PT 160

Association of Genetic Polymorphisms with Response to Placebo Treatment in Patients with Osteoarthritis Knee Pain

Richard H. Gracely,[1] Shad B. Smith,[1] Gary Slade,[1] Luda Diatchenko,[1] Roger B. Fillingim,[1] Bonnie Fijal,[2] Eli Frakes,[2] Samuel A. McLean,[1] Kevin Piezer,[2] Alexandra N. Heinloth,[3] William Maixner,[1] John P. Houston[2]

[1]Algynomics Inc., Chapel Hill, NC, USA; [2]Eli Lilly and Company and/or any of its subsidiaries, Indianapolis, IN, USA; [3]i3 Statprobe, Ann Arbor, MI, USA

ABSTRACT

Aim of investigation: Identification of genetic polymorphisms associated with therapeutic response to placebo or duloxetine in patients with painful osteoarthritis of the knee.

Methods used: Used were data from patients who consented to genetic testing in 2 placebo-controlled, 13-week studies examining the efficacy of duloxetine 60 to 120 mg/day in treatment of knee osteoarthritis. Factor analysis using Maximum Likelihood Extraction and Oblique (oblimin) Rotation identified factors (Depression and Pain Factors) that captured the highest proportion of variance among 25 variables measuring symptom, function, and comorbidity related to osteoarthritis. Forty-four single nucleotide polymorphisms (SNPs) in 12 candidate genes (prioritized for analysis from a larger set of genes selected for exploratory analysis) were analyzed for associations with post-treatment change in factor scores. Associations were evaluated separately in duloxetine (N=82) and placebo (N=83) groups using analysis of covariance with adjustment for baseline factor.

Results: Two factors, one reflecting depression (Depression Factor) and the other reflecting pain (Pain Factor), accounted for >75% of variance in measured variables. In the placebo group, change in the Depression Factor was statistically significantly associated with 2 *HTR3A* SNPs (rs1176752, p<0.001; rs1150226, p<0.001) and 1 *COMT* SNP (rs174696, p<0.001). Genetic associations were not significant (p>0.0018) for the Depression Factor in the duloxetine group, nor for the Pain Factor in either treatment group.

Conclusions: Genetic polymorphisms in *HTR3A* and *COMT* may influence placebo response of depression symptoms in patients with osteoarthritis knee pain.

INTRODUCTION

- An epidemiological study with data from North Carolina suggests that the life-time risk for symptomatic knee osteoarthritis is 44.7% in the general population, with an increase to 56.8% in patients with a history of a knee injury.[1]
- The efficacy of duloxetine, a dual norepinephrine/serotonin reuptake inhibitor, in the treatment of osteoarthritis knee pain has been demonstrated in 2 double-blind, placebo-controlled clinical trials.[2,3]
- Genetic risk factors for the development of knee osteoarthritis have been identified,[4] but no studies have examined how genetic factors might influence treatment outcome in patients with osteoarthritis knee pain.

OBJECTIVE

To test the association of potentially functional single nucleotide polymorphisms (SNPs) in select candidate genes with response to treatment with duloxetine and placebo in patients with osteoarthritis knee pain.

METHODS

- **Patients and Study Design**
  - Used were data from white patients who consented to genetic testing while participating in either one of 2 clinical trials examining the efficacy and safety of treatment with duloxetine in patients with osteoarthritis knee pain.
  - Patients with Major Depressive Disorder were excluded from both studies.
  - Both clinical trials were double-blind and placebo-controlled; patients received treatment with duloxetine 60 to 120 mg/day.
- **Measures Used to Assess Changes in Pain**
  - 11-point Likert scale patient diary
  - Brief Pain Inventory (BPI)
- **Measures Used to Assess Changes in Depression**
  - Beck Depression Inventory II (BDI-II)
  - Hospital Anxiety Depression Scale (HADS)
  - 36-item Short-Form Health Survey (SF-36)
- **Genotyping**
  - Genotyping was performed by Beckman-Coulter Genomics (Morrisville, NC) on the Algynomics Pain Research Panel, a proprietary candidate gene SNP array using Affymetrix MegAllele technology. Additional SNPs were genotyped with the Sequenom MassArray platform as previously described.[5]
  - All SNPs used in the current analysis had ≥95% usable calls across patients, and only SNPs with minor allele frequencies of ≥1% were examined.

METHODS (continued)

- **Statistical Analysis**
  - Factor analysis with Maximum Likelihood Extraction and Oblique (oblimin) Rotation was used to capture the highest proportion of variance among 25 variables measuring symptom, function, and comorbidity related to osteoarthritis.
  - Associations were evaluated separately in duloxetine and placebo groups using analysis of covariance with adjustment for the baseline factor (Depression or Pain Factor). For these analyses, the difference between week 13 and baseline values were evaluated using last observation carried forward (LOCF) methodology.
  - The Spectral Decomposition Method of Nyholt[6] was used to estimate the effective number of independent SNPs tested, accounting for linkage disequilibrium between neighboring SNPs.
  - The threshold for statistical significance (p=0.0018) was determined by applying a Bonferroni correction based on testing 28 independent SNPs.
- **SNPs in Candidate Genes**
  - Associations of 44 SNPs in 12 candidate genes with improvement in measures of pain and depression were examined. The 12 candidate genes are critical elements of the norepinephrine/serotonin pathways, which are known to strongly contribute to pain and depression. The genes included *ADRA2A, ADRA2B, ADRA2C, ADRB2, COMT, DRD2, GCH1, HTR2A, HTR3A, SLC6A2, SLC6A4,* and *TPH1.*
  - Within a chosen gene locus, SNPs situated in potentially functional regions (promoters, exons, areas of conservation) were examined.

RESULTS

Table 1. Factor Loading

Depression Factor	Pain Factor
BDI-II Total Score (0.77)	BPI – worst pain (0.73)
HADS Anxiety Subscale Score – Odd numbered items (0.72)	BPI – least pain (0.80)
HADS Anxiety Subscale Score – Even numbered items (0.73)	BPI – average pain (0.93)
SF-36 Mental Health Transformed Score (-0.81)	BPI – pain now (0.61)
SF-36 Vitality Transformed Score (-0.55)	

Abbreviations: BDI-II=Beck Depression Inventory II; BPI=Brief Pain Inventory; HADS=Hospital Anxiety Depression Scale; SF-36=36-item Short-Form Health Survey.
Values in parentheses indicate standard regression coefficients from the rotated factor pattern.

Table 2. Patient Baseline Characteristics

	Placebo (N=83)	Duloxetine (N=82)
Female gender, n (%)	69 (83.1)	56 (68.3)
Age (years), mean (SD)	62.7 (9.0)	63.1 (8.3)
BMI, mean (SD)	31.0 (4.9)	29.6 (4.2)
Self-identified White Ethnicity, %	100	100
BDI-II Total Score, mean (SD)	4.3 (5.1)	4.5 (6.9)
Depression Factor, mean (SD)	0.0 (0.9)	0.0 (1.1)
Pain Factor, mean (SD)	0.01 (0.9)	0.03 (1.1)

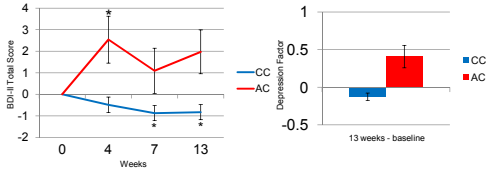
Abbreviations: BDI-II=Beck Depression Inventory II; BMI=body mass index; n=number of patients affected; N=total number of self-identified white patients who agreed to genetic testing; SD=standard deviation.

Table 3. SNPs Associated with Change in Depression Factor in the Placebo Group

Gene	SNP	Unadjusted p-Value	
		Placebo Group	Duloxetine Group
HTR3A	rs1176752	***0.001***	0.876
	rs1150226	***0.001***	0.271
COMT	rs174696	***0.001***	0.034

Abbreviation: SNP=single nucleotide polymorphism. Results from generalized linear model, where the dependent variable was change in depression factor from baseline (Week 0) to Week 13.
Italic and Bold indicates p-values that reached statistical significance.

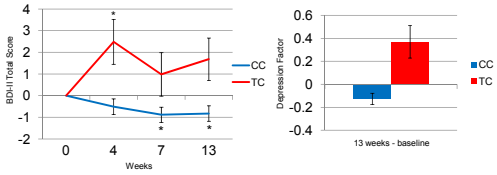
Figure 1. LS Mean Changes in BDI-II Total Score and Depression Factor Stratified by *HTR3A* SNP rs1176752 Genotype in the Placebo Group



Week	Baseline BDI-II Total Score	0	4	7	13
rs1176752, CC genotype	4.5	n=91	n=90	n=89	n=85
rs1176752, AC genotype	3.6	n=10	n=11	n=11	n=11
rs1176752, AA genotype	NA	n=0	n=0	n=0	n=0

*Abbreviations: BDI-II=Beck Depression Inventory II; LS Mean=least squared mean; n=number of patients; NA=not available; SNP=single nucleotide polymorphism. A is minor allele, C is major allele. Error bars indicate standard error; * p<0.05 for BDI-II change from baseline. LS Mean refers to least-squares means for endpoints from analysis of covariance model.*

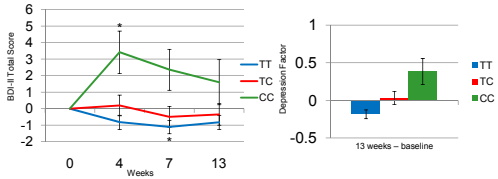
Figure 2. LS Mean Changes in BDI-II Total Score and Depression Factor Stratified by *HTR3A* SNP rs1150226 Genotype in the Placebo Group



Week	Baseline BDI-II Total Score	0	4	7	13
rs1150226, CC genotype	4.5	n=90	n=89	n=88	n=84
rs1150226, TC genotype	3.5	n=11	n=12	n=12	n=12
rs1150226, TT genotype		n=0	n=0	n=0	n=0

*Abbreviations: BDI-II=Beck Depression Inventory II; LS Mean=least squared mean; n=number of patients; SNP=single nucleotide polymorphism. T is minor allele, C is major allele. Error bars indicate standard error; * p<0.05 for BDI-II change from baseline. LS Mean refers to least-squares means for endpoints from analysis of covariance model.*

Figure 3. LS Mean Changes in BDI-II Total Score and Depression Factor Stratified by *COMT* SNP rs174696 Genotype in the Placebo Group



Week	Baseline BDI-II Total Score	0	4	7	13
rs174696, TT genotype	4.5	n=64	n=64	n=65	n=63
rs174696, TC genotype	3.9	n=29	n=29	n=27	n=26
rs174696, CC genotype	6.1	n=7	n=7	n=7	n=6

*Abbreviations: BDI-II=Beck Depression Inventory II; LS Mean=least squared mean; n=number of patients; SNP=single nucleotide polymorphism. C is minor allele, T is major allele. Error bars indicate standard error; * p<0.05 for BDI-II change from baseline. LS Mean refers to least-squares means for endpoints from analysis of covariance model.*

Results Summary

- The Depression and Pain Factors explained >75% of variance in the 25 endpoint measures.
- A total of 3 SNPs in 2 genes, *COMT* and *HTR3A*, were statistically significantly associated with change in the Depression Factor in the placebo group.
- At the end of treatment in the placebo-treated group
  - BDI-II was significantly decreased from baseline for *HTR3A* major homozygotes but not for heterozygotes.
  - BDI-II was not significantly decreased from baseline within individual *COMT* genotypes, although baseline BDI-II appeared greater for patients with the minor allele for each of the two genotypes.
- No statistically significant associations between candidate SNPs and change in the Depression Factor were detected in the duloxetine group.
- None of the examined SNPs were statistically significantly associated with change in the Pain Factor in either group.

Limitations

- Overall, patients in this study had very low depression scores – similar to a healthy population.
- Only white patients were included in the current analyses (too few study participants of other ethnicities).
- Power to detect associations was limited.
- Limited coverage was present for some candidate genes.
- High number of tests.
- Lack of replication.

CONCLUSIONS

- Change in the Depression Factor was statistically significantly associated with rs1176752 and rs1150226 in *HTR3A* and with rs174696 in *COMT* in patients with osteoarthritis knee pain treated with placebo.
- Genetic associations were not significant (p>0.0018) for the Depression Factor in the duloxetine group, nor for the Pain Factor in either treatment group.
- A prior study in clinically depressed patients showed significant associations between *COMT* SNP rs174696 and decreases in depression scores during treatment with duloxetine, with the strongest decreases observed in carriers of the minor homozygote genotype.[7] Here, we observed higher baseline depression (BDI-II) scores in carriers of the minor homozygote genotype of *COMT* SNPs rs174696 in patients with osteoarthritis knee pain but no significant changes in BDI-II within any of the *COMT* genotypes at the end of placebo treatment.
- Due to the very low level of depression observed in the current population, the clinical significance of these results require further investigation.

References:

1. Murphy L, et al. *Arthritis Rheum* 2008;59(9):1207-1213
2. Chappell AS, et al. *Pain* 2009;146(3):253-260
3. Chappell AS, et al. *Pain Pract* 2010 Jul 5 [Epub ahead of print]
4. Valdes AM and Spector TD. *Curr Opin Rheumatol* 2010;22(2):139-143
5. Nicolini H, et al. *Psychol Med* 2009;39(2):267-276
6. Nyholt DR. *Am J Hum Genet* 2004;74(4):765-769
7. Perlis RH, et al. *Biol Psychiatry* 2009;65(9):785-791

Disclosures

This study was sponsored by Eli Lilly and Company and/or any of its subsidiaries. Drs. Fijal, Frakes, Gray, Piezer, Liu, and Houston are fulltime employees of Eli Lilly and/or any of its subsidiaries, and minor stockholders of Eli Lilly and Company. Drs. Maixner, Fillingim, Smith, Diatchenko, Gracely, McLean, and Slade are consultants and stockholders of Algynomics.
Dr. Heinloth is a fulltime employee of i3 Statprobe, a division of Ingenix, which is a subsidiary of United Health Group.