Functional relevance of naturally occurring mutations in adhesion G protein-coupled receptor ADGRD1 (GPR133)

Liane Fischer, Caroline Wilde, Torsten Schöneberg* and Ines Liebscher*

Abstract

Background: A large number of human inherited and acquired diseases and phenotypes are caused by mutations in G protein-coupled receptors (GPCR). Genome-wide association studies (GWAS) have shown that variations in the ADGRD1 (GPR133) locus are linked with differences in metabolism, human height and heart frequency. ADGRD1 is a G protein-coupled receptor belonging to the class of adhesion GPCRs.

Results: Analysis of more than 1000 sequenced human genomes revealed approximately 9000 single nucleotide polymorphisms (SNPs) in the human ADGRD1 as listed in public data bases. Approximately 2.4 % of these SNPs are located in exons resulting in 129 non-synonymous SNPs (nsSNPs) at 119 positions of ADGRD1. However, the functional relevance of those variants is unknown. In-depth characterization of these amino acid changes revealed several nsSNPs (A448D, Q600stop, C632fs [frame shift], A761E, N795K) causing full or partial loss of receptor function, while one nsSNP (F383S) significantly increased basal activity of ADGRD1.

Conclusion: Our results show that a broad spectrum of functionally relevant ADGRD1 variants is present in the human population which may cause clinically relevant phenotypes, while being compatible with life when heterozygous.

Keywords: GPR133, ADGRD1, Adhesion GPCR, Mutations, SNP, Database

Background

More than three dozen human inherited and acquired diseases are caused by mutations in G protein-coupled receptors (GPCRs) [1–4]. At the molecular level, mutational alteration in GPCRs can have two major consequences – the gain and loss of receptor function. Most mutations causative for human diseases have been found in rhodopsin-like GPCRs, which comprise the largest family of GPCRs, but only a few mutations in the second largest family; the adhesion GPCRs (aGPCRs). Examples are ADGRG1 (GPR56) and ADGRV1 (VLGR1), where gene mutations cause brain malformation (bilateral frontoparietal polymicrogyria) [5] and a form of Usher syndrome [6], respectively.

ADGRD1, a member of the aGPCR family, was among the first aGPCRs identified in genome-wide association studies (GWAS) where SNPs in intronic regions were associated with human height in Sorbs and Korean populations [7, 8]. A GWAS with over 1000 subjects revealed an association of ADGRD1 with the postprandial levels of very low density lipoproteins (VLDL) [9]. Marroni et al. found two SNPs associated with RR duration leading to an increase of one beat per second in heart rate [10]. Recently, the methylation status of the ADGRD1 locus was associated with body weight in humans [11] which is in agreement with an earlier study connecting SNPs in this gene and body weight in mice [12].

Our data base analysis of more than 1000 sequenced human genomes including the 1000 Genomes [13], exome sequencing projects (NHLBI-ESP [14], ClinSeq Project [15]) and the human haplotype map [16], revealed approximately 9000 single nucleotide polymorphisms (SNPs) in the human ADGRD1 gene. Out of those 2.4 % SNPs are located in exons leading to 129 amino acid

* Correspondence: schoberg@medizin.uni-leipzig.de; liebscher@medizin.uni-leipzig.de
From the Section of Molecular Biochemistry, Institute of Biochemistry, Medical Faculty, University of Leipzig, Johannisallee 30, 04103 Leipzig, Germany

© 2016 The Author(s). Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
changes at 119 positions (Fig. 1a). Compared to other GPCR genes [17] the number of SNPs in ADGRD1 is not significantly different. However, a functional relevance of most SNPs can only be evaluated by experimental testing. In general, their large size, the lack of agonists and knowledge about their signal transduction, until recently, rendered the functional characterization of aGPCRs very difficult.

ADGRD1, like most members of the aGPCR family, displays a characteristically complex and long N terminus. The ectodomain contains a pentraxin-like (PTX) domain (as a part of the Laminin G3 domain (LamG3)) as well as a GPCR autoproteolysis inducing (GAIN) domain. The structure of the GAIN domain, which also harbors the GPCR proteolysis site (GPS), has been resolved [18], showing that it is divided into two subdomains: the up to six α-helices containing subdomain A and the 13 β-strands containing subdomain B. A proposed autoproteolytic event at the GPS [19] cleaves the full length receptor protein in an extracellular N-terminal fragment (NTF) and an intracellular C-terminal fragment (CTF), which contains the seven transmembrane region [20, 21].

We have recently shown that ADGRD1 couples to the Gs protein/adenylyl cyclase signaling pathway [22] and is activated by a tethered agonist [23]. This now allows for characterization of natural ADGRD1 variants found in the human population. We identified several inactivating and constitutively activating mutations in the human ADGRD1 gene. This will provide the molecular basis of understanding phenotypes related to ADGRD1 variants and will initiate in-depth clinical characterization of individuals carrying such receptor variants.

Results and discussion
Allelic variation is comparable to rhodopsin-like GPCR and variations can occur in all parts of ADGRD1
According to NCBI database of Single Nucleotide Polymorphisms (dbSNP) nearly 9000 single nucleotide polymorphisms were identified in the ADGRD1 gene (ADGRD1, Gene ID: 283383, chromosomal position [GRCh38 Primary Assembly, human Chr 12, NT_029419.13] 130953907..131141465) [24]. Approximately 96 % of SNPs are intronic, while the remaining 4 % cluster in untranslated regions (UTR) and exons. The SNPs within exons led to 88 synonymous changes and 129...
non-synonymous exchanges including 3 frameshifts and 1 stop mutation (Table 1). Additional file 1: Table S1 summarizes characteristics and origin of 109 nsSNPs that were analyzed in this study. ADGRD1 displays a SNP frequency of ~24.8/100 codons, which is comparable to those of rhodopsin-like GPCRs (Table 1) [17]. Notably, there is no ‘hotspot’ of nsSNPs within the coding region (Fig. 1a). One should consider that sequencing errors might be responsible for some of the listed nsSNPs and that all functionally relevant mutations occurred only heterozygously at low frequency in the populations investigated. However, reevaluation of sequencing data of some inactivation mutations revealed considerable coverage of the detected nsSNPs in those individuals (personal communication Dr. Biesecker), thereby verifying some of the most striking variants.

**nsSNPs in ADGRD1 change receptor activity on a broad scale**

Receptor function can be evaluated at the levels of signaling activity and of membrane expression, latter as a measure of proper folding and translocation. For example, most of the loss-of-function mutations in the vasopressin type 2 receptor, which lead to nephrogenic diabetes insipidus, display significant reduction of cell surface expression [25]. Gain-of-function GPCR mutants exhibit mostly lower cell surface levels as a result of reduced phosphorylation, structural instability and enhanced internalization and degradation but show simultaneously significant increases in basal signaling activity [4, 26–28].

ADGRD1 is a G	extsubscript{s} protein-coupled receptor with high basal activity and high expression levels at the cell surface [22, 23, 29]. To efficiently evaluate the functional relevance of the natural occurring nsSNPs we initially took advantage of the high basal activity of ADGRD1 as a sensitive measure for its G	extsubscript{s}-protein coupling ability. Thus, we examined 109 nsSNPs in an assay that uses cAMP response element (CRE) to trigger the secretion of an alkaline phosphatase (CRE-SeAP assay) to assess signaling activity, while cell surface and whole cell expression was monitored in parallel through ELISAs (Additional file 2). Supported by experiences with other GPCRs [1–4] we considered the combination of these assays to be sufficient to identify most mutants with impaired or significantly increased basal activity and/or altered cell surface trafficking. Based on the function (basal CRE levels) and expression levels of the human wt ADGRD1 a twofold standard deviation (SD) was set as arbitrary threshold to define “normal” function. This is a reasonable range as shown in studies evaluating the functional distribution of GPCR orthologs [30, 31].

Except for two nsSNPs (M1T, N795K), all examined nsSNPs displayed very similar total cell expression levels compared to wt ADGRD1 (Additional file 2). To identify those nsSNPs, which cause alteration of basal CRE-level and/or of cell surface expression, all variants were plotted in respect to these parameters (Fig. 1b). Almost 60 % of the analyzed receptor mutants exhibited wt CRE-SeAP levels, 12.5 % showed more than and 11.6 % less than 2-fold SD different CRE-response from wt (Fig. 1b; Additional file 2). The remaining variants were within the 1-fold SD. With 80 % of the variants displaying wt cell surface expression levels this parameter was less effected (Fig. 1b; Additional file 2). Activity-altering nsSNPs were found evenly distributed throughout the receptor (Table 2).

**nsSNPs within ADGRD1 change basal activity levels in a domain-specific manner**

The majority of nsSNPs in the N terminus that alter receptor activity levels were located either at the very N terminus or within the GAIN domain (Fig. 2a). The very N terminus includes the signal peptide, a PTX-like domain (as a part of the LamG3 domain) and a 112 amino acid-long spacer region. In this receptor region, nsSNPs mainly affected the receptor expression level with a subsequent decrease in CRE activity levels. M1T (rs144264048, T > C) was found heterozygously in a European American population (Additional file 1). Interestingly, this missense mutation was expressed in whole cell and can be detected at low levels at the cell membrane. However, relevant basal CRE activity was not observed (Fig. 2a). These findings are in accordance with a report by Kozak, who found that a one-base change of

| Table 1 | Comparison of exon SNPs of human ADGRD1 with Rhodopsin-like GPCRs |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|               | Human ADGRD1 a  | Rhodopsin-like GPCRs (Stäubert et al., 2014 [17]) | Absolute n | n/100 codons | n/100 codons |
| Synonymous      | 88              | 10              | 10              |
| Non-synonymous  | 129             | 15              | 15              |
| Frameshift      | 3               | 0.3             | 0.3             |
| Stop mutation   | 1               | 0.1             | 0.5             |

* a Data collection: 21.04.2015

| Table 2 | Domain distribution of functional relevant nsSNPs |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | AA              | n               | Inspected nsSNPs n | ±2SD | n (% inspected nsSNPs) |
| Whole receptor  | 874             | 107             | 23 (21.5) |
| N terminus      | 570             | 76              | 17 (22.4) |
| PTX             | 111             | 15              | 3 (20.0)  |
| GAIN            | 295             | 43              | 9 (20.9)   |
| 7TM             | 243             | 21              | 4 (19.0)   |
| C terminus      | 61              | 10              | 2 (20.0)   |

Exclusion of stop and frameshift mutants
AUG can function as initiator codon [32]. The efficiency for initiation of ACG, however, is under 3% compared to AUG [32]. There have been reports of an existing AUG-ACG mutation in the human α globulin gene, which reduces gene expression resulting in clinical thalassemia [33].

Two mutations, E78K [rs267603378, G > A] and G141D [rs142759046, G > A], in the spacer region, led to significant increase in activity, which cannot be explained by an associated elevated membrane expression (Fig. 2a). E78K was identified by Y. Samuels (NHGRI/NIH; http://www.ncbi.nlm.nih.gov/clinvar/RCV000062447/) as one out of three nsSNPs within ADGRD1 that are found in malignant melanoma tissue [34]. G141D was found in two heterozygous genotypes out of 2201 in African Americans by NHLBI ESP and by 1000 Genomes (Additional file 1).

There were two mutations, Y85C [rs199848650, A > G] and F110L [rs148928637, C > G], in the spacer region that led to significant decrease in activity (Fig. 2a). Y85C appeared in the ClinSeq study that analyzed 1306 chromosomes from 662 people of a European population. Of note, ClinSeq includes healthy and disease-
affected participants and had an initial focus on cardiovascular disease [15]. Yet, the individual carrying this SNP showed no obvious clinical phenotype (personal communication Dr. Biesecker).

W174S (rs141606054, G > C), E178K (rs148148477, G > A) and G195R (rs267603379, G > A) are located within two alpha helical structures of the PTX-like domain, as predicted via hydrophobicity plot according to Kyte- Doolittle with Protein 7.1.0 (DNAStar, Lasergene). The latter two SNPs have also been identified by Samuels’ laboratory (NHGRI/NIH) as being found in samples from malignant melanoma. Amino acid changes at these positions significantly decrease cell surface expression, which results in an even more pronounced reduction of CRE levels (Fig. 2a). W174 and G195 are 100 % conserved in 74 vertebrate ADGRD1 orthologs whereas the position E178 is substituted by aspartate in 12 % of orthologous sequences but not by lysine (Additional file 2).

The GAIN domain is considered an important structural and functional element of aGPCRs [18]. Nine out of 43 inspected nsSNPs within the GAIN domain change ADGRD1-mediated CRE levels, the majority of them leading to constitutive activation of the receptor (Fig. 2a). This is most pronounced in case of F383S (rs200232576, T > C), which increased receptor activity by 262 %. Considering that ADGRD1 has been associated with changes in heart frequency [10] it is striking to see that the most prominent activity increasing SNP origins from a cohort that includes participants with potential heart maladies. Nonetheless, F383S is found in a single person of the ClinSeq project [15] that does not show signs of coronary artery disease (personal communication Dr. Biesecker). The position F383 is conserved and only substituted by tyrosine in orthologs (Additional file 2). Within the GAIN domain only A448D (rs200173874, C > A) reduced receptor activity most likely because of reduced plasma membrane expression. This functional relevance of A448 is in agreement with its high evolutionary conservation (Additional file 2). Interestingly, out of the six investigated nsSNPs within the highly conserved GPS region, there was none that significantly changed receptor activity.

Several nsSNPs within the highly conserved 7TM core (S667L (rs377401276, C > T) in ICL2, A761E (rs369201469 C > A) and V764M (rs149434203, G > A) in TM6 and N795K (rs369853823, C > A) in TM7) led to a reduction in receptor-function (Fig. 2b). N795K completely abolished the CRE receptor signal. Even introduced into the constitutive active P2Y12-CTF chimera [23], N795K completely abolished the function of this construct (Fig. 2c). N795K was found to be heterozygous in European Americans (NHLBI ESP) with a minor allele frequency of 0.000077 (Additional file 1). The position is highly conserved in vertebrates (Additional file 2).

The importance of cleavage for aGPCR function is still a matter of debate, yet GPR133-cleavage deficient mutants have been shown to display similar cell surface and cAMP levels compared to the wt receptor [22]. In line with this, a selection of activating, inactivating and control SNPs with wt function throughout the GPR133 structure did not display any changes in occurrence of cleavage (Additional file 2).

Our analysis includes data from three studies with a pathophysiological focus: (1) coronary artery disease (ClinSeq study); [15]), (2) malignant melanoma [34] and (3) heart, lung and blood disorders (the NHLBI-ESP study; [14]). So far, for none of the functionally altered variants a direct attribution to an affected individual could be found. However, two nsSNPs, with significantly reduced, and one nsSNP, with increased signaling abilities, were found in samples with malignant melanoma.

Since all variants were found in adults our data indicate that inactivation or constitutive activation of only one ADGRD1 allele is compatible with life and does not result in a clinically relevant phenotype in humans. However, association of nsSNPs in ADGRD1 with common human diseases cannot be excluded. Of note, the nsSNP A446V, which was previously identified by a study searching for genes influencing human height [8] displayed wt function in our assays.

**Application of the Stachel-derived peptide agonist p13 can restore wt activity levels of selected nsSNPs**

To evaluate the capability of these receptor variants to be activated through the tethered peptide agonist of ADGRD1, we stimulated selected receptor mutants with 500 μM of the synthetic peptide agonist p13 [23] (Fig. 2d). As a functional read-out a luciferase based reporter gene assay under the control of CREB was used. Half of the analyzed nsSNPs showed wt-equivalent activation levels upon peptide application despite reduced basal activity levels, indicating that these mutants are capable of exerting wt function. Some nsSNPs with significantly reduced activation levels through p13 (A448D, V764M, N795K and A816T) were expressed at lower levels in the cell membrane, accounting for the observed lack in stimulation (Fig. 2a and d). However, there are also nsSNPs (F110L, W174S, E178K, G195R) which showed significantly reduced cell surface expressions but wt typical peptide responses (Fig. 2a and d). It seems that peptide stimulation can compensate lack of membrane expression as long as the mutation is within the very N-terminal region of the receptor. Of note, all nsSNPs that do not reach wt activation levels upon p13 are located either in the GAIN domain or the 7TM core. It is conceivable from this data that there are natural variations within the binding pocket of the tethered agonist, which is yet to be defined. Specifically, position
A761 in TM6 seems to be an interesting candidate since the reduction in cell surface expression is not low enough to explain the deficiency in activation through p13 (Fig. 2b and d). N795K, as part of TM7 most likely disrupts proper protein folding as plasma membrane expression is severely disrupted. Therefore, neither basal nor peptide-induced activity was detected (Fig. 2c and d).

The constitutively active F383S-mutant, as a part of the second β-strand in subdomain B of the GAIN domain [18], exhibited an even more pronounced p13-induced activation (Fig. 2d). Here additive effects are likely the cause.

Characterization of ORF-disrupting ADGRD1 variants
The natural variant Q600stop (rs200986302, C > T) leads to a premature truncation of ADGRD1 in the first intracellular loop (Fig. 3a). Nonetheless, the protein was expressed at cell surface ELISA (Fig. 3b). As expected, truncation of the last six transmembranes resulted in a complete lack of basal and peptide-induced signal transduction (Fig. 3c). This unusual SNP was also found heterozygously in a single person in the ClinSeq study. Again no clear sign of coronary artery disease was evident (personal communication Dr. Biesecker).

There is a natural variation of ADGRD1 that causes an extension of the reading frame (Fig. 3a). In C632fs (rs34176886, />C)), an insertion of one base pair in extracellular loop one leads to a frameshift which results in a 78-amino acid longer receptor. Even though it seems that this protein is produced in the cell, it lacks expression in the plasma membrane. Therefore, no second messenger activity was detected (Fig. 3b). To verify the frameshift, the Flag epitope was set into frame of this mutant right upstream of the newly gained stop codon. A pronounced signal in the sandwich ELISA indicated the translation of this frameshift-caused artificial protein (Fig. 3b).

In sum, there are at least two naturally occurring nsSNPs that lead to non-functional ADGRD1 due to gross alteration of the receptor protein chain. In contrast to classical rhodopsin-like GPCR, where truncation after the first transmembrane domain would be considered dysfunctional, aGPCRs are thought to have bimodal functions [35–37]. Here, the CTF mediates the intracellular signal cascade, called ‘cis-signaling’ while the NTF resolves the ‘trans-signaling’ to other cells. Since there is no report on ADGRD1-deficient animal models available the individual functions and the physiological relevance of NTF vs. CTF are unknown yet.

Conclusion
Our analysis of naturally occurring SNPs within the ADGRD1 gene showed that this receptor displays mutation frequencies that are similar to a prototypic GPCR. While functional consequences of the nsSNPs with respect to intracellular signal transduction are highly variable, cleavage does not seem to be affected. It seems that a range between complete abolishment of up to a 3-fold...
increase in ADGRD1 function is compatible with human life and development at least with only one allele being affected. Gene-deficient animal models will shed light on the physiologic relevance of this evolutionarily conserved GPCR. However, the existence of clinically healthy carriers for functionally relevant ADGRD1 variants will eventually lead to the identification of homozygous / compound heterozygous individuals exposing the phenotypic relevance also in humans.

Methods
If not stated otherwise, all standard substances were purchased from Sigma-Aldrich (Taufkirchen, Germany), Merck (Darmstadt, Germany), and C. Roth GmbH (Karlsruhe, Germany). Cell culture material and primers were obtained from Thermo Fisher Scientific Inc., (Darmstadt, Germany).

Data base search for single nucleotide polymorphisms
Non-synonymous SNPs (nsSNPs) found in human ADGRD1 were extracted (latest update 21-04-2015) from the NCBI database of Single Nucleotide Polymorphisms (dbSNP) [24], which combines results from 1000Genomes [13], exome sequencing projects (NHBLI-ESP [14], ClinSeq Project [15]), the human haplotype map (HAP-Map [16]) as well as smaller sequencing projects (e.g. exome, Illumina Chip). A novel nsSNP (A446V) was found by an exome sequencing study which identified alterations influencing human height [8] and was also included.

Generation of wild type (wt) and mutant ADGRD1 constructs
The human ADGRD1 was N-terminally tagged with a haemagglutinin (HA) epitope and C-terminally tagged with a FLAG epitope based on [22]. nsSNPs were generated through site-directed mutagenesis strategy. One of the reported nsSNPs results in a frameshift and prolongs the protein by 78 amino acids (AA). To generate this frameshift mutant (C632fs), it was necessary to clone the FLAG-epitope upstream of the stop signal and expression in percentage of each trial. All amino acid positions are based on wt ADGRD1 variant NP_942122.2. Graphical and statistical analyses were done using Lipofectamine™ 2000 (Thermo Fisher Scientific Inc., Darmstadt, Germany) according to the manufacture’s protocol.

All plasmids were introduced into the eukaryotic expression vector pcDps. All generated constructs were confirmed by sequencing.

Cell culture and transfection
COS-7 and HEK293T cells were cultured in Dulbecco’s minimum essential medium (DMEM) containing 10 % fetal bovine serum (FBS), 100 units/ml penicillin and 100 μg/ml streptomycin at 37 °C and 5 % CO₂ in a humidified atmosphere. One day prior to transfection, COS-7 cells were split into 48-well plates (3 × 10⁴ cells/well for cell surface ELISA), or 6-well plates (3 × 10⁵ cells/well for sandwich ELISA and Western Blot). For secreted alkaline phosphatase (SeAP) and luciferase assays HEK293T cells were split into 96-well plates (3 × 10⁴ cells/well). Transfection of cells was done using Lipofectamine™ 2000 (Thermo Fisher Scientific Inc., Darmstadt, Germany) according to the manufacture’s protocol.

Functional assays
Because ADGRD1 is a Gα-coupled receptor function was measured with cAMP response element (CRE) secreted alkaline phosphatase (SeAP) and CRE binding protein (CREB) luciferase assays. SeAP plasmid (Takara Bio Europe SAS, Saint-Germain-en-Laye, France) and wt or mutant ADGRD1 constructs were co-transfected with each 75 ng of DNA/well. Basal and peptide agonist induced luciferase as well as SeAP levels were measured after 5 h of incubation. For SeAP experiments endogenously expressed alkaline phosphatases were heat inactivated for 2 h right before the measurement. 10 μM forskolin was used as positive control for CRE and CREB activities. As an additional read-out for cAMP formation we measured luciferase activity under the control of CREB. Thus, co-transfection of 50 ng receptor plasmid and 50 ng luciferase plasmid (PathDetect trans-reporting System) was performed using HEK293T cells. Luciferase activity was detected using steadylite plus (PerkinElmer, Rodgau, Germany).

Expression
To detect cell surface and whole cell expression in transfected COS-7 cells ELISAs were used as described previously [38].

Data analysis
Measurements were performed at least three times in triplicates. After normalization to mock-transfection (empty vector (EV)) data were compared to wt function and expression in percentage of each trial. All amino acid positions are based on wt ADGRD1 variant NP_942122.2. Graphical and statistical analyses were done using Prism version 5.01 (GraphPad Software Inc., San Diego, CA). Statistical methods are described in the figure legends.
Additional files

Additional file 1: Table S1. Here, for all inspected nsSNPs descriptive information’s can be found (e.g. Ref. SNP accession numbers, Chr. position, genotype frequencies, references) as well as raw data of the functional analysis and degree of conservation. (XLSX 38 KB)

Additional file 2: Here, all suppl. tables (suppl. tab. S1-S3), figures (suppl. fig. S1 and S2) and methods (Western Blot, only used for supplementary data) are listed. (DOCX 1357 KB)

Abbreviations

aGPCR, adhesion G protein-coupled receptor; CRE, CAMP response element; CREB, CRE binding protein; CTP, C-terminal fragment; dbSNP, NCBI database of Single Nucleotide Polymorphisms; DMEM, Dulbecco’s minimum essential medium; ELISA, enzyme-linked immunosorbent assay; EV, empty vector; GAIN, GPCR autoproteolysis inducing; GPS, GPCR proteolysis site; GWAS, Genome-wide association study; HA, haemagglutinin; ICL1, intracellular loop 1; LamG3, Laminin G3; nsSNP, non-synonymous SNP; NTF, N-terminal fragment; PTX, pentraxin; SD, standard deviation; SeAP, secretion of alkaline phosphatase; SNP, single nucleotide polymorphism; UTR, untranslated regions; VLDL, very low density lipoproteins; wt, wildtype

Acknowledgement

We thank Susanne Lautenschläger and Kay-Uwe Simon for excellent technical assistance. We are grateful to Sven Rodhe for peptide synthesis.

Funding

This work was financially supported by Deutsche Forschungsgemeinschaft (FOR2149) and the BMBF (IFB Adipositas/Diabetes Leipzig ADI-K767). The design of the study and collection, analysis and interpretation of data and writing of the manuscript was done by the authors (specified in section ‘Authors’ contributions’).

Availability of data and materials

The data sets supporting the results of this article are included within the article and its additional files. Raw data for the functional analysis of inspected nsSNPs can be found in Additional file 2, as well as in Figs. 2 and 3.

Authors’ contributions

LF, TS and IL designed experiments; LF, CW and IL performed experiments; LF, CW and IL analyzed results; LF, TS and IL wrote the paper; all authors edited and approved of the manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

Received: 20 November 2015 Accepted: 14 July 2016

References

1. Vassart G, Costagliola S, G protein-coupled receptors: mutations and endocrine diseases. Nat Rev Endocrinol. 2011;7:362–72.
2. Thompson MD, Hendy GN, Percy ME, Bichet DG, Cole, David EC. G protein-coupled receptor mutations and endocrine disease. Methods Mol Biol. 2014;1175:153–87.
3. Spiegel AM. Inherited endocrine diseases involving G proteins and G protein-coupled receptors. Endocr Dev. 2007;11:33–44.
4. Schönberg T, Schub A, Biebmann H, Hermisdorff T, Römpler H, Sangkuhl K. Mutant G-protein-coupled receptors as a cause of human diseases. Pharmacol Ther. 2004;104:173–206.
5. Piao X, Hill RS, Bodel A, Chang BS, Basel-Vanagaite L, Straubsgger R, Dobyns WB, Qasrawi B, Winter RM, Innes AM, Voit T, Ross ME, Michaud JL, Descarie J, Barkovich AJ, Walsh CA. G protein-coupled receptor-dependent development of human frontal cortex. Science (New York, NY). 2004;303:2033–6.
6. Weston MD, Lujendjik, Mirjani WM, Humphrey KD, Moller C, Kimberling WJ. Mutations in the VGLR1 gene implicate G-protein signaling in the pathogenesis of Usher syndrome type II. Am J Hum Genet. 2004;74:357–66.
7. Tönjes A, Koriath M, Schleinitz D, Dietrich K, Bocchter Y, Rayner NW, Almgren P, Enigk B, Richter O, Rohrn S, Fischer-Roinskiy A, Pfeiffer A, Hoffmann K, Krohn K, Aust G, Spranger J, Groop L, Blüher M, Koivu P, Stumvoll M. Genetic variation in GPR133 is associated with height: genome wide association study in the self-contained population of Sorbs. Hum Mol Genet. 2009;18:4662–8.
8. Kim J, Park Y, Baik K, Choi H, Yang G, Koh I, Hwang J, Lee J, Lee Y, Rhee H, Kwon TS, Han B, Heath KE, Inoue H, Yoo H, Park K, Lee J. Ezone sequencing and subsequent association studies identify five amino acid-altering variants influencing human height. Hum Genet. 2012;131:1471–8.
9. Kraja AT, Berecki IB, Tsai MY, Or dovos JM, Hopkins PN, Lai C, Frazier-Wood AC, Straka RJ, Hixson JE, Province MA, Amett DK. Genetic Analysis of 16 NMR-Lipoprotein Fractions in Humans, the GOLDSN Study. Lipids. 2013;48:155–65.
10. Mannoni F, Pfeuffer A, Aulchenko VS, Franklin CS, Isaacs A, Pichler I, Wild SH, Oostra BA, Wright AF, Campbell H, Wittmann JC, Kaab S, Hicks AA, Gyllensten U, Rudan I, Meitinger T, Pattaro C, van Duijn CM, Wilson JF, Pramstaller PP. A genome-wide association scan of RR and QT interval duration in 3 European genetically isolated populations. the EUROSPAN project. Circ Cardiovasc Genet. 2009;2:322–8.
11. Mansego ML, Milagro FI, Zulet MÁ, Moreno-Aliaga MJ, Martínez JA. Differential DNA methylation in relation to age and health risks of obesity. Int J Mol Sci. 2015;16:16818–32.
12. Chan YT, Jones FC, McConnell E, Bryk J, Bünger L, Tautz D. Parallel selection mapping using artificially selected mice reveals body weight control loci. Curr Biol. 2012;22:794–800.
13. Abecasis GR, Anton A, Brooks LD, DePristo MA, Durbin RM, Handsaker RE, Kang HM, Martínez GT, McVean GA. An integrated map of genetic variation from 1,092 human genomes. Nature. 2012;491:656–9.
14. Tennesen JA, Bigham AW, O’Connor TD, Fu W, Kenny EE, Gravel S, McGee S, Do R, Liu X, Jun G, Kang HM, Jordan D, Leal SM, Gabriel S, Rieder MJ, Abecasis G, Altshuler D, Nickerson DA, Boerwinkle E, Sunyaev S, Bustamante CD, Bamshad MJ, Akey JM. Evolution and functional impact of rare coding variation from deep sequencing of human exomes. Science (New York, NY). 2013;337:64–9.
15. Biesecker LG, Mulllkin JC, Facio FM, Turner C, Cherukuri PF, Blakeleys RW, Bouffard GG, Chines PS, Cruz P, Hansen NF, Teer JK, Masiki D, Young AC, Manolillo TA, Wilson AF, Finkel T, Hwang P, Arai A, Remaley AT, Sachdev V, Shamburek R, Cannon RO, Green ED. The ClinSeq Project: piloting large-scale genome sequencing for research in genomics medicine. Genome Res. 2009;19:1665–74.
16. Altshuler DM, Gibbs RA, Peltonen L, Dermitzakis E, Schaffner SF, Yu F, Bonnen PE, de Bakker, Paul IW, Deloukas P, Gabriel SB, Glirrriam W, Hunt S, Inouye M, Jia X, Palotie A, Parkin M, Whittaker P, Chang K, Hawes A, Lewis LR, Ren Y, Wheeler D, Muzny DM, Bames C, Darvishi K, Hurles M, Kom JM, Kristiansson K, Lee C, McCarrol SA, et al. Integrating common and rare genetic variation in diverse human populations. Nature. 2010;465:52–8.
17. Staudert C, Le Duc D, Schönberg T. Examining the dynamic evolution of G protein-coupled receptors. In: Stevens CW, editor. G protein-coupled receptor genetics. Totowa: Humana Press; 2014. p. 23–43. Methods in Pharmacology and Toxicology.
18. Aray D, Boucard AA, Bolliger MF, Nguyen J, Solts SM, Südhof TC, Brunger AT. A novel evolutionarily conserved domain of the AAdhesion GPCRs mediates autoproteolysis. EMBO J. 2012;31:1364–74.
19. Lin H, Chang G, Davies JQ, Stacey M, Harris J, Gordon S. Autocatalytic cleavage of the EMR2 receptor occurs at a conserved G protein-coupled receptor proteolytic site motif. J Biol Chem. 2004;279:1823–32.
20. Liebscher I, Schönberg T, Prömel S. Progress in demystification of adhesion class G protein-coupled receptors take the stage. Sci Signal. 2013;6:re3.
21. Bohnekamp J, Schönberg T. Cell adhesion receptor GPR133 couples to Gs protein-coupled receptors. In: Stevens CW, editor. G protein-coupled receptors take the stage. Sci Signal. 2013;6:re3.
22. Bohnekamp J, Schönberg T. Cell adhesion receptor GPR133 couples to Gs protein-coupled receptors. J Biol Chem. 2011;286:41912–6.
23. Liebscher I, Schön J, Petersen SC, Fischer L, Auerbach N, Dernberg LM, Mogha A, Cöster M, Simon K, Rothermund S, Monk KR, Schönberg T, A.
tethered agonist within the ectodomain activates the adhesion G protein-coupled receptors GPR126 and GPR133. Cell Rep. 2014;9:2018–26.

24. Sherry ST, Ward MH, Kholodov M, Baker J, Phan L, Smigielski EM, Sirotkin K. dbSNP: the NCBI database of genetic variation. Nucleic Acids Res. 2001;29:308–11.

25. Bößelt I, Tramma D, Kalamhou S, Niemeyer T, Nykanen P, Graf K, Krude H, Marensi KS, Di Candia S, Schöneberg T, Schulz A. Functional characterization of novel loss-of-function mutations in the vasopressin type 2 receptor gene causing nephrogenic diabetes insipidus. Nephrol Dial Transplant. 2012;27:1521–8.

26. Seifert R, Wenzel-Seifert K. Constitutive activity of G-protein-coupled receptors: cause of disease and common property of wild-type receptors. Naunyn Schmiedeberg’s Arch Pharmacol. 2002;366:381–416.

27. Parrot C, Miserey-Lenkei S, Bardin S, Convol P, Clauser E. Lessons from constitutively active mutants of G protein-coupled receptors. Trends Endocrinol Metab. 2002;13:336–43.

28. Janovick JA, Pogozheva ID, Mosberg HI, Cornea A, Conn PM. Rescue of misrouted GnRH receptors reveals its constitutive activity. Mol Endocrinol. 2012;26:179–88.

29. Gupte J, Swaminath G, Danoa J, Tian H, Li Y, Wu X. Signaling property study of adhesion G-protein-coupled receptors. FEMS Lett. 2012;586:1214–9.

30. Cöster M, Wittkopf D, Kreuchwig A, Kleinau G, Thor D, Krause G, Schöneberg T. Using ortholog sequence data to predict the functional relevance of mutations in G-protein-coupled receptors. FASEB J. 2012;26:3273–81.

31. Böselt I, Römler H, Hermsof T, Thor D, Busch W, Schulz A, Schöneberg T. Involvement of the V2 vasopressin receptor in adaptation to limited water supply. PLoS ONE. 2009;4:e5573.

32. Kozak M. Context effects and inefficient initiation at non-AUG codons in eukaryotic cell-free translation systems. Mol Cell Biol. 1989;9:5073–80.

33. Pirastu M, Saglio G, Chang X, Cao A, Kan YW. Initiation codon mutation as a cause of alpha thalassemia. J Biol Chem. 1984;259:12315–7.

34. Wei X, Walla V, Lin J, Teer JK, Prickett TD, Gattner J, Davis S, Sternke-Hale K, Davies MA, Genshenwald JE, Robinson W, Robinson S, Rosenberg SA, Samuels Y. Exome sequencing identifies GRIN2A as frequently mutated in melanoma. Nat Genet. 2011;43:442–6.

35. Petersen SC, Luo R, Liebscher I, Giera S, Lomsberg M, Orum J, Schöneberg T. Using ortholog sequence data to predict the functional relevance of mutations in G-protein-coupled receptors. FASEB J. 2012;26:3273–81.

36. Patra C, van Amerongen MJ, Ghosh S, Ricciardi F, Sajjad A, Novoyateleva T, Mogha A, Monk KR, Mehrdad C, Engel FB. Organ-specific function of adhesion G protein-coupled receptor GPR126 is domain-dependent. Proc Natl Acad Sci. 2013;110:16896–903.

37. Prömel S, Waller-Evans H, Dixon J, Zahn D, Colledge WH, Doran J, Carlton MBL, Grosse J, Schöneberg T, Rusi AP, Langenhan T. Characterization and functional study of a cluster of four highly conserved orphan adhesion-GPCR in mouse. Dev Dyn. 2012;241:1591–602.

38. Schöneberg T, Schulz A, Biebermann H, Grüters A, Grimm T, Hübschmann K, Filler G, Gudermann T, Schulz G. V2 vasopressin receptor dysfunction in nephrogenic diabetes insipidus caused by different molecular mechanisms. Hum Mutat. 1998;12:196–205.