Job Stress, Gene Polymorphism of \( \beta_2 \)-AR, and Prevalence of Hypertension

SHAN-FA YU\textsuperscript{a,}\textsuperscript{1}, WEN-HUI ZHOU\textsuperscript{a}, KAI-YOU JIANG\textsuperscript{a}, GUI-ZHENG GU\textsuperscript{a}, AND SHENG WANG\textsuperscript{a,2}

\textsuperscript{a}Department of Occupational and Environmental Health, School of Public Health, Peking University, Beijing 100083, China; \textsuperscript{b}Henan Provincial Institute of Occupational Medicine, Zhengzhou 450052, Henan, China

Objective To study the interactive effect of job stress and genetic susceptibility (or gene polymorphism) on hypertension.

Methods A cross-sectional epidemiological study was conducted in 452 workers from a thermal power plant in China. Extrinsic effort, occupational reward, and over-commitment were measured. Hypertensive patients were defined by three phases of screening, reexamination, and final diagnosis. \( \beta_2 \)-AR genotypes and allele frequencies at amino acid positions 16 (\( \beta_2 \)-AR-16: Arg \( \rightarrow \)Gly) and 27 (\( \beta_2 \)-AR-27: Gln \( \rightarrow \)Glu) were identified by PCR-RFLP.

Results Job stress was related with the prevalence of hypertension in males (\( P < 0.05 \)), whereas no significant relationship was found in females (\( P > 0.05 \)). Differences in genotypes and allele frequencies of the \( \beta_2 \)-AR-16 were statistically significant between the hypertension and control groups (\( P < 0.05 \)), whereas those of \( \beta_2 \)-AR-27 were not (\( P > 0.05 \)). The prevalence of hypertension was higher in individuals carrying Gly16 allele than in those carrying Arg16 allele of the high job stress group (\( P < 0.01 \) or 0.05).

Conclusion High job stress and polymorphism of \( \beta_2 \)-AR-16 have an interactive effect on the prevalence of hypertension in male workers.

Key words: Job stress; Hypertension; \( \beta_2 \)-AR; Gene polymorphism

INTRODUCTION

Hypertension is a widespread polygenic human disease caused by complex interaction of genetic factors and environmental conditions. Psycho-emotional stress, diet, and life style play an important role in its pathogenesis. Stress response is controlled mainly by the hypothalamus-pituitary-adrenal system (HPA), the sympathetic-catecholamine system in particular. In most cases occurrence and maintenance of hypertension are accompanied with alterations in functions of the sympathetic-catecholamine system. Thus, it is important to investigate the operation of the sympathetic-catecholamine system both at body and molecular levels for the understanding of the causes for stress-related hypertension. The gene for adrenergic receptor (AR) is one of the important genes determining the function of the sympathetic-catecholamine system. Its excitomotor consists of several hormones, including adrenalin and noradrenalin hormones as the main stress hormones\textsuperscript{[1]}. Some recent studies on animal model experiments of stress-induced hypertension and laboratory tests of hypertensive patients and healthy people who received stimulus of stressors have shown that repeated exposure to stress in combination with genetic susceptibility might be related to the prevalence of hypertension\textsuperscript{[2,3,4,5]}. However, no study on the interactive effect of psychosocial factors at work (job stress) and gene polymorphism on hypertension has been reported. Therefore, the present study was to analyze the relationship of job stress and gene polymorphism with the prevalence of hypertension by linking job stress with polymorphism of \( \beta_2 \)-AR-16 and \( \beta_2 \)-AR-27, which are important candidate gene polymorphisms for studying the genetic susceptibility of hypertension.

MATERIALS AND METHODS

Subjects

Participants in this study were 452 workers from a thermal power plant whose length of service was more than two years, and who were residents of Han nationality in Henan province and willing to take part...
in a medical examination and questionnaire survey. Six participants (1.3%) were excluded because of one or more items missing responses. The remaining 446 workers (98.7%) were considered samples for analysis in the present study.

A breakdown of the demographic characteristics of the sample showed that 281 (63.0%) of the respondents were male and 165 (37.0%) female. Age ranged from 22 to 58 years ($\bar{x} = 37.0, \ s = 6.5$). The mean length of service was 16.1 years ($s = 6.3$) and 100% of the respondents worked on a full-time basis. Most of them (85.7%) were married. Their education backgrounds were: 138 (30.9%) with college education and over, 271 (60.8%) with high school education, and 37 (8.3%) with primary school education. Of the participants 233 (52.2%) served as workplace inspectors, 70 (15.7%) as maintenance workers, 49 (11.0%) as visual display terminal (VDT) operators in the control center, and 94 (21.1%) as auxiliary workers.

**Job Stress Measurement**

The effort-reward imbalance (ERI) questionnaire was used in this study [6-8], consisting of the following three scales: extrinsic effort (6 items), occupational reward (11 items), and overcommitment (29 items). The questionnaire was also formulated by means of translation and back-translation. Extrinsic effort was evaluated by measuring the psychosocial workload. Occupational reward focused on the worker’s financial status (i.e. salary), self-esteem, and career opportunity (e.g. promotion prospects and job security). Overcommitment as a personal (intrinsic) component was defined as a set of attitudes, behaviors, and emotions reflecting excessive striving along with a strong desire for recognition and esteem. Cronbach’s alpha of effort, reward, and overcommitment scales were 0.78, 0.65, and 0.84, respectively. Furthermore, according to the theoretical formulation, a ratio of effort/reward=0.5454 was calculated to assess the degree of imbalance between high effort and low reward at work where a value equaling 1.0 indicated the critical condition, >1.0 indicated high job stress and <1.0 indicated low job stress.

**Medical Examination**

The medical examination included assessments of height, weight, blood pressure, medical history, and personal health habits. All physiological assessments were obtained in a clinic setting during normal working hours (i.e., 8 am-5 pm). In the process of reexamination, three standardized blood pressure measurements were taken by trained nurses according to the standard protocols. All readings were obtained from the right arm with standard mercury manometers after participants were at the supine position for 30 min rest at least. Three measurements were performed at least 1 min intervals. Systolic and diastolic BP were defined according to Korotkoff I and V. Participants were classified as hypertensive if (1) the mean of their three systolic readings was equal to or greater than 140 mmHg, or (2) the mean of their three diastolic readings was equal to or greater than 90 mmHg, or (3) they were currently on antihypertensive therapy.

After removing the cases of secondary hypertension, diabetic patients, liver or kidney disease, 84 workers (58 males and 26 females) were diagnosed as hypertension. The prevalence of hypertension was 18.8%, with 26.3% in men and 3.4% in women.

**Measurement of Covariables**

Biobehavioral factors potentially related to hypertension and confounding factors evaluated in this study included: age, sex, education, marital status, income, length of service, body mass index (weight (kg) / height (m)^2), family hypertensive history, salt intake, smoking (pack, years), caffeine and alcohol consumption, and physical exercise.

**Biochemical Detection**

After overnight fasting, blood specimens were collected by venous puncture and stored under appropriate conditions for analysis of serum total cholesterol and triglyceride. Triglyceride was detected by glycerophosphoric acid oxidase endpoint method, linear range $\leq 11.4$ mmol/L, while cholesterol was detected by cholesterol oxidase endpoint method, linear range $\leq 12.9$ mmol/L. Triglyceride and cholesterol kits were purchased from Sichuan MAKER Science Technology CO. LTD.

**Molecular Biology Research**

$\beta_2$-AR16, 27 genotypes and allele frequencies were detected in 58 male hypertensive patients and 58 normotensive subjects under field epidemiological survey. Patients and normotensive subjects were matched with age, length of service, education level, and type of work.

**Reagents**

A DNA extractor kit for isolation of genomic DNA was purchased from Axygen Biosciences. dNTP mixture, TaKaRa Taq™, 50 and 100 bp DNA
loading marker were obtained from Takara Biotechnology Co. Ltd (Dalian, China). PCR primers were synthesized by AuGCT Biotechnology Synthesis Laboratory. The PCR products, including positions 16 and 27, were generated using the forward primer $\beta_2$-AR-16, 27 F (5'-GCCTTCTTGCTGACCCCAT-3') and the reverse primer $\beta_2$-AR-16, 27 R (5'-CAGACGCTCGAACTTGCCCATG-3'). Agarose, acrylamide, N,N'-methylene bisacrylamide, and argent nitrate, ethidium bromide from Sigma were used for electrophoresis and stain. All other materials were of reagent grade and commercially available.

**Isolation of Genomic DNA and Determination of $\beta_2$-AR Genotypes**

Genomic DNA obtained from peripheral blood was isolated using A DNA extractor kit (Fig. 1). $\beta_2$-AR genotypes were determined by a combination of primer-induced restriction site and restriction fragment assay. PCR amplification of genomic DNA was performed in a final volume of 25 µL consisting of approximately 250 ng of genomic DNA, 1 unit of TaKaRa Taq TM, 2 µL dNTP mixture, and 0.5 µmol/L of each primer in the reaction buffer. The amplification conditions were denaturation at 95°C for 10 min, followed by 30 cycles at 95°C for 30 s, at 63°C for 45 s, at 72°C for 60 s for 30 cycles, and a final extension at 72°C for 7 min.

The size of the PCR product was 168 bp (Fig. 2). For detection of the $\beta_2$-AR-16 polymorphism, 5 µL of PCR products was completely digested with 1 U of NcoI (Takara Biotechnology (Dalian) Co. Ltd.) in the appropriate basal buffer (final volume, 10 µL) at 37°C for 2 h. A 22 bp fragment was cut from the 3'-end of the 168 bp with NcoI. The PCR products were obtained from alleles and an 18 bp fragment was obtained from the 5'-end of the Gly16 allele. The restriction digests were electrophoresed on a 12% polyacrylamide gel and visualized under natural illumination with argent nitrate staining (Fig. 3). The $\beta_2$-AR-27 genotypes were identified using another aliquot of the same PCR product, 0.5 U of BbvI (New England BioLabs) was added to the basal buffer (final volume, 10 µL) at 37°C for 1 h. BbvI digested only the Gln27 allele to produce 105 and 63 bp fragments which were separated from the uncut Glu27 alleles on a 2% agarose gel and visualized under ultraviolet illumination with ethidium bromide staining (Fig. 4).

**Statistical Analyses**

The crude relation between job stress (ERI) and
prevalence of hypertension was subjected to univariate logistic analysis followed by multivariate logistic analysis including effort, reward, overcommitment and ERI variables as the main effects, and the potential 14 confounding factors. Gender difference in the relation between job stress (ERI) and prevalence of hypertension was analyzed by Chi-square test. Representation of samples was analyzed by Hardy-Weinberg equilibrium test, β2-AR-16, 27 genotypes and allele frequencies were compared by Chi-square test, or exact probability. Statistical analyses were performed with SPSS 13.0. The significance level for all statistical analyses was set at a probability of less than 0.05 (two-tailed test).

RESULTS

Correlation Between Job Stress and Prevalence of Hypertension

Univariate analyses revealed that not only some common factors such as parents’ hypertensive history, triglyceride, cholesterol, BMI, and school education levels, but also job stress (ERI), overcommitment and effort were significantly correlated to hypertension (P<0.05), whereas no association was observed between reward and the prevalence of hypertension (P>0.05). In order to further determine the impact of job stress (ERI) on the prevalence of hypertension, we performed multivariate logistic regression for variables associated with hypertension in univariate analyses. The risk of hypertension for workers who have a high job stress was about two-fold that for workers with a low job stress (OR=2.12) (Table 1).

To examine whether an interactive effect exists on job stresses (ERI) and sex, we analyzed the effect of gender differences on the relation between ERI and hypertension by stratification analysis. Job stress in males were correlated to the prevalence of hypertension (P<0.01) with an odds ratio of 3.13, while in females no such correlation was observed in females (Table 2).

### TABLE 1

<table>
<thead>
<tr>
<th>Univariate and Multivariate Logistic Analyses of Job Stress and Prevalence of Hypertension</th>
<th>Standard Partial Regression Coefficient</th>
<th>Odds Ratio (95%CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Univariate Analyses</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parents Hypertension History</td>
<td>0.95</td>
<td>2.57 (1.57-4.21)</td>
<td>0.000</td>
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<tr>
<td>Job Stress (ERI)</td>
<td>0.75</td>
<td>2.11 (1.28-3.48)</td>
<td>0.004</td>
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<tr>
<td>Overcommitment</td>
<td>0.56</td>
<td>1.74 (1.04-2.91)</td>
<td>0.033</td>
</tr>
<tr>
<td>Effort</td>
<td>0.31</td>
<td>1.37 (1.02-1.83)</td>
<td>0.038</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>0.37</td>
<td>1.45 (1.09-1.93)</td>
<td>0.010</td>
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<tr>
<td>Triglyceride (mmol/L)</td>
<td>0.30</td>
<td>1.35 (1.14-1.61)</td>
<td>0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.15</td>
<td>1.15 (1.07-1.25)</td>
<td>0.000</td>
</tr>
<tr>
<td>Education Levels</td>
<td>−0.49</td>
<td>0.61 (0.40-0.95)</td>
<td>0.023</td>
</tr>
<tr>
<td><strong>Multivariate Analyses</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Parents Hypertension History</td>
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<td>2.65 (1.56-4.53)</td>
<td>0.000</td>
</tr>
<tr>
<td>Job Stress (ERI)</td>
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</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.11</td>
<td>1.12 (1.02-1.22)</td>
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<tr>
<td>Triglyceride (mmol/L)</td>
<td>0.29</td>
<td>1.33 (1.11-1.59)</td>
<td>0.002</td>
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<tr>
<td>Education</td>
<td>−0.48</td>
<td>0.62 (0.40-0.98)</td>
<td>0.038</td>
</tr>
</tbody>
</table>

### TABLE 2

| Effect of Gender on the Relation Between Job Stress (ERI) and Hypertension |
|--------------------------------------------------|-----------------------------------|----------------|---------|
| Job Stress Level                                  | Hypertension Patients | Control | Odds Ratio (95%CI) | P-value |
| **Male (n=281)**                                 |                      |        |                    |        |
| High Job Stress Level                             | 29                   | 54     | 3.13 (1.72-5.70)   | 0.000   |
| Low Job Stress Level                              | 29                   | 169    |                    |        |
| **Female (n=165)**                               |                      |        |                    |        |
| High Job Stress Level                             | 4                    | 31     | 0.63 (0.20-1.98)   | 0.602   |
| Low Job Stress Level                              | 22                   | 108    |                    |        |
Relation Between Polymorphism of $\beta_2$-AR and Prevalence of Hypertension

Table 3 displays the genotypes and allele frequencies of $\beta_2$-AR-16 and 27 in the 58 hypertension patients and 58 control subjects. Allele frequencies of $\beta_2$AR-16 were Arg16:Gly16=55.2%:44.8% in the hypertension group and 68.1%:31.9% in the control group. Allele frequencies of $\beta_2$-AR-27 were Gln27:Glu27=87.1%:12.9 in the hypertension group and 94.0%:6.0% in the control group. Hardy-Weinberg test showed that both genotypes and allele frequencies of $\beta_2$-AR-16 and 27 met the genetic equilibrium.

Chi-square test revealed that there was a statistically significant difference in genotypes and allele frequencies of $\beta_2$-AR-16 between the two groups ($P<0.05$), but no statistically significant difference in those of $\beta_2$-AR-27 between the two groups ($P>0.05$).

Interactive Effect of Job Stress and Polymorphism of $\beta_2$-AR-16 on Hypertension

To explore the interactive effect of job stress and polymorphism of $\beta_2$-AR-16 on hypertension, we analyzed the relation between polymorphism of $\beta_2$-AR-16 and hypertension under different job stress levels by stratification analysis. As shown in Table 4, ArgGly and GlyGly genotypes were related to the prevalence of hypertension in the high job stress group ($P<0.01$ or 0.05), with an odds ratio of 3.65 and 3.47, respectively. Moreover, in subjects carrying Gly16 allele in the high job stress group, the risk of hypertension was about two-fold as high as that in subjects carrying Arg16 allele, whereas in the low job stress group no statistically significant difference in the risk of hypertension was found between them ($P>0.05$).
DISCUSSION

The effort-reward imbalance model assumes that exposure to work places with a specific task profile (high efforts in combination with low rewards) leads to poor health, especially to stress-related cardiovascular diseases, such as hypertension, coronary heart disease, and myocardial infarction\[9-13\]. This model is based on the norm of reciprocity of work contracts. Accordingly, effort at work is reciprocated by socially-defined rewards including money, esteem, and status control in terms of promotion prospects and job security. The effort-reward imbalance model can be applied in a wide range of occupations and populations with diverse socio-demographic profiles. In the past decades, studies showed that the model works cross-culturally not only in Western/Northern Europe, but also in Central/Eastern Europe, North America, and Japan\[14-15\]. Its application has spread over populations in the United States and China with diverse socio-cultural backgrounds. There is evidence that the model works for either sex\[14-15\].

Job stress was defined by the Chinese version of the ERI model in this study, and the results of logistic analyses showed that job stress was related to hypertension after adjustment for confounding factors, indicating that job stress may be a risk factor for hypertension. The results support most prospective and case-control studies on increased risk and morbidity of cardiovascular diseases in employees exposed to a stressful psychosocial work environment as measured by the ERI model\[9-13\]. In recent years, many researches have focused on whether gender affects the relationship between health conditions and stress, but no definite conclusion is available\[12, 16-17\]. Our findings indicate that job stress caused by imbalance between efforts and rewords is probably a risk factor for hypertension in male subjects.

It was reported that the β2-AR gene has nine forms of polymorphism, of which three are probably related to cardiovascular diseases, namely β2AR-16 with replacement of arginine (Arg16) for glycine (Gly16), β2-AR-27 with replacement of glutamine (Gln27) for glutamic acid (Glu27), and β2AR-164 with replacement of threonine (Thr164) for isoleucine (Ile164)\[18\]. Green et al.\[19\] reported that both Arg16Gly and Gln27Glu play a role in the down-regulation of β2-adrenergic receptor. Cellular transfection assays showed that the Gly16 isoform could significantly down-regulate β2-adrenergic receptor in response to the β2-agonist isoproterenol compared with the Arg16 isoform. Such a response could likely produce an increased blood pressure due to the decreased agonist sensitivity. Conversely, the Glu27 isoform could not down-regulate β2-adrenergic receptor compared to the Gln27 isoform in cell transfection experiments, suggesting that the sensitivity of Gln27 isoform to adrenergic agonists is increased in cells carrying this allele. These researches may provide evidence of β2-AR polymorphism affecting β2-AR function for the ultimate determination of blood pressure and hypertensive condition. It was reported that both Arg16Gly and Gln27Glu are significantly associated with the prevalence of hypertension\[12, 20-21\]. The Glu27 allele is more frequently found in hypertension individuals and is significantly associated with the occurrence of hypertension compared with the Gln27 allele, so is the Gly16 allele\[20-21\]. However, others studies reported that polymorphism of β2-AR-27 may not be related to hypertension\[22-23\] probably due to the differences in geographic conditions, ethnic, and races, as well as methods of statistical analyses. Our results show that there were statistically significant differences in genotypes and allele frequencies of β2-AR-16 between the hypertension and control groups (P<0.05), and individual carriers of Gly16 allele were more likely to show a positive response to hypertension than those of Ary16 allele. However, no association was observed between gene polymorphism of β2-AR-27 and hypertension (P>0.05), indicating that polymorphism of β2-AR-16 is related with genetic susceptibility to hypertension, but polymorphism of β2-AR-27 is otherwise. Our results are in agreement with the reported findings in β2-AR-16 and β2-AR-27\[18-22\].

At present, studies on genetic architecture change of stress-induced hypertension in animal experiments are increasing. Khvorostova\[3\] reported that expression of the proopiomelanocortin (POMC) gene in NISAG rats with inherited stress-induced arterial hypertension is 3.4 times higher than that in non-stressed normotensive rats of the inbred same strain Wistar Albino rats by reverse transcription-competitive PCR method. Furthermore, the basic transcription level of the POMC gene in NISAG rats is higher than that in WAG rats. There is evidence that it is possible to localize the genes responsible for stress-induced arterial hypertension in ISIAH rats at the Atp1a1 locus, chromosome 2 and the Lngfr gene locus, chromosome 10\[41\]. In addition, analyses of renin-angiotensin-aldosterone system gene variants in blood pressure at rest and during behavioral stress in young normotensive subjects demonstrated that the RAAS genetic modifications may regulate the dynamic BP in response to behavioral stress.
compared with the static blood pressure\textsuperscript{[5]}. However, no study on the interactive effect of job stress and genetic susceptibility of individuals on hypertension is reported. Our results show that individuals carrying β\textsubscript{2}-AR-16: Arg→Gly high job stress developed hypertension more often, suggesting that job stress may induce the development of hypertension, and moreover, individuals carrying hypertension predisposing genes may have hypertension more often.

Several limitations need to be taken into account in the study. Firstly, this study was a cross-sectional observation, which could result in information and selection bias and either over or under estimation of the association. Lack of evaluation of the effect of job stress due to cross-sectional data used could lead to an underestimation of true association. The use of self-reported questionnaire to measure job stress may have response bias. However, no reliable objective measurement of job stress is available at present. Secondly, we just selected the two polymorphism loci of a single gene to analyze the association of job stress and polymorphism with the prevalence of hypertension. However, hypertension is a polygenic human disease, and interactions between genes are inevitable. Therefore, our findings may be affected interactively by other genes. Finally, our sample size was relatively small and might not represent the total workforce in China and some associations might appear by chance in our analyses. To the best of our knowledge, this is the first study to explore the interactive effect of job stress and β\textsubscript{2}-AR gene polymorphism on hypertension. Its findings require replication in other designs and settings to confirm their validity.

In conclusion, job stress and β\textsubscript{2}-AR gene polymorphism are associated with the prevalence of hypertension, which may explain the link between job stresses and increased cardiovascular disease risk.

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