

## Alzheimer tau test and detergent cellulase Made by genetic engineering (No. 9 in a series of articles to promote a better understanding of the use of genetic engineering)

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### 1. Towards an earlier diagnosis of Alzheimer's disease

#### 1.1. Introduction

For the last half century, advances in public health and biomedicine have resulted in better health and increased longevity for many millions of persons. This has inevitably led to a general shift in the patterns of morbidity and mortality from the acute diseases of childhood to the neoplastic and degenerative conditions of older age. As a direct consequence of the gradual aging of our populations, Alzheimer's disease (AD) has emerged as the most important of the neurodegenerative disorders—not only because of its frequent occurrence but due to its devastating consequences for the patient, family and society as a whole.

In Europe alone, it is now estimated that between 1 and 1.5 million persons suffer from this progressively debilitating disorder. Up to 7% of the population above the age of 65, and 15% of those over 85 years are now thought to be affected. Given these facts, it is not surprising that research into early diagnosis and effective treatment of AD are major public health priorities.

#### 1.2. Clinical manifestations

The clinical course of the disease is well known. At its onset, AD is characterized by memory-related disturbances. Increasingly serious emotional changes soon follow. Most patients exhibit steady deterioration with a survival of about 8–10 years after the initial symptoms. By the time the condition is diagnosed, irreversible neurological damage has occurred. The patient usually dies from an intercurrent disease in a state of total helplessness. Expensive institutional care is usually needed long before the end.

Dementia is by far the major cause of long-term disability in older persons. Of those affected, AD is responsible for more than 50% of the cases referred to hospital. It is therefore imperative to differentiate AD from other dementing conditions which may be treatable or even partially reversible such as pseudo-dementia or vascular dementia. Current diagnostic tests to identify AD patients make use of a comprehensive but complex set of social, psychological, medical, psychiatric, and neurological examinations. Unfortunately, such a battery of tests cannot detect the early insidious, neurodegenerative changes in AD patients until clinical symptoms become apparent.

Table 1  
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Product	Alzheimer tau test	Detergent Cellulase
Principal trade names	INNOTEST hTAU Antigen	Puradax®
Principal uses	Diagnosis of Alzheimer's disease	Laundry cleaning
Manufacturers	Innogenetics N.V.	Genencor International
Donor organism	Human gene	Alkaliphilic <i>Bacillus</i>
Host organism	<i>Escherichia coli</i>	<i>Bacillus subtilis</i>
Advantages	First Alzheimer's disease diagnosis test Early Alzheimer's disease diagnosis	Reduced pilling of clothes during washing Anti greying of clothes Longer life for textile fabrics Safe production at high yield

### 1.3. AD pathogenesis

In addition to the neuronal cell loss which occurs predominantly in the hippocampus and temporal lobe of the cerebral cortex, Alzheimer's neuropathology is characterized by abundant senile plaques and neurofibrillary lesions in these same brain regions. Beta-amyloid is the main component of extracellular amyloid plaques, whereas the microtubule-associated protein tau in a hyperphosphorylated state is the main fibrous constituent of the intracellular neurofibrillary lesions.

These pathological changes were discovered through the use of classical silver impregnation techniques. The cloning in 1987 of the amyloid precursor protein (APP) led to the development of newly highly sensitive antibodies which improved our knowledge as to how senile plaques might be formed. In 1988, tau protein was found in neurofibrillary tangles and, in 1991, it was recognized that an abnormal phosphorylated form of tau was the major component of these structures.

Both the 'amyloid' and 'tau' approaches to understanding the pathogenesis of AD have been vigorously pursued by researchers world-wide for over a decade. Yet despite considerable efforts, their attempts to develop a non-invasive diagnostic test have, until recently, largely been in vain.

### 1.4. Tau-based diagnostic markers

Within the last years, however, this bleak picture has begun to change. Innogenetics was the first to break the ice by successfully developing an ELISA kit for the detection of tau proteins in human cerebrospinal fluid (CSF).

Based on initial tests with this kit, tau CSF levels were measured in 103 Alzheimer patients and 28 age-matched controls free of neurological disease. While 96% of the controls had tau levels below 200 pg ml<sup>-1</sup>, more than 90% of the Alzheimer group showed tau concentrations above this level. These results, indicating that elevated tau concentrations can be found in Alzheimer CSF, have now been confirmed in several multicenter studies. Moreover, initial results from ongoing trials indicate that tau is elevated before the onset of the clinical symptoms of dementia.

In addition to the INNOTEST hTau antigen kit (Table 1), Innogenetics developed a broad range of monoclonal antibodies recognizing different forms of the tau protein. Among these, AT8 is highly specific for the abnormal phosphorylated form of tau predominantly found in AD brains. The exact phosphorylated residues needed for AT8 recognition were determined by in vitro phosphorylation of site-directed mutagenized recombinant tau protein. This antibody is able to

detect neurofibrillary changes, before the appearance of neurofibrillary tangles.

It is well-established that neurofibrillary lesions correlate well with the clinical symptoms of the disease, and the major component of these lesions is abnormally phosphorylated tau protein. Normally six isoforms of the Tau protein are found in the brain which serve to stabilize the microtubular network in the axons. In AD, these different tau isoforms are progressively hyper-phosphorylated and correlate well with the presence of neurofibrillary lesions, abundantly present in AD.

### 1.5. Amyloid-based diagnostics

Important inroads have also been made to develop amyloid-based diagnostics. Last year, U.S.-based Athena Neurosciences announced the availability of a service for amyloid determinations in body fluids, including the CSF. More specifically, with an ELISA-based test they measure the levels of the pathological beta-amyloid 1–42 form of the peptide, which is known to be linked with the development of neuritic plaques. A decrease in the CSF concentrations of beta-amyloid 1–42 has been correlated with progression of the disease.

### 1.6. The genetic approach

Alzheimer's disease can also be differentiated clinically into two forms based on the onset of disease symptoms: the so-called early-onset forms comprise 5–10% of case, while the majority of patients (90–95%) are affected by the late-onset form. Current research combining genetic linkage and biochemical studies indicates that the risk of developing the adult late-onset form is increased when the E4 allele of the gene encoding apolipoprotein E (ApoE4) is present, at least in AD with an age of onset below 70 years. However, assessment of risk factors does not confirm that the disease is actually present in a given patient. E4 typing assays are now available for research and clinical studies.

Finally, linkage analysis studies have revealed the presence of two genes, presenilin-1 and presenilin-2, that are mutated in about half of all familial AD cases (5% of all AD). It has been

hypothesized that the presenilin genes are part of the amyloidogenic cascade of biochemical events in the brain leading to AD pathology. Research tests for presenilin gene mutations may well be incorporated into Alzheimer testing schemes in the near future.

In conclusion, important breakthroughs in biotechnology-driven AD research have emerged making an earlier laboratory diagnosis of AD a real possibility. The first biochemical markers are now available (Tau, Beta-amyloid, etc.) for use by researchers, clinicians, and the pharmaceutical industry. Possible combinations of test markers may also help optimize this biochemical approach. At first, diagnostic tests will help differentiate non-treatable from treatable forms of dementia. Once effective drugs become available for AD, such biochemical tests will not only be used as a tool for the early diagnosis of AD, but also for monitoring the effectiveness of treatment.

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## 2. Brighter appearance to coloured textiles thanks to a new cellulase from an extremophilic bacterium

The domestic laundry consists largely of cotton fabrics. Cotton is 100% cellulose, a substrate for cellulase. Cellulases are useful cleaning additives because they remove the fibrils (pills) from cotton textiles and prevent the accumulation of new pills on the surface of textiles. These effects contribute to a brighter appearance to coloured textiles despite the normal wear and tear resulting from frequent washing. In addition, redeposition of secondary soils, which lead to a greyish appearance of fabrics, is prevented, resulting in a significant anti-greying or whiteness retention effect perceived in consumer trials. These positive consumer benefits all have to be achieved with low fibre damage.

Table 2  
Summary of products published in the series 'Made by genetic engineering'

Product	Lipase	Hepatitis B vaccine
Author/manufacture	B. Diderichsen, Novo Nordisk, Denmark	P. Crooy, SmithKline Beecham Biologicals, Belgium
Made by genetic engineering No. 1, 1991. <i>Biotech Forum Europe</i> 8, 246–247.		
Product	Human insulin	Human growth hormone
Author/manufacture	E. Rasmussen, Novo Nordisk, Denmark	L. Fryklund, KabiPharmacia, Sweden
Made by genetic engineering No. 2, 1992. <i>Biotech Forum Europe</i> 9, 144–145.		
Product	Protein G	Interferon alfa-2a
Author/manufacture	R. Hjorth, Pharmacia LKB Biotechnology, Sweden	S. Ryser, F. Hoffmann-La Roche AG, Switzerland
Made by genetic engineering No. 3, 1992. <i>Biotech Forum Europe</i> 9, 641–642.		
Product	AIDS test	$\alpha$ -Amylase
Author/manufacture	E. Baumann, F. Hoffmann-La Roche AG, Switzerland	B. Diderichsen, Novo Nordisk, Denmark
Made by genetic engineering No. 4, 1995. <i>J. Biotechnol.</i> 38, 193–197.		
Product	Erythropoietin	Interferon beta-1b
Author/manufacture	C. Kionka, Boehringer Mannheim, Germany	T. Petri, Schering AG, Germany
Made by genetic engineering No. 5, 1995. <i>J. Biotechnol.</i> 43, 73–77.		
Product	Interferon gamma	Rabies vaccine
Author/manufacture	E. Falkner and I. Maurer-Fogy, Bender and Co GesmbH/Boehringer Ingelheim Vienna, Austria	J. Terré, G. Chappuis, M. Lombard and P. Desmetre, Rhone Mérieux, France
Made by genetic engineering No. 6, 1996. <i>J. Biotechnol.</i> 46, 155–159.		
Product	Tissue plasminogen activator (rt-PA)	Granulocyte-macrophage colony-stimulating factor (GM-CSF)
Author/manufacture	W. Werz and R.G. Werner, Dr. Karl Thomae GmbH / Boehringer Ingelheim Pharma, Germany	R. Till, Novartis / Schering Plough International, Switzerland
Made by genetic engineering No. 7, 1998. <i>J. Biotechnol.</i> 61, 157–161.		
Product	Human coagulation factor VII	Folicle stimulating hormone (FSH)
Author/manufacture	U. Hedner and T. Lund-Hansen, Novo Nordisk A/S, Denmark	J.C. Heikoop and W. Olijve, N.V. Organon, Oss, The Netherlands
Made by genetic engineering No. 8, 1998. <i>J. Biotechnol.</i> 61, 231–236.		
Product	Alzheimer tau test	Detergent Cellulase
Author/manufacture	E. Vanmechelen and H. Vanderstichele, Innogenetics N.V., Belgium	B. Jones and W. Quax, Genencor International, The Netherlands
Made by genetic engineering No. 9, 1998. <i>J. Biotechnol.</i> 66, 229–233.		

Most available cellulases function at an acidic pH and are optimized for total digestion of cellulose. As detergents operate at high alkaline pH there is a need for an alkaline cellulase which does not degrade intact fibres.

Soda lakes are highly alkaline extreme environments. The soda lakes of the East African Rift Valley are typical examples where permanent or semi-permanent standing bodies of water with

pHs in the range from 8 to > 12 have formed in closed drainage basins exposed to high evaporation rates. Many of the lakes are very shallow and solar heating results in water temperatures often in the range 30–50°C. Furthermore, the Rift Valley is a tectonically active region and some of the lakes are fed by hot springs, at temperatures between 50°C and boiling, arising from deep ground-water aquifers. Surprisingly for such a

harsh environment these lakes comprise a remarkably rich diversity of micro-organisms. Systematic studies have shown that many of the microbes are obligatory alkaliphilic or at least alkali-tolerant. They can be cultivated under special laboratory conditions. The organisms, mainly prokaryotes, that live out their lives under extreme conditions of temperature or pH for example, are collectively referred to as extremophiles.

The conditions in laundry detergents and during the washing cycle have much in common with those in a soda lake. Most of the traditional enzymes applied in laundry detergents are not derived from organisms selected from particularly alkaline environments since these extreme microbes have not yielded to growth on an industrial scale. With the introduction of genetic engineering it now has become possible to use the genetic information from the extremophiles to develop a fermentation process using established industrial expression hosts, such as bacilli.

Recently, Genencor International launched Puradax<sup>®</sup>, an endo-cellulase especially adapted for detergent applications (Table 1). This enzyme evolved naturally in a soda lake environment to operate in alkaline detergent conditions and because of its extremophile background Puradax<sup>®</sup> is compatible with detergent ingredients, including bleaches, bleach activators, surfactants, builders and the alkaline pH during the wash. In application tests Puradax<sup>®</sup> removes the fibrils (pills) from cotton textiles and prevents the accumulation of new pills on the surface of textiles during many wash cycles. In addition, these positive consumer benefits are all achieved with low

fibre damage which is an essential factor for a detergent cellulase.

The extremophile organism secreting Puradax<sup>®</sup> was especially selected from a new species of obligatory alkaliphilic *Bacillus* (BCE103) representing a novel *Bacillus* RNA group. In common with many extremophiles *Bacillus* BCE103 has poor growth properties for industrial fermentation and it does not secrete sufficient amounts of the enzyme to develop an economic production process. Therefore the endo-cellulase gene was cloned and overexpressed in an established industrial expression host: *Bacillus subtilis*.

*Bacillus subtilis* was chosen as a host because it is non-pathogenic and is able to produce large amounts of secreted enzymes, such as proteases. As an established industrial production host the yield of product from *Bacillus subtilis* is high, resulting in a reduced consumption of raw materials and energy. The extracellular localization of the cellulase simplifies the recovery of the enzyme from the fermentation broth. By using recombinant DNA technology the Puradax<sup>®</sup> cellulase is now affordable for use in detergent compositions. As a result clothes retain their smart appearance for a longer period.

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The products published in this series so far are summarized in Table 2.