An Asn to Lys Polymorphism in the Third Intracellular Loop of the Human α2A-Adrenergic Receptor Imparts Enhanced Agonist-promoted G/ Coupling* 

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α2A-Adrenergic receptors (α2AR) are presynaptic autoinhibitory receptors of noradrenergic neurons in the central and peripheral sympathetic nervous systems, which act to dynamically regulate neurotransmitter release. Signaling through the G/Gα family of G-proteins, the receptor subserves numerous homeostatic and central nervous system functions. A single nucleotide polymorphism of this receptor, which results in an Asn to Lys substitution at amino acid 251 of the third intracellular loop, was identified in the human population. The frequency of Lys-251 was 10-fold greater in African-Americans than in Caucasians, but was not associated with essential hypertension. To determine the consequences of this substitution, wild-type and Lys-251 receptors were expressed in CHO and COS-7 cells. Expression, ligand binding, and basal receptor function were unaffected by the substitution. However, agonist-promoted [35S]GTPγS binding was ~40% greater with the Lys-251 receptor. This enhanced agonist function was observed with catecholamines, azepines, and imidazolines albeit to different degrees. In studies of agonist-promoted functional coupling to Gi, the polymorphic receptor displayed enhanced inhibition of adenyl cyclase (80 ± 4.4% versus 48 ± 4.1% inhibition) and markedly enhanced stimulation of MAP kinase (57 ± 9% versus 15 ± 2-fold increase over basal) compared with wild-type α2AR. The potency of epinephrine in stimulating inositol phosphate accumulation was increased ~4 fold with the Lys-251 receptor. Unlike previously described variants of G-protein-coupled receptors, where the minor species causes either a loss of function or increased non-agonist function, Lys-251 α2AR represents a new class of polymorphism whose phenotype is a gain of agonist-promoted function.

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α2-Adrenergic receptors (α2AR)1 are cell surface receptors for catecholamines that couple to the G/Gα family of G-proteins. Cellular effects of α2AR activation include inhibition of adenylyl cyclase, activation of inwardly rectifying K+ channels, inhibition of voltage-gated Ca2+ channels, activation of phospholipase C, and stimulation of mitogen-activated protein kinase (MAP kinase) signaling (1, 2). α2ARs are widely expressed and participate in a broad spectrum of physiologic functions, including metabolic, cardiac, vascular, and central and peripheral nervous systems, via presynaptic and postsynaptic mechanisms. Three human α2AR subtypes have been cloned and characterized (α2A, α2B, and α2C), with the α2AAR being the most extensively expressed of the subtypes. At peripheral sites, α2AR acts to inhibit insulin secretion by pancreatic β cells, contract vascular smooth muscle, inhibit lipolysis in adipocytes, modulate water and electrolyte flux in renal cells, and aggregate platelets (3). The α2AAR is the principal presynaptic inhibitory autoreceptor of central and peripheral sympathetic nerves (4). Such inhibition of neurotransmitter release in the brain by the α2AAR is the basis for the central hypotensive, sedative, anesthetic-sparing, and analgesic responses of α2AR agonists (5, 6). Indeed, α2AR agonists such as clonidine and guanabenz are potent antihypertensive agents, which act via central presynaptic α2ARs (7). The blood pressure and other responses to α2AR agonists and antagonists, however, are subject to interindividual variation in the human population (7–9). Such variation, of course, could be due to genetic variation in the structure of the receptor itself, its cognate G-proteins, the effectors, or downstream intracellular targets. Of particular interest are physiologic (7–9) and genetic (10, 11) studies, which suggest that altered α2AR function predisposes individuals to essential hypertension. Thus, what has been shown with βAR polymorphisms (12), a functional polymorphism of the α2AR may act as a risk factor for disease, act to modify a given disease, or alter the therapeutic response to agonists or antagonists.

The above has prompted us to examine the α2AR coding sequence for polymorphic variation in cohorts of hypertensive and normotensive individuals from two ethnic backgrounds. Here we report a single nucleotide polymorphism of the α2AR that results in an Asn to Lys change at amino acid 251 in a coupling domain within the third intracellular loop of the receptor. This polymorphism was much more common in African-Americans than in Caucasians but was not associated with essential hypertension in either group. Surprisingly, recombinant studies revealed that the Lys-251 polymorphism confers significantly increased agonist-promoted binding to Gαi, manifested functionally as enhanced coupling to the inhibition of adenylyl cyclase, activation of MAP kinase signaling, and stimulation of inositol phosphate accumulation.
**Materials and Methods**

**Polymorphism Detection.** The intronless human α2A-AR gene (GenBank* accession number AF281308, which includes the sequence corrections illuminated by Guyer (13)) was amplified by overlapping PCR reactions from genomic DNA derived from blood samples. The 1350-bp coding sequence as well as 341-bp 5′-UTR and 174-bp 3′-UTR were examined. In this paper the adenine of the initiator ATG codon is designated as nucleotide 1, and amino acid 1 is the encoded methionine. The human receptor consists of 450 amino acids. For initial examination, DNA from 27 hypertensive individuals was utilized. Overlapping PCR products encompassing the α2A gene were designated fragments 1–5 and were generated using the following primers: Fragment 1 (600 bp), 5′-TTTACCATCGGCTTCCCTAC-3′ (sense) and 5′-GAGAGACGAGAAGGTGTTGG3′ (antisense); Fragment 2 (467 bp), 5′-TGCTTCATCACTGGCGTGGTC-3′ (sense) and 5′-CGTACCCTTCTGGTGTTGATC-3′ (antisense); Fragment 3 (556 bp), 5′-GCCATCATCATGCACTCCTGGTC-3′ (sense) and 5′-GGCTCGCTCGGCGCTGCTTGG-3′ (antisense); Fragment 4 (436 bp), 5′-GACCTGGAGAGCCGTCGTTCTT-3′ (sense) and 5′-TGACCGGGTTCAACGAGCTGTTG-3′ (antisense); and Fragment 5 (353 bp), 5′-GCCAGACAAGCTTTTAACAC-3′ (sense) and 5′-TGTAAAACGACGGTTTCAGTA-3′ (antisense). The 5′-end of each sense and antisense primer also contained sequence corresponding to the M13 Forward (5′-TGTAAAACGACGGTTTCAGTA-3′) and M13 Reverse (5′-CAGGAAACAGCTATGACC-3′) universal sequencing primers, respectively. The PCR consisted of 20–100 ng of genomic DNA, 5 pmol of each M13 primer, 0.8 mM dNTPs, 10% dimethyl sulfoxide, 2.5 units of Platinum Taq DNA polymerase (Life Technologies, Inc.), 20 μl of 5× buffer A (Invitrogen) in a 100-μl reaction volume. Reactions were started by an initial incubation at 94 °C for 4 min, followed by 35 cycles of 94 °C for 30 s, denaturation for 30 s, and 72 °C for 1 min, followed by a final extension at 72 °C for 7 min. The denaturation temperature was 56 °C for fragments 1 and 5, 58 °C for fragments 2 and 4, and 60 °C for fragment 3. PCR reactions were purified using the QiAquick PCR purification system (Qiagen), and automated sequencing of both strands of each PCR product was performed using an Applied Biosystems sequencer using dye primer methods. As discussed below, a C to G transversion at nucleotide 753 creates a unique restriction endonuclease site in PCR fragment 3, and the presence or absence of this polymorphism in additional samples was studied by Styl digestion of fragment 3 PCR products (see Fig. 1D). This rapid detection technique was applied to additional DNA samples providing genotypes at this locus from a total of 376 individuals (normotensive: 125 Caucasian and 99 African-American; hypertensive: 75 and 77, respectively). Normotensive and hypertensive patients were selected as described previously (14).

** Constructs and Cell Transfection.** To create the polymorphic α2A-LSy-251 construct, a portion of the coding region of the α2A-AR gene containing a G at nucleotide position 753 was amplified from a homozygous individual using fragment 2 sense and fragment 4 antisense primers (see PCR conditions described above). This fragment was digested with and sub cloned into the BglII and SacII sites of the wild-type α2A-AR sequence in the expression vector pBC12B1. Chinese hamster ovary cells (CHO-K1) were permanently transfected by a calcium phosphate precipitation technique as described previously using 30 μg of each receptor construct and 3.0 μg of pSVneo to provide for G418 resistance (15). Selection of positive clones was carried out in 1.0 mg/ml G418, and expression of the α2A-AR from individual clonal lines was determined by radioligand binding as described below. Cells were grown in monolayers in Ham’s F-12 medium supplemented with 10% fetal calf serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 80 μg/ml G418 (to maintain selection pressure) at 37 °C in a 5% CO2 atmosphere. COS-7 cells were co-transfected with 1–10 μg of each α2A-AR construct and 5 μg of G418 using a DEAE-dextran method essentially as described previously (16). These transfections also included 5 μg of the large T antigen-containing plasmid, pRSVT (17), to maximize expression of the α2A-AR gene from the SV40 promoter of pBC12B1.

**Adenylyl Cyclase Activities.** α2A-AR inhibition of adenylyl cyclase was determined in membrane preparations from CHO cells stably expressing the two receptors using methods similar to those previously described (18). Reactions consisted of 20-μg cell membranes, 2.7 mM phosphoenolpyruvate, 50 μM GTP, 0.1 mM cAMP, 0.12 mM ATP, 50 μM myokinase, 0.05 mM ascorbic acid, and 2 μCi of [32P]ATP in a buffer containing 40 mM HEPES, pH 7.4, 1.6 mM MgCl2, and 0.8 mM EDTA for 30 min at 37 °C. Reactions were terminated by the addition of a stop solution containing excess ATP and cAMP and ∼100,000 dpm of [3H]cAMP. Labeled cAMP was isolated by gravity chromatography over alumina columns with [3H]cAMP used to quantitate column recovery. Activities were measured in the presence of water (basal), 5 μM...
forskolin, and 5 \textmu M forskolin with the indicated concentrations of ago-nists. Results are expressed as percentage inhibition of forskolin-stim-u-lated activity.

[35S]GTPγS Binding—Receptor-G-protein interaction was also quan-titated by radiolabeled guanosine 5'-3-O-(thio)triphosphate ([35S]GTPγS) binding in COS-7 cells transiently transfected with each a2AAR construct and Gia2. Briefly, cell membranes (~20 \textmu g) were incubated in buffer containing 25 mM Tris-HCl, pH 7.4, 5 mM MgCl2, 1 mM EDTA, 1 mM dithiothreitol, 100 mM NaCl, 1 \textmu M GDP, 2 nM [35S]GTPγS and the indicated agonist in a 100-\textmu l reaction volume for 15 min at room temperature. Incubations were terminated by dilution with 4 volumes of ice-cold 10 mM Tris-HCl buffer (pH 7.4) and vacuum filtration over Whatmann GF/C glass fiber filters. Nonspecific binding was measured in the presence of 10 \textmu M GTPγS.

MAP Kinase Activation—Activation of p44/42 MAP kinase was de-termined by quantitative immunoblotting using specific antibodies to identify phosphorylated and total MAP kinase expression. Briefly, confluent cells were incubated overnight in serum-free media prior to treatment with media alone (basal), epinephrine (10 \textmu M), or thrombin (1 unit/ml) for 5 min. Cells were washed three times with phosphate-buffered saline (PBS) then lysed in RIPA (20 mM Tris, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40, 0.5% deoxycholate, 0.1% SDS, and 5 mM NaF) containing protease inhibitors (10 \muM benzamidine, 10 \muM soybean trypsin inhibitor, 10 \muM aprotinin, and 5 \muM leu-}

TABLE I

| Receptor  | Radioligand Binding | Adenylyl cyclase activity |
|-----------|---------------------|--------------------------|
|           | Bmax [fmol/mg]     | Kd [nM] | Epinephrine Ki [nM] | Basal [pmol/min/mg] | Forskolin [pmol/min/mg] |
| Asn-251   | 2360 ± 263         | 3.4 ± 0.21 | 593 ± 65 | 11.9 ± 2.3 | 32.7 ± 4.0 |
| Lys-251   | 2590 ± 140         | 3.6 ± 0.25 | 734 ± 31 | 13.9 ± 1.6 | 31.6 ± 6.5 |

**Fig. 2.** Location of the Lys-251 a2AAR polymorphism and alignment of flanking amino acid residues of the third intracellular loop from various species. The locations of the Lys-251 amino acid polymorphism in the third intracellular loop as well as two synonymous single nucleotide polymorphisms are indicated by the black circles. Alignment of a2AAR amino acid sequence from various species shows that the region around residue 251 is highly conserved and that Asn at position 251 is invariant in all mammalian species reported to date except for human where we have noted the Lys polymorphism. For clarity, the mid-portion of the third intracellular loop (white dots) is not shown.
was determined using saturation binding assays as described (22) with 12 concentrations (0.5–30 nM) of \([^3H]yohimbine and 10 \mu M\) phentolamine used to define nonspecific binding. For competition studies, membranes were incubated in 50 mM Tris-HCl, pH 7.4, 10 mM MgSO\(_4\), 0.5 mM EDTA, with 2.0 nM \([^3H]yohimbine and 18 concentrations of the indicated competitor in the presence of 100 \mu M\ Gpp(NH)p for 30 min at 37 °C. Reactions for the above radioligand binding studies were terminated by dilution with 4 volumes of cold 10 mM Tris-HCl buffer (pH 7.4) and vacuum filtration over Whatman GF/C glass fiber filters.

**Miscellaneous**—Protein determinations were by the copper bicinchoninic acid method (23). Data from adenylyl cyclase and radioligand binding assays were analyzed by iterative least-square techniques using Prism software (GraphPad, San Diego, CA). Agreement between genotypes observed and those predicted by the Hardy-Weinberg equilibrium \(p\) alleles was not different than that predicted from Hardy-Weinberg analogy to human 251 in all mammalian a region thought to be important for G-protein interaction (24).

A portion of the third intracellular loop of the third intracellular loop. The pocket composed of the transmembrane spanning domains is the receptor in CHO cells. Saturation radioligand binding studies were analyzed in cell lines expressing the wild-type Asn-251 receptor (Fig. 1). This consisted of a C to G transversion at nucleotide 753 that changed amino acid 251 from Asn to Lys.

**RESULTS AND DISCUSSION**

Sequence analysis of the entire coding region of the \(\alpha_2A\)AR gene from 54 chromosomes revealed one nonsynonymous sequence variant located within the third intracellular loop of the receptor (Fig. 1). This consisted of a C to G transversion at nucleotide 753 that changed amino acid 251 from Asn to Lys (Fig. 2). Although the Lys-251 receptor is relatively rare, it is 10-fold more common in African-Americans than in Caucasians, with an allele frequency of 0.05 as compared with 0.004 \(p = 0.01\). The distribution of homozygous and heterozygous alleles was not different than that predicted from Hardy-Weinberg equilibrium \(p > 0.9\). Two previously unreported synonymous single nucleotide polymorphisms were also identified at nucleic acids 849 (C to G) and 1093 (C to A). Considering the role of the \(\alpha_2A\)AR in regulating blood pressure, we also determined the frequency of this polymorphism in patients with essential hypertension. Our analysis of 99 normotensive and 75 hypertensive African-Americans as well as 125 normotensive and 75 hypertensive Caucasians showed no differences in the frequency of this polymorphism in patients with essential hypertension in either group.

The consequences of this polymorphism on ligand binding and receptor function were evaluated by permanently expressing the human wild-type \(\alpha_2A\)AR and the Lys-251 polymorphic receptor in CHO cells. Saturation radioligand binding studies revealed essentially identical dissociation binding constants for the \(\alpha_2A\)AR antagonist \([^3H]yohimbine \((K_d = 3.4 \pm 0.21 \text{ versus } 3.6 \pm 0.25 \text{ nM}, \text{respectively, } n = 4)\), and competition binding assays showed no differences in binding of the agonist \((\sim)\) epinephrine \((K_i = 593 \pm 65 \text{ versus } 734 \pm 31 \text{ nM}, \text{respectively, } n = 3, \text{Table I})\). These data confirm that the ligand binding pocket composed of the transmembrane spanning domains is not perturbed by the presence of Lys at amino acid 251 in the third intracellular loop.

The Lys-251 polymorphism occurs in a highly conserved portion of the third intracellular loop of the \(\alpha_2A\)AR (Fig. 2), in a region thought to be important for G-protein interaction (24).

Indeed, as shown in Fig. 2, Asn is present in the position analogous to human 251 in all mammalian \(\alpha_2A\)ARs reported to date. To assess whether this polymorphism affects G-protein coupling, functional studies examining agonist-promoted inhibition of forskolin-stimulated adenylyl cyclase activities were carried out in cell lines expressing the wild-type Asn-251 receptor and the polymorphic Lys-251 receptor at levels of 2360 ± 263 and 2590 ± 140 fmol/mg \((n = 5, p > 0.05\), respectively. As shown in Table I, basal and 5.0 \mu M forskolin-stimulated adenylyl cyclase activities were not different between Asn-251- and Lys-251-expressing cell lines, indicating that non-agonist-dependent function is equivalent with the two receptors. However, activation of the polymorphic Lys-251 receptor with the full agonist epinephrine resulted in enhanced inhibition of adenylyl cyclase activity compared with wild-type \(\alpha_2A\)AR. Maximal inhibition of adenylyl cyclase was 60 ± 4.4% with the variant receptor compared with 46 ± 4.1% with wild-type \(n = 5, p < 0.005, \text{Fig. 3A}\). Similar results were also found when receptors were activated by the partial agonist oxymetazoline, with the Lys-251 having an \(\sim 40\%\) augmented function compared with the Asn-251 receptor \((50 \pm 6.6\%\text{ versus } 35 \pm 4.7\%\text{ inhibition}, n = 5, p < 0.05, \text{Fig. 3B}\). No significant differences in the EC\(_{50}\) values for epinephrine \((583 \pm 196 \text{ nM versus } 462 \pm 145 \text{ nM})\) or for oxymetazoline \((54.0 \pm 7.3 \text{ nM versus } 67.3 \pm 15.7 \text{ nM})\) were observed.

This enhanced function was also found by quantitating agonist-promoted receptor-Gi interaction with \([^{35}S]G\text{TP}\text{S}\text{ binding. In these experiments, Asn-251 and Lys-251 receptors were transiently co-expressed in COS-7 cells (2.3 \pm 0.3 \text{ versus } 2.2 \pm 1.4 \text{ pmol/mg}) along with G\text{i22.} Of note, in the absence of co-
transfected Gs, epinephrine promoted [35S]GTPγS binding was only 12 ± 2.6% over basal, compared with 57 ± 11% over basal when G112 was co-expressed. So, these studies primarily quantitate α2AR-G112 coupling as opposed to G-proteins, which are endogenously expressed in these cells. As is shown in Fig. 4, the Lys-251 receptor displayed increased [35S]GTPγS binding in response to all agonists tested, albeit to varying degrees. Basal [35S]GTPγS binding was equivalent. Stimulation with the full agonists UK-14304, epinephrine, and norepinephrine resulted in ~40% enhanced GTPγS binding for the Lys-251 receptor as compared with the Asn-251 receptor. On the other hand, partial agonists displayed from 45% (BHT-933) up to 72% (guanabenz) enhancement of [35S]GTPγS binding with the Lys-251 receptor. These results are consistent with the adenyl cyclase activity studies, which also showed enhanced function of the polymorphic receptor. In addition, they indicate that the gain-of-function phenotype is more pronounced with some, but not all, partial agonists compared with full agonists.

We next investigated agonist-mediated modulation of MAP kinase by wild-type and the Lys-251 receptor. α2AR act to stimulate MAP kinase activity and thus can potentially regulate cell growth and differentiation (2). Although noting that regulation of MAP kinase activity is both receptor and cell-type specific, MAP kinase activation by α2AR receptors appears to be initiated by βγ released from Gs (2). To investigate the extent of MAP kinase activation in CHO cell lines expressing both the Asn-251 and Lys-251 receptors, quantitative immunoblots using an antibody specific to the activated (phosphorylated) form of ERK 1/2 were performed. Although the total amount of MAP kinase was not different (Fig. 5A), agonist-promoted stimulation of MAP kinase activity was markedly different between the two cell lines (Fig. 5, A and B). Activation of the Lys-251 receptor with 10 μM epinephrine resulted in a 57 ± 9-fold increase in MAP kinase activity over basal as compared with 15 ± 2.1-fold increase with the Asn-251 receptor (n = 3, p < 0.05).

Finally, coupling of these two receptors to the stimulation of inositol phosphate production was examined. Such α2AR signaling is a complex response due to activation of phospholipase C by βγ released from activated Gs, and G (25). Maximal epinephrine-stimulated accumulation of inositol phosphates was not found to be different between Lys-251- and Asn-251-expressing cells (Fig. 6). However, the EC50 was lower with the Lys-251 receptor (119 ± 36 nM) than the wild-type α2AR (493 ± 21 nM, p < 0.05). Thus signaling to PLC is also enhanced with the polymorphic receptor based on this increase in agonist potency.

α2ARs are widely expressed throughout the nervous system and peripheral tissues. Recent work with relatively selective agonists and antagonists, radiolabels, and specific molecular probes in several species, including genetically engineered mice, have begun to elucidate specific functions for the various α2AR subtypes (26). The latter studies have been particularly useful in identifying subtype-specific functions. Mice lacking α2AR have higher resting systolic blood pressures and more rapidly develop hypertension with sodium loading after subtotal nephrectomy than wild-type mice (27). Furthermore, these α2AR knock-out mice fail to display a hypotensive response to the agonist dexmedetomidine (5). Heart rates in these mice were increased at rest, which correlated with increased [3H]norepinephrine release from cardiac sympathetic nerves. These data thus indicate 1) that the presynaptic inhibition of neurotransmitter release in cortical and cardiac nerves serves important homeostatic functions in blood pressure and cardiac function, and 2) that the physiologic effects of therapeutic agonists such as clonidine reduce blood pressure by specifically acting at the α2AR subtype. The lack of a hypotensive effect of α2AR agonists has also been shown in genetically altered (hit-and-run) mice expressing a dysfunctional α2AR (D79N) (28). These mice also responded poorly to α2AR agonists for several other physiologic functions (6). Dexmedetomidine failed to reduce rotorod latency or induce prolongation of sleep time, to enhance the efficacy of halothane, or to attenuate thermally induced pain in these mice. Thus the sedative, anesthetic-sparing, and analgesic effects of α2AR agonists are due to activation of the α2AR subtype. Indeed, these physiologic defects correlated with absent α2AR regulation of inwardly rectifying K+ channels of locus ceruleus neurons and voltage-gated Ca2+ channels of these same neurons, as well as those of the superior cervical ganglion (6).

The above studies indicate that a polymorphism resulting in a markedly depressed α2AR function in humans would likely be of physiologic importance. Indeed, such a polymorphism might well be a significant risk factor for hypertension. How-
ever, the one coding block polymorphism that we found in Caucasians and African-Americans is not associated with essential hypertension and in fact its phenotype is a gain of function. It should be noted that with our sample size we have the power to detect a polymorphism with an allele frequency of 0.04 with a statistical certainty of 90%, thus it is unlikely that we have failed to detect another polymorphism that is common in any of the cohorts. Based on the phenotype of the Lys-251 receptor, and the known physiologic function of the \( \alpha_{2A} \)AR, one might predict that the polymorphism would predispose to autonomic dysfunction characterized by hypotension and bradycardia. Similarly, patients with essential hypertension who have the polymorphism may have milder disease or display enhanced efficacy of antihypertensive agents such as clonidine or guanabenz. Interestingly, these individuals may display more pronounced central nervous system side-effects from these agents, such as sedation, which could ultimately limit their therapeutic utility. Finally, the hyperfunctioning polymorphism would be predicted to result in less norepinephrine release from cardiac sympathetic nerves, thereby potentially providing protection against the deleterious effects of catecholamines in patients with heart failure. These scenarios await explicit testing.

Mutations of G-protein-coupled receptors are the basis of a number of rare diseases (29). In contrast, polymorphisms (allele frequencies >1%) of these receptors have been identified, which can be minor risk factors for complex diseases (30), but more importantly act as disease modifiers (31) or alter response to therapeutic agents targeting the receptor (32). Interestingly, when such mutations or polymorphisms have been found to alter function, the minor allelic variant (i.e. the least common form of the receptor) results in either decreased agonist-promoted function or increased non-agonist-dependent function (i.e. constitutive activation). An example of the former is the Ile-164 polymorphisms of the \( \beta_2 \)AR, which imparts defective agonist-promoted coupling to \( G_s \) (33). Constitutive activation results in receptors adopting a mutation-induced agonist-bound like state and thus signaling becomes independent of agonist. Such persistent activation is the pathologic basis for diseases such as male precocious puberty, which is due to a mutation in the leutinizing hormone receptor (34). In the current report we show that the Lys-251 receptor does not exhibit constitutive activation, based on wild-type \([^35]GTP\gamma S\) binding, adenylyl cyclase, and MAP kinase activities in the absence of agonist. Instead, the phenotype that we observed was one of increased agonist-promoted function. To our knowledge this is the first delineation of a polymorphism of a pharmacogenetic locus of any G-protein-coupled receptor where the minor allele displays this property, and thus this represents a new class of polymorphism for the superfamily.

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