Change in mRNA Expression after Atenolol, a Beta-adrenergic Receptor Antagonist and Association with Pharmacological Response

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ABSTRACT

Aims. Genetic determinants of variability in response to β-blockers are poorly characterized. We defined changes in mRNA expression after a β-blocker to identify novel genes that could affect response and correlated these with inhibition of exercise-induced tachycardia, a measure of β-blocker sensitivity.

Methods. Nine subjects exercised before and after a single oral dose of 25mg atenolol and mRNA gene expression was measured using an Affymetrix GeneChip Human Gene 1.0 ST Array. The area under the heart rate-exercise intensity curve (AUC) was calculated for each subject; the difference between post- and pre-atenolol AUCs (∆AUC), a measure of β-blocker response, was correlated with the fold-change in mRNA expression of the genes that changed more than 1.3-fold.

Results. Fifty genes showed more than 1.3-fold increase in expression; 9 of these reached statistical significance (P < 0.05). Thirty-six genes had more than 1.3-fold decrease in expression after atenolol; 6 of these reached statistical significance (P < 0.05). Change in mRNA expression of FGFBP2 and Probeset ID 8118979 was significantly correlated with atenolol response (P = 0.03 and 0.02, respectively).

Conclusion. The expression of several genes not previously identified as part of the adrenergic signaling pathway changed in response to a single oral dose of atenolol. Variation in these genes could contribute to unexplained differences in response to β-blockers.

Key Words. Atenolol; mRNA expression; Microarray

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Introduction

Beta-blockers are frequently prescribed to treat ischemic heart disease, heart failure, hypertension, and arrhythmias [1–5]. They block the effects of agonists acting on β-adrenergic receptors (ARs) and influence downstream signaling pathways. There are substantial interindividual and ethnic differences in response to β-blockers that are partly accounted for by variation in the genes encoding the β1-AR (ADRB1) and mediators of downstream signaling pathways [6,7]. The relationship between variability in ADRB1 and other candidate genes and variability in response to β-blockers has been extensively evaluated [6,8], but much of the variability remains unexplained.

Another approach to identifying the mechanisms underlying interindividual variability in response would be to identify additional candidate
genes by examining the changes in messenger RNA (mRNA) that occur after exposure to a β-blocker. There is no information regarding such an approach.

Therefore, we carried out this exploratory study to identify novel genes that may regulate response to a β-blocker by measuring changes in mRNA expression after oral administration of a β-blocker (atenolol) to subjects who underwent an exercise test to determine β-blocker sensitivity. We also studied the correlation between change in mRNA expression after atenolol administration and β-blocker sensitivity, assessed by attenuation of exercise-induced tachycardia [9].

Methods

Subjects

This study was approved by the Institutional Review Board of Vanderbilt University Medical Centre, Nashville, TN, and all subjects gave written informed consent. We enrolled 9 unrelated subjects; one subject was excluded from analysis because the post-treatment sample could not be hybridized. Subjects were eligible to participate if they were between 18–40 years of age and had no clinically significant abnormality based on medical history, physical examination, electrocardiogram, and routine laboratory testing. Subjects reported their ethnicity and that of their parents and grandparents using checkboxes to choose among “Caucasian”, “African–American”, “Hispanic”, “Chinese”, “Japanese”, and “other” (the latter to be specified). Multiple choices were permitted. A patient was assigned to an ethnic group when both parents and at least three out of four grandparents were of the same ethnicity. Patients were free of medications and dietary supplements for at least 1 week and received a controlled alcohol-free and caffeine-free diet (providing 150 mmol of sodium, 70 mmol of potassium, and 600 mmol of calcium daily) for 5 days before the study.

Protocol

Details of the exercise protocol have been described in detail elsewhere [6]. Briefly, after an overnight fast a 20 G intravenous cannula was inserted into an antecubital arm vein for blood sampling and after 30 minutes of supine rest, a baseline blood sample was drawn for mRNA analysis into two PAXgene Blood mRNA Tubes (PreAnalytiX/Qiagen Inc., Valencia, CA), incubated at room temperature for 2 hours, and then stored at −20°C. Then, subjects exercised on an electronically braked supine bicycle ergometer at sequentially increasing workloads of 25, 50, and 75 watts for 2 minutes each. Then, 10 minutes after completion of exercise, subjects swallowed a 25 mg tablet of atenolol. A second blood sample for mRNA expression was collected 2.5 hours after atenolol (to coincide with peak atenolol concentrations) and immediately after the blood draw a second exercise test was performed as per previously described protocol.

mRNA

Total mRNA was extracted from whole blood using PAXgene Blood mRNA Kit (Qiagen, Valencia, CA) and then subjected to DNase treatment according to the manufacturer’s instructions (Qiagen, Valencia, CA). The mRNA were assessed for concentration by spectrophotometry and integrity using the Agilent Bioanalyzer (Agilent Technologies, Palo Alto, CA), and then stored at −20°C.

Microarray

Following quality control, the mRNA was prepared for microarray analysis using the GeneChip Whole Transcript (WT) Sense Target Labeling Assay protocol (Affymetrix Inc, Santa Clara, CA). Briefly, a total of 100ng of total mRNA was reverse transcribed to cDNA T7-random primers followed by second-strand synthesis. The double-stranded cDNA was then used as template in an in vitro transcription reaction followed by cDNA synthesis, fragmentation of the single stranded cDNA and labeling through a terminal deoxytransferase reaction. The biotinylated cDNA (5 μg) was fragmented and hybridized to an Affymetrix GeneChip Human Gene 1.0 ST Array (Affymetrix Inc, Santa Clara, CA).

Data Analysis

Following scanning, CEL files were imported into Partek Genomic Suitev6.4 (Partek Inc, St Louis, MO) and robust multi-chip average (RMA) normalized. A paired-sample t-test was performed between the pre-treatment and post-treatment groups. A 1.3-fold change in gene expression was considered potentially significant [10].

Demographic data are expressed as mean ± standard deviation (SD). We used two-sample Wilcoxon rank-sum (Mann-Whitney) test to compare outcomes before and after atenolol. A response-feature approach was used to model multiple heart rate measurements in the same subjects.
In these analyses, the response feature was the area under the heart rate-exercise intensity curve (AUC) for each subject. The difference between post- and pre-atenolol heart rate AUCs (ΔAUC) was calculated to determine the response to atenolol and this was correlated with the fold-change in mRNA expression of the genes that were significantly upregulated and downregulated (≥1.3-fold) using a non-parametric measure of correlation (Spearman’s rank correlation coefficient). Analyses were performed using the statistical software STATA v.10.0 (StataCorp, College Station, TX) and Partek (Partek Inc, St Louis, MO).

Results

Subjects

The demographic characteristics of the study subjects (n = 8) are described in Table 1.

| Characteristic (N = 8) | Mean ± SD  |
|-----------------------|------------|
| Age (years)           | 28.3 ± 6.3 |
| Sex (Male/Female)     | 5/3        |
| Ethnicity: Caucasian/African American/Hispanic/Asian | 2/1/1/4 |
| Weight (kg)           | 70.5 ± 12.0 |
| Height (m)            | 1.72 ± 0.08 |

Atenolol Effect

Atenolol significantly reduced the resting heart rate (mean reduction = 6.5 ± 6.8 beats/minute; \( P = 0.02 \)), and heart rate at all the exercise stages (mean reduction = 13 ± 10.2, 13.1 ± 9.9, 19.0 ± 9.5 beats/minute at 25, 50, and 75W of exercise, respectively; all \( P < 0.02 \)). Heart rate-AUC was also significantly reduced (mean reduction = 1050 ± 658 beats/minute.watt; \( P = 0.02 \)).

Microarray

There were 50 genes upregulated more than 1.3-fold (Table 2). Change in mRNA expression for 9 of these genes (TXN, SLC04C1, LOC339240, SNRPN, CLEC2B, SNORA49 and Probeset IDs 8142763, 7984008, 7906751) reached statistical significance (\( P < 0.05 \)) (Figure 1). A range of other genes including WD repeat domain 74, small cajal body-specific mRNA 7, killer cell lectin-like receptor subfamily F, S100 calcium binding protein A12, and fibroblast growth factor binding protein 2 were also upregulated. Change in mRNA expression for fibroblast growth factor binding protein 2 (FGFBP2) correlated significantly with atenolol effect (ΔAUC) (Spearman coefficient = −0.76; \( P = 0.03 \)).

Thirty-six genes were downregulated at least 1.3-fold after atenolol administration (Table 3); the decrease in mRNA expression for 6 of these
| Genes upregulated more than 1.3-fold |
|------------------------------------|
| Gene symbol | Gene title | Ref seq ID | Function | Fold change ± SE | P value* | Spearman’s rho | P value** |
|-------------|------------|------------|----------|------------------|----------|----------------|----------|
| RPL34       | Ribosomal Protein L34 (4q25) | CR542242 | Ribosomal protein: component of the 60S subunit. | 1.52 ± 1.39 | 0.25 | −0.45 | 0.26 |
| WDR74       | WD repeat domain 74 (11q12.3) | AK29330 | | 1.50 ± 1.2 | 0.06 | 0.05 | 0.91 |
| SCARNA7     | Small Cajal body-specific RNA 7 (3q25.22) | NR_003001 | | 1.48 ± 1.34 | 0.22 | −0.31 | 0.45 |
| SNRPN       | Small Nuclear Ribonucleoprotein polypeptide N (15q11.2) | NR_003239 | Role in pre-mRNA processing, possibly tissue-specific alternative splicing events. | 1.47 ± 1.19 | 0.07 | −0.24 | 0.56 |
| TXN         | Thioredoxin (9q31) | NM_003299 | Role in pre-mRNA processing, possibly tissue-specific alternative splicing events. | 1.47 ± 1.16 | 0.04* | −0.05 | 0.91 |
| SNRPN       | Small Nuclear Ribonucleoprotein Polypeptide N (15q11.2) | NR_003318 | | 1.47 ± 1.22 | 0.09 | −0.48 | 0.22 |
| RPS3A       | Ribosomal Protein S3A (4q31.2-q31.3) | NM_001006 | Ribosomal protein: component of the 40S subunit. | 1.45 ± 1.31 | 0.21 | −0.24 | 0.57 |
| hCG_1983332 | hCG1983332 (7q11.21) | NR_003536 | Ribosomal protein: component of the 40S subunit. | 1.45 ± 1.18 | 0.06 | −0.39 | 0.33 |
| 8144569     | Similar to Large Subunit Ribosomal Protein L36a (12p13.31) | NM_001101383 | | 1.45 ± 1.22 | 0.10 | 0.28 | 0.49 |
| RPL26       | Ribosomal Protein L26 (17p13) | NM_000987 | Ribosomal protein: component of the 60S subunit. | 1.45 ± 1.43 | 0.33 | −0.31 | 0.45 |
| KLRF1       | Killer Cell Lectin-like Receptor Subfamily F, member 1 (12p13.31) | NM_016523 | | 1.45 ± 1.31 | 0.21 | −0.24 | 0.57 |
| 8142763     | Ribosomal Protein L9 (4p13) | NM_001024921 | Ribosomal protein: component of the 60S subunit. | 1.41 ± 1.13 | 0.03* | 0.28 | 0.49 |
| RPL9        | Ribosomal Protein L9 (4p13) | NM_000661 | Ribosomal protein: component of the 60S subunit. | 1.41 ± 1.31 | 0.24 | −0.19 | 0.65 |
| S100A12     | S100 Calcium Binding Protein A12 (1q21) | NM_005621 | Involved in the regulation of cell cycle progression, differentiation and calcium-dependent signal transduction pathways. | 1.41 ± 1.25 | 0.16 | 0.02 | 0.95 |
| SNRPN       | Small Nuclear Ribonucleoprotein Polypeptide N (15q11.2) | NR_003316 | Pre-mRNA processing, possibly tissue-specific alternative splicing events. | 1.41 ± 1.19 | 0.10 | −0.36 | 0.38 |
| LSM3        | LSM3 Homolog, U6 Small Nuclear mRNA associated (S. cerevisia) | NM_014463 | Pre-mRNA splicing. | 1.40 ± 1.22 | 0.13 | −0.36 | 0.38 |
| 7984008     | Fibroblast Growth Factor Binding Protein 2 (4p16) | NM_031950 | Secreted by cytotoxic lymphocytes and involved in cytotoxic lymphocyte-mediated immunity. | 1.40 ± 1.13 | 0.03* | −0.26 | 0.53 |
| FGFBP2      | Similar to Large Subunit Ribosomal Protein L36a (12p13.31) | NM_001101383 | | 1.40 ± 1.16 | 0.06 | −0.76 | 0.03** |
| hCG_1787519 | Similar to Large Subunit Ribosomal Protein L36a (12p13.31) | NM_004387 | | 1.39 ± 1.16 | 0.06 | −0.48 | 0.23 |
| SLC04C1     | Solute Carrier Organic Anion Transporter Family, member (5q21.2) | NM_180991 | | 1.39 ± 1.10 | 0.01* | −0.14 | 0.73 |
| LOC339240   | Keratin Pseudogene (17p11.2) | ENST00000332088 | Involved in the regulation of cell cycle progression and differentiation. | 1.39 ± 1.11 | 0.02* | −0.31 | 0.45 |
| S100A8      | S100 Calcium Binding Protein A8 (1q21) | NM_002964 | Type II membrane protein. | 1.39 ± 1.26 | 0.20 | −0.38 | 0.35 |
| KLRB1       | Killer Cell Lectin-Like Receptor Subfamily B, member 1 (12p13.31) | NM_002258 | | 1.39 ± 1.20 | 0.11 | −0.45 | 0.26 |
| Gene            | Description                                                                 | Probeset ID | Fold Change | P-value 1 | P-value 2 | 2-tailed P-value |
|----------------|------------------------------------------------------------------------------|-------------|-------------|-----------|-----------|-----------------|
| SNRPN          | Small Nuclear Ribonucleoprotein Polypeptide N (15q11.2)                      | NR_003330   | 1.38 ± 1.12 | 0.02*     | 0.52      | 0.17            |
| CLEC2B         | C-type Lectin Domain Family 2, member B (12p13.31)                           | NM_005127   | 1.37 ± 1.09 | 0.007*    | 0.04      | 0.91            |
| 8102728        | Chromosome 15 Open Reading Frame 15 (15q21)                                 | NM_016304   | 1.37 ± 1.31 | 0.28      | 0.57      | 0.14            |
| 8107940        | Hypothetical Protein BC007307 (19q13.41)                                     | ENST00000357015 | 1.31 ± 1.13 | 0.07      | 0.31      | 0.45            |
| SNORD28        | Small Nuclear RNA, C/D box 28 (11q13)                                       | NR_002562   | 1.35 ± 1.27 | 0.25      | 0.12      | 0.78            |
| SNORD29        | Ribosomal Protein L17-Like (15q11.2)                                        | NM_006144   | 1.35 ± 1.20 | 0.14      | 0.19      | 0.65            |
| SNORD30        | Ribosomal Protein L36a-Like (14q21)                                         | NM_001001   | 1.35 ± 1.15 | 0.07      | 0.55      | 0.16            |
| SNORD31        | Ribosomal Protein L17-Like (15q21)                                         | NM_001093733 | 1.34 ± 1.31 | 0.31      | 0.24      | 0.57            |
| SNORD32        | Ribosomal Protein L36a-Like (14q21)                                         | NM_001001   | 1.33 ± 1.14 | 0.30      | 0.24      | 0.57            |
| SF3B14         | Splicing Factor 3B, 14 kDa subunit (2p23.3)                                 | NM_016047   | 1.31 ± 1.33 | 0.12      | 0.02      | 0.95            |
| 8103222        | Ribosomal Protein L24 (3q12)                                                | NM_000986   | 1.31 ± 1.30 | 0.35      | 0.24      | 0.57            |
| 8103070        | Ribosomal Protein L36a-Like (14q21)                                         | NM_001001   | 1.30 ± 1.17 | 0.05*     | 0.09      | 0.82            |

*P value represents the change in mRNA comparing before and after atenolol; **P value represents the significance of Spearman's correlation coefficient examining the association between fold-change in mRNA expression and attenuation of exercise-induced tachycardia by atenolol.
Table 3  Genes downregulated more than 1.3-fold

| Gene symbol | Gene title                                      | Ref seq ID | Function                                                                                     | Fold change ± SE | P value* | Spearman’s Rho | P value** |
|-------------|-------------------------------------------------|------------|--------------------------------------------------------------------------------------------|------------------|----------|-----------------|----------|
| SLC4A1      | Solute Carrier Family 4, Anion Exchanger, Member 1 (17q21.31) | NM_000342  | Erythrocyte chloride/bicarbonate exchanger involved in carbon dioxide transport.               | −1.53 ± 1.29     | 0.14     | −0.12           | 0.78     |
| HBZ         | Hemoglobin, Zeta (16p13.3)                       | NM_005332  | Alpha-like hemoglobin. Synthesized in the yolk sac of the early embryo.                       | −1.53 ± 1.26     | 0.11     | −0.53           | 0.18     |
| FKBP5       | FK506 Binding Protein 5 (6p21.3-p21.2)           | NM_004117  | Role in immunoregulation and calcineurin inhibition.                                          | −1.51 ± 1.16     | 0.03*    | 0.17            | 0.69     |
| 7996260 (Probeset ID) |                                                |            |                                                                                             | −1.51 ± 1.17     | 0.04*    | −0.29           | 0.49     |
| 8169638 (Probeset ID) |                                                |            |                                                                                             | −1.48 ± 1.24     | 0.11     | −0.43           | 0.28     |
| TRIM58      | Tripartite Motif-Containing 58 (1q44)            | NM_015431  |                                                                                             | −1.47 ± 1.22     | 0.09     | −0.24           | 0.56     |
| SELENBP1    | Selenium Binding Protein 1 (1q21-q22)            | NM_003944  | Selenium-binding protein family.                                                             | −1.45 ± 1.25     | 0.14     | −0.2            | 0.64     |
| ALAS2       | Delta-Aminolevulinate Synthase 2 (Xp11.21)       | NM_00032   | Erythroid-specific mitochondrially located enzyme.                                           | −1.41 ± 1.26     | 0.18     | −0.19           | 0.64     |
| GMPR        | Guanosine Monophosphate Reductase (6p23)         | NM_006877  | Catalyzes the irreversible NADPH-dependent reductive deamination of guanosine monophosphate (GMP) to inosine monophosphate (IMP). | −1.40 ± 1.20     | 0.11     | −0.19           | 0.64     |
| EPB49       | Erythrocyte Membrane Protein band 4.9 (dematin) (8p21.1) | NM_001978  |                                                                                             | −1.40 ± 1.27     | 0.20     | −0.21           | 0.61     |
| SLC38A5     | Solute Carrier Family 38, member 5 (Xp11.23)      | NM_03518   | Mediates Na(+)−coupled transport of neutral amino acids.                                     | −1.40 ± 1.25     | 0.18     | −0.43           | 0.28     |
| ALS2CR2     | Amyotrophic Lateral Sclerosis 2 (juvenile) Chromosome region (2q33.1) | NM_018571  |                                                                                             | −1.38 ± 1.28     | 0.23     | −0.2            | 0.64     |
| SNCA        | Alpha Synuclein (non A4 component of amyloid precursor) (4q22.1) | NM_000345  | Inhibits phospholipase D2.                                                                  | −1.38 ± 1.26     | 0.20     | −0.19           | 0.65     |
| SLC6A8      | Solute Carrier Family 6 (neurotransmitter transporter) (Xq28) | NM_005629  | Transports creatine into and out of cells.                                                   | −1.37 ± 1.12     | 0.03*    | −0.38           | 0.34     |
| OR2W3       | Olfactory Receptor, Family 2, Subfamily W, Member 3 (1q44) | NM_001001957 |                                                                                             | −1.36 ± 1.17     | 0.09     | −0.41           | 0.31     |
| Gene Name | Description | Probeset ID | Change Over Baseline | P-value | Fold Change | Fold Change | Fold Change | Fold Change |
|-----------|-------------|-------------|-----------------------|---------|-------------|-------------|-------------|-------------|
| C16orf35  | Chromosome 16 Open Reading Frame 35 (6p13.3) | NM_001077350 | -1.36 ± 1.22          | 0.17    | -0.55       | 0.15        |
| GYP/C     | Glycophorin C (Gerbich blood group) (2q14-q21) | NM_002101 | -1.36 ± 1.21          | 0.15    | 0.77        | 0.02**      |
| CS/DA     | Cold Shock Domain Protein A (12p13.1) | NM_003651 | -1.35 ± 1.24          | 0.21    | -0.40       | 0.32        |
| SLC25A39  | Solute Carrier Family 25, Member 39 (17q12) | NM_016016 | -1.34 ± 1.24          | 0.21    | -0.34       | 0.41        |
| WDR40A    | WD Repeat Domain 40A (9p13.3) | NM_015397 | -1.34 ± 1.22          | 0.19    | -0.19       | 0.65        |
| SLC25A39  | Solute Carrier Family 25, Member 39 (17q12) | NM_138578 | -1.34 ± 1.22          | 0.19    | -0.19       | 0.65        |
| GYPA      | Glycophorin A (MNS blood group) (4q28.2-q31.1) | NM_002099 | -1.33 ± 1.18          | 0.13    | -0.14       | 0.73        |
| TMOD1     | Tropomodulin 1 (9q22.3) | NM_003275 | -1.33 ± 1.25          | 0.25    | 0.13        | 0.76        |
| TMEM63B   | Transmembrane Protein 63B (6p21.1) | NM_0018426 | -1.33 ± 1.34          | 0.37    | 0.62        | 0.1         |
| PDZK1I/P1 | PDZK1 Interacting Protein 1 (1p33) | NM_005764 | -1.32 ± 1.21          | 0.18    | -0.34       | 0.41        |
| GYPA      | Glycophorin A (MNS blood group) (4q28.2-q31.1) | NM_002099 | -1.32 ± 1.16          | 0.11    | 0.31        | 0.45        |
| PDZK1I/P1 | PDZK1 Interacting Protein 1 (1p33) | NM_005764 | -1.32 ± 1.14          | 0.08    | 0.05        | 0.90        |
| TMOD1     | Tropomodulin 1 (9q22.3) | NM_003275 | -1.31 ± 1.30          | 0.32    | -0.02       | 0.95        |

*P-value represents the change in mRNA comparing before and after atenolol; **P-value represents the significance of Spearman’s correlation coefficient examining the association between fold-change in mRNA expression and attenuation of exercise-induced tachycardia by atenolol.
Thirty-six genes are downregulated at least 1.3-fold after atenolol administration, and the decrease in mRNA expression for 6 of these genes (FKBP5, SLC6A8 and Probeset IDs 7996260, 8084810, 8003857, 8125461) is statistically significant. These genes can be categorized into 3 broad ontogenic groups based on the proteins that they code: transport proteins, ion channels and cytoskeletal proteins. FKBPs codes for a chaperone protein that has been implicated in stress related disorders [16,17]. The other downregulated genes code for a wide variety of proteins like solute carrier family 4, anion exchanger, hemoglobin zeta, tripartite motif-containing 58, and δ-aminolevulinate synthase 2. As is the case with the upregulated genes, the role of these downregulated genes in adrenergic signaling is not known.

 change in mRNA expression of Probeset ID 8118979 is correlated significantly with attenuation of exercise-induced tachycardia.

Discussion

There is no information about the effect of a β-blocker on mRNA expression, thus our finding that several genes are upregulated or downregulated are of interest. There are 50 genes upregulated more than 1.3-fold, and change in mRNA expression for 9 of these genes is statistically significant (P < 0.05). Many of these genes code for ribosomal proteins, small nuclear ribonucleoprotein polypeptides and signal transduction pathways that have not previously been associated with β-blocker signaling. One of the significantly upregulated genes (TXN) codes for thioredoxin. Thioredoxins act as antioxidants by facilitating the reduction of other proteins by cysteine thiol-disulfide exchange [12,13]. The thioredoxins are kept in the reduced state by the flavoenzyme thioredoxin reductase, in a NADPH-dependent reaction [14]. Thioredoxin reductase activity is indirectly regulated by β2-ARs in human cutaneous tissue; its activity in human melanoma cells is stimulated by calcium, and calcium exchange between these cells and surrounding skin is stimulated by β2-ARs [15]. Another upregulated gene, SLC04C1, codes for an organic anion transporter that is expressed on the basolateral membrane of renal proximal tubular cells. It transports cardiac glycosides, thyroid hormone, cAMP, and methotrexate in a sodium-independent manner [16]. However, no clear role has been identified for these significantly upregulated genes in the adrenergic signaling pathway.

Similarly, no clear role has been identified in adrenergic signaling for other upregulated genes such as WD repeat domain 74, small cajal bodiespecific mRNA 7, killer cell lectin-like receptor subfamily F, S100 calcium binding protein A12, fibroblast growth factor binding protein 2, solute carrier organic anion transporter family, and keratin pseudogene.

This study has several limitations. Subjects exercised before atenolol was administered, and the effect of atenolol on mRNA expression could potentially have been altered by the preceding exercise. However, the majority of genes that changed more than 1.3-fold were not influenced by exercise in previous studies [20–25]. Another potential limitation is that we used whole blood for analysis of mRNA expression, and changes in mRNA expression can be lower than those obtained from isolated cells [26,27]. We studied change in mRNA expression 2.5 hours after administration of a single dose of atenolol, when peak atenolol concentrations are reached. The pattern of gene expression after atenolol may vary with time, and after chronic administration of drug. However, ethnic and genetic differences in sensitivity to atenolol measured as inhibition of exercise-induced tachycardia can be detected 2.5 hours after a single dose [6]. Therefore, the gene
expression profile at this time is of interest. We administered a single dose of atenolol (25 mg) to our subjects. It is possible that the change in the pattern of mRNA expression with higher doses or with multiple doses may differ. Also, we did not correct for multiple comparisons but included only those genes that were upregulated or downregulated more than 1.3 fold to limit the false discovery rate; there is little consensus on the optimum method of correction for multiple comparisons in gene expression assays [28], and the analysis should be regarded as exploratory and hypothesis-generating.

In conclusion, in this preliminary study many genes not known to be involved with adrenergic signaling were upregulated or downregulated in response to atenolol. Change in mRNA expression for 2 of these genes is significantly correlated with atenolol-mediated attenuation of exercise-induced tachycardia. Additional studies to determine the reproducibility of the findings and the effects of chronic therapy may provide novel insights into the mechanisms of actions of \( \beta \)-blockers.

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