Diagnosis of Alzheimer's disease through measurement of anti-beta amyloid antibody in patient's serum

Ji Hoon Sohn[1], Hee Kim[2], Jung On So[1], Ji Yeon Huh[1], Jong Won Kim[3], Man Ho Kim[1], Inhee Mook-Jung[1], Young Ho Kim[2]

[1]Seoul National University College of Medicine; [2]Digitalbiotech. Inc.; [3]Sung Kyun Kwan University School of Medicine

Abstract

Amyloid beta (Aβ) is believed one of the major pathogens of Alzheimer's disease (AD), and the reduction of Aβ is considered a primary therapeutic target. Immunization with Aβ can reduce Aβ burden and pathological features in transgenic AD model mouse. This means anti-Aβ autoantibodies may have a role in AD pathology. Recent findings suggest anti-Aβ autoantibodies level decrease in AD patients. The early detection of AD is important for treatment of this disease. However, diagnosis on AD has only been possible through limited methods such as neuropsychological examination or MRI. To investigate whether it was possible to detect the presence and different levels of naturally occurring anti-Aβ autoantibodies in the plasma of patients with AD and age-matched controls. An advanced ELISA was performed to detect levels of naturally occurring anti-Aβ autoantibodies in the plasma. The level of anti-Aβ auto-antibodies from patients with idiopathic Parkinson's disease or stroke and from normal controls were compared to that of AD patients. Our results showed a significantly lower anti-Aβ autoantibodies level in AD compared to those with other neurological diseases or control. The level of anti-Aβ autoantibodies in the serum may be used to diagnose the presence of AD.

Diagnosis of Alzheimer's disease through measurement of anti-Aβ autoantibodies in serum

Young Ho Kim Ph.D.
Digitalbiotech, Inc.
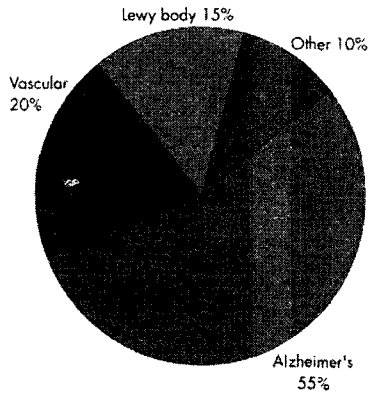
DIGITAL BIOTECH

Unmet needs of Alzheimer's disease

- Increase AD patients with high age society
- no curable medicine ever developed

Disease	no. of patients	medical expenses/year
Alzheimer's	**5,000,000**	**100 billion dollar**
Parkinson	1,000,000	5 - 10 billion dollar
stroke	3,000,000	50 billion dollar
spinal injury	500,000	5 billion dollar
schizophrenia	1,500,000	40 billion dollar

(ref: NIH, Voluntary Organization, 1990, 1997, USA; NINDS Congressional Report 2001)

DIGITAL BIOTECH

Dementia and Alzheimer's disease

Lewy body 15%
Other 10%
Vascular 20%
Alzheimer's 55%

No. of AD patients	
USA	4M
World	1.5M

DIGITAL BIOTECH

Genes associated with Alzheimer's disease

Disease Onset	Gene Product	Chromosome
Early	Amyloid precursor protein (APP)	21
	Presenilin 1 (PS1)	14
	Presenilin 2 (PS2)	1
Late	Apolipoprotein E	19
	LDL receptor-related protein (LRP)	12
	a_2-Macroglobulin (a_2M)	12
	FE65	11
	Chromosome 12 gene product distinct from LRP and a_2M	12
	Chromosomal loci	10

DIGITAL BIOTECH

Neuropathology of AD
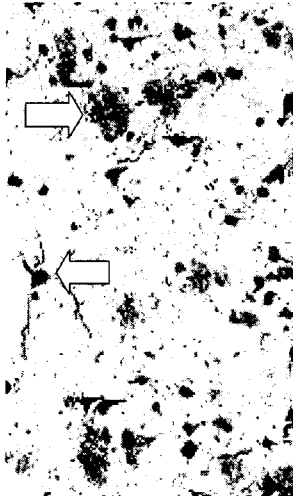
❖ **Neuritic plaques**

   beta amyloid

❖ **Neurofibrillary tangles**

   tau protein

DIGITAL BIOTECH

Clinical diagnosis of AD

Imaging from MRI

normal   Alzheimer

DIGITAL BIOTECH

Clinical diagnosis of AD

Biological markers

blood / CSF

Molecular diagnosis - APP. PS1. PS2. ApoE e4.

Blood test   - beta amyloid. Tau protein.

CSF test   - beta amyloid. Tau protein. Neuronal thread protein

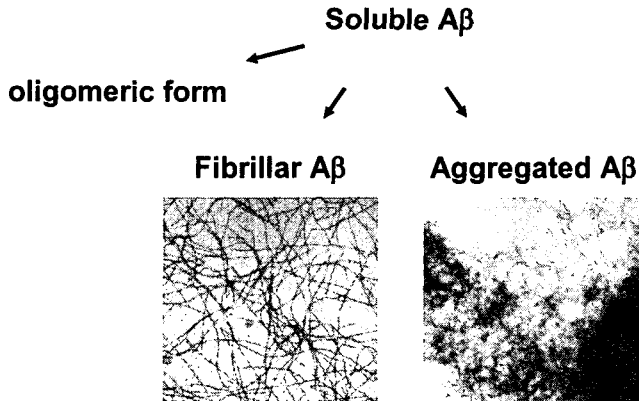**Others**   - skin amyloid. Tropicamide. Olfactory biopsy

DIGITAL BIOTECH

Schematic structure of APP

Extracellular   Membrane

N | SP | Cysteine-rich | Acidic | KPI | OX 2 | | Aβ | | C

β α $A\beta_{1\text{-}42}$ γ I,G,F

--- SEVKM | DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA | TVIVITLVM L ---

NL   G   V   P

Swedish FAD missense   Flemish & Dutch FAD missense   British, Indiana, etc. FAD missense
(β cleavage, ↑)   (aggregability, altered α cleavage ↑)   (γ_{42} cleavage ↑)
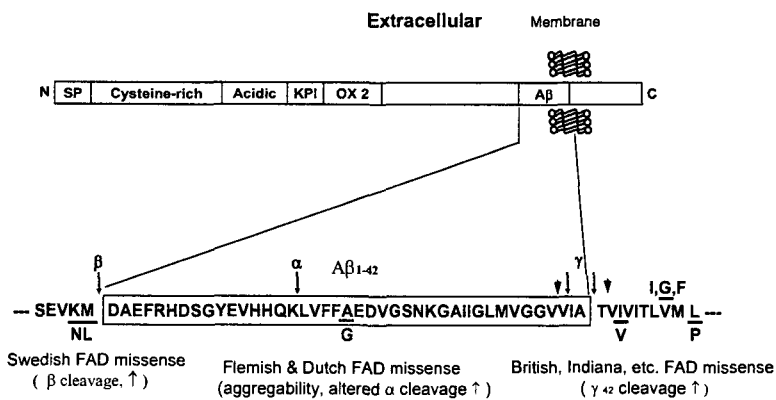
DIGITAL BIOTECH

Beta amyloid as causative reagent for AD

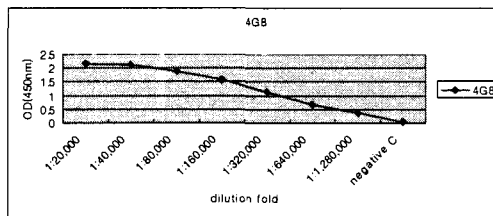Different forms of Aβ

Soluble Aβ

oligomeric form

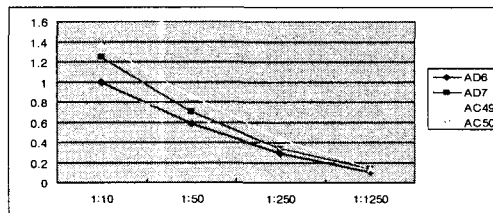Fibrillar Aβ   Aggregated Aβ

DIGITAL BIOTECH

Development of an advanced ELISA test

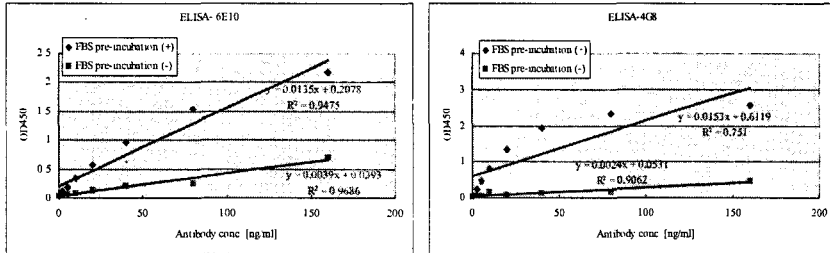Determination of standard material (comparison mouse Mab vs. human serum)

<mouse monoclonal>

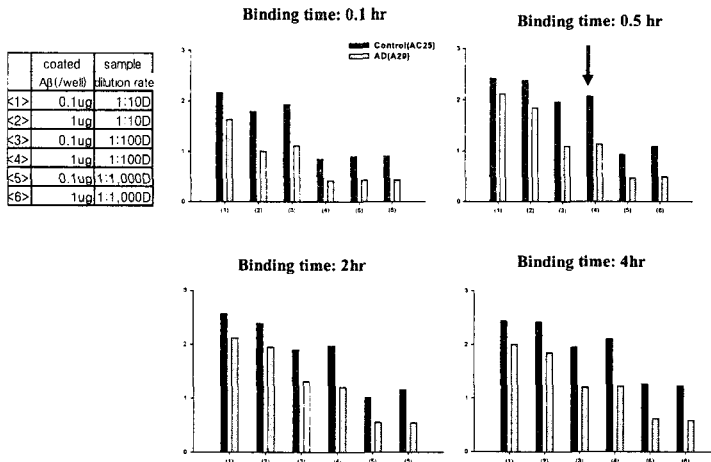<human serum>   DIGITAL BIOTECH

Development of an advanced ELISA test

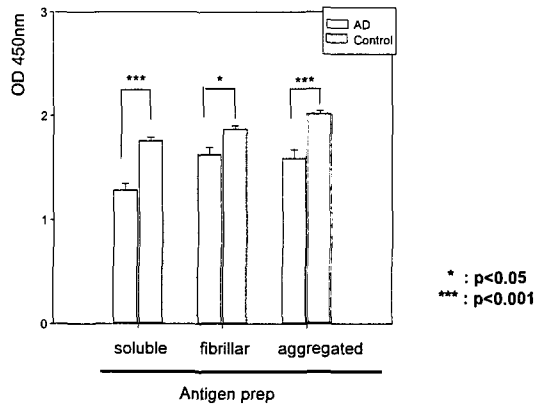Determination of standard material

Development of an advanced ELISA test
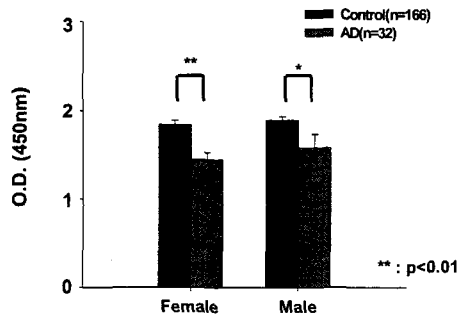
Determination of incubation time and coating concentration

Anti-Aβ autoantibodies in the serum of AD patients recognized different forms of Aβ

AD patients vs. Control
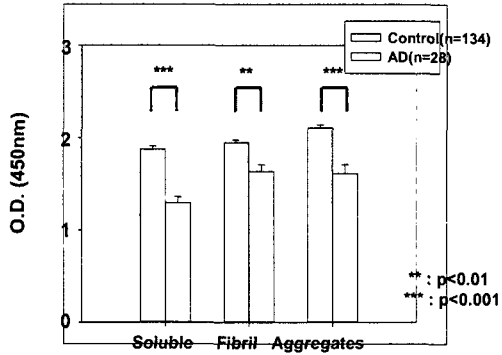
OD 450nm

AD
Control

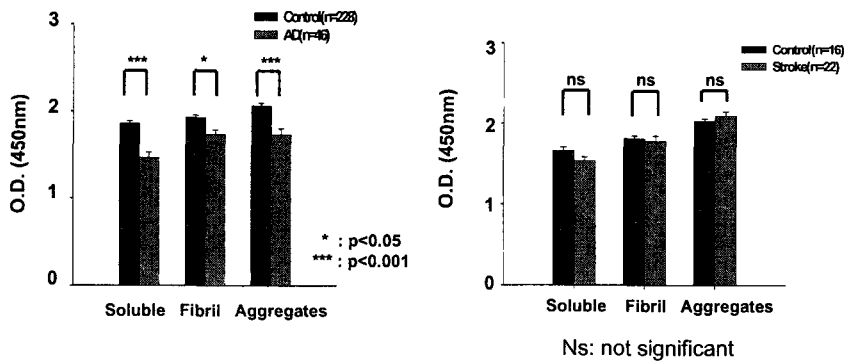soluble   fibrillar   aggregated

Antigen prep

* : p<0.05
*** : p<0.001

DIGITAL BIOTECH

Female vs. Male in AD patients

O.D. (450nm)

Control(n=166)
AD(n=32)

Female   Male

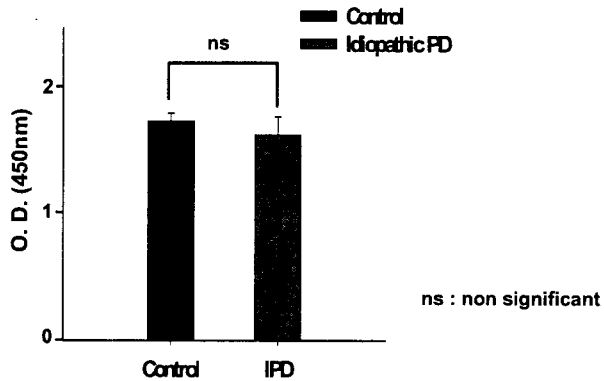** : p<0.01

DIGITAL BIOTECH

61~80 year old group

Anti-Ab antibodies in serums of AD and Stroke patients

Ns: not significant

Idiopathic Parkinson's Disease vs. Control

(IPD n=11, control n=28)

ns : non significant

* Idiopathic Parkinson's Disease : the most common form of Parkinsonism, a group of movement
(IPD)   disorders that have similar features and symptoms.

DIGITAL BIOTECH

Results of Clinical Field Test

Results	AD samples (272)		Normal samples (478)	
	positive	negative	positive	negative
No. of samples	226	46	167	311
analysis	sensitivity		specificity	
	226/272*100= 83.1%		311/478*100= 65.1%	

DIGITAL BIOTECH

148

Development of diagnostic ELISA kit for Alzheimer's disease

Digitalbiotech, Inc. cooperate with Standard Diagnostic, Inc.

DIGITAL BIOTECH

Acknowledgement

Seoul National University
College of Medicine

Inhee Mook-Jung, Ph.D.

Man Ho Kim, MD, Ph.D.

Sung Kyun Kwan University
School of Medicine

Jong Won Kim, MD, Ph.D.

DIGITAL BIOTECH