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# Genotype-independent decrease in plasma dopamine beta-hydroxylase activity in Alzheimer's disease

Maja Mustapic [a], Paola Presecki [b], Nela Pivac [a], Ninoslav Mimica [c], Patrick R. Hof [d], Goran Simic [e], Vera Folnegovic-Smalc [c], Dorotea Muck-Seler [a,*]

[a] Division of Molecular Medicine, Rudjer Boskovic Institute, Bijenicka 54, HR-10000 Zagreb, Croatia
[b] Psychiatric Hospital Sveti Ivan, Jankomir 11, pp 68, HR-10090 Zagreb, Croatia
[c] University Psychiatric Hospital Vrapce, Bolnicka cesta 32, HR-10090 Zagreb, Croatia
[d] Fishberg Department of Neuroscience and Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA
[e] Department of Neuroscience, Croatian Institute for Brain Research, Medical School University of Zagreb, Salata 12, HR-10000 Zagreb, Croatia

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ABSTRACT

The noradrenergic system is involved in the etiology and progression of Alzheimer's disease (AD) but its role is still unclear. Dopamine beta-hydroxylase (DBH) as a catecholamine-synthesizing enzyme plays a central role in noradrenaline (NA) synthesis and turnover. Plasma DBH (pDBH) activity shows wide inheritable interindividual variability that is under genetic control. The aim of this study was to determine pDBH activity, *DBH* (C-970T; rs1611115) and *DBH* (C1603T; rs6271) gene polymorphisms in 207 patients with AD and in 90 healthy age-matched controls. Plasma DBH activity was lower, particularly in the early stage of AD, compared to values in middle and late stages of the disease, as well as to control values. Two-way ANOVA revealed significant effect of both diagnosis and *DBH* (C-970T) or *DBH* (C1603T) genotypes on pDBH activity, but without significant diagnosis × genotype interaction. No association was found between AD and *DBH* C-970T (OR = 1.08, 95% CI 1.13–4.37; $p = 0.779$) and C1603T (OR = 0.89; 95% CI 0.36–2.20; $p = 0.814$) genotypes controlled for age, gender, and *ApoE4* allele. The decrease in pDBH activity, found in early phase of AD suggests that alterations in DBH activity represent a compensatory mechanism for the loss of noradrenergic neurons, and that treatment with selective NA reuptake inhibitors may be indicated in early stages of AD to compensate for loss of noradrenergic activity in the locus coeruleus.



## 1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by memory loss and cognitive impairment. Because aging is one of the main risk factors for AD and due to the generalized increase in life expectancy, the prevalence of AD among individuals above 65 years of age is rapidly increasing and AD represents a major public health problem worldwide (Alzheimer's Association, 2010).

None of the many hypotheses of the etiology of AD has successfully explained all of the features of the disease. AD is a multifactorial disease with a complex interplay of many genetic and environmental factors resulting in a network of interactions that still have to be deciphered. Neuroimaging studies have revealed brain atrophy in the neocortex, hippocampus, and several other brain regions (Cummings, 2004). Dysfunction in cholinergic system was recognized early (Coyle et al., 1983; Giacobini, 2003; Lyness et al., 2003; Wenk, 2003). The loss of serotonergic and noradrenergic nuclei in the brainstem has also been reported in patients with AD (Garcia-Alloza et al., 2005; Herrmann et al., 2004) implying a role for these neurotransmitter systems in the etiology of AD (Lyness et al., 2003).

The noradrenergic system has two main projections, one originating from noradrenergic cells bodies in the ventrolateral tegmental area that is involved in sexual and feeding behaviors, and the other, originating from the locus coeruleus (LC), associated with learning, memory and cognitive functions (Grudzien et al., 2007; Heneka et al., 2010). The loss of noradrenergic neurons from LC correlates with the increase of extracellular amyloid β protein (Aβ) deposition in mice (Heneka et al., 2010), neurofibrillary abnormalities in early stage of AD (Grudzien et al., 2007), onset (American Psychiatric Association, 1994; Counts and Mufson, 2010), and duration of dementia (Counts and Mufson, 2010; Forstl et al., 1994). In AD, reduced concentration of noradrenaline (NA) has been reported in many brain regions (Herrmann et al., 2004; Hoogendijk et al., 1999). In addition, increases in cerebrospinal fluid

*Abbreviations:* Aβ, amyloid β protein; AD, Alzheimer's disease; ANOVA, one-way analysis of variance; CSF, cerebrospinal fluid; DBH, dopamine beta-hydroxylase; *DBH*, dopamine beta-hydroxylase gene; LC, locus coeruleus; MHPG, 3-methoxy-4-hydroxyphenylglycol; MMSE, Mini-Mental State Examination; NA, noradrenaline; pDBH, plasma dopamine beta-hydroxylase.

* Corresponding author at: Laboratory of Molecular Neuropsychiatry, Division of Molecular Medicine, Rudjer Boskovic Institute, Bijenicka 54, HR-10000 Zagreb, Croatia. Tel.: +385 1 4571 207; fax: +385 1 4561 010.
*E-mail address:* seler@irb.hr (D. Muck-Seler).

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(CSF) NA levels (Elrod et al., 1997; Raskind et al., 1999) in AD support the hypothesis that increased noradrenergic activity represents a compensatory mechanism for both cholinergic and noradrenergic deficits (Giubilei et al., 2004; Herrmann et al., 2004). There are several mechanisms providing evidence for the role of NA in AD as not merely a risk factor but as an actual etiological factor (Counts and Mufson, 2010; Fitzgerald, 2010; Weinshenker, 2008). Neuronal plasticity resulting in hyperinnervation of the forebrain regions and noradrenergic sprouting to reinnervate brain regions marked by loss of cholinergic neurons might be mechanisms that account for this compensation (McMillan et al., 2011; Szot et al., 2006). Apart from its role as a neurotransmitter, NA may act as an endogenous anti-inflammatory agent by inhibition of inflammatory activation of microglial cells (Feinstein et al., 2002; Heneka and O'Banion, 2007). Therefore, it has been suggested that cell death in LC and the loss of NA-mediated anti-inflammatory protection could exacerbate inflammation and contribute to the pathogenesis of AD.

The enzyme dopamine beta-hydroxylase (DBH) catalyzes the oxidative hydroxylation of dopamine to NA. DBH is present in noradrenergic neurons in the central nervous system, peripheral postganglionic sympathetic neurons, and adrenal medulla (Kim et al., 2002). It is the only catecholamine-synthesizing enzyme within synaptic vesicles, where it exists in soluble and membrane-bound forms (Lewis and Asnani, 1992). DBH is co-released by exocytosis together with NA and can be found in CSF, plasma, and serum (Weinshilboum et al., 1971). The enzymatic activity of pDBH is characterized by wide interindividual variation regulated by genetic inheritance (Cubells and Zabetian, 2004). The enzymatic activity of DBH corresponds to the plasma level of DBH protein (O'Connor et al., 1994; Weinshilboum et al., 1973), is quite stable, and does not change after physical activity (Cubells and Zabetian, 2004). Single nucleotide polymorphism in the promoter region C-970T of the *DBH* gene (rs1611115, formerly called C-1021T) accounts for 30 to 50% of the variance in DBH activity, and the T-970 allele contributes to decreased pDBH activity through co-dominant inheritance (Zabetian et al., 2003). The next plausible variant at *DBH* locus accounting for the variance in pDBH activity, but with considerably less effect is the C1603T polymorphism in intron 11 (Tang et al., 2005; Zabetian et al., 2003).

Although DBH modulates NA levels (Burke et al., 1999; McMillan et al., 2011; Szot et al., 2006) little is known about the effect of DBH activity and/or NA turnover on the development and progression of AD. In patients with AD, reduced DBH activity was found in hippocampus and neocortex postmortem (Cross et al., 1981; Perry et al., 1981). It is possible that the −970T allele and consequently lowered DBH activity in patients with AD could be responsible for the reduced synthesis of NA and loss of its neuroprotective role (Heneka et al., 2010; Mateo et al., 2006; Weinshenker, 2008; Wenk et al., 2003).

Our hypothesis was that pDBH activity could be a biomarker for the development and cognitive dysfunction in AD. Because pDBH activity is genotype-controlled, the aim of the present study was to determine pDBH activity and C-970T and C1603T *DBH* gene polymorphisms in patients with AD and healthy age-matched control subjects. As so far no study has analyzed the relationship between pDBH activity and cognitive function in patients with AD, we measured pDBH activity in early, middle, and late phases of AD, to elucidate the possible involvement of DBH, and consequently of NA in the progress of AD.

## 2. Methods

### 2.1. Study population

The present study included 297 unrelated Croatian Caucasian subjects: 207 patients with probable sporadic late onset AD (mean age 80 ± 7.1; range 65–98 years; 185 women), who were recruited from the Psychiatric Clinic Vrapce, Zagreb, Croatia, and a control group of 90 healthy, elderly volunteers (mean age 77.0 ± 7.9; range 60–90 years; 67 women) from local elder living communities. The diagnosis of probable AD was made according to the DSM-IV (American Psychiatric Association, 1994) and the criteria of the National Institute of Neurologic and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINDS–ADRDA) (McKhann et al., 1984). All subjects underwent a clinical interview to rule out for Axis I disorders and to evaluate current and past medical status. Exclusion criteria for patients and controls were the diagnoses of severe somatic diseases (heart disease, epilepsy, brain trauma, cancer), major functional psychiatric disorders (depression, schizophrenia), hypertension, smoking, and alcoholism. Patients were treated with acetylcholinesterase inhibitors, and some received antipsychotics, but were medication-free for at least one week before blood sampling. Their cognitive status was evaluated with the Mini-Mental State Examination (MMSE) (Folstein et al., 1975) that was translated and validated to the Croatian population (www.parinc.com; Boban et al., 2012). MMSE scores (expressed as mean ± standard deviations) were 28.56 ± 1.94 (range 26–30) and 11.69 ± 8.14 (range 0–24) in controls and in patients with AD, respectively. Patients were additionally subdivided according to the MMSE scores into three groups: 47 patients in the early (20.67 ± 1.3; range 19–24), 80 patients in the middle (14.27 ± 2.1; range 10–18) and 80 patients in the late (1.58 ± 2.4; range 0–9) phases of AD. The study was approved by the local Ethics Committee of the University Psychiatric Hospital Vrapce. All of the patients or their proxies and the healthy controls gave informed consent. The study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

### 2.2. Biochemical and molecular analyses

Blood samples (4 ml) were taken in a plastic syringe with 1 ml of acid citrate dextrose as an anticoagulant. Plasma was obtained after centrifugation of blood at 5000 ×*g* for 10 min, and stored at −20 °C until assayed using modified method of Nagatsu and Udenfriend (1972). Briefly, DBH converts substrate tyramine to octopamine, which is oxidized to p-hydroxybenzaldehyde, and measured photometrically at 330 nm. The full protocol is available at http://hypertension.ucsd.edu/list_of_protocols/25_DBH_assay.htm.

Genomic DNA was extracted from blood samples using standard salting out procedure (Miller et al., 1988). Genotyping of *DBH* (C-970T; rs1611115), *DBH* (C1603T; rs6271), and *ApoE* (rs7412, rs429358) polymorphisms were performed in ABI Prism 7000 Sequencing Detection System apparatus (ABI, Foster City, CA USA) using a TaqMan-based allele-specific polymerase chain reaction assays, according to the procedure described by Applied Biosystems (ABI, Foster City, CA, USA). The primers and probes were purchased from ABI Assay ID numbers are available upon request.

### 2.3. Statistical analysis

The results were expressed as means ± standard deviations. Differences in pDBH activity among groups were evaluated with one-way analysis of variance (ANOVA) and Tukey's test. Because the data for pDBH activity were not normally distributed, they were natural logarithm-transformed. Two-way ANOVA was used to test the effect and interaction of diagnosis (AD and healthy controls) and genotype (CC, CT, TT) on plasma DBH activity. The correlation between age and pDBH activity was determined by a Spearman's coefficient of correlation. The deviations from Hardy–Weinberg equilibrium, and genotype and allele distributions were performed by the $\chi^2$ test. Logistic regression was applied to test the association of AD and *DBH* (C-970T) and *DBH* (C1603T) genotypes TT + CT vs. CC, controlled for age, gender, and ApoE4 allele. Power was set to 0.8. The determination of the minimum sample size required to achieve a desired power were evaluated using Sigma Stat 3.5. The statistical packages used were GraphPad