# Interleukin-1ζ Gene Polymorphism Influences the Severity of Neurodegeneration in Alzheimer's Disease

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## ABSTRACT

**In subjects with Alzheimer's disease (AD) homozygous for the interleukin (IL)-1ζ -889 *1 allele significantly more of amyloid-η and hyperphosphorylated-ο and more of microglia cells (not significant) were seen when compared to subjects homozygous for the IL-1ζ 2 allele. The parallel increases in AD related lesions and microglia cells suggest that neuroinflammation might be of importance in progression of AD in a subset of subjects with a genetic predisposition (homozygous for IL-1ζ 1 allele).**

**Keywords:** IL-1ζ, gene, amyloid, microglia, Alzheimer's disease, homozygous

## Introduction

The interleukin(IL)-1 ζ 2 allele has been reported to be associated with an increased risk for Alzheimer's disease (AD)[1,2,3] though a lack of association have also been reported[4]. Furthermore the cognitive decline was found to be more rapid in subjects homozygous for the IL-1ζ 1 allele[5]. The mode of action of the IL-1ζ genotype in AD is not known.

IL-1, a proinflammatory cytokine, is expressed in glial cells and is responsible for mediating a variety of processes in the host response to inflammatory diseases. IL-1 levels are elevated in AD brains, and overexpression of IL-1 is associated with amyloid Aη plaque progression[6].

In a subject with AD, the brain tissue is packed with Aη aggregates and with hyperphosphorylated tau (HP-ο). Additionally numerous glial cells are seen[7]. An association between the number of astrocytes in the tissue and Aη protein aggregates and between the number of microglial cells and HP-ο has been reported[8] implying that glial cells might be involved in the pathogenesis of AD.

The objectives of this study were to analyze whether or not the proposed risk factor, the polymorphism in the IL 1ζ allele influences the pace in cognitive decline and the neuropathological lesions of AD including the gliosis.

## Material and methods

Sixty two patients with sporadic histopathologically verified AD were included in this study.

The determination of APOE genotype was carried out as described earlier[9] For the determination of the 1 to 2 transition polymorphism at –889 in the IL-1ζ locus, a upstream primer was designed (5'-ATC ACA CCT AGT TCA TTT CC-3') The downstream primer (5'-TTA CAT ATG AGC CTT CCA TG-3') and the RFLP protocol were the same as described previously[10].

The evaluation of the extent of lesions was carried out as reported previously[7,8]. The Aη aggregates, HP-ο expression, astrocytes and microglia were visualized utilizing immunohistochemical (IHC) methodology. Monoclonal antibody to human Aη (DAKO, M872), monoclonal antibody to human HP-ο(Innogenetics BR-03), monoclonal antibody to human histocompatibility class II complex (microglia) (DAKO M775) and polyclonal antibodies to glial fibrillary acidic protein (astrocytes) (DAKO Z0334) were used.

The quantification of Aη expression was performed under light microscopy using NIH Image system for PC[8] and the Aη expression is given as percent of stained area. The HP-οexpression was quantified under light microscopy

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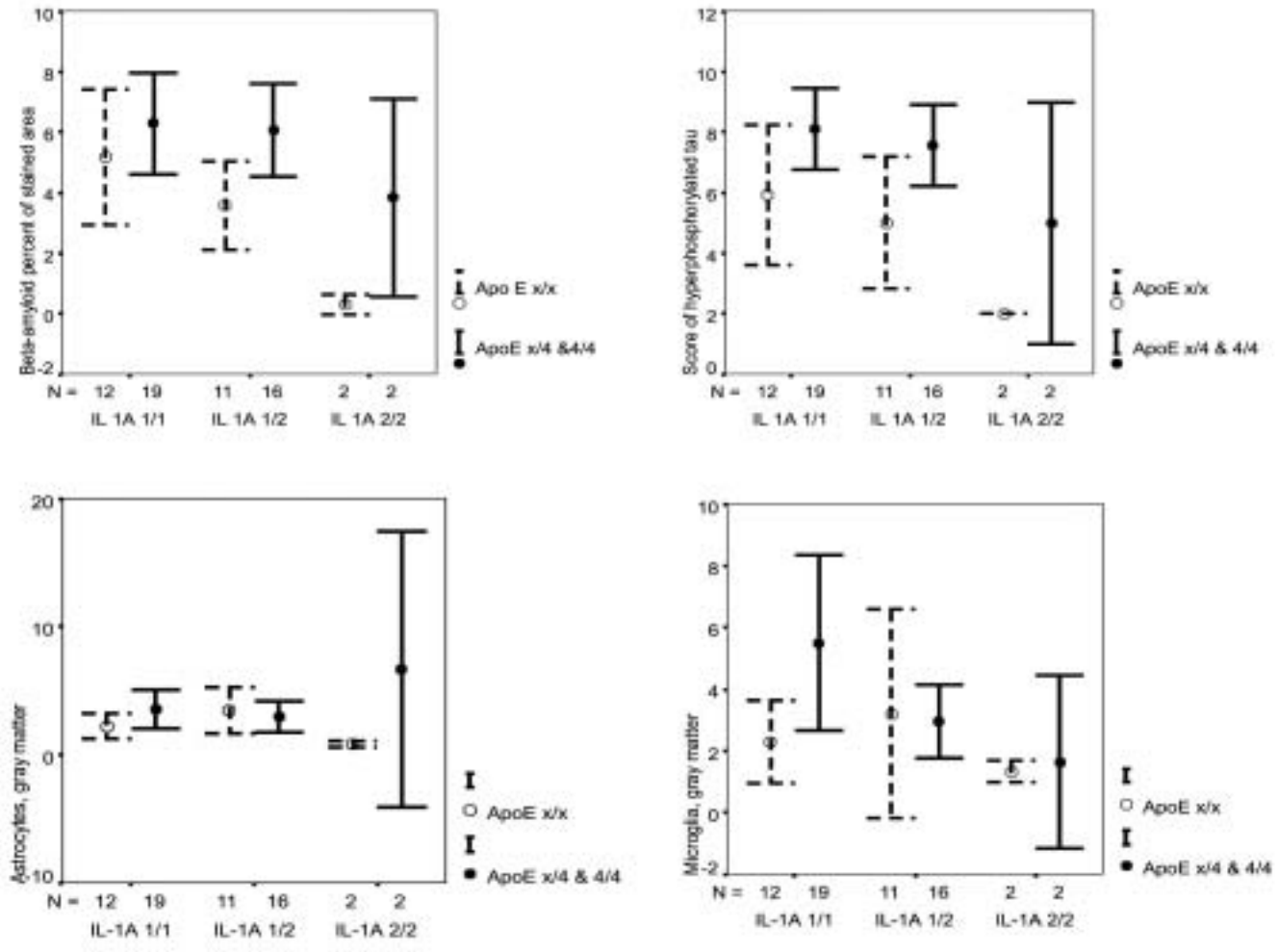


**Figure 1.**

**a)** Aη load **b)** score of hyperphosphorylated-ϱ **c)** number of astrocytes in gray and **d)** number of microglial cells in gray matter in subjects with various IL-1ζ and ApoE genotypes. Aη load, number of astroglia and microglia are given as percent of stained area. Results are given as mean +/- standard error. Student t-test was used for comparisons.

and scored on a four step scale from 0 to 3 and the results are reported as the sum of scores in frontal, temporal and parietal cortices and hippocampus. The quantification of GFAP, HLA DR -protein expression was performed using Quantimet 570 Image Analysis system (Leica Cambridge Ltd, Cambridge, England) and the results are given as percent of stained area[7].

SPSS program for Windows was used for statistical analysis. The differences were verified statistically by means of Student t-test and the correlation between individual variables was estimated using Pearson's correlation test.

## Results

The clinical information is given in table 1. The duration of the disease did not differ significantly comparing different IL-1ζ genotypes. Those homozygous for the IL-1ζ 2 allele were oldest both with respect to the age at onset as well as the age at death. The age at death was significantly lower in subjects homozygous for IL-1ζ 1 allele compared to those carrying one or two copies of IL-1ζ allele 2. The cognitive impairment was most severe in subjects with IL-1ζ 1/1 genotype, significantly so compared to subjects with the IL-1ζ 2/2 genotype. A significant negative correlation ($r = -0.7$, $n = 31$, $p < 0.001$) was found between the MMSE value and duration of the disease in subjects with one or two copies of IL-1ζ 2 allele. No such significance was found in subjects homozygous for IL-1ζ 1 allele ($r = -0.3$, $n = 30$, $p < 0.2$) who had the lowest age at onset and the shortest duration of the disease.

There were significantly more Aη aggregates and significantly higher score of HP-oin patients with IL-1ζ 1/1 genotype, compared to patients with IL-1ζ 2/2 genotype (Table 2). Similarly, there were more microglial cells, however not significantly, in subjects with the IL-1ζ 1/1 genotype. The number of astroglia cells was not influenced by the IL-1ζ genotype.

**Table 1.** Clinical information M.Overmyer et al

| IL-1ζ genotype | Number | Gender Fe/Ma | ApoE κ4 no/yes | MMSE mean ± SE | Age at onset mean ± SE (range) | Age at death mean ± SE (range) | Duration Mean ± SE |
|---|---|---|---|---|---|---|---|
| 1/1 | 31 | 27/ 4 | 12/19 | 2.4 ± 0.8* | 71.6 ± 1.9(45 – 86) | 80.6 ± 1.8 (54 – 98) | 8.9 ± 0.8 |
| 1/2 | 27 | 22/ 5 | 11/16 | 3.2 ± 1.0 | 74.5 ± 1.7 (54 – 92) | 85.4 ± 1.6 (69 – 102) | 10.9 ± 0.9 |
| 2/2 | 4 | 3/ 1 | 2/ 2 | 6.0 ± 3.6* | 82.0 ± 2.7 (76 – 89) | 91.3 ± 3.2 (86 – 100) | 9.3 ± 1.8 |
| — | 62 | 52/10 | 25/37 | 3.0 ± 0.6 | 73.6 ± 1.3 (45 – 92) | 83.4 ± 1.2 (54 – 102) | 9.8 ± 0.6 |

IL – interleukin, MMSE – mini mental state examination, SE – standard error. For statistics Student t-test was used * $p < 0.03$

**Table 2.** Neuropathological findings M.Overmyer et al

| IL-1ζ genotype | BW gram | Immunohistochemistry (IHC) ηA | HT-ο | Astrocytes (IHC) gray | white | Microglia (IHC) gray | white |
|---|---|---|---|---|---|---|---|
| 1/1 | 1123 ± 27 | 5.8 ± 0.7* | 7.3 ± 0.6* | 3.0 ± 0.5 | 7.8 ± 0.7 | 4.2 ± 0.9 | 7.5 ± 1.8 |
| 1/2 | 1076 ± 30 | 5.1 ± 0.6 | 6.5 ± 0.6 | 3.2 ± 0.5 | 7.9 ± 0.8 | 3.0 ± 0.8 | 5.9 ± 1.2 |
| 2/2 | 1189 ± 52 | 2.1 ± 1.2* | 3.5 ± 1.2* | 3.7 ± 2.8 | 10.1 ± 0.9 | 1.5 ± 0.6 | 3.2 ± 1.4 |
| — | 1107 ± 19 | 5.3 ± 0.4 | 6.7 ± 0.4 | 3.1 ± 0.4 | 8.1 ± 0.5 | 3.5 ± 0.6 | 6.6 ± 1.1 |

Values given as mean ∂ standard error. IL – interleukin, BW – brain weight, ηA – beta amyloid, HT-ο– hyperphosphorylated tau (results given as percent of stained area). For statistics Student t-test was used * $p < 0.05$

When the subjects were grouped according to their ApoE genotype, the influence of the IL 1ζ genotype was still noted. Both in individuals with and without the ApoE E κ4 allele, (Figure 1 a-d) with addition of IL-1ζ allele 2, the extent of lesions i.e., ηA load, HP-ο score and number of microglia cells decreased. The decrease was however not significant.

## Discussion

Our data suggest that the IL-1ζ allele 1 is associated with more pronounced neurodegeneration in AD. Subjects homozygous for IL-1ζ 1 allele had significantly more Aη aggregates and a significantly higher score of HP-ο when compared to subjects homozygous for IL 1ζ 2 allele. Moreover, subjects homozygous for IL-1ζ 1 allele had the most severe impairment (significant) in relation to the shortest duration of the disease. In line with our results, in a clinical study, Murphy et al[5] reported that in subjects homozygous for the IL-1ζ 1 allele, the decline in memory impairment was more rapid than in others. The mean values for microglial cells were highest (not significant) in subjects with IL-1ζ 1/1 genotype. The number of microglial cells has been shown to correlate with the extent of neuronal degeneration seen as neurofibrillary tangles[8]. Also in current study, an increase in the score of HP-ο was seen in parallel with the increase in the number of microglial cells. Based on our results, the neuroinflammation (number of microglia) as well as the neurotoxicity (higher loads of Aη and HP-ο) was more intense in subjects homozygous for the IL-1ζ 1 allele than in subjects carrying the IL-1ζ 2 allele.

The IL 1ζ allele 2 may be, as has been recently reported, a risk factor for initiating the development of AD, whereas according to our results it is the IL-1ζ 1 allele that is associated with the hallmark lesions of AD. The parallel increase in the extent of hallmark lesions of AD and microglia cells further suggest that neuroinflammation might be of importance in the pathogenesis and progression of AD, especially in a subset of subjects with genetic predisposition i.e. the IL-1ζ 1/1 genotype.

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