Published in final edited form as:
Am J Med Genet B Neuropsychiatr Genet. 2006 July 5; 141B(5): 449–462. doi:10.1002/ajmg.b.30324.

Three major haplotypes of the β_2 adrenergic receptor define psychological profile, blood pressure, and the risk for development of a common musculoskeletal pain disorder

Luda Diatchenko[1], **Amy D. Anderson**[2], **Gary D. Slade**[3], **Roger B. Fillingim**[4], **Svetlana A. Shabalina**[5], **Tomas Higgins**[1], **Swetha Sama**[1], **Inna Belfer**[6,7], **David Goldman**[6], **Mitchell B. Max**[7], **Bruce S. Weir**[2], and **William Maixner**[1]

[1] University of North Carolina, Center for Neurosensory Disorders, Columbia St., CB#7455, Chapel Hill, NC, 27599

[2] North Carolina State University, Bioinformatics Research Center, Raleigh, USA

[3] University of Adelaide, Australian Research Centre for Population Oral Health, SA 5005, Adelaide, Australia

[4] University of Florida College of Dentistry, Gainesville, USA

[5] NCBI, NIH, Bethesda, MD 20894, USA

[6] Laboratory of Neurogenetics, NIAAA, NIH, Rockville, MD, 20852, USA

[7] NIDCR, NIH, Pain & Neurosensory Mechanisms Branch, Bethesda, MD 20892-1258, USA

Abstract

Adrenergic receptor β_2 (ADRB2) is a primary target for epinephrine. It plays a critical role in mediating physiological and psychological responses to environmental stressors. Thus, functional genetic variants of *ADRB2* will be associated with a complex array of psychological and physiological phenotypes. These genetic variants should also interact with environmental factors such as physical or emotional stress to produce a phenotype vulnerable to pathological states. In this study, we determined whether common genetic variants of *ADRB2* contribute to the development of a common chronic pain condition that is associated with increased levels of psychological distress and low blood pressure, factors which are strongly influenced by the adrenergic system. We genotyped 202 female subjects and examined the relationships between three major *ADRB2* haplotypes and psychological factors, resting blood pressure, and the risk of developing a chronic musculoskeletal pain condition - Temporomandibular Joint Disorder (TMD). We propose that the first haplotype codes for lower levels of ADRB2 expression, the second haplotype codes for higher ADRB2 expression, and the third haplotype codes for higher receptor expression and rapid agonist-induced internalization. Individuals who carried one haplotype coding for high and one coding for low ADRB2 expression displayed the highest positive psychological traits, had higher levels of resting arterial pressure, and were about 10 times less likely to develop TMD. Thus, our data suggest that either positive or negative imbalances in ADRB2 function increase the vulnerability to chronic pain conditions such as TMD through different etiological pathways that imply the need for tailored treatment options.

Corresponding author: Luda Diatchenko, University of North Carolina at Chapel Hill, Center for Neurosensory Disodrers, Columbia St., CB#7455, 2120 Old Dental Bld., Chapel Hill, NC, 27599, USA. phone: 919-843-2549, fax: 919-966-3683; email: lbdiatch@email.unc.edu.

Keywords

adrenergic receptor β_2; haplotype; SNPs; chronic pain; blood pressure; somatization; depression; anxiety; negative moods

Introduction

Persistent pain is an unpleasant sensory and emotional experience that is associated with several chronic disease processes and clinical conditions. One very common chronic musculoskeletal pain condition that results from maladaptive biological and psychological factors is myogenous temporomandibular disorder (TMD), which is characterized by persistent facial pain, impaired oral function, and several comordid medical conditions (NIH, National Ambulatory Medical Care Survey, 1979;John, Miglioretti et al., 2003;Von Korff et al., 1993). In the United States, this condition impacts 5 to 15% of the adult population and incurs ~ $1B annually in healthcare costs (NIH, National Ambulatory Medical Care Survey, 1979). Risk determinants for TMD include biological factors that contribute to increases in pain sensitivity (Fillingim, Fillingim et al., 1998; Maixner, Fillingim et al., 1995;Maixner, Sigurdsson et al., 1995;Harkins et al., 1989;Sarlani et al., 2004) as well as psychological factors which contribute to high levels of somatization, depression and anxiety (Ohrbach and Dworkin, 1998;Okeson et al., 1996;Epker et al., 1999;Epker and Gatchel, 2000;Dworkin et al., 1990;Carlson et al., 1993;Keefe and Dolan, 1986;Vassend et al., 1995;Yap et al., 2003).

The profile of risk factors for TMD is consistent with the view that impairments in CNS regulatory processes contribute to the enhanced pain sensitivity and psychological dysfunction commonly seen in TMD patients (Fillingim, Fillingim et al 1998;Maixner, Fillingim, Booker, and Sigurdsson, 1995;Maixner, Sigurdsson et al., 1995;Harkins et al., 1989;Sarlani et al., 2004). We have proposed that genetic polymorphisms which influence adrenergic receptor mediated responses, when coupled with environmental factors such as physical or emotional stress, can produce a clinical phenotype that is vulnerable to TMD and related comorbid conditions (Diatchenko et al., 2005). Our recent studies have shown that genetic variations in catechol-*O*-methyltransferase (COMT), an enzyme that metabolizes catecholamines and catechol substances such as catecholestrogen, substantially influences pain perception and the risk of developing TMD (Diatchenko et al., 2005). Women who possessed COMT haplotypes that code for low levels of COMT activity show enhanced sensitivity to experimental pain and were 2.3 times more likely to develop TMD when compared to women who have COMT haplotypes coding for high levels of COMT activity (Diatchenko et al., 2005). COMT haplotypes were not found to be associated with the psychological factors that predicted TMD onset, suggesting that there are unidentified genetic variations which influence pain related psychological factors.

One possible candidate gene that might influence the psychological factors which contribute to TMD risk is the gene that codes for β2-adrenergic receptor. Alterations in β-adrenergic receptor function have been implicated in several psychiatric diseases and psychological disorders - including those associated with chronic pain conditions. Reductions in the density of β-adrenergic receptors are associated with depression and panic-anxiety (Magliozzi et al., 1989). Panic disorder, with agoraphobia, is associated with decreased lymphocyte β-adrenergic receptor density and diminished stimulation-evoked cAMP production (Maddock et al., 1993). β-adrenergic desensitization is correlated with the severity of psychomotor agitation seen in depressed patients (Mann et al., 1985) and the density of ADRB2 is negatively correlated with anxiety, depression, and hostility assessed with Profile of Mood States (POMS) questionnaire (Yu et al., 1999). Recent studies have

also shown that the stimulation of central ADRB2 produces anti-depressive effects in rodents (Zhang et al., 2003). In rats, central administration of the non-selective β-adrenergic receptor agonist isoproterenol produces a dose-dependent increase in defensive withdrawal, supporting the involvement of CNS β-adrenergic receptors in stress-related behavioral changes (Gorman and Dunn, 1993).

ADRB2 activity also regulates resting arterial blood pressure (Small et al., 2003), which is related to human pain sensitivity and central nervous system pain regulatory systems. In general, higher levels of resting arterial blood pressure are associated with diminished sensitivity to noxious thermal, mechanical, and ischemic stimuli (Bruehl and Chung, 2004;Fillingim, Maixner et al., 1998;Fillingim and Maixner, 1996;Maixner et al., 1997;Pfleeger et al., 1997;Maixner, 1991;Randich and Maixner, 1984;Sheps et al., 1992). Recently it has been shown that individuals with higher resting arterial pressure have a lower prevalence of chronic musculoskeletal pain complaints (Hagen et al., 2005).

Taking into account the central role of ADRB2 in epinephrine mediated responses, we hypothesize that functional genetic variants of *ADRB2* will be associated with a complex array of psychological and physiological phenotypes. Because specific psychological and physiological signs and symptoms (i.e., phenotypes) are associated with and predict TMD development, we hypothesized that certain genetic polymorphisms in *ADRB2* will contribute to the risk of developing TMD. Based on our findings, and the current knowledge related to ADRB2 cell biology, physiology, pharmacology and molecular genetics, we have developed a heuristic model that depicts the putative properties of the receptor physiology corresponding to the three common haplotypes examined in this study. Our model conceptually explains the relationship between the three *ADRB2* haplotypes with resting blood pressure, measures of psychological distress, and the risk of TMD development.

Materials and Methods

Subject recruitment

Data for this study were collected from 210 healthy female Caucasians aged 18 to 34 years who participated in an prospective study examining risk factors for TMD (Diatchenko et al., 2005). Only females were included in this study as they exhibit a higher prevalence of TMD than males (Carlsson and Le Resche, 1995). At the time of recruitment, subjects completed a baseline assessment comprised of psychological questionnaires, resting arterial blood pressure, and a clinical examination of the head and neck. Blood samples were collected and used for the genotyping of 8 single nucleotide polymorphisms of the *ADRB2* gene. Subjects were subsequently followed for up to 3 years, by both quarterly interviews and annual physical examinations, to identify newly developed cases of TMD. The study received ethical and safety approval from the University of North Carolina at Chapel Hill's Institutional Review Board.

Psychological questionnaires

Four psychological questionnaires that assessed depression, anxiety and somatization, which represent three major psychological domains that are consistently associated with chronic pain conditions, were completed by the participants. The questionnaires were not used to provide diagnoses of a psychiatric condition and we did not endeavor to make such diagnoses in this study. The following questionnaires were used: The *Profile of Mood States- Bi-Polar (POMS-Bi)* consists of 72 mood-related items that assess both positive and negative affective dimensions (Lorr and McNair, 1988). This instrument provides measures of mood over the previous week in six categories: agreeable-hostile, elated-depressed, confident-unsure, energetic-tired, clearheaded-confused, composed-anxious. Summary

scores were computed for two of six symptoms: depression and anxiety. High scores indicate positive mood. The *Brief Symptom Inventory (BSI)* is a short form of the Symptom Checklist 90 Revised and consists of 53 items that assess a feeling or thought. It is scored on a 5 point scale from 0 (no such problem) to 4 (severe problem). It provides ratings of psychological distress in nine symptom areas: somatization, obsessive-compulsive, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid ideation, and psychoticism (Derogatis and Melisaratos, 1983). Summary scores were computed for three of nine symptoms: somatization, depression and anxiety. High scores indicate great psychological distress. The *Pennebaker Inventory for Limbic Languidness (PILL)* assesses the frequency of occurrence of 54 common physical symptoms and sensations and appears related to the construct of somatization or to the general tendency to perceive and endorse physical symptoms. A total score is computed by summing all items. It has been reported to have high internal consistency (alpha = 0.88) and adequate test-retest reliability (0.70 over two months) (NIH, National Ambulatory Medical Care Survey, 1979). Recently it has been used as a measure of hypervigilance in fibromyalgia patients (McDermid et al., 1996). These patients demonstrated lower pressure pain thresholds and tolerances and higher scores on the PILL compared to arthritis patients and pain-free controls. The *State-Trait Anxiety Inventory (STAI)* contains 20 statements evaluating levels of state and trait anxiety (Spielberger et al., 1983). The STAI is comprised of two forms, one measuring general propensity to experience anxiety (Trait Anxiety) and the other measures the subject's anxiety level at the time of questionnaire completion (State Anxiety). Summary scores for either State and Trait Anxiety is computed by summing all items for each forms. Higher scores indicate greater anxiety level. Each of these instruments is widely used in clinical research and has good psychometric properties.

Blood pressure measurements

Resting systolic and diastolic blood pressures were assessed on the right arm with an automatic blood pressure monitor (Dinamap). Five measures obtained at 2 minute intervals after a 15 minute rest period were averaged to derive measures of resting systolic and diastolic arterial blood pressure.

Genotyping

Two hundred two enrollees consented to genotyping. Genomic DNA was purified from 198 subjects using QIAamp™ 96 DNA Blood Kit (Qiagen, Valencia, CA, USA) and used for 5′exonuclease assay (Shi et al., 1999). The primer and probes were used as described in (Belfer et al., 2004). The genotyping error rate was directly determined and was <0.005. Genotype completion rate was 95%. The PHASE program (Stephens et al., 2001;Stephens and Donnelly, 2003) was used for haplotypes reconstruction.

Statistical analyses

Distributions of phenotype scores in the sample were evaluated to assess normality. All examined variables appeared to be distributed approximately normal with the exception of the BSI scores. The distributions of the BSI variables were bimodal. For all BSI variables examined, approximately one third of the individuals in the study answered every question in the negative and hence received a score of 30. The remaining individuals had scores that were approximately normally distributed between the values of 49 and 75. As there was no acceptable way to transform these bimodal response variables to normality, these values were reduce to a binary form and data were recorded as to whether or not each individual had a score above 30.

Our primary analysis for each of our psychological and physiological traits was a regression in which we compared the number of copies of each haplotype with the response variable.

For our explanatory variables, we defined *tij* to be the number of copies of haplotype i possessed by individual *j*. So, for example, if individual *j* was heterozygous with haplotypes H1 and H2, we assumed $t_{1j} = 1$, $t_{2j} = 1$, and $t_{3j} = 0$. Similarly, if individual *j* was homozygous for haplotype H1, we assumed $t_{1j} = 2$, $t_{2j} = 0$, and $t_{3j} = 0$.

We chose to parameterize the linear model as follows:

$$y_j=\gamma_1+\gamma_2 t_{2j}+\gamma_3 t_{3j}+\gamma_{12} t_{1j} t_{2j}+\gamma_{13} t_{1j} t_{3j}+\gamma_{23} t_{2j} t_{3j}+\varepsilon_j, \quad (1)$$

where, for the normal phenotypes, y_j is the trait value for individual *j*. For the binary BSI variables, these analyses were performed according to the analogous logistic model.

The mean trait values for each diplotype using the parameterization in Equation 1 are given in Table 1. Note that, when all six parameters are allowed to vary, this model fits a separate mean to each of the 6 diplotypes and, for the normal variables, this is merely a reparameterization of the one-way ANOVA on the diplotypes. To determine which pairs of diplotypes had significantly different mean trait values, we used Tukey's Honestly Significant Difference post hoc test in the case of the normal variables. For the binary variables, we used likelihood ratio tests with a Bonferroni correction for these comparisons.

In addition when examining the differences between the mean trait values for each pair of diplotypes, we attempted to determine how each haplotype behaved in a copy or dose-effect manner by comparing the trait values of individuals with 0, 1, and 2 copies of each haplotype. As with the comparisons between diplotypes, these were assessed via an ANOVAs with Tukey's HSD test for the normal variables and were assessed directly from the regression as likelihood ratio tests with a Bonferroni correction for the BSI variables.

The main advantage in parameterizing the model as a regression (as shown in Equation 1) rather than as an ANOVA is that the regression model allows more direct way to examine interactions between the haplotypes. Of note is that haplotype H1 plays a different role in this parameterization than the other two haplotypes. The mean trait value for H1/H1 individuals is given by a single parameter, γ_1, which serves as a baseline value in this parameterization of the model. We chose this parameterization because it permits interactions (additivity, dominance, etc.) between H1 and the other haplotypes to be easily tested (see below). A second aspect of this model is that departures from additivity between the haplotypes are captured in the interaction parameters γ_{12}, γ_{13}, and γ_{23}. When these are all zero, we have a strictly additive model in which the mean heterozygous trait values fall exactly midway between the mean homozygous trait values.

We assumed in our analyses that the default model should be an additive model, so we first tested for interactions using an F-test (likelihood ratio test for the BSI variables) in which we compared the fit of the full model given in Equation 1 to the additive model in which γ_{12}, γ_{13}, and γ_{23} are constrained to be equal to zero. If this test showed insufficient evidence for non-additivity, that is, if the full model did not show a significantly better fit to the data than the additive model, we halted our analysis at this point.

For the variables that showed evidence of non-additivity, we continued with our analyses. We used the Akaike Information Criterion (Akaike, 1974) to find the best fitting model of the form shown in Equation 1. The form of this “best-fit” model depended upon the trait being examined, but generally looked like Equation 1 with one or more of the γ_{ij} parameters set to zero.

Once we had obtained the “best-fit” model, we investigated the relationship between those pairs of variables that had an interaction term that was significantly different than zero. The parameterization shown in Equation 1 is well suited for this type of testing. For example, interactions between haplotypes H1 and H2 are captured in the parameter γ_{12}, as can be seen by comparing the mean trait values for H1/H1, H1/H2, and H2/H2 in Table 1. When $\gamma_{12} = 0$, the relationship between H1 and H2 is additive. When $\gamma_{12} = -\gamma_2$, H1 is dominant to H2. When $\gamma_{12} = \gamma_2$, H1 is recessive to H2. Overdominance and underdominance occur when $|\gamma_{12}| > |\gamma_2|$. Thus, with this parameterization, relationships such as additivity, dominance, recessiveness, and over/underdominance are hypotheses that can be expressed in terms of the model's parameters and hence can be easily tested. The relationship that was of particular interest to us was that of over/underdominance and we tested for this type of relationship using a likelihood ratio test in which we compared the maximum obtainable likelihood under the best-fit model with that under the constraint that $|\gamma_{12}| \leq |\gamma_2|$.

Investigations of the relationship between haplotypes H1 and H3 proceeded with this model analogously to the procedure for testing relationships between H1 and H2. Interactions between H2 and H3 are less neatly summarized by the parameters in Equation 1. For those comparisons, we reparameterized the model, exchanging the roles of H1 and H2 so that H2/H2 individuals served as the baseline.

As a final note, this was a preliminary study with a small sample size (ultimately about 38 H1/H1, 50 H1/H2, 25 H1/H3, 25 H2/H2, 32 H2/H3 and 10 H3/H3 individuals, but these numbers varied slightly depending upon which variable was being examined). Since our intention was to gain insight, we were more concerned with Type II than Type I error. Therefore, at each step where our protocol required us to proceed with follow-up testing only if a previous test was significant, we set our criterion of significance at p ~0.10.

EST database analysis

In order to examine if there is a significant difference in the expression of ADRB2 mRNA driven by allelic combinations at the promoter region of the three identified haplotypes, we analysed existing expressed sequence tag (EST) databases. Since sequencing of each EST clone is a random non-selective process, and there are substantial numbers of EST sequences in the NCBI EST data base, the frequency of gene-specific ESTs is a measure of its RNA expression level (Bortoluzzi and Danieli, 1999). This approach has been used in several studies to estimate the relative abundance of specific RNA transcripts (Lee et al., 1995;Okubo et al., 1992;Comeron, 2004;Castillo-Davis et al., 2002), as well as it's tissue or stage-specific levels (Lee et al., 1995;Comeron, 2004;Shen, He et al., 2005). It has also been shown that the relative RNA expression values between SAGE, EST and microarray databases are comparable (Comeron, 2004).

Computer analysis of all SNP combinations in the human EST database (dbEST release 030405, 6,053,112 - human entries) was performed using the BLAST program. Complete nucleotide sequence of *ADRB2* gene was analyzed (length - 2015 nucleotides, accession number - NM_000024). We used a custom designed program, which was programmed in C and contained a Bash shell script, to generate BLAST program output files. Our program produces an output file (*.csv) in Excel format that contains a complete list of nucleotide variation (SNPs) and their combinations for the selected gene in the EST database. We restricted the analysis of nucleotide variations to known common SNPs with frequencies >10%. Only hits with >95% of similarity over 100 nt to the original sequence were considered. A Chi-Square analysis was used for statistical assessment of the EST data.

TMD incidence cases

From the initial 202 enrolled subjects that were genotyped, 181 (~ 90%) completed the 3-year observation period and 15 enrollees developed TMD. We investigated the relationship of the incidence of TMD in our study population with different diplotypes by calculating the relative risk of TMD onset. Since our sample sizes were small compared to the TMD incidence levels, we calculated confidence intervals for our relative risks using Koopman's method (Koopman, 1984).

Results

Common haplotypes of ADRB2

Genomic DNA from peripheral blood samples was genotyped for SNPs within the *ADRB2* gene locus. Eight SNPs were chosen that display a high frequency of polymorphism in the human population (> 20% prevalence), and form one haploblock (Belfer, et al., 2004) (Fig. 1). The first five examined SNPs (G-7127A, rs11958940, rs1432622, rs1432623 and rs2400707) are located in the promoter region of the gene. The next three SNPs (rs1042713, rs1042714 and rs1042717) are located within the coding region for gene. Variations $Arg^{16}Gly$ (rs1042713) and $Gln^{27}Glu$ (rs1042714) are well-studied common nonsynonymous polymorphisms. SNP $Leu^{84}Leu$ (rs1042714) is a synonymous polymorphism. The known functional SNP $Thr^{164}Ile$ was also assessed, however none of the examine subjects possessed the minor allele.

In agreement with previous reports (Belfer et al., 2004;Drysdale et al., 2000), three major haplotypes were determined, representing 97.4% of all haplotypes observed in this study (Fig. 1B). Only five subjects carried haplotypes that were different from the three major ones. Each of these five haplotypes was different from one of the major haplotypes at only one or two SNP position and was considered in subsequent analyses as one of the major corresponding haplotypes.

Association of ADRB2 polymorphism with blood pressure and psychological traits

Descriptive statistics from the full 6-parameter (6 common diplotypes) regression model for each response variable are presented in Figure 2. Of the ten trait values examined, three (BSI anxiety, STAI state anxiety, and POMS elated-depressed) showed no associations and were not examined further. We also analyzed the effect of haplotype copy number (i.e., dose-effect) on the tested traits. We found a significant association of the number of copies of haplotype H1 with both resting systolic and diastolic blood pressures, and an effect of haplotype H2 on resting diastolic blood pressure (Fig. 3A). Subjects with no copies of H1 had significantly lower resting blood pressure than those who had one copy (Fig. 2A). Consistent with this observation, subjects who carried two copies of H2 showed significantly lower resting diastolic blood pressure than those who carried no copies of H1 (Fig. 2A). H1 heterozygotes, with either H2 or H3, displayed the highest systolic blood pressures, and H1/H3 heterozygotes displayed the highest diastolic blood pressure, while H2 homozygotes and H2/H3 heterozygotes had the lowest systolic and diastolic blood pressures (Fig.2A). Thus, our data suggest an overdominant relationship between H1 and H3 and possibly between H1 and H2 haplotypes: subjects with one copy of H1 had higher systolic and diastolic blood pressures than subjects homozygous for either haplotype (Fig. 3A).

The H2 haplotype was significantly associated with somatization scores (Fig. 3B). Subjects bearing two copies of H2 had significantly higher levels of somatization (both PILL and BSI scores) than subjects possessing only one or no copies of H2 (Fig. 3B). H2 homozygotes displayed the highest somatization score among all diplotypes (Fig. 2B). H2 was also significantly associated with trait anxiety: subjects with no H2 reported higher trait anxiety

levels than those bearing one copy of H2 (Fig. 3B). Consistent with this result, homozygotes for H1 and homozygotes for H3 had the highest trait anxiety scores, while H2 heterozygotes had the lowest scores, although these results must be tempered by the fact that the overall association between the ADRB2 haplotypes and this trait was weak (Fig. 2B, Table 2). H3 was strongly associated with the POMS composed-anxious score (lower scores correspond to more negative characteristics) and BSI depression. H3 homozygotes had significantly higher state-dependent anxiety (POMS composed-anxious), and lower BSI depression scores (Fig.3C).

Haplotype interactions

Table 2 summarizes the findings from assessments of interactions between haplotypes. For systolic blood pressure, diastolic blood pressure, and BSI depression scores a comparison between the additive model and the full model, which included all possible interaction, indicated that the model with interactions provided a better fit to the data. There was some indication that this is also the case for somatization (PILL; P=0.1042), and the POMS measure of “composed-anxious” (P=0.1133) scores.

Systolic and diastolic blood pressure variables had a best-fit to the model that included significant interactions between H1 and H2 and between H1 and H3. Further analyses did not support the hypothesis that H1 and H2 showed over/underdominance (P=0.341, P=0.862) for either of the blood pressure phenotypes. However, the interaction between haplotypes H1 and H3 was significant (P=0.045) for diastolic blood pressure and tend to support an overdominance effect for systolic blood measure (P=0.082).

For BSI depression, the best-fit to model included a significant interaction between H1 and H3. A likelihood ratio test for over/underdominance in the relationship between H1 and H3 gave a p-value of 0.044, supporting an overdominant relationship.

ADRB2 polymorphism and RNA expression

The vast majority of published studies that have examined *ADRB2* polymorphism describe associations with the nonsynonymous $Arg^{16}Gly$ (rs1042713) and $Gln^{27}Glu$ (rs1042714) SNPs. It has been consistently observed that $Arg^{16}Gly$ polymorphism is associated with agonist-induced internalization of the receptor (Small et al., 2003). Although several phenotypes have been associated with $Gln^{27}Glu$ polymorphism, the functional effects of this SNP remain unclear (Small et al., 2003). We hypothesized that since $Gln^{27}Glu$ polymorphism is in strong linkage disequilibrium (LD) with SNPs in the promoter region (rs11958940, rs14326222, rs14326223, rs2400707; Fig. 1) that a set of these SNPs defines the efficiency of RNA transcription, and haplotype H1 codes for reduced efficiency of transcription compared to H2 and H3.

To examine if there is a significant difference in the level of expression of ADRB2 mRNA driven by allelic combinations at the promoter region of three haplotypes, we estimated the relative expression level of *ADRB2* mRNAs on the basis of the relative abundance of ESTs, as described by Castilo-Davis and coworkers (2002). We assigned each EST in available EST libraries to one of the three identified haplotypes based on their EST sequence (Table 3). If each haplotype codes at similar transcription efficiencies, we would expect the haplotype-specific ESTs to appear in the databases in proportion to the population haplotype frequencies, whereas, if transcription efficiencies vary between the different haplotypes, we would expect those haplotypes that produce more transcripts to be over-represented.

Fifty-three *ADRB2* EST were found. Twenty-four of these had a sequence in the 5′region of the gene that permitted the identification of the corresponding haplotype. Five ESTs corresponded to H1, 8 ESTs corresponded to H2, and 11 ESTs corresponded to H3 (Table

3). We compared haplotype-specific EST frequencies with the haplotypes frequencies in our cohort (Fig. 1), as well as haplotype frequencies reported by others (Belfer et al., 2004). The distribution of EST frequencies was statistically different from the distribution of haplotype frequencies in both cohorts ($\chi 2$ analysis; P's <0.05). After normalization to haplotype frequencies in human population, the amounts of H2- and H3-specific ESTs relative to H1 were 1.8 and 4.4 respectively (Table 3), which suggests that H1 codes for a lower efficiency of transcription compared to H2 and H3. Since the allelic combination of H1 in the promoter region of the gene and *Gln*^{27}*Glu* SNPs is opposite to the allelic combination associated with H2 and H3 (haplotype AAAGG versus TGGAC for rs11958940, rs14326222, rs14326223, and rs2400707, *Gln*^{27}*Glu*, respectively; Fig. 1) our data suggest that H1 codes for a lower efficiency of transcription while H2 and H3 code for high efficiency of transcription.

ADRB2 haplotypes and risk of TMD

Next, we determined the clinical significance of *ADRB2* genetic variants by examining whether *ADRB2* polymorphism is related to the incidence of TMD onset. We predicted that since *ADRB2* genotype is associated with somatization, depression, trait anxiety and low blood pressure, which are phenotypic characteristics commonly associated with chronic pain conditions, these genotypes should also be associated with TMD development. Because none of the three major haplotypes showed a simple relationship with all of these psychological characteristics or blood pressure, but instead showed a complicated interplay of associations, we suggest that there are complex interactions between different haplotypes and associated molecular properties of the corresponding receptor variants. We hypothesised that specific combinations of receptor variants contribute to the development of TMD more than others.

Based on the analysis of the *ADRB2* EST databases, as well as previously published literature (Small et al., 2003), we suggest there are two primary functional sites within the human *ADRB2* gene locus: one that influences the level of receptor expression and one that influences that ability of the receptor to internalize in response to agonist stimulation. Based on these assumptions, haplotypes were subdivided for those coding for lower ADRB2 expression (H1 haplotypes, low *ADRB2* expression, “Lo”, Table 4A), and those that code for high ADRB2 expression (i.e., H2 and H3 haplotypes, high ADRB2 expression, “Hi”, Table 4A). Similarly, H1 and H3 haplotypes, coding for Gly16 associated with efficient internalization of the receptor, were clustered into group “I” and H2 coding for Arg16 was identified as group “N” (Table 4B).

Of 181 subjects followed over the three year observational time period, 15 were diagnosed with first-onset TMD, yielding an overall incidence of 8.3%. TMD incidence was highest among the 25 H2/H2 homozygotes (5/25 = 20% (0.200), Table 4). The lowest TMD incidence was among H1/H2 and/or H1/H3 heterozygotes. Although the “Hi/Lo” reference group of H1 heterozygotes represented almost half of the initially tested cohort ((n=76), Table 4A) only one TMD case was observed in this group, yielding a TMD incidence of 1.3%. Compared with the reference group of “Hi/Lo” H1 heterozygotes, there was a significantly elevated risk of developing TMD for “Lo” homozygotes (relative risk [RR] = 8.0, 95% confidence interval [CI] = 1.2 – 52.2 and 99%CI = 0.815-79.7) and a significantly elevated risk for the “Hi” homozygotes (RR=11.3, 95%CI = 1.95-67.9, and 99%CI = 1.38-102.1). Grouping subjects based on receptor internalization characteristics yielded lower RRs. Specifically, comparing the N/N group with the heterozygotes I/N group as a reference group, revealed an RR=3.32, with a 95% CI = 1.08-9.83, and 99%CI = 0.794-13.2.

These results strongly suggest a significant influence of receptor expression level on risk of TMD development. The lowest TMD incidence rate of the subjects caring one copy of H1 (i.e., heterozygous for H1-H2 or H1-H3, “Hi/Lo” group) is consistent with the evidence for

an overdominance effect of H1, when coupled with either H2 or H3 (Table 2), more positive psychological characteristics and higher resting blood pressure for Hi/Lo heterozygotes (Fig. 2 and 3), which are characteristics that appear to protect from the development of TMD.

Discussion

A heuristic model of functional variants of ADRB2

Our conceptual model of the complex interplay of associations between ADRB2 haplotypes, human psychological traits and blood pressure are presented in Fig.4. We analyzed our association data taking into account the following reported properties of the receptor stimulation: i). a high level of receptor activity has been reported to produce antidepressant--like effects in rodents (Zhang, Huang, and O'Donnell, 2003); ii). reductions in the density of β-adrenergic receptors are associated with depression- and anxiety- related clinical conditions (Magliozzi et al.;Maddock et al.; Mann et al.) and the density of ADRB2 is negatively correlated with anxiety and depression assessed with unidirectional POMS questionnaire (Yu, Dimsdale, and Mills, 1999); iii). chronic stimulation of ADRB2 enhances epinephrine-mediated physiological arousal and increases somatic awareness across multiple systems (e.g., gastrointestinal, cardiorespiratory, etc.) (Kopin, 1984;Easton and Sherman, 1976;Lader, 1988); iv). stimulation of ADRB2 lead to decreased blood pressure (Brodde and Michel, 1992;Iaccarino et al., 2002;Gratze et al., 1999;Snapir et al., 2003). Furthermore, we considered haplotype-specific differences in the ability of receptor to be internalized in response to agonist stimulation: H1 and H3 haplotypes code for a rapid internalization of receptor, as these two haplotypes carry the *G* allele at SNP rs1042713 (*Arg*^{16}*Gly*), which codes for Gly16 (Fig. 1) (Small et al., 2003). Finally, we took into account haplotype-specific level of receptor expression, assuming that H1 codes for the lowest amount of *ADRB2* RNA and H3 codes for the highest amount of *ADRB2* RNA (table 3). Based on these assumptions we propose a model that explains how different haplotypes produce different phenotypes (Fig. 4).

In the resting physiological state, H1 homozygotes express several times fewer receptors than either H2 or H3 homozygous (Fig. 4A). Chronically diminished ADRB2 function in the CNS, as we proposed for H1 homozygotes, would produce psychological traits such as depression and anxiety, which has been observed in some patients treated with non-selective ADRB receptor blockers (Thiessen et al., 1990). In contrast, H2 codes for higher receptor expression (Table 3) coupled with low efficiency of agonist-induced receptor internalization (Small et al., 2003). Chronically enhanced ADRB2 function, as we proposed for H2 homozygotes, would display psychological characteristics of physiological arousal and enhanced awareness of bodily functions (i.e., somatization, Fig 2B, 3B) evoked by stimulation of central ADRB2 and a reduction in arterial blood pressure by stimulation ADRB2 on peripheral blood vessels (Fig.2A, 3A). In agreement with these observations, H2 demonstrated a protective role against elevated trait anxiety because subjects without H2 reported significantly higher trait anxiety scores, although the lowest trait anxiety scores were reported by subjects with one copy of H2, rather then with two copies of H2 (Fig. 2B and 3B).

Endogenous stimulants, such as epinephrine released from the adrenal glands in response to a stressful situation, should lead to a rapid internalization of receptor variants H1 and H3. For the H3 homozygotes, but not H1 homozygotes, this would lead to a significant reduction in receptor density producing a stress-evoked state-dependent anxiety (Fig. 2C and 3C; Fig. 4B), as H3 expresses the highest level of ADRB2 (Table 3). Furthermore, H3 homozygotes reported the lowest level of BSI depression (BSI) (Fig. 2C and 3C). This is in agreement with the evidence that H3 codes for the highest level ADRB2 expression (Table 3), which mediates an anti-depression effect (Gorman and Dunn, 1993). On the other hand, we

proposed that the efficient epinephrine-dependent internalization of the receptor, which should be associated with H3 homozygotes, will lead to highly dynamic changes in receptor density resulting in high psychological reactivity and low depression scores. Based on this model, we propose that the relative rank order of ADRB2 receptor function in response to agonist stimulation is the following: H2 (high expression/low rate of internalization) similar to H3 (high expression/high rate of internalization), both of which are greater than H1 (low expression/ high rate of internalization).

We observed some inconsistency in association pattern of ADRB2 polymorphism with different measures of depression and anxiety. Specifically, the derived measure of depression from the POMS questionnaire failed to show an association with *ADRB2*, while BSI depression score did. Similarly, different measures of anxiety were associated with different haplotypes in our study. We suggest that this reflects differences in the parameters of anxiety and depression measured by different questionnaires. For example, the STAI Trait Anxiety scale measures trait or chronic levels of perceived anxiety and based on our model is negatively correlated with the haplotype that produce and maintain high levels of ADRB2 expression (i.e., H2). Anxiety assessed with the POMS scale assesses the level of anxiety experienced in the ‘here and now” (Yu et al., 1999), and based on our model, should be dynamically associated with the efficiency of agonist-induced receptor internalization that occurs in response to acutely stressful environmental events (i.e., H3). STAI State anxiety and BSI anxiety measures may tap into a combination of these two anxiety constructs and that is why these measures were not associated significantly with either haplotype. Consistent with our findings, it has been reported that increased POMS measures of anxiety, but not STAI Anxiety measures, are highly associated with the down-regulation of ADRB on human lymphocytes (Yu et al., 1999).

Our heuristic model (Fig. 4) demonstrates why it is necessary to consider haplotypes rather than single SNPs. Each haplotype codes for two basic receptor properties - the level of expression and the internalization responses produced by agonist stimulation. Both of these properties influence physiological responses and phenotypes associated with ADRB2. Consistent with our model, Drysdale and coworkers (2000) reported a complex relationship between the three major ADRB2 haplotypes and bronchodilator responses to a ADRB2 agonist. Mean responses was significantly related to the haplotype pairs ($P = 0.007$) but not to individual SNPs.

We would like to stress that the proposed heuristic model does not explain the entire complexity of ADRB2 biology and its role in chronic pain conditions and related phenotypes. There are multiple mechanisms that contribute to ADRB2 regulation, including the bioavailability of the agonist, posphorylation, endocytosis and recycling of the receptor, and receptor expression and translation. Each process is regulated by different pathways and depends on the biological activities of a complex gene network. Future studies designed to investigate the interaction between genetic variants of ADRB2 and the genetic variants of other genes are required that will more comprehensively elucidate our understanding of individual variations in ADRB2-mediated phenotypes.

ADRB2 polymorphism and resting blood pressure

Although several studies have examined the association between resting arterial blood pressure and *ADRB2* polymorphism, the published results vary and are often difficult to interpret. The associations between resting blood pressure and the three major ADRB2 haplotypes observed in our study reveal a complex pattern of associations, which can be explained by a combination of putative effects: i). haplotype-specific differences in the amount of expressed receptor; ii). haplotype-specific differences in the ability of receptor to be internalized in response to agonist stimulation; iii). an overdominance effect associated

with H1; and iv). tissue-specific differences in ADRB2 mediated vasodilatory responses, such that stimulation of CNS ADRB2 results in elevations in arterial blood pressure while stimulation of peripheral ADRB2 results in a reduction in arterial blood pressure.

It is well know that the stimulation of vascular ADRB2 produces peripheral vasodilation, decreases peripheral vascular resistance, and lowers arterial blood pressure (Brodde and Michel, 1992;Iaccarino et al., 2002;Gratze et al., 1999;Snapir et al., 2003). Thus, it is generally accepted that decreased peripheral vascular resistance and lower resting arterial blood pressure results when relatively higher amounts of ADRB2s are expressed on peripheral blood vessels. In contrast, stimulation of ADRB2 in the CNS produces an increase in peripheral vascular resistance and arterial blood pressure (Routledge and Marsden, 1987;Ward-Routledge et al., 1988). Thus, tissue specific regulation of receptor expression, which occurs in a haplotype specific manner, may differentially influence resting arterial blood pressure.

A substantial number of publications have shown that the magnitude and direction of agonist-induced peripheral vasodilation is associated with *ADRB2* polymorphisms. The intravenous infusion of epinephrine produces effects on blood pressure in a manner that depends on the *ADRB2* genotype (Snapir, et al., 2003). Individuals with at least one *A* allele at SNP rs1042713 ($Arg^{16}Gly$), which codes for Arg16 and is a specific marker for H2, show reduced diastolic blood pressure in response to a graded epinephrine infusion. In contrast, individuals carrying the Glu27 allele, which is specific to H1 (Bray et al., 2000) (low expression/rapid internalization) or Gly16 (Kotanko et al., 1997), which is specific for both H1 (low expression) and H3 (rapid internalization), have been associated with essential hypertension and diminished peripheral vasodilatory capacity in response to ADRB2 stimulation (Gratze et al., 1999;Hoit et al., 2000).

Our findings are in agreement with these reports and we propose that variations in resting arterial blood pressure can be explained by the effects of *ADRB2* polymorphism on receptor expression and/or receptor internalization. For example, resting arterial diastolic blood pressure is associated with the number of copies of H2 (Fig. 2A). H2 homozygotes (high expression/slow internalization) had the lowest resting diastolic pressure and subjects without a H2 haplotype (subjects with combinations of H1 and H3, rapid receptor internalization) had the greatest resting diastolic pressures (Fig.2A, 3A). An even stronger effect was observed for H1 (Fig. 2A, 3A). Subjects with no H1 (subjects with combinations of H2 and H3, high receptor expression) had the lowest systolic and diastolic blood pressure (Fig. 2A, 3A). Although homozygotes for H1 had higher resting arterial blood pressure, the highest resting arterial systolic and diastolic blood pressures were seen with H1-H2 and H1-H3 heterozygotes (Fig. 3A), suggesting a H1 overdominance (Table 2). It is noteworthy that these same individuals were less likely to develop TMD (Table 4), which is consistent with the substantial literature that pain sensitivity and the risk of developing chronic musculoskeletal conditions is inversely related to resting arterial blood pressure (Hagen et al., 2005;Bruehl and Chung, 2004;Fillingim, Maixner, et al., 1998;Fillingim and Maixner, 1996;Maixner et al., 1997;Pfleeger et al., 1997;Maixner, 1991;Randich and Maixner, 1984;Sheps et al., 1992).

Haplotype-dependent ADRB2 expression

Our analysis of EST databases suggests that H1 codes for a lower amount of RNA expression compared to H2 and H3. The outcomes of our association study, and known effects of genetic variations in ADRB2 on various physiological functions such as blood pressure (Bray et al., 2000) and airway resistance (Tattersfield and Hall, 2004), support this conclusion. Two cell biology-based studies that examined the relationship between ADRB2 expression and common polymorphisms did not provide congruent results (McGraw et al.,

2000), which may be due to tissue specific effects on haplotype expression. Future cell culture studies are required to accurately assess haplotype-specific expression in different tissue-specific cell lines, which will more directly test our proposed model (see below) that H1 codes for a lower level of expression of the receptor compared to H2 and H3, particularly in the CNS.

Overdominance associated with ADRB2 H1 haplotype

An overdominance of H1 over H2 and H3 is another feature of *ADRB2* genetics that complicates the interpretation of association studies. Heterozygotes for H1-H2 and H1-H3 show the highest level of resting arterial blood pressure (Fig. 2A). Although the H1-H2 and H1-H3 heterozygotes represented almost half of the initially tested cohort, only one TMD case was observed in this group, yielding a significantly elevated risk of developing TMD for both “Lo” and “Hi” homozygotes and suggests that the presence of one copy of H1 is protective against TMD onset. An overdominance was further confirmed by a linear statistical model that investigated the interactions between haplotypes. The best-fit model for resting blood pressure revealed interactions between the H1 with H2 and H3, but only the H1-H3 interaction revealed a significant overdominance, which is likely due to the limited sample size. The H1-H3 interaction also showed overdominance when associated with BSI assessed depression phenotype (Table 2): H1-H3 heterozygotes showed the highest BSI assessed depression score while H3 homozygotes showed the lowest BSI depression score (Fig. 3). Consistent with our findings, other investigators have observed non-linear relationships between *ADRB2* haplotype dosage and corresponding mean values of assessed phenotypes (Drysdale et al., 2002).

An overdominance has been described in crop genetics (Syed and Chen, 2004) and there is accumulating evidence that overdominance is a common occurrence in humans, with heterozygotes expressing phenotypic values that are either substantially above or below both homozygotes. While the mechanism(s) underlying the phenomenon of overdominance is poorly understood, we suggest that the overdominance effect of H1 haplotype, when combined with H2 and H3, on blood pressure and psychological associations results from the integration of opposite effects associated with central vs peripheral ADRB2 activation (Brodde and Michel, 1992;Iaccarino, et al., 2002;Gratze, et al., 1999;Snapir, et al., 2003;Routledge and Marsden, 1987;Ward-Routledge et al., 1988). Another possible explanation is that the dose-effects evoked by a ADRB2 stimulant produces an inverted U-shaped dose-response curve. U-shaped responses are commonly seen under normal physiological conditions. For example, U-shaped behavioral responses are seen in response to graded stimulation of the locus coeruleus, a region of the brain important in attention, autonomic function, arousal, anxiety, and goal directed behaviors (Aston-Jones et al., 1999). The third possibility is tissue specific effects on haplotype expression. In fact, it has been shown in a recent study analyzing more than 140 polymorphisms involved in the regulation of 107 genes that single-locus tissue-specific overdominance is very common and may represent a selective mechanism for the maintenance of *cis*-regulatory variation (Rockman and Wray, 2002). Thus, additional studies are required to identify the mechanism(s) of the observed overdominance effect.

Limitations associated with this study

We recognized that our study was conducted on a limited number of subjects. As a consequence, we did not consider each genetic variant independently, but instead haplotype groups based on their likely biological properties: receptor expression level and internalization efficiency. The grouping of haplotypes supports the suggestion that receptor expression level strongly influences the risk of TMD development with Lo/Hi heterozygotes are protected from developing TMD. However, it is possible that relative risk of

development of TMD will be largely different among the subjects with different diplotypes once a larger cohort is examined.

Clinical relevance of the observed associations with ADRB2 haplotypes

Our results are of considerable clinical significance and are the first to demonstrate an association between a genetic polymorphism that correlates with psychological traits, resting blood pressure, and the risk for developing a common chronic pain condition. The observed genetic risk associated with *ADRB2* polymorphism is substantially greater than that associated with other risk factors such as estrogen exposure, history of chronic pain at other body sites (John et alk., 2003;Von Korff et al., 1993), and genetic variations in the gene that codes for COMT (Diatchenko et al., 2005). The clinical relevance of these findings is best quantified by the measure of population attributable risk for being homozygous for either high or low expression of ADRB2, which was 82% in this cohort of women, indicating that more than eight of ten myogenous TMD cases can be predicted by these *ADRB2* haplotypes.

Individuals with relatively high ADRB2 function (H2 and H3) had a high likelihood of developing TMD (Table5). H2 homozygotes showed the highest TMD incidence rate (0.200). These subjects showed high somatization score and low blood pressure, which are factors that appear to influence the development chronic musculoskeletal pain conditions (Maixner et al., 1997;Bruehl and Chung, 2004;Macfarlane et al., 2004). H2-H3 heterozygotes and H3 homozygotes, where ADRB2 function should be slightly diminished, showed TMD incidence rates of 0.125 and 0.100 respectively, which was still higher than the average TMD incidence rate of 0.083. The homozygotes for low ADRB2 function also show increased risk for TMD development with incidence rate of 0.105. These findings suggest that subjects with either high or low ADRB2 receptor activity are at risk of developing a chronic pain condition *via* different etiological pathways. H1 heterozygotes, those who carry one haplotype copy that codes for high ADRB2 expression (H2 or H3) and one copy of H1 that codes for low ADRB2 expression and rapid internalization were protected from the development of TMD. Only one subject who was H1-H2 heterozygote developed TMD, even though almost half of all the variants observed in our cohort were H1-H2 or H1-H3 heterozygotes (Table 4). Thus, our data suggest that either positive or negative imbalances in ADRB2 function increase the vulnerability to TMD. Collectively 14 of 15 TMD (93%) patients were associated with a putative hyperfunction (10/15) or hypofunction of (4/15) of ADRB2 (Table 4).

Our findings also have potentially important treatment implications. If ADRB2 hyperfunction contributes to TMD, then a relatively high percentage of these patients (~ 60-70%) should respond to treatment with an ADRB2 antagonist, like propranolol. In contrast, approximately 25 to 30% of TMD cases should have a hypofunction of ADRB2 (H1/H1) and should not respond to treatment with ADRB2 antagonist. In fact, treatment of this group with such an agent may actually worsen their signs and symptoms. Thus, it may be possible to predict treatment outcomes to ADRB2 blockade by determining the specific haplotype profile for patients with TMD and related disorders.

Acknowledgments

Supported by grant DE016558, DE07509 and NS045688

Reference List

1. Report of the Panel on Communicative Disorders and Stroke Council. Washington, D.C.: Public Health Service, NIH. National Ambulatory Medical Care Survey; Jun 1. 1979 No:81-1914
2. The Psychology of Physical Symptoms. Springer Verlag Inc; New York: 1982.

3. Akaike H. A new look at statistical model identification. IEEE Trans Biomed Engin. 1974; AU-19:716–722.
4. Aston-Jones G, Rajkowski J, Cohen J. Role of locus coeruleus in attention and behavioral flexibility. Biol Psychiatry. 1999; 46:1309–1320. [PubMed: 10560036]
5. Belfer I, Buzas B, Evans C, Hipp H, Phillips G, Taubman J, Lorincz I, Lipsky RH, Enoch MA, Max MB, Goldman D. Haplotype structure of the beta adrenergic receptor genes in US Caucasians and African Americans. Eur J Hum Genet. 2004
6. Bortoluzzi S, Danieli GA. Towards an in silico analysis of transcription patterns. Trends Genet. 1999; 15:118–119. [PubMed: 10203810]
7. Bray MS, Krushkal J, Li L, Ferrell R, Kardia S, Sing CF, Turner ST, Boerwinkle E. Positional genomic analysis identifies the beta(2)-adrenergic receptor gene as a susceptibility locus for human hypertension. Circulation. 2000; 101:2877–2882. [PubMed: 10869257]
8. Brodde OE, Michel MC. Adrenergic receptors and their signal transduction mechanisms in hypertension. J Hypertens Suppl. 1992; 10:S133–S145. [PubMed: 1291648]
9. Bruehl S, Chung OY. Interactions between the cardiovascular and pain regulatory systems: an updated review of mechanisms and possible alterations in chronic pain. Neurosci Biobehav Rev. 2004; 28:395–414. [PubMed: 15341037]
10. Busjahn A, Freier K, Faulhaber HD, Li GH, Rosenthal M, Jordan J, Hoehe MR, Timmermann B, Luft FC. Beta-2 adrenergic receptor gene variations and coping styles in twins. Biol Psychol. 2002; 61:97–109. [PubMed: 12385671]
11. Carlson CR, Okeson JP, Falace DA, Nitz AJ, Curran SL, Anderson D. Comparison of psychologic and physiologic functioning between patients with masticatory muscle pain and matched controls. Jour of Orofacial Pain. 1993; 7:15–22. [PubMed: 8467294]
12. Carlsson, GE.; Le Resche, L. Epidemiology of temporomandibular disorders. In: Sessle, BJ.; Bryant, PS.; Dionne, RA., editors. Temporomandibular Disorders and Related Pain Conditions. IASP Press; Seattle: 1995. p. 211-226.
13. Castillo-Davis CI, Mekhedov SL, Hartl DL, Koonin EV, Kondrashov FA. Selection for short introns in highly expressed genes. Nat Genet. 2002; 31:415–418. [PubMed: 12134150]
14. Comeron JM. Selective and mutational patterns associated with gene expression in humans: influences on synonymous composition and intron presence. Genetics. 2004; 167:1293–1304. [PubMed: 15280243]
15. Derogatis LR, Melisaratos N. The Brief Symptom Inventory: an introductory report. Psychol Med. 1983; 13:595–605. [PubMed: 6622612]
16. Diatchenko L, Slade GD, Nackley AG, Bhalang K, Sigurdsson A, Belfer I, Goldman D, Xu K, Shabalina SA, Shagin D, Max MB, Makarov SS, Maixner W. Genetic basis for individual variations in pain perception and the development of a chronic pain condition. Hum Mol Genet. 2005:135–143. [PubMed: 15537663]
17. Drysdale CM, McGraw DW, Stack CB, Stephens JC, Judson RS, Nandabalan K, Arnold K, Ruano G, Liggett SB. Complex promoter and coding region beta 2-adrenergic receptor haplotypes alter receptor expression and predict in vivo responsiveness. Proc Natl Acad Sci USA. 2000; 97:10483–10488. [PubMed: 10984540]
18. Dworkin SF, Huggins KH, Le Resche L, Von Korff M, Howard J, Truelove E, Sommers E. Epidemiology of signs and symptoms in temporomandibular disorders: clinical signs in cases and controls. JADA. 1990; 120:273–281. [PubMed: 2312947]
19. Easton JD, Sherman DG. Somatic anxiety attacks and propranolol. Arch Neurol. 1976; 33:689–691. [PubMed: 973806]
20. Epker J, Gatchel RJ. Coping profile differences in the biopsychosocial functioning of patients with temporomandibular disorder. Psychosom Med. 2000; 62:69–75. [PubMed: 10705913]
21. Epker J, Gatchel RJ, Ellis E III. A model for predicting chronic TMD: practical application in clinical settings. J Am Dent Assoc. 1999; 130:1470–1475. [PubMed: 10570591]
22. Fillingim RB, Fillingim LA, Hollins M, Sigurdsson A, Maixner W. Generalized vibrotactile allodynia in a patient with temporomandibular disorder. Pain. 1998; 78:75–78. [PubMed: 9822214]

23. Fillingim RB, Maixner W. The influence of resting blood pressure and gender on pain responses. Psychosomatic Med. 1996; 58:326–332.
24. Fillingim RB, Maixner W, Bunting S, Silva S. Resting blood pressure and thermal pain responses among females: effects on pain unpleasantness but not pain intensity. International Journal of Psychophysiology. 1998; 30:313–318. [PubMed: 9834887]
25. Gorman AL, Dunn AJ. Beta-adrenergic receptors are involved in stress-related behavioral changes. Pharmacol Biochem Behav. 1993; 45:1–7. [PubMed: 8100069]
26. Gratze G, Fortin J, Labugger R, Binder A, Kotanko P, Timmermann B, Luft FC, Hoehe MR, Skrabal F. beta-2 Adrenergic receptor variants affect resting blood pressure and agonist-induced vasodilation in young adult Caucasians. Hypertension. 1999; 33:1425–1430. [PubMed: 10373227]
27. Hagen K, Zwart JA, Holmen J, Svebak S, Bovim G, Stovner LJ. Does hypertension protect against chronic musculoskeletal complaints? The Nord-Trondelag Health Study. Arch Intern Med. 2005; 165:916–922. [PubMed: 15851644]
28. Harkins SW, Price DD, Braith J. Effects of extraversion and neuroticism on experimental pain, clinical pain, and illness behavior. Pain. 1989; 36:209–218. [PubMed: 2919101]
29. Hoit BD, Suresh DP, Craft L, Walsh RA, Liggett SB. beta2-adrenergic receptor polymorphisms at amino acid 16 differentially influence agonist-stimulated blood pressure and peripheral blood flow in normal individuals. Am Heart J. 2000; 139:537–542. [PubMed: 10689270]
30. Iaccarino G, Cipolletta E, Fiorillo A, Annecchiarico M, Ciccarelli M, Cimini V, Koch WJ, Trimarco B. Beta(2)-adrenergic receptor gene delivery to the endothelium corrects impaired adrenergic vasorelaxation in hypertension. Circulation. 2002; 106:349–355. [PubMed: 12119252]
31. John MT, Miglioretti DL, LeResche L, Von Korff M, Critchlow CW. Widespread pain as a risk factor for dysfunctional temporomandibular disorder pain. Pain. 2003; 102:257–263. [PubMed: 12670667]
32. Keefe FJ, Dolan E. Pain behavior and pain coping strategies in low back pain and myofascial pain dysfunctional patients. Pain. 1986; 24:49–56. [PubMed: 2937006]
33. Koopman PAR. Confidence intervals for the ratio of two binomial proportions. Biometrics. 1984; 40:513–517.
34. Kopin IJ. Avenues of investigation for the role of catecholamines in anxiety. Psychopathology. 1984; 17 1:83–97. [PubMed: 6369370]
35. Kotanko P, Binder A, Tasker J, DeFreitas P, Kamdar S, Clark AJ, Skrabal F, Caulfield M. Essential hypertension in African Caribbeans associates with a variant of the beta2-adrenoceptor. Hypertension. 1997; 30:773–776. [PubMed: 9336371]
36. Lader M. Beta-adrenoceptor antagonists in neuropsychiatry: an update. J Clin Psychiatry. 1988; 49:213–223. [PubMed: 2897959]
37. Lee NH, Weinstock KG, Kirkness EF, Earle-Hughes JA, Fuldner RA, Marmaros S, Glodek A, Gocayne JD, Adams MD, Kerlavage AR. Comparative expressed-sequence-tag analysis of differential gene expression profiles in PC-12 cells before and after nerve growth factor treatment. Proc Natl Acad Sci USA. 1995; 92:8303–8307. [PubMed: 7667285]
38. Lorr, M.; McNair, DM. Profile of Mood States: Bipolar Form. Educational and Industrial Testing Service; San Diego, CA: 1988.
39. Macfarlane TV, Blinkhorn AS, Davies RM, Kincey J, Worthington HV. Predictors of outcome for orofacial pain in the general population: a four-year follow-up study. JDR. 2004; 83:712–719. [PubMed: 15329378]
40. Maddock RJ, Carter CS, Magliozzi JR, Gietzen DW. Evidence that decreased function of lymphocyte beta adrenoreceptors reflects regulatory and adaptive processes in panic disorder with agoraphobia. Am J Psychiatry. 1993; 150:1219–1225. [PubMed: 8392297]
41. Magliozzi JR, Gietzen D, Maddock RJ, Haack D, Doran AR, Goodman T, Weiler PG. Lymphocyte beta-adrenoreceptor density in patients with unipolar depression and normal controls. Biol Psychiatry. 1989; 26:15–25. [PubMed: 2541807]
42. Maixner W. Interactions between cardiovascular and pain modulatory systems: physiological and pathophysiological implications. J Cardiovas Electrophysiol. 1991; 2(Supplement):S2–S12.

43. Maixner W, Fillingim R, Booker D, Sigurdsson A. Sensitivity of patients with painful temporomandibular disorders to experimentally evoked pain. Pain. 1995; 63:341–351. [PubMed: 8719535]
44. Maixner W, Fillingim RB, Kincaid S, Sigurdsson A, Harris MB. Relationship between pain sensitivity and resting arterial blood pressure in patients with painful temporomandibular disorders. Psychosomatic Med. 1997; 59:503–511.
45. Maixner, W.; Sigurdsson, A.; Fillingim, R.; Lundeen, T.; Booker, D. Regulation of acute and chronic orofacial pain. In: Fricton, JR.; Dubner, RB., editors. Orofacial Pain and Temporomandibular Disorders. Raven Press Ltd.; New York: 1995. p. 85-102.
46. Mann JJ, Brown RP, Halper JP, Sweeney JA, Kocsis JH, Stokes PE, Bilezikian JP. Reduced sensitivity of lymphocyte beta-adrenergic receptors in patients with endogenous depression and psychomotor agitation. N Engl J Med. 1985; 313:715–720. [PubMed: 2993884]
47. McDermid AJ, Rollman GB, McCain GA. Generalized hypervigilance in fibromyalgia: evidence of perceptual amplification. Pain. 1996; 66:133–144. [PubMed: 8880834]
48. McGraw DW, Forbes SL, Kramer LA, Liggett SB. Polymorphisms of the 5′ leader cistron of the human beta2-adrenergic receptor regulate receptor expression. J Clin Invest. 1998; 102:1927–1932. [PubMed: 9835617]
49. Ohrbach R, Dworkin SF. Five-year outcomes in TMD: relationship of changes in pain to changes in physical and psychological variables. Pain. 1998; 74:315–326. [PubMed: 9520246]
50. Okeson, JP.; Adler, RC.; Anderson, GC.; Baragona, PM.; Broker, EB.; Falace, DA.; Graff-Radford, SB.; Kaplan, AS.; McDonald, C.; McNeill, C.; Milliner, EK.; Rosenbaum, RS.; Seligman, DA. Differential Diagnosis and Management Considerations of Temporomandibular Disorders. In: Okeson, JP., editor. Orofacial Pain. Quintessence; Chicago: 1996. p. 113-184.
51. Okubo K, Hori N, Matoba R, Niiyama T, Fukushima A, Kojima Y, Matsubara K. Large scale cDNA sequencing for analysis of quantitative and qualitative aspects of gene expression. Nat Genet. 1992; 2:173–179. [PubMed: 1345164]
52. Parola AL, Kobilka BK. The peptide product of a 5′ leader cistron in the beta 2 adrenergic receptor mRNA inhibits receptor synthesis. J Biol Chem. 1994; 269:4497–4505. [PubMed: 8308019]
53. Pfleeger M, Straneva P, Fillingim RB, Maixner W, Girdler SS. Menstrual cycle, blood pressure and ischemic pain sensitivity in women. Int J Beh Med. 1997 Submitted.
54. Randich A, Maixner W. Interactions between cardiovascular and pain regulatory systems. Neurosci Biobehav Rev. 1984; 8:343–367. [PubMed: 6095151]
55. Rockman MV, Wray GA. Abundant raw material for cis-regulatory evolution in humans. Mol Biol Evol. 2002; 19:1991–2004. [PubMed: 12411608]
56. Routledge C, Marsden CA. Adrenaline in the CNS: in vivo evidence for a functional pathway innervating the hypothalamus. Neuropharmacology. 1987; 26:823–830. [PubMed: 2889157]
57. Sarlani E, Grace EG, Reynolds MA, Greenspan JD. Evidence for up-regulated central nociceptive processing in patients with masticatory myofascial pain. J Orofac Pain. 2004; 18:41–55. [PubMed: 15029872]
58. Shen D, He J, Chang HR. In silico identification of breast cancer genes by combined multiple high throughput analyses. Int J Mol Med. 2005; 15:205–212. [PubMed: 15647832]
59. Sheps DS, Bragdon EE, Gray TF, Ballenger M, Usedom JE, Maixner W. Relationship between systemic hypertension and pain perception. Am J Cardiol. 1992; 70:3F–5F.
60. Shi MM, Bleavins MR, de la iglesia FA. Technologies for detecting genetic polymorphisms in pharmacogenomics. Mol Diagn. 1999; 4:343–351. [PubMed: 10671645]
61. Small KM, McGraw DW, Liggett SB. Pharmacology and physiology of human adrenergic receptor polymorphisms. Annu Rev Pharmacol Toxicol. 2003; 43:381–411. [PubMed: 12540746]
62. Snapir A, Koskenvuo J, Toikka J, Orho-Melander M, Hinkka S, Saraste M, Hartiala J, Scheinin M. Effects of common polymorphisms in the alpha1A-, alpha2B-, beta1- and beta2-adrenoreceptors on haemodynamic responses to adrenaline. Clin Sci (Lond). 2003; 104:509–520. [PubMed: 12519093]
63. Spielberger, CD.; Gorusch, RL.; Lushene, R.; Vagg, PR.; Jacobs, GA. Manual for the State-Trait Anxiety Inventory (Form Y1). Consulting Psychologists Press; Palo Alto, CA: 1983.

64. Stephens M, Donnelly P. A comparison of bayesian methods for haplotype reconstruction from population genotype data. Am J Hum Genet. 2003; 73:1162–1169. [PubMed: 14574645]

65. Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. Am J Hum Genet. 2001; 68:978–989. [PubMed: 11254454]

66. Syed NH, Chen ZJ. Molecular marker genotypes, heterozygosity and genetic interactions explain heterosis in Arabidopsis thaliana. Heredity. 2004

67. Tattersfield AE, Hall IP. Are beta2-adrenoceptor polymorphisms important in asthma--an unravelling story. Lancet. 2004; 364:1464–1466. [PubMed: 15500871]

68. Thiessen BQ, Wallace SM, Blackburn JL, Wilson TW, Bergman U. Increased prescribing of antidepressants subsequent to beta-blocker therapy. Arch Intern Med. 1990; 150:2286–2290. [PubMed: 1978648]

69. Vassend O, Krogstad BS, Dahl BL. Negative affectivity, somatic complaints, and symptoms of temporomandibular disorders. J Psychosom Res. 1995; 39:889–899. [PubMed: 8636921]

70. Von Korff M, Le Resche L, Dworkin SF. First onset of common pain symptoms: a prospective study of depression as a risk factor. Pain. 1993; 55:251–258. [PubMed: 8309712]

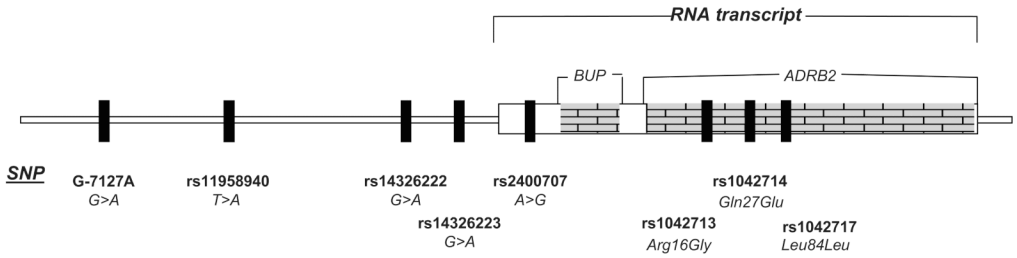
71. Ward-Routledge C, Marshall P, Marsden CA. Involvement of central alpha- and beta-adrenoceptors in the pressor response to electrical stimulation of the rostral ventrolateral medulla in rats. Br J Pharmacol. 1988; 94:609–619. [PubMed: 2840166]

72. Yap AU, Dworkin SF, Chua EK, List T, Tan KB, Tan HH. Prevalence of temporomandibular disorder subtypes, psychologic distress, and psychosocial dysfunction in Asian patients. J Orofac Pain. 2003; 17:21–28. [PubMed: 12756927]

73. Yu BH, Dimsdale JE, Mills PJ. Psychological states and lymphocyte beta-adrenergic receptor responsiveness. Neuropsychopharmacology. 1999; 21:147–152. [PubMed: 10379529]

74. Zhang HT, Huang Y, O'Donnell JM. Antagonism of the antidepressant-like effects of clenbuterol by central administration of beta-adrenergic antagonists in rats. Psychopharmacology (Berl). 2003; 170:102–107. [PubMed: 12898120]

A



B

Common haplotypes									Number of subjects	Frequency
#1	G	A	A	A	G	G	G	G	160	0.41
#2	A	T	G	G	A	A	C	G	140	0.36
#3	G	T	G	G	A	G	C	A	81	0.21
Others									5	0.013

Figure 1.

(a) Schematic diagram of *ADRB2* genomic organization, SNP positions and distribution percentages. The human *ADRB2* is an intronless gene that spans ~5,500 kb on chromosome 5q31-32. *ADRB2* transcript codes for two independent peptides showed as break blocks: β_2 adrenergic receptor protein and β_2 adrenergic receptors upstream protein (BUP) that inhibits receptor translation (Parola and Kobilka, 1994). **(b) Estimated frequencies of the *ADRB2* haplotypes.** The sequence of alleles in each haplotype reflects the order of occurrence from 5′ to 3′ in the *ADRB2* gene locus (SNPs: G-7127A, rs11958940, rs1432622, rs1432623, rs2400707, rs1042713, rs1042714 and rs1042717, respectively).

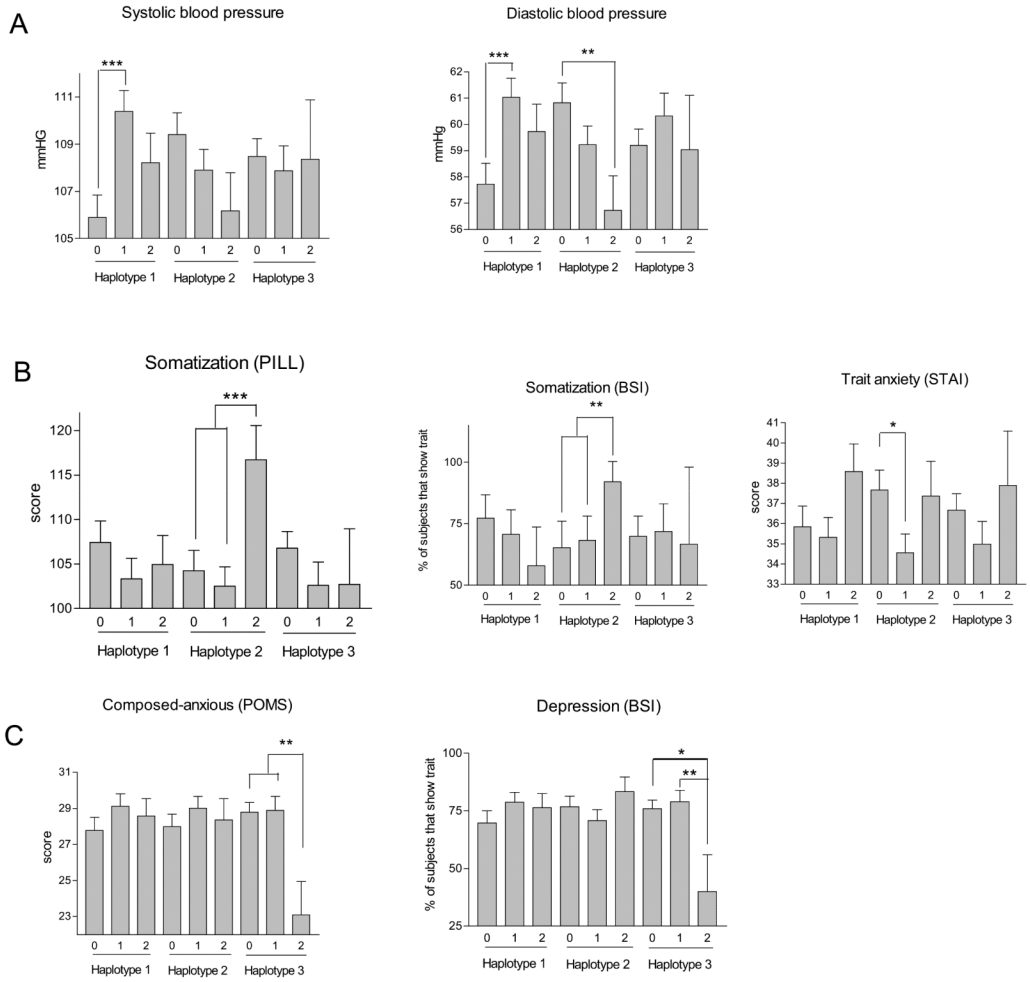


Figure 2. Psychological scores and resting arterial blood pressure categorized by six major *ADRB2* diplotypes

The major effects of six major *ADRB2* diplotypes are presented. The following diplotypes are shown: homozygotes for H1 (1,1), homozygotes for H2 (2,2), homozygotes for H3 (3,3), heterozygotes H1-H2 (1,2), H1-H3 (1,3), and H2-H3 (2,3). Each value represents the mean of each variable with associated SEM. Greater positive values for PILL, BSI and Trait Anxiety scores reflect more negative psychological characteristics. The greater values for measured obtained from the POMS scale reflect more positive psychological characteristics: composed. PILL, STAI and POMS scores were measured in relative unites, blood pressure was measured in mm of mercury (mmHg), BSI depression and somatization presented as percent of subjects that show trait (subjects, scored at 30 and corresponded to individuals that answered all questions negatively, were treated as a group that showed no signs of depression or somatization). ***P < 0.01, **P < 0.05 and *P < 0.1 different from the indicated groups.

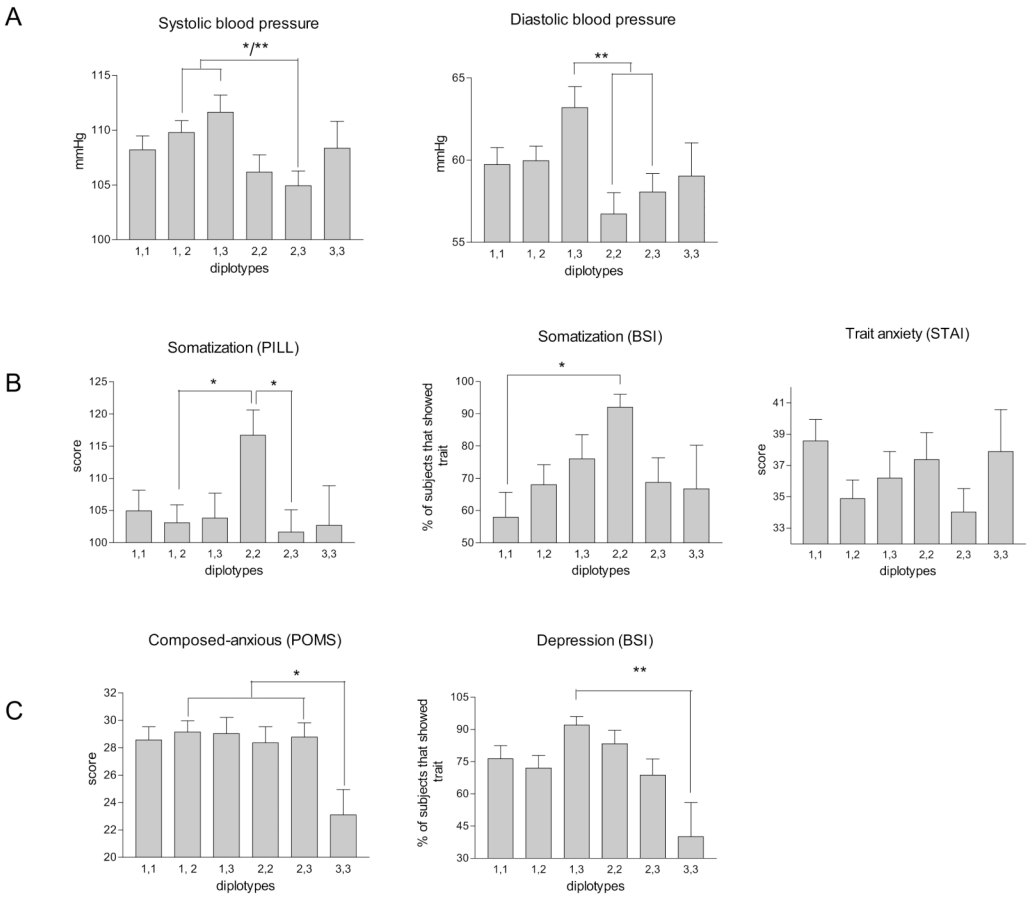


Figure 3. The effect of *ADRB2* haplotypes on psychological scores and resting arterial blood pressure

The major effects of the number of copies of haplotype 1 (A), haplotype 2 (B) or haplotype 3 (C) are presented. The following haplotype dose-effects are shown: no corresponding haplotype (0), one copy (1) or two copies (2) of the corresponding haplotype. Each value represents the mean of each variable with associated SEM. Greater positive values for PILL, BSI and Trait Anxiety scores reflect more negative psychological characteristics. The greater values for measures obtained from the POMS scale reflect more positive psychological characteristics: composed. PILL, STAI and POMS scores were measured in relative unites, blood pressure was measured in mm of mercury (mmHg), BSI depression and somatization presented as percent of subjects that show trait (subjects, scored at 30 and corresponded to individuals that answered all questions negatively, were treated as a group that showed no sings of depression or somatization). ***P < 0.01, **P < 0.05 and *P < 0.1 different from the indicated groups.

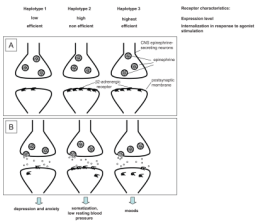


Figure 4. Proposed functional variants of ADRB2 corresponding to the three major haplotypes

Putative haplotype-specific expression of ADRB2 on the postsynaptic membrane of CNS neurons at (A) resting state and (B) following stimulation with an agonist. In the periphery, epinephrine is released from adrenal glands and binds peripheral ADRB2, for example, smooth muscle.

Table 1
Haplotypes interactions: Mean trait values

Diplotype	Mean trait value
H1/H1	γ_1
H1/H2	$\gamma_1 + \gamma_2 + \gamma_{12}$
H1/H3	$\gamma_1 + \gamma_3 + \gamma_{13}$
H2/H2	$\gamma_1 + 2\gamma_2$
H2/H3	$\gamma_1 + \gamma_2 + \gamma_3 + \gamma_{23}$
H3/H3	$\gamma_1 + 2\gamma_3$

Am J Med Genet B Neuropsychiatr Genet. Author manuscript; available in PMC 2008 October 22.

Table 2

Haplotypes interactions: linear model analysis

				Best-fit model				
Trait	**FMP***	**FAP****	**Model P val**	**Parameter**	**Parameter Estimate**	**Standard Error**	**Pval (H_0 : =0)**	**P val****
Systolic blood pressure	0.016	0.022	0.013	γ_1	108.22	1.26	0	
				γ_2	-1.39	0.96	0.150	
				γ_3	-0.81	1.15	0.484	
				γ_{12}	2.95	1.45	0.043	0.341
				γ_{13}	4.24	1.93	0.030	0.082
Diastolic blood pressure	0.012	0.066	0.005	γ_1	59.73	1.027	0	
				γ_2	-1.48	0.79	0.062	
				γ_3	-0.28	0.94	0.768	
				γ_{12}	1.71	1.18	0.151	
				γ_{13}	3.75	1.58	0.019	0.045
Somatization (PILL)	0.064	0.104	0.033	γ_1	104.94	3.04	0	
				γ_2	5.89	2.47	0.018	
				γ_3	-1.13	3.20	0.725	
				γ_{12}	-7.73	3.71	0.039	0.651
				γ_{23}	-8.05	4.66	0.086	0.862
Somatization (BSI)	0.065	0.175	0.067	γ_1	0.36	0.29	0.209	
				γ_2	0.60	0.26	0.023	
				γ_3	0.22	0.29	0.446	
Trait Anxiety (STAI)	0.213							
State Anxiety (STAI)	0.369							
Anxiety (POMS)	0.658							
Composed-anxious (POMS)	0.100	0.113	0.064	γ_1	28.83	0.86	0	
				γ_2	-0.04	0.75	0.953	
				γ_3	-2.86	1.02	0.006	
				γ_{13}	3.08	1.55	0.050	0.882

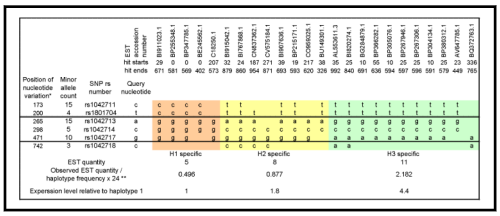
				Best-fit model				
Trait	**FMP***	**FAP****	**Model P val**	**Parameter**	**Parameter Estimate**	**Standard Error**	**Pval (H_0 : =0)**	**P val****
				γ_{23}	2.86	1.48	0.054	0.975
Depression (BSI)	0.032	0.016	0.013	γ_1	1.00	0.33	0.003	
				γ_2	0.21	0.29	0.464	
				γ_3	-0.58	0.29	0.046	
				γ_{13}	2.03	0.80	0.011	0.044
Anxiety (BSI)	0.655							

*full model P value

**P value for the full model vs additive model comparison

Table 3

Relative EST abundance



Position of nucleotide variation*	Minor allele count	SNP rs number	EST accession number	BI911023.1	BP258348.1	BP347780.1	BE348582.1	C18326.1	BI915042.1	BI767668.1	CN837362.1	CV578184.1	BI907636.1	BP215171.1	CO869228.1	BU149391.1	AL835611.3	BI830274.1	BG284479.1	BP268282.1	BP265078.1	BP267946.1	BP267306.1	BP264134.1	BP265312.1	AV647785.1	BG372783.1
			hit starts	29	0	0	0	207	32	24	187	271	39	19	217	38	35	25	10	55	10	25	25	10	25	23	336
			hit ends	671	581	569	402	573	879	860	954	871	693	593	620	326	992	840	691	636	594	597	596	591	579	449	765
			Query nucleotide																								
173	15	rs1042711	c	c	c	c	c		t	t			t	t		t	t	t	t	t	t	t	t	t	t	t	
200	4	rs1801704	t	c	c	c	c		t	t	t		t	t		t	t	t	t	t	t	t	t	t	t	t	
265	15	rs1042713	a	g	g	g	g	g	a	a	a		a	a	a	a	g	g	g	g	g	g	g	g	g	g	
296	5	rs1042714	c	g	g	g	g	g	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	
471	10	rs1042717	g	g	a	g		g	g	g	g	g	g	g	g		a	a	a	a	a	a	a	a	a		a
742	3	rs1042718	c						c	c	c	c					a	a									a
EST quantity					H1 specific					H2 specific											H3 specific					
					5					8											11					
Observed EST quantity / haplotype frequency x 24 **					0.496					0.877											2.182					
Expression level relative to haplotype 1					1					1.8											4.4					

Table 4

Table 4A: Relative risk of TMD development among ADRB2 diplotype groups based on receptor expression level.

Haplotype Combination	TMD Incidence	Groups*	Groups TMD Incidence	Group Estimated RR	95% lower bound	95% upper bound	99% lower bound	99% upper bound
H1/H1	4/38 (0.105)	Lo/Lo	4/38 (0.105)	8.08	1.2	52.2	0.815	79.7
H2/H2	5/25 (0.200)	Hi/Hi						
H3/H3	1/10 (0.100)							
H2/H3	4/32 (0.125)		10/67 (0.149)	11.3	1.95	67.9	1.38	102.1
H1/H2	1/51 (0.020)	Hi/Lo						
H1/H3	0/25 (0.000)		1/76 (0.013)	1 (reference)	NA	NA	NA	NA
All subjects	15/181 (0.083)		15/181 (0.083)					

Table 4B: Relative risk of TMD development among ADRB2 diplotype groups based on receptor internalization characteristics.

Haplotype Combination	TMD Incidence	Groups*	Groups TMD Incidence	Group Estimated RR	95% lower bound	95% upper bound	99% lower bound	99% upper bound
H2/H2	5/25 (0.200)	N/N	5/25 (0.200)	3.32	1.08	9.83	0.794	13.2
H1/H1	4/38 (0.105)	I / I						
H3/H3	1/10 (0.100)							
H1/H3	0/25 (0.000)		5/73 (0.0.68)	1.14	0.364	3.54	0.267	4.82
H2/H3	4/32 (0.125)	I/R						
H1/H2	1/51 (0.020)		5/83 (0.060)	1 (reference)	NA	NA	NA	NA
All subjects	15/181 (0.083)		15/181 (0.083)					

*H1 was considered to code for low levels of RNA expression (Lo) and H2 and H3 to code for high levels of RNA expression (Hi).

H1 and H3 were considered to code for receptor variants that rapidly internalizes in response to agonist (I) and H2 to code for receptor variant that slowly internalizes in response to agonist (N).

Am J Med Genet B Neuropsychiatr Genet. Author manuscript; available in PMC 2008 October 22.