# Relevance of defensin β 2 and α defensins (HNP1-3) in Alzheimer's disease

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## Abstract

The DEFB4 gene copy numbers were investigated in 206 AD patients and in 250 controls. The levels of the human defensin β 2 (hBD2) and α defensins (HNP 1-3) in the sera and in the cerebrospinal fluid (CSF) of the patiens and the controls were determined .

Higher copy numbers of the DEFB4 gene was observed in AD patients as compared with the controls. The levels of hBD-2 and HNP 1-3 were significantly elevated in the sera and in the CSF of the AD patients

These data suggest that both defensin β 2 and α defensins have potential role in the development of AD.

## 1. **Introduction**

Initiating event in AD is related to the abnormal processing of beta-amyloid (A ), ultimately leading to the formation of A plaques in the brain (Jack et al., 2010). It was recently postulated that brain infections involving bacteria or viruses may play an initiating role in amyloid plaque formation and the development of AD (Hill et al., 2014); (Maheshwari and Eslick, 2015); (Mawanda and Wallace, 2013). Persistent subclinical CNS infections have been reported for AD patients, caused by various pathogens such as *Chlamydia pneumoniae*, *Helicobacter pylori*, *Herpes simplex virus* I, spirochetes or even fungi (Shima et al., 2010; Löwheim et al.,2015; Miklossy 2011 ; Alonso et al, 2014)

It is presumed, that neuropathological alterations might be associated with abnormal expression and regulatory function of antimicrobial peptides (AMPs), including defensins (Foster and McVey Neufeld, 2013); (Williams et al., 2012). Human β defensins have been suggested to play a role in chronic inflammation-associated brain injury, a risk factor for AD. In this respect, the literature reports (Williams et al., 2012); (Welling et al., 2015) lead us to hypothesize that upregulation of the production of defensins might trigger the aggregation of amyloid in the brain.

Human α defensins include human neutrophil peptide 1-4 (HNP1-4) and intestinal human defensins (HD-5 and HD-6), produced by Paneth cells. Human β defensins make up another family of antimicrobial peptides (Ganz, 2003); (Pazgier et al., 2006). Besides their antibacterial and antiviral effects, defensins exert numerous immunological effects (Oppenheim and Yang, 2005); (Yang et al., 2002). While the expression of human defensin beta-1 (hBD1) is generally constitutive, the levels of

human defensin beta-2 (hBD2) are inducible by proinflammatory cytokines and bacteria.

Defensin genes have been mapped to 8p22-p23 (Linzmeier and Ganz, 2005). A role of the DEFB4 gene in determining human β-defensin-2 (hBD2) as potential modifier in AD has not been hypothesized previously.

We therefore considered it of interest to investigate the relevance of the copy number (CN) polymorphism of the DEFB4 genes in AD. We additionally investigated the levels of human β-defensin-2 (hBD 2) in the cerebrospinal fluid (CSF) and the sera of the patients with AD. The study was supplemented with the measurement of human α-defensin (Human Neutrophil Peptide 1-3; HNP1-3) in the circulation and in the CSF.

## 2. Materials and methods

### 2.1 Patients and controls

A total of 206 AD patients (69 men and 137 women, average age and standard deviation (S.D.) 76.42 ± 4.21 years, average onset at 65 years) were enrolled in the study. The clinical diagnosis of AD was based on neurological and psychiatric examinations, together with the assessment of psychometric tests to confirm cognitive impairment. Additionally, a brain CT scan or MRI was performed in each case. All participants fulfilled criteria of NINCDS–ADRDA (McKhann et al., 1984); (Dubois et al., 2007). The cognitive evaluation of AD patients was based on the AD Assessment Scale – Cognitive Subscale (ADAS-Cog) (Rosen et al., 1984); (Pákáski et al., 2012), the Mini-Mental State Exam (MMSE) (Folstein et al., 1975); (Janka et al., 1988), and the Clock Drawing Test (CDT) (Kálmán et al., 1995). To exclude pseudodementia caused by depression, mood was scored via the Beck Depression Inventory (Beck et al., 1961),which might be a limitation (Wagle et al., 2000), but patients having less than 12 BDI scores have only been included to the study.

The control group comprised 250 healthy volunteers (92 men and 158 women, average age ± S.D. 72.69 ± 6.82 years) with normal cognition. (MMSE score higher than 28)

All cases and controls were of Hungarian ethnic origin. Informed consent was obtained from all patients or responsible guardian in case of incapacity and controls. All patients were treated in accordance with the Patient Right's Protection Act of our institutions and according to international guidelines.
The local Ethics Committee of the Hungarian Investigation Review Board gave prior approval to the study.

2.2 Determination of DEFB4 Gene Copy Number (CN)
Genomic DNA was extracted from peripheral blood anticoagulated with EDTA in accordance with the manufacturer's instructions (High Pure PCR Template Preparation Kit; Roche Diagnostic GmbH, Mannheim, Germany, Cat.no: 1796828).
A TaqMan real-time PCR assay, specifically for amplification of genomic DEFB4, was performed as described previously (Tiszlavicz et al., 2012). Briefly, quantitative DEFB4 amplification data were normalized to ABL [FAM-lebeled albumin (Applied Biosystems, Cat. No. 4331182)], as a standard reference gene considered to be present only in 2 copies in the genom. The reference gene was used as an internal standard (Bentley et al., 2010) added to each single tube. Quantification was evaluated by the comparative CT (Threshold Cycle) method (Szilagyi et al., 2006).

2.3. Determination of human β-defensin 2 (hBD2) and HNP1-3 concentrations

ELISA of hBD2 (Alpha Diagnostic San Antonio, TX, USA) was used to test for the occurrence of the human β-defensin 2 peptide in the sera and in CSF of the controls and patients, on the basis of manufacturer's instructions.

The HNP1-3 concentrations in the sera and CSF were determined by ELISA (Hycult-Biotech
HK324, Uden, The Netherlands) according to the instructions of the manufacturer.

### 2.4. Statistical analysis

The significance of the differences was analyzed by the Mann-Whitney test. The GraphPad prism 5 statistical program was used for all statistical calculations (GraphPad Software Inc. San Diego, CA, USA).

## 3. RESULTS

### 3.1. Copy number (CN) polymorphism of DEFB4

The determination of CN was performed in 206 patients in the AD group and in 250 controls. The median CN in the controls was 4, the 75% percentile 5, and the 25% percentile was 3. In the AD patients the median was 5, with a 75 % percentile of 6, and with a 25% percentile of 4; $p<0.001$ with the Mann-Whitney test (Fig.1.insert).

### 3.2. Levels of hBD2 in sera and in cerebrospinal fluids (CSF)

The levels of human β-defensin 2 were determined in the sera of 52 AD patients and 45 controls. Significantly higher levels of hBD2 were measured in AD patients than in the controls (Fig.1a.). The median levels were 265.5 pg/ml in AD vs. 169.4 pg/ml respectively ($p < 0.01$).

The median levels of β-defensin 2 in the CSF of 43 AD patients were 8.6 pg/ml vs 1.201 pg/ml in the control group ($n=40$), $p < 0.001$ .Fig.1b.

### 3.3. Levels of HNP1-3 (α-defensin) in sera and in cerebrospinal fluids (CSF)

The median serum concentrations of α-defensin in 43 AD patients were 147.3 ng/ml, significantly higher than that in 40 controls: 122.3 ng/ml ($p < 0.05$). Fig.1c.

The median concentration of α-defensin in the CSF of 52 AD patients was significantly higher: 85.16 ng/ml than that in 40 controls: 1.23 ng/ml, $p<0.001$). Fig.1d.

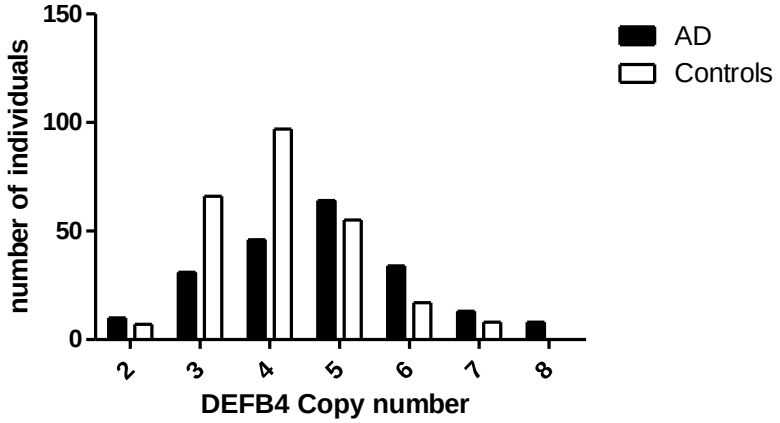
## 4. DISCUSSION

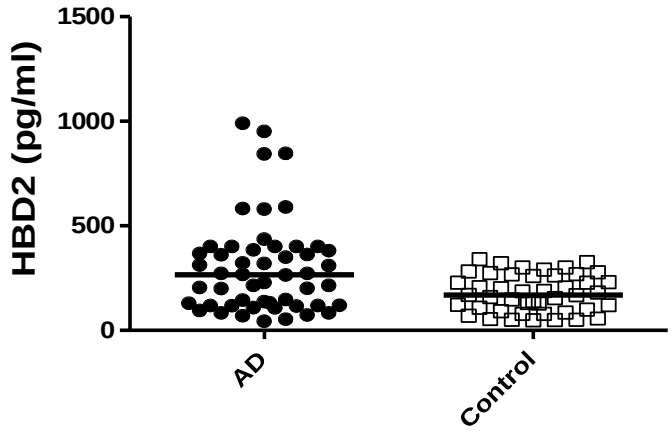
It has been postulated, that chronic infections might be initial events in the pathogenesis of AD, which can give rise to persistent inflammatory stimuli. The inflammatory response thereafter may indirectly lead to the upregulation of amyloid β production (Welling et al., 2015). Not only the levels of the inducible hBD-2 were elevated in the cerebrospinal fluid and sera of AD patients, but also those of HNP1-3. Our data are in accordance with recent findings (Watt et al., 2015), reporting that the levels of peripheral α-defensins are elevated in Alzheimer's disease. The copy number polymorphism of the DEFB4 gene has been reported to influence the production of hBD2 (Linzmeier and Ganz, 2005); (Hollox et al., 2008); (Jansen et al., 2009; Tiszlavicz et al., 2012);). Accordingly, in our study a significant linear correlation was found between the DEFB4 CN and the serum levels ($p = 0.002$; $r^2 = 0.196$) or CSF levels ($p = 0.001$; $r^2 = 0.222$) respectively. The secretion of HNP 1-3 seems to be independent of the copy number of DEFA gene (Németh et al., 2014), and we therefore did not investigate it.

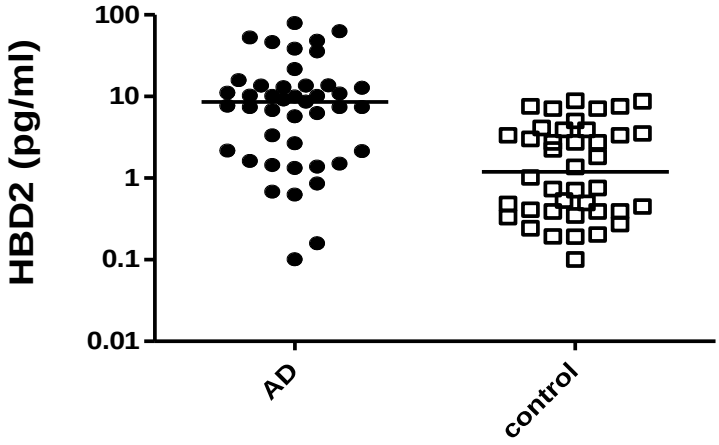
The present study supports the view of the potential roleof antimicrobial peptides such as human α and β-defensins as pathogen-targeting agents in brain infections with respect to the pathology of AD. Whether the AD condition is the consequence of high levels of defensins which induce neurodegeneration and A formation, or the elevated levels of defensins are the consequence of AD is currently unknown. Further investigations are necessary to elucidate the regulatory functions of defensins in the pathomechanism of AD.

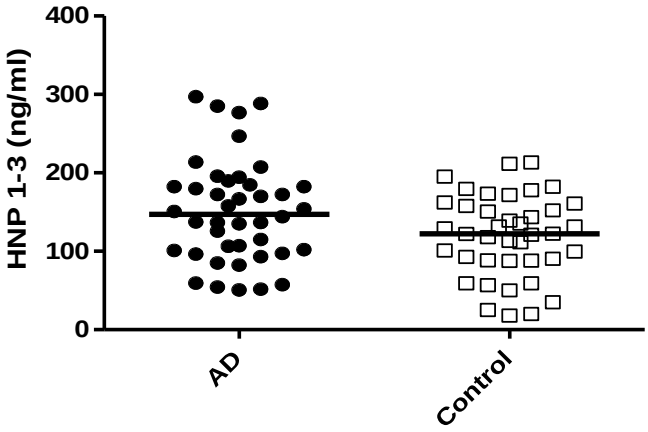
**Legend**

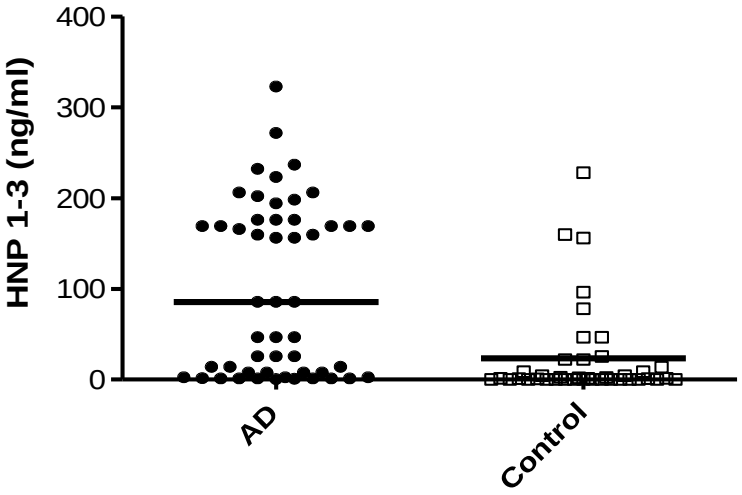
Levels of hBD2 in the sera (Fig1a) and in the cerebrospinal fluids (Fig1b); levels of HNP1-3 in the sera (Fig 1c) and in the cerebrospinal fluids (Fig1d) of AD patients and controls. Horizontal lines represents medians. Insertion: Individual variation in copy numbers of DEFB4.



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