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Gene polymorphisms of stress hormone and cytokine receptors associate with immunomodulatory profile and psychological measurement

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ABSTRACT

Objective: We sought to identify whether stable single nucleotide polymorphisms (SNPs) of various endocrine and immune molecules could be used as biomarkers associated with specific immune alterations and chronic stress measures in normal humans.

Methods: A total of 207 volunteer participants answered stress questionnaire and gave peripheral blood cells for identification of SNPs in genes coding for glucocorticoid receptor (GR), beta 2 adrenergic receptor (B2AR), interferon-gamma receptors (IFNGR1, IFNGR2), and interleukin-4 receptor (IL4R). Immunoregulatory profiles were measured by flow cytometry and genotyping assays were performed by allelic discrimination real-time PCR.

Results: Several significant differences were revealed in associations between stress marker and immune indicators based on SNP categories. For instance, Th1 levels of the minor alleles of GR TthIIII (AA) and IFNGR2 Q64R (Arg/Arg) groups were positively associated with chronic stress (PSS) ($p = 0.024$ and 0.005, respectively) compared with wild type (WT) and negatively associated with PSS in the heterozygous genotypes of GR BclI and IL4R Ile50Val ($p = 0.040$ and $p = 0.052$, respectively). Treg levels of the minor alleles of BclI (GG) and IFNGR1 T-56C (CC) groups were positively associated with PSS ($p = 0.045$ and $p = 0.010$, respectively) and negatively associated in the minor allele (Val/Val) of IL4R Ile50Va and the heterozygous genotype of IL4R Q576R ($p = 0.041$ and $p = 0.017$, respectively) compared to WT.

Conclusion: The data support the notion that gene polymorphisms from various components of the psychoneuroendocrine-immune network may be useful as biomarkers to categorize individual stress-associated immune responses.

Introduction

It is well established in human studies that the immune system changes in response to both acute and chronic psychological stress [1–3]. Stress hormones influence immune function by binding to glucocorticoid receptor (GR) and beta 2 adrenergic receptor (β2AR), which are expressed on many T helper (Th) and regulatory T cells (Treg) [4,5]. In vitro studies from us and others have documented that stress hormones, namely, glucocorticoids (GC) and catecholamines (CT), can influence immunomodulatory responses including changes in number of regulatory T cells (Treg), Th1/Th2 cytokine production, and costimulatory molecule mRNA expression [6–8]. Since stress hormone receptors including GR and β2AR are present on immune effector (Th1 and Th2) and regulatory (Treg, Tr1, and Th3) cells, corticosteroids (CS) and catecholamines (CT) will bind their receptors to alter immune responses when encountering stressful situation.

Although associations between psychological stress and altered immune responses have been extensively studied, it is unclear why individual immune responses differently significantly to the same stressor. Previous studies suggest that genetic characteristics of each individual may contribute to these differences [9,10]. SNPs of GR and β2AR have been reported to identify stress susceptibility measured by changes in control of asthma, diabetes, and cardiovascular health [11,12]. Multiple SNPs of cytokine receptors and disease activity have been reported for the endocrine and immune biomarkers including glucocorticoids, catecholamines, IFNγ, and IL-4 [13–16]. These SNPs have been associated with disease risk, response to pharmacological interventions including corticosteroids, β agonists, leukotriene modifiers, and even newer biological therapies [17]. Thus, we selected a set of SNPs from genes of stress hormone receptors and cytokine receptors associated with a known immune imbalance (asthma) similar to the imbalance that

Abbreviations: β2AR, beta 2 adrenergic receptor; GR, glucocorticoid receptor; IFNGR, interferon-γ receptor; IL4R, interleukin-4 receptor; PBMC, peripheral blood mononuclear cells; PSS, Perceived Stress Scale; SNP, single nucleotide polymorphism.

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we and others have previously demonstrated to be associated with stress. These were used as representative SNPs in a proof of concept study. We recently reported associations between single nucleotide polymorphisms (SNPs) of stress hormone receptor and cytokine receptors and immune parameters in normal human subjects [18,19]. For example, Th2 levels were significantly higher in the Arg/Arg group (minor allele) of β2AR Gly16Arg SNP compared to wild-type (WT) and Th1 was higher in Glu/Glu group of β2AR Gln27Glu SNP compared to WT [18]. For interferon-γ receptor 1 (IFNGR1), Th1 levels were significantly higher in the heterozygous (CT) of SNP T-56C compared to WT [19]. In addition, previous studies showed that the minor allele (CC) of IFNGR1 T-56C was associated with high susceptibility to *H. pylori* infection, protection against severe malaria, and pulmonary tuberculosis [20–22]. It was also reported that SNP Q64R of IFNGR2 is a fully functional polymorphism in an *in vitro* study [23] and associated with total IgE levels [24].

We aimed to investigate in a secondary analysis of a previously published pilot study (18) whether genetic variants in various receptors of stress hormone and cytokine in the stress-immune network may have potential as biomarkers for the associations between psychological stress and immune parameters in normal humans. Peripheral blood samples from 207 normal participants were used in genotyping assays for GR, β 2AR, IFNGR1, IFNGR2, and interleukin-4 receptor (IL4R) genes. Immunomodulatory profiles from PBMC and psychological questionnaires were collected and segregated by genotype. We hypothesized that various SNPs of stress hormone receptor and/or cytokine receptor would have distinct relationships between immune and stress measures. If distinct associations between stress and immune measures could be categorized by specific SNP patterns, this could provide the needed evidence to expand this line of investigation of both number of subjects and number of candidate genes, and top prospectively predict responses to experimental and natural stressors.

Materials and methods

Human subjects

The current study is a secondary analysis of a study investigating stress hormone receptor polymorphisms (18). A total of 207 healthy volunteers were recruited after obtaining written informed consent according to a University of Mississippi Medical Center IRB-approved protocol. Subjects were excluded for any past medical history of smoking, psychiatric illnesses, cardiovascular diseases, autoimmune diseases, or recent medical use of glucocorticoids, catecholamines, and/or adrenergic antagonists.

Psychological measurement

To measure levels of perceived stress and anxiety, participants completed self-report psychological questionnaires at the study visit. Stress perception was measured using the 10-item Perceived Stress Scale (PSS), which includes ratings of feeling overwhelmed, out of control, and stressed, over the previous month, and has been extensively validated [25]. Respondents rated how often they felt or thought a certain way ranging from 0 (never) to 5 (very often). Normal values are based on an L. Harris Poll that gathered information using the PSS-10 with 2387 respondents in the United States [26].

Blood collection and PBMC isolation

Venous blood was collected into heparinized tubes for peripheral blood mononuclear cells (PBMC) isolation and into EDTA-coated tubes for genetic assessment from all subjects between 8:00 and 10:00 AM to control for diurnal variation. No participant had eaten within 2 h of having the blood sample collected. PBMC were isolated using a Ficoll-Hypaque gradient as previously described [27]. Briefly, cells were centrifuged at 650 × *g* for 30 min, washed twice in HBSS, and then resuspended in 1 ml cRPMI supplemented with 5% FBS. Cell counts were obtained using an automated hematology analyzer (Coulter).

Flow cytometry

T cell populations in PBMC were analyzed by flow cytometry according to previously described methods [28]. Briefly, 1×10^6 PBMC were incubated with the following surface antibodies: CD3-APC, CD4- or CD8-PerCP, or CD25-PE. Cells were then washed, fixed with 4% paraformaldehyde (PFA) for 10 min at RT, followed by an additional wash. Fixed cells were then permeabilized with 0.5% saponin in PBS (PERM) and incubated with antibodies for the following intracellular antibodies: FoxP3-FITC, IL10-FITC, or TGFβ-PE. For the detection of IFNγ and IL4, cells were first stimulated with 10 ng/ml phorbol myristate acetate (PMA), 1 μg/ml ionomycin, and 3 μM monensin for 4 h at 37 ° C and 5% CO_2. Stimulated cells were then fixed with 4% PFA for 10 min at RT, washed, permeabilized as described above, and incubated with CD3-APC, CD8-Per-CP, IL4-PE, and IFNγ-FITC for 1 h at RT. Appropriate isotype controls were used to define positive and negative staining during the initial setup of the T cell profiles of interest. Data were collected using the BD FACSCalibur flow cytometer and analyzed with the CellQuest software. Cell populations were defined as follows: Th1, $CD3^+CD8^-IFN\gamma^+IL4^-$; Th2, $CD3^+CD8^-IFN\gamma^-IL4^+$; Treg, $CD3^+CD4^+CD25^{hi}FoxP3^+$; Tr1, $CD3^+CD8^-IL10^+$; Th3, $CD3^+CD8^-TGF\beta^+$. Peripheral blood mononuclear cells were gated using forward and side scatter. For Th1, Th2, Tr1, and Th3 populations, IFNγ, IL4, IL10, and TGFβ concentrations, respectively, were quantified from the PBMCs gated for $CD3^+CD8^-$. For the Treg cells, the percentage of FoxP3 + cells in the CD4 + population are reported.

Genomic DNA preparation and genotyping assay

DNA was extracted from whole blood sample using commercial Gentra Puregene Blood Kit (QIAGEN, Valencia, CA, USA), and 20 ng of DNA was used in each assay. Genotyping assays for GR, β2AR, IFNGR1, IFNGR2, and IL4R were performed by allelic discrimination real-time PCR using Applied Biosystems 7300 Real-Time PCR System with TaqMan SNP assay reagents and genotyping analyzed by SDS 2.1 package (Applied Biosystems, Foster City, CA). Pre-designed primer/probe sets were available from Applied Biosystems (Table 1).

Statistical analysis

Multiple linear regressions was used to test main effects and interaction between outcome (Th1, Th2, Th1/Th2, Th3,Treg, and Tr1) and psychological stress (PSS) for levels of genotype compared to wild type for each SNP, reporting difference of slopes (Δβ) and 95% confident intervals also plots were generated depicting those differences. Models were adjusted for age, gender, BMI, and race. Huber–White robust standard errors were computed. Analyses were performed after log transformations were applied to skewed data (Th1/Th2, Treg, Tr1, and Th3) to satisfy models assumptions. Two subjects with the highest Th1/Th2 ratio scores (97.48 and 78.5) and one individual with the lowest Tr1 (0.04) were found. Sensitivity analysis examining potentially influential data point found similar qualitative results.

Heat map was generated using slopes (β) from the same models to describe the relationship between outcomes and psychological stress (PSS) for each level of genotype. A p-value of <0.05 was deemed statistically significant. Statistical analysis was performed using STATA (version 13; StataCorp, College Station, TX, USA).