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Novel plasma biomarker of atenolol-induced hyperglycemia identified through a metabolomics-genomics integrative approach

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Abstract

Introduction—While atenolol is an effective antihypertensive agent, its use is also associated with adverse events including hyperglycemia and incident diabetes that may offset the benefits of blood pressure lowering. By combining metabolomic and genomic data acquired from hypertensive individuals treated with atenolol, it may be possible to better understand the pathways that most impact the development of an adverse glycemic state.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval The research outlined in this manuscript stems from human subjects research. As indicated, the research protocol was approved by the Institutional Review Board at all of the enrolling sites, and all patients provided written, voluntary informed consent prior to participation in any research procedures.

Objective—To identify biomarkers that can help predict susceptibility to blood glucose excursions during exposure to atenolol.

Methods—Plasma samples acquired from 234 Caucasian participants treated with atenolol in the Pharmacogenomic Evaluation of Antihypertensive Responses trial were analyzed by gas chromatography Time-Of-Flight Mass Spectroscopy. Metabolomics and genomics data were integrated by first correlating participant's metabolomic profiles to change in glucose after treatment with atenolol, and then incorporating genotype information from genes involved in metabolite pathways associated with glucose response.

Results—Our findings indicate that the baseline level of β-alanine was associated with glucose change after treatment with atenolol (Q = 0.007, β = 2.97 mg/dL). Analysis of genomic data revealed that carriers of the G allele for SNP rs2669429 in gene *DPYS*, which codes for dihydropyrimidinase, an enzyme involved in β-alanine formation, had significantly higher glucose levels after treatment with atenolol when compared with non-carriers (Q = 0.05, β = 2.76 mg/dL). This finding was replicated in participants who received atenolol as an add-on therapy (P = 0.04, β = 1.86 mg/dL).

Conclusion—These results suggest that β-alanine and rs2669429 may be predictors of atenolol-induced hyperglycemia in Caucasian individuals and further investigation is warranted.

Keywords

Pharmacometabolomics; Pharmacogenomics; β-blockers; Atenolol; Hyperglycemia; β-alanine

1 Introduction

Hypertension is a global health concern that affects an increasingly high percentage of the worldwide population and is predicted to affect around 1.5 billion people by 2025 (Kearney et al. 2005). The use of β-blockers as a first-line treatment option for uncomplicated hypertension has decreased in part due to the association of this drug class with incidence of stroke and new-onset diabetes (Fathallah et al. 2015; Lindholm et al. 2005). Nonetheless, β-blockers remain among the most prescribed drugs in the United States, surpassing 160 million prescriptions per year (IMS Institute for Healthcare Informatics 2012), and are a preferred choice for hypertensive patients with ischemic heart disease and heart failure (Akbar and Alorainy 2014; O'Gara et al. 2013; Yancy et al. 2013).

The prodiabetic effect of some β-blockers is concerning since diabetes increases the risk of adverse cardiovascular outcomes in hypertensive patients (Heart Outcomes Prevention Evaluation (HOPE) Study Investigators 2001; UK Prospective Diabetes Study (UKPDS) Group 1998). Studies have shown that patients being treated with β-blockers have as much as 32 % higher risk of developing diabetes when compared with individuals taking no drugs (Gress et al. 2000; Messerli et al. 2008). Furthermore, results from the Heart Outcomes Prevention Evaluation (HOPE) study reported that diabetic hypertensive patients have a similar risk of cardiovascular outcomes as those patients with a prior myocardial infarction (Yusuf et al. 2000).

Given the increased risk of adverse cardiovascular outcomes with diabetes, the development of methods to identify those individuals who are at risk of dysglycemia during β-blocker exposure would allow safer therapies to be recommended to the at-risk population before the onset of these adverse drug reactions.

Recent advances in biotechnology tools have allowed the acquisition of high-throughput biological data from patients, including metabolomic and genomic profile information. These data have the potential to be used to personalize medication prescribing. In this study, we utilized a metabolomic-genomic integrative approach to identify biomarkers associated with glucose change after treatment with the β-blocker atenolol.

2 Materials and methods

2.1 Study cohort

Caucasian participants with HTN who were treated with atenolol in the Pharmacogenomic Evaluation of Antihypertensive Responses (PEAR) study are included in this analysis. PEAR was undertaken to evaluate the pharmacogenomic determinants of the antihypertensive and adverse metabolic responses to atenolol and hydrochlorothiazide in HTN participants without a history of heart disease or diabetes (Johnson et al. 2009). In PEAR, participants between the ages of 17–65 years, of any race or gender and with clinic measured diastolic blood pressure (BP) ≥90 mmHg, were recruited at the University of Florida (Gainesville, FL), Mayo Clinic (Rochester, MN), and Emory University (Atlanta, GA). If BP was very elevated (systolic BP > 180 mm Hg or diastolic BP > 110 mmHg), or there was a history of diabetes or documented cardiovascular disease (including history of angina pectoris, heart failure, cardiac pacemaker, myocardial infarction, stroke), or other complications were present (pregnancy, renal diseases, sleep apnea, etc.), participants were excluded. The Institutional Review Boards at each study site reviewed and approved the protocol and all participants provided written informed consent. Additionally, the PEAR study was conducted in accordance with the Declaration of Helsinki. Details regarding study design have been previously published (Johnson et al. 2009) and the PEAR study is registered at clinicaltrials.gov (NCT00246519). Briefly, after an initial 3–8 week washout period, participants were randomized to receive HCTZ 12.5 mg or atenolol 50 mg daily monotherapy for 9 weeks and doses were titrated to HCTZ 25 mg or atenolol 100 mg daily in individuals whose blood pressure remained over 120/70 mmHg. Following monotherapy response, participants had fasting blood plasma samples collected. After monotherapy response, participants with blood pressure >120/70 had the alternate drug added to their regimen for an additional 9 weeks and went through similar dose titration steps as well as biological sample collection procedures as described in the monotherapy phase. The current study focuses on the monotherapy response to atenolol. Participants who received atenolol as an add-on treatment to HCTZ were included in a replication analysis.

2.2 Glucose measurement

At baseline (before atenolol therapy) and after completion of 6–9 weeks atenolol therapy, fasting blood samples were collected for glucose analyses. Plasma glucose levels were measured on a Hitachi 911 Chemistry Analyzer (Roche Diagnostics, Indianapolis IN) at the

Central Laboratory at Mayo Clinic. Samples were measured in duplicate and the means of the duplicates were used in the analyses. Outliers were excluded if regression residual value exceeded four standard deviations of the mean. Change in glucose was defined as the difference between the post-atenolol level and the baseline level.

2.3 GC-TOF mass spectrometry

Compared with other chromatographic systems, gas chromatography (GC) offers better resolution, sensitivity and reproducibility when used with a thermal detector (David 2012), which makes GC–mass spectrometry (MS) a prominent method for analysis of biological samples, which are complex by nature (Schauer et al. 2005). Therefore, GC–MS was the platform utilized to identify metabolites in the PEAR study. Mass spectrometry data and metabolite identification procedures have been previously reported (Rotroff et al. 2015). Briefly, plasma samples were divided in aliquots and stored at −80 °C. At the time of analysis, 30 μL of plasma were thawed, extracted and derivatized; 30 μL aliquots were extracted with 1 mL of degassed acetonitrile:isopropanol:water (3:3:2) at −20 °C and then centrifuged, decanted and later evaporated. Extracts were cleaned with acetonitrile/water (1:1) to remove triglycerides and cell membrane lipids. The resulting supernatant was evaporated again. Internal standards C8–C30 FAMEs were added and sample was derivatized using methoxyamine hydrochloride and afterwards by *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (Sigma-Aldrich) for trimethylsilylation of acidic protons.

Mass spectrometry was performed using an Agilent gas chromatograph and a Leco Pegasus IV Time-Of-Flight (TOF) spectrometer with 280 °C transfer line temperature, electron ionization at −70 V and an ion source temperature of 250 °C. Mass spectra were acquired from *m/z* 85 to 500 at 20 spectra/s and 1750 V detector voltage. Results files were processed by the metabolomics BinBase database. Database entries were matched against the Fiehn mass spectral library using retention index and mass spectrum information or the 05 commercial library. Quantitative data were normalized to the sum intensities of all known metabolites. For statistical analyses the metabolite peak height ratios were standardized to a mean of 0 and standard deviation of 1.

2.4 Genotyping and quality control

Single nucleotide polymorphism (SNP) genotyping was done on the Illumina Omni1 M Quad GWAS Beadchip (Illumina, San Diego, CA) using the company's Infinium II Assay. Genotypes were called using BeadStudio software and GenTrain2 calling algorithm, also from Illumina. Participants with sample genotype call rates below 95 % were excluded and SNPs were excluded if genotype call rates were below 95 %. Sex/gender mismatches were assessed from X chromosome genotype data. Cryptic relatedness was estimated by pairwise identity-by-descent analysis using PLINK (http://pngu.mgh.harvard.edu/purcell/plink/). Genotypes were imputed from HapMap III utilizing MaCH software (Minor Allele Frequency >3 % and $r^2 > 0.3$). Hardy–Weinberg Equilibrium was determined in PLINK using an exact test. SNPs out of Hardy–Weinberg Equilibrium or with Minor Allele Frequency <3 % were excluded from analyses. After quality control procedures, the total SNP call rate of the remaining individuals was 99.86 %. Principal Component Analysis

(PCA) was performed using GWAS data to assess the individuals' ancestral backgrounds and self-identified race information was confirmed with PCA of data for genetic ancestry.

2.5 Statistical analysis

The primary outcome of interest is change in glucose during treatment with atenolol. We have previously reported that participants with lower baseline glucose measures exhibited the largest variation in glucose after antihypertensive drug exposure (Moore et al. 2014). Therefore, participants with impaired fasting glucose (IFG), defined as baseline fasting glucose levels ≥100 mg/dL, were excluded from our metabolomics analysis in order to observe the maximum effect of antihypertensive treatment on glucose level. Association between baseline levels of 212 identified metabolites and glucose change after treatment with atenolol was assessed by multivariable linear regression adjusted for age, gender, baseline glucose and waist circumference. Analyses were conducted in SAS version 9.3 (SAS Institute Inc., Cary, NC). False discovery rate (FDR) procedure was utilized to account for multiple comparisons, with a significance threshold of $Q < 0.05$.

A targeted genomics analysis approach was utilized based on those genes involved in the pathway of metabolites identified in the metabolomics step which also had published evidence that supported involvement in glucose dysregulation. For this, a search in literature databases was conducted to identify metabolites that had been previously associated with glucose imbalance. This step was conducted to establish a candidate gene approach based on genes likely to be involved with glucose metabolism. The metabolic pathway of identified metabolites was assessed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) and genes directly involved in the breakdown or production (up to two reactions from the metabolite being analyzed) were selected to be included in this analysis. SNPs within gene transcripts ±2 kb were extracted using PLINK (Purcell et al. 2007). Linkage Disequilibrium-based pruning was conducted in PLINK, using a window size of 50 SNPs, a window shift parameter of five SNPs and a VIF threshold of two. Remaining SNPs had their association with glucose response tested through linear regression adjusted for age, gender, baseline glucose, waist circumference and principal components 1 and 2 using PLINK. The genomics analyses included all 234 PEAR atenolol participants. Statistical significance of SNP associations with glucose change was determined after FDR adjustment for multiple comparisons. Replication of genomic and metabolomic findings was conducted in an additional 228 participants treated with atenolol as an add-on therapy in PEAR. SNPs were considered replicated if the one-sided P value was <0.05 with the same direction of effect.

Sensitivity analysis was performed by testing the association between rs2669429 and glucose change while excluding individuals who presented with IFG at baseline. We also tested the sensitivity of the results by conducting a meta-analysis between the discovery and replication cohorts including all 234 participants as well as excluding those who presented IFG at baseline.

3 Results

3.1 Study population

The population was limited to Caucasian participants and includes 130 in the metabolomics analysis and 234 in the genomics analysis, as summarized in Fig. 1. Baseline characteristics of the metabolomics and genomics cohorts are summarized in Tables 1 and 2, respectively. Among those included in the metabolomics analysis, characteristics were similar comparing the discover and the replication sample sets, with the exception of waist circumference, with average waist circumference of 97 and 106 cm in the discovery and replication cohorts, respectively ($P < 0.0001$). Participants included in the metabolomics analysis had an average age of 50 years and consisted of 55 % females and average fasting glucose level in the discovery and replication cohorts was 87.7 and 89.5 mg/dL, respectively.

In the genomics analysis the discovery and replication cohorts did not differ in terms of baseline characteristics. Participants included in the genomics analysis were obese, with average waist circumference of 97.6 and 98.7 cm in the discovery and replication cohorts, respectively.

3.2 Metabolomics analysis

A total of 489 metabolites were detected and the 212 metabolites which were successfully matched to known compounds were used in the current analysis. A list of the metabolites identified is presented in Supplemental Table 1. The known metabolites have a microsatellite instability index (MSI) classification of 1 (verified with standards, mass spectra and retention times), with the exception of 25 metabolites with a MSI classification of 2, which were not verified by standards but with good mass spectral matches. After exclusion of participants with IFG at baseline, a total of 106 individuals remained in the metabolomics analysis. Linear regression analysis revealed that higher baseline levels of β-alanine were associated with increase in fasting glucose after treatment with atenolol ($P < 0.0001$; Q-value = 0.007, β = +2.97 mg/dL). Each unit increment in β-alanine standard deviation was associated with an average increase of 2.97 mg/dL in glucose after atenolol treatment. In the replication cohort, which consisted of participants who received atenolol add-on treatment, association of baseline β-alanine level and change in glucose was observed to be in the same direction, however, did not achieve statistical significance ($P = 0.89$, β = +0.81 mg/dL). Given previous evidence in the literature associating the β-alanine pathway with glucose imbalance (Cheng et al. 2012; Zhou et al. 2013), β-alanine was pursued in the subsequent genomics analysis.

3.3 Genomics analysis

Figure 2 depicts the workflow of metabolic pathway analysis and SNP selection conducted in the genomics analysis. Assessment of β-alanine pathway in KEGG database resulted in 19 genes directly involved in β-alanine metabolism. Figure 3 shows a diagram of β-alanine pathway with genes involved in synthesis and degradation of the metabolite. Given the fact that hepatic tissue is the main site of β-alanine production (Trexler et al. 2015), genes that lack expression in the liver according to the Tissue-specific Gene Expression and Regulation (TiGER) database (http://bioinfo.wilmer.jhu.edu/tiger/) were removed from analysis, leaving

a total of 14 genes. Extraction of SNPs within these genes resulted in 404 SNPs. LD-based pruning of SNPs conducted in PLINK determined 72 SNPs in 14 genes of the β-alanine pathway. A complete list of these genes and SNPs is shown in Supplement Table 2. Linear regression between SNPs and glucose change revealed that SNP rs2669429 in *DPYS* was significantly associated with an increase in fasting glucose after treatment with atenolol in our discovery cohort (P = 0.0006; Q = 0.05, β = +2.76 mg/dL; Fig. 3). These results indicate that for each copy of the G allele of rs2669429 the individual's glucose level is predicted to increase by 2.76 mg/dL after treatment with atenolol. This finding was successfully replicated in our atenolol add-on cohort (one-sided P = 0.04, β = +1.86 mg/dL; Fig. 4). Figure 4 shows the mean glucose change in the discovery and replication cohorts according to rs2669429 genotypes.

Sensitivity analyses were conducted with several different approaches. When participants with IFG at start of treatment with atenolol were excluded, the direction and magnitude of the effect was almost identical to what was observed in the main analysis, however the FDR corrected Q-value of the association between SNP rs2669429 did not reach significance (Q = 0.18, β = 2.65 mg/dL). Meta-analyses were also conducted for evaluation of sensitivity. When the entire cohorts of 234 and 228 participants in the discovery and replication cohorts were used, the meta-analysis of rs2669429 and glucose change resulted in a statistically significant Q-value of 0.02 and an effect size of 2.33 mg/dL. When individuals with baseline IFG were excluded from the meta-analysis, the magnitude of effect was similar (Q = 0.11, β = 2.17 mg/dL).

4 Discussion

In this study, we integrated metabolomics and genomics data from hypertensive individuals in order to identify potential biomarkers that can be used to predict an individual's glucose response during treatment with atenolol. The metabolomics step in our analyses revealed that the amino acid β-alanine was significantly associated with glucose change after treatment with atenolol. From the genomics analysis, we identified a gene in the pathway of β-alanine (*DPYS*) that was also associated with glucose response.

β-alanine is a non-essential amino-acid that has recently gained much attention due to its use as a performance enhancer by high-intensity athletes (Blancquaert et al. 2015). β-alanine intake leads to an increase of intramuscular carnosine, a histidine-containing dipeptide that acts as an intracellular buffer, protecting myocytes from acid accumulation and thus improving exercise performance and duration. β-alanine has been previously associated with diabetic status in a study looking at amino acid alterations in obesity and diabetes (Zhou et al. 2013). Furthermore, *N*-carbamoyl-β-alanine, an intermediate of β-alanine production, was associated with several cardiometabolic risk factors, including body mass index, waist circumference, insulin levels and insulin resistance (Cheng et al. 2012).

A recent study conducted in a cohort of 35 Caucasian males found higher levels of muscle carnosine, a peptide derived from β-alanine, in obese and glucose intolerant individuals (Stegen et al. 2015). In the present study we found an increase in β-alanine levels that is associated with an increase of blood glucose levels after treatment with atenolol.

Considering that β-alanine is the rate-limiting precursor of carnosine synthesis in muscle (Harris et al. 2006), our results could point to a role of β-alanine-derived carnosine in the dysregulation of glucose metabolism after treatment with a β-blocker.

However, the evidence concerning carnosine levels in diabetic state is contradictory, given that both increased and decreased levels of carnosine have been observed in studies analyzing human muscle tissue samples (Gualano et al. 2012; Srikanthan et al. 2012). Since carnosine was not present among the metabolites identified in our metabolomics platform, a separate study to quantify this compound in our cohort would be necessary to confirm if an association (whether positive or negative) exists between this metabolite and glucose change during treatment with atenolol.

We further assessed the involvement of β-alanine with our phenotype by moving it forward in the metabolomics-genomics integrative analysis. Genomic data revealed that SNP rs2669429 is significantly associated with increased levels of blood glucose after treatment with atenolol. In our study population, individuals carrying the rs2669429 G allele presented increased glucose change after treatment with atenolol when compared to individuals with the A allele. SNP rs2669429 is located within the first intron of the gene *DPYS*, which codes for the enzyme dihydropyrimidinase (DHP). DHP is involved in the reductive degradation of pyrimidines and catalyzes the second step in the formation of β-alanine from uracil. SNP rs2669429 has been previously reported to correlate with DHP-mediated fluoropyrimidine metabolism (Fidlerova et al. 2010). Furthermore, rs2669429 was found to be located in a binding site of the hepatic transcription factor HNF1 (Thomas et al. 2007). HFN1 is an important modulator of gene expression in the liver and pancreatic β-cells. HNF1 has been reported to control the expression of glucose and insulin transporters (Evans et al. 2008) and mutations in this gene have been associated with Maturity Onset Diabetes of the Young (MODY) (Kaisaki et al. 1997).

This study has some limitations worthy of mention. Our analyses were restricted to Caucasian participants from PEAR and the generalizability of our findings to other race groups should be assessed in future studies. Although many sources in the literature report an association of antihypertensive therapy with atenolol and development of diabetes, the present study found only modest increases in fasting blood glucose after 6–9 weeks of treatment with this β-blocker. This observation possibly stems from the short period participants were exposed to atenolol, since short-term treatment with the drug might not be sufficient to elicit full adverse glucose reactions. Nonetheless, previous studies have shown that minor increases in fasting glucose levels before diabetes threshold is reached are also associated with increased chances of diabetes onset (Nichols et al. 2008). Furthermore, fasting blood glucose was shown to be linearly correlated with increased risk of adverse cardiovascular outcomes, even at glucose levels considered normal (Sung et al. 2009). Sensitivity analyses indicate our study suffered from low sample size, which in turn decreased the power of our analyses. Specifically, our genomics signal, rs2669429, was not significant after participants with IFG were removed from the analysis, but maintained the same direction and similar effect sizes when meta-analysis including the discovery and replication cohorts was conducted.

5 Concluding remarks

In summary, by utilizing a metabolomics-genomics integrative approach we were able to identify β-alanine and SNP rs2669429 in gene *DPYS* as potential biomarkers associated with increased levels of blood glucose after treatment with the β-blocker atenolol. Our data suggest that β-alanine and rs2669429 might be important biomarkers to identify individuals who are at higher risk of developing dysglycemia and diabetes if exposed to atenolol. This could allow healthcare providers to choose alternative therapy strategies prior to the onset of adverse effects, reducing risk to patients and financial costs associated with treatment.

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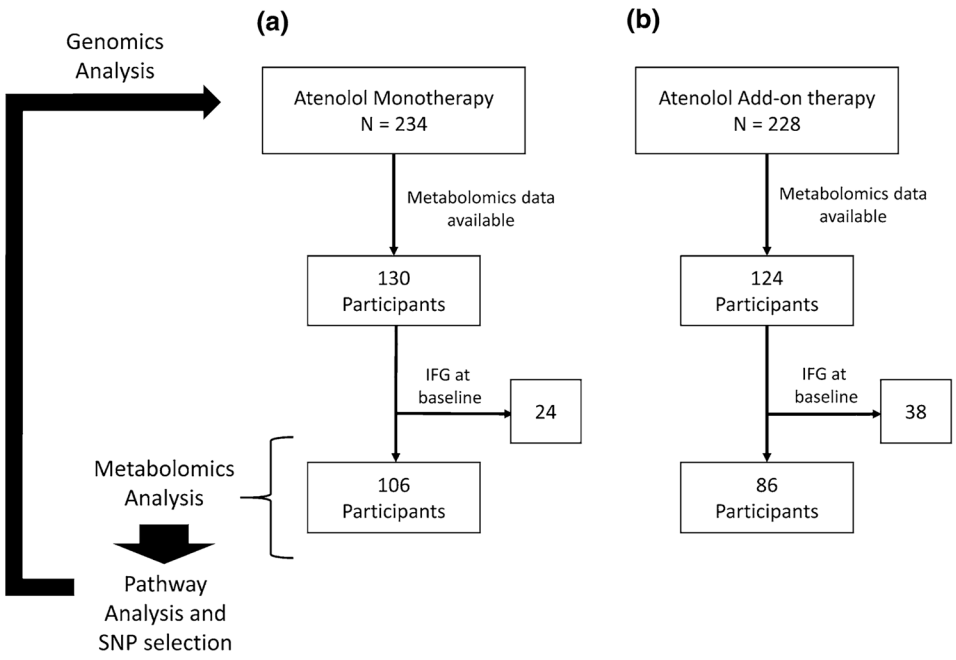

Fig. 1.

Flow diagram of participants included in metabolomics and genomics analyses in the discovery cohort (**a**) and in the replication cohort (**b**). IFG impaired fasting glucose

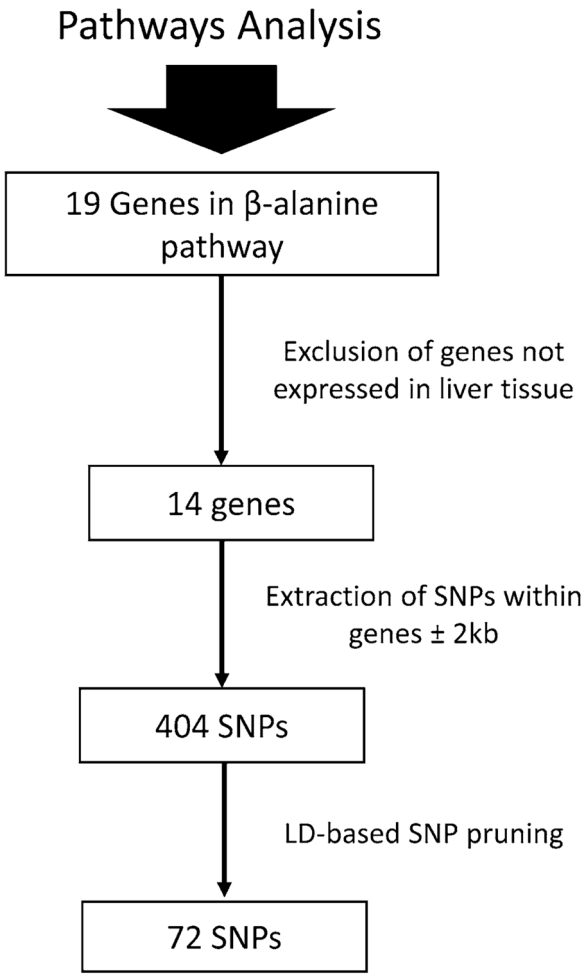

Fig. 2.

Flow diagram of the steps conducted in the genomics analyses. After identification of genes involved in β-alanine metabolism, SNPs were extracted and LD-based pruning ($r^2 = 0.3$) was conducted using PLINK

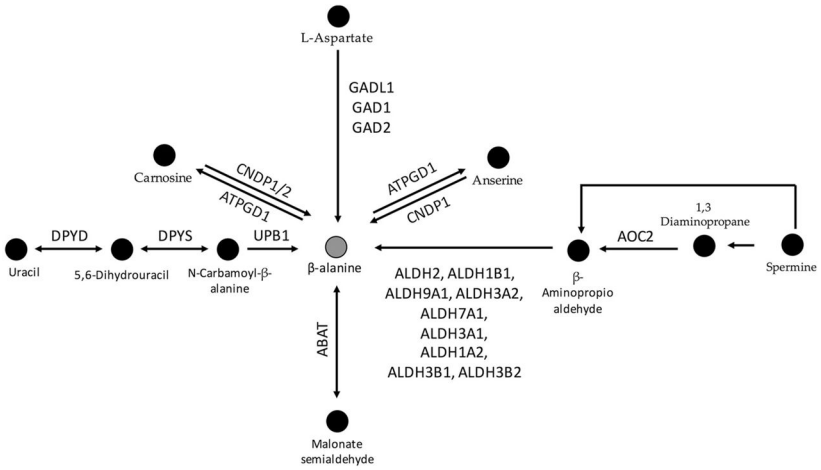

Fig. 3.

Diagram of β-alanine pathway analyzed in KEGG Pathway. *Gene symbols in italic* represent genes included in the genomics analysis

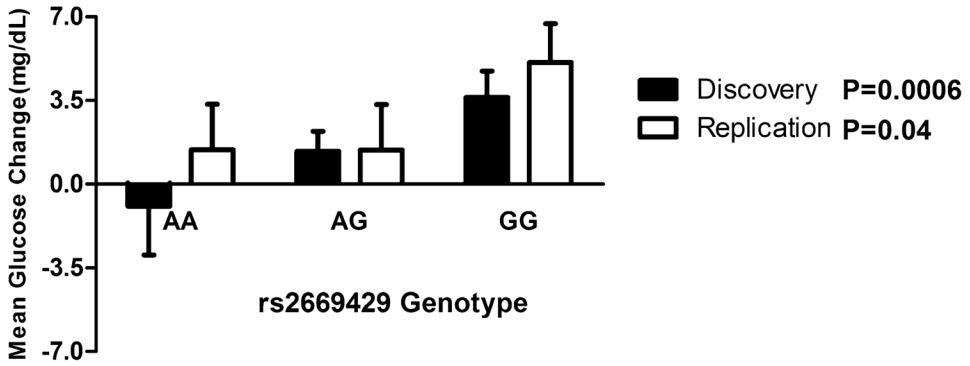

Fig. 4.

Mean glucose change (in mg/dL) after treatment with atenolol according to genotype groups, in the discovery and replication cohorts. *P*-values for the discovery ($P = 0.0006$) and replication ($P = 0.04$) analyses are included

Table 1

Baseline characteristics of participants included in the metabolomics analysis

Characteristic	Discovery (n = 106)	Replication (n = 86)	*P*[*]
Age (years)[a]	50.3 ± 10	49.9 ± 9.1	0.94
Sex (%)	Female (54.7 %)	Female (48.3 %)	0.13
Waist circumference (cm)[a]	97.0 ± 13.1	106.7 ± 11	< 0.0001
Baseline glucose (mg/dL)[a]	87.7 ± 6.9	89.5 ± 7.2	0.07
Glucose change (mg/dL)[a]	4.7 ± 8.4	3.2 ± 9.6	0.23
Fasting insulin (mIU/mL)[a]	8.5 ± 6.1	10 ± 6.7	0.10

[*] *P*-values from two-sample *t*-test for quantitative variables or Chi-Square test for categorical variables

[a] Values are presented as mean ± standard deviation

Table 2

Baseline characteristics of participants included in the genomics analysis

Characteristic	Discovery (n = 234)	Replication (n = 228)	*P*[*]
Age (years)[a]	49.5 ± 9.5	50.0 ± 9.4	0.64
Sex (%)	Female (46.9 %)	Female (39.9 %)	0.13
Waist circumference (cm)[a]	97.6 ± 12.7	98.7 ± 13.6	0.37
Baseline glucose (mg/dL)[a]	91.9 ± 11.6	94.2 ± 13.2	0.44
Glucose change (mg/dL)[a]	1.7 ± 10.1	1.5 ± 11.7	0.78
Fasting insulin (mIU/mL)[a]	10.0 ± 9.9	10.4 ± 9.6	0.46

[*] *P*-values from two-sample *t*-test for quantitative variables or Chi-Square test for categorical variables

[a] Values are presented as mean ± standard deviation