Novel Probes Establish Mas-Related G Protein-Coupled Receptor X1 Variants as Receptors with Loss or Gain of Function

Daniel Heller, Jamie R. Doyle, Venkata S. Raman, Martin Beinborn, Krishna Kumar, and Alan S. Kopin

Molecular Pharmacology Research Center, Molecular Cardiology Research Institute, Tufts Medical Center, Boston, Massachusetts (D.H., J.R.D., M.B., A.S.K.); Department of Chemistry, Tufts University, Medford, Massachusetts (V.S.R., K.K.)

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ABSTRACT

The Mas-related G protein-coupled receptor X1 (MrgprX1) is a human seven transmembrane-domain protein with a putative role in nociception and pruritus. This receptor is expressed in dorsal root ganglion neurons and is activated by a variety of endogenous peptides, including bovine adrenal medulla peptide (BAM) and \( \gamma \)-melanocyte-stimulating hormone (\( \gamma \)-2-MSH). In the present work, we study how naturally occurring missense mutations alter the activity of MrgprX1. To characterize selected receptor variants, we initially used the endogenous peptide ligand BAM8-22. In addition, we generated and characterized a panel of novel recombinant and synthetic peptide ligands. Our studies identified a mutation in the second intracellular loop of MrgprX1, R131S, that causes a decrease in both ligand-mediated and constitutive signaling. Another mutation in this region, H133R, results in a gain of function phenotype reflected by an increase in ligand-mediated signaling. Using epitope-tagged variants, we determined that the alterations in basal and ligand-mediated signaling were not explained by changes in receptor expression levels. Our results demonstrate that naturally occurring mutations can alter the pharmacology of MrgprX1. This study provides a theoretical basis for exploring whether MrgprX1 variability underlies differences in somatosensation within human populations.

INTRODUCTION

The Mas-related G protein-coupled receptor X1 (MrgprX1) is a human G protein-coupled receptor (GPCR) expressed in dorsal root ganglia neurons (Dong et al., 2001; Lembo et al., 2002). The endogenous ligands bovine adrenal medulla peptide 8–22 (BAM8-22) and \( \gamma \)-melanocyte-stimulating hormone (\( \gamma \)-2-MSH) activate this receptor and trigger \( \text{G}_{\text{o}} \)-mediated signaling (Lembo et al., 2002; Tatemoto et al., 2006; Solinski et al., 2014). The existing literature suggests that in mouse models the receptors in the Mrgpr family modulate nociception and prurition in vivo (Liu et al., 2009; Guan et al., 2010; Solinski et al., 2014). A recent report showed that in humans BAM8-22 produces itching sensations through a histamine-independent pathway (Sikand et al., 2011). Despite these studies, there still remain many unanswered questions regarding the precise role of MrgprX1 in mediating somatosensory signals. Analysis of the coding region of the MrgprX1 gene has revealed genetic variation among humans (NHLBI GO Exome Sequencing Project), outlined in Fig. 1A and Table 1. To our knowledge, however, the potential impact of naturally occurring missense mutations on the pharmacologic properties of MrgprX1 has not yet been studied.

In the past, naturally occurring variants of GPCRs have proved helpful in understanding differences in susceptibility to disease. For example, loss of function mutations in the melanocortin 4 receptor may account for up to 6% of individuals with severe, early-onset obesity (Loos, 2011). Additionally, polymorphisms in chemokine receptors CCR5 and CCR2 have been linked to delayed progression of AIDS after human immunodeficiency virus (HIV) infection (Reiche et al., 2007). Furthermore, changes in chemosensory response to sweet and savory tastes are associated with single-nucleotide polymorphisms in the GPCRs TAS1R1 and TAS1R3 (Hayes et al., 2013). In an era where genetic information is becoming more accessible to patients and physicians, the link between functional abnormalities in gene products (MrgprX1) and clinical conditions (pruritus and nociception) can be more readily explored. As an initial step, we have examined the extent to which MrgprX1 missense mutations alter the pharmacologic response to the endogenous ligand BAM8-22.

To enable more detailed characterization of signaling differences among MrgprX1 variants, we developed a series of novel agonists (Fig. 1B). Previous work in our laboratory has shown that peptide ligands may be anchored to the cell surface using recombinant DNA technology. Such membrane-
tethered ligands (MTLs) provide a complementary tool to explore GPCR function (Fortin et al., 2011, 2009; Harwood et al., 2013). Conversion of these recombinant ligands into synthetic membrane-anchored ligands (SMALs), in which a peptide is covalently coupled to a flexible linker and a lipid moiety, yields potent, soluble ligands that anchor to the cell surface and activate the corresponding GPCR (Fortin et al., 2011; Doyle et al., 2014). The potential advantages of such ligands include increased potency and prolonged stability (Zhang and Bulaj, 2012).

In our current study, we use this expanded panel of ligands to characterize a series of MrgprX1 missense mutations with an allele frequency exceeding 0.1% (Table 1). An illustration of MrgprX1 (Fig. 1A) highlights the location of each variant residue. We demonstrate that two mutations in MrgprX1, R131S and H133R, alter receptor-mediated signaling, resulting in loss and gain of function, respectively. We propose that these variants should be assessed in human populations to determine whether they modify susceptibility to histamine-independent itch and/or nociception.

**Materials and Methods**

**Generation of Receptor cDNA Constructs.** The MrgprX1 cDNA, in pcDNA 3.1, was generously provided by Dr. Xinzhong Dong (Johns Hopkins University School of Medicine, Baltimore, MD). The construct was subcloned into pcDNA1.1 (Invitrogen). Naturally occurring missense mutations were chosen using data from the National Heart, Lung, and Blood Institute (NHLBI) GO ESP Exome Variant Server (ESV, Seattle; http://evs.gs.washington.edu/EVS/). Oligonucleotide-directed site-specific mutagenesis (as in Fortin et al., 2009; Doyle et al., 2014) was used to generate the receptor cDNA constructs (Fortin et al., 2009; Doyle et al., 2014). DNA sequences corresponding to the peptide ligands were each sequentially replaced with those encoding BAM8-22 (VGRPEWWMDYQKRYG) and γ2-MSH (YVMGHFRWDRFG) (Lembo et al., 2002) using oligonucleotide-directed site-specific mutagenesis, producing both type I and type II MTLs for each peptide (Fortin et al., 2009; Harwood et al., 2013).

**Generation of SMAL Constructs.** Reagents for peptide synthesis were purchased from Chem-Impex (Wood Dale, IL). N-Fmoc-PEG8-proopionic acid and palmitic acid were obtained from AAPPtec (Louisville, KY) and Sigma-Aldrich (St. Louis, MO), respectively. Peptides were assembled on 4-hydroxymethyl phenylacetamido-methyl resin using the in situ neutralization protocol for 4-Noc chemistry with 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate as the activating agent on a 0.25-mmol scale (Schnölzer et al., 2007). Peptide coupling reactions were performed with a 4-fold excess of the protected amino acid (1 mmol). A GGK linker was added to the N terminus of the ligand (Fortin et al., 2011, 2009; Harwood et al., 2013). Peptides were cleaved from the resin by using high hydrogen fluoride conditions (90% anhydrous hydrogen fluoride/10% anisole at 0°C for 1.5 hours), and precipitated using cold diethyl ether. Crude peptides were purified by reversed phase high-pressure liquid chromatography.

**TABLE 1**

<table>
<thead>
<tr>
<th>Variant</th>
<th>dbSNP Reference ID</th>
<th>EA Frequency</th>
<th>AA Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>I36V</td>
<td>rs11024885</td>
<td>0.63%</td>
<td>10.17%</td>
</tr>
<tr>
<td>A46T</td>
<td>rs78179510</td>
<td>17.69%</td>
<td>19.24%</td>
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<td>R50L</td>
<td>rs55954376</td>
<td>0.01%</td>
<td>3.42%</td>
</tr>
<tr>
<td>R131S</td>
<td>rs111448117</td>
<td>1.19%</td>
<td>0.23%</td>
</tr>
<tr>
<td>H133R</td>
<td>rs140351170</td>
<td>0.33%</td>
<td>0.07%</td>
</tr>
<tr>
<td>H137R</td>
<td>rs143702818</td>
<td>0.01%</td>
<td>0.41%</td>
</tr>
<tr>
<td>F273L</td>
<td>rs138263314</td>
<td>2.44%</td>
<td>0.53%</td>
</tr>
</tbody>
</table>

AA, African American; EA, European American; SNP, single-nucleotide polymorphism.
and the purities were determined by analytical high-pressure liquid chromatography (Vydac, C18, 5 μm, 4.6 mm × 250 mm) with a linear gradient of solvent B over 20 minutes at a flow rate of 1.5 ml/min. Elution was monitored by absorbance at 230 nm. Purities of peptides ranged from 90% to 95%. Peptides were analytically characterized by matrix-assisted laser desorption/ionization–time-of-flight (MALDI-TOF) mass spectrometry. MarvinSketch version 14.9.1 (ChemAxon, Cambridge, MA) was used for drawing and displaying chemical structures (Table 2).

**Transfection and Luciferase Reporter Gene Assay.** A luciferase reporter-based assay was used as an index of receptor-mediated signaling (as in Doyle et al., 2013). Human kidney cells (HEK293), grown in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin were seeded in 96-well plates and grown to 80% confluence. Using polyethyleneimine (2.0 μg/ml in serum-free Dulbecco’s modified Eagle’s medium), cells were transiently transfected with cDNAs encoding 1) wild-type (WT) or variant MrgprX1 (3 ng/well); 2) a serum response element (SRE)-luciferase reporter construct (SRE<sub>5x</sub>-Luc-PEST), which includes five SRE repeats, a luciferase reporter gene, and the protein degradation sequence hPEST (catalog no. E1340; Promega, Madison, WI) (25 ng/well); and 3) a cytomegalovirus promoter-degradation sequence hPEST (catalog no. E1340; Promega, Madison, WI) (25 ng/well). Twenty-four hours after transfection, cells were stimulated with soluble BAM8-22 for 4 hours. Luciferase activity was quantified and normalized relative to the WT receptor signal (maximum stimulation = 100%). The results are shown as mean ± S.E.M. ****P < 0.0001 versus WT (at 10<sup>−5</sup> M).

**Enzyme-Linked Immunosorbent Assay.** An enzyme-linked immunosorbent assay (ELISA) was used to assess total and surface receptor expression (as in Doyle et al., 2013). In brief, HEK293 cells were grown and seeded as previously described using 96-well plates pretreated with poly-L-lysine. When 80% confluent, the cells were transfected with HA-tagged receptor constructs. After 24 hours, the cells were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) for 10 minutes. To measure total expression levels, 0.1% Triton X-100 in PBS was applied to permeabilize the cell membrane. To assess surface expression, treatment with Triton X-100 was omitted. Cells were washed with PBS/100 mM glycine and then incubated in PBS/20% fetal bovine serum for 30 minutes. Immunoblot analysis was performed as previously described (as in Doyle et al., 2013). The R131S MrgprX1 variant shows reduced endogenous ligand mediated signaling. HEK293 cells were transfected with cDNA encoding either WT or variant MrgprX1, an SRE-luciferase reporter construct, and β-galactosidase. After 24 hours, cells were stimulated with soluble BAM8-22 for 4 hours. Luciferase activity was quantified and normalized relative to β-galactosidase expression. Three independent experiments were performed in triplicate, and the data are expressed relative to the WT receptor signal (maximum stimulation = 100%). The results are shown as mean ± S.E.M. ****P < 0.0001 versus WT (at 10<sup>−5</sup> M).
minutes to block nonspecific antibody binding. A horseradish peroxidase-conjugated antibody directed against the HA epitope tag (Roche, catalog #12013819001) was diluted 1:500 and added to the cells for 3 hours. Cells were then washed 5 times with PBS. The horseradish peroxidase substrate BM-blue (3,3'-5,5'-tetramethylbenzidine; Roche Applied Science, Indianapolis, IN) was added at 50 μl per well. After 30 minutes, 50 μl of 2.0 M sulfuric acid was added to each well to stop the reaction. The concentration of the colorimetric product was quantified by measuring absorbance at 450 nm using a SpectraMax microplate reader (Molecular Devices).

**Data Analysis.** GraphPad Prism software, version 6.0 (GraphPad Software), was used for sigmoidal curve fitting of ligand concentration–response curves, linear regression, and statistical analysis. EC50 and pEC50 values were calculated for each independent experiment as an index of ligand potency. Reported values represent the mean of three independent experiments. Statistical comparisons

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**Fig. 3.** Anchored ligands as tools to study MrgprX1 signaling. Type I tethered BAM8-22 (A) and type II tethered γ2-MSH (B) are active on the WT receptor. (C) Lipidated BAM8-22 and (D) lipidated γ2-MSH exhibit increased potency compared with the corresponding soluble peptides. To determine MTL activity, HEK293 cells were transfected with increasing amounts of cDNA encoding either tethered BAM8-22 or tethered γ2-MSH, as well as a fixed amount of cDNA encoding WT MrgprX1, an SRE-luciferase reporter construct, and β-galactosidase. To determine synthetic membrane-anchored ligand activity, similar methodology was used with the tether cDNA omitted. Twenty-four hours after transfection, the cells were stimulated with lipidated BAM8-22 or lipidated γ2-MSH for 4 hours. Luciferase activity was quantified and normalized relative to β-galactosidase expression. The data shown represent at least two independent experiments performed in triplicate. The results were expressed relative to the WT receptor signal (maximum = 100%) and graphed as mean ± S.E.M.

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**Fig. 4.** The R131S variant exhibits decreased response to stimulation with BAM8-22 analogs. The R131S variant displays negligible signaling levels with tethered BAM8-22 (A) as well as reduced signaling with lipidated BAM8-22 (B). To measure MTL activity, HEK293 cells were transfected with cDNAs encoding tethered BAM8-22, either WT or variant MrgprX1, an SRE-luciferase reporter construct, and β-galactosidase. The empty vector, pcDNA1.1, was transfected instead of receptor cDNA as a control. To measure synthetic membrane anchored ligand activity, a similar methodology was used with the tether cDNA omitted. Cells were stimulated 24 hours after transfection with lipidated BAM8-22 for 4 hours. Luciferase activity was quantified and normalized relative to β-galactosidase expression. For each receptor, three independent experiments were performed in triplicate. Data are expressed relative to the maximum signal achieved at the WT receptor. Results are shown as the mean ± S.E.M. ****P < 0.0001 variant receptor versus WT (at 10⁻⁶ M in B). All variants except R131S are statistically significantly different from pcDNA1.1 (P < 0.05).
should be noted that HEK293 cells transfected with an empty vector control show no activity after treatment with ligand (data not shown).

Characterization of Novel Recombinant and Synthetic MrgprX1 Ligands. As additional tools for structure–function studies, MTLs incorporating one of two endogenous peptide ligands for MrgprX1, BAM8-22 and γ2-MSH, were generated. The activities of MTL constructs in both orientations (type I, with an extracellular N terminus of the ligand; type II, with an extracellular C terminus) were assessed using a luciferase-based reporter assay as described in Materials and Methods. When expressed in HEK293 cells together with MrgprX1, a subset of MTL constructs activated the receptor in a cDNA concentration-dependent manner (Fig. 3, A and B). Active MTLs included type I tethered BAM8-22 (free extracellular N terminus) and type II tethered γ2-MSH (free extracellular C terminus). These constructs were therefore used in subsequent experiments.

Previous studies have shown that the activity of recombinant MTLs can be recapitulated using SMALs, which integrate into the cellular membrane via a lipid moiety (Fortin et al., 2011; Doyle et al., 2014). Lipidated constructs were generated corresponding to the two active MrgprX1 MTLs. Guided by the MTL results, PEG8 and palmitic acid were covalently attached to the C-terminus of BAM8-22 and the N terminus of γ2-MSH to generate corresponding SMALs (Table 2). When compared with the endogenous soluble form, both lipidated BAM8-22 and lipidated γ2-MSH displayed significantly increased potency (Fig. 3, C and D).

In a parallel set of experiments (data not shown), signaling levels at saturating concentrations of the four novel MrgprX1 ligands were assessed at the WT receptor. Tethered BAM8-22, tethered γ2-MSH, and lipidated γ2-MSH signaling represented 38.4% ± 5.4%, 12.9% ± 2.0%, and 67.9% ± 2.4% (mean ± S.E.M.) of maximum lipidated BAM8-22 signaling (at 10⁻⁶ M), respectively.

Select MrgprX1 Missense Mutations Result in Altered Ligand-Mediated Signaling. The activity of tethered and lipidated BAM8-22 at each of the seven MrgprX1

![Fig. 5](https://example.com/figure5.png)

**TABLE 3**

Comparison of signaling induced by lipidated BAM8-22 at selected MrgprX1 variants

<table>
<thead>
<tr>
<th>Variant</th>
<th>EC₅₀ (nM)</th>
<th>pEC₅₀ᵃ</th>
<th>Curve Maximumᵇᵇ</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>12.0</td>
<td>7.92 ± 0.065</td>
<td>101.3% ± 2.7</td>
</tr>
<tr>
<td>I36V</td>
<td>9.3</td>
<td>8.03 ± 0.12</td>
<td>108.9% ± 5.4</td>
</tr>
<tr>
<td>A46T</td>
<td>9.5</td>
<td>8.02 ± 0.112</td>
<td>111.6% ± 5.2</td>
</tr>
<tr>
<td>R55L</td>
<td>10.2</td>
<td>7.99 ± 0.142</td>
<td>107.5% ± 6.4</td>
</tr>
<tr>
<td>R131S</td>
<td>57.1</td>
<td>7.24 ± 0.117</td>
<td>40.4% ± 2.8⁵</td>
</tr>
<tr>
<td>H133R</td>
<td>8.6</td>
<td>8.06 ± 0.103</td>
<td>110.2% ± 4.6</td>
</tr>
<tr>
<td>H137R</td>
<td>7.9</td>
<td>8.10 ± 0.089</td>
<td>109.2% ± 4.0</td>
</tr>
<tr>
<td>F273L</td>
<td>12.1</td>
<td>7.92 ± 0.076</td>
<td>95.9% ± 3.1</td>
</tr>
</tbody>
</table>

ᵃShown as mean ± S.E.M. ᵇᵇCurve maxima are extrapolated from the best-fit curve. Luciferase signal at the WT receptor achieved at 10⁻⁶ M lipidated BAM8-22 is defined as 100%.

ᵇᵇP < 0.0001 (versus WT).
variants was assessed (Fig. 4). After stimulation with either the recombinant or the synthetic BAM8-22 analog, the R131S variant consistently displayed attenuated levels of signaling. In addition to decreased efficacy, a statistical analysis of calculated EC50 values for all seven variants suggests that only the R131S mutation significantly decreases the potency and efficacy of lipidated BAM8-22 (Table 3).

The R131S variant also displayed decreased ligand-mediated signaling with either tethered or lipidated γ2-MSH (Fig. 5). Additionally, the H133R mutation significantly increased tethered and lipidated γ2-MSH mediated signaling, an effect not observed with lipidated or tethered BAM8-22. A moderate decrease in signaling with the R55L and F273L variants was observed with both tethered BAM8-22 and tethered γ2-MSH, although this decrease only reached statistical significance with tethered γ2-MSH.

The R131S Missense Mutation Reduces the Basal Activity of MrgrprX1. To explore whether changes in receptor-mediated signaling levels in part reflect altered basal activity, ligand-independent signaling of the R131S and the H133R variants was assessed (Fig. 6). WT MrgrprX1 exhibited significant basal activity, approximating 6% of the maximum BAM8-22 stimulated level of signaling (at 10 μM). The H133R variant showed basal activity levels comparable to WT. In contrast, the R131S variant showed markedly attenuated ligand-independent activity.

Expression Levels of the R131S and H133R Variants Are Comparable to Wild Type. We next explored the possibility that the observed differences in ligand-dependent and ligand-independent signaling were the result of altered receptor expression. An ELISA was used for this analysis. We generated epitope-tagged versions of WT MrgrprX1 and of the R131S and H133R variants. Each receptor was expressed in HEK293 cells. Both the R131S and H133R variants exhibited levels of total and surface expression comparable to WT MrgrprX1 (Fig. 7). These data suggest that observed differences in signaling are not attributable to changes in receptor expression.

Discussion

Initial analysis of naturally occurring MrgrprX1 variants with the endogenous ligand BAM8-22 identified R131S as a potential loss-of-function mutation (Fig. 2). To further investigate ligand-mediated signaling of this variant as well as other receptor mutants, we generated MTL and SMAL analogs of BAM8-22 and γ2-MSH. In addition to confirming the loss of function resulting from the R131S mutation, use of these recombinant and synthetic ligands revealed that the H133R substitution conferred a ligand-dependent gain of function phenotype (Figs. 4 and 5). Defining how missense mutations in this receptor alter pharmacologic function is an important first step toward understanding the potential role of natural variants in altering somatosensation and/or the response to drugs targeting MrgrprX1 in vivo.

There are multiple mechanisms through which missense mutations may affect GPCR function. Some variants affect the active/inactive state equilibrium and may in turn have systematic effects on ligand-mediated signaling (Samama et al., 1993; Kopin et al., 2003; Beinborn et al., 2004). Other mutations alter ligand interaction with the receptor, either directly or indirectly through changes in receptor tertiary structure. (Bond et al., 1998; Fortin et al., 2010).

The data presented in this report suggest that the R131S mutation decreases both ligand-mediated and ligand-independent activity of MrgrprX1.

![Fig. 6. The MrgrprX1 variant R131S exhibits decreased ligand-independent signaling. To measure constitutive activity, HEK293 cells were transfected with cDNAs encoding the corresponding MrgrprX1 variant, an SRE-luciferase reporter construct, and β-galactosidase. After 24 hours, luciferase activity was quantified and normalized relative to β-galactosidase expression. Three independent experiments were performed in triplicate. The data are expressed relative to the maximum signal on WT MrgrprX1 at 3 ng of cDNA, achieved by stimulating with 10^{-5} M soluble BAM8-22 for 4 hours. The results are shown as mean ± S.E.M., and lines are fitted with linear regression. ***P < 0.001 (versus WT, at 8 ng cDNA).](#)

![Fig. 7. The R131S and H133R variants are expressed at levels comparable to WT. HEK293 cells were transfected with increasing amounts of cDNA encoding the respective N-terminally HA epitope tagged MrgrprX1 variant. After 24 hours, surface (A) and total (B) expression levels were assessed by ELISA using nonpermeabilized and permeabilized cells, respectively. Differences between the expression levels of the WT receptor and the R131S and H133R variants are not statistically significant (P > 0.05). After subtraction of the background signal (no cDNA transfected), the data were expressed relative to maximum WT MrgrprX1 expression in permeabilized cells (total expression). The results are shown as mean ± S.E.M., and lines are fitted with linear regression.](#)
independent (basal) activity of MrgprX1. These properties place it in the former group of mutations. Notably, these differences in receptor activity levels cannot be explained by changes in receptor expression (Fig. 7). The location of residue R131 in the second intracellular loop, a domain that has been established as important in G protein binding (Hu et al., 2010), suggests that this mutation could be affecting the ability of MrgprX1 to interact with G proteins and/or shift MrgprX1 from the active to the inactive state.

The H133R mutation does not affect basal activity and slightly increases the efficacy of a subset of ligands (i.e., tethered and lipidated γ2-MSH but not tethered or lipidated BAM8-22). This suggests that H133R is not a systematic modulator and therefore belongs to the latter group of mutations (as described previously). Like with R131S, these changes in ligand-mediated receptor activity are not accompanied by changes in receptor expression. Given its location in the second intracellular loop, H133R may represent a mutation that impacts the ligand-receptor interaction indirectly (e.g., by slightly altering the orientation of residues that interact with the ligand).

The purported role of MrgprX1 in mediating pain and somatosensation, in particular histamine-independent itch (Sikand et al., 2011; Bader et al., 2014; Solinski et al., 2014), suggests that the unique signaling properties of the R131S and H133R variants may have important implications for the development and use of therapeutics targeting this receptor. Missense variants have also proven important in understanding differences in somatosensation in the past. For example, the N40D mutation in the human μ-opioid receptor may alter susceptibility to pain (Lötsch and Geisslinger, 2005) and pruritus (Tsai et al., 2010). Similarly, missense mutations in the sodium channel Na\(_1\).7 have been linked to pain-related disorders (Fertleman et al., 2006; Dreith and Waxman, 2007) and altered pain perception (Reimann et al., 2010).

The possibility that MrgprX1 variants may be linked to a specific phenotype highlights the need for data collection that will allow for matching of the MrgprX1 genotype with sensitivity to MrgprX1-mediated somatosensation. This should be feasible particularly with the R131S variant, which has an allele frequency of greater than 1%. Future studies may reveal that mutations such as R131S are linked to decreased nociception or pruritus. Extending beyond the coding region of the gene, variations in upstream regulatory sequences may also play a role in altering susceptibility to histamine-independent itch by altering MrgprX1 expression (Wray, 2007).

Although mutational analysis with the naturally occurring agonist-receptor pair is most physiologically relevant, MTLs and their lipidated counterparts can provide powerful molecular probes to explore pharmacologic differences between receptor variants. As illustrated, such modified peptide ligands exhibit enhanced effective concentration and thus provide experimental tools that facilitate the pharmacologic characterization of GPCRs. In addition, MTLs can be expressed as transgenic constructs enabling exploration of corresponding receptor functionality. In addition, our data exemplify constructs enabling exploration of corresponding receptor functionality and important differences in both basal and ligand-induced signaling that may contribute to somatosensory variability in the human population.

Taken together, our experiments illustrate how naturally occurring missense variants may markedly alter the pharmacologic properties of a GPCR. In addition, our data exemplify how MTLs and SMALs provide complementary tools to differentiate receptor variants that are systematic modulators from mutations that preferentially affect a subset of receptor agonists. As with a growing number of GPCRs (Rana et al., 2001; Thompson et al., 2014), MrgprX1 receptor variants display important differences in both basal and ligand-induced signaling that may contribute to somatosensory variability in the human population.

**References**


