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Response to citalopram is not associated with SLC6A4 genotype in African-Americans and Caucasians with major depression

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Abstract

Aims—Ethnic differences in genotype frequency provide a natural condition for assessing the contribution of gene variations to the causes and treatments of disease. Accordingly, the purpose of this study was to determine whether ethnic variations in allele frequencies of the serotonin transporter gene-linked polymorphic region (5-HTTLPR) of the SLC6A4 gene were related to the response to the treatment of depression.

Main methods—African-Americans (n=101) and Caucasians (n=100) with major depressive disorder were treated with the antidepressant citalopram (20–60 mg/day) for 8 weeks. Genotyping for the long (L) and short (s) alleles (LL, Ls, and ss) of the SLC6A4 gene was performed and the association between genotype and treatment response was assessed.

Key findings—Subjects in both ethnic groups showed a significant reduction in depression scores over time (p<.0001). However, in spite of a significantly greater frequency of the L allele in African-Americans as compared to Caucasians, a comparable clinical response between the two

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Conflict of Interest Statement

All other authors declare that there are no conflicts of interest.

groups was found with 5-HTTLPR polymorphism not significantly associated with clinical response in either ethnic group.

Significance—The results are consistent with a previous finding and in accord with most of the results obtained in Caucasian subjects that SLC6A4 genotype is not related, at least by itself, to a response to treatment in either ethnic group to any clinically significant degree.

Keywords

depression; ethnicity; genotype; pharmacogenomics; African-American

Introduction

A seminal study by Smeraldi et al. 1998 reported that subjects homozygous for the short (ss) allele of the 5-HTTLPR polymorphism in the serotonin transporter gene (SLC6A4) were less likely to respond to fluvoxamine, a selective serotonin re-uptake inhibitor. As reviewed (Kato and Serretti 2010; Taylor et al. 2010), during the past decade and a half, more than 35 prospective and retrospective studies further assessed the possible association between the serotonin transporter genotype and clinical response (Porcelli et al. 2012), with mixed results. Initial studies suggested the long (L) variant of the allele conferred a greater response, whereas the short (s) variant conferred a lesser response. Meta-analyses of existing data, coupled with more recent results, do not support the initial more robust findings, although a modest association between the L variant and clinical response appears to remain (Porcelli et al. 2012).

The preponderance of data in these studies was obtained from non-Hispanic white [Caucasian (C)/European] subjects. Considerably less data are available from other ethnic and racial groups. Response to medications is complex, with ethnicity and culture being two of the more prominent influencers. Since almost two-thirds of the world's population are "non-white," it is imperative to study other ethnic and racial groups in order to assess whether the findings in whites generalize to other populations and, if not, why not.

To our knowledge, there is only one report on the relationship between SLC6A4 genotype and response to treatment in African-Americans (AA) with depression. In the STAR*D study, 299 AA were treated with citalopram (CIT) (Kraft et al. 2007; Mrazek et al. 2009). No significant association between SLC6A4 genotype and either response to treatment or remission with CIT were found. The lack of association in the AA group might have been due to racial stratification in that the subjects only were required to self-identify as AA. Based on the allele frequencies for the serotonin transporter gene, with AA having a greater allele frequency than C for the L variant (Lotrich et al. 2003; Noskova et al. 2008), coupled with the use of more rigorous criteria to define ethnicity, it was hypothesized that AA subjects would respond better to CIT than C subjects.

Material and methods

Subjects

We reported the results of a modest size prospective open-label clinical trial comparing the efficacy of CIT with dose escalation (20–60 mg/day; the 60 mg dosage was used prior to the FDA warnings regarding potential cardiac adverse events) in subjects with non-psychotic major depression (Lesser et al. 2010). The parent study consisted of 301 screened subjects (169 AA and 132 C), of whom 241 (127 AA and 114 C) were genotyped and considered intent-to-treat (ITT) as defined by one week of treatment with citalopram, with 201 of these (101 AA and 100 C) completing the study (completers) (80% of AA completed, while

88% of C completed). Subjects were between 18 and 70 years of age, self-identified as AA or C ethnic background, and reported that both of their parents and all four of their grandparents also self-identified with the same ethnic group.

Procedures

After obtaining written informed consent, subjects were screened as described (Lesser et al. 2010). Psychiatric diagnoses were obtained by utilizing the Structured Clinical Interview for DSM-IV Disorders (SCID) (First et al. 1994). Depression severity was measured by administering a structured interview for the 21-item Hamilton Rating Scale for Depression (HAM-D) (Hamilton 1967), and subjects also completed the self-report Beck Depression Inventory (BDI) (Beck et al. 1961). All subjects met DSM-IV criteria for current major depression and had a pretreatment HAM-D score of ≥ 15 . At screening, 20 ml of blood was collected for immortalization of lymphocytes (Anderson and Gusella 1984). Chronic stress was assessed with the Chronic Burden Scale (Gurung et al. 2004). Social support and undermining were measured as described by Vinokur and van Ryn 1993.

All eligible patients were entered into the active treatment phase of the study after a 1-week washout period during which patients were given placebo pills physically identical to the active medication. Visits were scheduled at weekly intervals for nine weeks. Symptom severity data were collected at each visit. CIT was begun at a starting dose of 20 mg/day and could be increased to 40 mg at week 4 and to 60 mg/day at week 7 if there was lack of efficacy and tolerable side effects. Response also was measured by the % change in HAM-D score from baseline to week 8 of treatment. Subjects were classified as responders to CIT if their HAM-D score decreased by $\geq 50\%$ by the end of the study compared to their baseline values. Subjects were classified as remitters if their HAM-D score were ≤ 7 at the end of the study.

SLC6A4 genotyping for the L and s alleles (LL, Ls, and ss) was performed as originally described by Lesch et al. 1996. Alleles were designated according to the number of tandem copies of the repeated segment inferred from size correspondence to the alleles (Gelernter et al. 1997). Unbeknownst to the laboratory, 10% of the samples were randomly chosen, re-coded and re-analyzed to assess reliability of the genotyping. There was 100% concordance between the initial and repeat results.

All data were verified and screened for outliers. In order to identify possible covariates, t-tests, Chi-Square analyses and the Fisher's exact test were used. For ITT, the last observation carried forward (LOCF) method was used. Data were analyzed by mixed-model repeated measures ANOVA and ANCOVA and Chi-Square analyses. Due to ethnic differences in allele frequency, data from all subjects were analyzed using ethnicity as a covariate or with each ethnic group analyzed separately. P values $\leq .05$ were considered statistically significant. Power analyses were performed to assess the chances of a type II statistical error as described previously (Cohen 1977; Poland et al. 1980).

Results

Table 1 shows the key demographic and clinical characteristics of the study subjects, divided by ethnicity. Significant differences between ethnic groups are denoted. Only the ITT subject data are shown (n=241), as all results were comparable to those found for the completers (n=201). There was no ethnic difference in dropout rate between ITT vs. completers ($\chi^2 = 2.4$, df = 1, $p = .12$). Table 2 shows the genotype and allele frequencies with the sample size for the SLC6A4 gene in AA and C subjects. The frequency for the L allele was significantly greater in AA as compared to C ($p = .002$). However, both groups

were in Hardy-Weinberg equilibrium [$\chi^2 = 1.64$ and 0.05 ($p = ns$) for AA and C, respectively].

Analysis of the entire dataset by two-way repeated measures ANCOVA, with ethnicity as a covariate and genotype as the between-subjects factor, showed a significant effect of treatment over time ($F_{5,1181} = 113$, $p = 0.0001$), but no effect of ethnicity ($F_{5,1181} = 1.6$, $p = 0.16$), genotype ($F_{10,1181} = 1.1$, $p = 0.4$) or presence of significant interactions ($F_{1,1181} = 1.3$, $p = 0.2$). Further analyses within each ethnic group revealed comparable results. Inclusion of BMI, education, burden/stress and social support, which were significantly different between the two groups (see Table 1), as additional covariates in the analyses did not modify the results either in the entire sample or in each ethnic group analyzed separately. For illustration purposes, Figure 1 shows the HAM-D scores stratified by genotype in the combined group (AA+C) (top), in AA (middle) and in C (bottom) ($n=241$). As shown in Table 3, comparable results were found using % change in HAM-D as the response criterion. None of the aforementioned covariates affected the findings (all p values >0.3).

Table 4 shows SLC6A4 genotype and associated response/remission rates for AA and C subjects at week 8. There were no significant differences in remission or response rates in relation to ethnicity, genotype or genotype $\times$ ethnicity at week 8, with similar results obtained for response at weeks 4 and 6 (all p values $> .4$). Results based on BDI scores were quite comparable to the HAM-D findings (data not shown).

Discussion

The purpose of this study was to investigate a possible ethnic difference in response to CIT that might be attributable to variations in 5-HTTLPR. The comparable clinical response independent of genotype in this subset of patients is consistent with our findings in the larger parent study (Lesser et al. 2010). L allele frequency was higher (0.74) in AA than in C (0.60), consistent with previous studies in which SLC6A4 L allele frequencies have been as high as 87% in AA, whereas C typically have allele frequencies of 45–65% (Lotrich et al. 2003; Noskova et al. 2008). Despite this ethnic difference in allele frequency, coupled with our utilization of more stringent inclusion criteria to define ethnicity than was done previously, no difference in clinical response between AA and C in general, or between AA and C stratified by SLC6A4 genotype, was detected. The trend for a slower response to treatment in the ITT AA subjects with the ss allele (Figure 1) was due to two AA subjects with high HAM-D scores who dropped out of the study early on. This trend for a slower response disappeared in the completer analyses since data from these two subjects would not have been included (not shown).

Although there are numerous reports on the role of SLC6A4 genotype in response to antidepressants, most of these studies have been performed in C subjects. Based upon meta-analyses of extant data and more recent reports, polymorphism of the serotonin transporter gene does not appear to be strongly associated with treatment response to SSRIs, particularly in C subjects (Porcelli et al. 2012). Considerably fewer studies have been performed in other ethnic groups, so the role of SLC6A4 in these groups remains unclear.

Other than C, the largest ethnic group studied to date is Asians, where somewhat conflicting results have been reported. In fact, and in opposition to most of the findings in C, treatment response in some Asian subjects was found to be better in those with the s allele (see Kato and Serretti 2010). There also have been some studies in Hispanic/Spanish subjects and, consistent with the findings in C, no relationship between SLC6A4 variation and response to CIT or fluoxetine was found (Mrazek et al. 2009; Dong et al. 2009).

In order to assess genotype-environmental interactions, baseline measures of social support and stress were measured. Some studies have suggested that psychosocial status, working in conjunction with SLC6A4 genotype, determines response to treatment (Narasimhan et al. 2011; Keers and Uher 2012; McGuffin et al. 2011). Although baseline ethnic differences in social support and stress were found, neither of these variables significantly influenced the response to CIT. We did not, however, assess the subjects for a history of child abuse and/or neglect, measures that might more strongly interact with genetics and subsequent environment to predispose individuals to both depression and treatment response (McGuffin et al. 2011; Keers and Uher 2012).

Conclusion

Our results are consistent with the STAR*D findings (Kraft et al. 2007; Mrazek et al. 2009) and, in accord with many reports, that SLC6A4 genotype is not strongly related, at least by itself, to response to CIT in either AA or C subjects to any clinically significant degree. The modest sample sizes in the present study might not have conferred sufficient power to detect all differences in response with confidence. If AAs were to respond better to CIT as hypothesized, with a sample size of 100/group, coupled with the observed variability, the study had sufficient power to detect a further reduction in the HAM-D to 71% or more in the AA group (as compared to the 56–58% decrease actually observed in both groups), which would have been both statistically and clinically significant. Conversely, with sample sizes for each of the genotypes ranging from 11 to 71, it is unlikely that small differences in effect size could be consistently discerned. Additionally, these studies do not exclude the possibility that rare variants that could impact treatment response in selected individuals might exist. Exploring this possibility would require sequencing of SLC6A4 (Mrazek et al. 2009). Future studies also should include the characterization of genetic ancestry.

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Figure 1.

Effect of treatment with CIT (20–60 mg/day) for eight weeks on HAM-D scores in African-American (AA) ($n = 127$) and Caucasian (C) ($n = 114$) subjects with major depression stratified by SLC6A4 genotype (LL, Ls, ss); The graphs are for the ITT group ($n=241$). There were no significant ethnic by genotype differences in treatment response in either the combined group or in each ethnic group analyzed separately (all p values >1.0) (see results section for details). Top graph shows HAM-D scores in AA and C subjects combined separated by genotype; Middle graph shows HAM-D scores in AA subjects separated by genotype; Bottom graph shows HAM-D scores in C subjects by genotype.

Table 1

Demographic and psychosocial variables for the study subjects in the ITT groups (n=241)

	African American	Caucasian
Sample Size	127	114
Age	41.7±10.7	42.5±11.6
Male/Female	48/79	53/61
BMI	31.6±7.7	28.0±6.5 ^a
Baseline HAM-D	20.1±5.6	20.3±4.5
Baseline BDI	25.7±9.7	26.8±8.3
Education (years)	13.8±2.0	14.5±2.8 ^b
Employed	40%	56% ^c
Psych History	33%	42%
Family Psych History	35%	34%
Current Physical Illness	49%	38%
History Physical Illness	43%	43%
Burden/Stress	38.9±9.3	35.2±7.9 ^d
Social Support	68.8±18.8	63.0±19.6 ^e
Social Undermining	31.1±22.2	26.0±18.5
Final Dose (mg/d)	28.6±13.0	28.1±13.4
Final HAM-D	8.2±6.7	8.9±7.1
Final BDI	10.6±10.7	11.1±9.0
Reduction in HAM-D (%)	-58.1±32.2	-56.3±34.1
Reduction in BDI (%)	-59.1±34.1	-58.3±31.5
Response Rates	81/127 (68.8%)	77/114 (67.5%)
Remission Rates	63/127 (49.6%)	50/114 (43.9%)

AA vs. C (p values):

^a .03;^b .001;^c .02;^d .002;^e 0.03;

Genotype and allele frequencies (%) with number of subjects (n) for the SLC6A4 gene in African American and Caucasian subjects

Table 2

	Genotype Frequency (n)			Allele Frequency (%)	
	LL	Ls	ss	L	s
African American (127)	55.9 (71)	35.4 (45)	8.7 (11)	74 *	26
Caucasian (114)	36.0 (41)	48.2 (55)	15.8 (18)	60	40

(* 0.002, AA vs. C)

Table 3

Mean ($\pm$ sd) percent (%) change in HAM-D score from baseline to the end of study as stratified by genotype and ethnicity. Number of subjects (n) in each group also is shown.

Group	Genotype	% Change in HAM-D
African-American (n=127)	LL (71)	-59.3 ± 32.3
	Ls (45)	-56.9 ± 34.0
	Ss (11)	-55.7 ± 26.5
Caucasian (n=114)	LL (41)	-63.3 ± 30.8
	Ls (55)	-50.6 ± 37.8
	ss (18)	-57.8 ± 26.9

Table 4

SLC6A4 genotype and associated response/remission rates (%) for AA and C subjects at week 8 of treatment *. Number of subjects (n) in each group also is shown.

Genotype	African-American	Caucasian
LL	64.7/49.3 (71)	77.9/53.7 (41)
Ls	62.2/53.5 (45)	61.8/38.2 (55)
ss	63.6/36.4 (11)	61.0/38.9 (18)

(*For each cell, response rate is in the numerator/remission rate is in the denominator)