rodriguez JM, Frankish A, Diekhans M, Harrow J, Vazquez J, Valencia A, and Tress ML (2014) Multiple evidence strands suggest that there may be as few as 19,000 human protein-coding genes. *Hum Mol Genet* **23**:5866–5878.
Fares S, Spiess K, Olesen ETB, Zuo J, Jackson S, Kledal TN, Wills MR, and Rosenkilde MM (2019) Distinct roles of extracellular domains in the epstein-barr virus-encoded

BILF1 receptor for signaling and major histocompatibility complex class I downregulation. *MBio* **10**:e01707-18.
Fares-Taie L, Gerber S, Tawara A, Ramirez-Miranda A, Douet JY, Verdin H, Guilloux A, Zenteno JC, Kondo H, Moisset H, et al. (2015) Submicroscopic deletions at 13q32.1 cause congenital microcoria. *Am J Hum Genet* **96**:631–639.
Feldman BJ, Rosenthal SM, Vargas GA, Fenwick RG, Huang EA, Matsuda-Abedini M, Lustig RH, Mathias RS, Portale AA, Miller WL, et al. (2005) Nephrogenic syndrome of inappropriate antidiuresis. *N Engl J Med* **352**:1884–1890.
Feldman GJ, Parvizi J, Levenstien M, Scott K, Erickson JA, Fortina P, Devoto M, and Peters CL (2013) Developmental dysplasia of the hip: linkage mapping and whole exome sequencing identify a shared variant in CX3CR1 in all affected members of a large multigeneration family. *J Bone Miner Res* **28**:2540–2549.
Fillingim RB, Kaplan L, Staud R, Ness TJ, Glover TL, Campbell CM, Mogil JS, and Wallace MR (2005) The A118G single nucleotide polymorphism of the mu-opioid receptor gene (OPRM1) is associated with pressure pain sensitivity in humans. *J Pain* **6**:159–167.
Firouzabadi SG, Kariminejad R, Vameghi R, Darvish H, Ghaedi H, Banihashemi S, Firouzkouhi Moghaddam M, Jamali P, Mofidi Tehrani HF, Dehghani H, et al. (2017) Copy number variants in patients with autism and additional clinical features: report of VIPR2 duplication and a novel microduplication syndrome. *Mol Neurobiol* **54**:7019–7027.
Firth HV, Richards SM, Bevan AP, Clayton S, Corpas M, Rajan D, Van Vooren S, Moreau Y, Pettett RM, and Carter NP (2009) DECIPHER: DatabasE of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources. *Am J Hum Genet* **84**:524–533.
Fischer L, Wilde C, Schöneberg T, and Liebscher I (2016) Functional relevance of naturally occurring mutations in adhesion G protein-coupled receptor ADGRD1 (GPR133). *BMC Genomics* **17**:609.
Frederiksen AL, Duno M, and Welinder LG (2013) Blue cone monochromatism in a female due to skewed X-inactivation. *Ophthalmic Genet* **34**:101–104.
Fröjmark AS, Schuster J, Sobol M, Entesarian M, Kilander MBC, Gabrikova D, Nawaz S, Baig SM, Schulte G, Klar J, et al. (2011) Mutations in Frizzled 6 cause isolated autosomal-recessive nail dysplasia. *Am J Hum Genet* **88**:852–860.
Fu C, Wang J, Luo S, Yang Q, Li Q, Zheng H, Hu X, Su J, Zhang S, Chen R, et al. (2016) Next-generation sequencing analysis of TSHR in 384 Chinese subclinical congenital hypothyroidism (CH) and CH patients. *Clin Chim Acta* **462**:127–132.
Fukami M, Suzuki E, Izumi Y, Torii T, Narumi S, Igarashi M, Miyado M, Katsumi M, Fujisawa Y, Nakabayashi K, et al. (2017) Paradoxical gain-of-function mutant of the G-protein-coupled receptor PROKR2 promotes early puberty. *J Cell Mol Med* **21**:2623–2626.
Gao Z, Waggoner D, Stephens M, Ober C, and Przeworski M (2015) An estimate of the average number of recessive lethal mutations carried by humans. *Genetics* **199**:1243–1254.
Garcia G III, Raleigh DR, and Reiter JF (2018) How the ciliary membrane is organized inside-out to communicate outside-in. *Curr Biol* **28**:R421–R434.
Garg A, Sankella S, Xing C, and Agarwal AK (2016) Whole-exome sequencing identifies *ADRA2A* mutation in atypical familial partial lipodystrophy. *JCI Insight* **1**:e86870.
Gassmann M, Shaban H, Vigot R, Sansig G, Haller C, Barbieri S, Humeau Y, Schuler V, Müller M, Kinzel B, et al. (2004) Redistribution of GABAB(1) protein and atypical GABAB responses in GABAB(2)-deficient mice. *J Neurosci* **24**:6086–6097.
Gavalas NG, Gottumukkala RV, Gawkrodger DJ, Watson PF, Weetman AP, and Kemp EH (2009) Mapping of melanin-concentrating hormone receptor 1 B cell epitopes predicts two major binding sites for vitiligo patient autoantibodies. *Exp Dermatol* **18**:454–463.
Gazzo AM, Daneels D, Cilia E, Bonduelle M, Abramowicz M, Van Dooren S, Smits G, and Lenaerts T (2016) DIDA: a curated and annotated digenic diseases database. *Nucleic Acids Res* **44**:D900–D907.
Gelling RW, Du XQ, Dichmann DS, Romer J, Huang H, Cui L, Obici S, Tang B, Holst JJ, Fledelius C, et al. (2003) Lower blood glucose, hyperglucagonemia, and pancreatic alpha cell hyperplasia in glucagon receptor knockout mice. *Proc Natl Acad Sci USA* **100**:1438–1443.
Gelpi AP and King MC (1976) Association of Duffy blood groups with the sickle cell trait. *Hum Genet* **32**:65–68.
Generoso SF, Giustiniano M, La Regina G, Bottone S, Passacantilli S, Di Maro S, Cassese H, Bruno A, Mallardo M, Dentice M, et al. (2015) Pharmacological folding chaperones act as allosteric ligands of Frizzled4. *Nat Chem Biol* **11**:280–286.
Gershengorn MC and Neumann S (2012) Update in TSH receptor agonists and antagonists. *J Clin Endocrinol Metab* **97**:4287–4292.
Gordon CT, Weaver KN, Zechi-Ceide RM, Madsen EC, Tavares AL, Oufadem M, Kurihara Y, Adameyko I, Picard A, Breton S, et al. (2015) Mutations in the endothelin receptor type A cause mandibulofacial dysostosis with alopecia. *Am J Hum Genet* **96**:519–531.
Gorlov IP, Kamat A, Bogatcheva NV, Jones E, Lamb DJ, Truong A, Bishop CE, McElreavey K, and Agoulnik AI (2002) Mutations of the GREAT gene cause cryptorchidism. *Hum Mol Genet* **11**:2309–2318.
Gorvin CM, Babinsky VN, Malinauskas T, Nissen PH, Schou AJ, Hanyaloglu AC, Siebold C, Jones EY, Hannan FM, and Thakker RV (2018) A calcium-sensing receptor mutation causing hypocalcemia disrupts a transmembrane salt bridge to activate β-arrestin-biased signaling. *Sci Signal* **11**:eaan3714.
Gottumukkala RV, Waterman EA, Herd LM, Gawkrodger DJ, Watson PF, Weetman AP, and Kemp EH (2003) Autoantibodies in vitiligo patients recognize multiple domains of the melanin-concentrating hormone receptor. *J Invest Dermatol* **121**:765–770.
Grasberger H and Refetoff S (2017) Resistance to thyrotropin. *Best Pract Res Clin Endocrinol Metab* **31**:183–194.
Greenwald DL, Cashman SM, and Kumar-Singh R (2013) Mutation-independent rescue of a novel mouse model of Retinitis Pigmentosa. *Gene Ther* **20**:425–434.
Gribouval O, Gonzales M, Neuhaus T, Aziza J, Bieth E, Laurent N, Bouton JM, Feuillet F, Makni S, Ben Amar H, et al. (2005) Mutations in genes in the renin-angiotensin system are associated with autosomal recessive renal tubular dysgenesis. *Nat Genet* **37**:964–968.
Gribouval O, Morinière V, Pawtowski A, Arrondel C, Sallinen SL, Saloranta C, Clericuzio C, Viot G, Tantau J, Blesson S, et al. (2012) Spectrum of mutations in the renin-angiotensin system genes in autosomal recessive renal tubular dysgenesis. *Hum Mutat* **33**:316–326.
Grillo G, Turi A, Licciulli F, Mignone F, Liuni S, Banfi S, Gennarino VA, Horner DS, Pavesi G, Picardi E, et al. (2010) UTRdb and UTRsite (RELEASE 2010): a collection of sequences and regulatory motifs of the untranslated regions of eukaryotic mRNAs. *Nucleic Acids Res* **38**:D75–D80.
Grosse R, Schöneberg T, Schultz G, and Gudermann T (1997) Inhibition of gonadotropin-releasing hormone receptor signaling by expression of a splice variant of the human receptor. *Mol Endocrinol* **11**:1305–1318.
Grüters A, Schöneberg T, Biebermann H, Krude H, Krohn HP, Dralle H, and Gudermann T (1998) Severe congenital hyperthyroidism caused by a germ-line neo mutation in the extracellular portion of the thyrotropin receptor. *J Clin Endocrinol Metab* **83**:1431–1436.
Gudermann T, Kalkbrenner F, and Schultz G (1996) Diversity and selectivity of receptor-G protein interaction. *Annu Rev Pharmacol Toxicol* **36**:429–459.
Gudermann T, Schöneberg T, and Schultz G (1997) Functional and structural complexity of signal transduction via G-protein-coupled receptors. *Annu Rev Neurosci* **20**:399–427.
Guergueltcheva V, Azmanov DN, Angelicheva D, Smith KR, Chamova T, Florez L, Bynevelt M, Nguyen T, Cherninkova S, Bojinova V, et al. (2012) Autosomal-recessive congenital cerebellar ataxia is caused by mutations in metabotropic glutamate receptor 1. *Am J Hum Genet* **91**:553–564.
Guerin K, Gregory-Evans CY, Hodges MD, Moosajee M, Mackay DS, Gregory-Evans K, and Flannery JG (2008) Systemic aminoglycoside treatment in rodent models of retinitis pigmentosa. *Exp Eye Res* **87**:197–207.
Guerrini R, Bonanni P, Patrignani A, Brown P, Parmeggiani L, Grosse P, Brovedani P, Moro F, Aridon P, Carrozzo R, et al. (2001) Autosomal dominant cortical myoclonus and epilepsy (ADCME) with complex partial and generalized seizures: a newly recognized epilepsy syndrome with linkage to chromosome 2p11.1-q12.2. *Brain* **124**:2459–2475.
Gurevich VV and Gurevich EV (2019) Arrestin mutations: some cause diseases, others promise cure. *Prog Mol Biol Transl Sci* **161**:29–45.
Gutkind JS, Novotny EA, Brann MR, and Robbins KC (1991) Muscarinic acetylcholine receptor subtypes as agonist-dependent oncogenes. *Proc Natl Acad Sci USA* **88**:4703–4707.
Gwinn MR, Sharma A, and De Nardin E (1999) Single nucleotide polymorphisms of the N-formyl peptide receptor in localized juvenile periodontitis. *J Periodontol* **70**:1194–1201.
Hai L, McGee SR, Rabideau AC, Paquet M, and Narayan P (2015) Infertility in female mice with a gain-of-function mutation in the luteinizing hormone receptor is due to irregular estrous cyclicity, anovulation, hormonal alterations, and polycystic ovaries. *Biol Reprod* **93**:16.
Hamann J, Aust G, Araç D, Engel FB, Formstone C, Fredriksson R, Hall RA, Harty BL, Kirchhoff C, Knapp B, et al. (2015) International Union of Basic and Clinical Pharmacology. XCIV. Adhesion G protein-coupled receptors. *Pharmacol Rev* **67**:338–367.
Hamdan FF, Myers CT, Cossette P, Lemay P, Spiegelman D, Laporte AD, Nassif C, Diallo O, Monlong J, Cadieux-Dion M, et al.; Deciphering Developmental Disorders Study (2017) High rate of recurrent de novo mutations in developmental and epileptic encephalopathies. *Am J Hum Genet* **101**:664–685.
Hauser AS, Attwood MM, Rask-Andersen M, Schiöth HB, and Gloriam DE (2017) Trends in GPCR drug discovery: new agents, targets and indications. *Nat Rev Drug Discov* **16**:829–842.
Hauser AS, Chavali S, Masuho I, Jahn LJ, Martemyanov KA, Gloriam DE, and Babu MM (2018) Pharmacogenomics of GPCR drug targets. *Cell* **172**:41–54.e19.
Hauser AS, Gloriam DE, Bräuner-Osborne H, and Foster SR (2020) Novel approaches leading towards peptide GPCR de-orphanisation. *Br J Pharmacol* **177**:961–968.
Hedrick PW and Verrelli BC (2006) "Ground truth" for selection on CCR5-Delta32. *Trends Genet* **22**:293–296.
Helsmoortel C, Swagemakers SM, Vandeweyer G, Stubbs AP, Palli I, Mortier G, Kooy RF, and van der Spek PJ (2016) Whole genome sequencing of a dizygotic twin suggests a role for the serotonin receptor HTR7 in autism spectrum disorder. *Am J Med Genet B Neuropsychiatr Genet* **171**:1049–1056.
Hernandez PA, Gorlin RJ, Lukens JN, Taniuchi S, Bohinjec J, Francois F, Klotman ME, and Diaz GA (2003) Mutations in the chemokine receptor gene CXCR4 are associated with WHIM syndrome, a combined immunodeficiency disease. *Nat Genet* **34**:70–74.
Hertzberg L, Vendramini E, Ganmore I, Cazzaniga G, Schmitz M, Chalker J, Shiloh R, Iacobucci I, Shochat C, Zeligson S, et al. (2010) Down syndrome acute lymphoblastic leukemia, a highly heterogeneous disease in which aberrant expression of CRLF2 is associated with mutated JAK2: a report from the International BFM Study Group. *Blood* **115**:1006–1017.
Hilger D, Masureel M, and Kobilka BK (2018) Structure and dynamics of GPCR signaling complexes. *Nat Struct Mol Biol* **25**:4–12.
Hirata T, Kakizuka A, Ushikubi F, Fuse I, Okuma M, and Narumiya S (1994) Arg60 to Leu mutation of the human thromboxane A2 receptor in a dominantly inherited bleeding disorder. *J Clin Invest* **94**:1662–1667.
Ho C, Conner DA, Pollak MR, Ladd DJ, Kifor O, Warren HB, Brown EM, Seidman JG, and Seidman CE (1995) A mouse model of human familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism. *Nat Genet* **11**:389–394.
Ho KWD, Wallace MR, Staud R, and Fillingim RB (2020) OPRM1, OPRK1, and COMT genetic polymorphisms associated with opioid effects on experimental pain: a randomized, double-blind, placebo-controlled study. *Pharmacogenomics J* **20**:471–481.
Hollopeter G, Jantzen HM, Vincent D, Li G, England L, Ramakrishnan V, Yang RB, Nurden P, Nurden A, Julius D, et al. (2001) Identification of the platelet ADP receptor targeted by antithrombotic drugs. *Nature* **409**:202–207.
Hou ZS and Tao YX (2019) Mutations in GPR101 as a potential cause of X-linked acrogigantism and acromegaly. *Prog Mol Biol Transl Sci* **161**:47–67.

Hou ZS, Ulloa-Aguirre A, and Tao YX (2018) Pharmacoperone drugs: targeting misfolded proteins causing lysosomal storage-, ion channels-, and G protein-coupled receptors-associated conformational disorders. *Expert Rev Clin Pharmacol* **11**: 611–624.

Huang P, Chen C, Mague SD, Blendy JA, and Liu-Chen LY (2012) A common single nucleotide polymorphism A118G of the μ opioid receptor alters its N-glycosylation and protein stability. *Biochem J* **441**:379–386.

Huszar D, Lynch CA, Fairchild-Huntress V, Dunmore JH, Fang Q, Berkemeier LR, Gu W, Kesterson RA, Boston BA, Cone RD, et al. (1997) Targeted disruption of the melanocortin-4 receptor results in obesity in mice. *Cell* **88**:131–141.

Imamura F, Arimoto I, Fujiyoshi Y, and Doi T (2000) W276 mutation in the endothelin receptor subtype B impairs Gq coupling but not Gi or Go coupling. *Biochemistry* **39**: 686–692.

Inglehearn CF, Bashir R, Lester DH, Jay M, Bird AC, and Bhattacharya SS (1991) A 3-bp deletion in the rhodopsin gene in a family with autosomal dominant retinitis pigmentosa. *Am J Hum Genet* **48**:26–30.

Insel PA, Sriram K, Wiley SZ, Wilderman A, Katakia T, McCann T, Yokouchi H, Zhang L, Corriden R, Liu D, et al. (2018) GPCRomics: GPCR expression in cancer cells and tumors identifies new, potential biomarkers and therapeutic targets. *Front Pharmacol* **9**:431.

Insel PA, Tang CM, Hahntow I, and Michel MC (2007) Impact of GPCRs in clinical medicine: monogenic diseases, genetic variants and drug targets. *Biochim Biophys Acta* **1768**:994–1005.

Issa S, Bondurand N, Faubert E, Poisson S, Lecerf L, Nitschke P, Deggouj N, Loundon N, Jonard L, David A, et al. (2017) EDNRB mutations cause Waardenburg syndrome type II in the heterozygous state. *Hum Mutat* **38**:581–593.

Jaberi E, Rohani M, Shahidi GA, Nafissi S, Arefian E, Soleimani M, Moghadam A, Arzenani MK, Keramatian F, Klotzle B, et al. (2016) Mutation in ADORA1 identified as likely cause of early-onset parkinsonism and cognitive dysfunction. *Mov Disord* **31**:1004–1011.

Janovick JA, Patny A, Mosley R, Goulet MT, Altman MD, Rush TS III, Cornea A, and Conn PM (2009) Molecular mechanism of action of pharmacoperone rescue of misrouted GPCR mutants: the GnRH receptor. *Mol Endocrinol* **23**:157–168.

Jaquette J and Segaloff DL (1997) Temperature sensitivity of some mutants of the lutropin/choriogonadotropin receptor. *Endocrinology* **138**:85–91.

Jean-Alphonse F, Perkovska S, Frantz MC, Durroux T, Méjean C, Morin D, Loison S, Bonnet D, Hibert M, Mouillac B, et al. (2009) Biased agonist pharmacochaperones of the AVP V2 receptor may treat congenital nephrogenic diabetes insipidus. *J Am Soc Nephrol* **20**:2190–2203.

Jiang YC, Wu HM, Cheng KH, and Sunny Sun H (2012) Menstrual cycle-dependent febrile episode mediated by sequence-specific repression of poly(ADP-ribose) polymerase-1 on the transcription of the human serotonin receptor 1A gene. *Hum Mutat* **33**:209–217.

Jin C, Zhou Z, Li W, Zhang F, Yuan J, Liu X, and Cheng Z (2016) Analysis of the association of VIPR2 polymorphisms with susceptibility to schizophrenia. *Psychiatry Res* **241**:104–107.

Jobert AS, Zhang P, Couvineau A, Bonaventure J, Roume J, Le Merrer M, and Silve C (1998) Absence of functional receptors for parathyroid hormone and parathyroid hormone-related peptide in Blomstrand chondrodysplasia. *J Clin Invest* **102**:34–40.

Kamenický P, Bouligand J, and Chanson P (2015) Gigantism, acromegaly, and GPR101 mutations. *N Engl J Med* **372**:1264.

Karaca E, Buyukkaya R, Pehlivan D, Charng WL, Yaykasli KO, Bayram Y, Gambin T, Withers M, Atik MM, Arslanoglu I, et al. (2015) Whole-exome sequencing identifies homozygous GPR161 mutation in a family with pituitary stalk interruption syndrome. *J Clin Endocrinol Metab* **100**:E140–E147.

Karczewski KJ, Francioli LC, Tiao G, Cummings BB, Alföldi J, Wang Q, Collins RL, Laricchia KM, Ganna A, Birnbaum DP, et al.; Genome Aggregation Database Consortium (2020) The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature* **581**:434–443.

Katagiri S, Iwasa M, Hayashi T, Hosono K, Yamashita T, Kuniyoshi K, Ueno S, Kondo M, Ueyama H, Ogita H, et al. (2018) Genotype determination of the OPN1LW/OPN1MW genes: novel disease-causing mechanisms in Japanese patients with blue cone monochromacy. *Sci Rep* **8**:11507.

Kaur H, Carvalho J, Looso M, Singh P, Chennupati R, Preussner J, Günther S, Albarrán-Juárez J, Tischner D, Classen S, et al. (2017) Single-cell profiling reveals heterogeneity and functional patterning of GPCR expression in the vascular system. *Nat Commun* **8**:15700.

Kayikci M, Venkatakrishnan AJ, Scott-Brown J, Ravarani CNJ, Flock T, and Babu MM (2018) Visualization and analysis of non-covalent contacts using the Protein Contacts Atlas. *Nat Struct Mol Biol* **25**:185–194.

Kemp EH, Waterman EA, Hawes BE, O'Neill K, Gottumukkala RV, Gawkrodger DJ, Weetman AP, and Watson PF (2002) The melanin-concentrating hormone receptor 1, a novel target of autoantibody responses in vitiligo. *J Clin Invest* **109**:923–930.

Kerem E, Konstan MW, De Boeck K, Accurso FJ, Sermet-Gaudelus I, Wilschanski M, Elborn JS, Melotti P, Bronsveld I, Fajac I, et al.; Cystic Fibrosis Ataluren Study Group (2014) Ataluren for the treatment of nonsense-mutation cystic fibrosis: a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet Respir Med* **2**:539–547.

Khamaysi Z, Bochner R, Indelman M, Magal L, Avitan-Hersh E, Sarig O, Sprecher E, and Bergman R (2016) Segmental basal cell naevus syndrome caused by an activating mutation in smoothened. *Br J Dermatol* **175**:178–181.

Kifor O, Moore FD Jr, Delaney M, Garber J, Hendy GN, Butters R, Gao P, Cantor TL, Kifor I, Brown EM, et al. (2003) A syndrome of hypocalciuric hypercalcemia caused by autoantibodies directed at the calcium-sensing receptor. *J Clin Endocrinol Metab* **88**:60–72.

Kim P and Zhou X (2019) FusionGDB: fusion gene annotation DataBase. *Nucleic Acids Res* **47**:D994–D1004.

Kim SE, Lei Y, Hwang SH, Wlodarczyk BJ, Mukhopadhyay S, Shaw GM, Ross ME, and Finnell RH (2019) Dominant negative GPR161 rare variants are risk factors of human spina bifida. *Hum Mol Genet* **28**:200–208.

Kleinau G, Claus M, Jaeschke H, Mueller S, Neumann S, Paschke R, and Krause G (2007) Contacts between extracellular loop two and transmembrane helix six determine basal activity of the thyroid-stimulating hormone receptor. *J Biol Chem* **282**:518–525.

Klepstad P, Rakvåg TT, Kaasa S, Holthe M, Dale O, Borchgrevink PC, Baar C, Vikan T, Krokan HE, and Skorpen F (2004) The 118 A > G polymorphism in the human mu-opioid receptor gene may increase morphine requirements in patients with pain caused by malignant disease. *Acta Anaesthesiol Scand* **48**:1232–1239.

Knapp B and Wolfrum U (2016) Adhesion GPCR-related protein networks. *Handb Exp Pharmacol* **234**:147–178.

Knierim AB, Röthe J, Çakir MV, Lede V, Wilde C, Liebscher I, Thor D, and Schöneberg T (2019) Genetic basis of functional variability in adhesion G protein-coupled receptors. *Sci Rep* **9**:11036.

Kou Ikuyo, Takahashi Yohei, Johnson Todd A, Takahashi Atsushi, Guo Long, Dai Jin, Qiu Xusheng, Sharma Swarkar, Takimoto Aki, Ogura Yoji, et al. (2013) Genetic variants in GPR126 are associated with adolescent idiopathic scoliosis. *Nat Genet* **45**:676–679.

Kovacs P and Schöneberg T (2016) The relevance of genomic signatures at adhesion GPCR loci in humans. *Handb Exp Pharmacol* **234**:179–217.

Kremer H, Kraaij R, Toledo SP, Post M, Fridman JB, Hayashida CY, van Reen M, Milgrom E, Ropers HH, Mariman E, et al. (1995) Male pseudohermaphroditism due to a homozygous missense mutation of the luteinizing hormone receptor gene. *Nat Genet* **9**:160–164.

Kreuchwig A, Kleinau G, and Krause G (2013) Research resource: novel structural insights bridge gaps in glycoprotein hormone receptor analyses. *Mol Endocrinol* **27**: 1357–1363.

Kühnen P, Clément K, Wiegand S, Blankenstein O, Gottesdiener K, Martini LL, Mai K, Blume-Peytavi U, Grüters A, and Krude H (2016) Proopiomelanocortin deficiency treated with a melanocortin-4 receptor agonist. *N Engl J Med* **375**:240–246.

Kustanovich V, Ishii J, Crawford L, Yang M, McGough JJ, McCracken JT, Smalley SL, and Nelson SF (2004) Transmission disequilibrium testing of dopamine-related candidate gene polymorphisms in ADHD: confirmation of association of ADHD with DRD4 and DRD5. *Mol Psychiatry* **9**:711–717.

Lania AG, Mantovani G, and Spada A (2006) Mechanisms of disease: mutations of G proteins and G-protein-coupled receptors in endocrine diseases. *Nat Clin Pract Endocrinol Metab* **2**:681–693.

Laugwitz KL, Allgeier A, Offermanns S, Spicher K, Van Sande J, Dumont JE, and Schultz G (1996) The human thyrotropin receptor: a heptahelical receptor capable of stimulating members of all four G protein families. *Proc Natl Acad Sci USA* **93**:116–120.

Lede V, Meusel A, Garten A, Popkova Y, Penke M, Franke C, Ricken A, Schulz A, Kiess W, Huster D, et al. (2017) Altered hepatic lipid metabolism in mice lacking both the melanocortin type 4 receptor and low density lipoprotein receptor. *PLoS One* **12**:e0172000.

Lee RJ, Xiong G, Kofonow JM, Chen B, Lysenko A, Jiang P, Abraham V, Doghramji L, Adappa ND, Palmer JN, et al. (2012) T2R38 taste receptor polymorphisms underlie susceptibility to upper respiratory infection. *J Clin Invest* **122**:4145–4159.

Lee YS, Poh LK, and Loke KY (2002) A novel melanocortin 3 receptor gene (MC3R) mutation associated with severe obesity. *J Clin Endocrinol Metab* **87**:1423–1426.

Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, O'Donnell-Luria AH, Ware JS, Hill AJ, Cummings BB, et al.; Exome Aggregation Consortium (2016) Analysis of protein-coding genetic variation in 60,706 humans. *Nature* **536**: 285–291.

Leppek K, Das R, and Barna M (2018) Functional 5′ UTR mRNA structures in eukaryotic translation regulation and how to find them. *Nat Rev Mol Cell Biol* **19**: 158–174.

Lester DH, Inglehearn CF, Bashir R, Ackford H, Esakowitz L, Jay M, Bird AC, Wright AF, Papiha SS, and Bhattacharya SS (1990) Linkage to D3S47 (C17) in one large autosomal dominant retinitis pigmentosa family and exclusion in another: confirmation of genetic heterogeneity. *Am J Hum Genet* **47**:536–541.

Li P, Zhang HZ, Huff S, Nimmakayalu M, Qumsiyeh M, Yu J, Szekely A, Xu T, and Pober BR (2006) Karyotype-phenotype insights from 11q14.1-q23.2 interstitial deletions: FZD4 haploinsufficiency and exudative vitreoretinopathy in a patient with a complex chromosome rearrangement. *Am J Med Genet A* **140**:2721–2729.

Li Y, Peng J, Li J, Zhang Q, Li J, Zhang X, Fei P, She K, and Zhao P (2018) The characteristics of digenic familial exudative vitreoretinopathy. *Graefes Arch Clin Exp Ophthalmol* **256**:2149–2156.

Liaw CW and Connolly DT (2009) Sequence polymorphisms provide a common consensus sequence for GPR41 and GPR42. *DNA Cell Biol* **28**:555–560.

Liebscher I, Schöneberg T, and Prömel S (2013) Progress in demystification of adhesion G protein-coupled receptors. *Biol Chem* **394**:937–950.

Lin HV, Efanov AM, Fang X, Beavers LS, Wang X, Wang J, Gonzalez Valcarcel IC, and Ma T (2016) GPR142 controls tryptophan-induced insulin and incretin hormone secretion to improve glucose metabolism. *PLoS One* **11**:e0157298.

Liu C, Widen SA, Williamson KA, Ratnapriya R, Gerth-Kahlert C, Rainger J, Alur RP, Strachan E, Manjunath SH, Balakrishnan A, et al.; UK10K Consortium (2016) A secreted WNT-ligand-binding domain of FZD5 generated by a frameshift mutation causes autosomal dominant coloboma. *Hum Mol Genet* **25**:1382–1391.

Liu R, Paxton WA, Choe S, Ceradini D, Martin SR, Horuk R, MacDonald ME, Stuhlmann H, Koup RA, and Landau NR (1996) Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. *Cell* **86**:367–377.

Lotta LA, Mokrosinski J, Mendes de Oliveira E, Li C, Sharp SJ, Luan J, Brouwers B, Ayinampudi V, Bowker N, Kerrison N, et al. (2019) Human gain-of-function MC4R variants show signaling bias and protect against obesity. *Cell* **177**:597–607.e9.

Lu S, Liu S, Wietelmann A, Kojonazarov B, Atzberger A, Tang C, Schermuly RT, Gröne HJ, and Offermanns S (2017) Developmental vascular remodeling defects and postnatal kidney failure in mice lacking Gpr116 (Adgrf5) and Eltd1 (Adgrl4). *PLoS One* **12**:e0183166.

Luo J, Sun P, Siwko S, Liu M, and Xiao J (2019) The role of GPCRs in bone diseases and dysfunctions. *Bone Res* **7**:19.

Luo R, Jin Z, Deng Y, Strokes N, and Piao X (2012) Disease-associated mutations prevent GPR56-collagen III interaction. *PLoS One* **7**:e29818.

Lv X, Liu J, Shi Q, Tan Q, Wu D, Skinner JJ, Walker AL, Zhao L, Gu X, Chen N, et al. (2016) In vitro expression and analysis of the 826 human G protein-coupled receptors. *Protein Cell* **7**:325–337.
MacArthur DG, Balasubramanian S, Frankish A, Huang N, Morris J, Walter K, Jostins L, Habegger L, Pickrell JK, Montgomery SB, et al.; 1000 Genomes Project Consortium (2012) A systematic survey of loss-of-function variants in human protein-coding genes. *Science* **335**:823–828.
Mackie DI, Al Mutairi F, Davis RB, Kechele DO, Nielsen NR, Snyder JC, Caron MG, Kliman HJ, Berg JS, Simms J, et al. (2018) h*CALCRL* mutation causes autosomal recessive nonimmune hydrops fetalis with lymphatic dysplasia. *J Exp Med* **215**: 2339–2353.
Makita N, Sato J, Manaka K, Shoji Y, Oishi A, Hashimoto M, Fujita T, and Iiri T (2007) An acquired hypocalciuric hypercalcemia autoantibody induces allosteric transition among active human Ca-sensing receptor conformations. *Proc Natl Acad Sci USA* **104**:5443–5448.
Makita N, Sato T, Yajima-Shoji Y, Sato J, Manaka K, Eda-Hashimoto M, Ootaki M, Matsumoto N, Nangaku M, and Iiri T (2016) Analysis of the V2 vasopressin receptor (V2R) mutations causing partial nephrogenic diabetes insipidus highlights a sustainable signaling by a non-peptide V2R agonist. *J Biol Chem* **291**:22460–22471.
Malik V, Rodino-Klapac LR, Viollet L, Wall C, King W, Al-Dahhak R, Lewis S, Shilling CJ, Kota J, Serrano-Munuera C, et al. (2010) Gentamicin-induced readthrough of stop codons in Duchenne muscular dystrophy. *Ann Neurol* **67**:771–780.
Marano LA, Marcorin L, Castelli EC, and Mendes-Junior CT (2017) Evaluation of MC1R high-throughput nucleotide sequencing data generated by the 1000 Genomes Project. *Genet Mol Biol* **40**:530–539.
Marchese A, Nguyen T, Malik P, Xu S, Cheng R, Xie Z, Heng HH, George SR, Kolakowski LF Jr, and O'Dowd BF (1998) Cloning genes encoding receptors related to chemoattractant receptors. *Genomics* **50**:281–286.
Marcinkowski P, Hoyer I, Specker E, Furkert J, Rutz C, Neuenschwander M, Sobottka S, Sun H, Nazare M, Berchner-Pfannschmidt U, et al. (2019) A new highly thyrotropin receptor-selective small-molecule antagonist with potential for the treatment of graves' orbitopathy. *Thyroid* **29**:111–123.
Marín YE and Chen S (2004) Involvement of metabotropic glutamate receptor 1, a G protein coupled receptor, in melanoma development. *J Mol Med (Berl)* **82**:735–749.
Markovic D and Challiss RA (2009) Alternative splicing of G protein-coupled receptors: physiology and pathophysiology. *Cell Mol Life Sci* **66**:3337–3352.
Martin AL, Steurer MA, and Aronstam RS (2015) Constitutive activity among orphan class-A G protein coupled receptors. *PLoS One* **10**:e0138463.
Martin MM, Garcia JA, McFarland JD, Duffy AA, Gregson JP, and Elton TS (2003) Translation of the human angiotensin II type 1 receptor mRNA is mediated by a highly efficient internal ribosome entry site. *Mol Cell Endocrinol* **212**:51–61.
Martínez-Cadenas C, López S, Ribas G, Flores C, García O, Sevilla A, Smith-Zubiaga I, Ibarrola-Villaba M, Pino-Yanes MdelM, Gardeazabal J, et al. (2013) Simultaneous purifying selection on the ancestral MC1R allele and positive selection on the melanoma-risk allele V60L in south Europeans. *Mol Biol Evol* **30**:2654–2665.
Matharu N, Rattanasopha S, Tamura S, Maliskova L, Wang Y, Bernard A, Hardin A, Eckalbar WL, Vaisse C, and Ahituv N (2019) CRISPR-mediated activation of a promoter or enhancer rescues obesity caused by haploinsufficiency. *Science* **363**:eaau0629.
Matsumoto M, Straub RE, Marenco S, Nicodemus KK, Matsumoto S, Fujikawa A, Miyoshi S, Shobo M, Takahashi S, Yarimizu J, et al. (2008) The evolutionarily conserved G protein-coupled receptor SREB2/GPR85 influences brain size, behavior, and vulnerability to schizophrenia. *Proc Natl Acad Sci USA* **105**:6133–6138.
Matsumoto S, Yamazaki C, Masumoto KH, Nagano M, Naito M, Soga T, Hiyama H, Matsumoto M, Takasaki J, Kamohara M, et al. (2006) Abnormal development of the olfactory bulb and reproductive system in mice lacking prokineticin receptor PKR2. *Proc Natl Acad Sci USA* **103**:4140–4145.
May LT, Avlani VA, Sexton PM, and Christopoulos A (2004) Allosteric modulation of G protein-coupled receptors. *Curr Pharm Des* **10**:2003–2013.
Mayeur H, Roche O, Vêtu C, Jaliffa C, Marchant D, Dollfus H, Bonneau D, Munier FL, Schorderet DF, Levin AV, et al. (2006) Eight previously unidentified mutations found in the OA1 ocular albinism gene. *BMC Med Genet* **7**:41.
Mayr C (2016) Evolution and biological roles of alternative 3'UTRs. *Trends Cell Biol* **26**:227–237.
Mayr C (2017) Regulation by 3′-untranslated regions. *Annu Rev Genet* **51**:171–194.
McCormack SE, Li D, Kim YJ, Lee JY, Kim SH, Rapaport R, and Levine MA (2017) Digenic inheritance of PROKR2 and WDR11 mutations in pituitary stalk interruption syndrome. *J Clin Endocrinol Metab* **102**:2501–2507.
McFarland KC, Sprengel R, Phillips HS, Köhler M, Rosemblit N, Nikolics K, Segaloff DL, and Seeburg PH (1989) Lutropin-choriogonadotropin receptor: an unusual member of the G protein-coupled receptor family. *Science* **245**:494–499.
McGee J, Goodyear RJ, McMillan DR, Stauffer EA, Holt JR, Locke KG, Birch DG, Legan PK, White PC, Walsh EJ, et al. (2006) The very large G-protein-coupled receptor VLGR1: a component of the ankle link complex required for the normal development of auditory hair bundles. *J Neurosci* **26**:6543–6553.
McLysaght A, Makino T, Grayton HM, Tropeano M, Mitchell KJ, Vassos E, and Collier DA (2014) Ohnologs are overrepresented in pathogenic copy number mutations. *Proc Natl Acad Sci USA* **111**:361–366.
McManus KF, Taravella AM, Henn BM, Bustamante CD, Sikora M, and Cornejo OE (2017) Population genetic analysis of the DARC locus (Duffy) reveals adaptation from standing variation associated with malaria resistance in humans. *PLoS Genet* **13**:e1006560.
Medeiros-Neto G, de Lacerda L, and Wondisford FE (1997) Familial congenital hypothyroidism caused by abnormal and bioinactive TSH due to mutations in the beta-subunit gene. *Trends Endocrinol Metab* **8**:15–20.
Miller LJ, Holicky EL, Ulrich CD, and Wieben ED (1995) Abnormal processing of the human cholecystokinin receptor gene in association with gallstones and obesity. *Gastroenterology* **109**:1375–1380.
Miller LM, Gragg M, Kim TG, and Park PS (2015) Misfolded opsin mutants display elevated β-sheet structure. *FEBS Lett* **589**:3119–3125.
Milligan G, Ward RJ, and Marsango S (2019) GPCR homo-oligomerization. *Curr Opin Cell Biol* **57**:40–47.
Mogha A, Benesh AE, Patra C, Engel FB, Schöneberg T, Liebscher I, and Monk KR (2013) Gpr126 functions in Schwann cells to control differentiation and myelination via G-protein activation. *J Neurosci* **33**:17976–17985.
Mogil JS, Ritchie J, Smith SB, Strasburg K, Kaplan L, Wallace MR, Romberg RR, Bijl H, Sarton EY, Fillingim RB, et al. (2005) Melanocortin-1 receptor gene variants affect pain and mu-opioid analgesia in mice and humans. *J Med Genet* **42**:583–587.
Moia S, Godi M, Walker GE, Roccio M, Agretti P, Tonacchera M, Berardi R, Bellone S, Prodam F, Giordano M, et al. (2013) The W520X mutation in the TSHR gene brings on subclinical hypothyroidism through an haploinsufficiency mechanism. *J Endocrinol Invest* **36**:716–721.
Möller I, Murali R, Müller H, Wiesner T, Jackett LA, Scholz SL, Cosgarea I, van de Nes JA, Sucker A, Hillen U, et al. (2017) Activating cysteinyl leukotriene receptor 2 (CYSLTR2) mutations in blue nevi. *Mod Pathol* **30**:350–356.
Monk KR, Hamann J, Langenhan T, Nijmeijer S, Schöneberg T, and Liebscher I (2015) Adhesion G protein-coupled receptors: from in vitro pharmacology to in vivo mechanisms. *Mol Pharmacol* **88**:617–623.
Monk KR, Naylor SG, Glenn TD, Mercurio S, Perlin JR, Dominguez C, Moens CB, and Talbot WS (2009) A G protein-coupled receptor is essential for Schwann cells to initiate myelination. *Science* **325**:1402–1405.
Montaner S, Kufareva I, Abagyan R, and Gutkind JS (2013) Molecular mechanisms deployed by virally encoded G protein-coupled receptors in human diseases. *Annu Rev Pharmacol Toxicol* **53**:331–354.
Moore AR, Ceraudo E, Sher JJ, Guan Y, Shoushtari AN, Chang MT, Zhang JQ, Walczak EG, Kazmi MA, Taylor BS, et al. (2016) Recurrent activating mutations of G-protein-coupled receptor CYSLTR2 in uveal melanoma. *Nat Genet* **48**:675–680.
Morello JP, Salahpour A, Petäjä-Repo UE, Laperrière A, Lonergan M, Arthus MF, Nabi IR, Bichet DG, and Bouvier M (2001) Association of calnexin with wild type and mutant AVPR2 that causes nephrogenic diabetes insipidus. *Biochemistry* **40**: 6766–6775.
Morimura H, Saindelle-Ribeaudeau F, Berson EL, and Dryja TP (1999) Mutations in RGR, encoding a light-sensitive opsin homologue, in patients with retinitis pigmentosa. *Nat Genet* **23**:393–394.
Morri M, Sanchez-Romero I, Tichy AM, Kainrath S, Gerrard EJ, Hirschfeld PP, Schwarz J, and Janovjak H (2018) Optical functionalization of human Class A orphan G-protein-coupled receptors. *Nat Commun* **9**:1950.
Mouillac B and Mendre C (2018) Pharmacological chaperones as potential therapeutic strategies for misfolded mutant vasopressin receptors. *Handb Exp Pharmacol* **245**: 63–83.
Mullighan CG, Collins-Underwood JR, Phillips LA, Loudin MG, Liu W, Zhang J, Ma J, Coustan-Smith E, Harvey RC, Willman CL, et al. (2009) Rearrangement of CRLF2 in B-progenitor- and Down syndrome-associated acute lymphoblastic leukemia. *Nat Genet* **41**:1243–1246.
Mundell SJ, Rabbolini D, Gabrielli S, Chen Q, Aungraheeta R, Hutchinson JL, Kilo T, Mackay J, Ward CM, Stevenson W, et al. (2018) Receptor homodimerization plays a critical role in a novel dominant negative P2RY12 variant identified in a family with severe bleeding. *J Thromb Haemost* **16**:44–53.
Mutyam V, Du M, Xue X, Keeling KM, White EL, Bostwick JR, Rasmussen L, Liu B, Mazur M, Hong JS, et al. (2016) Discovery of clinically approved agents that promote suppression of cystic fibrosis transmembrane conductance regulator nonsense mutations. *Am J Respir Crit Care Med* **194**:1092–1103.
Naash MI, Hollyfield JG, al-Ubaidi MR, and Baehr W (1993) Simulation of human autosomal dominant retinitis pigmentosa in transgenic mice expressing a mutated murine opsin gene. *Proc Natl Acad Sci USA* **90**:5499–5503.
Nagel-Wolfrum K, Möller F, Penner I, Baasov T, and Wolfrum U (2016) Targeting nonsense mutations in diseases with translational read-through-inducing drugs (TRIDs). *BioDrugs* **30**:49–74.
Nathans J and Hogness DS (1984) Isolation and nucleotide sequence of the gene encoding human rhodopsin. *Proc Natl Acad Sci USA* **81**:4851–4855.
Nathans J, Maumenee IH, Zrenner E, Sadowski B, Sharpe LT, Lewis RA, Hansen E, Rosenberg T, Schwartz M, Heckenlively JR, et al. (1993) Genetic heterogeneity among blue-cone monochromats. *Am J Hum Genet* **53**:987–1000.
Neumann S, Huang W, Eliseeva E, Titus S, Thomas CJ, and Gershengorn MC (2010a) A small molecule inverse agonist for the human thyroid-stimulating hormone receptor. *Endocrinology* **151**:3454–3459.
Neumann S, Raaka BM, and Gershengorn MC (2010b) Constitutively active thyrotropin and thyrotropin-releasing hormone receptors and their inverse agonists. *Methods Enzymol* **485**:147–160.
Newton CL and Anderson RC (2018) Pharmacoperones for misfolded gonadotropin receptors. *Handb Exp Pharmacol* **245**:111–134.
Ng PC and Henikoff S (2003) SIFT: predicting amino acid changes that affect protein function. *Nucleic Acids Res* **31**:3812–3814.
Nikolaev SI, Garieri M, Santoni F, Falconnet E, Ribaux P, Guipponi M, Murray A, Groet J, Giarin E, Basso G, et al. (2014) Frequent cases of RAS-mutated Down syndrome acute lymphoblastic leukaemia lack JAK2 mutations. *Nat Commun* **5**: 4654.
Noda H, Guo J, Khatri A, Dean T, Reyes M, Armanini M, Brooks DJ, Martins JS, Schipani E, Bouxsein ML, et al. (2020) An inverse agonist ligand of the PTH receptor partially rescues skeletal defects in a mouse model of Jansen's metaphyseal chondrodysplasia. *J Bone Miner Res* **35**:540–549.
Nomura Y, Onigata K, Nagashima T, Yutani S, Mochizuki H, Nagashima K, and Morikawa A (1997) Detection of skewed X-inactivation in two female carriers of vasopressin type 2 receptor gene mutation. *J Clin Endocrinol Metab* **82**:3434–3437.
Novembre J, Galvani AP, and Slatkin M (2005) The geographic spread of the CCR5 Delta32 HIV-resistance allele. *PLoS Biol* **3**:e339.
Okashah N, Wan Q, Ghosh S, Sandhu M, Inoue A, Vaidehi N, and Lambert NA (2019) Variable G protein determinants of GPCR coupling selectivity. *Proc Natl Acad Sci USA* **116**:12054–12059.
Olesen ET, Rützler MR, Moeller HB, Praetorius HA, and Fenton RA (2011) Vasopressin-independent targeting of aquaporin-2 by selective E-prostanoid receptor agonists alleviates nephrogenic diabetes insipidus. *Proc Natl Acad Sci USA* **108**:12949–12954.

O'Reilly M, Palfi A, Chadderton N, Millington-Ward S, Ader M, Cronin T, Tuohy T, Auricchio A, Hildinger M, Tivnan A, et al. (2007) RNA interference-mediated suppression and replacement of human rhodopsin in vivo. *Am J Hum Genet* **81**:127–135.
Panigrahi P, Jere A, and Anamika K (2018) FusionHub: a unified web platform for annotation and visualization of gene fusion events in human cancer. *PLoS One* **13**: e0196588.
Pantel J, Legendre M, Cabrol S, Hilal L, Hajaji Y, Morisset S, Nivot S, Vie-Luton MP, Grouselle D, de Kerdanet M, et al. (2006) Loss of constitutive activity of the growth hormone secretagogue receptor in familial short stature. *J Clin Invest* **116**:760–768.
Parasol N, Reid M, Rios M, Castilho L, Harari I, and Kosower NS (1998) A novel mutation in the coding sequence of the FY*B allele of the Duffy chemokine receptor gene is associated with an altered erythrocyte phenotype. *Blood* **92**:2237–2243.
Park D, Tosello-Trampont AC, Elliott MR, Lu M, Haney LB, Ma Z, Klibanov AL, Mandell JW, and Ravichandran KS (2007) BAI1 is an engulfment receptor for apoptotic cells upstream of the ELMO/Dock180/Rac module. *Nature* **450**:430–434.
Park PS (2014) Constitutively active rhodopsin and retinal disease. *Adv Pharmacol* **70**:1–36.
Park PS (2019) Rhodopsin oligomerization and aggregation. *J Membr Biol* **252**: 413–423.
Parma J, Duprez L, Van Sande J, Cochaux P, Gervy C, Mockel J, Dumont J, and Vassart G (1993) Somatic mutations in the thyrotropin receptor gene cause hyperfunctioning thyroid adenomas. *Nature* **365**:649–651.
Parmentier M, Libert F, Maenhaut C, Lefort A, Gérard C, Perret J, Van Sande J, Dumont JE, and Vassart G (1989) Molecular cloning of the thyrotropin receptor. *Science* **246**:1620–1622.
Parnot C, Miserey-Lenkei S, Bardin S, Corvol P, and Clauser E (2002) Lessons from constitutively active mutants of G protein-coupled receptors. *Trends Endocrinol Metab* **13**:336–343.
Parola AL and Kobilka BK (1994) The peptide product of a 5′ leader cistron in the beta 2 adrenergic receptor mRNA inhibits receptor synthesis. *J Biol Chem* **269**: 4497–4505.
Parry DA, Smith CE, El-Sayed W, Poulter JA, Shore RC, Logan CV, Mogi C, Sato K, Okajima F, Harada A, et al. (2016) Mutations in the pH-sensing G-protein-coupled receptor GPR68 cause amelogenesis imperfecta. *Am J Hum Genet* **99**:984–990.
Parsons SJH, Wright NB, Burkitt-Wright E, Skae MS, and Murray PG (2017) A heterozygous microdeletion of 20p12.2-3 encompassing PROKR2 and BMP2 in a patient with congenital hypopituitarism and growth hormone deficiency. *Am J Med Genet A* **173**:2261–2267.
Pasternack SM, von Kügelgen I, Al Aboud K, Lee YA, Rüschendorf F, Voss K, Hillmer AM, Molderings GJ, Franz T, Ramirez A, et al. (2008) G protein-coupled receptor P2Y5 and its ligand LPA are involved in maintenance of human hair growth. *Nat Genet* **40**:329–334.
Patat O, Pagin A, Siegfried A, Mitchell V, Chassaing N, Faguer S, Monteil L, Gaston V, Bujan L, Courtade-Saïdi M, et al. (2016) Truncating mutations in the adhesion G protein-coupled receptor G2 gene ADGRG2 cause an X-linked congenital bilateral absence of vas deferens. *Am J Hum Genet* **99**:437–442.
Patra C, van Amerongen MJ, Ghosh S, Ricciardi F, Sajjad A, Novoyatleva T, Mogha A, Monk KR, Mühlfeld C, and Engel FB (2013) Organ-specific function of adhesion G protein-coupled receptor GPR126 is domain-dependent. *Proc Natl Acad Sci USA* **110**:16898–16903.
Peachey NS, Ray TA, Florijn R, Rowe LB, Sjoerdsma T, Contreras-Alcantara S, Baba K, Tosini G, Pozdeyev N, Iuvone PM, et al. (2012) GPR179 is required for depolarizing bipolar cell function and is mutated in autosomal-recessive complete congenital stationary night blindness. *Am J Hum Genet* **90**:331–339.
Pei B, Sisu C, Frankish A, Howald C, Habegger L, Mu XJ, Harte R, Balasubramanian S, Tanzer A, Diekhans M, et al. (2012) The GENCODE pseudogene resource. *Genome Biol* **13**:R51.
Peng Y, Meng Y, Wang Z, Qin M, Li X, Dian Y, and Huang S (2009) A novel GPR143 duplication mutation in a Chinese family with X-linked congenital nystagmus. *Mol Vis* **15**:810–814.
Petersen SC, Luo R, Liebscher I, Giera S, Jeong SJ, Mogha A, Ghidinelli M, Feltri ML, Schöneberg T, Piao X, et al. (2015) The adhesion GPCR GPR126 has distinct, domain-dependent functions in Schwann cell development mediated by interaction with laminin-211. *Neuron* **85**:755–769.
Piao X, Hill RS, Bodell A, Chang BS, Basel-Vanagaite L, Straussberg R, Dobyns WB, Qasrawi B, Winter RM, Innes AM, et al. (2004) G protein-coupled receptor-dependent development of human frontal cortex. *Science* **303**:2033–2036.
Ploier B, Caro LN, Morizumi T, Pandey K, Pearring JN, Goren MA, Finnemann SC, Graumann J, Arshavsky VY, Dittman JS, et al. (2016) Dimerization deficiency of enigmatic retinitis pigmentosa-linked rhodopsin mutants. *Nat Commun* **7**:12832.
Plotz PH (2003) The autoantibody repertoire: searching for order. *Nat Rev Immunol* **3**:73–78.
Pollak MR, Brown EM, Chou YH, Hebert SC, Marx SJ, Steinmann B, Levi T, Seidman CE, and Seidman JG (1993) Mutations in the human Ca(2+)-sensing receptor gene cause familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism. *Cell* **75**:1297–1303.
Pollak MR, Brown EM, Estep HL, McLaine PN, Kifor O, Park J, Hebert SC, Seidman CE, and Seidman JG (1994) Autosomal dominant hypocalcaemia caused by a Ca(2+)-sensing receptor gene mutation. *Nat Genet* **8**:303–307.
Prömel S, Frickenhaus M, Hughes S, Mestek L, Staunton D, Woollard A, Vakonakis I, Schöneberg T, Schnabel R, Russ AP, et al. (2012) The GPS motif is a molecular switch for bimodal activities of adhesion class G protein-coupled receptors. *Cell Rep* **2**:321–331.
Puffenberger EG, Hosoda K, Washington SS, Nakao K, deWit D, Yanagisawa M, and Chakravart A (1994) A missense mutation of the endothelin-B receptor gene in multigenic Hirschsprung's disease. *Cell* **79**:1257–1266.
Puhl HL III, Won YJ, Lu VB, and Ikeda SR (2015) Human GPR42 is a transcribed multisite variant that exhibits copy number polymorphism and is functional when heterologously expressed. *Sci Rep* **5**:12880.
Purcell RH, Toro C, Gahl WA, and Hall RA (2017) A disease-associated mutation in the adhesion GPCR BAI2 (ADGRB2) increases receptor signaling activity. *Hum Mutat* **38**:1751–1760.
Qu X, Xiao D, and Weber HC (2004) Transcriptional activation of the human gastrin-releasing peptide receptor gene in gastrointestinal and prostatic epithelial cancer cells. *J Mol Neurosci* **22**:75–82.
Rao VR, Cohen GB, and Oprian DD (1994) Rhodopsin mutation G90D and a molecular mechanism for congenital night blindness. *Nature* **367**:639–642.
Rappaport N, Twik M, Plaschkes I, Nudel R, Iny Stein T, Levitt J, Gershoni M, Morrey CP, Safran M, and Lancet D (2017) MalaCards: an amalgamated human disease compendium with diverse clinical and genetic annotation and structured search. *Nucleic Acids Res* **45**:D877–D887.
Ravenscroft G, Nolent F, Rajagopalan S, Meireles AM, Paavola KJ, Gaillard D, Alanio E, Buckland M, Arbuckle S, Krivanek M, et al. (2015) Mutations of GPR126 are responsible for severe arthrogryposis multiplex congenita. *Am J Hum Genet* **96**: 955–961.
Rees Smith B, McLachlan SM, and Furmaniak J (1988) Autoantibodies to the thyrotropin receptor. *Endocr Rev* **9**:106–121.
Reiter E, Ahn S, Shukla AK, and Lefkowitz RJ (2012) Molecular mechanism of β-arrestin-biased agonism at seven-transmembrane receptors. *Annu Rev Pharmacol Toxicol* **52**:179–197.
Restagno G, Maghtheh M, Bhattacharya S, Ferrone M, Garnerone S, Samuelly R, and Carbonara A (1993) A large deletion at the 3′ end of the rhodopsin gene in an Italian family with a diffuse form of autosomal dominant retinitis pigmentosa. *Hum Mol Genet* **2**:207–208.
Ridge KD, Lee SS, and Yao LL (1995) In vivo assembly of rhodopsin from expressed polypeptide fragments. *Proc Natl Acad Sci USA* **92**:3204–3208.
Riedmayr LM, Böhm S, Biel M, and Becirovic E (2020) Enigmatic rhodopsin mutation creates an exceptionally strong splice acceptor site. *Hum Mol Genet* **29**:295–304.
Rivero-Müller A, Chou YY, Ji I, Lajic S, Hanyaloglu AC, Jonas K, Rahman N, Ji TH, and Huhtaniemi I (2010) Rescue of defective G protein-coupled receptor function in vivo by intermolecular cooperation. *Proc Natl Acad Sci USA* **107**:2319–2324.
Robbins LS, Nadeau JH, Johnson KR, Kelly MA, Roselli-Rehfuss L, Baack E, Mountjoy KG, and Cone RD (1993) Pigmentation phenotypes of variant extension locus alleles result from point mutations that alter MSH receptor function. *Cell* **72**: 827–834.
Robinson A, Escuin S, Doudney K, Vekemans M, Stevenson RE, Greene ND, Copp AJ, and Stanier P (2012) Mutations in the planar cell polarity genes CELSR1 and SCRIB are associated with the severe neural tube defect craniorachischisis. *Hum Mutat* **33**:440–447.
Robitaille J, MacDonald ML, Kaykas A, Sheldahl LC, Zeisler J, Dubé MP, Zhang LH, Singaraja RR, Guernsey DL, Zheng B, et al. (2002) Mutant frizzled-4 disrupts retinal angiogenesis in familial exudative vitreoretinopathy. *Nat Genet* **32**:326–330.
Rodd C, Millette M, Iacovazzo D, Stiles CE, Barry S, Evanson J, Albrecht S, Caswell R, Bunce B, Jose S, et al. (2016) Somatic GPR101 duplication causing X-linked acrogigantism (XLAG)-diagnosis and management. *J Clin Endocrinol Metab* **101**: 1927–1930.
Rodien P, Brémont C, Sanson ML, Parma J, Van Sande J, Costagliola S, Luton JP, Vassart G, and Duprez L (1998) Familial gestational hyperthyroidism caused by a mutant thyrotropin receptor hypersensitive to human chorionic gonadotropin. *N Engl J Med* **339**:1823–1826.
Römpler H, Schulz A, Pitra C, Coop G, Przeworski M, Pääbo S, and Schöneberg T (2005) The rise and fall of the chemoattractant receptor GPR33. *J Biol Chem* **280**: 31068–31075.
Rosenthal W, Seibold A, Antaramian A, Lonergan M, Arthus MF, Hendy GN, Birnbaumer M, and Bichet DG (1992) Molecular identification of the gene responsible for congenital nephrogenic diabetes insipidus. *Nature* **359**:233–235.
Rudenko O, Shang J, Munk A, Ekberg JP, Petersen N, Engelstoft MS, Egerod KL, Hjorth SA, Wu M, Feng Y, et al. (2019) The aromatic amino acid sensor GPR142 controls metabolism through balanced regulation of pancreatic and gut hormones. *Mol Metab* **19**:49–64.
Saal HM, Prows CA, Guerreiro I, Donlin M, Knudson L, Sund KL, Chang CF, Brugmann SA, and Stottmann RW (2015) A mutation in FRIZZLED2 impairs Wnt signaling and causes autosomal dominant omodysplasia. *Hum Mol Genet* **24**: 3399–3409.
Saleheen D, Natarajan P, Armean IM, Zhao W, Rasheed A, Khetarpal SA, Won HH, Karczewski KJ, O'Donnell-Luria AH, Samocha KE, et al. (2017) Human knockouts and phenotypic analysis in a cohort with a high rate of consanguinity. *Nature* **544**: 235–239.
Salvatori R, Fan X, Mullis PE, Haile A, and Levine MA (2002) Decreased expression of the GHRH receptor gene due to a mutation in a Pit-1 binding site. *Mol Endocrinol* **16**:450–458.
Samocha KE, Robinson EB, Sanders SJ, Stevens C, Sabo A, McGrath LM, Kosmicki JA, Rehnström K, Mallick S, Kirby A, et al. (2014) A framework for the interpretation of de novo mutation in human disease. *Nat Genet* **46**:944–950.
Samson M, Libert F, Doranz BJ, Rucker J, Liesnard C, Farber CM, Saragosti S, Lapoumeroulie C, Cognaux J, Forceille C, et al. (1996) Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. *Nature* **382**:722–725.
Sandrock M, Schulz A, Merkwitz C, Schöneberg T, Spanel-Borowski K, and Ricken A (2009) Reduction in corpora lutea number in obese melanocortin-4-receptor-deficient mice. *Reprod Biol Endocrinol* **7**:24.
Sangkuhl K, Schulz A, Römpler H, Yun J, Wess J, and Schöneberg T (2004) Aminoglycoside-mediated rescue of a disease-causing nonsense mutation in the V2 vasopressin receptor gene in vitro and in vivo. *Hum Mol Genet* **13**:893–903.
Santos-Cortez RL, Faridi R, Rehman AU, Lee K, Ansar M, Wang X, Morell RJ, Isaacson R, Belyantseva IA, Dai H, et al.; University of Washington Center for Mendelian Genomics (2016) Autosomal-recessive hearing impairment due to rare missense variants within S1PR2. *Am J Hum Genet* **98**:331–338.
Sarfati J, Fouveaut C, Leroy C, Jeanpierre M, Hardelin JP, and Dodé C (2013) Greater prevalence of PROKR2 mutations in Kallmann syndrome patients from the Maghreb than in European patients. *Eur J Endocrinol* **169**:805–809.
Satoh M, Aso K, Ogikubo S, Yoshizawa-Ogasawara A, and Saji T (2015) Hypothyroidism caused by the combination of two heterozygous mutations: one in the TSH

receptor gene the other in the DUOX2 gene. *J Pediatr Endocrinol Metab* **28**: 657–661.
Schernthaner-Reiter MH, Adams D, Trivellin G, Ramnitz MS, Raygada M, Golas G, Faucz FR, Nilsson O, Nella AA, Dileepan K, et al. (2016) A novel AVPR2 splice site mutation leads to partial X-linked nephrogenic diabetes insipidus in two brothers. *Eur J Pediatr* **175**:727–733.
Schipani E, Kruse K, and Jüppner H (1995) A constitutively active mutant PTH-PTHrP receptor in Jansen-type metaphyseal chondrodysplasia. *Science* **268**:98–100.
Schmidt P, Ritscher L, Dong EN, Hermsdorf T, Cöster M, Wittkopf D, Meiler J, and Schöneberg T (2013) Identification of determinants required for agonistic and inverse agonistic ligand properties at the ADP receptor P2Y12. *Mol Pharmacol* **83**: 256–266.
Scholz N, Guan C, Nieberler M, Grotemeyer A, Maiellaro I, Gao S, Beck S, Pawlak M, Sauer M, Asan E, et al. (2017) Mechano-dependent signaling by Latrophilin/CIRL quenches cAMP in proprioceptive neurons. *eLife* **6**:e28360.
Schöneberg T, Kleinau G, and Brüser A (2016) What are they waiting for?-Tethered agonism in G protein-coupled receptors. *Pharmacol Res* **108**:9–15.
Schöneberg T, Liebscher I, Luo R, Monk KR, and Piao X (2015) Tethered agonists: a new mechanism underlying adhesion G protein-coupled receptor activation. *J Recept Signal Transduct Res* **35**:220–223.
Schöneberg T, Liu J, and Wess J (1995) Plasma membrane localization and functional rescue of truncated forms of a G protein-coupled receptor. *J Biol Chem* **270**: 18000–18006.
Schöneberg T, Meister J, Knierim AB, and Schulz A (2018) The G protein-coupled receptor GPR34 - the past 20 years of a grownup. *Pharmacol Ther* **189**:71–88.
Schöneberg T, Sandig V, Wess J, Gudermann T, and Schultz G (1997) Reconstitution of mutant V2 vasopressin receptors by adenovirus-mediated gene transfer. Molecular basis and clinical implication. *J Clin Invest* **100**:1547–1556.
Schöneberg T, Schulz A, Biebermann H, Grüters A, Grimm T, Hübschmann K, Filler G, Gudermann T, and Schultz G (1998) V2 vasopressin receptor dysfunction in nephrogenic diabetes insipidus caused by different molecular mechanisms. *Hum Mutat* **12**:196–205.
Schöneberg T, Schulz A, Biebermann H, Hermsdorf T, Römpler H, and Sangkuhl K (2004) Mutant G-protein-coupled receptors as a cause of human diseases. *Pharmacol Ther* **104**:173–206.
Schöneberg T, Yun J, Wenkert D, and Wess J (1996) Functional rescue of mutant V2 vasopressin receptors causing nephrogenic diabetes insipidus by a co-expressed receptor polypeptide. *EMBO J* **15**:1283–1291.
Schulz A, Sangkuhl K, Lennert T, Wigger M, Price DA, Nuuja A, Grüters A, Schultz G, and Schöneberg T (2002) Aminoglycoside pretreatment partially restores the function of truncated V(2) vasopressin receptors found in patients with nephrogenic diabetes insipidus. *J Clin Endocrinol Metab* **87**:5247–5257.
Seifert R and Wenzel-Seifert K (2002) Constitutive activity of G-protein-coupled receptors: cause of disease and common property of wild-type receptors. *Naunyn Schmiedebergs Arch Pharmacol* **366**:381–416.
Seminara SB, Messager S, Chatzidaki EE, Thresher RR, Acierno JS Jr, Shagoury JK, Bo-Abbas Y, Kuohung W, Schwinof KM, Hendrick AG, et al. (2003) The GPR54 gene as a regulator of puberty. *N Engl J Med* **349**:1614–1627.
Shenker A, Laue L, Kosugi S, Merendino JJ Jr, Minegishi T, and Cutler GB Jr (1993) A constitutively activating mutation of the luteinizing hormone receptor in familial male precocious puberty. *Nature* **365**:652–654.
Shi D, Komatsu K, Hirao M, Toyooka Y, Koyama H, Tissir F, Goffinet AM, Uemura T, and Fujimori T (2014) Celsr1 is required for the generation of polarity at multiple levels of the mouse oviduct. *Development* **141**:4558–4568.
Shi MW, Zhang NA, Shi CP, Liu CJ, Luo ZH, Wang DY, Guo AY, and Chen ZX (2019) SAGD: a comprehensive sex-associated gene database from transcriptomes. *Nucleic Acids Res* **47**:D835–D840.
Shim SH, Shim JS, Min K, Lee HS, Park JE, Park SH, Hwang E, and Kim M (2014) Siblings with opposite chromosome constitutions, dup(2q)/del(7q) and del(2q)/dup(7q). *Gene* **534**:100–106.
Shimomura Y, Wajid M, Ishii Y, Shapiro L, Petukhova L, Gordon D, and Christiano AM (2008) Disruption of P2RY5, an orphan G protein-coupled receptor, underlies autosomal recessive woolly hair. *Nat Genet* **40**:335–339.
Shin MK, Russell LB, and Tilghman SM (1997) Molecular characterization of four induced alleles at the Ednrb locus. *Proc Natl Acad Sci USA* **94**:13105–13110.
Shrimpton AE, Braddock BR, Thomson LL, Stein CK, and Hoo JJ (2004) Molecular delineation of deletions on 2q37.3 in three cases with an Albright hereditary osteodystrophy-like phenotype. *Clin Genet* **66**:537–544.
Simiele F, Recchiuti A, Mattoscio D, De Luca A, Cianci E, Franchi S, Gatta V, Parolari A, Werba JP, Camera M, et al. (2012) Transcriptional regulation of the human FPR2/ALX gene: evidence of a heritable genetic variant that impairs promoter activity. *FASEB J* **26**:1323–1333.
Smallwood PM, Wang Y, and Nathans J (2002) Role of a locus control region in the mutually exclusive expression of human red and green cone pigment genes. *Proc Natl Acad Sci USA* **99**:1008–1011.
Smith E, Janovick JA, Bannister TD, Shumate J, Scampavia L, Conn PM, and Spicer TP (2016) Identification of potential pharmacoperones capable of rescuing the functionality of misfolded vasopressin 2 receptor involved in nephrogenic diabetes insipidus. *J Biomol Screen* **21**:824–831.
Smoleń-Dzirba J, Rosińska M, Janiec J, Beniowski M, Cycoń M, Bratosiewicz-Wąsik J, and Wąsik TJ (2017) HIV-1 infection in persons homozygous for CCR5-Δ32 allele: the next case and the review. *AIDS Rev* **19**:219–230.
Sohara E, Rai T, Yang SS, Uchida K, Nitta K, Horita S, Ohno M, Harada A, Sasaki S, and Uchida S (2006) Pathogenesis and treatment of autosomal-dominant nephrogenic diabetes insipidus caused by an aquaporin 2 mutation. *Proc Natl Acad Sci USA* **103**:14217–14222.
Song X, Vishnivetskiy SA, Gross OP, Emelianoff K, Mendez A, Chen J, Gurevich EV, Burns ME, and Gurevich VV (2009) Enhanced arrestin facilitates recovery and protects rods lacking rhodopsin phosphorylation. *Curr Biol* **19**:700–705.
Spiegel AM and Weinstein LS (2004) Inherited diseases involving g proteins and g protein-coupled receptors. *Annu Rev Med* **55**:27–39.
Sposini S, Caltabiano G, Hanyaloglu AC, and Miele R (2015) Identification of transmembrane domains that regulate spatial arrangements and activity of prokineticin receptor 2 dimers. *Mol Cell Endocrinol* **399**:362–372.
Srinivasan S, Lubrano-Berthelier C, Govaerts C, Picard F, Santiago P, Conklin BR, and Vaisse C (2004) Constitutive activity of the melanocortin-4 receptor is maintained by its N-terminal domain and plays a role in energy homeostasis in humans. *J Clin Invest* **114**:1158–1164.
Sriram K, Moyung K, Corriden R, Carter H, and Insel PA (2019) GPCRs show widespread differential mRNA expression and frequent mutation and copy number variation in solid tumors. *PLoS Biol* **17**:e3000434.
Steagall WK, Barrow BJ, Glasgow CG, Mendoza JW, Ehrmantraut M, Lin JP, Insel PA, and Moss J (2007) Beta-2-adrenergic receptor polymorphisms in cystic fibrosis. *Pharmacogenet Genomics* **17**:425–430.
Stenson PD, Ball EV, Mort M, Phillips AD, Shaw K, and Cooper DN (2012) The Human Gene Mutation Database (HGMD) and its exploitation in the fields of personalized genomics and molecular evolution. *Curr Protoc Bioinformatics* **Chapter 1**: Unit1.13.
Stephens JC, Reich DE, Goldstein DB, Shin HD, Smith MW, Carrington M, Winkler C, Huttley GA, Allikmets R, Schriml L, et al. (1998) Dating the origin of the CCR5-Delta32 AIDS-resistance allele by the coalescence of haplotypes. *Am J Hum Genet* **62**:1507–1515.
Stoy H and Gurevich VV (2015) How genetic errors in GPCRs affect their function: possible therapeutic strategies. *Genes Dis* **2**:108–132.
Styrkarsdottir U, Thorleifsson G, Sulem P, Gudbjartsson DF, Sigurdsson A, Jonasdottir A, Jonasdottir A, Oddsson A, Helgason A, Magnusson OT, et al. (2013) Nonsense mutation in the LGR4 gene is associated with several human diseases and other traits. *Nature* **497**:517–520.
Sun H, Guo Y, Lan X, Jia J, Cai X, Zhang G, Xie J, Liang Q, Li Y, and Yu G (2020) PhenoModifier: a genetic modifier database for elucidating the genetic basis of human phenotypic variation. *Nucleic Acids Res* **48**:D977–D982.
Sunthornthepvarakul T, Gottschalk ME, Hayashi Y, and Refetoff S (1995) Brief report: resistance to thyrotropin caused by mutations in the thyrotropin-receptor gene. *N Engl J Med* **332**:155–160.
Swords FM, Baig A, Malchoff DM, Malchoff CD, Thorner MO, King PJ, Hunyady L, and Clark AJ (2002) Impaired desensitization of a mutant adrenocorticotropin receptor associated with apparent constitutive activity. *Mol Endocrinol* **16**:2746–2753.
Tamasauskas D, Powell V, Saksela K, and Yazdanbakhsh K (2001) A homologous naturally occurring mutation in Duffy and CCR5 leading to reduced receptor expression. *Blood* **97**:3651–3654.
Tan JK, McKenzie C, Mariño E, Macia L, and Mackay CR (2017) Metabolite-sensing G protein-coupled receptors-facilitators of diet-related immune regulation. *Annu Rev Immunol* **35**:371–402.
Tang CM and Insel PA (2005) Genetic variation in G-protein-coupled receptors--consequences for G-protein-coupled receptors as drug targets. *Expert Opin Ther Targets* **9**:1247–1265.
Tang H, Shu C, Chen H, Zhang X, Zang Z, and Deng C (2019) Constitutively active BRS3 is a genuinely orphan GPCR in placental mammals. *PLoS Biol* **17**:e3000175.
Tang X, Jin R, Qu G, Wang X, Li Z, Yuan Z, Zhao C, Siwko S, Shi T, Wang P, et al. (2013) GPR116, an adhesion G-protein-coupled receptor, promotes breast cancer metastasis via the Gαq-p63RhoGEF-Rho GTPase pathway. *Cancer Res* **73**:6206–6218.
Tao YX (2008) Constitutive activation of G protein-coupled receptors and diseases: insights into mechanisms of activation and therapeutics. *Pharmacol Ther* **120**: 129–148.
Tao YX and Conn PM (2014) Chaperoning G protein-coupled receptors: from cell biology to therapeutics. *Endocr Rev* **35**:602–647.
Tao YX and Conn PM (2018) Pharmacoperones as novel therapeutics for diverse protein conformational diseases. *Physiol Rev* **98**:697–725.
Taqi MM, Faisal M, and Zaman H (2019) *OPRM1* A118G polymorphisms and its role in opioid addiction: implication on severity and treatment approaches. *Pharm Genomics Pers Med* **12**:361–368.
Tarnow P, Schöneberg T, Krude H, Grüters A, and Biebermann H (2003) Mutationally induced disulfide bond formation within the third extracellular loop causes melanocortin 4 receptor inactivation in patients with obesity. *J Biol Chem* **278**:48666–48673.
Teles MG, Bianco SD, Brito VN, Trarbach EB, Kuohung W, Xu S, Seminara SB, Mendonca BB, Kaiser UB, and Latronico AC (2008) A GPR54-activating mutation in a patient with central precocious puberty. *N Engl J Med* **358**:709–715.
Themmen APN and Huhtaniemi IT (2000) Mutations of gonadotropins and gonadotropin receptors: elucidating the physiology and pathophysiology of pituitary-gonadal function. *Endocr Rev* **21**:551–583.
Thompson MD, Cole DE, Capra V, Siminovitch KA, Rovati GE, Burnham WM, and Rana BK (2014) Pharmacogenetics of the G protein-coupled receptors. *Methods Mol Biol* **1175**:189–242.
Tischner D, Grimm M, Kaur H, Staudenraus D, Carvalho J, Looso M, Günther S, Wanke F, Moos S, Siller N, et al. (2017) Single-cell profiling reveals GPCR heterogeneity and functional patterning during neuroinflammation. *JCI Insight* **2**:e95063.
Tissir F, Bar I, Jossin Y, De Backer O, and Goffinet AM (2005) Protocadherin Celsr3 is crucial in axonal tract development [published correction appears in *Nat Neurosci* (2006) 9:147]. *Nat Neurosci* **8**:451–457.
Tissir F, Qu Y, Montcouquiol M, Zhou L, Komatsu K, Shi D, Fujimori T, Labeau J, Tyteca D, Courtoy P, et al. (2010) Lack of cadherins Celsr2 and Celsr3 impairs ependymal ciliogenesis, leading to fatal hydrocephalus. *Nat Neurosci* **13**:700–707.
Topaloglu AK, Reimann F, Guclu M, Yalin AS, Kotan LD, Porter KM, Serin A, Mungan NO, Cook JR, Imamoglu S, et al. (2009) TAC3 and TACR3 mutations in familial hypogonadotropic hypogonadism reveal a key role for Neurokinin B in the central control of reproduction. *Nat Genet* **41**:354–358.
Tournamille C, Colin Y, Cartron JP, and Le Van Kim C (1995) Disruption of a GATA motif in the Duffy gene promoter abolishes erythroid gene expression in Duffy-negative individuals. *Nat Genet* **10**:224–228.
Trivellin G, Daly AF, Faucz FR, Yuan B, Rostomyan L, Larco DO, Schernthaner-Reiter MH, Szarek E, Leal LF, Caberg JH, et al. (2014) Gigantism and acromegaly due to Xq26 microduplications and GPR101 mutation. *N Engl J Med* **371**:2363–2374.

Trivellin G, Sharwood E, Hijazi H, Carvalho CMB, Yuan B, Tatton-Brown K, Coman D, Lupski JR, Cotterill AM, Lodish MB, et al. (2018) Xq26.3 duplication in a boy with motor delay and low muscle tone refines the X-linked acrogigantism genetic locus. *J Endocr Soc* **2**:1100–1108.
Tsigos C, Arai K, Hung W, and Chrousos GP (1993) Hereditary isolated glucocorticoid deficiency is associated with abnormalities of the adrenocorticotropin receptor gene. *J Clin Invest* **92**:2458–2461.
Turki J, Pak J, Green SA, Martin RJ, and Liggett SB (1995) Genetic polymorphisms of the beta 2-adrenergic receptor in nocturnal and nonnocturnal asthma. Evidence that Gly16 correlates with the nocturnal phenotype. *J Clin Invest* **95**:1635–1641.
Vaisse C, Clément K, Guy-Grand B, and Froguel P (1998) A frameshift mutation in human MC4R is associated with a dominant form of obesity. *Nat Genet* **20**:113–114.
Valverde P, Healy E, Jackson I, Rees JL, and Thody AJ (1995) Variants of the melanocyte-stimulating hormone receptor gene are associated with red hair and fair skin in humans. *Nat Genet* **11**:328–330.
van der Westhuizen ET, Valant C, Sexton PM, and Christopoulos A (2015) Endogenous allosteric modulators of G protein-coupled receptors. *J Pharmacol Exp Ther* **353**:246–260.
Vargas-Poussou R, Huang C, Hulin P, Houillier P, Jeunemaître X, Paillard M, Planelles G, Déchaux M, Miller RT, and Antignac C (2002) Functional characterization of a calcium-sensing receptor mutation in severe autosomal dominant hypocalcemia with a Bartter-like syndrome. *J Am Soc Nephrol* **13**:2259–2266.
Vassart G and Costagliola S (2011) G protein-coupled receptors: mutations and endocrine diseases. *Nat Rev Endocrinol* **7**:362–372.
Venkatakrishnan AJ, Deupi X, Lebon G, Heydenreich FM, Flock T, Miljus T, Balaji S, Bouvier M, Veprintsev DB, Tate CG, et al. (2016) Diverse activation pathways in class A GPCRs converge near the G-protein-coupling region. *Nature* **536**:484–487.
Verheij JB, Kunze J, Osinga J, van Essen AJ, and Hofstra RM (2002) ABCD syndrome is caused by a homozygous mutation in the EDNRB gene. *Am J Med Genet* **108**:223–225.
Vezain M, Lecuyer M, Rubio M, Dupé V, Ratié L, David V, Pasquier L, Odent S, Coutant S, Tournier I, et al. (2018) A de novo variant in ADGRL2 suggests a novel mechanism underlying the previously undescribed association of extreme microcephaly with severely reduced sulcation and rhombencephalosynapsis. *Acta Neuropathol Commun* **6**:109.
Vischer HF, Siderius M, Leurs R, and Smit MJ (2014) Herpesvirus-encoded GPCRs: neglected players in inflammatory and proliferative diseases? *Nat Rev Drug Discov* **13**:123–139.
Wada A, Kunieda T, Nishimura M, Kakizoe-Ishida Y, Watanabe N, Ohkawa K, and Tsudzuki M (2005) A nucleotide substitution responsible for the tawny coat color mutation carried by the MSKR inbred strain of mice. *J Hered* **96**:145–149.
Wajnrajch MP, Gertner JM, Harbison MD, Chua SC Jr, and Leibel RL (1996) Nonsense mutation in the human growth hormone-releasing hormone receptor causes growth failure analogous to the little (lit) mouse. *Nat Genet* **12**:88–90.
Walter C and Lötsch J (2009) Meta-analysis of the relevance of the OPRM1 118A>G genetic variant for pain treatment. *Pain* **146**:270–275.
Wang Y, Huso D, Cahill H, Ryugo D, and Nathans J (2001) Progressive cerebellar, auditory, and esophageal dysfunction caused by targeted disruption of the frizzled-4 gene. *J Neurosci* **21**:4761–4771.
Wang Y, Macke JP, Merbs SL, Zack DJ, Klaunberg B, Bennett J, Gearhart J, and Nathans J (1992) A locus control region adjacent to the human red and green visual pigment genes. *Neuron* **9**:429–440.
Watanabe M, Houten SM, Mataki C, Christoffolete MA, Kim BW, Sato H, Messaddeq N, Harney JW, Ezaki O, Kodama T, et al. (2006) Bile acids induce energy expenditure by promoting intracellular thyroid hormone activation. *Nature* **439**:484–489.
Watson LM, Bamber E, Schnekenberg RP, Williams J, Bettencourt C, Lickiss J, Jayawant S, Fawcett K, Clokie S, Wallis Y, et al. (2017) Dominant mutations in GRM1 cause spinocerebellar ataxia type 44. *Am J Hum Genet* **101**:451–458.
Weber S, Thiele H, Mir S, Toliat MR, Sozeri B, Reutter H, Draaken M, Ludwig M, Altmüller J, Frommolt P, et al. (2011) Muscarinic acetylcholine receptor M3 mutation causes urinary bladder disease and a prune-belly-like syndrome. *Am J Hum Genet* **89**:668–674.
Weinstein LS, Chen M, Xie T, and Liu J (2006) Genetic diseases associated with heterotrimeric G proteins. *Trends Pharmacol Sci* **27**:260–266.
Weis WI and Kobilka BK (2018) The molecular basis of G protein-coupled receptor activation. *Annu Rev Biochem* **87**:897–919.
Weitl N and Seifert R (2008) Distinct interactions of human beta1- and beta2-adrenoceptors with isoproterenol, epinephrine, norepinephrine, and dopamine. *J Pharmacol Exp Ther* **327**:760–769.
Weitz CJ, Miyake Y, Shinzato K, Montag E, Zrenner E, Went LN, and Nathans J (1992) Human tritanopia associated with two amino acid substitutions in the blue-sensitive opsin. *Am J Hum Genet* **50**:498–507.
Welch EM, Barton ER, Zhuo J, Tomizawa Y, Friesen WJ, Trifillis P, Paushkin S, Patel M, Trotta CR, Hwang S, et al. (2007) PTC124 targets genetic disorders caused by nonsense mutations. *Nature* **447**:87–91.
Weng Z, Situ C, Lin L, Wu Z, Zhu J, and Zhang R (2019) Structure of BAI1/ELMO2 complex reveals an action mechanism of adhesion GPCRs via ELMO family scaffolds. *Nat Commun* **10**:51.
Weston MD, Luijendijk MW, Humphrey KD, Möller C, and Kimberling WJ (2004) Mutations in the VLGR1 gene implicate G-protein signaling in the pathogenesis of Usher syndrome type II. *Am J Hum Genet* **74**:357–366.
Winderickx J, Sanocki E, Lindsey DT, Teller DY, Motulsky AG, and Deeb SS (1992) Defective colour vision associated with a missense mutation in the human green visual pigment gene. *Nat Genet* **1**:251–256.
Wise H (2012) The roles played by highly truncated splice variants of G protein-coupled receptors. *J Mol Signal* **7**:13.
Wonerow P, Schöneberg T, Schultz G, Gudermann T, and Paschke R (1998) Deletions in the third intracellular loop of the thyrotropin receptor. A new mechanism for constitutive activation. *J Biol Chem* **273**:7900–7905.
Wootten D, Christopoulos A, Marti-Solano M, Babu MM, and Sexton PM (2018) Mechanisms of signalling and biased agonism in G protein-coupled receptors. *Nat Rev Mol Cell Biol* **19**:638–653.
Wu S, Wilson MD, Busby ER, Isaac ER, and Sherwood NM (2010) Disruption of the single copy gonadotropin-releasing hormone receptor in mice by gene trap: severe reduction of reproductive organs and functions in developing and adult mice. *Endocrinology* **151**:1142–1152.
Wu V, Yeerna H, Nohata N, Chiou J, Harismendy O, Raimondi F, Inoue A, Russell RB, Tamayo P, and Gutkind JS (2019) Illuminating the Onco-GPCRome: novel G protein-coupled receptor-driven oncocrine networks and targets for cancer immunotherapy. *J Biol Chem* **294**:11062–11086.
Xie J, Murone M, Luoh SM, Ryan A, Gu Q, Zhang C, Bonifas JM, Lam CW, Hynes M, Goddard A, et al. (1998) Activating Smoothened mutations in sporadic basal-cell carcinoma. *Nature* **391**:90–92.
Xiong HY, Alipanahi B, Lee LJ, Bretschneider H, Merico D, Yuen RK, Hua Y, Gueroussov S, Najafabadi HS, Hughes TR, et al. (2015) RNA splicing. The human splicing code reveals new insights into the genetic determinants of disease. *Science* **347**:1254806.
Xu G, Rabadan-Diehl C, Nikodemova M, Wynn P, Spiess J, and Aguilera G (2001) Inhibition of corticotropin releasing hormone type-1 receptor translation by an upstream AUG triplet in the 5′ untranslated region. *Mol Pharmacol* **59**:485–492.
Xu Q, Wang Y, Dabdoub A, Smallwood PM, Williams J, Woods C, Kelley MW, Jiang L, Tasman W, Zhang K, et al. (2004) Vascular development in the retina and inner ear: control by Norrin and Frizzled-4, a high-affinity ligand-receptor pair. *Cell* **116**:883–895.
Yagi H, Tokano H, Maeda M, Takabayashi T, Nagano T, Kiyama H, Fujieda S, Kitamura K, and Sato M (2007) Vlgr1 is required for proper stereocilia maturation of cochlear hair cells. *Genes Cells* **12**:235–250.
Yeo GS, Farooqi IS, Aminian S, Halsall DJ, Stanhope RG, and O'Rahilly S (1998) A frameshift mutation in MC4R associated with dominantly inherited human obesity. *Nat Genet* **20**:111–112.
Yi T, Weng J, Siwko S, Luo J, Li D, and Liu M (2014) LGR4/GPR48 inactivation leads to aniridia-genitourinary anomalies-mental retardation syndrome defects. *J Biol Chem* **289**:8767–8780.
Yoo Y, Jung J, Lee YN, Lee Y, Cho H, Na E, Hong J, Kim E, Lee JS, Lee JS, et al. (2017) GABBR2 mutations determine phenotype in rett syndrome and epileptic encephalopathy. *Ann Neurol* **82**:466–478.
Young D, Waitches G, Birchmeier C, Fasano O, and Wigler M (1986) Isolation and characterization of a new cellular oncogene encoding a protein with multiple potential transmembrane domains. *Cell* **45**:711–719.
Yu T, Li J, Li N, Liu R, Ding Y, Chang G, Chen Y, Shen Y, Wang X, and Wang J (2016) Obesity and developmental delay in a patient with uniparental disomy of chromosome 2. *Int J Obes* **40**:1935–1941.
Yun J, Schöneberg T, Liu J, Schulz A, Ecelbarger CA, Promeneur D, Nielsen S, Sheng H, Grinberg A, Deng C, et al. (2000) Generation and phenotype of mice harboring a nonsense mutation in the V2 vasopressin receptor gene. *J Clin Invest* **106**: 1361–1371.
Zhang J, Zhang K, Gao ZG, Paoletta S, Zhang D, Han GW, Li T, Ma L, Zhang W, Müller CE, et al. (2014) Agonist-bound structure of the human P2Y12 receptor. *Nature* **509**:119–122.
Zhao J, Li Y, Wang C, Zhang H, Zhang H, Jiang B, Guo X, and Song X (2020) IRESbase: a comprehensive database of experimentally validated internal ribosome entry sites. *Genomics Proteomics Bioinformatics* DOI: 10.1016/j.gpb.2020.03.001 [published ahead of print].
Zhao N, Peacock SO, Lo CH, Heidman LM, Rice MA, Fahrenholtz CD, Greene AM, Magani F, Copello VA, Martinez MJ, et al. (2019) Arginine vasopressin receptor 1a is a therapeutic target for castration-resistant prostate cancer. *Sci Transl Med* **11**: eaaw4636.
Zheng M, Mitra RN, Weiss ER, and Han Z (2020) Rhodopsin genomic loci DNA nanoparticles improve expression and rescue of retinal degeneration in a model for retinitis pigmentosa. *Mol Ther* **28**:523–535.
Zhou C, Dhall D, Nissen NN, Chen CR, and Yu R (2009) Homozygous P86S mutation of the human glucagon receptor is associated with hyperglucagonemia, alpha cell hyperplasia, and islet cell tumor. *Pancreas* **38**:941–946.
Zhou L, Bar I, Achouri Y, Campbell K, De Backer O, Hebert JM, Jones K, Kessaris N, de Rouvroit CL, O'Leary D, et al. (2008a) Early forebrain wiring: genetic dissection using conditional Celsr3 mutant mice. *Science* **320**:946–949.
Zhou P, Wang Z, Zhang J, Hu L, and Kong X (2008b) Identification of a novel GPR143 deletion in a Chinese family with X-linked congenital nystagmus. *Mol Vis* **14**: 1015–1019.
Zuo J, Currin A, Griffin BD, Shannon-Lowe C, Thomas WA, Ressing ME, Wiertz EJ, and Rowe M (2009) The Epstein-Barr virus G-protein-coupled receptor contributes to immune evasion by targeting MHC class I molecules for degradation. *PLoS Pathog* **5**:e1000255.

Supplements

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

Torsten Schöneberg and Ines Liebscher

Rudolf Schönheimer Institute of Biochemistry, Molecular Biochemistry, Medical Faculty, Johannisallee 30, 04103 Leipzig, Germany

*To whom correspondences should be addressed:
Torsten Schöneberg, schoberg@medizin.uni-leipzig.de

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 ϕGPR33). 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)****

References

Barnes MJ, Li CM, Xu Y, An J, Huang Y and Cyster JG (2015) The lysophosphatidylserine receptor GPR174 constrains regulatory T cell development and function. *J Exp Med* **212**:1011-1020.

Davies B, Baumann C, Kirchhoff C, Ivell R, Nubbemeyer R, Habenicht UF, Theuring F and Gottwald U (2004) Targeted deletion of the epididymal receptor HE6 results in fluid dysregulation and male infertility. *Mol Cell Biol* **24**:8642-8648.

Engel KM, Schrock K, Teupser D, Holdt LM, Tonjes A, Kern M, Dietrich K, Kovacs P, Krugel U, Scheidt HA, Schiller J, Huster D, Brockmann GA, Augustin M, Thiery J, Bluher M, Stumvoll M, Schoneberg T and Schulz A (2011) Reduced food intake and body weight in mice deficient for the G protein-coupled receptor GPR82. *PLoS One* **6**:e29400.

Hein L, Barsh GS, Pratt RE, Dzau VJ and Kobilka BK (1995) Behavioural and cardiovascular effects of disrupting the angiotensin II type-2 receptor in mice. *Nature* **377**:744-747.

Ichiki T, Labosky PA, Shiota C, Okuyama S, Imagawa Y, Fogo A, Niimura F, Ichikawa I, Hogan BL and Inagami T (1995) Effects on blood pressure and exploratory behaviour of mice lacking angiotensin II type-2 receptor. *Nature* **377**:748-750.

Incerti B, Cortese K, Pizzigoni A, Surace EM, Varani S, Coppola M, Jeffery G, Seeliger M, Jaissle G, Bennett DC, Marigo V, Schiaffino MV, Tacchetti C and Ballabio A (2000) Oa1 knock-out: new insights on the pathogenesis of ocular albinism type 1. *Hum Mol Genet* **9**:2781-2788.

Ivanova EA, Bechtold DA, Dupre SM, Brennand J, Barrett P, Luckman SM and Loudon AS (2008) Altered metabolism in the melatonin-related receptor (GPR50) knockout mouse. *Am J Physiol Endocrinol Metab* **294**:E176-182.

Karczewski KJ, Francioli LC, Tiao G, Cummings BB, Alfoldi J, Wang Q, Collins RL, Laricchia KM, Ganna A, Birnbaum DP, Gauthier LD, Brand H, Solomonson M, Watts NA, Rhodes D, Singer-Berk M, England EM, Seaby EG, Kosmicki JA, Walters RK, Tashman K, Farjoun Y, Banks E, Poterba T, Wang A, Seed C, Whiffin N, Chong JX, Samocha KE, Pierce-Hoffman E, Zappala Z, O'Donnell-Luria AH, Minikel EV, Weisburd B, Lek M, Ware JS, Vittal C, Armean IM, Bergelson L, Cibulskis K, Connolly KM, Covarrubias M, Donnelly S, Ferriera S, Gabriel S, Gentry J, Gupta N, Jeandet T, Kaplan D, Llanwarne C, Munshi R, Novod S, Petrillo N, Roazen D, Ruano-Rubio V, Saltzman A, Schleicher M, Soto J, Tibbetts K, Tolonen C, Wade G, Talkowski ME, Genome Aggregation Database C, Neale BM, Daly MJ and MacArthur DG (2020) The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature* **581**:434-443.

Kawahara Y, Grimberg A, Teegarden S, Mombereau C, Liu S, Bale TL, Blendy JA and Nishikura K (2008) Dysregulated editing of serotonin 2C receptor mRNAs results in energy dissipation and loss of fat mass. *J Neurosci* **28**:12834-12844.

Lan H, Vassileva G, Corona A, Liu L, Baker H, Golovko A, Abbondanzo SJ, Hu W, Yang S, Ning Y, Del Vecchio RA, Poulet F, Laverty M, Gustafson EL, Hedrick JA and Kowalski TJ (2009) GPR119 is required for physiological regulation of glucagon-like peptide-1 secretion but not for metabolic homeostasis. *J Endocrinol* **201**:219-230.

Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, O'Donnell-Luria AH, Ware JS, Hill AJ, Cummings BB, Tukiainen T, Birnbaum DP, Kosmicki JA, Duncan LE, Estrada K, Zhao F, Zou J, Pierce-Hoffman E, Berghout J, Cooper DN, Deflaux N, DePristo M, Do R, Flannick J, Fromer M, Gauthier L, Goldstein J, Gupta N, Howrigan D, Kiezun A, Kurki MI, Moonshine AL, Natarajan P, Orozco L, Peloso GM, Poplin R, Rivas MA, Ruano-Rubio V, Rose SA, Ruderfer DM, Shakir K, Stenson PD, Stevens C, Thomas BP, Tiao G,

Tusie-Luna MT, Weisburd B, Won HH, Yu D, Altshuler DM, Ardissino D, Boehnke M, Danesh J, Donnelly S, Elosua R, Florez JC, Gabriel SB, Getz G, Glatt SJ, Hultman CM, Kathiresan S, Laakso M, McCarroll S, McCarthy MI, McGovern D, McPherson R, Neale BM, Palotie A, Purcell SM, Saleheen D, Scharf JM, Sklar P, Sullivan PF, Tuomilehto J, Tsuang MT, Watkins HC, Wilson JG, Daly MJ, MacArthur DG and Exome Aggregation C (2016) Analysis of protein-coding genetic variation in 60,706 humans. *Nature* **536**:285-291.

Liebscher I, Muller U, Teupser D, Engemaier E, Engel KM, Ritscher L, Thor D, Sangkuhl K, Ricken A, Wurm A, Piehler D, Schmutzler S, Fuhrmann H, Albert FW, Reichenbach A, Thiery J, Schoneberg T and Schulz A (2011) Altered immune response in mice deficient for the G protein-coupled receptor GPR34. *J Biol Chem* **286**:2101-2110.

Liu L, Huang D, Matsui M, He TT, Hu T, Demartino J, Lu B, Gerard C and Ransohoff RM (2006) Severe disease, unaltered leukocyte migration, and reduced IFN-gamma production in CXCR3-/- mice with experimental autoimmune encephalomyelitis. *J Immunol* **176**:4399-4409.

Maekawa A, Austen KF and Kanaoka Y (2002) Targeted gene disruption reveals the role of cysteinyl leukotriene 1 receptor in the enhanced vascular permeability of mice undergoing acute inflammatory responses. *J Biol Chem* **277**:20820-20824.

Ohki-Hamazaki H, Watase K, Yamamoto K, Ogura H, Yamano M, Yamada K, Maeno H, Imaki J, Kikuyama S, Wada E and Wada K (1997) Mice lacking bombesin receptor subtype-3 develop metabolic defects and obesity. *Nature* **390**:165-169.

Oyoshi MK, He R, Kanaoka Y, ElKhal A, Kawamoto S, Lewis CN, Austen KF and Geha RS (2012) Eosinophil-derived leukotriene C4 signals via type 2 cysteinyl leukotriene receptor to promote skin fibrosis in a mouse model of atopic dermatitis. *Proc Natl Acad Sci U S A* **109**:4992-4997.

Preissler J, Grosche A, Lede V, Le Duc D, Krugel K, Matyash V, Szulzewsky F, Kallendrusch S, Immig K, Kettenmann H, Bechmann I, Schoneberg T and Schulz A (2015) Altered microglial phagocytosis in GPR34-deficient mice. *Glia* **63**:206-215.

Robaye B, Ghanem E, Wilkin F, Fokan D, Van Driessche W, Schurmans S, Boeynaems JM and Beauwens R (2003) Loss of nucleotide regulation of epithelial chloride transport in the jejunum of P2Y4-null mice. *Mol Pharmacol* **63**:777-783.

Saleheen D, Natarajan P, Armean IM, Zhao W, Rasheed A, Khetarpal SA, Won HH, Karczewski KJ, O'Donnell-Luria AH, Samocha KE, Weisburd B, Gupta N, Zaidi M, Samuel M, Imran A, Abbas S, Majeed F, Ishaq M, Akhtar S, Trindade K, Mucksavage M, Qamar N, Zaman KS, Yaqoob Z, Saghir T, Rizvi SNH, Memon A, Hayyat Mallick N, Ishaq M, Rasheed SZ, Memon FU, Mahmood K, Ahmed N, Do R, Krauss RM, MacArthur DG, Gabriel S, Lander ES, Daly MJ, Frossard P, Danesh J, Rader DJ and Kathiresan S (2017) Human knockouts and phenotypic analysis in a cohort with a high rate of consanguinity. *Nature* **544**:235-239.

Smallwood PM, Olveczky BP, Williams GL, Jacobs GH, Reese BE, Meister M and Nathans J (2003) Genetically engineered mice with an additional class of cone photoreceptors: implications for the evolution of color vision. *Proc Natl Acad Sci U S A* **100**:11706-11711.

Sumida H, Noguchi K, Kihara Y, Abe M, Yanagida K, Hamano F, Sato S, Tamaki K, Morishita Y, Kano MR, Iwata C, Miyazono K, Sakimura K, Shimizu T and Ishii S (2010) LPA4 regulates blood and lymphatic vessel formation during mouse embryogenesis. *Blood* **116**:5060-5070.

Sun YG and Chen ZF (2007) A gastrin-releasing peptide receptor mediates the itch sensation in the spinal cord. *Nature* **448**:700-703.

Wada E, Watase K, Yamada K, Ogura H, Yamano M, Inomata Y, Eguchi J, Yamamoto K, Sunday ME, Maeno H, Mikoshiba K, Ohki-Hamazaki H and Wada K (1997) Generation and characterization of mice lacking gastrin-releasing peptide receptor. *Biochem Biophys Res Commun* **239**:28-33.

Yun J, Schoneberg T, Liu J, Schulz A, Ecelbarger CA, Promeneur D, Nielsen S, Sheng H, Grinberg A, Deng C and Wess J (2000) Generation and phenotype of mice harboring a nonsense mutation in the V2 vasopressin receptor gene. *J Clin Invest* **106**:1361-1371.

Zhao R, Chen X, Ma W, Zhang J, Guo J, Zhong X, Yao J, Sun J, Rubinfien J, Zhou X, Wang J and Qi H (2020) A GPR174-CCL21 module imparts sexual dimorphism to humoral immunity. *Nature* **577**:416-420.