

Evaluation of How Gene-Job Strain Interaction Affects Blood Pressure in the PAMELA Study

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Objective: To see whether there is interaction between single nucleotide polymorphisms (SNPs) belonging to candidate genes for hypertension and job strain and its components (job demand and job control) in an accurately phenotyped Italian cohort (Pressioni Arteriose Monitorate E Loro Associazioni). **Methods:** From the Pressioni Arteriose Monitorate E Loro Associazioni cohort, we sampled 924 employed men and women who had completed a modified version of the Karasek questionnaire and for whom deoxyribonucleic acid information was available. We selected 1,510 common tagging and putative functional SNPs in 92 candidate autosomal genes involved in sympathetic nervous system, oxidative stress, renin-angiotensin-aldosterone system, and sodium balance. The interaction of each candidate SNP with the perceived job stress scores on ambulatory systolic and diastolic blood pressures was evaluated by adding product terms to a multiple regression linear model, adjusted for sex, age, total cholesterol, and body mass index. **Results:** Among all the SNPs tested, the interaction of SNP rs11210278 located on the endothelin 2 gene and job control was statistically significant after controlling for multiple testing, using false discovery rate (unadjusted $p = .0000661$; p adjusted for false discovery rate = .0085), indicating an inverse association with systolic blood pressure for the homozygous wild-type allele (C/C) and a positive association for the variant genotypes (C/T and T/T). **Conclusions:** We show that the hypertensive response to job strain is present only in carriers of the rs11210278 wild-type genotype. The phenomenon is discussed in relationship to the controversial results of field studies on job strain and hypertension. **Key Words:** job strain, blood pressure, candidate genes, stress perception, psychophysiology.

BMI = body mass index; **BP** = blood pressure; **CI** = confidence interval; **DBP** = diastolic blood pressure; **EDN2** = endothelin 2; **FDR** = false discovery rate; **MONICA** = MONItoring Cardiovascular diseases; **MOPSY** = MONica PSYchosocial; **PAMELA** = Pressioni Arteriose Monitorate E Loro Associazioni; **SBP** = systolic blood pressure; **SNP** = single nucleotide polymorphism.

INTRODUCTION

Psychosocial stress is considered to be one of the major risk factors that may contribute to the development of essential hypertension (1). Perceived work-related stress (job strain) seems to play a double role on increasing cardiovascular risk for its ubiquitous nature and long-term impact (2,3). It may act indirectly by inducing unhealthy life-styles, such as smoking, reduced physical activities, increased calorie intake (4,5), and/or it may act directly by affecting major regulatory systems and contributing to increase arterial pressure (3,5,6).

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The biological plausibility of job strain hypothesis in the genesis of hypertension is loosely confirmed in a variety of field studies. Positive findings, especially among men, have been found in several studies, using mainly ambulatory blood pressure (BP) (7–15) and the Karasek model for perceived stress (16). However, not everyone who develops hypertension reports job strain and vice versa. The traditional psychological explanation is that some people may not perceive stress because of a denial attitude, a noncomplaining tendency, or alexithymia, which unconsciously transfer anxiety into a somatic disorder (17). Another possible explanation is that job strain operates on a background of genetic susceptibility, as suggested by Imumorin et al. (18) in the gene-environment interaction model of stress-induced hypertension. Borderline significant interactions between two single nucleotide polymorphisms (SNPs) and job strain have been shown in men, both for systolic (SBP) and diastolic blood pressures (DBP) (19,20).

We (21) have previously shown an association between job strain and ambulatory BP in the Pressioni Arteriose Monitorate E Loro Associazioni (PAMELA) Italian cohort. This association was also observed with clinic BP in four general population samples recruited in “Area Brianza,” near Milan, Italy, as part of the World Health Organization (WHO) “MONItoring Cardiovascular diseases” (MONICA) project (22).

In this study, we tested the hypothesis that genetic variants in genes involved in pathways of BP regulation and cardiovascular risk may interact with job strain and its dimensions and, thus, influence BP.

METHODS

PAMELA Study

The PAMELA study design has been reported elsewhere (23). Briefly, 2051 people were selected randomly from the Monza population in 1991 to represent gender and age decades (25–74 years), according to the criteria used in the WHO MONICA study, conducted in the same geographic area (24). Participants were invited to come to the outpatient clinic of the local hospital (San

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Gerardo) in the morning of a work day (Monday through Friday) where detailed phenotypic data were collected.

The study was approved by the ethics committee of the San Gerardo Hospital in Monza, Italy. To test the SNP \times job strain interaction, we selected only men and women who were employed (currently working with a stable job), who completed the MONICA PSYchosocial (MOPSY) questionnaire, and for whom deoxyribonucleic acid was available ($n = 990$). After excluding those on BP-lowering treatment ($n = 66$), we had a final sample comprising 924 people. All patients were Caucasian origin.

Phenotype Definitions

Data relevant to the present study include three sphygmomanometric BP measurements with the subject in the sitting position, starting 10 minutes after the beginning of the medical visit and 24-hour ambulatory BP monitoring, using an oscillometric device (90207, Spacelabs Healthcare, Issaquah, Washington), with BP readings set at 20-minute intervals. After the device was checked for accuracy, the participants were sent home with instructions to participate in their usual activities and to come back the next morning for device removal. Sitting BP measures were taken before ambulatory monitoring. For the present analysis, we averaged the ambulatory SBP and DBP values for 24 hours. We concentrated on the averaged ambulatory BP measures, as those have been previously shown to better correlate with stress perception, with a similar behavior during work, leisure time, and night—“carry over effect” (25). Additional data included two home BP measurements (approximately at 7 AM and 7 PM) made with a semiautomatic device (HP 5331, Philips, Andover, Massachusetts) on the arm contralateral to that used for ambulatory monitoring and three more sphygmomanometric sitting BP measurements taken after removal of the ambulatory BP device.

Information (history and physical examination) on cardiovascular risk factors, including overweight, smoking habits, serum cholesterol, blood glucose, diabetes mellitus, antihypertensive treatments, and history of previous cardiovascular morbid events, were obtained.

Job Strain Assessment

Each employed participant was asked to complete the MOPSY questionnaire, in which a short job strain scale derived from the Karasek questionnaire was included. The scale (set up at the WHO Regional Office for Europe, within the framework of the MONICA Project) was composed of 13 items, of which six items dealt with job control and five items dealt with perceived job demand (26). The remaining two items on social support were not considered for the present analysis. The Italian version of the MOPSY questionnaire was validated at the time of the first MONICA survey (27): Internal consistency (Cronbach α coefficient) resulted in 0.72 for job control and 0.6 for job demand; in addition, the two dimensions resulted in an orthogonal relationship, as foreseen by the model, with a Pearson correlation coefficient varying from 0.01 in men to 0.05 in women.

Job strain evaluation was obtained through the traditional quadrant term approach (28), using as cutoff points the overall sample medians of the two considered scores. The participants were classified into the low strain group (group 1) if they scored equal or below the median of the job demand scale and above the median of the job control scale. Similarly, the passive condition (group 2) was determined by scores equal or below the sample median for both dimensions; the active condition (group 3) by demand and control scores above the correspondent sample medians; and the high strain (group 4) condition by levels of job demand above the sample median and job control equal or below the sample median. In addition, according to the levels of each job strain score, participants were classified into tertile levels of job demand (cutoff points: low tertile, <27 ; medium tertile, 27–30; and high tertile, ≥ 31) and job control (cutoff points: low tertile, <69 ; medium tertile, 69–75; and high tertile, ≥ 75).

SNP Selection and Genotyping

SNP selection and genotyping were done as previously described (29). We selected 1,510 common tagging and putative functional SNPs in 92 candidate autosomal genes with high biological plausibility in the development and progression of cardiovascular disease. The genes chosen were mainly involved in sympathetic nervous system, oxidative stress, renin-angiotensin-aldosterone system, and sodium balance. We selected those SNPs that tagged common variants (minor allele frequency, ≥ 0.1) in the HapMap Caucasian European reference panel with $r^2 \geq 0.8$ by assessing each gene, including 10-kb flanking region within the HapMap database (<http://www.hapmap.org/>). We also

added to the list common potentially functional SNPs from SeattleSNPs (<http://pga.gs.washington.edu/>). All SNPs were tested against the predicted score of success provided by Illumina (<http://www.illumina.com/>), and the final selected SNPs fulfilled the following parameters—minor allele frequency, ≥ 0.05 ; r^2 , ≥ 0.8 ; Illumina score, >0.6 ; and no tag SNPs within 60 bp of each other. We had a 100% coverage for all the genes selected.

The list of candidate genes, along with their functional class, chromosome location, and number of SNPs is shown in Supplemental Digital Content 1 (<http://links.lww.com/PSYMED/A33>). Genotyping was performed on the Illumina BeadChip platform (<http://www.illumina.com/>).

Statistical Methods

Quality checking included manual review of all cluster plots, genotype frequency, call rate, and deviation from Hardy-Weinberg equilibrium, using the χ^2 test as previously described (29).

Analysis of variance and the χ^2 test were used to compare continuous and categorical variables, respectively, between job strain quadrant term and/or genotype categories.

The interaction of each candidate SNP with the perceived job stress scores (job strain quadrant term, tertiles of job demand, tertiles of job control) on ambulatory SBP and DBP was evaluated by adding product terms to a multiple regression linear model, adjusted for the following covariates: sex, age, total cholesterol, and body mass index (BMI), using an additive model. The significant results were additionally tested with clinic and home BP readings correcting for the same covariates.

Job stress scores were treated as categorical variables. *F* test was used to assess the significance of the interaction terms. Because testing multiple SNPs could lead to false-positive associations, we estimated the false discovery rate (FDR) and used an FDR threshold of 0.05, indicating that we should expect only 5% of the declared discoveries below this threshold to be false (30). We applied the FDR multiple testing correction to 1,324 tests for each of the 3×2 combinations of job stress and outcome variables separately to balance the loss of power that would result if we assumed independence between outcomes and/or stress dimensions. The assumption underlying the classical method of Benjamini and Hochberg (30) that we used for FDR could lead to very conservative inference, as it assumes that all the hypotheses tested are independent or weakly correlated. In our study, the independence assumption could be considered true for the very great majority of the 1,324 SNPs tested, but it does not apply to the two outcome measures (SBP and DBP) and/or to the three job stress dimensions (job control score, job demand score, and job strain quadrant).

RESULTS

The characteristics of the PAMELA study subjects included in this analysis are presented in Table 1. Men accounted for 62% of the total cohort. Participants scored above the sample median of the demand scale (ie, high strain + active quadrants) were younger than those who scored below the median (ie, low strain + passive quadrants), both in men ($p = .02$) and in women ($p = .006$). In women, BMI and total cholesterol were significantly different across stress perception categories. In our study sample, ambulatory SBP and ambulatory DBP did not differ significantly between stress perception categories, both in men ($p = .38$ for ambulatory SBP; $p = .99$ for ambulatory DBP) and in women ($p = .76$ for SBP; $p = .61$ for ambulatory DBP). The same was observed for all the clinic and home readings.

As previously reported (29), of the 1,510 autosomal SNPs selected, after quality control, 105 were excluded because of poor clustering, 55 were excluded because of Minor Allele Frequency <0.01 , 26 were excluded because of deviation from Hardy-Weinberg equilibrium; for the final analysis, 1,324 (86%) SNPs were included. Genotyping was successful in 99.48% of individuals.

TABLE 1. Participant Characteristics by Stress Perception Category and Sex (n = 924)

	Stress Perception				p
	Low Strain	Active	Passive	High Strain	
Men, n	156	154	166	97	
Age, years	45.1 (9.9)	42.5 (10.4)	44.6 (11.1)	41.6 (9.7)	.02
Body mass index, kg/m ²	26 (3.8)	25.4 (3.5)	25.3 (3.3)	25.9 (3.7)	.24
Total cholesterol, mg/dL	223.7 (41.3)	218.8 (45.6)	215.7 (45.2)	215.4 (41.1)	.34
HDL cholesterol, mg/dL	50 (13.8)	49.8 (15.1)	48.5 (13.5)	51.7 (12.6)	.35
Serum triglyceride, mg/dL	131.6 (95.9)	122.8 (72.4)	122.8 (75.5)	114.4 (65.5)	.40
Current smokers, n (%)	54 (34.6)	50 (32.5)	66 (39.8)	36 (37.1)	.57
Current alcohol drinkers, n (%)	101 (64.7)	97 (63.4)	107 (64.5)	61 (63.5)	.99
Ambulatory SBP, mm Hg	120.7 (10.2)	120 (10.1)	121.5 (10.2)	121.9 (9.2)	.38
Ambulatory DBP, mm Hg	76.2 (7.2)	76.1 (6.3)	76.3 (7.1)	76.1 (6.9)	.99
Clinic SBP, mm Hg	128.4 (16.3)	126.5 (14.7)	129.7 (16.8)	128.2 (14.4)	.59
Clinic DBP, mm Hg	84.9 (9.9)	84.2 (8.9)	85.1 (9.7)	84.1 (9.8)	.76
Home SBP, mm Hg	125.3 (14.3)	121.7 (14.3)	125.1 (15.9)	125.2 (13.3)	.68
Home DBP, mm Hg	77.8 (8.4)	76.1 (8.9)	77.5 (9.6)	77.2 (8.8)	.83
Women, n	77	55	130	89	
Age, years	42.5 (10.4)	38.6 (9.9)	41.2 (10.1)	37.6 (9.0)	.006
Body mass index, kg/m ²	24.7 (4.6)	23.8 (3.7)	23 (3.3)	24 (5.1)	.03
Total cholesterol, mg/dL	218.8 (53.9)	210.7 (47.2)	210.2 (34.5)	198 (42)	.02
HDL cholesterol, mg/dL	61.9 (16.9)	58.3 (16)	63.4 (13.4)	61.6 (14.7)	.20
Serum triglyceride, mg/dL	88.5 (43.9)	85 (56)	76.8 (38.1)	74.7 (42.3)	.13
Current smokers, n (%)	22 (28.6)	14 (25.5)	33 (25.4)	22 (24.7)	.95
Current alcohol drinkers, n (%)	27 (35.1)	17 (30.9)	33 (25.4)	29 (32.9)	.45
Ambulatory SBP, mm Hg	114.7 (10.5)	114.3 (7.7)	113.4 (9.8)	114.3 (8.3)	.76
Ambulatory DBP, mm Hg	71.6 (7.1)	71.8 (6.7)	70.7 (6)	71.3 (5.9)	.61
Clinic SBP, mm Hg	122.2 (18.1)	116.6 (14.4)	119.9 (17.4)	118.2 (14.2)	.24
Clinic DBP, mm Hg	78.8 (10.2)	77.4 (10.6)	78.2 (10.6)	77.7 (10.2)	.61
Home SBP, mm Hg	113.9 (15.7)	109.7 (13.5)	111.1 (16.4)	111.1 (14.1)	.34
Home DBP, mm Hg	72.1 (9.7)	68.6 (9.5)	71.4 (10.4)	71.1 (10.2)	.94

Data are reported as mean (standard deviation) values.

HDL = high-density lipoprotein; SBP = systolic blood pressure; DBP = diastolic blood pressure.

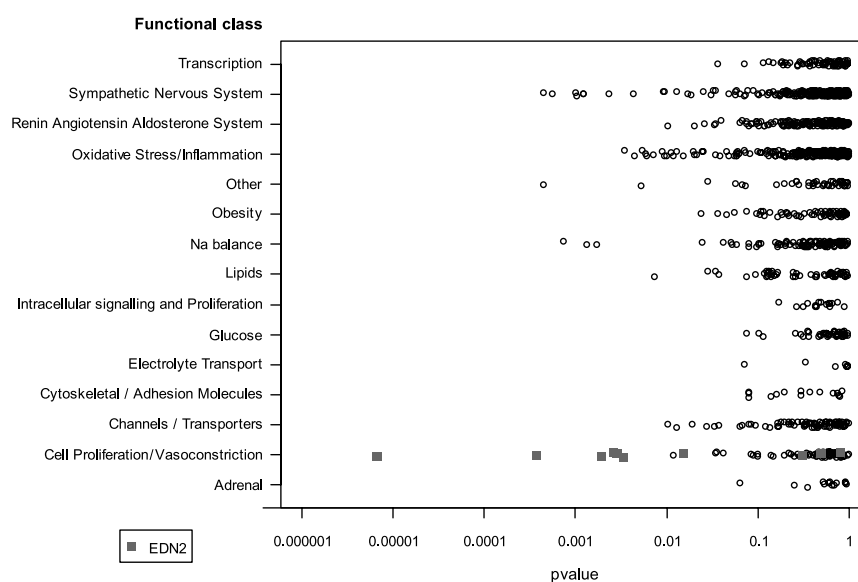


Figure 1. Plot of the p values from the F test measuring single nucleotide polymorphism (SNP)-job control interaction effect on ambulatory systolic blood pressure, by genetic functional class. The 11 SNPs on endothelin 2 (EDN2) gene are marked with red squares. Na = sodium.

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TABLE 2. Ambulatory SBP and DBP Adjusted Means by rs11210278 Genotype and Job Control Levels

rs11210278 Genotype	Job Control (Score Tertiles)	Ambulatory SBP		Ambulatory DBP	
		Adjusted Mean ^a	95% CI	Adjusted Mean ^a	95% CI
Wild-type (C/C)	Low (n = 215)	119.5	118.2–120.7	74.6	73.7–75.4
	Medium (n = 245)	118.6	117.5–119.8	74.5	73.7–75.3
	High (n = 160)	115.9	114.5–117.3	73.1	72.2–74.1
Heterozygous (C/T)	Low (n = 87)	117.1	115.6–118.7	73.9	72.9–74.9
	Medium (n = 95)	118.7	117.2–120.2	74.5	73.5–75.6
	High (n = 82)	120.2	118.6–121.9	75.5	74.4–76.6
Homozygous mutant (T/T)	Low (n = 15)	114.8	111.7–117.9	73.2	71.1–75.3
	Medium (n = 13)	118.8	115.7–121.9	74.6	72.4–76.7
	High (n = 12)	124.5	121.2–127.9	77.9	75.6–80.2
Interaction <i>p</i> value ^b		0.00000661		0.0031	

^a Adjusted for age, sex, body mass index, and total cholesterol in a multiple linear regression model. Adjusted means are evaluated at the mean level of the adjusting covariates.

^b Interaction in multiple linear regression model was defined as the product terms of the dummy independent variables: tertile of job control (Low, Medium, or High) and genotype (coded as an ordinal variable with levels 1, 2, 3). The *p* value is based on the *F* test (2 days) for the interaction between job control and genotype. SBP = systolic blood pressure; DBP = diastolic blood pressure; CI = confidence interval.

Figure 1 shows the *p* values from the *F* test measuring SNP-job control interaction effect by genetic functional class. When studying ambulatory SBP, the only interaction with an FDR of <5% was found for SNP rs11210278 on the intronic region of the endothelin 2 (EDN2) gene on chromosome 1 and job control score (unadjusted *p* = .00000661; *p* adjusted for FDR = .0085). This association remains borderline significant if we use an FDR correction for all the 1324 × 2 × 3 = 7944 tests (*p* adjusted for FDR = .0525). Even though only the interaction of SNP rs11210278 and job control result was significant after multiple testing correction, we saw a trend for 6 of the remaining 10 SNPs on gene EDN2 that passed quality checking (Fig. 1).

No significant interactions were found for job strain quadrant term and job demand score. When studying ambulatory DBP, we did not find any interactions with an FDR of <5%.

Main effects on ambulatory SBP values estimated, using a multiple linear regression model, are shown in Supplemental Digital Content 2 (<http://links.lww.com/PSYMED/A34>). Table 2 summarizes the effects of job strain on ambulatory BP levels by rs11210278 genotype. After adjustment for age, sex, BMI, and total cholesterol, we found a significant decrease in ambulatory SBP with increasing job strain in individuals with the C/C genotype and an increase in those with C/T and T/T genotypes.

Figure 2 shows the EDN2 gene with all the SNPs tested, the LD blocks and the interaction *p* values. As shown in Figure 3, stratification by gender did not alter the association. We found similar results with clinic and home SBP measurements (clinic SBP, unadjusted *p* = .00498; home SBP, unadjusted *p* = .00159). A similar pattern was observed for ambulatory DBP (unadjusted *p* = .00310).

DISCUSSION

The main finding of this study is that there is a statistically significant interaction for ambulatory SBP between job control

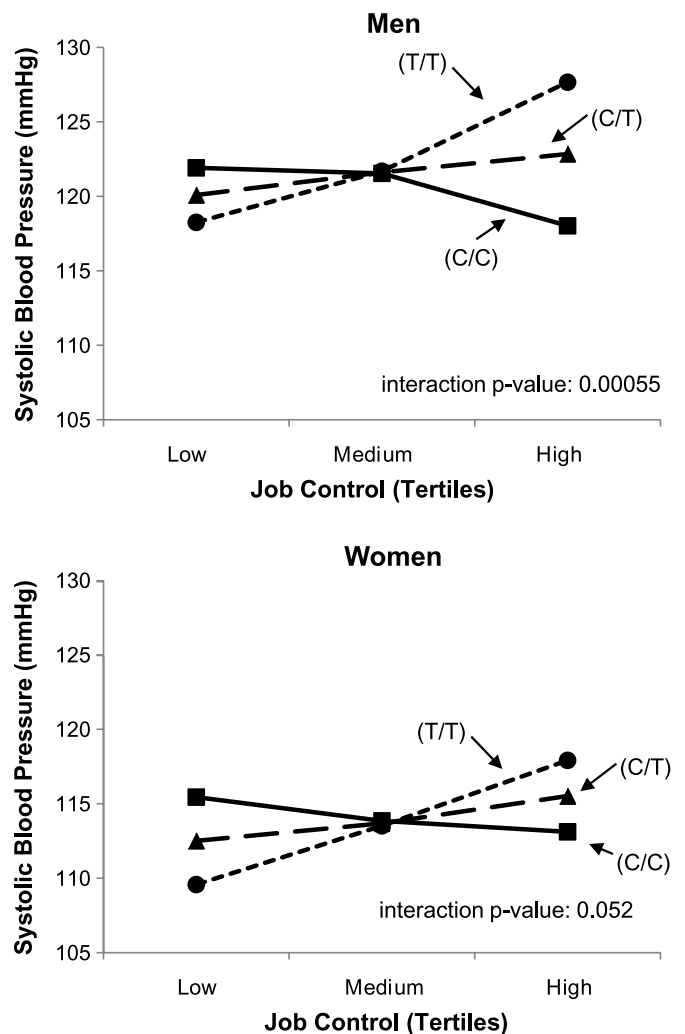


Figure 2. LD plot for the endothelin 2 gene and interaction *p* values from the *F* test measuring single nucleotide polymorphism-job control interaction.

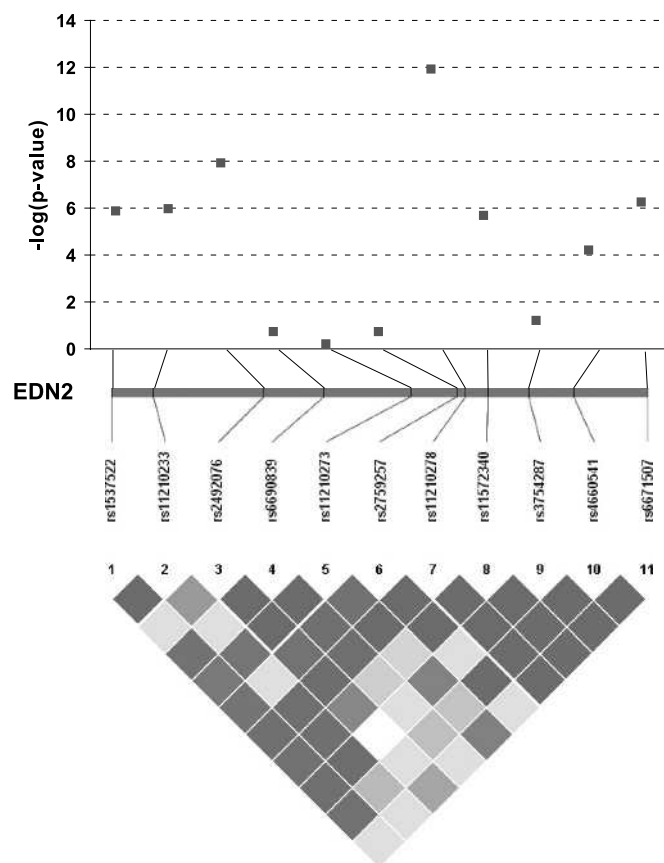


Figure 3. Interaction plot of the estimated ambulatory systolic blood pressure levels according to job control tertile and rs1210278 genotype by gender. *EDN2* = endothelin 2.

and SNP rs121078 located in the intronic region of the *EDN2* gene on chromosome 1. This result is confirmed when studying men and women separately and is further validated when looking at clinic and home readings and correcting for the same covariates.

Job strain has been prospectively linked to an increase of BP because of its “control” dimension (9,32), so our finding is in line with the existing literature. It has been argued that low control is typical of jobs characterized by poor content and less remuneration, which are common among people of low socioeconomic status. This could imply that low job control does not reflect only an unsatisfactory workplace and interpersonal relationship at work but is rather an index of a more complex social discomfort produced by the interplay of work with variables like education, income, housing, and others (33). In addition, the pattern of response and significance is similar in men and women. This represents a new finding, as the latter group does not usually show significant BP responses associated with the job content scales (10,15).

The endothelial system has been proposed as a mediator of the effect of job strain (18). The *EDN2* gene is located on chromosome 1p34 and belongs to a family of highly potent vasoconstrictive peptides (*EDN1* on chromosome 6p24, *EDN3* on chromosome 20q13). *EDN2* encodes a preproprotein, which is processed to a short mature form that functions as a ligand for

the endothelin receptors. Endothelin receptors are widely expressed in all tissues, including nonvascular structures like epithelial cells, glia, and neurons. The principal physiological role of endothelin receptors is the maintenance of vascular tone, mitogenic activity, and potentiating the effects of other growth factor, such as platelet-derived growth factor (34). Imumorin et al. (18) suggested that the role of the behavioral stress is pivotal in mediating the adverse effects of the endothelin genes on vascular function.

Carriers of the C/C genotype (homozygous wild-type) respond to an increase in stress perception with an expected increase in ambulatory SBP, whereas both heterozygous and homozygous mutant (C/T and T/T) respond with a decrease. Having a copy of the T allele cannot simply be considered as protective in stress susceptibility because both heterozygous and homozygous mutant show an increase of BP as stress perception decreases. Such a response is very similar to what is psychologically known as denial, mentioned in the Introduction as a noncomplaining tendency with somatization. Our results suggest that denial might have—in addition to psychological—genetic components, which influence reciprocally each other, making the biology of the stress phenomenon not understandable without a deeper analysis.

Genetic predisposition plays a role in BP regulation. It is estimated that around 30% of variation in BP is due to genetic factors (35) and that BP is more closely correlated in monozygotic, rather than dizygotic, twins (36). Also, there are individual differences in stress response stereotypy, in which BP increase is a frequent expression (9). Since 1957 (37), different stress exposure has been demonstrated to produce different cognitive emotional response, to which, in rats, a behaviorally induced gene expression seems to correspond (38). In our population study, the genetic component associated with BP increase does not regard a central nervous system expression but a peripheral mechanism (cell proliferation/vasoconstriction). A possible hypothesis is that the peripheral mechanism in the variant genotype of the *EDN2* gene is induced, almost as compensation, by the inability to appreciate stress adequately, which has, at least initially, a positive function in the adaptive process (39). Further research and replications are needed to evaluate this hypothesis and many others that can be suggested as regard the relationship between the perception of the environment and the peripheral control of a fundamental physiological function like BP.

Our study has many strengths. First, the PAMELA study is a large sample of an extensively phenotyped general population that enables studies of gene and environmental effects and allows us to account for several potential confounders (eg, BMI, total and high-density lipoprotein cholesterol, smoking, and alcohol consumption). We selected a set of genes with high biological plausibility in cardiovascular traits and selected SNPs that tagged all variants in the genes and their flanking 10-kb regions (40).

Our study has some limitations. First, because of the cross-sectional nature of the study design, no inferences can be made with respect to causation. Second, for the same reason, we did

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not have a time dimension to control for prolonged exposure to job strain. Third, we had to exclude those who did not work or had retired during the study; this exclusion reduced our sample size. Finally, we recognize that power is a major issue in the testing of gene-environment interactions, and our study was underpowered. However, this is an exploratory analysis. We think that our result is novel and interesting, as it provides new insights into how environmental factors, such as job strain, can influence gene-phenotype associations.

The next step would be to validate our result with replication on multiple independent samples. Still, the lack of many large epidemiological studies, which include both a measure of job strain and genotypic data, makes replication challenging.

REFERENCES

1. Beilin LJ, Puddey IB, Burke V. Lifestyle and hypertension. *Am J Hypertens* 1999;12:934–45.
2. Kivimaki M, Leino-Arjas P, Luukkonen R, Riihimaki H, Vahtera J, Kirjonen J. Work stress and risk of cardiovascular mortality: prospective cohort study of industrial employees. *BMJ* 2002;325:857.
3. Lucini D, Riva S, Pizzinelli P, Pagani M. Stress management at the worksite: reversal of symptoms profile and cardiovascular dysregulation. *Hypertension* 2007;49:291–7.
4. Berkman LF. Tracking social and biological experiences: the social etiology of cardiovascular disease. *Circulation* 2005;111:3022–4.
5. McEwen BS. Protective and damaging effects of stress mediators. *N Engl J Med* 1998;338:171–9.
6. Rozanski A, Blumenthal JA, Davidson KW, Saab PG, Kubzansky L. The epidemiology, pathophysiology, and management of psychosocial risk factors in cardiac practice: the emerging field of behavioral cardiology. *J Am Coll Cardiol* 2005;45:637–51.
7. Schnall PL, Schwartz JE, Landsbergis PA, Warren K, Pickering TG. A longitudinal study of job strain and ambulatory blood pressure: results from a three-year follow-up. *Psychosom Med* 1998;60:697–706.
8. Schnall PL, Landsbergis PA, Baker D. Job strain and cardiovascular disease. *Annu Rev Public Health* 1994;15:381–411.
9. Pickering T. The effects of occupational stress on blood pressure in men and women. *Acta Physiol Scand Suppl* 1997;640:125–8.
10. Ohlin B, Berglund G, Rosvall M, Nilsson PM. Job strain in men, but not in women, predicts a significant rise in blood pressure after 6.5 years of follow-up. *J Hypertens* 2007;25:525–31.
11. Theorell T, Karasek RA. Current issues relating to psychosocial job strain and cardiovascular disease research. *J Occup Health Psychol* 1996;1:9–26.
12. Landsbergis PA, Schnall PL, Pickering TG, Warren K, Schwartz JE. Life-course exposure to job strain and ambulatory blood pressure in men. *Am J Epidemiol* 2003;157:998–1006.
13. Kawakami N, Haratani T, Araki S. Job strain and arterial blood pressure, serum cholesterol, and smoking as risk factors for coronary heart disease in Japan. *Int Arch Occup Environ Health* 1998;71:429–32.
14. Kang MG, Koh SB, Cha BS, Park JK, Baik SK, Chang SJ. Job stress and cardiovascular risk factors in male workers. *Prev Med* 2005;40:583–8.
15. Guimont C, Brisson C, Dagenais GR, Milot A, Vezina M, Masse B, Moisan J, Laflamme N, Blanchette C. Effects of job strain on blood pressure: a prospective study of male and female white-collar workers. *Am J Public Health* 2006;96:1436–43.
16. Karasek RA. Job demands, job decision latitude and mental strain: implication for job redesign. *Administrative Science Quarterly* 1979;24:285–307.
17. Greiner BA, Krause N, Ragland D, Fisher JM. Occupational stressors and hypertension: a multi-method study using observer-based job analysis and self-reports in urban transit operators. *Soc Sci Med* 2004;59:1081–94.
18. Imumorin IG, Dong Y, Zhu H, Poole JC, Harshfield GA, Treiber FA, Snieder H. A gene-environment interaction model of stress-induced hypertension. *Cardiovasc Toxicol* 2005;5:109–32.
19. Ohlin B, Berglund G, Nilsson PM, Melander O. Job strain, decision latitude and alpha2B-adrenergic receptor polymorphism significantly interact, and associate with higher blood pressures in men. *J Hypertens* 2007;25:1613–9.
20. Ohlin B, Berglund G, Nilsson PM, Melander O. Job strain, job demands and adrenergic beta1-receptor-polymorphism: a possible interaction affecting blood pressure in men. *J Hypertens* 2008;26:1583–9.
21. Cesana G, Ferrario M, Segna R, Milesi C, De VG, Mancina G, Zanchetti A. Job strain and ambulatory blood pressure levels in a population-based employed sample of men from northern Italy. *Scand J Work Environ Health* 1996;22:294–305.
22. Cesana G, Segna R, Ferrario M, Chiodini P, Corrao G, Mancina G. Job strain and blood pressure in employed men and women: a pooled analysis of four northern Italian population samples. *Psychosom Med* 2003;65:558–63.
23. Mancina G, Segna R, Bravi C, De Vito G, Valagussa F, Cesana G, Zanchetti A. Ambulatory blood pressure normality: results from the PAMELA study. *J Hypertens* 1995;13:1377–90.
24. The World Health Organization MONICA Project (monitoring trends and determinants in cardiovascular disease): a major international collaboration. WHO MONICA Project Principal Investigators. *J Clin Epidemiol* 1988;41:105–14.
25. Belkic KL, Landsbergis PA, Schnall PL, Baker D. Is job strain a major source of cardiovascular disease risk? *Scand J Work Environ Health* 2004;30:85–128.
26. WHO Regional Office for Europe. MONICA psychosocial optional study: MOPSY suggested measurement and instruments. Copenhagen: WHO Regional Office for Europe, draft 3037H MRC; 1988.
27. Cesana G, Poncato E, Duzioni F. Indagine pilota sulla affidabilità di un questionario per lo studio dei fattori psico-socio-occupazionali in relazione alla insorgenza di coronaropatia. *Arch Sci Lav* 1987;3:151–70.
28. Landsbergis PA, Schnall PL, Warren K, Pickering TG, Schwartz JE. Association between ambulatory blood pressure and alternative formulations of job strain. *Scand J Work Environ Health* 1994;20:349–63.
29. Padmanabhan S, Menni C, Lee WK, Laing S, Brambilla P, Segna R, Perego R, Grassi G, Cesana G, Delles C, Mancina G, Dominiczak AF. The effects of sex and method of blood pressure measurement on genetic associations with blood pressure in the PAMELA study. *J Hypertens* 2010;28:465–77.
30. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc B* 1995;57:289–300.
31. Lu X, Perkins DL. Re-sampling strategy to improve the estimation of number of null hypotheses in FDR control under strong correlation structures. *BMC Bioinformatics* 2007;8:157.
32. Steptoe A, Willemsen G. The influence of low job control on ambulatory blood pressure and perceived stress over the working day in men and women from the Whitehall II cohort. *J Hypertens* 2004;22:915–20.
33. Marmot MG, Bosma H, Hemingway H, Brunner E, Stansfeld S. Contribution of job control and other risk factors to social variations in coronary heart disease incidence. *Lancet* 1997;350:235–9.
34. NCBI Entrez Gene. Gene ID 1907. Available at http://www.ncbi.nlm.nih.gov/gene/1907?ordinalpos=2&itool=EntrezSystem2.PEntrez.Gene.Gene_ResultsPanel.Gene_RVDocSum. 2010. Accessed December 15, 2010.
35. Beevers G, Lip GY, O'Brien E. ABC of hypertension: the pathophysiology of hypertension. *BMJ* 2001;322:912–6.
36. Luft FC. Twins in cardiovascular genetic research. *Hypertension* 2001;37:350–6.
37. Levine S. Infantile experience and resistance to physiological stress. *Science* 1957;126:405.
38. Meaney MJ, Bhatnagar S, Diorio J, Larocque S, Francis D, O'Donnell D, Shanks N, Sharma S, Smythe J, Viau V. Molecular basis for the development of individual differences in the hypothalamic-pituitary-adrenal stress response. *Cell Mol Neurobiol* 1993;13:321–47.
39. Cesana G, Menni C. Cardiovascular risk as a paradigm of the negative consequences of stress at work: a “conflicting evidence.” In: Sher L, editor. *Psychological Factors and Cardiovascular Disorders: The Role of Stress and Psychosocial Influences*. Hauppauge, New York: Nova Science Publishers; 2009.
40. Padmanabhan S, Melander O, Hastie C, Menni C, Delles C, Connell JM, Dominiczak AF. Hypertension and genome-wide association studies: combining high fidelity phenotyping and hypercontrols. *J Hypertens* 2008;26:1275–81.