ORIGINAL RESEARCH

Key Words: Pharmacogenetics, SSRI, Adverse Drug Reactions, ADRB1, Beta-1 Receptor

A Pilot Study of the Pharmacodynamic Impact of SSRI Drug Selection and Beta-1 Receptor Genotype (*ADRB1*) on Cardiac Vital Signs in Depressed Patients: A Novel Pharmacogenetic Approach

By Kelan L.H. Thomas, Vicki L. Ellingrod, Jeffrey R. Bishop, Michael J. Keiser

ABSTRACT ~ Objective: *The adrenergic beta-1 receptor gene* (ADRB1) *Ser49Gly and Arg389Gly variants differentially affect blood pressure response to beta-blocker therapy. Binding site prediction results for fluoxetine and paroxetine in a bioinformatics model estimated that each of these particular selective serotonin reuptake inhibitors (SSRIs) have high receptor affinity as an "Adrenergic (beta) Blocker," which was confirmed in vitro. This pilot study was conducted to understand the relationship between these "beta-blocking" SSRIs (fluoxetine and paroxetine) and cardiac vital signs (systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR)), when subjects are stratified by* ADRB1 *genotype. Previously ascertained DNA and clinical data was examined from 122 subjects recruited for a cross-sectional study of health and well being during SSRI pharmacotherapy. A multivariate linear regression analysis was used to determine which variables affected cardiac vital signs. There was a significant interaction between Arg389Gly variant status and "beta-blocking" SSRIs [p = 0.0353] in relation to SBP. Specifically in homozygous Arg389 subjects, those receiving "beta-blocking" SSRIs had significantly lower SBP (mean 104 mmHg) compared to the group taking other SSRIs (mean 122 mmHg) [p = 0.0437]. In these same homozygous Arg389 subjects, those receiving "beta-blocking" SSRIs also had lower HR (mean 60 bpm) compared to the other SSRIs (mean 79 bpm) [p = 0.00877]. Future prospective studies of this phenomenon are necessary to identify all genetic markers that can predict SSRI-associated cardiovascular effects that may be related to the SSRI Discontinuation Syndrome and potentially influence pharmacotherapy decisions.* Psychopharmacology Bulletin. *2010;43(1):11–22.*

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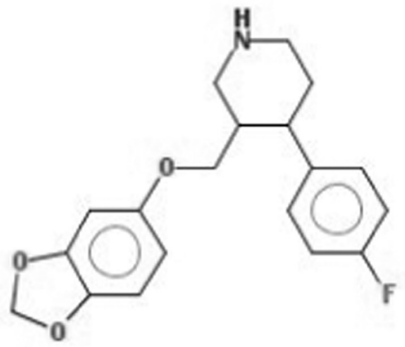
INTRODUCTION

In the field of psychiatry, relatively few pharmacogenetic studies have used medication-related adverse drug reactions (ADRs) as the primary outcome and most only consider candidate genes already identified by efficacy studies.[1] While this approach is a logical starting point, psychiatric medications often bind to many different receptors that could be completely unrelated to the targets mediating efficacy. Two of the authors

FIGURE 1

SCREENSHOT OF SEA SEARCH RESULTS FOR PAROXETINE

sea viewer

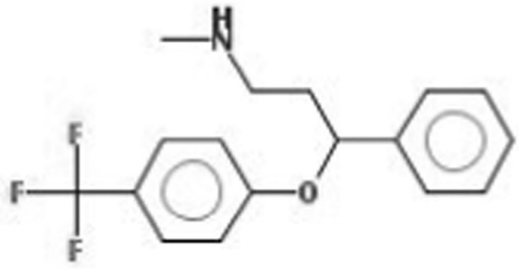


	Code	#Ligands	Reference Name	E-value	MaxTC	Molecules
1	31250	212	Adrenergic (beta) Blocker	3.11e-7	0.49	view >>
2	06245	1042	5 HT Reuptake Inhibitor	3.66e-5	1.00	view >>
3	31656	203	Endothelin ETB Antagonist	6.62e-4	0.39	view >>
4	12342	1761	Antimigraine	5.38e-3	0.58	view >>
5	06240	565	5 HT1A Antagonist	1.48e-2	0.49	view >>
6	11200	35	Antidyskinetic	2.22e-2	0.33	view >>
7	31655	387	Endothelin ETA Antagonist	1.12e-1	0.39	view >>
8	07712	421	Sigma Antagonist	4.28e-1	0.34	view >>
9	10000	3082	Anticonvulsant	4.53e-1	0.58	view >>
10	31650	828	Endothelin Antagonist	5.01e-1	0.39	view >>
11	09229	87	Nicotinic Agonist	2.63e+0	0.33	view >>
12	08000	6458	Antidepressant	5.64e+0	1.00	view >>
13	33456	2121	Antiischemic, Cerebral	5.78e+0	0.58	view >>

FIGURE 2

SCREENSHOT OF SEA SEARCH RESULTS FOR FLUOXETINE

sea viewer



	Code	#Ligands	Reference Name	E-value	MaxTC	Molecules
1	08415	382	Norepinephrine Uptake Inhibitor	1.15e-29	0.55	view >>
2	06245	1042	5 HT Reuptake Inhibitor	9.68e-28	1.00	view >>
3	31250	212	Adrenergic (beta) Blocker	9.41e-15	0.55	view >>
4	35100	1500	Urinary Incontinence, Agent for	4.64e-9	0.54	view >>
5	06240	565	5 HT1A Antagonist	4.85e-9	0.55	view >>
6	08000	6458	Antidepressant	5.03e-8	1.00	view >>
7	33456	2121	Antiischemic, Cerebral	3.07e-3	0.62	view >>
8	01200	5490	Analgesic, Non-Opioid	1.79e-2	0.54	view >>
9	31500	1663	Calcium Channel Blocker	1.14e-1	0.59	view >>
10	09280	250	Antiamyloidogenic Agent	1.66e-1	0.55	view >>
11	06200	7121	Anxiolytic	1.03e+0	1.00	view >>
12	12342	1761	Antimigraine	2.38e+0	0.62	view >>
13	09221	809	Acetylcholinesterase Inhibitor	4.57e+0	0.41	view >>
14	42731	1820	Substance P Antagonist	5.78e+0	0.36	view >>
15	10000	3082	Anticonvulsant	6.36e+0	0.62	view >>

(MK and KT) have recently published a pharmacology prediction bioinformatics model and confirmed that several medications bind unappreciated secondary targets, which may provide better explanations of ADRs.[2] Predicting drug binding to these targets and understanding how ADRs relate to genetic variation in receptors could aid our understanding of ADR pharmacology. A greater understanding of these unknown pharmacology targets could thereby facilitate the advancement of personalized medicine into clinical practice.

The selective serotonin reuptake inhibitor (SSRI) class of antidepressants is generally well tolerated overall, but there are still several ADRs that render the medication intolerable to some patients.[1] The SSRI Discontinuation Syndrome is an ADR that can be problematic when patients lower the dose or decide to stop taking their SSRI without tapering. Most interesting though, not all SSRIs are associated with this Discontinuation Syndrome, which may be due to subtle differences in each drug's pharmacokinetic and pharmacodynamic profile. By using a novel bioinformatics tool for binding site prediction, the profiles for the antidepressants, fluoxetine and paroxetine both suggested a strong affinity for the adrenergic beta-1 receptor (ADRB1) (http://sea.docking.org/search) (Figure 1 & 2), which was confirmed [paroxetine (K_i = 10 μM) and fluoxetine (K_i = 4.4 μM)].[2]

Previous research has suggested a pharmacogenomic relationship between cardiac vital signs, heart rate (HR), systolic and diastolic blood pressure (SBP & DBP) and the *ADRB1* gene. Two common single nucleotide polymorphisms (SNPs) in linkage disequilibrium on the *ADRB1* gene have been well characterized and are known to be associated with differential cardiovascular response to beta-blockers. The relationships between these variants and cardiovascular differences seen with SSRI use have not been previously studied. Thus, the purpose of this pilot project was to begin exploring the relationship between the *ADRB1* Ser49Gly [rs1801252] and Arg389Gly [rs1801253] SNPs and cardiovascular measures in a depression cohort currently being treated with antidepressants. Our hypothesis was that cardiac vital signs would be lower in subjects receiving "beta-blocking" (fluoxetine or paroxetine) SSRIs who also possessed the *ADRB1* 49Gly and Arg389 beta-blocker responsive alleles.

MATERIALS AND METHODS

Bioinformatics Tool

Recently, two authors (MK and KT) have demonstrated the ability of *in silico* modeling to predict unappreciated secondary targets for drug molecules.[2] The "Similarity Ensemble Approach" (SEA), can recognize two sets of ligands as similar even though no single identical compound is shared between them. The search for unappreciated SSRI targets was conducted using this bioinformatics tool for each individual compound's canonical simplified molecular input line entry specification (SMILES) via the online SEA Search Tool at http://sea.docking.org/search. This tool converts each SSRI to a Scitegic ECFP_4 molecular fingerprint, which it then compares against a database of fingerprints for 65,241

other pharmaceutical compounds, organized into sets for 246 therapeutic targets.[2] Then it calculates SEA E-values as previously described and outputs the top-scoring therapeutic targets predicted for each SSRI, as in Figures 1 & 2. The fingerprint database is derived from the MDL® Drug Data Report (MDDR), a drug compound database covering the patent literature, journals, meetings and congresses.[2]

Participants

Subjects included in this study were originally recruited for a point prevalence "health and well-being study" of SSRI use.[3] In the original study, 122 outpatients treated for depression with an SSRI were recruited through local advertisements. Potential research subjects were screened by telephone for eligibility. If inclusion and exclusion criteria were met, they were then interviewed to determine the efficacy and tolerability of current antidepressant therapy where a buccal cell-derived DNA sample was also obtained.

The inclusion criteria for the parent study were designed to include relatively healthy patients taking an SSRI for depression, who did not have prior cardiovascular disease or medications affecting cardiovascular function. This makes the sample optimal for investigating differences in blood pressure potentially caused by SSRIs and subsequently investigating gene variants affecting these outcomes. Subjects were included if they had been treated with an SSRI for at least six weeks, were between ages 18–40, and did not have problems with sexual desire or functioning before starting the medication. Potential subjects were excluded if they were taking another medication for depression or any other medications known to affect sexual functioning (positively or negatively) including beta-blockers and other cardiovascular active drugs, had any other documented primary Axis I diagnosis, cardiovascular disease, neurological disorder, diabetes mellitus (Type I or II), genitourinary disease, or reported frequent urinary tract infections. Chart reviews were conducted to confirm that subjects met above inclusion criteria and carried a diagnosis of depression. There were a total of thirteen subjects excluded from the final analysis; seven due to inclusion/exclusion criteria and six who later withdrew consent.

Assessments

Subjects were assessed in person at the University of Iowa General Clinical Research Center (GCRC) where they were consented for the study, using an informed consent document approved by the University of Iowa Institutional Review Board. The general assessment included

vital signs, height, weight, and sociodemographic variables previously associated with sexual well-being (i.e. age, marital status, number of children, years of education, alcohol consumption, smoking status, or illicit drug use). Trained GCRC nurses assessed cardiac vital signs after participants had been seated for approximately 15 minutes.

Genotyping

Genomic DNA was isolated from buccal cells collected with cheek brushes (Cyto-pak, Medical Packaging Corp, Camarillo, CA) using a previously described protocol.[4] Genotyping for the *ADRB1* SNPs was done with Pyrosequencing™ Technology.[5] Duplicates were also used to verify replicable results during genotyping. The PCR primers used for amplification and genotyping of each variant were as follows: for the Ser49Gly [rs1801252] assay, the primers were forward 5'AGCCCG-GTAACCTGTCGT, reverse 5' Biotin – CGCTGTCCACTGCT-GAGA, and sequencing 5'CCTCCCGCCAGCGAA; and for the Arg389Gly [rs1801253] assay, the primers were forward 5' CAACTCG-GCCTTCAACCC, reverse 5' Biotin – GCTCGTCCAGGCTC-GAGTC, and sequencing 5' CCGCAAGGCCTTCCAG. Forty-five PCR cycles were done for reactions in a 30 μl volume with 1.5 mM Mg^{2+} and 10 pmol of each primer according to the following specifications: initial denaturation for 5 min at 94°C, 94°C for 30 s, 56°C for 30 s, 62°C for 30 s, and a final extension at 72°C for 10 min.

PCR products were visualized by electrophoresis on 1.8% agarose gels stained with ethidium bromide before Pyrosequencing.

Data Analysis

Subjects were stratified into four groups based on both genotype and the type of SSRI they were receiving. To compare subject characteristics and clinical outcomes across genotype groups, subjects were divided according to their *ADRB1* Ser49Gly or Arg389Gly status, where Ser49 or 389Gly carriers formed one group and the more functional 49Gly and Arg389 homozygotes formed the other. To compare subject characteristics and clinical outcomes by SSRI groups, subjects receiving fluoxetine or paroxetine were defined as "beta-blocking" SSRIs, while those receiving citalopram, sertraline, or escitalopram, were defined as non-"beta-blocking" SSRIs. The "beta-blocking" SSRI group was then compared to the non-"beta-blocking" SSRI group to determine the difference in average cardiac vital signs within each genotype group. Differences between any group comparisons of cardiac vital signs averages were determined by the use of Welch's t-tests for comparing

means. A multivariate linear regression analysis was carried out to compare *ADRB1* dysfunctional allele carriers (Ser49 or 389Gly) to homozygotes of the other more functional allele (49Gly/49Gly or Arg389/Arg389) for effects on cardiac vital signs.

All statistical analyses were performed with the use of R software (version 2.7.1; R Foundation for Statistical Computing, Vienna, Austria) or Haploview[6] for genetic analysis. A p-value of less than 0.05 was considered statistically significant.

RESULTS

A total of 109 participants were analyzed from the cohort. Of these 109 subjects, 84 were female (77%), 100 were Caucasian (92%) and the mean age (± s.d.) was 26 ± 5.7, with a total of 36 subjects (33%) receiving "beta-blocking" SSRIs. The frequencies of the Ser49 and 389Gly alleles in our study population were 0.87 and 0.66, respectively. The alleles for both *ADRB1* SNPs were in linkage disequilibrium (D' = 1.00) and both sets of genotypes did not deviate from Hardy-Weinberg equilibrium (p = 0.95 and 0.97 respectively). The Ser49Gly variant status was eliminated from the final statistical analyses since there were only 2 out of the 109 subjects homozygous for 49Gly. Subject characteristics were stratified by *ADRB1* Arg389Gly genotype to test for any difference in means, illustrated in Table 1.

Overall, there were significant associations between SBP and those receiving "beta-blocking" SSRIs across *ADRB1* Arg389Gly variant status. After controlling for gender [t = 2.240, p = 0.0273] and body mass index (BMI) [t = 3.666, p = 0.000392] covariates in the regression

TABLE 1

SUBJECT CHARACTERISTICS STRATIFIED BY *ADRB1* ARG389GLY SNP GENOTYPE

		ARG389GLY	
Genotype (n = 109)	Arg/Arg (n = 13)	Gly allele carriers (n = 96)	p-value
Hardy-Weinberg Equilibirum	Arg allele freq = 0.34	Gly allele freq = 0.66	0.97
MEASURE	MEAN(SD)	MEAN (SD)	P-VALUE
Age (years)	27 (7.3)	26 (5.5)	0.64
BMI (kg/m^2)	27.6 (7.1)	27.3 (6.5)	0.89
SBP (mm Hg)	116 (18.5)	121 (12.3)	0.36
DBP (mmHg)	70 (8.6)	70 (9.7)	1.0
HR (n = 66) (bpm)	72 (13.0)	80 (16.1)	0.12

(*continued*)

TABLE 1 (*CONTINUED*)

MEASURE	N (%)	N (%)	P-VALUE
Female (n = 84, 77%)	11 (85)	73 (76)	0.49
Male (n = 25)	2 (15)	23 (24)	0.49
Citalopram (n = 13)	2 (15)	11 (12)	0.68
Escitalopram (n = 33)	2 (15)	31 (32)	0.21
Sertraline (n = 27)	5 (39)	22 (23)	0.22
Fluoxetine (n = 25)	3 (23)	22 (23)	0.99
Paroxetine (n = 11)	1 (8)	10 (10)	0.76
"Beta-blocking" (n = 36, 33%)	4 (31)	32 (33)	0.85

model, there was a significant interaction between Arg389Gly variant status and "beta-blocking" SSRIs [t = 2.133, p = 0.0353]. Specifically, amongst homozygous Arg389 subjects, those receiving "beta-blocking" SSRIs had significantly lower SBP (mean 104 mmHg) compared to those receiving the other SSRIs (mean 122 mmHg) [t = 2.278, p = 0.0437]. In these same homozygous Arg389 subjects, those receiving "beta-blocking" SSRIs also had lower HR (mean 60 bpm) compared to the other SSRIs (mean 79 bpm) [t = 3.612, p = 0.00877]. There was no difference between these groups in mean DBP [t = 1.448, p = 0.181].

DISCUSSION

As part of this pilot investigation we found that specific cardiovascular measures (i.e. SBP and HR) were statistically lower in subjects receiving paroxetine or fluoxetine ("beta-blocking" SSRIs) who also genetically possessed the *ADRB1* Arg389 allele, which has been associated with greater response to beta-blockade. This study is the first (to our knowledge) to examine the relationship between *ADRB1* genetic variability and differences in SBP and HR related to SSRI use. Our ability to determine these differences and test our hypothesis rests upon the deliberate convergence of several different fields of study as well as this new approach to pharmacology prediction.

Candidate Gene Selection

When investigating a drug's pharmacologic pathway, the strongest evidence of a secondary target is the K_i value measured by *in vitro* binding experiments. Lack of resources and time limit this *in vitro* approach, whereas *in silico* algorithms allow for exponentially more comparisons to elucidate new secondary targets. The SEA search tool illustrates a new approach to uncovering secondary targets that could prove useful in candidate gene selection. Secondary pharmacologic targets predicted for

medications may explain why individual drugs in the class tend to have a greater incidence or severity of certain ADRs than others. Variants at each of these off-site target proteins may contribute to a genetically predisposed ADR phenotype induced by treatment with a specific drug.

Candidate SNPs in the pharmacologic pathways may provide additional explanations for the inter-individual variation seen with ADR phenotypes. After selecting candidate SNPs, a pharmacologic mechanism can be hypothesized to explain how each polymorphism may relate to an ADR phenotype. In most cases this is expected to be the rare variant allele, however there could be some polymorphisms for which the common allele is actually related to the ADR phenotype depending on its functional consequences and how it relates to a drug's pharmacologic profile. Therefore, the functional significance of each individual variant on pharmacology would also inform selection of risk alleles for an association analysis with a particular ADR phenotype.

BETA-1 Receptor Polymorphisms

Sandilands and O'Shaughnessy have already functionally characterized these two prominent polymorphisms in *ADRB1* resulting in nonsynonymous mutations, Ser49Gly and Arg389Gly.[7] They expressed the four possible diplotypes in HEK cells and generated concentration-response curves for isoprenaline-stimulated cAMP production.[7] In these experiments, the haplotypes with the Ser49 and 389Gly alleles in *ADRB1* encoded for beta-1 receptors with drastically reduced agonist-stimulated coupling to G_S and therefore less cAMP generation.[7] The Ser49/389Gly diplotype had the lowest cAMP generation while the 49Gly/Arg389 diplotype had the highest cAMP generation and therefore dysfunctional beta-1 adrenergic signaling is found in subjects who are carriers of Ser49 and 389Gly, while normal baseline function is maintained for subjects homozygous for 49Gly/49Gly and Arg389/Arg389.

In one Chinese study of 223 subjects with essential hypertension, subjects carrying the 389Gly variant were nonresponders to metoprolol antihypertensive monotherapy after four weeks, while subjects with the homozygous Arg389/Arg389 genotype experienced a 10% average decrease in SBP from baseline.[8] This study demonstrated that Arg389Gly SNP status determined beta-blocker response and that carrying one copy of the 389Gly variant resulted in no SBP change after four weeks of metoprolol treatment. Therefore, introduction of a drug with beta-receptor antagonism or inverse agonism was hypothesized to decrease blood pressure in subjects with the normal Arg389 homozygous genotype, while subjects carrying the less functional 389Gly variant

would remain unaffected by beta-blockade. These findings are consistent with the average SBP group differences seen between Arg389 homozygotes (mean SBP 104 mmHg) and 389Gly carriers (mean SBP 122 mmHg) treated with "beta-blocking SSRIs" in this pilot project. Therefore, these differences in beta-receptor activity could be related to the risk specific ADRs in subsequent studies.

SSRI Discontinuation Syndrome

In a study by Hindmarch and colleagues, paroxetine was found to be the only SSRI with significant deterioration in health and functioning after abrupt discontinuation compared to fluoxetine, citalopram and sertraline; the authors hypothesized this was due to paroxetine's short half-life and exceptionally high potency for both muscarinic receptors and the serotonin transporter.[9] In another study of 107 subjects treated with fluoxetine, paroxetine or sertraline for depression, abrupt withdrawal of paroxetine resulted in an increased standing heart rate in double blind placebo-controlled trials.[10] This finding is counterintuitive to the expected outcome of muscarinic receptor and serotonin transporter (SERT) blockade interruption; which should abruptly increase vagal tone and decrease serotonergic activity, thereby simultaneously lowering heart rate and blood pressure. However, if paroxetine does indeed decrease blood pressure via beta-1 antagonism, this abrupt discontinuation may result in mild rebound tachycardia due to beta-1 receptor sensitization. Beta-blockers are already well known for causing this reversible increase in beta-1 receptor density, which then allows for greater adrenergic stimulation via norepineprhine and subsequent tachycardia after the beta-blocking agent is abruptly removed. Therefore, this SSRI-induced beta-receptor sensitization provides a novel mechanistic theory for the more severe SSRI Discontinuation Syndrome seen after paroxetine treatment. Even though fluoxetine probably also results in beta-1 receptor sensitization, the SSRI Discontinuation Syndrome may not occur due to this drug's extended half-life and subsequent self-tapering effect, which minimizes rebound cardiovascular response by allowing a slow receptor density normalization.

In subjects carrying only the fully functional Arg389 allele, use of these "beta-blocking" SSRIs was associated with significantly lower SBP and HR compared to use of other medications in the SSRI class. This outcome is consistent with metoprolol pharmacogenetics studies demonstrating that Arg389/Arg389 homozygotes had up to 3-fold greater reductions in SBP compared to 389Gly carriers after four weeks of monotherapy.[8,11] There were no significant independent associations between the *ADRB1* genotypes and cardiac vital signs in a main effects

model, therefore this is likely a true pharmacogenetic interaction where phenotype was only unmasked by the introduction of the "beta-blocking" SSRI. The clinical relevance of this finding is that paroxetine and fluoxetine may exhibit mild beta-blockade and this may provide a novel pharmacologic explanation for why paroxetine tends to have a more severe Discontinuation Syndrome than other SSRIs.

Limitations

A major limitation of this pilot study was its retrospective nature as a secondary point prevalence analysis with only a single measurement of cardiac vital signs as the primary outcome. The pilot's sample size was small, but allowed for detecting statistically significant group differences due to the large effect size observed in this hypothesis-driven proof of principle analysis. A large prospective cohort with equal representation of each medication group, designed to specifically monitor abrupt placebo-controlled withdrawal effects would be a superior design to determine if *ADRB1* genotype can help predict cardiovascular outcomes or SSRI Discontinuation Syndrome. Most importantly, it is necessary to recognize that other gene variants in *ADRB1* or other related genes may be relevant to investigate along with additional adrenergic beta-receptor subtypes.

CONCLUSION

We report results from a pilot project that subjects taking a "beta-blocking" SSRI, like fluoxetine or paroxetine, may observe decreased systolic blood pressure and heart rate if they are homozygous Arg389/Arg389 at the Arg389Gly *ADRB1* SNP. To our knowledge this is the first investigation to examine the relationship between SSRI-associated cardiac vital signs differences and genetic variability within the *ADRB1* gene. Thus, more research in the area of SSRIs and cardiovascular pharmacology is required to determine if cardiovascular health is clinically relevant when selecting appropriate antidepressant pharmacotherapy regimens. Future prospective studies of this phenomenon are necessary to identify genetic markers that can reliably predict SSRI-associated cardiovascular effects and influence pharmacotherapy decisions. ✤

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