

DOI: 10.20098/j.cnki.1001-5132.2023.0414

Uric acid mediated hypomethylation of *OPRD1* gene affects Alzheimer's disease

CHEN Yao^{1,2}, CHEN Yong^{1*}, YING Ying¹, FAN Jianbo³, GENG Baoqing¹, ZHENG Chengying⁴,
XU Ting⁴, FU Haoyuan⁵, ZHANG Yunge⁶, CHEN Ji⁶

(1.Ningbo No.2 Hospital, Ningbo 315010, China; 2.Taizhou Hospital of Zhejiang, Taizhou 317099, China; 3.Zhenhai Longsai Hospital, Ningbo 315299, China; 4.Ningbo Kangning Hospital, Ningbo 315201, China; 5.The Third People's Hospital Health Care Group of Cixi (Chang He Branch), Ningbo 315325, China; 6.Health Science Center, Ningbo University, Ningbo 315211, China)

Abstract: The present study was aimed to investigate the correlation between *OPRD1* gene methylation, uric acid and Alzheimer's disease. It also examined whether uric acid could affect *OPRD1* gene methylation *in vitro*. The venous blood of the patients with Alzheimer's disease, the hyperuricemia group and the healthy control group was collected. The cells were successively treated with different concentrations of uric acid and the methylation level of *OPRD1* gene was subsequently determined. The methylation levels of *OPRD1* at pos2 and pos4 in the hyperuricemia group were lower than those of the healthy control group; while the methylation level of pos4 in the Alzheimer's disease group was higher than that of the healthy control group. Uric acid levels were negatively correlated with *OPRD1* gene methylation levels. *In vitro*, the methylation levels at pos2, pos3 and pos4 sites of *OPRD1* gene in cells with high concentration of uric acid were lower even further. The present study suggested that uric acid may affect the onset of Alzheimer's disease by mediating the hypomethylation modification at the pos4 site of *OPRD1* gene.

Key words: uric acid; key enzymes; *OPRD1* gene; methylation; Alzheimer's disease

CLC number: R741

Document code: A

Article ID: 1001-5132 (2023) 04-0078-11

1 Introduction

Alzheimer's disease (AD), the most common neurodegenerative disease leading to senile dementia, is a major public health problem worthy of the attention of the whole society. Recent studies have found that hypermethylation modification of the *OPRD1* gene is

associated with the risk of AD^[1]. The δ -opioid receptor (DOR) encoded by the *OPRD1* gene is involved in protecting neurons from hypoxic/ischemic stress. A research displays that the distribution of DOR in the brain of patients with AD is significantly reduced^[2]. In addition, quantitative autoradiography found that the binding of DOR in the amygdala complex and ventral

收稿日期: 2023-04-13.

宁波大学学报(理工版)网址: <http://journalg.nbu.edu.cn/>

基金项目: 浙江省医学科学研究基金(2020KY328, 2020KY268).

第一作者: 陈瑶(1997-), 女, 浙江台州人, 硕士/住院医师, 主要研究方向: 风湿免疫. E-mail: chenyl0290@enzemed.com

*通信作者: 陈勇(1968-), 男, 浙江宁波人, 主任医师, 主要研究方向: 风湿免疫. E-mail: nbdeyycy@163.com

putamen in the brain of patients with AD decreased^[3]. This indicates that the *OPRD1* gene has a strong correlation with DOR and AD, which may be due to the down-regulation of *OPRD1* gene expression in the hypermethylated state and the inhibition of DOR coding, leading to an increased risk of AD.

Uric acid is the end product of purine metabolism in the human body. At present, there is increasing evidence that uric acid may play a certain neuroprotective role in the occurrence and development of AD. A meta-analysis in 2016 found that serum uric acid levels in patients with AD were lower than those in the healthy control group, and high uric acid levels were significantly associated with a reduced risk of AD^[4]. In addition, a study on uric acid, AD and cognitive dysfunction has shown that high levels of uric acid can slow the progression of cognitive decline, especially statistically significant in patients with mild cognitive impairment and AD^[5].

In the early stage of the research group, methylation microarray screening was performed on the genome of patients with gout and hyperuricemia^[6]. The results showed that in patients with gout, the pos3 site in the promoter region of the *OPRD1* gene was hypomethylated, and in patients with hyperuricemia, the pos2 and pos4 sites in the promoter region of *OPRD1* gene were hypomethylated. And through a large sample experiment of human peripheral blood, it was confirmed that the *OPRD1* gene is hypomethylated and the *OPRD1* protein is highly expressed in gout patients. Therefore, we have reason to believe that uric acid may be related to the hypomethylation of the *OPRD1* gene.

To sum up, the purpose of this study is to confirm the correlation between *OPRD1* gene methylation level and uric acid level as well as AD in humans, and explore the correlation between uric acid and methylation modification at *OPRD1* gene locus in cell experiments.

The clinical significance of this study is to explore the pathogenesis of AD with uric acid level as the source, which provides a new idea and a theoretical basis for the

treatment of AD.

2 Materials and methods

2.1 Experiment 1: Correlation between *OPRD1* gene methylation and uric acid and AD

2.1.1 Research objects

45 patients with AD were enrolled in the study. The diagnosis was consistent with the 1994 "American Diagnostic and Statistical Manual of Mental Disorders". A total of 100 patients with hyperuricemia were enrolled, all of whom met the criteria of serum uric acid $>420 \mu\text{mol}\cdot\text{L}^{-1}$ in men and postmenopausal women, and $>360 \mu\text{mol}\cdot\text{L}^{-1}$ in premenopausal women. 100 healthy cases were included in the control group, excluding primary gout, hyperuricemia, AD, hypertension, diabetes, hyperlipidemia, coronary atherosclerotic heart disease, liver and kidney dysfunction, and malignant tumor.

The basic information and laboratory indexes of the patients were recorded, including age, gender, uric acid (UA), aspartate transaminase (AST), alanine transaminase (ALT), creatinine (Cr), fasting blood glucose (GLU), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride (TG) and C-reactive protein (CRP).

This study has been approved by the Hospital Ethics Committee. Participants were fully informed of the content and purpose of the study and informed consent was obtained before the study.

2.1.2 Experimental process

The whole genome DNA of peripheral blood cells was extracted with a DNA kit (QIAamp, US) and modified with sulfite using a DNA methylation modification kit (Zymo, US). The PCR primer synthesis was designed by PyroMark Assay Design 2.0. The appropriate cycle number, time, and temperature were explored, and the study sequence was amplified by PCR. Finally, the methylation level of the *OPRD1* gene was detected by pyrophosphate detector (PyroMark Q96 ID, QIAGEN). The expression level of *OPRD1* protein in

plasma was detected according to the ELISA kit instructions (Enzyme-linked Biotechnology Co. Ltd. Shanghai). The methylation level of *OPRD1* gene and the level of OPRD1 protein in plasma were compared among the three groups.

2.1.3 Statistical analysis

The data were analyzed by SPSS 25.0 software, the differences among the three groups were compared by ANOVA, and the correlation was analyzed by Pearson test, where $P < 0.05$ indicates the difference being statistically significant.

2.2 Experiment 2: Uric acid reduces *OPRD1* gene methylation by regulating key enzymes

2.2.1 Research objects

Human HEK293T cells.

2.2.2 Experimental process

The concentrations of uric acid were respectively adjusted to $720 \mu\text{mol}\cdot\text{L}^{-1}$ (high concentration), $120 \mu\text{mol}\cdot\text{L}^{-1}$ (low concentration), and $720 \mu\text{mol}\cdot\text{L}^{-1}$ (high concentration) for successively culturing HEK293T cells. The specific experimental groups are shown in Fig. 1. In addition, the control group of HEK293T cells was cultured in complete medium without uric acid.

The whole genome DNA of HEK293T cells was extracted by DNA kit, the DNA was modified by sulfite with DNA methylation modification kit, and the

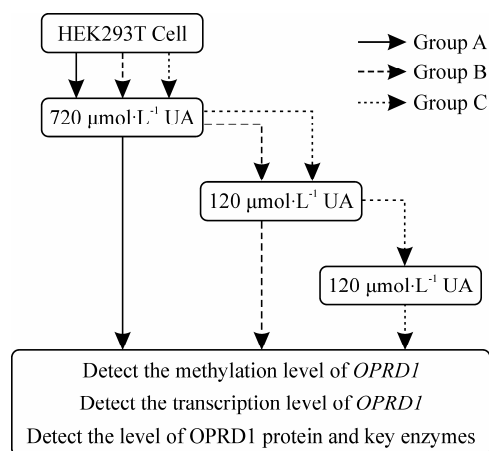


Fig. 1 Cell experiment process

methylation level was detected by a pyrophosphate detector. Total RNA was extracted from cells according to the instructions of the RNA extraction kit. The total RNA was reversely transcribed into cDNA according to the instructions of the reverse transcription kit, and PCR amplification was performed. The gene expression changes were analyzed by real-time fluorescence quantitative method, and the mRNA was relatively quantified by $2^{-\Delta C_t}$ method. The levels of OPRD1 protein and DNA methyltransferase (DNMT1, DNMT3A and DNMT3B) and demethylase (TET1 hydroxylase, AID deaminase and TDG glycosidase) were detected according to the ELISA kit instructions.

The differences of *OPRD1* gene methylation level,

Table 1 Laboratory indicators of AD, HUA and control group

Laboratory indicator	AD group	HUA group	Control group
Age ¹⁾	82.39±8.66	55.50 (39.25, 66.00)	39.50 (32.00, 53.00)
UA/($\mu\text{mol}\cdot\text{L}^{-1}$)	292.46±91.68 ²⁾	457.70±54.70	276.90 (244.50, 341.58) ²⁾
AST/($\text{U}\cdot\text{L}^{-1}$)	20.00 (17.00, 26.00)	22.00 (19.00, 27.00)	19.50 (17.00, 24.00) ²⁾
ALT/($\text{U}\cdot\text{L}^{-1}$)	15.00 (7.50, 19.00) ²⁾	21.00 (16.25, 36.00)	17.00 (12.00, 23.00) ²⁾
Cr/($\mu\text{mol}\cdot\text{L}^{-1}$)	65.00 (54.60, 85.68) ³⁾	74.84±14.07 ³⁾	56.70 (49.30, 70.65)
GLU/($\text{mmol}\cdot\text{L}^{-1}$)	5.33 (4.55, 7.23)	5.04 (4.59, 5.59)	5.00±0.39
TC ¹⁾ /($\text{mmol}\cdot\text{L}^{-1}$)	3.66 (2.98, 4.58)	5.22±1.10	4.56 (4.14, 4.97)
HDL ¹⁾ /($\text{mmol}\cdot\text{L}^{-1}$)	1.02±0.24	1.28 (1.13, 1.46)	1.55±0.31
LDL/($\text{mmol}\cdot\text{L}^{-1}$)	2.12±0.74 ²⁾	3.29±0.86	2.60 (2.25, 2.86) ²⁾
TG ¹⁾ /($\text{mmol}\cdot\text{L}^{-1}$)	1.06 (0.79, 1.67)	1.60 (1.14, 2.25)	0.84 (0.66, 1.06)
CRP ¹⁾ /($\text{mg}\cdot\text{L}^{-1}$)	3.05 (0.80, 9.55)	1.12 (0.53, 2.15)	0.65 (0.35, 1.19)

Note: 1) The indexes of the three groups are compared with each other, and the P values are all < 0.05 ; 2) Compared with HUA group, $P < 0.05$; 3) Compared with the control group, $P < 0.05$.

gene expression (*OPRD1* mRNA) level, protein level and key enzyme level in cells cultured in different concentrations of uric acid environment were compared.

2.2.3 Statistical analysis

The data were analyzed by SPSS 25.0 software, the differences among the four groups were compared by ANOVA with $P < 0.05$ indicating that the difference was statistically significant.

3 Results

3.1 Experiment 1

Laboratory indicators of patients in the AD group, hyperuricemia group, and healthy control group were analyzed and compared (Table 1). The results showed that there were differences in age, TC, HDL, TG, and CRP among the three groups. In addition, the levels of UA, ALT and LDL in the hyperuricemia group were higher than those in the AD group. The levels of UA, ALT, AST and LDL in hyperuricemia group were higher than those in healthy control group. The levels of Cr in hyperuricemia group and AD group were higher than those in healthy control group. The above differences were statistically significant.

The methylation level of *OPRD1* gene promoter region in the three groups was detected by pyrosequencing. The results showed that the methylation level of *OPRD1* at pos2 (Chr1:28812599) and pos4 (Chr1:28811734) in the hyperuricemia group was lower than that in the healthy control group. The methylation level of *OPRD1* at pos4 locus in AD group was higher than that in healthy control group (Fig. 2). The results of the ELISA assay for the *OPRD1* protein level in the three groups showed that *OPRD1* protein level in the hyperuricemia group was higher than that in the healthy control group, and the *OPRD1* protein level in the AD group was lower than that in the healthy control group (Fig. 3). The difference was statistically significant.

The correlation test was conducted to analyze the relationship between uric acid and the methylation

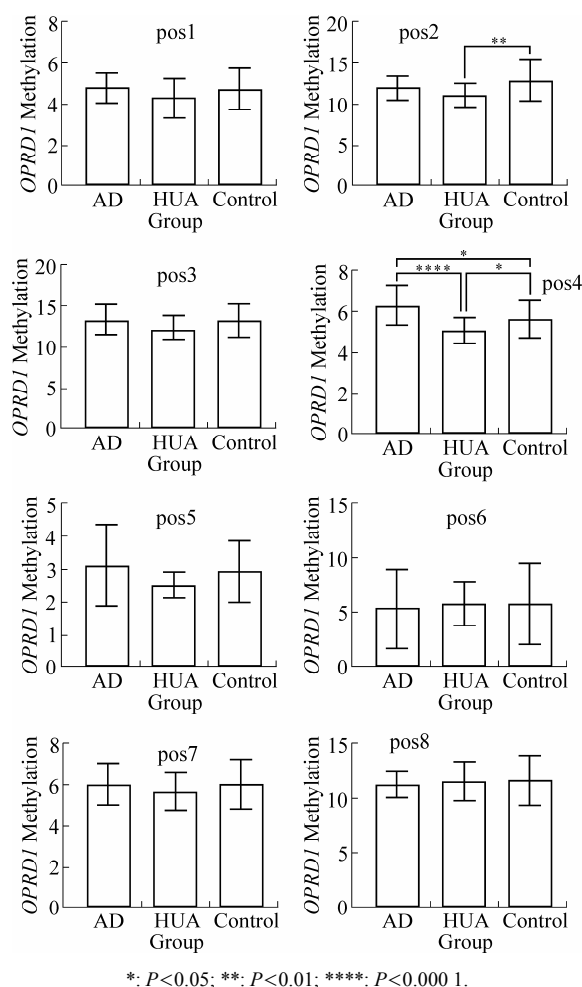


Fig. 2 The methylation level of *OPRD1* in AD, HUA and control group

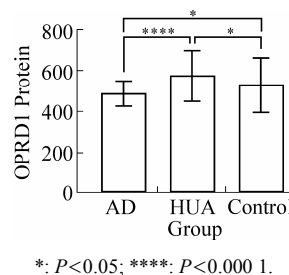


Fig. 3 *OPRD1* protein levels in AD, HUA and control group

levels at pos2 and pos4 of the *OPRD1* gene as well as the *OPRD1* protein level. It was found that uric acid level had a negative correlation with *OPRD1* methylation level (pos2: $r = -0.337$, $P = 0.001$; pos4: $r = -0.275$, $P = 0.009$), had a positive correlation with *OPRD1* protein level ($r = 0.257$, $P < 0.0001$), and after adjusting

for age, gender, AST, ALT, Cr, GLU, TC, HDL, LDL, TG, CRP and other confounding factors, the difference was still statistically significant. This indicates that high uric acid levels in humans are associated with hypomethylation at the pos2 and pos4 sites of the *OPRD1* gene promoter region and high expression of OPRD1 protein. Moreover, AD patients were hypermethylated at pos4, suggesting that uric acid might affect the pathogenesis of AD by mediating the hypomethylation modification at pos4 in the promoter region of the *OPRD1* gene.

There was no significant difference in uric acid levels between the AD group and the healthy control group. However, the *OPRD1* methylation level in the AD group was higher than that in the healthy control group at the pos4 locus, suggesting that *OPRD1* gene methylation might be related to other factors. Therefore, correlation analysis was performed on age, gender, and the above laboratory indicators with the methylation level at the pos4 site and OPRD1 protein level. The results showed that age was significantly positively correlated with the methylation level of pos4 locus of *OPRD1* gene ($r=0.454$, $P<0.001$), and negatively correlated with the expression level of OPRD1 protein ($r=-0.291$, $P<0.001$). Besides, it still had statistical significance after other confounding factors were controlled. This suggests that the increased methylation of the *OPRD1* gene in AD patients may be closely related to age.

3.2 Experiment 2

The results of cell experiments showed that after the cells were adjusted from high uric acid concentration environment to low uric acid concentration environment, the methylation levels of pos2 (Chr1:28812599), pos3 (Chr1:28812605) and pos4 (Chr1:28811734) in the promoter region of *OPRD1* gene were higher than before, while the mRNA level and protein level of OPRD1 were lower than before. After the cells were cultured again under high concentration of uric acid, the methylation level decreased again, and the mRNA level and protein level increased again, with statistically

significant differences (Fig. 4, 5, 6). This indicates that the high uric acid level in HEK293T cells is related to the hypomethylation of the *OPRD1* gene at pos2, pos3,

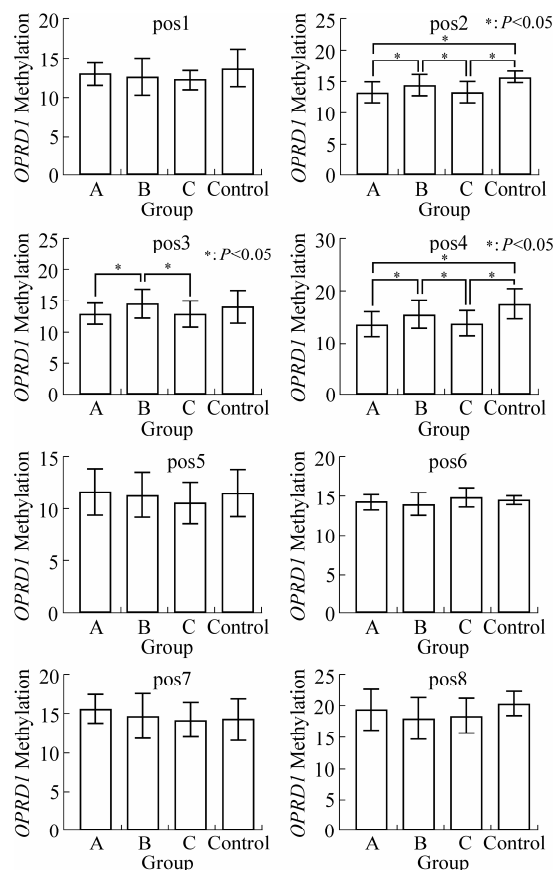


Fig. 4 Methylation levels of *OPRD1* in HEK293T cultured with different concentrations of UA

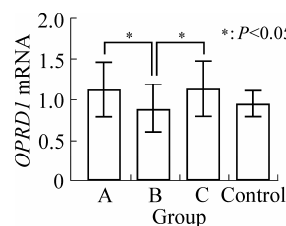


Fig. 5 Transcription levels of *OPRD1* in HEK293T cultured with different concentrations of UA

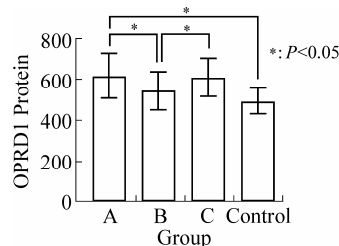


Fig. 6 OPRD1 protein levels in HEK293T cultured with different concentrations of UA

and pos4, as well as high transcription of the *OPRD1* gene and high expression of the *OPRD1* protein.

ELISA method was used to detect the levels of methylation and demethylation key enzymes, and it was found that the DNMT3B level increased when the cells were transferred from the high uric acid environment to the low uric acid environment, and it decreased after being adjusted to the high uric acid environment again, with a statistically significant difference (Fig. 7). This indicated that uric acid might affect the methylation process of *OPRD1* gene by regulating the level of DNMT3B.

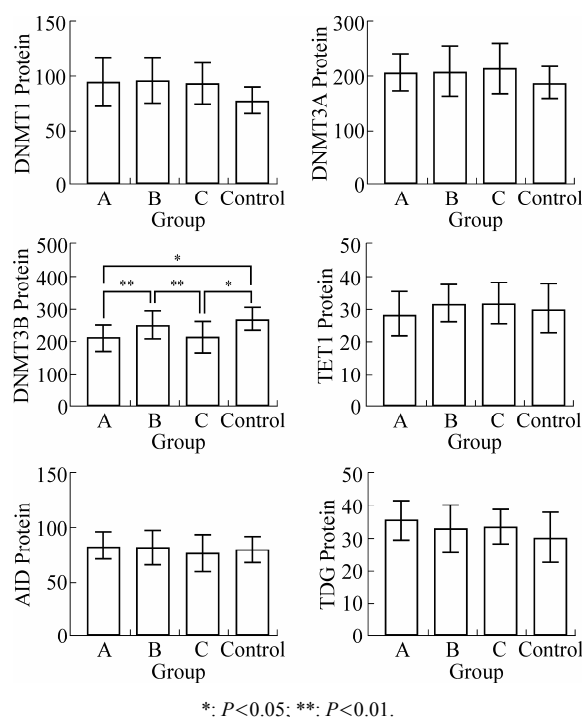


Fig. 7 Key enzyme levels in HEK293T cultured with different concentrations of UA

4 Discussion

The above study found that high uric acid level was associated with hypomethylation of pos2 and pos4 sites in the promoter region of *OPRD1* gene, as well as with high transcription and high expression of the *OPRD1* gene. And the pos4 locus in the promoter region of *OPRD1* gene in patients with AD was hypermethy-

lated. In addition, the level of DNMT3B, a key enzyme regulating DNA methylation, decreased with the increase of uric acid level. This suggested that uric acid might mediate the hypomethylation modification at the pos4 site in the promoter region of the *OPRD1* gene by regulating the level of DNMT3B, thereby affecting the pathogenesis of AD. The specific mechanism may be related to the important role played by DNA methylation in the pathogenesis of AD and the neuroprotective effects of *OPRD1*-encoded δ -opioid receptor.

4.1 DNA methylation participates in the pathogenesis of AD

DNA methylation, one of the most common epigenetic modifications, is a process in which S-adenosylmethionine (SAM) is used as a methyl donor to methylate cytosine under the action of methyltransferases (DNMTs) to synthesize 5-methylcytosine (5mC). Among them, DNMT3A and DNMT3B are responsible for introducing methyl groups into unmethylated DNA, and DNMT1 is responsible for introducing methyl groups into newly synthesized sub-chains^[7]. DNA demethylation includes passive demethylation and active demethylation. Passive demethylation refers to the inability to maintain modification of newly synthesized chains due to the inactivation of DNMT1, resulting in DNA methylation that cannot be inherited from the fundamental chain to the sub-chain. The mechanism of active demethylation is that 5mC generates 5-hydroxymethylcytosine (5hmC) under the oxidation of TET1 hydroxylase, and then deaminates by AID deaminase, followed by TDG glycosidase-mediated cleavage and initiation of base excision repair, to finally generate an unmodified cytosine to complete the active demethylation of DNA^[8].

Research shows levels of 5mC and 5hmC in the middle frontal and temporal gyrus were significantly increased in AD patients compared with age-matched controls^[9]. They were positively correlated with each other and with the AD marker tau protein. This indicates an overall high level of DNA methylation in the brain of

AD patients as compared to their peers. Although the specific pathogenesis between DNA methylation and AD is still unclear at present, it is reasonable to believe that DNA methylation is closely related to the occurrence and development of AD, which may be related to the methylation changes of whole genes or specific genes.

One of the main causes affecting the onset of AD is the hyperphosphorylation of tau protein. Under normal physiological conditions, the phosphorylation and dephosphorylation of tau protein are in dynamic equilibrium. The key enzyme regulating tau phosphorylation is glycogen synthase kinase 3 β (GSK-3 β), and the key enzyme regulating tau dephosphorylation is protein phosphatase-2a (PP2A). Iwata et al^[10] detected the methylation status of GSK-3 β in postmortem brain specimens of AD patients, and found that the demethylation level of GSK-3 β promoter region increased, resulting in the overexpression of GSK-3 β .

In addition, studies have found that vitamin B deficiency can lead to a hypermethylation modification in the PP2A promoter region and a hypomethylation modification in the GSK-3 β promoter region, resulting in inhibition of PP2A expression and up-regulation of GSK-3 β expression, which together participate in the hyperphosphorylation of tau protein^[11]. The results of the above studies indicated that abnormal methylation or demethylation of the promoter regions of key enzyme genes involved in the phosphorylation and dephosphorylation of tau protein might be one of the risk factors for the development of AD.

It has been recognized that oxidative stress plays an important role in the progression of AD. 8-Hydroxydeoxyguanosine (8-OH-DG) is the main oxidative stress marker of DNA oxidative damage, and it has been found that this component can be detected in the brain and peripheral blood of AD patients^[12]. Studies have suggested, 8-OH-DG can reduce the ability of DNMTs to methylate cytosines^[13]. This suggests that oxidative stress may be involved in the progression of AD by affecting DNA methylation levels.

There is increasing evidence that apoptosis is related to the pathological mechanism of AD, and the expression of genes involved in the apoptotic pathway is also affected by DNA methylation modification. Cell death receptor-4 (DR4) is involved in the activation of classical apoptotic pathways and the induction of apoptosis. It was found that in AD patients and cells cultured in a folic acid-deficient environment, the DR4 promoter was hypomethylated, the expression level of DR4 was increased, and the mRNA levels of DNMT1 and DNMT3A were increased^[14]. This indicates that folate deficiency might induce apoptosis by regulating the hypomethylation of DR4 promoter, increasing DR4 expression and up-regulating DNMTs expression in AD, which might be related to folate deficiency-induced DNA damage.

At present, studies have found that there is an inflammatory process from activated microglia to cytokine and complement cascades in the brain of AD patients, and it has been proved that the inflammatory response is neurotoxic^[15]. More and more studies have shown that anti-inflammatory treatment can reduce the risk of AD.

Interleukin-1 (IL-1) and interleukin-6 (IL-6) are the main pro-inflammatory factors in the body. *IL-1* includes *IL-1 α* and *IL-1 β* genes. Studies have been conducted to analyze mRNA expression profiles of various genes involved in neuroinflammatory reactions in the brain of patients with delayed AD by functional genomics^[16]. The results showed that the gene expressions of *IL-1 β* and *IL-6* were affected by DNA methylation, and the hypomethylation of *IL-1 β* could occur at the early stage of the disease, while the methylation level of *IL-6* decreased progressively with the disease progression. The above studies have shown that inflammation-related factors involved in the pathogenesis of AD are also regulated by DNA methylation.

Studies have found that after adjusting for related confounding factors, higher DNA methylation levels in the Apolipoprotein E (APOE) promoter region will

increase the incidence of AD, and the higher the methylation level, the greater the risk of the disease^[17]. This suggests that the methylation modification of APOE gene may be an independent risk factor for AD.

The above studies have proved that DNA methylation is closely related to the pathogenesis of AD. It may play a role in affecting the phosphorylation or dephosphorylation of tau protein, affecting the oxidative damage of DNA, affecting apoptosis, inflammatory factors, and the expression of APOE-related genes, thereby affecting the occurrence and development of AD.

4.2 The neuroprotective effect of δ -opioid receptor encoded by *OPRD1*

Studies have found that the opioid system (including opioid receptors and opioid peptides) may be involved in the pathogenesis of AD by regulating the hyperphosphorylation of tau protein and related mechanisms such as neuroinflammation^[18]. Dysfunction of the opioid system can lead to hyperphosphorylation of tau protein and neuroinflammation, as well as cholinergic neuron degeneration and cognitive impairment. In addition, opioid receptors can also affect the regulation of neurotransmitters, which are usually closely related to the pathogenesis of AD. Therefore, opioid receptors have the potential to become new therapeutic targets for AD.

Classical opioid receptors mainly include three subtypes: μ , κ , and δ . Due to high sequence homology, the endogenous ligand orphanin FQ receptor is also classified as a member of the opioid receptor gene family. *OPRM1*, *OPRK1*, *OPRD1* and *OPRL1* encode μ opioid receptor, κ opioid receptor, δ opioid receptor and FQ receptor, respectively^[19]. *OPRD1*, *OPRM1*, *OPRK1* and *OPRL1* all belong to the G protein coupled receptor (GPCR) family. GPCR has been reported to be associated with many key neurotransmitters in the brain of AD patients^[20]. The correlation between GPCR and AD is also affected by the level of DNA methylation. Liu et al^[21] studied the association between *OPRM1* and

OPRM1 gene methylation levels and mild cognitive impairment in Xinjiang Uyghur and Han populations. The results showed that *OPRK1* gene was significantly hypermethylated in women with mild cognitive impairment in Xinjiang Uyghur and Han populations. Hypermethylation of CpG1 and hypomethylation of CpG2-4 in *OPRM1* gene were respectively associated with the risk of cognitive impairment in Xinjiang Uygur and Han populations. In addition, it has been reported in the literature that the methylation levels of *OPRK1*, *OPRM1* and *OPRL1* promoters in AD patients were significantly increased^[22]. Other studies also have found that the *OPRD1* promoter of AD patients is hypermethylated compared with the control group, suggesting that the *OPRD1* promoter may regulate the expression of *OPRD1* gene through hypermethylation modification, thereby increasing the risk of AD^[1]. This is consistent with our experimental results.

The study found that the δ -opioid receptor encoded by *OPRD1* is a unique neuroprotective agent against cerebral hypoxia/ischemic injury^[23]. Particularly, in the cerebral cortex, the up-regulation of DOR expression may increase the tolerance of neurons to hypoxia/ischemic stress. According to the duration and severity of cellular stress, DOR can trigger different mechanisms to mediate neuronal protection at multiple levels, including the stability of ionic homeostasis, the inhibition of the release of excitatory neurotransmitters, the increase of antioxidant capacity, and the regulation of the intracellular pathway, the inhibition of pro-apoptotic signals and the activation of pro-survival signaling pathways^[24].

In vivo experimental findings^[25], the activation of DOR could attenuate the disorder of K⁺ homeostasis caused by hypoxia or oxygen and glucose deprivation in the mouse cortex, and protect neurons from hypoxia/ischemic injury by regulating K⁺ homeostasis.

DOR can also provide neuroprotection by blocking the release of excitatory neurotransmitters. Zhang et al^[26] studied whether the activation of DOR could protect

neurons from glutamic acid-induced injury using neuronal cultures from rat neocortex. The results showed that the degree of glutamic acid-induced neuronal injury and cell death depended on the concentration of glutamic acid, exposure time, and culture days. However, the use of DOR agonists D-Ala2, D-Leu5-enkephali (DADLE) could reduce the glutamic acid-induced injury by nearly half, while the use of DOR antagonists could completely block the protective effect. This study showed that the activation of DOR could protect neocortical neurons from glutamate excitatory toxicity.

Studies have observed that DOR activation can increase the activity of antioxidant enzymes in cerebral ischemia models with middle cerebral artery occlusion^[27]. In addition, DOR also reduced the expression of caspase, which is a key enzyme in the apoptotic pathway and plays an important role in the apoptosis process. This indicates that the activation of DOR can reduce oxidative damage in ischemia/reperfusion by enhancing the antioxidant capacity and inhibiting apoptosis, which provides new insights into the neuroprotective mechanism of DOR.

Tian et al^[28] studied the effects of DOR-induced neuroprotection on the expression of brain derived neurotrophic factor (BDNF) and its receptor tyrosine protein kinase B (TrkB) in mice after long-term hypoxia. The results showed that hypoxia for 1 day had no significant effect on BDNF expression, while the expression of BDNF decreased gradually after hypoxia for 3 days and 10 days, and activation of DOR could partially reverse the hypoxia-induced decrease in BDNF and TrkB expressions. In addition, prolonged hypoxia (10 days) significantly increased the expression level of tumor necrosis factor- α (TNF- α), which could be completely reversed after DOR activation. Therefore, it can be concluded that long-term hypoxia can down-regulate BDNF-TrkB signaling, resulting in an increase in TNF- α in the cortex, and activation of DOR can up-regulate BDNF-TrkB signaling, thereby reducing

TNF- α levels in hypoxic cortex. This suggests that DOR may exert its neuroprotective role through the BDNF-TrkB pathway.

The neuroprotective effect of DOR provides a potential clue for finding therapeutic targets, and the methylation modification of the *OPRD1* gene encoding DOR is also found to be involved in the pathogenesis of AD. Therefore, it is of great clinical significance to identify the relevant factors that affect the methylation modification of the *OPRD1* gene.

4.3 Neuroprotective effect of uric acid on AD

Uric acid, as an important antioxidant, is considered to have the function of protecting nerve tissue and reducing oxidative damage. Euser et al^[29] conducted a prospective cohort study to assess the relationship between serum uric acid levels and dementia risk, and followed up participants without dementia to investigate cognitive function in later years. The results showed that higher uric acid concentrations were associated with a reduced risk of dementia, and in participants without dementia, higher baseline uric acid levels were associated with better cognitive function later in life. There are also prospective studies in China showing that higher baseline serum uric acid levels were associated with better cognition in later life^[30]. Khan et al^[31] conducted a meta-analysis of all published 46 studies on the relationship between uric acid and cognitive impairment. The results showed that uric acid levels were significantly negatively correlated with cognitive impairment in AD ($P < 0.001$), and uric acid played a neuroprotective role in AD.

The related mechanism of the neuroprotective effect of uric acid in AD is still unclear. In our study, we found that the promoter region of the *OPRD1* gene in AD patients was hypermethylated, which might be related to age.

The high uric acid level is closely related to the hypomethylation of the *OPRD1* gene, suggesting that uric acid may affect the pathogenesis of AD by mediating the hypomethylation of the *OPRD1* gene. The

related mechanism may be that the DOR encoded by the *OPRD1* gene has a neuroprotective effect, and the decline of uric acid level can increase the methylation status of the *OPRD1* gene, down-regulate the gene expression level, reduce the generation of DOR, and ultimately lead to the pathogenesis of AD. This provides a new idea and theoretical basis for the therapeutic targets of AD, and a new guiding significance for the clinical treatment of uric acid reduction, suggesting that better control of serum uric acid in a reasonable range is of positive significance for AD.

References:

- [1] Ji H, Wang Y, Liu G, et al. Elevated *OPRD1* promoter methylation in Alzheimer's disease patients[J]. PLoS One, 2017, 12(3):e0172335.
- [2] Jansen K, Faull R, Dragunow M, et al. Alzheimer's disease: Changes in hippocampal N-methyl-D-aspartate, quisqualate, neurotensin, adenosine, benzodiazepine, serotonin and opioid receptors — An autoradiographic study[J]. Neuroscience, 1990, 39(3):613-627.
- [3] Roussotte F, Jahanshad N, Hibar D, et al. A commonly carried genetic variant in the delta opioid receptor gene, *OPRD1*, is associated with smaller regional brain volumes: Replication in elderly and young populations[J]. Human Brain Mapping, 2014, 35(4):1226-1236.
- [4] Du N, Xu D, Hou X, et al. Inverse association between serum uric acid levels and Alzheimer's disease risk[J]. Molecular Neurobiology, 2016, 53(4):2594-2599.
- [5] Ye B, Lee W, Ham J, et al. Does serum uric acid act as a modulator of cerebrospinal fluid Alzheimer's disease biomarker related cognitive decline?[J]. European Journal of Neurology, 2016, 23(5):948-957.
- [6] Chen Y, Li X K, Ying Y, et al. Preliminary study on deoxyribonucleic acid methylation of breast cancer anti-resistance 1, phospho-diesterase 1C, opioid receptor delta 1 and neurexin 1 in gout and hyperuricemia[J]. Chinese Journal of Rheumatology, 2020, 24(10):676-680. (in Chinese)
- [7] Gujar H, Weisenberger D, Liang G. The roles of human DNA methyltransferases and their isoforms in shaping the epigenome[J]. Genes, 2019, 10(2):172.
- [8] Schuermann D, Weber A, Schär P. Active DNA demethylation by DNA repair: Facts and uncertainties[J]. DNA Repair, 2016, 44:92-102.
- [9] Coppieters N, Dieriks B, Lill C, et al. Global changes in DNA methylation and hydroxymethylation in Alzheimer's disease human brain[J]. Neurobiology of Aging, 2014, 35(6):1334-1344.
- [10] Iwata A, Nagata K, Hatsuta H, et al. Altered CpG methylation in sporadic Alzheimer's disease is associated with APP and MAPT dysregulation[J]. Human Molecular Genetics, 2014, 23(3):648-656.
- [11] Nicolai V, Fusco A, Cavallaro R, et al. B vitamin deficiency promotes tau phosphorylation through regulation of GSK3beta and PP2A[J]. Journal of Alzheimer's Disease, 2010, 19(3):895-907.
- [12] Zhao Y, Zhao B. Oxidative stress and the pathogenesis of Alzheimer's disease[J]. Oxidative Medicine and Cellular Longevity, 2013, 2013:316523.
- [13] Turk P, Laayoun A, Smith S, et al. DNA adduct 8-hydroxyl-2'-deoxyguanosine (8-hydroxyguanine) affects function of human DNA methyltransferase[J]. Carcinogenesis, 1995, 16(5):1253-1255.
- [14] Wang Y, Xu S, Cao Y, et al. Folate deficiency exacerbates apoptosis by inducing hypomethylation and resultant overexpression of DR4 together with altering DNMTs in Alzheimer's disease[J]. International Journal of Clinical and Experimental Medicine, 2014, 7(8):1945-1957.
- [15] McGeer P, Akiyama H, Itagaki S, et al. Immune system response in Alzheimer's disease[J]. The Canadian Journal of Neurological Sciences, 1989, 16:516-527.
- [16] Nicolai V, Cavallaro R, López-González I, et al. DNA methylation profiles of selected pro-inflammatory cytokines in Alzheimer disease[J]. Journal of Neuropathology and Experimental Neurology, 2017, 76(1):27-31.
- [17] Karlsson I, Ploner A, Wang Y, et al. Apolipoprotein E DNA methylation and late-life disease[J]. International Journal of Epidemiology, 2018, 47(3):899-907.
- [18] Cai Z, Ratka A. Opioid system and Alzheimer's disease [J]. Neuromolecular Medicine, 2012, 14(2):91-111.
- [19] Pradhan A, Befort K, Nozaki C, et al. The delta opioid receptor: An evolving target for the treatment of brain disorders[J]. Trends in Pharmacological Sciences, 2011, 32(10):581-590.
- [20] Thathiah A, De Strooper B. The role of G protein-coupled receptors in the pathology of Alzheimer's disease[J]. Nature Reviews Neuroscience, 2011, 12(2):73-87.
- [21] Liu G, Ji H, Liu J, et al. Association of *OPRK1* and *OPRM1* methylation with mild cognitive impairment in

- Xinjiang Han and Uygur populations[J]. Neuroscience Letters, 2017, 636:170-176.
- [22] Ji H, Wang Y, Liu G, et al. *OPRK1* promoter hypermethylation increases the risk of Alzheimer's disease[J]. Neuroscience Letters, 2015, 606:24-29.
- [23] Sheng S, Huang J, Ren Y, et al. Neuroprotection against hypoxic/ischemic injury: δ -opioid receptors and BDNF-TrkB pathway[J]. Cellular Physiology and Biochemistry: International Journal of Experimental Cellular Physiology, Biochemistry, and Pharmacology, 2018, 47(1):302-315.
- [24] He X, Sandhu H, Yang Y, et al. Neuroprotection against hypoxia/ischemia: δ -opioid receptor-mediated cellular/molecular events[J]. Cellular and Molecular Life Sciences, 2013, 70(13):2291-2303.
- [25] Chao D, Bazzay-Asaad A, Balboni G, et al. Activation of DOR attenuates anoxic K^+ derangement via inhibition of Na^+ entry in mouse cortex[J]. Cerebral Cortex, 2008, 18(9):2217-2227.
- [26] Zhang J, Haddad G, Xia Y. Delta-, but not mu- and kappa-, opioid receptor activation protects neocortical neurons from glutamate-induced excitotoxic injury[J]. Brain Research, 2000, 885(2):143-153.
- [27] Yang Y, Xia X, Zhang Y, et al. Delta-opioid receptor activation attenuates oxidative injury in the ischemic rat brain[J]. BMC Biology, 2009, 7:55.
- [28] Tian X, Hua F, Sandhu H, et al. Effect of δ -opioid receptor activation on BDNF-TrkB vs. TNF- α in the mouse cortex exposed to prolonged hypoxia[J]. International Journal of Molecular Sciences, 2013, 14(8): 15959-15976.
- [29] Euser S, Hofman A, Westendorp R, et al. Serum uric acid and cognitive function and dementia[J]. Brain, 2009, 132: 377-382.
- [30] Wang T, Wu Y, Sun Y, et al. A Prospective study on the association between uric acid and cognitive function among middle-aged and older Chinese[J]. Journal of Alzheimer's Disease, 2017, 58(1):79-86.
- [31] Khan A, Quinn T, Hewitt J, et al. Serum uric acid level and association with cognitive impairment and dementia: Systematic review and meta-analysis[J]. AGE, 2016, 38(1):16.

尿酸介导 *OPRD1* 基因低甲基化影响阿尔茨海默病

陈瑶^{1,2}, 陈勇^{1*}, 应颖¹, 范剑波³, 耿保庆¹, 郑成应⁴,
徐婷⁴, 傅浩源⁵, 张韵格⁶, 陈吉⁶

(1.宁波市第二医院, 浙江 宁波 315010; 2.浙江省台州医院, 浙江 台州 317099; 3.宁波市镇海龙赛医院, 浙江 宁波 315299; 4.宁波市康宁医院, 浙江 宁波 315201; 5.慈溪市第三人民医院医疗健康集团长河分院, 浙江 宁波 315325; 6.宁波大学 医学部, 浙江 宁波 315211)

摘要: 探讨 *OPRD1* 基因甲基化与尿酸及阿尔茨海默病的相关性, 并在细胞实验中证实尿酸可影响 *OPRD1* 基因甲基化水平. 采集阿尔茨海默病组、高尿酸血症组及健康对照组的静脉血, 检测 *OPRD1* 基因甲基化水平, 并探讨与尿酸水平的相关性. 细胞实验中, 采用不同尿酸浓度先后处理细胞, 分别检测 *OPRD1* 基因甲基化水平. 结果表明, 高尿酸血症组 *OPRD1* 基因 pos2、pos4 位点甲基化水平低于健康对照组, 阿尔茨海默病组 pos4 位点甲基化水平高于健康对照组, 尿酸水平与 *OPRD1* 基因甲基化水平负相关, 高浓度尿酸下细胞 *OPRD1* 基因 pos2、pos3、pos4 位点的甲基化水平更低. 由此得出尿酸可能通过介导 *OPRD1* 基因 pos4 位点的低甲基化修饰, 从而影响阿尔茨海默病的发病.

关键词: 尿酸; 关键酶; *OPRD1* 基因; 甲基化; 阿尔茨海默病

(责任编辑 章践立)