

# Altered features of neurotransmitters: NPY, $\alpha$ -MSH, and AgRP in type 2 diabetic patients with hypertension

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## Abstract

**Objective:** To investigate the features of neuropeptide Y (NPY),  $\alpha$ -melanocyte stimulating hormone ( $\alpha$ -MSH), and agouti-related protein (AgRP) in type 2 diabetes mellitus (T2DM) patients with hypertension.

**Methods:** Patients with T2DM ( $n = 384$ ) and healthy volunteers ( $n = 80$ ) were enrolled into this study. Serum NPY,  $\alpha$ -MSH, and AgRP levels were detected using ELISA.

**Results:** Significantly higher NPY and lower  $\alpha$ -MSH and AgRP levels were observed in patients with diabetes compared with those without diabetes, and the mean NPY levels increased, while  $\alpha$ -MSH and AgRP levels decreased, with the development of hypertension compared with diabetic patients without hypertension.  $\alpha$ -MSH and AgRP levels decreased with an increase in blood pressure in hypertension compared with the non-hypertension patients. Multiple stepwise linear regression analysis showed that NPY,  $\alpha$ -MSH, and AgRP levels were closely associated with blood pressure and glucose control. Receiver operating characteristic (ROC) curve analyses indicated that  $\alpha$ -MSH may be a better marker compared with NPY and AgRP for regulating glucose and blood pressure and to distinguish between T2DM patients with and without hypertension.

**Conclusion:** NPY,  $\alpha$ -MSH, and AgRP might play different roles and be closely related to the occurrence and development of diabetes and hypertension.

## Keywords

Type 2 diabetes mellitus, hypertension, NPY,  $\alpha$ -MSH, AgRP, glucose control

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## Introduction

Hypertension (HP) is an important problem for public health, and there has been a noticeable increase in its prevalence worldwide.<sup>1</sup> The pathogenesis of HP has not been completely clarified, but metabolic abnormalities have recently been recognized as trigger mechanisms for HP. It is generally known that cytokines have a wide range of biological functions, such as omentin, which participates in inflammation,<sup>2</sup> neuregulin, which affects insulin resistance,<sup>3</sup> and leptin, which regulates glucose and lipid metabolism.<sup>4</sup> Numerous studies have found that cytokines are closely related to diabetes.<sup>5-8</sup> However, recent studies have indicated that endogenously synthesized agents that are involved in energy metabolism play a key role in the development of HP.

Neuropeptide Y (NPY),  $\alpha$ -melanocyte stimulating hormone ( $\alpha$ -MSH), and agouti-related protein (AgRP) are secreted by the hypothalamus with definite effects on regulating the influence on energy intake and expenditure.<sup>9-11</sup> NPY is a potent orexigenic peptide in the brain and it is abundantly expressed in the arcuate nucleus (ARC) of the hypothalamus.<sup>12</sup> There is a large amount of evidence suggesting that central NPY administration resulted in weight gain and adiposity by increasing appetite and vagal activity as well as suppressing the thermogenic mechanism and sympathetic outflow, thereby regulating vasoconstriction and energy metabolism, which alters blood pressure and glucose.<sup>13</sup> Blood  $\alpha$ -MSH is a tridecanoic peptide that modulates appetite, glycometabolism, and lipid metabolism, and it was principally secreted by the hypothalamus, pituitary, and intestinal mucosa. Several lines of evidence suggested that  $\alpha$ -MSH increased the intracellular concentration of cyclic adenosine monophosphate (cAMP), activated protein kinase A via the

cAMP signaling pathways, and initiated pathways affecting sympathetic nerve activity to regulate blood pressure.<sup>14</sup> Therefore, the variations of  $\alpha$ -MSH in diabetic patients need to be further studied. AgRP is a long-lasting appetite stimulator. Similar to pharmacologic blockade of insulin signaling, mice with ablation of the insulin receptor in AgRP neurons showed impaired suppression of hepatic glucose production, demonstrating the significance of insulin action on these neurons in the maintenance of glucose homeostasis.<sup>10</sup> Another study suggested that the AgRP concentration was altered in patients with diabetes,<sup>12</sup> while the variation in HP was unstated.

The above studies revealed that many studies discussed the changes in NPY alone and with  $\alpha$ -MSH and AgRP in patients with diabetes or HP. Moreover, these factors also play a role in regulating energy metabolism by influencing food intake. However, the diagnostic value of serum NPY,  $\alpha$ -MSH, and AgRP, which act as neurotransmitters that regulate energy balance, and their changing characteristics in patients with diabetes combined with HP remain unclear. Therefore, this study was designed to evaluate the characteristics of energy balance neurotransmitters (NPY,  $\alpha$ -MSH, and AgRP) in patients with type 2 diabetes (T2DM) and HP, to determine the correlation between metabolic indexes, and to explore their diagnostic value in patients with diabetes mellitus and concurrent HP.

## Patients and methods

### Study population

The study included T2DM patients and healthy control subjects (NC group). All patients were clinically assessed using a detailed history (including age, duration of T2DM, and duration of HP) and physical examination. T2DM was diagnosed

based on the 1999 World Health Organization International Society of Diabetes Guidelines.<sup>15</sup> The inclusion criteria for patients with HP are based on the Hypertension Guidelines for patients diagnosed with HP.<sup>4</sup> T2DM patients were divided into the following four groups: DS, DH1, DH2, and DH3. The DS group included patients who had T2DM only. The DH1 group included patients with T2DM and grade 1 HP whose blood pressure ranged from a systolic blood pressure (SBP) of 140 to 159 mmHg and/or a diastolic blood pressure (DBP) of 90 to 99 mmHg. The DH2 group included patients with T2DM and grade 2 HP whose blood pressure ranged from a SBP of 160 to 179 mmHg and/or a DBP of 100 to 109 mmHg. The DH3 group included T2DM patients with grade 3 HP whose blood pressure was greater than or equal to a SBP of 180 mmHg and/or a DBP value of 110 mmHg. The exclusion criteria were previous duration of diabetes of more than 15 years; cardiovascular and/or cerebrovascular diseases; severe or acute complications of diabetes including diabetic ketoacidosis and hyperglycemic hyperosmolar status, severe hepatic and/or renal dysfunction; or mental disorders.

The study was approved by the research ethics committee of Baoding NO.1 Central Hospital (reference number: 2017003), and all subjects were informed about the study and provided written informed consent.

### *Physical examinations and laboratory tests*

The participants' height and weight were routinely measured. Body mass index (BMI) was calculated as body weight divided by the height squared ( $\text{kg}/\text{m}^2$ ). Heart rate (HR), SBP, and DBP were measured and recorded. Blood pressure and HR were measured three times in the sitting position using an automatic brachial sphygmomanometer. The participants were

seated and relaxed for 5 minutes before measurement.

Blood samples were drawn from each participant following an overnight fast of 8 to 10 hours. Venous blood samples were drawn into vacutainer tubes, were centrifuged at  $4000 \times g$  for 5 minutes, and serum samples were stored at  $-80^\circ\text{C}$  until the measurement was performed. All samples were analyzed within 3 months of inclusion in the study.

Serum fasting glucose (FBG), lipid profile (total cholesterol, triglycerides), alanine transaminase (ALT), and serum creatinine (Scr) were measured using a biochemical instrument (Hitachi 7600; Hitachi Medical Corporation, Tokyo, Japan). Glycosylated hemoglobin (HbA1c) was measured using liquid chromatography by saccharifying HA8180 instrument (ARKRAY corporation, Tokyo, Japan).

NPY,  $\alpha$ -MSH, and AgRP levels were measured in duplicate using enzyme-linked immunosorbent assay (ELISA) kits (Shanghai Enzyme-linked Biotechnology Co., Ltd., Shanghai, China). The manufacturer determined the within-run and between-run coefficients of variation to be 6% and 10%, respectively. The minimum detection limit for NPY was 5 ng/L and the maximum detection limit was 120 ng/L. The minimum detection limit for  $\alpha$ -MSH was 0.5 ng/mL, and the maximum detection limit was 15 ng/mL. The minimum detection limit for AgRP was 5 ng/L, and the maximum detection limit was 180 ng/L.

### *Statistical analysis*

Data were analyzed using the statistical program SPSS version 22.0 for Windows (IBM Corp., Armonk, NY, USA), and Microsoft EXCEL programs (Microsoft Corporation, Seattle, WA, USA). Normal distribution was tested using the Kolmogorov–Smirnov test. Values are expressed as the mean  $\pm$  standard deviation (SD) for normally distributed variables. For the comparison of

two means of independent samples, the least significant difference (LSD)-*t*-test (rank sum test) was used. To compare the homogeneity of variance in the parameters among the five groups, a one-way analysis of variance (ANOVA) was used. To identify the correlation between NPY,  $\alpha$ -MSH, AgRP, and other parameters, Pearson's correlation coefficient was assessed. This was followed by a multivariate linear regression. Additionally,  $p < 0.05$  was considered to be the statistically significant threshold.

## Results

### Clinical and laboratory characteristics of the study population

The study enrolled 384 T2DM patients and 80 healthy control subjects (NC group).

There were 88 patients in the DS group, 93 patients in the DH1 group, 98 patients in the DH2 group, and 105 patients in the DH3 group. The clinical and laboratory characteristics of the different study groups are summarized in Table 1. There were no differences in age, BMI, HR, TG, and TC among the studied groups, indicating that the data are feasible. SBP and DBP were highest in the DH3 group compared with the other groups ( $p < 0.05$ ), and the SBP and DBP in the DH1 group were lower compared with the DH2 group ( $p < 0.05$ ). Similarly, the SBP and DBP in the DS group were higher compared with the NC group, but the difference was not significant. Although DBP in the DS group was higher compared with NC group, the difference was not significant. Additionally, no remarkable variation tendency was

**Table 1.** Comparison of the clinical and laboratory characteristics between groups.

Variables	NC group (n = 80)	DS group (n = 88)	DH1 group (n = 93)	DH2 group (n = 98)	DH3 group (n = 105)
Age (years)	56.44 ± 8.36	57.41 ± 8.31	56.68 ± 8.30	57.21 ± 8.04	56.30 ± 7.95
BMI (kg/m <sup>2</sup> )	25.36 ± 1.46	25.73 ± 3.40	25.21 ± 3.27	26.08 ± 3.34	26.42 ± 3.11
HR (beats/minute)	71.31 ± 7.59	71.56 ± 7.59	72.14 ± 9.24	72.04 ± 9.24	72.66 ± 7.53
Duration of T2DM (years)	–	7.01 ± 2.71	6.40 ± 2.02	7.01 ± 2.70	6.82 ± 2.45
Duration of HP (years)	–	–	7.72 ± 1.31	7.59 ± 2.18	7.80 ± 2.05
SBP (mmHg)	124.66 ± 4.68	126.33 ± 9.76	147.44 ± 7.99 <sup>##</sup>	165.17 ± 10.68 <sup>##Δ</sup>	182.78 ± 10.60 <sup>##Δ▽</sup>
DBP (mmHg)	79.45 ± 6.07	80.06 ± 6.56	91.43 ± 5.57 <sup>##</sup>	98.64 ± 8.89 <sup>##Δ</sup>	109.44 ± 12.10 <sup>##Δ▽</sup>
FBG (mmol/L)	5.27 ± 0.62	9.26 ± 1.94 <sup>*</sup>	9.17 ± 1.65 <sup>*</sup>	8.79 ± 1.91 <sup>*</sup>	8.79 ± 1.91 <sup>*</sup>
HbA1c (%)	5.33 ± 0.40	9.46 ± 2.88 <sup>*</sup>	9.25 ± 3.28 <sup>*</sup>	8.91 ± 2.84 <sup>*</sup>	9.12 ± 3.79 <sup>*</sup>
TG (mmol/L)	1.69 ± 0.18	1.64 ± 0.29	1.69 ± 0.31	1.68 ± 0.28	1.76 ± 0.29
TC (mmol/L)	5.13 ± 0.55	5.03 ± 0.71	4.87 ± 0.77	5.11 ± 0.77	5.13 ± 0.61
Scr (mmol/L)	76.97 ± 15.72	73.60 ± 16.37	75.20 ± 14.25	79.50 ± 15.22	75.30 ± 14.19
ALT (mmol/L)	23.73 ± 5.40	23.36 ± 6.34	23.55 ± 6.52	22.91 ± 6.39	22.54 ± 6.58

Note: Values are presented as the mean ± SD.

NC group, normal control subjects; DS group, diabetes only; DH1 group, diabetes with grade 1 hypertension patients; DH2 group, diabetes with grade 2 hypertension patients; and DH3 group, diabetes with grade 3 hypertension patients. \* $P < 0.05$  compared with group NC, <sup>##</sup> $P < 0.05$  compared with group DS, <sup>Δ</sup> $P < 0.05$  compared with group DH1, <sup>▽</sup> $P < 0.05$  compared with group DH2.

Abbreviations: BMI, body mass index; HR, heart rate; DM, diabetes mellitus; HP, hypertension; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; HbA1c, glycosylated hemoglobin; TG, triacylglycerol; TC, total cholesterol; ALT, alanine transaminase; Scr, serum creatinine.

revealed for FBG and HbA1c in the DS, DH1, DH2, and DH3 groups. There was no difference in the HP course among the DH groups, and no difference in the T2DM course among the DS and DH groups.

### Comparison of NPY, $\alpha$ -MSH, and AgRP among the different groups

Plasma NPY levels were higher in the DS group compared with the NC group ( $P < 0.05$ ). Conversely, there was a distinct increase in NPY in hypertensive patients with T2DM compared with the DS group ( $p < 0.05$ ), and blood NPY in the DH3 group was the highest compared with the other groups, demonstrating that HP and diabetes had a synergistic effect on serum NPY levels. Details are shown in Table 2. Serum  $\alpha$ -MSH in the DS group decreased compared with the NC group ( $p < 0.05$ ), showing that  $\alpha$ -MSH in diabetes may decrease significantly. Compared with the NC and DS groups,  $\alpha$ -MSH in the DH1, DH2, and DH3 groups decreased significantly ( $p < 0.05$ ). AgRP in the DS group was lower compared with the NC group ( $p < 0.05$ ). Plasma AgRP levels in the DH groups decreased with the increase in blood pressure ( $p < 0.05$ ), and the lowest level was in the DH3 group. Additionally, serum AgRP in the DH1 group decreased compared with the DS group ( $p < 0.05$ ). AgRP

levels decreased with an increase in blood pressure, but  $\alpha$ -MSH decreased significantly only in patients with primary HP ( $p < 0.05$ ), and there was no significant decrease in patients with severe HP, suggesting that AgRP might have greater research value compared with  $\alpha$ -MSH.

### Correlations between NPY, $\alpha$ -MSH, AgRP, and other biochemical variables among the groups

To evaluate the correlations between NPY,  $\alpha$ -MSH, AgRP, and other biochemical variables, Pearson's correlation coefficients were calculated, and the data are presented in Table 3. Pearson's correlation analysis showed that NPY levels were positively correlated with SBP, DBP, duration of T2DM, duration of HP, FBG and HbA1c. NPY was negatively correlated with  $\alpha$ -MSH and AgRP. Additionally, NPY was not significantly correlated with age, BMI, TC, TG, ALT, and Scr. Plasma  $\alpha$ -MSH was negatively correlated with SBP, DBP, duration of T2DM, duration of HP, BMI, FBG, HbA1c, and NPY, indicating that  $\alpha$ -MSH was positively correlated with AgRP. There were also no evident correlations between  $\alpha$ -MSH and other relevant variables. AgRP levels showed a remarkable negative correlation with SBP, DBP, age, duration of T2DM, duration of HP, BMI, FBG,

**Table 2.** Comparison NPY,  $\alpha$ -MSH, and AgRP among the groups.

Variables	NC group (n=80)	DS group (n=88)	DH1 group (n=93)	DH2 group (n=98)	DH3 group (n=105)
NPY (ng/L)	8.15 ± 2.22	11.55 ± 3.40*	14.04 ± 2.39* <sup>##</sup>	14.72 ± 2.43* <sup>##</sup>	17.59 ± 4.22* <sup>##</sup> △ <sup>▽</sup>
$\alpha$ -MSH (ng/mL)	5.77 ± 0.95	3.36 ± 0.66*	2.16 ± 0.35* <sup>##</sup>	1.35 ± 0.22* <sup>##</sup> △	1.21 ± 0.18* <sup>##</sup> △
AgRP (ng/L)	53.65 ± 12.75	48.03 ± 9.60*	40.54 ± 10.20* <sup>##</sup>	35.85 ± 8.49* <sup>##</sup> △	30.30 ± 7.41* <sup>##</sup> △ <sup>▽</sup>

Note: Values are presented as the mean ± SD.

NC group, normal control subjects; DS group, diabetes only; DH1 group, diabetes with grade 1 hypertension patients; DH2 group, diabetes with grade 2 hypertension patients; and DH3 group, diabetes with grade 3 hypertension patients. \* $P < 0.05$  compared with group NC, <sup>##</sup> $P < 0.05$  compared with group DS, △ $P < 0.05$  compared with group DH1, <sup>▽</sup> $P < 0.05$  compared with group DH2.

Abbreviations: NPY, neuropeptide Y;  $\alpha$ -MSH,  $\alpha$ -melanocyte stimulating hormone; AgRP, agouti-related protein.

**Table 3.** Correlations between NPY,  $\alpha$ -MSH, and AgRP and other biochemical variables.

Variables	AgRP		$\alpha$ -MSH		NPY	
	r	P	r	P	r	P
HR (beats/min)	-0.082	0.078	-0.052	0.259	0.077	0.096
SBP (mmHg)	-0.589	<0.001	-0.751	<0.001	0.613	<0.001
DBP (mmHg)	-0.528	<0.001	-0.652	<0.001	0.529	<0.001
Age (years)	0.112	0.016	0.041	0.380	-0.051	0.277
Duration of T2DM (years)	-0.313	<0.001	-0.625	<0.001	0.403	<0.001
Duration of HP (years)	-0.529	<0.001	-0.766	<0.001	0.571	<0.001
BMI (kg/m <sup>2</sup> )	-0.132	0.004	-0.104	0.025	0.043	0.357
ALT (mmol/L)	-0.016	0.732	0.031	0.511	-0.049	0.292
TC (mmol/L)	0.013	0.778	0.035	0.447	0.004	0.923
TG (mmol/L)	-0.057	0.221	-0.053	0.257	0.028	0.553
Scr (mmol/L)	-0.049	0.289	-0.034	0.467	-0.001	0.981
FBG (mmol/L)	-0.242	<0.001	-0.494	<0.001	0.356	<0.001
HbA1c (%)	-0.190	<0.001	-0.354	<0.001	0.202	<0.001
AgRP (ng/L)	1.000	-	0.782	<0.001	-0.467	<0.001
$\alpha$ -MSH (ng/mL)	0.782	<0.001	1.000	-	-0.655	<0.001
NPY (ng/L)	-0.467	<0.001	-0.655	<0.001	1.000	-

r, Spearman correlation coefficient. P-value: correlation is significant at the 0.05 level.

BMI, body mass index; HR, heart rate; T2DM, type 2 diabetes mellitus; HP, hypertension; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; HbA1c, glycosylated hemoglobin; TG, triacylglycerol; TC, total cholesterol; ALT, alanine transaminase; Scr, serum creatinine; NPY, neuropeptide Y;  $\alpha$ -MSH,  $\alpha$ -melanocyte stimulating hormone; AgRP, agouti-related protein.

HbA1c, and NPY, while it was positively correlated with  $\alpha$ -MSH.

### Multiple linear regression analysis

Multiple linear regression analysis was performed to verify the factors affecting NPY,  $\alpha$ -MSH, and AgRP, and the results are presented in Table 4. Serum NPY was a dependent variable, with SBP, DBP, duration of T2DM, duration of HP, FBG, HbA1c,  $\alpha$ -MSH, and AgRP as independent variables. A multiple stepwise regression analysis showed that NPY was strongly correlated with SBP and  $\alpha$ -MSH. Concurrently, plasma  $\alpha$ -MSH acted as the dependent variable, while SBP, DBP, duration of T2DM, duration of HP, BMI, FBG, HbA1c, NPY, and AgRP were the independent variables. A multiple stepwise regression analysis showed that  $\alpha$ -MSH was strongly correlated with SBP, DBP, duration of T2DM,

duration of HP, FBG, HbA1c, NPY, and AgRP. The AgRP level was the dependent variable, and SBP, DBP, age, duration of T2DM, duration of HP, BMI, FBG, HbA1c, NPY, and  $\alpha$ -MSH were the independent variables. Multiple stepwise regression analysis showed that AgRP was strongly correlated with duration of T2DM, duration of HP, FBG, and  $\alpha$ -MSH. Thus, duration of T2DM, duration of HP, FBG, and HbA1c may be independent risk factors for transforming  $\alpha$ -MSH and AgRP.

### Receiver operating characteristic analysis

Receiver operating characteristic (ROC) analysis suggests that the plasma NPY level could distinguish between patients with T2DM and patients with T2DM combined with HP, with an area under the curve (AUC) for the ROC curve of 0.782

**Table 4.** Multiple linear regression analysis of factors affecting NPY,  $\alpha$ -MSH, and AgRP levels.

Dependent Variables	Independent Variable	$\beta$	SE	$\beta'$	t	95% CI		P
						min	max	
AgRP	SBP	0.004	0.025	0.007	0.148	-0.045	0.052	0.883
	DBP	-0.023	0.034	-0.026	-0.683	-0.091	0.031	0.495
	Age	0.084	0.040	0.054	2.106	0.006	0.163	0.036
	Duration of T2DM	0.965	0.130	0.260	7.402	0.709	1.221	<0.001
	Duration of HP	0.742	0.138	0.234	5.389	0.472	1.013	<0.001
	BMI	-0.196	0.106	-0.047	-1.847	-0.404	0.013	0.065
	FBG	0.724	0.179	0.126	4.055	0.373	1.076	<0.001
	HbA1C	0.199	0.110	0.051	1.812	-0.017	0.414	0.071
	$\alpha$ -MSH	9.066	0.424	1.220	21.389	8.233	9.899	<0.001
$\alpha$ -MSH	NPY	0.155	0.102	0.053	1.522	-0.045	0.356	0.129
	SBP	-0.008	0.002	-0.117	-4.339	-0.012	-0.005	<0.001
	DBP	-0.005	0.003	-0.045	-2.047	-0.011	0.000	0.041
	Duration of T2DM	-0.112	0.009	-0.224	-11.887	-0.130	-0.093	<0.001
	Duration of HP	-0.106	0.010	-0.248	-10.702	-0.126	-0.087	<0.001
	BMI	0.005	0.008	0.010	0.641	-0.011	0.022	0.522
	FBG	-0.090	0.014	-0.116	-6.606	-0.116	-0.063	<0.001
	HbA1C	-0.031	0.008	-0.060	-3.712	-0.048	-0.015	<0.001
	NPY	-0.032	0.008	-0.082	-4.104	-0.048	-0.017	<0.001
NPY	AgRP	0.055	0.003	0.411	21.569	0.050	0.060	<0.001
	SBP	0.042	0.011	0.233	3.728	0.020	0.064	<0.001
	DBP	0.026	0.016	0.084	1.637	-0.005	0.057	0.102
	Duration of T2DM	-0.005	0.063	-0.004	-0.083	-0.130	0.119	0.934
	Duration of HP	0.047	0.065	0.043	0.722	-0.081	0.175	0.471
	FBG	0.152	0.083	0.077	1.824	-0.012	0.315	0.069
	HbA1C	-0.029	0.051	-0.022	-0.578	-0.128	0.070	0.563
	AgRP	0.032	0.021	0.093	1.499	-0.010	0.074	0.135
	$\alpha$ -MSH	-1.121	0.271	-0.438	-4.139	-1.653	-0.589	<0.001

$\beta$ , unstandardized coefficients;  $\beta'$ , standardized coefficients; SE, standard error; t, significance test value; P, correlation is significant at the 0.05 level.

BMI, body mass index; HR, heart rate; T2DM, type 2 diabetes mellitus; HP, hypertension; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; HbA1c, glycosylated hemoglobin; TG, triacylglycerol; TC, total cholesterol; ALT, alanine transaminase; Scr, serum creatinine, NPY, neuropeptide Y;  $\alpha$ -MSH,  $\alpha$ -melanocyte stimulating hormone; AgRP, agouti-related protein; min, minimum; max, maximum.

(95% CI 0.726 to 0.838,  $p < 0.001$ ). The cut-off value was 11.65 ng/L, which was associated with sensitivity and specificity of 88.3% and 60.2%, respectively. Additionally, serum AgRP distinguished between patients with T2DM and patients with diabetes, with an AUC of 0.831 (95% CI 0.788 to 0.874,  $p < 0.001$ ). The cut-off value was 42.18 ng/L, with a sensitivity of 83.7% and a specificity of 69.4%.

Although NPY,  $\alpha$ -MSH, and AgRP were also identified as the predictors of T2DM and concurrent HP based on the ROC curve,  $\alpha$ -MSH presented a greater ability to distinguish between patients with T2DM and patients with T2DM and HP compared with NPY and AgRP. The optimal cut-off value of serum  $\alpha$ -MSH as an indicator for a diagnosis of diabetes combined with HP was projected to be 2.46 ng/mL, which yielded a

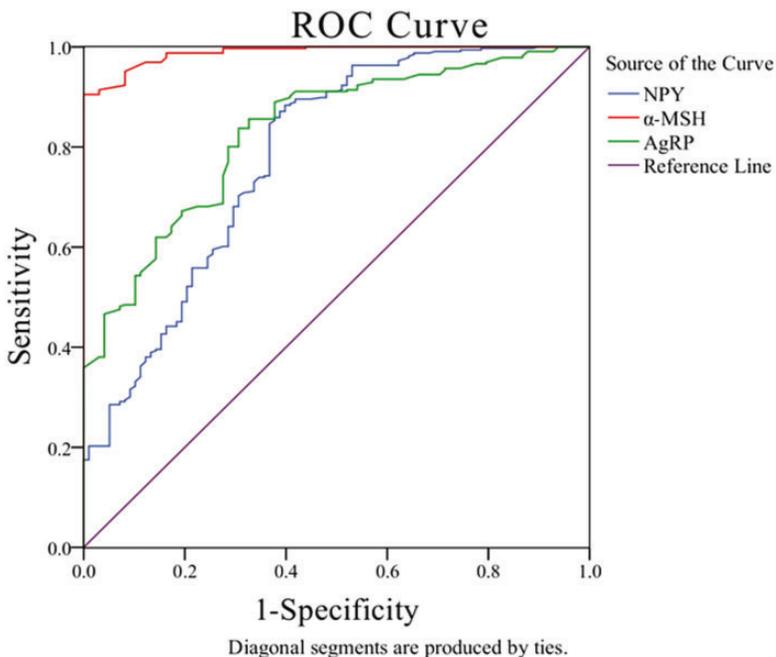
sensitivity of 92.3% and a specificity of 91.8%, with an AUC of 0.988 (95%CI 0.981 to 0.995,  $p < 0.001$ ; Figure 1).

## Discussion

HP and diabetes are closely related to energy metabolism. NPY,  $\alpha$ -MSH, and AgRP, as regulators of energy balance, can affect energy metabolism through different pathways.<sup>16,17</sup> This study explored the characteristics of changes in serum NPY,  $\alpha$ -MSH, and AgRP concentration in diabetic patients with different degrees of HP, and it showed the diverse trends of the three neurotransmitters. The important new findings are summarized as follows: 1) compared with healthy volunteers, patients with T2DM had an elevated level

of NPY and a lower level of  $\alpha$ -MSH and AgRP; 2) NPY levels in diabetes were significantly increased in T2DM patients with HP, but the  $\alpha$ -MSH and AgRP levels decreased in hypertensive diabetes; 3) T2DM patients showed a gradual increase in blood pressure with an increase in NPY level, but  $\alpha$ -MSH and AgRP levels both showed an opposite downward trend; and 4)  $\alpha$ -MSH was more valuable compared with NPY and AgRP for predicting the incidence of HP in diabetic patients.

Hypothalamic neuropeptides regulated a diverse array of physiological functions, among which the best characterized function was the maintenance of energy balance and insulin action.<sup>18</sup> NPY shows many polypeptides composed of 36 amino acid residues, which are principally located inside



**Figure 1.** Receiver operating characteristic curve of serum NPY,  $\alpha$ -MSH, and AgRP for predicting the occurrence of hypertension with T2DM.

Note: The ROC curve was described as a function of 1-specificity for predicting patients with T2DM and hypertension based on the serum concentrations of NPY,  $\alpha$ -MSH, and AgRP.

Abbreviations: NPY, neuropeptide Y;  $\alpha$ -MSH,  $\alpha$ -melanocyte stimulating hormone; AgRP, agouti-related protein.

sympathetic nerve endings, and it is a common central and peripheral nervous system neurotransmitter. NPY also participates in regulating the body's circadian rhythms and maintaining the memory process.<sup>19</sup> Previous studies have shown that changes in NPY could cause some diseases with vascular lesions, promote angiogenesis, and cause HP.<sup>20</sup>

Engström et al.<sup>21</sup> reported a pivotal role for NPY-dependent signaling in mediating the rapid feed-inducing effect and the acute glucose regulatory function that is governed by AgRP neurons. In our study, plasma NPY was visibly higher in T2DM with HP compared with T2DM patients without HP and normal individuals.

$\alpha$ -MSH and AgRP, which comprise the hypothalamus melanocortin neuronal circuits that regulate feeding and insulin secretion, transform blood glucose.<sup>22</sup> Recently, however, it was recognized that  $\alpha$ -MSH levels increased hepatic insulin sensitivity adequately, regulating feeding and energy consumption to influence glycometabolism.  $\alpha$ -MSH was also shown to be a factor that plays a significant regulatory role in obesity and diabetes.<sup>23</sup> Animal experiments have indicated that MC4R knock-out rats became obese without diabetes and showed a high degree of insulin resistance.<sup>24</sup> The study showed that the  $\alpha$ -MSH level in T2DM patients with different grades of HP decreased significantly compared with non-hypertensive patients with T2DM. The  $\alpha$ -MSH level was negatively correlated with SBP, DBP, duration of T2DM, duration of HP, FBG, HbA1c, NPY and AgRP. AgRP also showed a similar trend with respect to the variation in  $\alpha$ -MSH. Previous studies provided evidence that AgRP was only synthesized in NPY-containing cell bodies that were located in the ventromedial part of the arcuate nucleus in the hypothalamus.<sup>25</sup> The AgRP is an effective peptide that promotes appetite, and the appetite-stimulating effect of

AgRP was activated by the hormone ghrelin, which further increased food intake when AgRP was overexpressed.<sup>26</sup> Similarly, Zhang et al.<sup>27</sup> also showed that AgRP was closely associated with glucose homeostasis and insulin sensitivity. However, another recent study suggested that the change in AgRP mRNA expression was less pronounced in T2DM, and diabetic hyperphagia was regulated by central mechanisms in which the ghrelin-signaling pathway affected NPY and AgRP expression in the hypothalamus.<sup>28</sup> In our study, AgRP was markedly decreased in patients with T2DM only and in T2DM patients with HP compared with healthy volunteers. Pearson's correlation analysis revealed that AgRP was negatively correlated with SBP, DBP, age, duration of T2DM, duration of HP, BMI, FBG, HbA1c, and NPY, but positively correlated with  $\alpha$ -MSH. The specific mechanism needs to be studied in a large number of clinical and animal experiments.

The ROC curve assessed the diagnostic value of plasma NPY,  $\alpha$ -MSH, and AgRP that are used as energy balance regulators to identify their role in patients with T2DM with HP. Our analyses showed that serum NPY,  $\alpha$ -MSH, and AgRP may distinguish between T2DM patients with HP and those with T2DM only. Among them, serum  $\alpha$ -MSH had the highest differential diagnosis value. Therefore,  $\alpha$ -MSH is better compared with NPY and AgRP, and it could serve as a potential novel marker that regulates glucose and blood pressure to predict the occurrence of HP in T2DM patients.

Several limitations of our study should be addressed. First, more studies are needed to confirm our results because of the relatively small sample size in our study. Second, fundamental research and animal models are required to clarify the mechanisms of variation that might be considered to be other limitations of this study. Third, our study was a cross-sectional

design, and it can be difficult to accurately predict whether these factor levels preceded T2DM in HP patients. Thus, our findings require validation in a prospective long-term study.

The role of cytokines in regulating metabolism and inflammation has been confirmed by many studies, and metabolism and inflammation play an important role in the pathogenesis of diabetes or HP.<sup>29,30</sup> Whether there are other cytokines that also show significance requires further research.

In conclusion, above findings supported the hypothesis that the NPY level was increased but  $\alpha$ -MSH and AgRP levels were decreased in patients with T2DM and concurrent HP. However, there was a high degree of variation with the distinct grades of blood pressure in T2DM patients. Additionally, serum NPY,  $\alpha$ -MSH, and AgRP levels may play roles as biomarkers that regulate energy metabolism and identify hypertensive patients with T2DM.  $\alpha$ -MSH was the optimal diagnostic marker, and it could predict the occurrence of HP in T2DM patients. It can also provide some data for other studies on diabetes and HP or related research on energy metabolism-related factors. However, the clinical value of measuring NPY,  $\alpha$ -MSH, and AgRP levels requires further investigation.

#### Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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