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A polymorphism in the protein kinase C gene *PRKCB* is associated with α_2-adrenoceptor-mediated vasoconstriction

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Abstract

Objectives—α_2-Adrenoceptors (α_2-AR) mediate both constriction and dilatation of blood vessels. There is substantial inter-individual variability in dorsal hand vein (DHV) constriction responses to α_2-AR agonist activation. Genetic factors appear to contribute significantly to this variation. The present study was designed to identify genetic factors contributing to the inter-individual variability in α_2-AR-mediated vascular constriction induced by the selective α_2-AR agonist dexmedetomidine.

Methods—DHV constriction responses to local infusion of dexmedetomidine were assessed by measuring changes in vein diameter with a linear variable differential transformer. The outcome variable was log-transformed dexmedetomidine ED_{50} for constriction. A genome-wide association study (GWAS) of 433,378 single nucleotide polymorphisms (SNPs) was performed for the sensitivity of DHV responses in 64 healthy Finnish subjects. 20 SNPs were selected based on the GWAS results and their associations with the ED_{50} of dexmedetomidine were tested in an independent North American study population of 68 healthy individuals.

Results—In both study populations (GWAS and replication samples), the SNP rs9922316 in the gene for protein kinase C type β was consistently associated with dexmedetomidine ED_{50} for dorsal hand vein constriction (unadjusted p = 0.00016 for the combined population).

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Conclusions—Genetic variation in protein kinase C type β may contribute to the inter-individual variation in dorsal hand vein constriction responses to α_2-AR activation by the agonist dexmedetomidine.

Keywords

receptors, adrenergic, alpha; dorsal hand vein; GWAS; candidate genes; dexmedetomidine

Introduction

α_2-Adrenoceptors (α_2-ARs) are G-protein coupled receptors that mediate both vascular constriction [1] and dilatation [2]. Dorsal hand veins (DHV) allow measurement of local constriction and dilatation responses of human blood vessels in vivo in a minimally invasive manner that is free of the confounding effects that result from systemic doses of vasoactive agonists [3,4]. We have previously observed that there is substantial inter-individual variability in DHV constriction responses to α_2-AR agonist activation [5-7]; however, DHV responses are highly reproducible when measured over time in the same subject and in the right and left hand [8].

The substantial inter-individual differences in DHV sensitivity to α_2-AR activation have so far remained unexplained. Genetic factors may contribute significantly, as DHV sensitivity to constrict after infusion of norepinephrine is a familial trait [9,10]. Studies with mono- and dizygotic twin pairs and parents and their children have clearly demonstrated that genetic factors contribute significantly to the inter-individual differences in the responses of DHVs to norepinephrine, but did not further examine the genes involved [9,10].

Recently, we reported that factors such as gender, blood pressure, heart rate, blood lipids, plasma norepinephrine concentrations, hormonal contraception, menstrual cycle phase and blood haemoglobin concentration were not statistically significant determinants of DHV sensitivity to constriction after α_2-AR activation by dexmedetomidine, the most selective clinically available α_2-AR agonist [6]. Pharmacogenetic association studies of several candidate genes (i.e. *ADRA2B*, the α_{2B}-AR gene, 301-303 ins/del and other polymorphisms, and *ADRA1A*, the α_{1A}-AR gene, Arg347Cys polymorphism) [5,11] did not reveal significant contributions of these gene variants to the inter-individual variability in DHV responses to α-AR activation. Earlier studies also demonstrated that DHV responses to dexmedetomidine were not significantly different between black and white North American subjects [12].

Many molecular pathways have been implicated in α_2-AR-mediated cellular signalling. It is plausible that variation in the genes involved in α_2-AR-mediated signalling contributes significantly to the inter-individual variation in α_2-AR-mediated DHV constriction. α_2-ARs are known to couple to G_i-type G proteins that mediate inhibition of adenylyl cyclase activity and cAMP formation in smooth muscle cells. Activation of α_2-ARs is also known to increase intracellular Ca^{2+} levels resulting in vasoconstriction. In recombinant cell systems, α_2-ARs via G_q-type G-proteins activate phospholipase C resulting in the formation of inositol trisphosphate and Ca^{2+} release from the endoplasmic reticulum [13,14]. Interactions of α_2-ARs with voltage-sensitive Ca^{2+} channels have been shown to elicit extracellular Ca^{2+} influx [15,16], and α_2-ARs can also increase the activity of protein kinase C (PKC), which has been reported to contribute to membrane depolarization, Ca^{2+} influx, and smooth muscle contraction [14,17].

Previous DHV studies have suggested that genetic variation may contribute to the large inter-individual variability in responses to agonists, but so far, specific genetic variants have not been identified. The present combined whole-genome / candidate gene investigation was

designed to identify genetic factors contributing to the inter-individual variability in α_2-AR-mediated vascular constriction induced by dexmedetomidine.

Methods

We sought to identify possible associations of common genetic variants with the sensitivity of DHV constriction to dexmedetomidine, a potent and selective α_2-AR agonist. The study consisted of a discovery phase and a replication phase. In the discovery phase, we performed a genome-wide association study (GWAS) of 433,378 polymorphic gene loci with the sensitivity of DHV responses in 64 healthy Finnish subjects selected to represent the low and high ends of the sensitivity range to dexmedetomidine, as assessed by ED_{50} values for drug-induced DHV constriction. In the replication phase, we selected 20 SNPs identified by the GWAS and tested their associations with the ED_{50} of dexmedetomidine in an independent North American study population of 68 healthy individuals.

DHV constriction responses were assessed by measuring changes in dorsal hand vein diameter with a linear variable differential transformer method (LVDT). We set the log-transformed dexmedetomidine ED_{50} for DHV constriction as the dependent variable in the linear regression models used for the association analysis. The study was conducted in accordance with the Declaration of Helsinki (2000) of the World Medical Association. The discovery (GWAS) study was approved by the Ethics Committee of Southwest Finland Hospital District, Turku, Finland, and the replication study (candidate gene approach) was approved by the Institutional Review Board of the Vanderbilt University Medical Center, Nashville, TN, USA. All subjects gave their written informed consent.

Study Populations

The current study combines two study populations: one from Turku, Finland (discovery phase, GWAS) and one from Middle Tennessee, USA (replication phase, candidate gene approach). For the GWAS, non-smoking Caucasian men and women aged 18 to 40 years were eligible if they were unrelated and healthy as assessed by medical history, physical examination, 12-lead electrocardiogram, blood count and serum lipid profile. Information on this study population has been published in an earlier report that analyzed subject characteristics contributing to the inter-individual variation in DHV responses to dexmedetomidine [6]. From the 99 subjects of the earlier report, all of whom had provided DNA samples for eventual later analysis, we selected 64 subjects who represented the low and high ends of the response range, based on their DHV constriction responses to dexmedetomidine (ED_{50} values greater than 30 ng/min or less than 5 ng/min). One subject was excluded because the genetic analysis revealed that he was closely related to another study subject.

The subject recruitment process used at the Vanderbilt Medical Center for the replication population and the details of this population have been published earlier [5,7]. In short, male and female white or black residents of Middle Tennessee were eligible if they were 18–45 years of age and healthy based on medical history, physical examination and laboratory tests. Ethnicity was self-reported. Demographic data of the two study populations are presented in Table 1.

Measurement of Vascular Responses

The in vivo experiments of the discovery and replication phases of the study were performed independently by the two research groups. DHV responses were measured in supine subjects with a previously validated LVDT method, as described earlier [6,7]. In Turku, α_2-AR-mediated DHV constriction was elicited by graded infusions of dexmedetomidine

(Precedex®, Hospira, Lake Forest, IL, USA) into the investigated vein, with 8 consecutive dose rates ranging from 0.0128 to 1000 ng/min. Each of the 8 infusion phases lasted 5 min, and vein diameter was recorded during the last 3 min of each phase. At Vanderbilt, dexmedetomidine (Precedex®; 0.01–100 ng/min) was administered in increasing doses, with each dose infused for 7 min and with the DHV diameter recorded during the last 2 min of each infusion phase. Systemic drug effects were minimized by limiting the total dose of dexmedetomidine in Turku to 6.25 μg over 40 min and at Vanderbilt to 12.25 μg over 84 min, where, however, 94 % of the subjects achieved a plateau response (E_{max}) at cumulative doses below 6.59 μg over 70 min and therefore never received the higher dose rates.

Analysis of DHV responses to dexmedetomidine

DHV constriction responses to dexmedetomidine were expressed as the observed per cent reduction of vein diameter compared to a state of initial venous dilatation that was defined as the average of three stable baseline measurements. ED_{50} values of dexmedetomidine were determined using a sigmoidal dose–response model with variable slope (GraphPad Prism 5.01, GraphPad Software, San Diego, CA, USA).

GWAS genotyping and quality assurance

Genotyping and data analysis were performed at the Technology Centre of the Finnish Institute for Molecular Medicine (FIMM), University of Helsinki, Finland. The 64 discovery-phase study samples were genotyped using Illumina's Human660W-Quad BeadChips, iScan System, and with standard reagents and protocols provided by Illumina Inc. (San Diego, CA, USA). The genotypes were read and confirmed with Illumina's GenomeStudio v. 2009.1 software, in-house developed database tools, and the PLINK v1.07 toolset (http://pngu.mgh.harvard.edu/~purcell/plink/) [18].

Single nucleotide polymorphisms (SNP) with a genotyping success rate of < 0.95, minor allele frequency of < 0.10 or P-value of < 10^{-6} in an exact test for Hardy-Weinberg equilibrium were removed. Relatedness between the study subjects was assessed by estimating the pairwise identity-by-descent (IBD) for all subject pairs in the sample with PLINK. One pair of related individuals (estimated genome-wide IBD > 0.2) was identified and the individual with fewer successful genotype calls was removed from the study. A total of 433,378 SNPs passed the quality control and were included in the analysis.

Replication sample genotyping, quality assurance and SNP selection

The replication samples were genotyped at FIMM for 20 SNPs selected on the basis of the discovery phase results in two multiplex reactions using the iPlex assay on the MassARRAY System (Sequenom, San Diego, CA, USA) with standard reagents and protocols. The primer sequences are listed in Supplement table 1. Each individual sample was genotyped in duplicate. The concordance rate for all samples was 100 %.

20 SNPs from the GWAS were selected for the replication phase by including the top 5 loci of the GWAS probability ranking list (P-value range, $7.1 \cdot 10^{-6}$ to $5.2 \cdot 10^{-5}$) with no consideration of gene identity, and the top 15 loci (P-value range, 0.00011 to 0.018) from a pre-defined candidate gene set tagged by 7,227 SNPs. The candidate gene set was based on an extensive literature review and included altogether 256 genes implicated in α_2-AR-mediated signalling (see Supplement table 2). We retrieved the coordinates of the genes from the Ensembl database (genebuild 65) with BioMart (http://www.biomart.org) and converted them from GRCh37 to NCBI36 reference genome coordinates with the Ensembl assembly converter (http://www.ensembl.org/Homo_sapiens/UserData/SelectFeatures). To each gene in the set we assigned SNPs not more than 2000 base pairs away from the gene as well as all SNPs annotated as putative expression quantitative trait loci for the gene. The

latter annotation was extracted from the eQTL browser (http://eqtl.uchicago.edu/cgi-bin/gbrowse/eqtl/). We then ranked the candidate genes by the smallest P-values of the SNPs assigned to them and selected one SNP from each of the top 15 candidate genes for association analysis in the replication sample (see Table 2). For each gene, the most strongly associated SNP was selected. However, for *PRKCB* and *RGS20*, these were not accessible for the genotyping technology and the SNPs with second smallest P-values were used in their place.

Statistical analysis

The statistical tests for association were done with PLINK using linear regression and an additive genetic model. Log-transformed dexmedetomidine ED_{50} was set as the dependent variable with sex, age and temperature of the infusion hand finger tip (mean temperature from the last 25 min of the drug infusion (see [6]) (discovery phase) or sex and age (replication phase) used as covariates. The linear regression model requires the dependent variable to be normally distributed. The almost normal skewness (−0.025) and kurtosis (3.828) of the residual distribution of the discovery phase sample indicate that this assumption was not violated, despite the genotyped sample consisting of the lower and upper tertiles of the original phenotyped sample. The same was true also for the residual distribution of the replication phase sample, not selected for the extremes of the phenotype (skewness 0.339, kurtosis 3.164). The normality of the residual distributions was tested formally with Shapiro-Wilk's test yielding non-significant P-values for both the discovery sample (P = 0.766) and the replication sample (P = 0.642). The results from the two phases of the study were combined with PLINK using both fixed-effects and random-effects meta-analysis models. The fixed-effects model assumes that the true effect size is equal for each of the estimates to be combined and is commonly used in genetic association studies. The random-effects model assumes that the true effect sizes are different for each study sample and come from a distribution of true effect sizes [19]. We used PLINK to test and quantify the difference between the SNP effect size estimates from the discovery and replication phases with Cochran's Q-statistic and the I^2 heterogeneity index [20].

Results

In the discovery phase, we tested the association of 433,378 SNPs with dexmedetomidine ED_{50} for DHV constriction. The majority of the strongest GWAS association signals were relatively distant from known genes, the most significantly associated SNP rs1285441 ($p_{discovery} = 5.2 \cdot 10^{-6}$) being located more than 400 kb away from the nearest gene, *NXPH1*. None of the associations remained statistically significant after correction for multiple testing (see Table 3). We next constructed a candidate gene list of 256 genes known to be associated with α_2-AR signalling and ranked them in order of increasing P-values in the GWAS results. The 5 top hits from the unselected GWAS result list and the top 15 hits from the candidate gene list were included in the next phase of the study. These 20 SNPs were subjected for replication by association analysis in an independent sample, and the results were combined in a fixed-effects meta-analysis.

One of the 20 selected SNPs, rs9922316, a marker in the candidate gene encoding PKC type β (*PRKCB*), was consistently associated with dexmedetomidine ED_{50} for DHV constriction, both in the discovery sample and in the replication sample ($p_{combined} = 0.00016$, see Fig. 1 and 2 and Table 3). In this locus, allele A of rs9922316 was associated with higher dexmedetomidine ED_{50} in both datasets.

Discussion

The main finding of the present study is the association of the SNP rs9922316 in *PRKCB* with inter-individual variation in DHV responses to dexmedetomidine in two independent datasets. This is the first GWAS of the inter-individual variability of α_2-AR-mediated vasoconstriction responses. In previous studies, DHV response variability was not explained by selected markers in the α_1-AR and α_2-AR genes or in a limited set of other candidate genes [5,11,21].

Based on our Finnish cohort, we recently reported that DHV constriction to α_2-AR activation by dexmedetomidine was only weakly associated ($r^2 = 0.074$, $p = 0.018$) with a subject's DHV constriction response to the α_1-AR agonist phenylephrine, suggesting independent regulation of α_1-AR- and α_2-AR-mediated vasoconstriction [6]. Similarly, in the Vanderbilt cohort, α_1-AR- and α_2-AR-mediated DHV constriction responses appeared to be regulated independently [7].

The hypothesis of the present study was that genetic variation would contribute to the large inter-individual variability in vasoconstriction responses to α_2-AR agonists. A GWAS was first performed with sensitivity of α_2-AR-mediated DHV constriction as the dependent outcome variable. The 63 subjects included in the analysis represented the top and bottom tertiles of the ED_{50} range of a study population previously investigated in Turku [6]. The GWAS did not yield statistically significant hits after correction for multiple testing, even though many markers of biologically plausible genes were associated with dexmedetomidine ED_{50} with small unadjusted P-values. On the basis of the GWAS association data, 20 SNPs were selected for replication in the replication phase of the study. The replication included the top 5 loci from the GWAS (smallest P-values) and the top 15 loci (smallest P-values) from a pre-defined candidate gene list of 256 α_2-AR-associated genes (Table 2).

The three human α_2-AR subtypes are widely expressed in different tissues and organs, and they mediate many different physiological and pharmacological effects in the cardiovascular system. α_2-ARs have been shown to have importance in the regulation of vascular tone in humans at least in digital [22] and coronary [23] arteries and in large superficial veins [6]. Family studies on the inter-individual variability in DHV constriction responses to norepinephrine have suggested that multiple genetic polymorphisms may be involved [9,10], but the genetic determinants of DHV responsiveness to α_2-AR activation have not been identified.

Several G-protein-mediated signalling pathways of α_2-ARs coupled to different cellular regulatory mechanisms have been described. In the current study, we addressed the hypothesis that genetic variation explains a part of the difference between high- and low-responders to α_2-AR-mediated DHV constriction. Our results suggest that the A allele of the rs9922316 polymorphism in *PRKCB* is associated with decreased DHV responses to dexmedetomidine. PKC has been linked with the smooth muscle contraction cascade, including activation by increased diacylglycerol formation and interactions with the serine/threonine-specific protein kinases MEK and MAPK [24,25]. Interactions of α_2-ARs with PKC have been suggested to lead to Ca^{2+} influx and vasoconstriction [14,17]. Kim et al. recently reported that activation of α_2-ARs by dexmedetomidine resulted in a constriction response involving a Ca^{2+} sensitization mechanism mediated by Rho kinase, PKC, and phosphatidylinositol 3-kinase [26].

The two independent study populations were investigated in similar controlled clinical pharmacological settings previously found to be appropriate for examination of DHV

constriction responses. The subjects were healthy and similar in most respects, and comparable methods were used. The main difference between the populations was ethnic composition. Participants in the discovery phase of the study were Finns, whereas participants in the replication phase were black and white Americans. This limits the study's power to validate genetic variants unique to people of European descent. However, the alleles we investigated by GWAS are common and likely shared with comparable frequencies across various populations [27]. The observed minor allele frequencies in the study populations from Turku (Caucasians only) and Vanderbilt (white and black North American subjects) are shown in Supplement table 3. The minor allele of one of the SNPs, rs1540293 in chromosome 3, was very rare in the Vanderbilt material, and the replication analysis thus cannot be considered informative for this SNP.

It has been previously demonstrated that the employed method provides reproducible α_2-AR-mediated venoconstriction without marked systemic hemodynamic effects [4,8,12]. Similarly, α_1-AR activation by dexmedetomidine in this dose range is unlikely since DHV responses to dexmedetomidine and phenylephrine have been found to be independently regulated in individual subjects of both study populations [6,7].

The current finding of an association of rs9922316 in *PRKCB* provides only partial explanation of the large inter-individual DHV response variation. There is so far no evidence that rs9922316 is a functional polymorphism and therefore it should be considered as a marker for a functional gene polymorphism(s) in its vicinity. The assessment of the functionality of rs9922316 is beyond the scope of the present study. The small sample size sets limitations for result interpretation. Small effects of common variants may not be detected in this sample, and at the same time, effects of rare allelic variants may not be detected at all.

The candidate gene list of the replication phase was based on an extensive literature search, but it still represents an incomplete state of knowledge of α_2-AR signalling pathways. The canonical α_2-AR pathway of G_i-mediated adenylyl cyclase inhibition does not reliably account for constriction of different vessel types, and the suggested direct or indirect interactions of α_2-ARs with voltage-sensitive Ca^{2+} channels have been challenged numerous times in the history of pharmacology.

It is important to try to shed light on the details of genetic variation affecting vascular regulation and responses, since such knowledge may improve our understanding of cardiovascular functions in health and disease. However, we can only speculate on the impact of genetic variation of PKCβ and its heritability on other vessel types and diseases. The results of this pharmacogenetic study suggest that rs9922316 in *PRKCB* is associated with the venous constriction response to dexmedetomidine. We propose that PKCβ plays a significant role in the signalling pathways of dexmedetomidine to induce venous constriction.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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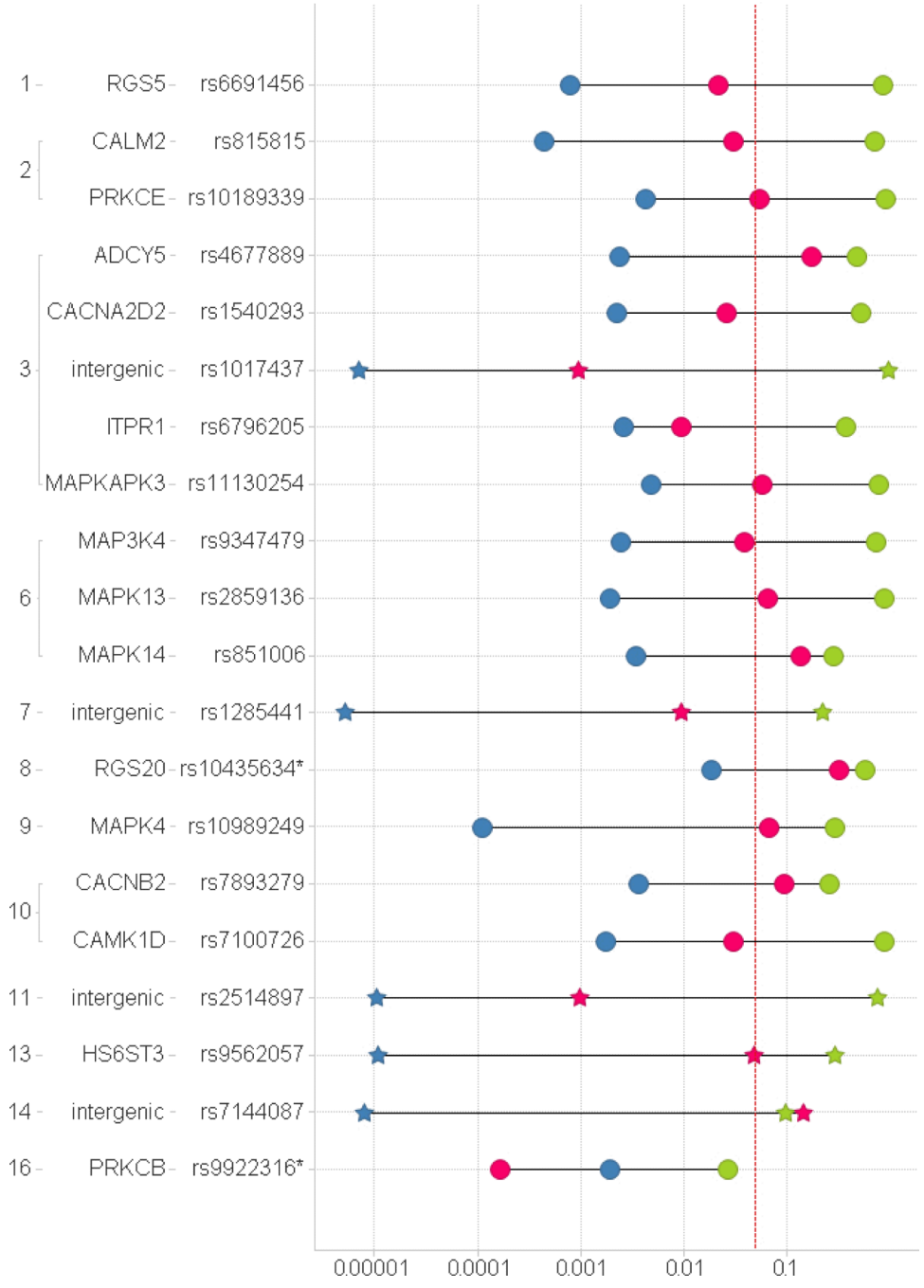

Fig. 1.

P-values (x axis) for the association between gene variants and ED_{50}. Green – replication (Vanderbilt); Blue – GWAS (discovery, Turku); Red – combined; Circle – candidate gene list; star - GWAS list. The dotted vertical line denotes a P-value of 0.05. The locations of the investigated SNPs in the human genome are indicated with the human chromosome number (far left) and the name of the gene. * PRKCB and RGS20 were not directly assessable by the employed genotyping technology and the second-best SNPs were used in their place.

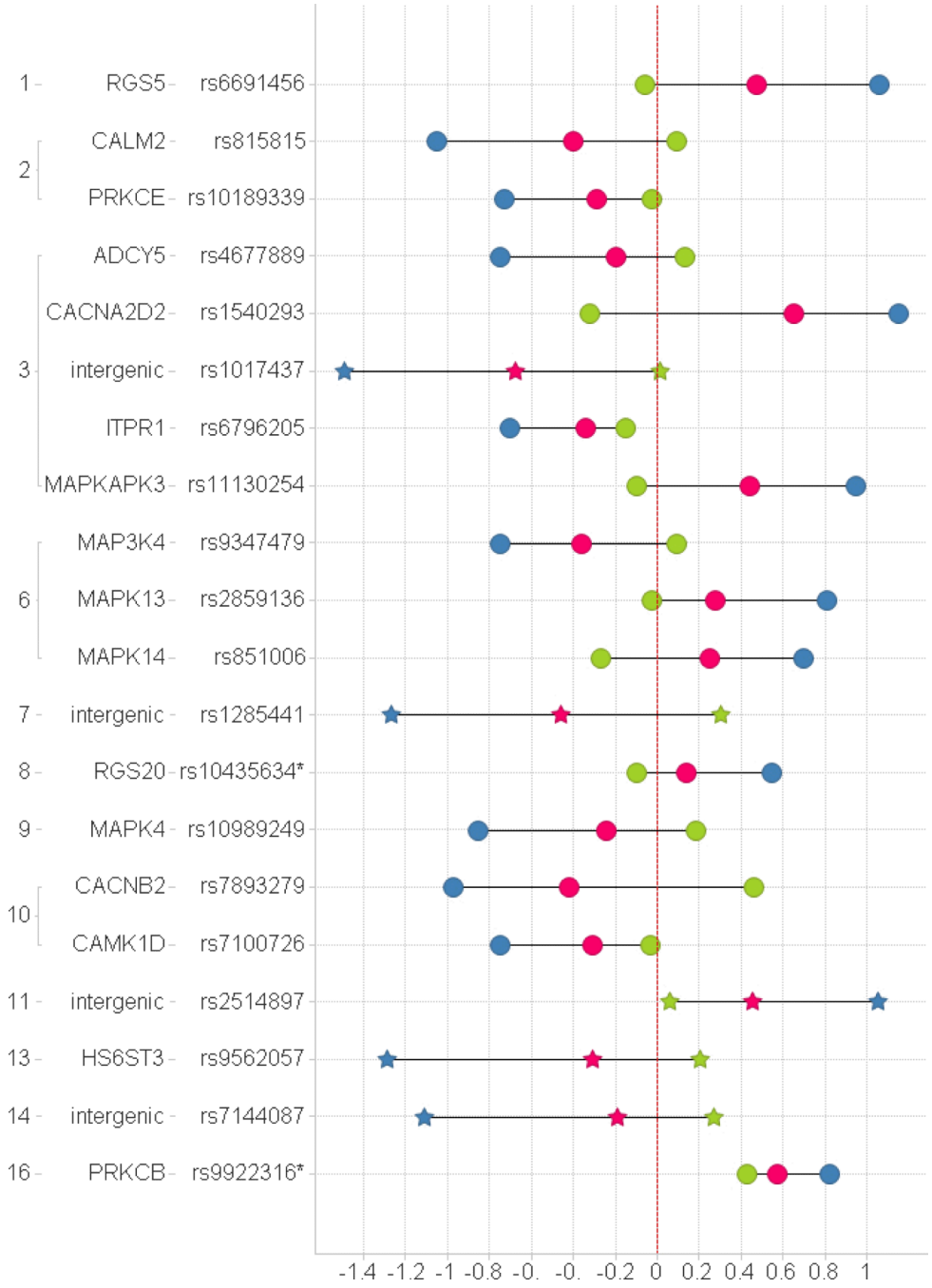

Fig. 2.

Beta coefficients (x axis). The coefficients describe the effect sizes of genes in the analysis. Green – replication (Vanderbilt); Blue – GWAS (discovery, Turku); Red - combined; Circle - candidate gene list; Star - GWAS list. The dotted vertical line denotes a beta coefficient of 0 (no effect). The locations of the investigated SNPs in the human genome are indicated with the human chromosome number (far left) and the name of the gene. * PRKCB and RGS20 were not directly assessable by the employed genotyping technology and the second-best SNPs were used in their place.

Table 1

Characteristics of the study populations

Characteristic	GWAS subjects (Turku)	Replication subjects (Vanderbilt)
n	64[1]	68
Gender: male : female	22 : 42	40 : 28
Ethnicity: African American : Caucasian	0 : 64	31 : 37
Age (years)	24.4 ± 5.6	28.1 ± 7.6

The values are numbers or means ± SD;

[1] one male subject aged 23 years was excluded from the genetic analysis because of relatedness to another subject

Table 2

Candidate genes for the replication phase. The replication analysis included the top 5 loci from the GWAS (smallest P-values) and top 15 loci (smallest P-values) from a pre-defined candidate gene list of 256 α_2-AR-associated genes

Gene	Protein	SNP	Source
HS6ST3	heparan sulfate 6-O-sulfotransferase 3	rs9562057	GWAS
intergenic	-	rs1017437	GWAS
intergenic	-	rs1285441	GWAS
intergenic	-	rs2514897	GWAS
intergenic	-	rs7144087	GWAS
ADCY5	adenylate cyclase 5	rs4677889	candidate
CACNA2D2	calcium channel, voltage-dependent, alpha 2/delta subunit 2	rs1540293	candidate
CACNB2	calcium channel, voltage-dependent, beta 2 subunit	rs7893279	candidate
CALM2	calmodulin 2 (phosphorylase kinase, delta)	rs815815	candidate
CAMK1D	calcium/calmodulin-dependent protein kinase ID	rs7100726	candidate
ITPR1	inositol 1,4,5-trisphosphate receptor, type 1	rs6796205	candidate
MAP3K4	mitogen-activated protein kinase kinase kinase 4	rs9347479	candidate
MAPK13	mitogen-activated protein kinase 13	rs2859136	candidate
MAPK14	mitogen-activated protein kinase 14	rs851006	candidate
MAPK4	mitogen-activated protein kinase 4	rs10989249	candidate
MAPKAPK3	mitogen-activated protein kinase-activated protein kinase 3	rs11130254	candidate
PRKCB	protein kinase C, beta	rs9922316*	candidate
PRKCE	protein kinase C, epsilon	rs10189339	candidate
RGS20	regulator of G-protein signaling 20	rs10435634*	candidate
RGS5	regulator of G-protein signaling 5	rs6691456	candidate

*PRKCB and RGS20 were not directly assessable by the employed genotyping technology and the second-best SNPs were used in their place. Gene and protein names obtained from The HUGO Gene Nomenclature Committee (HGNC) homepage (http://www.genenames.org)

Table 3

Results from the discovery and replication phases and their combined association outcome. The genes and SNPs are in descending order according to their combined P-value.

							Discovery (n = 63)			Replication (n = 68)			Combined (n = 131)	
Gene	**SNP**	**Source**	**P (R)**	**Beta (R)**	**P (Q)**	**I^2**	**Beta**	**SE**	**P**	**Beta**	**SE**	**P**	**Beta**	**P**
PRKCB	rs9922316*	candidate	0.002173	0.5892	0.2149	34.99	0.8205	0.2524	0.001921	0.4295	0.1889	0.02635	0.5699	0.00016
intergenic	rs1017437	GWAS	0.329	−0.7347	< 0.001	92.55	−1.492	0.3024	$7.12{\cdot}10^{-6}$	0.01319	0.2779	0.9623	−0.676	0.00095
intergenic	rs2514897	GWAS	0.2724	0.5463	< 0.001	91.99	1.052	0.2181	$1.05{\cdot}10^{-5}$	0.05636	0.1785	0.7532	0.4557	0.00097
intergenic	rs1285441	GWAS	0.5394	−0.4825	< 0.001	94.99	−1.27	0.253	$5.20{\cdot}10^{-6}$	0.3024	0.2445	0.2205	−0.4569	0.00935
ITPR1	rs6796205	candidate	0.143	−0.4055	< 0.05	74.94	−0.7034	0.2234	0.002591	−0.1482	0.1653	0.3734	−0.3446	0.0095
RGS5	rs6691456	candidate	0.3736	0.4979	< 0.01	86.28	1.061	0.2998	0.0008001	−0.05814	0.2863	0.8397	0.4757	0.0216
CACNA2D2	rs1540293	candidate	0.5348	0.4563	< 0.05	82.40	1.151	0.36	0.002249	−0.3216	0.5021	0.5241	0.651	0.02607
CAMK1D	rs7100726	candidate	0.295	−0.3751	< 0.05	83.62	−0.747	0.2274	0.001728	−0.03011	0.1802	0.8678	−0.3066	0.02992
CALM2	rs815815	candidate	0.4115	−0.4711	< 0.01	89.33	−1.053	0.2822	0.0004346	0.09441	0.2467	0.7033	−0.4026	0.03018
MAP3K4	rs9347479	candidate	0.4265	−0.335	< 0.05	83.03	−0.7513	0.2368	0.002414	0.09124	0.2538	0.7204	−0.3592	0.03803
HS6ST3	rs9562057	GWAS	0.4794	−0.5281	< 0.001	95.14	−1.286	0.2664	$1.08{\cdot}10^{-5}$	0.2075	0.1937	0.288	−0.309	0.04856
PRKCE	rs10189339	candidate	0.3059	−0.3601	< 0.05	80.75	−0.7297	0.2449	0.004211	−0.0253	0.1885	0.8937	−0.2874	0.05439
MAPKAPK3	rs11130254	candidate	0.4142	0.4259	< 0.05	80.29	0.9444	0.3225	0.004868	−0.09886	0.3325	0.7672	0.4387	0.05809
MAPK13	rs2859136	candidate	0.3682	0.3768	< 0.01	86.13	0.8121	0.2496	0.0019	−0.026	0.1874	0.8901	0.2761	0.0654
MAPK4	rs10989249	candidate	0.5235	−0.3313	< 0.001	93.29	−0.8571	0.2069	0.0001128	0.1817	0.1719	0.2944	−0.2425	0.06661
CACNB2	rs7893279	candidate	0.6993	−0.2768	< 0.01	87.01	−0.9722	0.3204	0.003609	0.4617	0.4054	0.2589	−0.4209	0.09405
MAPK14	rs851006	candidate	0.65	0.2198	< 0.01	87.92	0.6999	0.229	0.003386	−0.2691	0.247	0.2802	0.252	0.1335
intergenic	rs7144087	GWAS	0.5532	−0.4094	< 0.001	95.96	−1.109	0.2261	$7.99{\cdot}10^{-6}$	0.2719	0.1609	0.09583	−0.1923	0.1424
ADCY5	rs4677889	candidate	0.4987	−0.2974	< 0.01	88.58	−0.7503	0.2361	0.002378	0.1292	0.1805	0.477	−0.1952	0.1734
ARGS20	rs10435634*	candidate	0.5188	0.2083	<0.05	80.79	0.5487	0.2263	0.01846	−0.09789	0.1706	0.5682	0.1364	0.3166

P (R), P-value from a random-effects meta-analysis; Beta (R), beta coefficient estimate from a random-effects meta-analysis; P (Q), Cochran's Q -statistic P-value for a test of heterogeneity between the two study populations; I^2, heterogeneity index; Beta, beta coefficient; SE, standard error

Beta coefficients describe the effect sizes of genes in the analysis; they represent the magnitude of an independent variable's effect on the dependent variable in multiple regression analysis.

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