



No association between histamine N-methyltransferase functional polymorphism Thr105Ile and Alzheimer's disease

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ABSTRACT

Several abnormalities, including lower histamine levels in brain, elevated serum histamine and degeneration of histaminergic neurons in tuberomammillary nucleus, were described in the histaminergic system of patients with Alzheimer's disease (AD). Histamine is a central neurotransmitter with several functions in brain including regulation of memory, cognition, locomotion, and is degraded in part by histamine N-methyltransferase (HNMT). A common Thr105Ile polymorphism within HNMT gene results in decreased enzyme activity. The Thr105Ile polymorphism was associated with Parkinson's disease, essential tremor, attention-deficit hyperactivity disorder (ADHD), asthma and alcoholism, thus we tested possible association of HNMT functional polymorphism with AD. We have tested 256 AD cases and 1190 healthy controls of Croatian origin. Thr105Ile polymorphism was determined by TaqMan RT-PCR Genotyping Assay and EcoRV digestion. Prevalence of functional HNMT polymorphism among all tested groups was similar and frequency of less active Ile105 variant was 11.5% among AD patients and 13.4% for healthy controls ($p=0.26$, $\chi^2=1.25$). Our results indicate lack of the association of HNMT Thr105Ile functional polymorphism with Alzheimer's disease.

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the most prevalent form of dementia. Despite intensive research, the etiology, pathophysiology and neurobiological/molecular basis of AD is still unclear. The cognitive deficits, memory loss and behavioral disturbances, observed in patients with AD, could be associated with many factors including the alterations in neurotransmitter systems. Several studies have detected abnormalities in the histaminergic system of patients with AD. It was shown that level of histamine is significantly reduced in hypothalamus, hippocampus and temporal cortex of AD patients' brains [13]. Level of serum histamine was higher in AD patients than in healthy individuals and this was correlated with mental performance [3]. Also, pathological changes characteristic for

AD, neurofibrillary tangles of tau proteins, were found in tuberomammillary nucleus of the hypothalamus which is a sole site of histaminergic neurons in brain [2]. Loss of large neurons from the same brain area was documented, suggesting that degeneration of histaminergic neurons is important element of AD pathogenesis [11]. Based on these studies, it was suggested that histamine plays the important role in the pathogenesis of AD.

Histamine is a central neurotransmitter with several functions in brain including regulation of memory, cognition and locomotion [7]. Neuronal histamine is degraded to tele-methylhistamine by histamine N-methyltransferase (HNMT; EC 2.1.1.8). This methylation process is the main inactivation pathway for histamine in the brain. A common genetic polymorphism in exon 4 of the HNMT gene leads to change of threonine to isoleucine at position 105 (Thr105Ile polymorphism). Biochemical studies have shown that individuals heterozygous for Ile105 allele have 30–50% lower HNMT activity, while homozygous individuals have decreased enzyme activity of about 60% [8,14]. In healthy persons, carriers of the Ile105 allele, red blood cells HNMT activity was lower than in persons carrying the Thr105 allele [4]. So far, this polymorphism

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has been linked to many clinical conditions including Parkinson's disease [1], essential tremor [9], attention-deficit hyperactivity disorder [16], asthma [17], allergic rhinitis [6] and alcoholism [12].

Since histamine is a neurotransmitter that influences wide range of brain functions, especially cognition, that are affected by Alzheimer's disease, we have tested possible association of Thr105Ile functional polymorphism in HNMT gene with AD.

The study included 256 Caucasian patients with Alzheimer's disease of Croatian origin (mean age $\pm$ SD; 80.3 ± 7.1 years, range 65–98 years) from the Psychiatric Hospital Vrapce, Zagreb, Croatia. The diagnoses of AD were made according to DSM-IV, ICD-10 and NINCDS-ADRDA criteria [18]. The severity of dementia was evaluated using Mini Mental State Examination [5]. The mean MMSE scores in patients with AD were 10.7 ± 8.1 (range 0–24).

The control group consisted of 1190 Caucasian, elderly healthy subjects of Croatian origin (77.0 ± 7.9 ; range 60–90 years) from the local senior centers in Zagreb and Split. Control subjects did not show cognitive impairment, and their mean MMSE scores were 28.5 ± 1.2 (range 26–30). Local Ethic Committee of Psychiatric Hospital Vrapce in Zagreb and Medical School in Split approved the study. Informed consent was obtained from patients with AD or their guardians and healthy subjects.

Functional HNMT polymorphism Thr105Ile (rs11558538, C > T) was determined by TaqMan real-time PCR Genotyping Assay and EcoRV enzymatic digestion. Real-time PCR was performed with genomic DNA, using the TaqMan genotyping master mix (Applied Biosystems, USA) on a 7500 real-time PCR System (Applied Biosystems, USA). Single nucleotide polymorphism (SNP) genotyping was performed according to the manufacturer's instructions. Primers and probes were designed using Primer Express software, version 3.0 (Applied Biosystems, USA) and were obtained from Applied Biosystems. Genotyping results were additionally confirmed by restriction digestion with EcoRV for about 10% of the samples (Invitrogen, USA) and sequencing (Macrogen, South Korea). Digestion was performed for 6 h at 37°C and products were visualized by electrophoresis on 2% agarose gel and ethidium bromide staining.

The frequency of the genotypes and alleles was evaluated using a χ^2 test. The association was expressed as OR with 95% CI. The value of P less than 0.05 was considered statistically significant.

The genotype and allele frequencies for HNMT Thr105Ile polymorphism in healthy subjects and patients with AD are given in Table 1. There was no significant ($p > 0.05$) difference in the frequency of the Thr/Thr, Thr/Ile and Ile/Ile genotypes ($p = 0.52$; $\chi^2 = 1.28$; OR = 1.18, 95%CI = 0.86–1.64) and allele frequencies ($p = 0.26$; $\chi^2 = 1.25$; OR = 0.84, 95%CI = 0.63–1.13) or the combined Ile/Ile and Ile/Thr genotype versus the homozygous

Thr/Thr genotype ($p = 0.29$; $\chi^2 = 1.08$; OR = 0.84, 95%CI = 0.60–1.16) between patients with AD and healthy controls. Frequency of less active Ile105 variant was 11.5% among AD patients versus 13.4% for healthy controls. The Thr105Ile genotype distribution in patients with AD and in healthy controls was in the expected Hardy–Weinberg equilibrium.

In this research, we have tested association of Thr105Ile polymorphism that leads to decreased enzymatic activity of histamine N-methyltransferase, the only enzyme that degrades histamine in brain. Many previous studies indicated different histamine abnormalities in AD patients like lower histamine levels in brain [13], elevated serum histamine [3] and pathologic changes and degeneration of histamine producing neurons in tuberomammillary nucleus [2]. One possible explanation for the decline in histaminergic function is the lack of histamine due to the decrease in its synthesis. This presumption is in line with the decreased levels of histamine precursor histidine, and in the diminished activity of the synthesizing enzyme histidine decarboxylase, found in the postmortem frontal cortex of patients with AD [15]. Lower brain HNMT activity in the brain of patients with AD could represent a compensatory mechanism leading to the increase in histamine levels. Although Ile105 allele is connected with lower HNMT activity in vivo [4] and in vitro [8,14], we did not confirm the presumption of a higher frequency of Ile allele carriers among patients with AD.

To the best of our knowledge, this is the first study that evaluated HNMT Thr105Ile polymorphism in patients with AD. Since the results have shown similar distribution of alleles and genotypes of the HNMT Thr105Ile polymorphism between patients with AD and age-matched healthy controls, we have not confirmed our hypothesis that gene variants of the HNMT Thr105Ile polymorphism are associated with the etiology of AD and it is unlikely that diminished degradation of histamine is underlining most of the observed histamine changes in AD. However, we could not exclude the possibility that the HNMT activity in patients with AD is regulated by the other genetic and/or epigenetic factors. Examination of mtDNA shows that Southern Slavonic populations fall into the common West Eurasian mitochondrial haplogroups so we presume that similar distribution of HNMT alleles and genotypes can be expected in other European populations [10]. In contrast to Alzheimer's disease, HNMT Thr105Ile functional polymorphism was associated to neurological conditions such as Parkinson's disease [1,V.P. and J.T. unpublished observation], essential tremor [9] and attention-deficit hyperactivity disorder [16] and this discrepancy in association can be explained with different molecular mechanisms of pathogenesis for each of these disorders.

In conclusion, our results have shown that the functional Thr105Ile polymorphism of HNMT gene was not associated with AD

Table 1
The HNMT Thr105Ile genotypes and allele frequencies in patients with Alzheimer's disease (AD) and healthy controls.

HNMT Thr105Ile	Patients with AD N (%)	Healthy controls N (%)
Genotypes		
Thr/Thr	200 (78.1)	893 (75.0)
Thr/Ile	53 (20.7)	276 (23.2)
Ile/Ile	3 (1.2)	21 (1.8)
	$\chi^2 = 1.28$; $p = 0.52$; OR = 1.18, 95%CI = 0.86–1.64	
Alleles		
Thr	453 (88.5)	2062 (86.6)
Ile	59 (11.5)	318 (13.4)
	$\chi^2 = 1.25$; $p = 0.26$; OR = 0.84, 95%CI = 0.63–1.13	
Ile carriers (combined Ile/Ile + Thr/Ile genotypes) vs. homozygous Thr/Thr genotype		
Ile carriers (Ile/Ile + Thr/Ile)	56 (21.9)	297 (25.0)
Thr/Thr homozygous genotype	200 (78.1)	893 (75.0)
	$\chi^2 = 1.08$; $p = 0.29$; OR = 0.84, 95%CI = 0.60–1.16	

N is the number of subjects. Ile, isoleucine; Thr, threonine.

and consequently could not be a risk factor for this neurodegenerative disorder. Although degeneration of the histaminergic neurons in the tuberomammillary nucleus remains the most likely cause of histamine abnormalities in AD, our present findings do not support the hypothesis that the functional Thr105Ile polymorphism of HNMT contributed to the pathogenic effect of tuberomammillary nucleus degeneration and resulting cognitive impairment seen in AD.

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Disclosure statement

The authors have nothing to disclose.

Conflicts of interest

The authors have no potential conflicts of interest to report.

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