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Identification of single nucleotide polymorphisms of the human metabotropic glutamate receptor 1 gene and pharmacological characterization of a P993S variant

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ABSTRACT

mGluR1 receptors are believed to play major roles in the pathophysiology of diseases such as anxiety and chronic pain and are being actively investigated as targets for drug development. Sequence polymorphisms can potentially influence the efficacy of drugs in patient populations and are therefore an important consideration in the drug development process. To identify DNA sequence variants of the mGluR1 receptor, comparative DNA sequencing was performed on DNA samples (n = 186) from apparently healthy subjects representing two ethnic groups. In total, eight non-synonymous single nucleotide polymorphisms (SNPs) were identified and one SNP (c2977 > T) was found to be particularly common, this SNP results in a proline to serine substitution at residue 993 (P993S). The WT (P993) and S993 variants were expressed in an inducible system which allowed us to titrate gene expression to equivalent levels and were pharmacologically characterized. We determined the potency and affinity of standard antagonist compounds as well as the potency and efficacy of the endogenous ligand glutamate and other agonist compounds at both receptor variants. Agonist evoked increases in intracellular Ca^{2+} were measured by fluorometric imaging plate reader (FLIPR). The potency of mGluR1 antagonists was evaluated by their ability to inhibit quisqualate induced increases in intracellular Ca^{2+}, while their affinities were determined by radio-ligand binding studies. This study demonstrates that the Pro993Ser amino acid exchange is highly frequent in the human mGluR1 gene. This polymorphism however, does not appear to affect the potency of agonist compounds or the potencies or affinities of small molecule antagonist compounds.

1. Introduction

Glutamate is the major excitatory neurotransmitter in the central nervous system, it produces its excitatory effects by acting on two classes of cell surface receptors, ligand gated cation channels known as ionotropic glutamate receptors and G protein coupled receptors termed metabotropic glutamate receptors [1]. Metabotropic glutamate receptors form a family

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of eight subtypes (mGluR1-8) which on the basis of structural homology, pharmacology and signal transduction mechanisms are subdivided into three groups [2]. Group I receptors (mGluR1 and mGluR5) usually act postsynaptically to increase neuronal excitability [3]. Excessive glutamatergic neurotransmission has been shown to underlie many CNS diseases and to play an important role in the pathophysiology of diseases such as depression, anxiety, and chronic pain, which represent key areas of interest for drug development. Accordingly, mGluR group I antagonists could represent useful agents for the treatment of these conditions. Indeed, in preclinical models, mGluR I antagonists have been shown to be very effective agents in the treatment of prolonged and chronic pain [4–6] and to possess anxiolytic activity [7,8]. Recent data suggests that mGluR1 antagonists may also be useful therapeutic agents for treating drug dependence [9] and depression [10]. However, the involvement of mGluR1 in human CNS disorders has yet to be demonstrated, it is therefore important to carry out clinical proof of concept studies to understand the functions of mGluR1 in man. As a prelude to this type of activity it is important to understand the genetic variation within this receptor gene among human populations and within different ethnic groups.

Single nucleotide polymorphisms (SNPs), substitutions of a novel nucleotide for the wild type nucleotide within genomic DNA are the most common type of genetic variation and occur at a frequency of greater than 1% in the general population [11]. Within the coding region of a gene, non-synonymous SNPs, those that change the encoded amino acid sequence, can profoundly effect protein folding and disrupt post-translational modifications [12]. As far as GPCRs are concerned, there are a number of reports which show that non-synonymous SNPS may also alter the normal functioning of the receptor by disrupting ligand–receptor binding [13] or altering the normal coupling to G proteins [14,15]. In some cases, SNPs may lead to increased disease susceptibility or yield variable responses to therapeutic agents. Therefore, evaluating the pharmacogenetics of disease-related targets is an important step in the drug development process.

The current study reveals the frequency of polymorphisms in the human mGluR1 gene and pharmacologically characterizes the very frequent P993S receptor variant. Despite the fact that eight non-synonymous SNPs were identified, most were very rare and only the P993S variant was present at a frequency of greater than 5% in both populations. We have previously described the use of an inducible system, the Ecdysone system, to functionally express representative mGluR subtypes [16]. Expressing the WT and S993 mGluR1 variants in this system allowed us to tightly regulate gene expression, so that we were able to express the two forms of the receptor at almost identical expression levels facilitating the functional analysis of the receptor variants. It has previously been demonstrated that non-competitive antagonist compounds bind to the same binding pocket made from transmembrane helices IV–VII [17]. All of the mGluR1 selective antagonists tested displayed similar potencies and affinities at the two receptor variants, it is therefore unlikely that the identified genetic variations would significantly modify the effectiveness of mGluR1 antagonists in man.

2. Materials and methods

2.1. Compounds

Agonists. DHPG (3,5-dihydrophenylglycine); L-glutamate; quisqualic acid ((L)-(+)-a-amino-3,5-dioxo-1,2,4-oxadiazolidine-2-propanoic acid).

Antagonists. JNJ16259685 (3,4-dihydro-2*H*-pyrano[2,3]b-quinolinyl-7-yl)(*cis*-4-methoxycyclohexyl) methanone, LY456236 (4-methoxy-phenyl)-(6-methoxy-quinazolin-4-yl)-amine hydrochloride; all agonists and antagonists were purchased from Tocris Cookson (Bristol, UK), except for SCH1041222 which was synthesised by Schering Plough. Compounds were dissolved in HBSS or DMSO depending on their solubility. The final concentration of DMSO in all samples was compensated.

2.2. Research subjects

To identify SNPs, the mGluR1 gene was sequenced from anonymous Caucasian ($n = 47$) and African American ($n = 46$) individuals. Genomic DNA samples were obtained from the Caucasian and African American human variation panels collected from the Human Genetic Cell Repository of the National Institute for General Medical Sciences (Coriell Cell Repository; Camden, NJ, USA). All samples came from individuals who provided informed consent to be part of the DNA polymorphism Discovery Resource. Information on geographic origin and gender was collected for each individual in order to assemble the DNA polymorphism Discovery Resource, but all identifying and phenotypic information has been removed from the individual samples.

2.3. Polymerase chain reaction

The general strategy for SNP discovery is as previously described [18] with modifications as detailed. PCR primers were designed using the Primer3 software (http://www.genome.wi.mit.edu/cgi-bin/primer/primer3.cgi) to amplify 400–650 bp segments of the GRM1 coding region, as well as approximately 100 nucleotides flanking the intron/exon splice junctions as determined by gaped alignment of the GRM1 coding sequence (Genbank accession no. NM_000838) with a finished genomic contig from chromosome 6 using sequencher (v 4.05 Gene codes Corp., Ann Arbor, MI, USA). Forward and reverse primers were 5′ tailed with universal primers −21 M13 5′ TGTAAAACGACGGCCAGT and M13REV; CAGGAAACAGCTATGACC respectively. PCR reactions contained genomic DNA (24 ng in the presence of Platinum PCR supermix (100 mM dNTPs 1.5 mM $MgCl_2$, 0.1 U Platinum Taq polymerase (Invitrogen Corp., Carlsbad, CA, USA) and 0.2 pmol/ml forward and reverse primers in a 12 μl total volume. Thermocycling was performed in 96-well microplates on a PTC.200 thermocycler (MJ Research; Waltham, MA, USA) with an initial denaturation of 94 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 30 s, primer annealing for 30 s and primer extension at 72 °C for 1 min, after 35 cycles a final extension was carried out for 7 min at 72 °C.

2.4. DNA sequencing and analysis

Following DNA amplification, PCR amplicons were sequenced in the forward and reverse directions using ABI PRISM BigDye terminator v3.1 Cycle Sequencing DNA Sequencing Kit (Applied Biosystems, Foster City, CA) on an Applied Biosystems 3730XL DNA Analyzer. Chromatograms were transferred to a Unix workstation (DEC alpha, Compaq Corp.), base calling was performed with the Phred software (version 0.990722.g), sequences were assembled with the Phrap software (version 3.01) and scanned with the Polyphred software (version 3.5), and the results were viewed with the Consed software (version 9.0) (Phred, Phrap and Consed available at http://www.genome. washington.edu, PolyPhred is available at http://droog.mbt.washington.edu). Analysis parameters were all maintained at the software's default settings.

2.5. Cloning

c-DNA was synthesised from human hippocampal mRNA (Clontech, Mountain View, CA) using the superscript kit according to the manufacturers (Invitrogen, Paisley, UK) instructions. The coding sequence of mGluR1a was amplified from hippocampal c-DNA by PCR using a proofreading enzyme Pfu_{Turbo} (Stratagene, La Jolla, CA). The coding sequence for mGluR1a was isolated as two overlapping fragments using the following primer pairs, upstream Fwd 5′ ATGGTCGGGCTC-CTTTTGTTTTTTTTC 3′ (1–27), upstream Rev 5′ TTGGCAAT-GAGAGTGAATGGGCACA 3′ (1928–1952), downstream Fwd 5′ ATAGCCATCGCCTTTTCATGCCTGG 3′ (1783–1807), down-stream Rev 5′ TTACAGGGTGGAAGAGCTTTGCTTG 3′ (3561–3588), the 5′ and 3′ fragments were cloned into pCRScript and fully sequenced on both DNA strands. After sequence confirmation the amplified products were joined together using the restriction enzyme Sac1 which has a single site within the mGluR1a coding sequence at nucleotide position 1890. The entire coding sequence was sub-cloned into pCRScript and the junction region was sequenced. To create the expression construct, the mGluR1 coding sequence was amplified using the following primers, Fwd 5′ *gAATTC*gTCCTCACGACCATggTCgggCTCCTTTTgTTTTTTTTC 3′, reverse primer 5′ *gCggCCgC*ggATCCTTCCCCCTTACAgggTg-gAAgAgCTTTgCTTgTAgTCCC which contain an EcoR1 site and a full kozak sequence (forward primer) and a Not1 site (reverse primer). The mGluR1a coding sequence was sub-cloned into the expression vector pIND (Invitrogen, Paisley, UK) as an EcoR1–Not1 fragment, the construct was fully sequenced on both DNA strands.

2.6. Creation of polymorphic variant

The following primer pair was used to amplify a 1260 bp fragment at the 3′ end of the mGluR1a coding sequence from human hippocampal c-DNA, the amplified product extended from base pair 2340 to the end of the coding sequence 3588 bp, Fwd 5′ CCgCCACgTgCCCgCCAACTTCA 3′ Rev 5′ **gCggCCgc**TTACAgggTggAAgAgCTTTgCTT 3′, amplified products were cloned onto pCRScript (Stratagene, La Jolla, CA) and sequenced to determine clones with thymine residues at position 2977 in the coding sequence, the P993 version contains a cytosine residue, the C to T change converts proline into serine. The coding sequence of mGluR1 contains a unique site for the restriction enzyme Nde1 at position 2724 and plasmid pINDS993 containing *mGluR1a-S993* was made by replacing the Nde1–Not1 restriction fragment from pINDP993 with the equivalent fragment from plasmid pCRScript-S993. The expression construct was completely sequenced in the region that had undergone restriction fragment swapping to confirm that only the intended mutation had been introduced.

2.7. Isolation of clonal cell lines

The HEK-293_{EcR} cell line (Invitrogen, Paisley, UK), stably expressing the heterodimeric ecdysone receptor from the pVgRXR plasmid, was maintained in DMEM high glucose, supplemented with 10% foetal bovine serum (FBS), Pen/Strep, Glutamax™ (Invitrogen, Paisley, UK) and 400 μg/ml Zeocin™ (Invitrogen, Paisley, UK) at 37 °C, in an atmosphere containing 5% CO_2. Stable transfections with mGluR1a coding vectors, pINDP993 and pINDS993 were performed using calcium phosphate according to standard protocols. Stably transfected clones were obtained after selection with 550 μg/ml Geneticin® (Invitrogen, Paisley, UK). During the induction of the receptor, cells were maintained in essentially the same culture medium except for the substitution of 5% dialysed serum and the addition of 3 U glutamic–pyruvic transaminase (GPT) (Sigma) and 5 mM sodium pyruvate.

2.8. Binding

Saturation binding experiments to membrane preparations were carried out in a total volume of 125 μl of buffer (50 mM Tris–HCl pH 7.4, 1.2 mM $MgCl_2$, 2 mM $CaCl_2$, 0.1% (w/v) Bovine Serum Albumin) with a range of concentrations of [^{3}H] SCH1041222 (Schering-Plough Research Institute, 86.6 Ci/mmol). The reaction was conducted at 25 °C for 45 min and terminated by rapid vacuum filtration through GF/C filter-plates (Millipore, Italy) and three washing steps with ice-cold buffer. Dried filter-plates were counted with a Wallac Microbeta Trilux (Perkin Elmer, Wallac, Finland). Non-specific binding was defined in the presence of 10 μM unlabeled SCH1041222.

A [^{3}H] SCH1041222 displacement binding assay was performed to estimate the *in vitro* affinities of the reference compounds LY456236 and JNJ16259685. The [^{3}H] SCH1041222 concentration was set to 2 nM. K_i values were calculated using the Cheng–Prusoff equation [19]: $K_i = IC_{50}/[1 + ([C]/K_d)]$ (PRISM® software, version 4.02). Reported K_d and B_{Max} values are the results of nonlinear regression analysis using Graphpad PRISM (GraphPad Software; San Diego, CA, USA).

2.9. Measurement of intracellular calcium transients

Cells from clonal cell lines were seeded into black clear-bottom 96-well plates at a density of 60,000 cells/well, in DMEM high glucose supplemented with 5% dialysed FBS, 3 U/ml glutamic–pyruvic transaminase (GPT, Sigma, St. Louis, MO), 5 mM sodium pyruvate, 5 μM ponasterone A (Invitrogen, Paisley, UK). Following 24 h incubation, the cells were loaded with a fluorescent calcium indicator (Molecular Devices,

Sunnyvale, CA, USA). The dye was dissolved in the assay buffer which consisted of Hanks' balanced salt solution (Gibco Life Technologies) buffered with 20 mM Hepes solution (Sigma, St. Louis, MO). To avoid dye bleaching, 2.5 mM Probenecid (Sigma, St. Louis, MO) was added to the calcium indicator solution. Cells were loaded with the dye for 2 h at 37 °C in a CO_2 incubator. A fluorometric imaging plate reader (FLIPR 384; Molecular Devices, Sunnyvale, CA, USA) was used to measure intracellular calcium by increases in fluorescence upon agonist stimulation following 30 s baseline measurement or 10 min antagonist administration.

3. Results

3.1. Genetic variation of the human mGluR1 gene

The human mGluR1 gene consists of a 3582 nucleotide open reading frame (ORF) divided into 8 exons spanning 409 kbp on 6q24.3. Re-sequencing of the mGluR1 ORF, 5′ and 3′ UTRs and an average of 100 bp upstream and downstream flanking the intron/exon splice junctions from an anonymous panel of Caucasian ($n = 47$) and African American ($n = 46$) subjects identified 46 SNPs. 17 SNPs were identified in the protein coding region of the gene, eight of which resulted in an amino acid substitution (Table 1), of these, only one SNP c2977 > T (rs6923492:chr6:146797017 according to the March 2006 of the UCSC genome build) which results in an amino acid substitution P993S had an allele frequency of greater than 5% in both populations. This genetic variant occurred in exon 8 of the gene at a position corresponding to the C terminal intracellular tail of the receptor. The minor allele frequency was 28.3% in the African American samples, and 35.6% in the Caucasian samples.

3.2. Expression of WT and P993S variant mGluR1a receptors in HEK-293 cells

The coding sequence of the WT mGluR1a was isolated from hippocampal c-DNA and sub-cloned into the expression vector pIND. The C to T substitution at position 2977 of the mGluR1a c-DNA clone (corresponding to 405,804) was created by exchanging the Nde1–Not1 3′ fragment of the WT pINDp993 expression constructed with the equivalent fragment containing a thymine residue at position 2977. mGluR1 is a $G\alpha_q$-coupled receptor; its activation thus leads to an increase in intracellular calcium which can be measured using a FLIPR. Clonal cell lines expressing the WT (P993) or the S993 variant of mGluR1 were initially pre-screened for the stimulation of calcium mobilisation in response to quisqualate following 24 h induction with 5 μM ponasterone A. A single clonal cell line for each receptor variant referred to as P993 or S993 respectively was chosen for further studies. To determine how receptor density can be modulated in these clones, cells were induced for 24 h with increasing concentrations (1–3–5 μM) of ponasterone A and saturation binding studies were performed to determine relative expression levels. The WT clone (P993) gave mean B_{Max} values of 0.1, 0.7, 1.6, 1.9 pmol/ml, while the variant gave mean B_{Max} values of 0.1, 0.2, 0.7 and 1.55 pmol/ml respectively when induced with 0, 1, 3 and 5 μM ponasterone A (see Fig. 1 and Table 2). By varying levels of the inducer we were thus able to produce almost identical levels of expression for the WT and S993 cell line (mean B_{Max} = 1603 fmol/mg protein WT at 3 μM ponasterone A and 1550 fmol/mg protein S993 at 5 μM ponasterone A). A functional characterization of the WT and variant clones was carried out under these conditions.

3.3. Functional characterization of the WT and S993 variant

Saturation binding experiments performed using the non-competitive antagonist SCH1041222 [20], did not highlight significant differences in antagonist binding, mean K_d values determined by saturation binding of this ligand were 0.873 nM for the WT and 1.712 nM for the S993 cell line at the chosen B_{Max} values (1.60 pmol/mg WT and 1.55 pmol/mg S993), K_d values for both the WT and S993 cell lines at different B_{Max} values are shown in Table 2. Furthermore when heterologous displacement experiments were performed using standard non-competitive antagonists LY456236 [21] or JNJ16259685 [22], no significant differences in the affinity of these ligands were observed, mean K_i values of 1.6 nM WT and 3.7 nM S993 for the JNJ16259685 compound and 375 nM WT and 264 nM S993 for the LY456236 compound were determined (Fig. 2a and b). We also determined the potency of all of these non-competitive antagonist compounds in functional calcium assays, quisqualate-induced Ca_i^{2+} release was tested in the presence or absence of LY456236, JNJ16259685 and SCH1041222 (Fig. 3). When an agonist concentration corresponding to an EC_{80} value was utilised to stimulate the receptor, the addition of these reference antagonists resulted in a concentration-dependent

Table 1 – Position and allele frequencies of non-synonymous SNPs identified in the human mGluR1 receptor gene.

SNP	Position in c-DNA	Major allele	Minor allele	AA Change	African American		Caucasian	
					Major (%)	Minor (%)	Major (%)	Minor (%)
g.322791A > C	1345	A	C	K449Q	100	0	98.9	1.1
g.369607C > A	2185	C	A	P729T	100	0	97.7	2.3
g.404379G > A	2785	G	A	V929I	98.9	1.1	96.7	3.3
g.404571T > C	2977	C	T	P993S	71.7	28.3	64.4	35.6
g.404637C > T	3043	C	T	P1015S	98.9	1.1	100	0
g.404701G > T	3107	G	T	G1036V	98.9	1.1	100	0
g.404755G > A	3161	G	A	G1054D	98.9	1.1	100	0
g.404808C > G	3214	C	G	P1072A	96.7	3.3	100	0

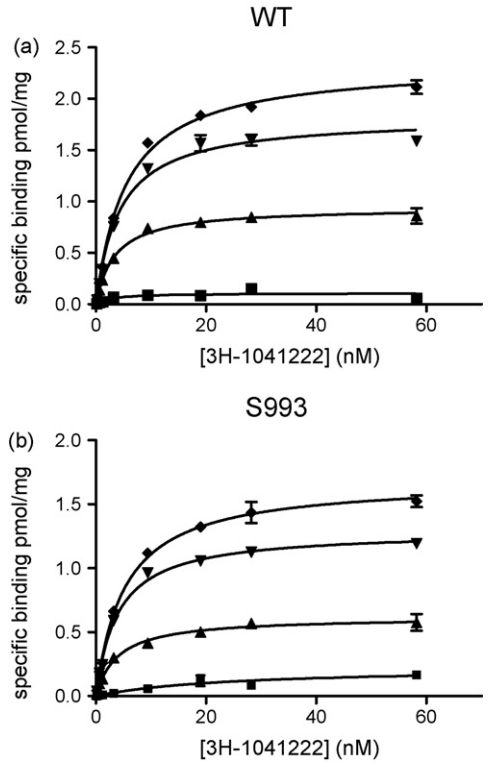

Fig. 1 – Saturation binding experiments for [^{3}H]SCH1041222, in the absence or presence of ponasterone A, (a) and (b) are saturation binding curves from representative experiments from the WT and S993 cell lines respectively, (■) un-induced, (▲) 1 μM, (▼) 3 μM and (◆) 5 μM of ponasterone A.

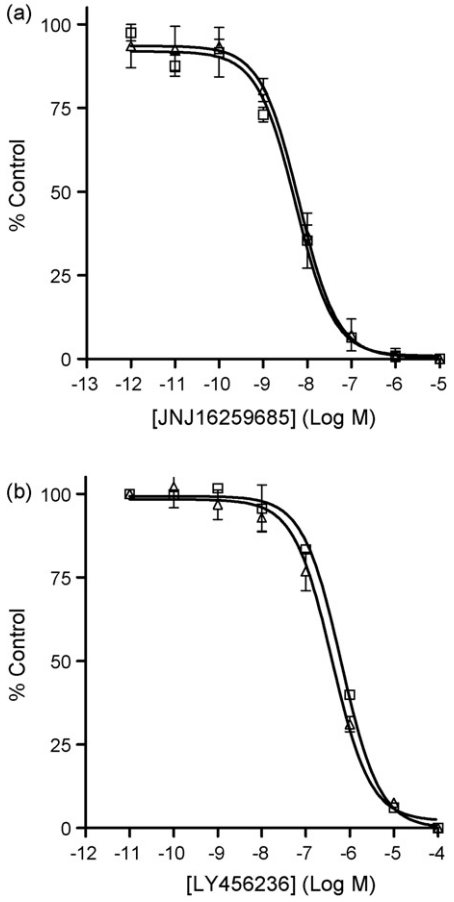

Fig. 2 – Competitive binding studies with selective mGluR1 antagonists: (a) JNJ16259685 and (b) LY456236, WT values are represented by an open square, and S993 values by an open triangle, results are the means of three independent experiments. Data are normalized to the specific binding of the radiolabeled ligand [^{3}H]SCH1041222 observed in the absence of competitor (% control).

inhibition of quisqualate elicited calcium signal with calculated IC_{50} values of 267 nM LY456236, 2.3 nM JNJ16259685 and 4.7 nM SCH1041222 for the WT cell line and 445 nM LY456236, 3.1 nM JNJ16259685 and 5.2 nM SCH1041222 for the S993 variant (Table 3). Here again we did not observe significant differences in the potencies of these compounds at the two receptor variants. The focus of the present study was to determine if the S993 polymorphism had an effect on the affinity or potency of antagonist compounds. However, given the fact that the polymorphic residue resides in the C terminal tail of the protein, it could effect G protein/receptor coupling we therefore performed functional calcium assays with a number of agonist compounds. Again there were no significant differences, in either the potency or the efficacy of any of the compounds tested (Fig. 4 for curves and Table 3).

4. Discussion

It is widely recognised that different patients respond in different ways to the same medication and there are now

Table 2 – Receptor densities and K_d values for the wild type and S993 clones at varying ponasterone A concentrations, results were generated using the radiolabeled ligand [^{3}H]SCH1041222 and are expressed as means (±S.E.M.) from three independent experiments.

	B_{Max} (fmol/mg protein)	K_d (nM)	B_{Max} (fmol/mg protein)	K_d (nM)
Ponasterone A (μM)				
0	136.7 (±67)	3.4 (±0.76)	145.8 (±50)	3.5 (±0.47)
1	799.4 (±147)	3.8 (±0.44)	217.2 (±87)	4.5 (±0.61)
3	1603 (±223)	4.0 (±0.51)	707.7 (±157)	4.8 (±0.67)
5	1951 (±354)	4.9 (±0.49)	1551 (±270)	3.8 (±0.51)

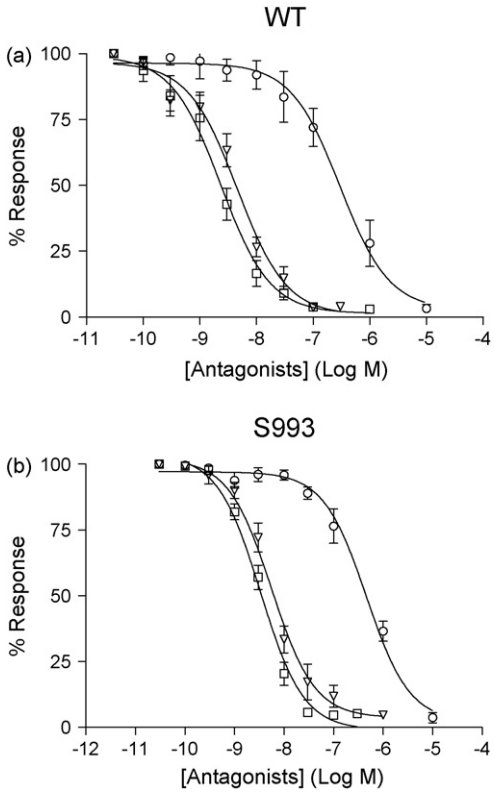

Fig. 3 – Antagonism by (○) LY456236, (□) JNJ16259685 and (▽) SCH1041222 of quisqualate stimulated calcium mobilisation in the WT and S993 expressing cell lines. Inhibition curves were constructed from the percentage responses pooled from three independent experiments performed in quadruplicate, values are means ± S.E.M. Data are normalized to the response generated by the agonist in the absence of the antagonists.

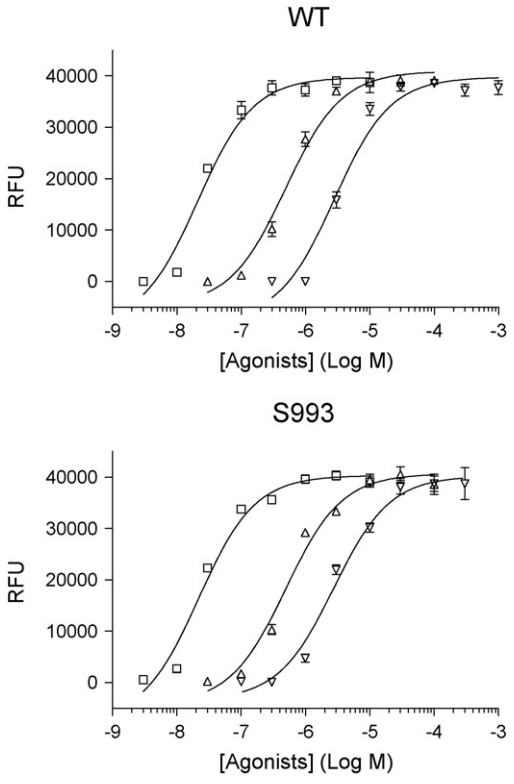

Fig. 4 – Concentration response curves for the effect of (□) quisqualate, (△) glutamate and (▽) DHPG on Ca^{2+} mobilisation in WT and S993 cell lines, values are means ± S.E.M. of quadruplicate determinations from a typical experiment.

numerous examples of cases in which inter-individual differences in drug response are due to sequence variants in genes encoding drug metabolizing enzymes, drug transporters or drug targets. Studies of genetic variation originally focused on drug metabolism but have now been extended to encompass the full spectrum of drug disposition, including a growing list of transporters that influence drug absorption, distribution and excretion [23]. Most drugs exert their pharmacological effects by interacting with specific target proteins, molecular studies have revealed that many of the genes which encode drug targets exhibit genetic polymorphism, which in many cases alters their sensitivity to specific medications. Well characterized examples include polymorphisms in β-adrenergic receptors and their sensitivity to β-agonists in asthmatics [24], the angiotensin converting enzyme (ACE) and its sensitivity to ACE inhibitors [25] and the 5-hydroxtryptamine receptor and response to neuroleptics such as clozapine [26]. There is now an extensive and growing list of targets where polymorphisms have been linked to altered drug sensitivity, ongoing and future studies will undoubtedly expand the number of such pharmacogenetic relationships.

Table 3 – Functional activities of agonists and antagonists examined at the WT or S993 variant respectively, data are the mean EC_{50} values of agonists and IC_{50} values of antagonists determined from three independent experiments performed in quadruplicate, and are expressed as means ± S.E.M.

	WT	S993
Agonist potencies (μM)		
Quisqualate	0.0215 (±0.012)	0.0241 (±0.009)
Glutamate	0.460 (±0.18)	0.340 (±0.14)
DHPG	1.79 (±0.73)	1.64 (±0.12)
Antagonist potencies (μM)		
JNJ1625968	0.0023 (±0.0008)	0.0031 (±0.0012)
SCH1041222	0.0047 (±0.0012)	0.0052 (±0.0014)
LY456236	0.267 (±0.120)	0.445 (±0.145)

The metabotropic glutamate receptor family belongs to the class C GPCRs, and is the subject of intense investigation by pharmaceutical companies, as it is widely believed that the glutamatergic system plays a major role in the pathophysiology of clinically important diseases. mGluR1 antagonists represent potential therapy in a wide range of disorders including chronic pain, depression and anxiety and the patent and the literature databases suggest that the development of such compounds is a very active field. However, studies investigating the occurrence of genetic variation within this

receptor gene and the comparison of agonist and antagonist potency at different mGluR1 variants have not been published.

This is the first report of polymorphisms in the human metabotropic receptor 1 gene. Re-sequencing of mGluR1 in 93 DNA samples from apparently healthy subjects of African American or Caucasian ancestry identified 46 SNPs and three insertion/deletion polymorphisms. Of the 46 SNPS identified, 17 were in the coding region of the gene and 8 of those resulted in an amino acid change. This study demonstrates that while there are a number of non-synonymous SNPs, most of these are very rare. Of the eight SNPs identified, four were only seen in the African American population and two were only observed in the Caucasian population, and all of these SNPs were present in less than 5% of their respective populations. Only two of the eight identified SNPs were present in both populations and only the c2977 > T (P993S) SNP was frequent in more than 5% of each population. This variant was observed in 28.3% of the African American and 35.6% of the Caucasian alleles respectively. The sample size used in this study was sufficiently powered to ensure identification of 99% of all SNPs present in either population at a minor allele freq of 5% or greater [11]. The polymorphism described here, S993, occurs in the intracellular C terminal part of the receptor. mGluRs bind glutamate and other agonists at an extracellular amino terminal domain [27], while non-competitive antagonists bind to a transmembrane portion of the receptor [17]. G protein/receptor coupling has been largely attributed to interactions between the second and third intracellular loops of the receptor and the C terminus of the G Protein [28,29], amino acids crucial for binding non-competitive antagonists of the human mGluR1 receptor reside in the transmembrane segments V–VII [17] and are thus located relatively far away from the polymorphism evaluated in this study. Although it cannot be excluded apriori that substitution of distant residues could evoke allosteric conformational changes in the antagonist binding pocket, it is not unduly surprising that the potency and affinity of these compounds does not differ between the S993 variant and the wild type receptor. Our results indicate that only the S993 polymorphism occurs frequently within the mGlu1 receptor and that this polymorphism does not significantly affect either antagonist affinities or potencies nor does it alter agonist potencies. However, it remains to be determined whether this SNP would play a role under pathological conditions or during neuronal plasticity. On balance, we believe that the identified genetic variations are unlikely to reduce the effectiveness of mGluR1 antagonists if and when such compounds eventually reach proof of concept studies in man.

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