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Genome-wide association study of hemodynamic response to mental stress

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Genome-wide association study of hemodynamic response to mental stress

By

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Bachelor of Medicine
Fudan University
2014

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An abstract of
A thesis submitted to the Faculty of the
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Abstract

Genome-wide association study of hemodynamic response to mental stress
By Xi Liu

Background: Elevated hemodynamic responses might play an important role in the progress of mental stress-induced ischemia (MSI). Former genetic studies have identified a number of genetic loci associated to baseline hemodynamic measurements including heart rate, blood pressure and their product. However, no robust genetic associations have been linked to hemodynamic response to mental stress. Our study aims to understand the genetic determinants of hemodynamic response to mental stress among the Caucasians and African Americans.

Methods: A sample of patients with coronary artery diseases (CAD), including 375 Caucasian and 161 African American patients, were investigated using a genome-wide association approach. Mental stress was induced by a designed public speaking task. Adjusted for age, sex, beta blocker use, pre-stress measure and population structure, multiple linear regression models were applied to examine the association between genome-wide SNPs and hemodynamic responses to mental stress. A meta-analysis was carried out to combine the results from African American and Caucasian participants.

Results: After correction for multiple testing, rs12229466a SNP located in TMEM312D, was significantly associated with heart rate response to mental stress (meta-analysis p-value=7.90×10^{-9}) with beta coefficients of 3.521 (p-value= 0.00015) in African Americans, and 3.692 (p-value= 2.63×10^{-5}) in Caucasians. SNP rs12229466 was not associated with heart rate response to physical stress. For systolic blood pressure and rate-pressure product, several SNPs were identified with suggestive association with their response to mental stress.

Conclusions: rs12229466 in TMEM312D was found to be associated with heart rate response to mental stress in CAD patients in both African American and Caucasian cohorts. Larger studies and independent replication studies in other population are needed to replicate this finding. Further functional studies to follow up this genetic locus may reveal the regulation mechanism of heart rate response to mental stress.

Keywords: Genome-wide association study, Hemodynamic response, Mental stress, Mental stress-induced ischemia, heart rate

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Contents

Introduction

Mental Stress-Induced Ischemia (MSI)

Ischemia results from an insufficient supply of oxygen by coronary circulation compared to the excessive demand for oxygen[1]. Many studies have showed that mental stress is associated with ischemia and adverse outcome in patients with coronary artery diseases (CAD)[2]. According to the meta-analysis conducted by Wei, mental stress induces ischemia in about one third to one half of patients with CAD [3], while rates of MSI in people without CAD are in the range of 16% to 21%[4]. Additionally, MSI is associated with up to 3-fold increased risk of cardiac events in the future.[5] Therefore, MSI is more common than previously noticed and thus might have potentially more serious effects on human health.

Clinical Importance of hemodynamic response

Many studies have discussed the mechanisms of mental stress-induced ischemia. The possible mechanisms of MSI include mental stress-induced autonomic changes, attenuated coronary blood flow, exaggerated systemic vascular resistance, and also increased hemodynamic responses[6]. Increased hemodynamic responses, including heart rate and blood pressure, might play an important role in the progress of MSI.

Hemodynamic response can affect and adjust to conditions of the body, and is associated with myocardial oxygen consumption. There are many different hemodynamic parameters, such as cardiac index, cardiac output, stoke volume and clinic blood pressure[7]. One of these hemodynamic parameters, the rate-pressure product (RPP = heart rate × systolic blood pressure), can be a reliable index of myocardial oxygen consumption[8] and thus can be a good indicator of

the heart's metabolic demands when facing stress. Other parameters, such as blood pressure and heart rate, are also related to the mechanism of ischemia and other cardiovascular diseases. Previous work has shown that changes in heart rate and RPP parallel changes in myocardial blood flow, which is an important indicator to evaluate the functional improvement in patients with ischemia[9]. A relationship between high blood pressure responses and increased cardiac events has been documented in cardiovascular disease patients[10]. Additionally, blood pressure plays a vital role in the mechanism of matching oxygen demand and perfusion of the myocardium [11], and thus is related to the mechanism of myocardial ischemia.

The mechanism of hemodynamic response to mental stress in MSI is still not crystal clear. However, there are some possible explanations. Experiences of mental stress results in activation of stress response systems, such as the sympathetic nervous system, and subsequent hemodynamic responses. When facing mental stress, heart rate and blood pressure often increase, and then increased cardiac output and vascular resistance sustain the increased blood pressure. Dimsdale stated that epinephrine response can be triggered by mental stress and is associated with increased hemodynamic response[12]. Schneider suggested that in β -adrenergic responses are associated with increased reactivity and delayed recovery of hemodynamic response (including blood pressure and heart rate) among people at genetic risk of hypertension[13], and these increased reactivity and delayed recovery might lead to intermediate phenotypes in the mechanism of hypertension. Other studies [14-16] have also supported the idea that β-adrenergic response is related to types of stress, such as hostility. These findings supported the hypothesis that the sympathetic nervous system, especially α- and β-adrenergic receptors, might play an important role in the mechanism of hemodynamic response to mental stress.

Whether the mechanism of hemodynamic response to mental stress is the same as that to physical stress remains to be determined. Some studies suggest that MSI is maintained by the same hemodynamic mechanisms as exercise-induced ischemia, as they all involve increased RPP and oxygen demand[4]. On the other hand, the majority of studies showed that the mechanism of hemodynamic response to mental stress is distinct from the mechanism due to physical stress because MSI occurs at a relatively lower level of RPP than that of exercise-induced ischemia[1]. Schiffer suggested the reason for this difference is that mental stress might decrease the supply of myocardial oxygen[17]. Other differences between the mechanisms of hemodynamic response to mental stress and physical stress may lie in β -adrenergic-mediated oxidative dysregulation. Exaggerated blood pressure responses to stress may be associated with adverse cardiac outcome because of β -adrenergic–mediated oxidative dysregulation [18]. Many researchers have suggested that epinephrine levels might rise when mental stress occurs[19], while norepinephrine levels tend to increase when doing physical exercise[20]. These findings further support the idea that the mechanisms of hemodynamic response to mental stress and that to physical stress are not exactly the same.

The mechanism of hemodynamic response to mental stress in MSI still remains to be determined. Further studies about the mechanisms and genetic association related hemodynamic response to mental stress are needed.

Genetic Association

With the move toward advanced genotyping technologies, genome-wide association studies (GWAS) have become an efficient way to reveal associations between novel loci and complex diseases and traits. Many studies have been conducted on the candidate genes related to

hemodynamic response to stress. These studies may contribute to research on biological mechanisms and genetic factors of hemodynamic response to mental stress and MSI.

Among all the candidate genes, α- and β-adrenergic receptors (ARs) are the most well studied and are recognized as being associated with hemodynamic response to stress and many other cardiovascular phenotypes. The genetic variability of ADRB1 has been well studied. ADRB1 includes two common polymorphisms. One is C→G substitution occurring at nucleotide 1165, which causes an Arg to Gly substitution at codon 389, and the other is a A→G substitution at nucleotide 14, causing a Ser to Gly substitution at codon 49 [21]. Hassan's study indicated an association between Gly49 form of the receptor and ischemic response to mental stress among cardiovascular disease patients [22]. In this study of 148 patients, the adjusted odds ratio for the potential effect of genotype (Ser/Ser vs Gly carriers) on MSI was 3.9 (95% CI: 1.2-12.5) (p-value=0.02). This genetic marker might play an important role in the mental stress response. Many studies have also been done on the association between Arg to Gly substitution at codon 389 and cardiovascular phenotypes. The results showed that Arg389Gly polymorphism affects early heart rate response to beta-blockers [23].

Other candidate genes are also considered. Some genes in the endothelial and the serotonin systems, the renin-angiotensin-aldosterone, and the sympathetic nervous might play a role in the regulation of hemodynamic response to mental stress. In Rocha's research, he found that the polymorphisms in the genes that encode for END-1, END-2 are related to hemodynamic reactivity, and the ENOS 894G→T polymorphism is associated to altered hemodynamic responses to mental stress occurring before and after exercise [24]. Additionally, there are several studies about the neurotransmitter serotonin (5HT). Williams suggested that a 44-base

pair insertion/deletion polymorphism in this region is associated with increased hemodynamic response to stress [25].

Although not directly related to mental stress, there are a great amount of genomic studies associated with blood pressure and heart rate, which may provide better understanding of the genetic effects related to the mechanism of hemodynamic response. According to Fox's study, rs2258119, which is located in C21orf91gene region, was the most significant Single Nucleotide Polymorphisms (SNP) associated with systolic blood pressure (p-value=4.7×10^{-8}) [26]. Newton's study[27] of 34,433 subjects of European ancestry identified association between blood pressure and common variants in eight regions near the CYP17A1 (p-value= 7×10^{-24}), CYP1A2 (p-value = 1×10^{-23}), FGF5 (p-value= 1×10^{-21}), SH2B3 (p-value = 3×10^{-18}), MTHFR (p-value = 2×10^{-13}), c10orf107 (p-value = 1×10^{-9}), ZNF652 (p-value = 5×10^{-9}) and PLCD3 (p-value = 1×10^{-8}) genes. In the genetic studies of resting heart rate, a significant level of association ($p<5 \times 10^{-8}$) was identified for SNPs in two genes expressed in the heart: CANX and MAML1 [28]. In a two-stage meta-analysis of GWAS of 181,171 observations [29], 14 new loci associated with heart rate were identified, and the associations with all 7 previously established loci (in CD46, MYH6 and FADS1 and near GJA1, ACHE, SLC35F1 and LINC00477) were confirmed.

These genetic association studies have identified genetic loci associated with baseline hemodynamic measurements including heart rate and blood pressure [26-29], and also on cardiovascular mechanisms related to stress response [22-24, 30] . However, genetic association studies focusing on hemodynamic response to mental stress are sparse. This GWAS may help to uncover the genetic determinants and biological mechanism of hemodynamic response to mental stress.

Methods

Study Population

695 patients with a diagnosis of CAD were enrolled from Emory affiliated hospitals and clinics from July 2009 to July 2014. Patients who had heart failure within the last two months, an acute coronary syndrome, or unsteady psychological conditions were excluded from the study. The CAD related risk factors, previous cardiovascular events and related medications were collected by questionnaires and reviews. After merging with genotype data the GWAS sample includes 375 Caucasian patients and 161 African American patients.

Mental-Stress Procedure

All the patients underwent a mental stress test. In a quiet, temperature-controlled room, after 30 minutes rest, mental stress was induced by a designed public speaking task. Blood pressure and heart rate were continuously measured and RPP was calculated. Hemodynamic response to mental stress was defined as the magnitude of increase in heart rate, blood pressure and RPP from baseline to highest values during the mental stress task. All procedures were conducted by experienced staff to ensure the quality of the mental stress test, and the staff paid special attention to the potential psychophysiological stress–induced factors of the mental stress test. Anti-ischemic medications, such as β-blockers, were stopped for about 24 to 48 hours. Medical history was collected from clinical information systems and demographic and behavioral data were collected using standard questionnaires. This study was approved by the Emory University Institutional Review Board. The consent of participation was gained from all patients enrolled.

GWAS Data Quality Control

We used Illumina's Multi-Ethnic Genotyping (MEG) chip, which is optimized for GWAS in multi-ethnic populations, particularly population with African ancestry. The MEG chip directly measures 1.7 million genetic markers including improved genome-wide coverage of non-European ancestry, over 400,000 exonic markers, more than 17,000 variants relevant to clinical and pharmacogenetic studies, and an additional 23,000 hand-curated variants selected for functional, immunological, oncological, ancestry, forensic, and common and rare disease applications. Observations with SNP call rate and individual call rate < 95% were removed. SNPs with minor allele frequency (MAF) < 0.05 and significant Hardy-Weinberg Equilibrium (HWE) results (HWE P value $<10^{-6}$) were also removed. Additional checks on the quality of genotyping included visual inspection of genotype cluster plots. After quality control there were 1,614,035 SNPs remaining in the dataset of this GWAS.

Statistical Methods

We examined the association between genetic polymorphisms and hemodynamic responses (heart rate, blood pressure and RPP) to mental stress. Statistical analysis was conducted in African American and Caucasian patients separately, and the two groups were combined for meta-analysis. A linear regression model was used to examine the association between genetic polymorphisms and hemodynamic responses. The increased hemodynamic response (post - baseline) was used as a dependent variable, and genotype status was the fixed factor. Based on previous studies, the covariates in this model included the baseline hemodynamic measurement, age, gender and use of Beta-blocker. Top ten principal components (PCs) of genome-wide SNP

data were used to address population structure within each ethnicity. The model was as the following:

Model 1:

$$Hemodynamic\ response = \alpha + \beta_1 SNP + \beta_2(Baseline\ measurement) + \beta_3 Age + \beta_4 Gender + \beta_5 Beta\ blocker + \sum_{i=1}^{10} \beta_{i+5} PC_i + \varepsilon,$$

where α denotes intersection term; βs are correlation coefficients for predictor variables; hemodynamic response is either changes in heart rate, systolic blood pressure or RPP; baseline measurement is either baseline heart rate, blood pressure or RPP corresponding to the hemodynamic response measures.

The statistical significance level was based on two-tailed tests, and p-values $\leq 5\times10^{-8}$ were considered to be significant. Bonferroni correction for multiple testing was conducted. Quantile-Quantile Plot (Q-Q plot) was generated to compare expected and observed probability distributions. Manhattan plots were generated to see the distribution of p-values through all the chromosomes except X/Y chromosomes. Regional plots were also generated for significant results. An inverse variance based meta-analysis was carried out using metal in Linux environment to combine the results from African American and Caucasian cohorts.

In addition to main analysis, other outcomes were used in this model to validate the result of SNP sites significantly associated with hemodynamic response. Hemodynamic response (maximum –minimum) to mental stress and hemodynamic response to physical stress were used instead of hemodynamic response (post - baseline). Stratified analyses were conducted to

determine if there is a difference between beta-blocker users and non-beta-blocker users or patients with MSI and patients without MSI.

Most of statistical analyses were performed in the R statistical environment version 3.2.0. Linear regression was performed using plink, and meta-analysis was conducted by metal.

Results

The average age of patients was 59.5 in African American and 64.5 in Caucasian cohorts, respectively. Among the African American and Caucasian cohorts, 62.1% and 76.5% were male, respectively. The summary statistics of age, smoking status, body mass index (BMI) and beta-blocker use are summarized in Table 1.

A combined result of GWAS of heart rate response to mental stress in both African American and Caucasian cohorts identified one SNP with significant association and 7 SNPs with suggestive associations (Table 2). The top significant SNP, rs12229466, showed a significant association with heart rate response to mental stress (p-value=7.90×10^{-9}). The C allele was coded as the effective allele for heart rate response in both African American and Caucasian cohorts. Participants carrying one additional copy of C allele increases heart rate response to mental stress of approximately 3.6 beat per minute. The effects of rs12229466 were consistent in both cohorts, with beta estimate= 3.521 (p-value= 0.00015) in African American cohort and beta estimate= 3.692 (p-value= 2.63×10^{-5}) in Caucasian cohort. In the results of each race separately, there were several suggestive significant SNPs, such as rs73640516 (p-value=1.71×10^{-7}) in Caucasians and JHU_3.649841 (p-value=1.79×10^{-6}) in African Americans. But their results were not accordant between two ethnicities, with opposite direction of effect ethnicity. In Figure 1A, Q-Q plot of the meta-analysis results compared the observed p-value distribution to the expected p-value distribution, and showed a moderate deflation (inflation factor = 0.99). The Manhattan plot (Figure 2A) for the GWAS for heart rate response to mental stress presented the notable significance level of a SNP (rs12229466) above the genome-wide significance threshold of 5×10^{-8} (shown as a horizontal red line). In the regional plot (Figure 3), the $-\log_{10}$ p-values for association with heart rate response to mental stress were plotted for each SNP genotyped in the

region around rs12229466. Within a 400 kb region overlapping with TMEM312D gene, there were 28 out of 481 SNPs with p-value<0.05. The second significant SNP in this region, rs12821109 (p-value=2.928×10^{-5}, effective allele G), is located within 1 kb range of rs12229466. These two SNPs are in moderate LD (r^2 of 0.2-0.4).

A combined result of GWAS of blood pressure response to mental stress in both African American and Caucasian cohorts identified no SNP significantly associated, but eight SNPs with suggestive association (Table 3). In the results of each race separately, there were several suggestive significant SNPs, such as rs2156672 (p-value=1.48×10^{-6}) in Caucasians and rs7686843 (p-value=3.9×10^{-7}) in African Americans. Two out of eight SNPs had results with opposite direction of effect ethnicity, while the results of other SNPs in both cohorts were consistent in directions. In Figure 1B, Q-Q plot of this meta-analysis results comparing the observed p-values to the expected p-values showed that the tests statistics are aligned with their normal distributions (inflation factor = 1.01). The Manhattan plot for the GWAS for blood pressure response to mental stress presented the distribution of significance level of SNPs (Figure 2B).

A combined result of GWAS of RPP response to mental stress in both African American and Caucasian cohorts identified 10 SNPs with suggestive associations (Table 4). The top significant SNP, rs12229466, had positive estimated beta coefficients in both cohorts, and its p-value is 4.09×10^{-7}. The levels of significance for rs12229466 were consistent between the two cohorts of African American and Caucasian, with beta estimate= 941.2 (p-value= 0.0001078) in African American cohort and beta estimate= 666.3 (p-value= 0.001215) in Caucasian cohort. In the results of each race separately, there were several suggestive significant SNPs, such as rs73640516 (p-value=6.3×10^{-7}) in Caucasian cohort and rs1454341 (p-value=1.43×10^{-6}) in

African American cohort. Two SNPs had results with opposite direction of effect ethnicity, while the results of other SNPs with suggestive associations in both cohorts were consistent in directions. Q-Q plot (Figure 1C) of this meta-analysis results comparing the observed p-values to the expected p-values showed that the tests statistics are aligned with their normal distributions (inflation factor = 0.98). The Manhattan plot (Figure 2C) for the GWAS for heart rate response to mental stress presented the distribution of significance level of SNPs.

To further assess the genetic effects of rs12229466, we conducted secondary analyses of additional phenotypes and in subgroups. The results of these genetic association analyses were summarized in Table 5. The identified SNP rs12229466, was similarly associated with another definition of hemodynamic response (e.g., difference between maximal level of heart rate after mental stress and minimal level of heart rate before mental stress: $HR_{MaxPost\text{-}stress}$-$HR_{MinPre\text{-}stress}$) to mental stress in both African American and Caucasian cohorts, with p-value= 0.000149 and 1.79×10^{-5}, respectively. While rs12229466 was not associated with hemodynamic response to physical stress in in both African American and Caucasian cohorts, with p-value=0.9825 and 0.8004, respectively. In the analysis in subgroups, the associations between rs12229466 and heart rate response to mental stress among patients with or without MSI were both suggestive significant, with both positive beta estimate. While the association between rs12229466 and heart rate response to mental stress was not statistically significant among non-beta-blocker users, there was a suggestive association among beta-blocker users.

Discussion

In this GWAS, one SNP was statistically associated with heart rate response to mental stress in CAD patients in both African American and Caucasian cohorts. The results of analysis using other measures of heart rate response and in subgroups also supported this association. This SNP, rs12229466, is located in gene encoding transmembrane protein 132D (TMEM132D).

Former studies [31-33] have suggested that TMEM132D might have a role in threat processing, and is related to panic disorder (PD). A GWAS found association between PD and a SNP (rs7309727) as well as a haplotype in TMEM132D[32]. Additionally, Inoue A[33] suggested that TMEM132D variants might increase vulnerability to panic. Another study provided evidence for association of PD with the gene TMEM132D in three independent German samples [31] . This study further supported the association of PD with the gene TMEM132D by a mouse model in which anxiety-related behavior was related to a TMEM132D SNP and expression of TMEM132D mRNA in the anterior cingulate cortex. These evidence supported the hypothesis that TMEM132D is relevant to the mechanism involved in the mental stress response. Thus the studies of TMEM132D and its possible association with mental stress-induced response are needed to further explore the mechanisms involved.

Genotype Tissue Expression project (GTex) analysis shows that TMEM132D is highly expressed across typhoid and whole blood tissue. There are 39 SNPs in TMEM132D region having significant or suggestive association with gene expression related to whole blood. For example, rs517020 is associated with gene expression related to whole blood, with p-value=4.0×10^{-8} and effect size=0.26. Giving TMEM132D's role in regulating gene expression, it is plausible that TMEM132D is related to hemodynamic response to mental stress via regulation of key pathways of heart rate control.

Replication is a critical strategy to reduce false-positive associations with traits in GWAS. In this study, internal replication was included by the study of the same phenotype in African American and Caucasian cohorts. The results of rs12229466 were of a similar magnitude of effect and significance in the same model and same direction in both cohorts. Future plans about independent replication would be extended to related phenotypes or in different populations. Additional replication studies with bigger sample size would be helpful to increase confidence in the consistency of the results.

This study had its limitations. Lack of information about gene function might be important limitations for this GWAS. In GWAS, conservative correction was used to correct for multiple testing, and this may lead to mask true positives. So some associations might be missing from the pre-setting threshold. Additionally, the sample size limited the ability of this study to identify smaller effects of genetic variations on hemodynamic response to mental stress. Besides, genotype imputation, which has been widely used in GWAS to boost power and facilitate the combination of results across meta-analysis, can be implemented in this study. Future studies of large sample size are needed to fully understand the effect of genetic variations on hemodynamic response to mental stress. The independent replication studies in other population are also needed, and genotype imputation can be used to facilitate these replication studies. Further studies focusing on identifying functional genetic variants and characterizing the gene function in relation to heart rate response to mental stress are of great importance to understand the mechanism of hemodynamic response to mental stress.

Tables and Figures

Table 1. Demographic information for the CAD patient cohorts stratified by race

	African American(N=161)	Caucasian(N=375)
Male	100(62.1%)	287(76.5%)
Age	59.5(9.0)	64.5(8.9)
Smoking	102(63.3%)	220(58.7%)
BMI	31.0(5.6)	29.5(5.2)
Beta-blocker	139(86.3%)	264(70.4%)

Table 2. SNPs related to heart rate response to mental stress in CAD patients

			Meta-Analysis			African American					White				
SNP	A1	CHR	Beta	P-value	Direction	MAF	Beta	SE	Stat	P-value	MAF	Beta	SE	Stat	P-value
rs12229466	C	12	3.61	7.90E-09	++	0.276	3.521	0.904	3.896	0.00015	0.2	3.692	0.867	4.259	2.63E-05
rs12458772	T	18	2.632	2.41E-06	-+	0.487	-2.021	0.909	-2.223	0.02783	0.399	3.002	0.707	4.245	2.79E-05
rs7952385	A	11	3.3	3.01E-06	++	0.125	2.927	1.235	2.371	0.01906	0.162	3.482	0.862	4.04	6.59E-05
JHU_12.129082071	T	12	3.152	4.55E-06	-+	0.432	-3.139	0.891	-3.523	0.000573	0.153	3.171	1.081	2.934	0.003564
rs547905	G	1	-2.637	5.27E-06	+-	0.357	1.983	0.867	2.287	0.02368	0.463	-3.164	0.778	-4.066	5.88E-05
JHU_1.183155716	C	1	-2.605	6.36E-06	+-	0.371	2.204	0.862	2.558	0.01158	0.451	-2.932	0.777	-3.772	0.000189
rs9442924	A	6	2.81	8.41E-06	++	0.363	3.623	0.868	4.173	5.19E-05	0.233	1.901	0.918	2.07	0.03921
JHU_15.41896256	T	15	3.199	8.55E-06	++	0.099	4.758	1.37	3.473	0.000682	0.275	2.607	0.844	3.088	0.002172

*A1: effective allele; CHR: chromosome; MAF: minor allele frequency.

Table 3. SNPs related to systolic blood pressure response to mental stress in CAD patients

			Meta-Analysis			African American					White				
SNP	A1	CHR	Beta	P-value	Direction	MAF	Beta	SE	Stat	P-value	MAF	Beta	SE	Stat	P-value
rs2349466	A	7	4.742	1.63E-06	++	0.3727	2.718	1.902	1.429	0.1553	0.3351	5.491	1.16	4.743	3.05E-06
JHU_8.20352422	G	8	-5.548	3.37E-06	++	0.4845	3.669	1.789	2.051	0.04205	0.1303	7.056	1.60	4.402	1.41E-05
rs11204117	T	8	5.459	3.44E-06	-+	0.4658	-3.737	1.732	-2.158	0.03259	0.1316	6.932	1.60	4.328	1.95E-05
rs2086329	T	15	4.556	4.09E-06	++	0.1863	3.397	2.333	1.456	0.1476	0.363	4.809	1.09	4.405	1.40E-05
rs4921720	T	8	5.495	4.26E-06	++	0.4907	3.542	1.793	1.976	0.05005	0.1303	7.056	1.60	4.402	1.41E-05
exm2266718	G	8	5.692	4.68E-06	-+	0.4037	-4.301	1.936	-2.222	0.02786	0.137	6.667	1.62	4.112	4.87E-05
rs1003979	A	6	4.128	8.22E-06	++	0.4845	6.2	1.741	3.561	0.000502	0.4481	3.312	1.09	3.031	0.002618
rs11099710	G	4	-6.384	8.90E-06	--	0.06211	-4.682	3.822	-1.225	0.2224	0.1383	-6.664	1.55	-4.297	2.23E-05

*A1: effective allele; CHR: chromosome; MAF: minor allele frequency.

Table 4. SNPs related to RPP response to mental stress in CAD patients

			Meta-Analysis			African American					White				
SNP	A1	CHR	Beta	P-value	Direction	MAF	Beta	SE	Stat	P-value	MAF	Beta	SE	Stat	P-value
rs12229466	C	12	784.4	4.09E-07	++	0.276	941.2	236.3	3.983	0.000108	0.2	666.3	204.32	3.261	0.00122
rs34150094	T	7	1025.7	1.25E-06	++	0.081	993.2	431.3	2.303	0.02273	0.133	1036	242	4.281	2.42E-05
kgp22768189	T	11	950.6	1.71E-06	++	0.115	1197	326.4	3.667	0.000346	0.138	805.7	249.44	3.23	0.00135
rs66587648	T	1	784.3	2.33E-06	++	0.146	830.7	313.6	2.649	0.008973	0.253	766.2	195.11	3.927	0.0001
rs1920244	G	3	722.9	2.93E-06	++	0.382	778.9	212	3.674	0.000337	0.177	659.2	225.14	2.928	0.00363
rs12585556	A	13	796.1	2.96E-06	++	0.357	975.9	225.4	4.329	2.81E-05	0.113	556.9	259.14	2.149	0.03227
JHU_11.104996021	C	11	-665.1	4.16E-06	+-	0.217	967.5	266.7	3.627	0.000398	0.463	-539.5	171.27	-3.15	0.00177
rs11626672	G	14	1040.6	4.53E-06	++	0.13	514.5	340.5	1.511	0.133	0.082	1461	303.3	4.817	2.16E-06
rs1454341	A	4	615.5	8.13E-06	+-	0.388	1120	222.5	5.033	1.43E-06	0.487	-300.6	175.17	-1.716	0.08708
rs9608852	T	22	661.1	9.94E-06	++	0.267	459.5	247.7	1.855	0.0657	0.265	776.9	187.07	4.153	4.11E-05

*A1: effective allele; CHR: chromosome; MAF: minor allele frequency.

Table 5. The summary of secondary analysis for the association between rs12229466 and heart rate response

		African American			Caucasian		
		N	Beta(SE)	p-value	N	Beta(SE)	p-value
Response to Physical Stress		161	0.054(2.455)	0.9825	375	0.349(1.378)	0.8004
Heart Rate Response (max-min)		161	4.092(1.050)	0.000149	375	4.509(1.037)	1.79E-05
Stratified Analysis							
By Beta-Blocker use	Yes	139	3.608(0.976)	0.000328	264	3.841(1.088)	0.000496
	No	21	-1.238(2.862)	0.6804	112	2.76(1.534)	0.07513
MSI	Yes	20	1.082(7.03)	0.8851	43	9.817(4.308)	0.03112
	No	139	3.128(1.028)	0.002881	327	2.486(0.812)	0.0024

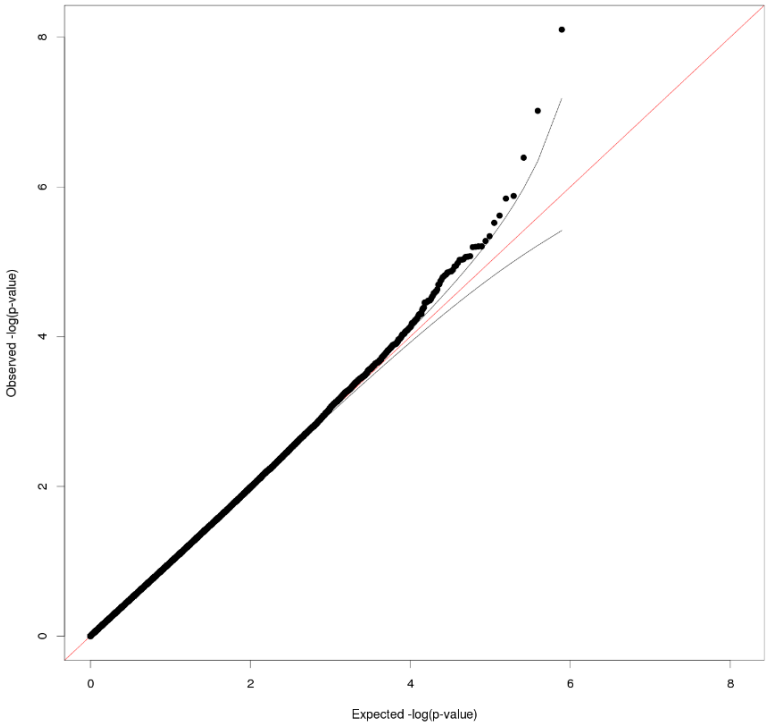

Figure 1A Q-Q plot for the meta-analysis for heart rate response

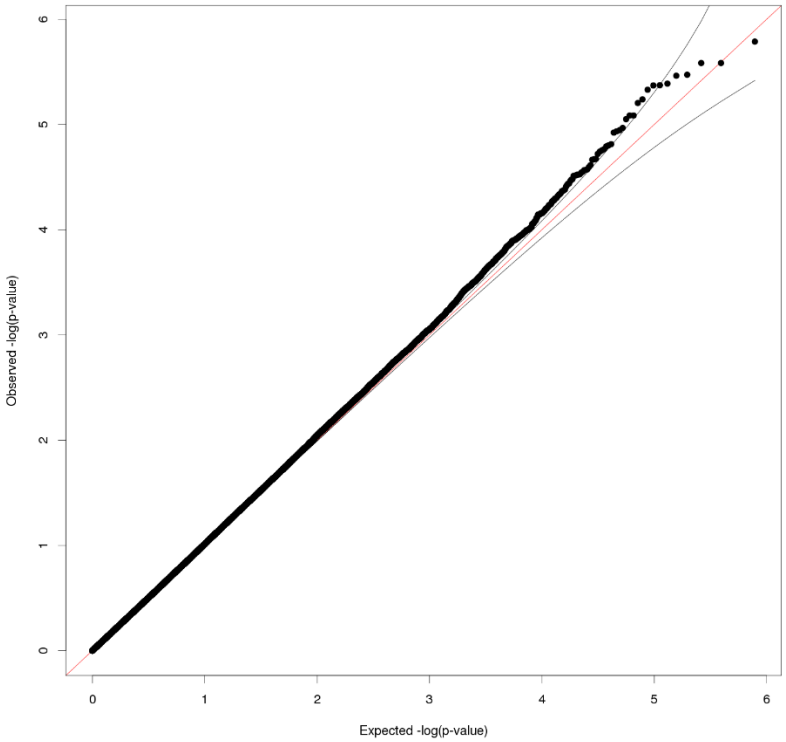

Figure 1B Q-Q plot for the meta-analysis for blood pressure response

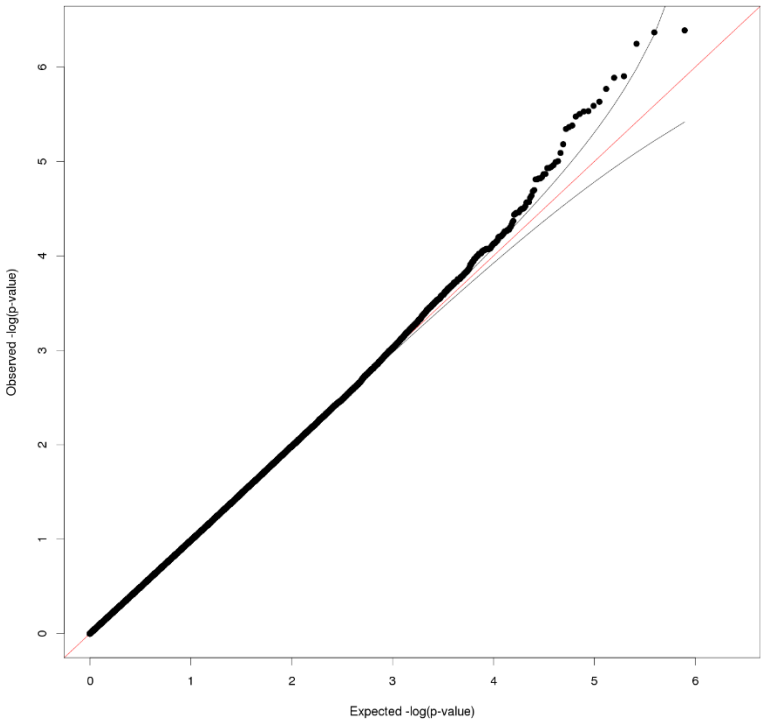

Figure 1C Q-Q plot for the meta-analysis for RPP response

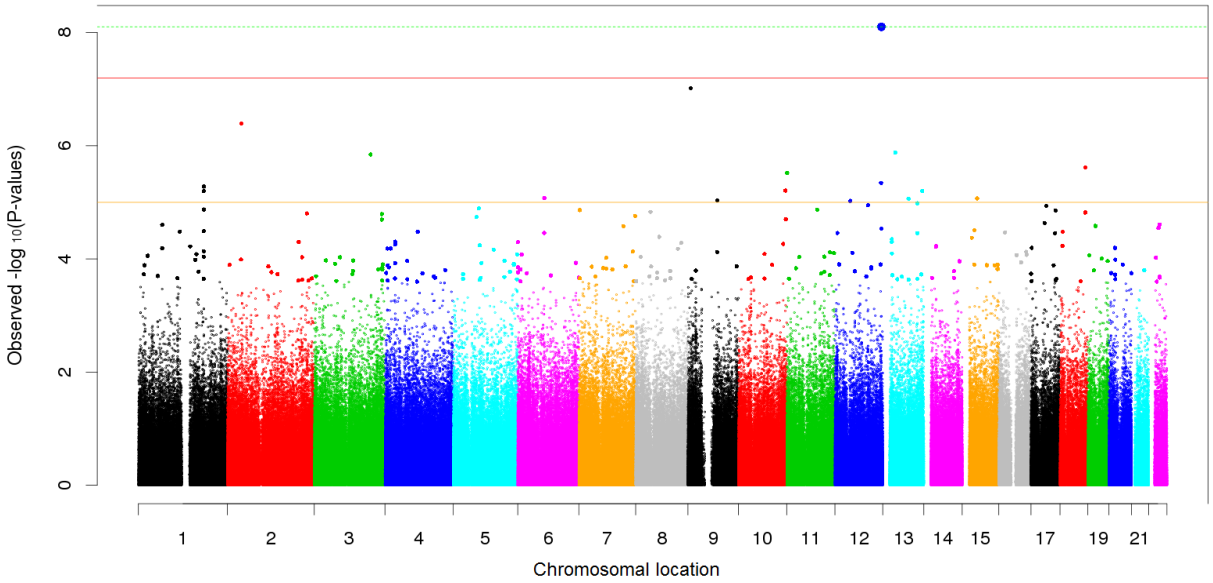

Figure 2A Manhattan plot of the association between SNPs and heart rate response

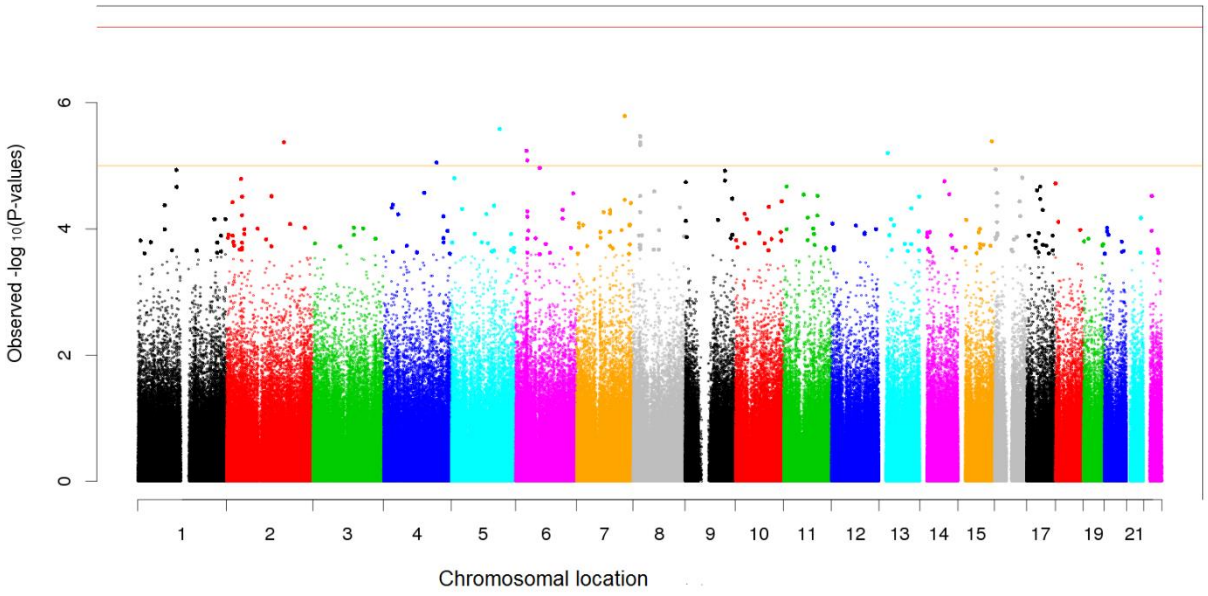

Figure 2B Manhattan plot of the association between SNPs and blood pressure response

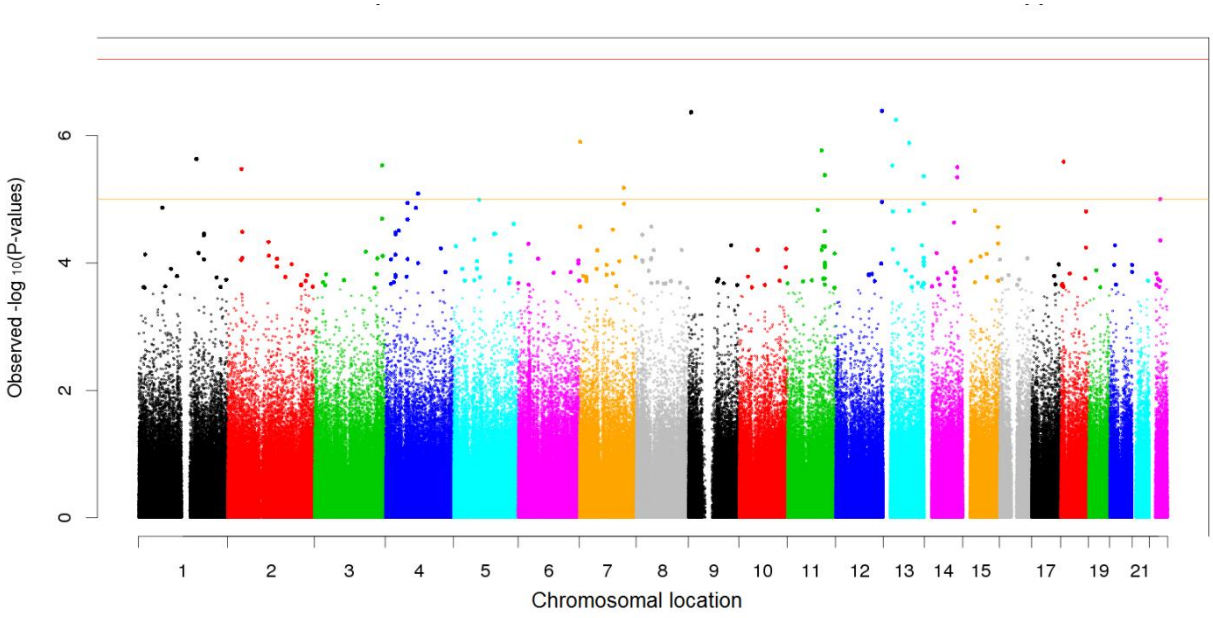

Figure 2C Manhattan plot of the association between SNPs and RPP response

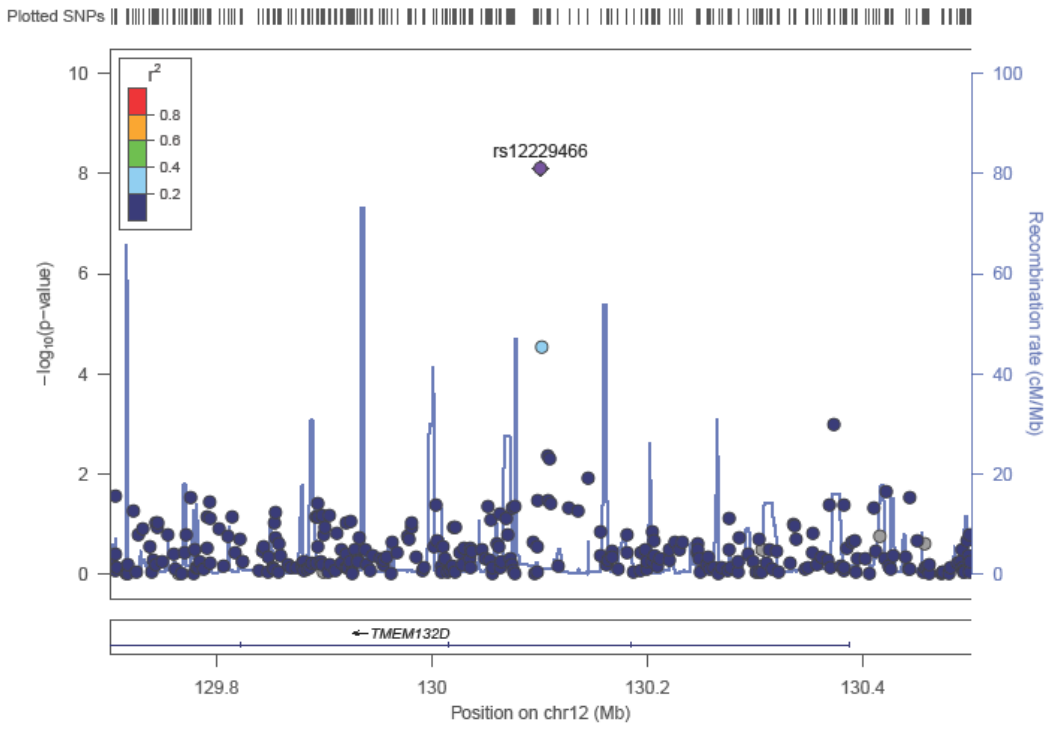

Figure 3A Regional plot of rs12224496 in TMEM312D gene with African reference

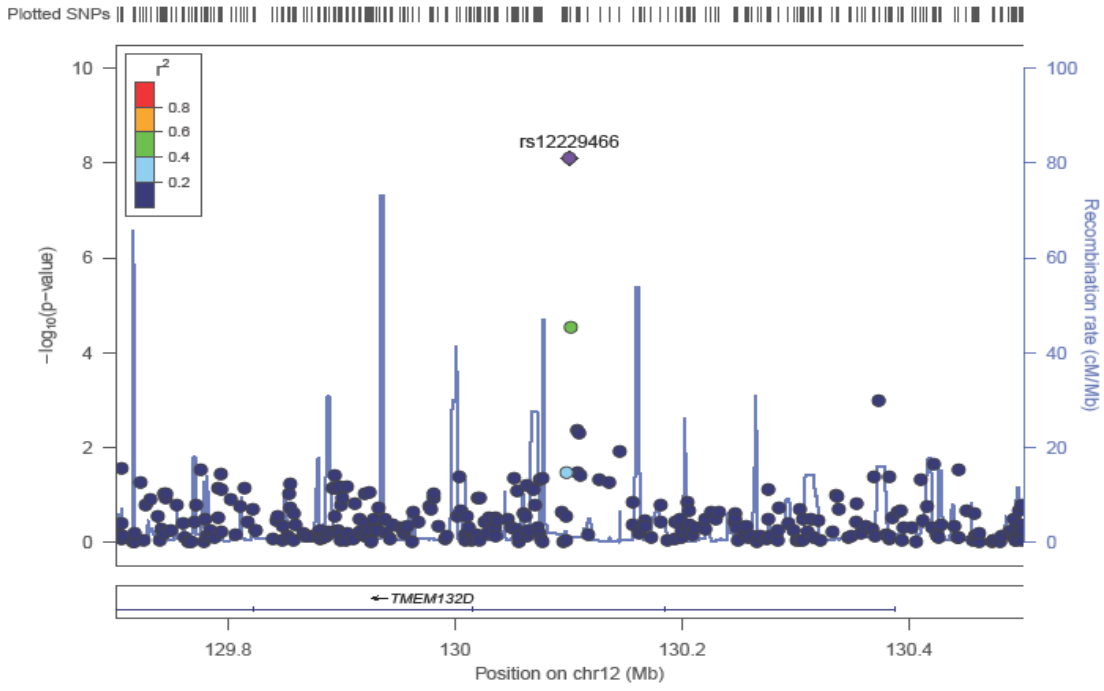

Figure 3B Regional plot of rs12224496 in TMEM312D gene with Europe reference

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