

## RESEARCH ARTICLE

# Association of Polymorphisms in Stress-Related TNF $\alpha$ and NPY Genes with the Metabolic Syndrome in Han and Hui Ethnic Groups

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### Abstract

**Background:** Metabolic syndrome (MS) is a cluster of complicated disorders caused by the interactive influencing factors of heredity and environment, which predisposes to many cancers. Results from epidemic research indicate that stress is tightly related to the pathogenesis of MS and neoplasia. This paper aims to investigate the association between psychological stress and MS with respect to the tumor necrosis factor alpha (TNF $\alpha$ ) and neuropeptide Y (NPY) genes in the Han and Hui ethnic groups. **Methods:** All subjects for this case-control study matched strict enrollment criteria (nationality, gender and age) and lived in the city of Wu Zhong of Ningxia Province in China. The enrolled group contained 102 matched pairs of Hui ethnic individuals and 98 matched pairs of Han ethnic individuals. Enrolled subjects completed the general Symptom Checklist-90 (SCL-90). The TNF $\alpha$ -308G/A variant and NPYrs16147 polymorphism were detected in case (81 males, 119 females) and control (81 males, 119 females) groups by polymerase chain reaction (PCR) amplification. **Results:** Nine factors of the SCL-90 were found to be statistically different ( $p < 0.05$ ) between case and control groups. The homozygous mutant genotype (AA) and the mutant allele (A) of the TNF $\alpha$ -308G/A gene were less frequently observed in the control population compared to the case group. The odds ratio (95% confidence interval) in "Allele" for MS was 2.28 (1.47–3.53),  $p = 0.0001$ , while "OR" was 1.11 (0.83–1.47),  $p = 0.15$ , for the NPYrs16147 gene polymorphism. **Conclusions:** Psychological stress has been positively associated with MS. A previous study from our group suggested there were differences in the level of psychological stress between Hui and Han ethnic groups. Furthermore, we found that the stress-related TNF $\alpha$  gene was associated with MS for both Han and Hui ethnic groups. In contrast, NPY may be a possible contributor to MS and associated cancer for the Han ethnic group.

**Keywords:** Metabolic syndrome - psychological stress - TNF $\alpha$ -308G/A - NPYrs16147 - SCL - 90 - Han and Hui ethnicity

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### Introduction

Metabolic syndrome (MS) is widely defined as a complex of interrelated risk factors, including dysglycemia, raised triglyceride (TC) levels, raised low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol levels, obesity and hypertension (Grundy et al., 2004; Ferrannini et al., 2006; McGrowder et al., 2012; Saukkonen et al., 2012; Song et al., 2012). With rapid changes in society and increasing demands of intense competition, modern life presents increasing exposure to different kinds of stress. Increasing stress is believed to play a critical role in the development of MS (Maier et al., 2000; Black et al., 2003; Almadi et al., 2013; Li et al., 2013) however, the relationship between stress and MS still remains uncertain, especially for different ethnic groups.

The tumor necrosis factor alpha (TNF $\alpha$ ) gene is

located in the 6p21.1-21.3 MHC-III chromosomal region, which is also the location of heredity susceptibility for schizophrenia (Peltonen et al., 1995; Teran-Garcia et al., 2007). An increasing number of researches consider TNF $\alpha$  to be a candidate gene for the development of MS (Vendrell et al., 2003; Sookoian et al., 2005; Nobili et al., 2012). A G/A substitution at position 308 upstream of the TNF $\alpha$  gene promoter increases the release of TNF- $\alpha$  protein, augmenting insulin resistance and playing an important role in the pathogenesis of diabetes (Vendrell et al., 2003; Sookoian et al., 2005; Li et al., 2012). However, a study suggested there was no specific evidence that variation in TNF $\alpha$  influences susceptibility to type 2 diabetes (Zeggini et al., 2005). In addition, another study found no association between TNF $\alpha$  gene polymorphism with characteristics of MS, including type 2 diabetes, hypertension, dyslipidemia and obesity in Hong Kong Chinese (Lee et al., 2000).

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Neuropeptide Y (NPY) is a 36 amino acid polypeptide (Gehlert, 1999) released from sympathetic nerves by various stressors, particularly chronic stress (Zukowska-Grojec, 1995; Morales-Medina et al., 2010; Gülsün et al., 2012). Increased NPY activity has been found to lead to vasoconstriction and results in high blood pressure (Haefliger et al., 1999; McDermott et al., 2007). NPY also affects the tendency for obesity, which is linked to the development of MS (Kuo et al., 2008; Ruohonen et al., 2009). Kalra et al suggested that NPY and its interacting proteins may be the basis for a new treatment modality to curb the pandemic of obesity and MS resulting from the suppression of NPY that occurs in age-related dietary obesity (Kalra et al., 2004). As the rs16147 single nucleotide polymorphism (SNP) of NPY, occurring in the promoter, has been shown to effect the expression of the gene (Itokawa et al., 2003; Zhou et al., 2008), our study was focused on the relationship between NPYrs16147 and MS.

Inter-individual variability in the regulation of the human stress system accounts, in part, for differences in the onset of stress-related diseases. These differences are also influenced by environmental and genetic factors. This study aimed to investigate the relationship between psychological stress and MS with respect to genetic susceptibility.

## Materials and Methods

### Subjects

Ningxia Province has the largest Hui population in China. This matched (1:1) case-control study was carried out in the city of Wu Zhong in Ningxia Province, China. All participants had been living in Wu Zhong for at least 5 years and were between 35 and 74 years of age. Patients were diagnosed according to the definition and criteria published by the International Diabetes Federation (IDF 2005), and participants were considered to have MS if they had central obesity (i.e., waist circumference, WC,  $\geq 90$  cm for Chinese men,  $\geq 80$  cm for Chinese women) and at least two of the following criteria: (1) TC  $\geq 1.7$  mmol/L; (2) reduced HDL-C levels  $< 1.03$  mmol/L for men and  $< 1.29$  mmol/l for women; (3) blood pressure of  $\geq 130/85$  mm Hg or previously diagnosed hypertension; and (4) raised fasting blood glucose (FBG) level of  $\geq 5.6$  mmol/L or previously diagnosed type 2 diabetes mellitus. The case group was comprised of 200 patients with MS (Hui 102, Han 98) and the control group of 200 healthy individuals (Hui 102, Han 98). Case-control pairs were individuals that were matched 1:1 with respect to gender, age and nationality. People with mental disease, history of drug abuse, metabolic diseases (such as diabetes mellitus), and other serious diseases were excluded from this study. The study was approved by The Medical Ethics Review Committee of Ningxia Medical University, and all patients agreed to participate and signed a consent form before the study was conducted.

### Symptom Checklist-90

The Symptom Checklist-90 (SCL-90), designed by Derogatis in 1975, is one of the most widely used measures

of psychological distress in clinical practice and research. The SCL-90 includes 90 items, which are evaluated on 5-point scale: 1, not at all; 2, a little bit; 3, moderately; 4, quite a bit; and 5, extremely. The SCL-90 measures nine primary distress dimensions (somatization, obsessive compulsive behavior, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid ideation and psychoticism). Four global indices of distress (global severity index, global severity index on equal, positive symptom total, factor score) were obtained from SCL-90-R scores. The subjects completed survey questions by entering the appropriate number which best describe their true feelings in the past one week, including the day of the survey.

### Anthropometric measurements

Anthropometric measurements, including height, weight, blood pressure, WC and hip circumference (HC), were recorded by trained physicians for all subjects. Body mass index (BMI) was calculated as  $\text{kg}/\text{m}^2$ . Standing height was measured once using a portable ruler (made in China), and body weight was measured on a digital scale while subjects were wearing light clothes and no shoes. Arterial blood pressure was measured three times in a sitting position.

### Laboratory measurements

Elbow vein blood was collected in two tubes (3-5 ml each) containing the anticoagulant ethylenediaminetetraacetic acid in the morning before participants had eaten. One blood sample was used to measure FBG, total cholesterol (TC), TG, LDL and HDL. Among them, FBG measurement should be finished in less than 1 h, the others in less than 3 h. Serum was separated from the second tube and stored at  $-20^\circ\text{C}$ .

### Genotyping assays

The region containing the G-to-A transition polymorphism of the TNF $\alpha$  gene (TNF $\alpha$ -308) and the C-to-T SNP of the NPY gene (NPYrs16147) were amplified by PCR using following primers: TNF $\alpha$ -308: forward 5'-AGGCAATAGGTTTTGAGGGCCAT-3' and reverse 5'-TCCTCCCTGCTCCGATTCCG-3'; NPYrs16147, forward 5'-TTGCCTCACTCCAACAGCG-3' and reverse 5'-ACAACACCAAAGCCCAAGTATCT-3'. DNA was amplified with the following conditions: initial denaturation of 5 min at  $94^\circ\text{C}$  (TNF $\alpha$ ) or  $95^\circ\text{C}$  (NPY); 35 (TNF $\alpha$ ) or 30 (NPY) cycles at  $94^\circ\text{C}$  for 30 s, annealing at  $60^\circ\text{C}$  (TNF $\alpha$ ) or  $64^\circ\text{C}$  (NPY) and  $72^\circ\text{C}$  for 1 min; and final extension at  $72^\circ\text{C}$  for 7 min. The PCR products were digested with NcoI (TNF $\alpha$ ) or BstXI (NPY), size-fractionated by agarose gel electrophoresis and stained with ethidium bromide for visualization. Genotypes were detected as follows. TNF $\alpha$ -308 polymorphism resulted in a single 107 bp fragment for the homozygous mutant (AA), fragments of 20 bp and 87 bp for the homozygous wild-type (GG) and fragments of 107 bp, 87 bp and 20 bp for the heterozygous mutant (GA). NPYrs16147 polymorphism resulted in a single 256 bp fragment for the homozygous mutant (CC), fragments of 144 bp and 112 bp for the homozygous wild-type (TT) and fragments of 256

bp, 144 bp and 112 bp for the heterozygous mutant (CT).

### Statistical analysis

All survey data were entered with and checked by EPIDATA 3.01. Statistical analysis was carried out using the Statistical Package for the Social Science (SPSS®) version 17.0 (IBM, IL, USA). Quantitative variables were analyzed by adopting t-test and one-way analysis of variance and presenting them as the mean  $\pm$  standard deviation (SD). All statistical tests were two-tailed, and  $p < 0.05$  was chosen as the level of significance. The chi-square test was employed to analyze the association

between genetic polymorphism and MS. Genotype frequencies of control groups were found to be in Hardy-Weinberg equilibrium.

## Results

### General subject characteristics

The anthropometric and biochemical characteristics of the case and control groups are shown in Table 1. Compared to the control group, the case group exhibited higher values for height, weight, BMI, WC, HC, systolic blood pressure, diastolic blood pressure, TC, TG, HDL and FBG ( $p < 0.05$ ), and these differences were statistically significant.

**Table 1. Anthropometric and Biochemical Characteristics of Case and Control Groups**

Parameters	Case (n=200)	Control (n=200)	T-value	P-value
Age (yrs.)	56.39 $\pm$ 9.90	56.12 $\pm$ 9.40	0.001	0.776
Height (cm)	164.53 $\pm$ 6.96	163.76 $\pm$ 7.04	1.221	0.27
Weight (kg)	71.07 $\pm$ 13.21	65.23 $\pm$ 10.99	23.259	<0.001*
BMI (kg/m <sup>2</sup> )	26.20 $\pm$ 4.24	24.28 $\pm$ 3.49	24.366	<0.001*
WC (cm)	94.03 $\pm$ 7.96	84.53 $\pm$ 9.38	119.162	<0.001*
HC (cm)	102.35 $\pm$ 7.29	95.93 $\pm$ 8.40	66.564	<0.001*
SBP (mmHg)	142.33 $\pm$ 19.21	125.57 $\pm$ 19.66	74.373	<0.001*
DBP (mmHg)	90.41 $\pm$ 12.67	81.54 $\pm$ 12.94	47.898	<0.001*
TC (mmol/L)	5.06 $\pm$ 2.53	4.55 $\pm$ 0.95	7.159	0.008
TG (mmol/L)	2.54 $\pm$ 2.07	1.49 $\pm$ 0.73	46.013	<0.001*
LDL (mmol/L)	2.69 $\pm$ 0.70	2.64 $\pm$ 0.68	0.586	0.444
HDL (mmol/L)	1.27 $\pm$ 0.31	1.48 $\pm$ 0.32	43.222	<0.001*
FPG (mmol/L)	5.73 $\pm$ 1.84	4.75 $\pm$ 1.05	42.731	<0.001*

Data given as Mean  $\pm$  SD; Abbreviations: BMI, body mass index; HC, hip circumference; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride; LDL, low density lipoprotein; HDL, high density lipoprotein; FPG, fasting plasma glucose; \*Significant value ( $p < 0.05$ )

**Table 2. Summary of SCL-90 Scores**

	Case (n=200)	Control (n=200)	T-value	P-value
Somatization	1.89 $\pm$ 0.72	1.41 $\pm$ 0.50	59.058	<0.001*
Compulsion	1.87 $\pm$ 0.74	1.43 $\pm$ 0.53	47.763	<0.001*
Interpersonal sensitivity	1.65 $\pm$ 0.68	1.32 $\pm$ 0.52	32.002	<0.001*
Depression	1.73 $\pm$ 0.70	1.36 $\pm$ 0.48	38.358	<0.001*
Anxiety	1.70 $\pm$ 0.68	1.31 $\pm$ 0.45	46.821	<0.001*
Hostility	1.72 $\pm$ 0.72	1.33 $\pm$ 0.52	37.396	<0.001*
Fear	1.55 $\pm$ 0.66	1.25 $\pm$ 0.48	25.827	<0.001*
Paranoia	1.59 $\pm$ 0.65	1.26 $\pm$ 0.47	34.095	<0.001*
Psychosis	1.56 $\pm$ 0.64	1.23 $\pm$ 0.42	36.834	<0.001*
Positive items	36.26 $\pm$ 24.0	20.11 $\pm$ 20.9	51.255	<0.001*

Data given as Mean  $\pm$  SD; \*Significant value ( $p < 0.05$ )

**Table 3. Distribution of TNF $\alpha$  Genotypes in Case and Control Groups**

Groups	Subjects	Genotype				Allele				$\chi^2$ for HWE in controls	
		AA (%)	GG (%)	GA (%)	P value	A (%)	G (%)	P value	OR (95% CI)	$\chi^2$	P value
HAN and HUI	Control (n=200)	4 (2.0)	188 (94.0)	8 (4.0)	0.001	16 (4)	384 (96)	0.0001	2.28 (1.47-3.53)	0.25	0.61
	Control (n=200)	11 (5.5)	162 (81.0)	27 (13.5)		49 (12.25)	351 (87.75)				
HUI	Control (n=102)	2 (1.96)	95 (93.1)	5 (4.95)	0.04	9 (4.42)	195 (95.58)	0.008	2.21 (1.21-4.04)	0.064	0.8
	Control (n=102)	8 (7.84)	80 (78.4)	14 (13.7)		30 (14.71)	174 (85.29)				
HAN	Control (n=98)	2 (2.04)	93 (94.9)	3 (3.06)	0.03	7 (3.57)	189 (96.43)	0.006	2.36 (1.26-4.44)	0.22	0.64
	Control (n=98)	3 (3.06)	82 (83.67)	13 (13.26)		19 (9.69)	177 (90.31)				

Data given as Mean  $\pm$  SD; \*Significant value ( $p < 0.05$ )

### Analysis of psychological status

Table 2 shows the SCL-90 scores (mean  $\pm$  SD) for nine factors. Significant differences ( $p < 0.05$ ) were observed for somatization, compulsion, interpersonal sensitivity, depression, anxiety, hostility, fear, paranoia and psychoticism between case and control groups. These results indicate that psychological stress was associated with MS.

### Analysis of TNF $\alpha$ -308 polymorphism

Distribution of TNF $\alpha$ -308 genotypes ( $\chi^2 = 13.39$ ,  $p = 0.001$ ) and alleles ( $\chi^2 = 14.32$ ,  $p = 0.001$ ) are presented in Table 3. Differences were statistically significant ( $p < 0.05$ ). The homozygous mutant genotype (AA) and the mutant allele (A) were less frequent in the control group compared to the case group. Table 3 presents the distribution of TNF $\alpha$ -308 genotypes and genotypic frequencies for case and control subjects of Han and Hui ethnicity. These differences were also statistically significant ( $p < 0.05$ ) for the Han and Hui ethnic groups. This result indicates that TNF $\alpha$ -308 is associated with MS.

### Analysis of NPYrs16147

Test demonstrated statistically significant differences for NPYrs16147 genotypes ( $\chi^2 = 3.67$ ,  $p = 0.16$ ) and alleles ( $\chi^2 = 2.07$ ,  $p = 0.15$ ) between case and control groups (Table 4). No statistical difference was observed for genotypes ( $\chi^2 = 2.42$ ,  $p = 0.300$ ) and genotypic frequencies ( $\chi^2 = 0.09$ ,  $p = 0.76$ ) for ethnic Hui subjects. For ethnic Han subjects, no statistically significant difference was observed for genotypes ( $\chi^2 = 4.77$ ,  $p = 0.09$ ). However, genotypic frequencies were statistically different ( $\chi^2 = 5.53$ ,  $p = 0.02$ ).

**Table 4. Distribution of NPYrs16147 Genotypes in Case and Control Groups**

Groups	Subjects	Genotype				Allele				$\chi^2$ for HWE in controls	
		CC (%)	CT (%)	TT (%)	P value	C (%)	T (%)	P value	OR (95% CI)	$\chi^2$	P value
HAN and HUI	Control (n=200)	79 (39.5)	90 (45.0)	31 (15.5)	0.16	248 (62.0)	152 (38.0)	0.15	0.81 (0.61-1.08)	0.405	0.52
	Case (n=200)	74 (37)	80 (40.0)	46 (23.0)		228 (57.0)	172 (43.0)				
HUI	Control (n=102)	36 (35.3)	40 (39.2)	15 (14.7)	0.29	123 (60.3)	81 (39.7)	0.76	1.06 (0.71-1.58)	0.199	0.65
	Case (n=102)	43 (42.2)	80 (78.4)	19 (18.6)		126 (61.8)	78 (38.2)				
HAN	Control (n=98)	43 (43.9)	39 (39.8)	16 (16.3)	0.09	125 (63.8)	71 (36.2)	0.01	1.62 (1.08-2.43)	1.885	0.169
	Case (n=98)	31 (31.6)	40 (40.8)	27 (27.6)		102 (52.0)	94 (48.0)				

Significant value ( $p < 0.05$ )

## Discussion

Differences in culture, lifestyle and heredity exist between the Hui and Han ethnic groups. We found that psychological stress was associated with MS, and results from our previous study that suggested differences in the level of psychological stress exist between Hui and Han ethnic groups. Further, we found the stress-related TNF $\alpha$  gene was associated with MS in the Hui and Han ethnic groups. In contrast, the NPY gene might possibly contribute to MS in the Han ethnic group. Metabolic syndrome has become a major public health problem within our society, several studies suggested that some components of the metabolic syndrome have been associated with the risk of breast cancer and prostate cancer (Ronco et al., 2012; Ozbek et al., 2014). Therefore, it is no time to delay to focus on the risk factors of MS

Jermendy et al performed an interesting twin study that explains differences between genetic and environmental factors in the pathogenesis of type 2 diabetes and cardiovascular diseases (Jermendy et al., 2011). A study investigating the prevalence of MS and its factors in a Dutch multi-ethnic cohort of obese and overweight children found that Turkish children had significantly higher prevalence of cardiometabolic risk factors relative to their peers of Moroccan descent (Van Vliet et al., 2009). Given racial differences in genetic susceptibility and aggravating stress levels, differential methods must be developed to treat and prevent MS for different groups.

Exposure to chronic psychological stress increased vulnerability to MS. Two major components mediate stress responses: the autonomic nervous system, encompassing the sympathetic and parasympathetic nervous systems, and the hypothalamus-pituitary-adrenocortical (HPA) axis (Bose et al., 2009). HPA has been widely recognized as a potential mechanism for coping with stress (McEwen, 1998). Dallman et al. suggest psychological stress leads to weight gain through behavioral pathways, such as increased food assumption and sedentary behavior, as well as through prolonged exposure to biological stress mediators such as cortisol (Dallman et al., 2010). In contrast, another study suggested that hypocortisolism caused by chronic stress might have beneficial effects for the individual (Fries et al., 2005). Analysis of psychological status in our study suggests that there is association between psychological stress and MS. Exploring the association of polymorphisms in the stress-related TNF $\alpha$  and NPY genes with metabolic syndrome in Han and Hui ethnic groups is very important.

Recent studies indicate that TNF $\alpha$  is related to insulin resistance, hypertension, leptin levels and hypercholesterolemia, which lead to MS (Moller 2000; Trayhurn et al., 2004; Lo et al., 2007). The G-to-A variant of TNF $\alpha$ -308 increased the excretion of cytokine TNF, which plays a critical role in the onset of MS (Dalziel et al., 2002; Gupta et al., 2012) However, TNF $\alpha$  exerts complex regulatory actions on the development of MS. Curti et al, by assessing the association of TNF $\alpha$ -308G/A with changes induced by lifestyle intervention, found that the TNF $\alpha$ -308 G/A SNP may predispose a better glucose metabolism response (Curti et al., 2012). However, several other studies suggest the TNF $\alpha$  G-308A polymorphism is unlikely to play an important role in the development of insulin resistance and obesity related to MS (Meirhaeghe et al., 2005). In our study, the mutant A allele was less frequent in the control group, and a significant association of the TNF $\alpha$ -308 G/A polymorphism with MS was observed.

Kuo et al found that exposure to stress lead to the release of NPY from sympathetic nerves, which increased white adipose tissue and mediated stress-induced obesity and MS (Kuo et al., 2007). In our study, NPY associated with MS only in ethnically Han individuals. Differences in these conclusions could be explained by differences in ethnicity as well as by the difference between the test organisms used (human and non-human animals). Differences in transcription factors regulating NPY expression has been observed for different races (Gallagher et al., 2010). For instance, feeding wild-type mice a high-fat diet for 2 weeks does not affect mRNA expression levels of NPY (Heijboer et al., 2005). However, a recent study of Spanish children confirmed the association between NPYrs16147 and obesity and demonstrated for the first time an association of NPYrs16131 with childhood obesity (Olza et al., 2013). Extreme stress combined with a high fat/sugar diet increases the endogenous release of NPY into visceral fat, causing the formation of new adipocytes and accelerating abdominal obesity and the development of MS (Rasmusson et al., 2010).

In conclusion, our findings suggest that psychological stress contributes to the development of MS. In addition, variants of the stress-related TNF $\alpha$  gene could constitute a useful predictive marker. We found that the NPY gene was associated with MS only in the Han ethnic group. To find potential and specific strategies to prevent and control the development of MS, a cohort study with subjects of different ethnicities that experience different levels of psychological stress is necessary to confirm and evaluate this study.

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