OCCUITY TECHNOLOGY

Early Detection of Alzheimer's Disease

By 2040, it is projected that the number of people living with dementia will have increased to 81 million. Much of the increase will be in developing countries. Already more than 60% of people with dementia live in developing countries, but by 2040 this will have increased to 71%. The fastest growth in the elderly population is taking place in China, India, and their south Asian and western Pacific neighbours.

Alzheimer's disease accounts for more than 50% of cases of dementia in Caucasian populations, but this may not apply to other national or ethnic groups, and more research is needed in this area. It is vital that, within this research remit, early and accurate detection is a priority. The major impediment to prevention or interventions to slow disease progression remains a lack of a positive and validated marker or the technology for early and accurate detection of the prodromal neurodegenerative processes. Occuity aims to address this by developing a device capable of detecting the build up of the Alzheimer's disease markers in the eye which when analysed using machine learning techniques is able to provide this screening tool.

Alzheimer's Disease belongs to a group of disorders that are characterised by a physical degeneration of brain or nerve tissue. Other well-known examples include Parkinson's disease, Huntington's chorea, and multiple sclerosis.

Although descriptions of dementia are found in literature from ancient Greece and through the Middle Ages, it is only really in the past 30 years that Alzheimer's has come into prominence. This is because increased longevity and, as the condition occurs more commonly in people in their 70s and 80s, the condition is now much more frequently reported in our ageing populations.

While dramatic progress has been made in unravelling the causes and nature of the neurodegeneration, brain changes mapped in some detail, and several biochemical and genetic defects discovered, it is clear that a lot of research still needs to be done. The discovery of specific genes that predispose people to Alzheimer's suggests the possibility of screening 'at-risk' people with options for a preventive treatment such as a vaccine. Less successful have been attempts to elucidate the environmental triggers for Alzheimer's despite several avenues of evidence to suggest that they exist.

Effects in Neural Anatomy

The normal healthy adult human brain weighs about 3 lbs. The outside is covered with a complex pattern of folds called convolutions, while inside, there is an outer layer of grey matter forming the cerebral cortex, an inner layer of white matter, and an interconnecting system of cavities called ventricles, which are full of cerebrospinal fluid. When floating in its cerebrospinal fluid, the brain only weighs about 14% of its weight in air, and is thus provided with buoyancy and a shock-resistant cushion.

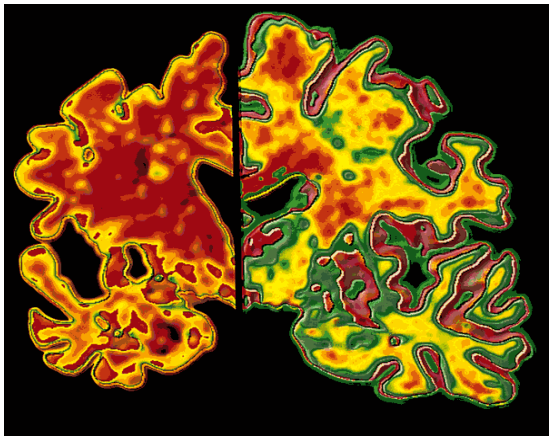

Figure 1: Vertical sections through a normal

(right) and a late stage Alzheimer's brain (left) showing massive tissue loss and enlargement of cavities

By comparison, a brain from a person who has died from Alzheimer's disease will have shrunk, the folds on the outside deepened and widened the proportions of white and grey matter and the internal cavities enlarged. By the time of death, an Alzheimer's brain is likely to have lost between 30%-50% of its weight (Figure1).

There is clear evidence from positron emission tomography scans that the brain of someone with Alzheimer's uses glucose and generates energy at a far lower level than that of a healthy person, thus reflecting the decline in mental function. However, in some people for reasons that remain unclear, these changes occur very slowly, while in others, they are much more rapid.

Evidence now suggests that Alzheimer's is a partly genetic, progressive condition characterised by:

- the development of neurofibrillary tangles inside neurons
- the formation of neurotoxic □-amyloid peptide and plaques which bind together
- death of neurons especially those using the neurotransmitter, acetylcholine and
- shrinkage of the brain due to this cell loss, especially in the areas concerned with memory, rational thought and speech.

Amyloid Formation and Detection

Beta-amyloid protein accumulates in the brains of patients with Alzheimer's disease, activating immune cells that try unsuccessfully to remove it. This triggers the release of poisons that ultimately kill nerve cells, leaving behind a trail of plaques and tangles which are the remains of nerve cells and fibres, clogged up with beta-amyloid.

There is evidence to suggest that Alzheimer's disease is not just a disease of neural tissue, but rather a systemic one that can manifest in the lens of the eye. The beta amyloid proteins that form plaques in the brain and impair cognitive function also build up near the edge of the lens, ultimately forming an unusual supranuclear cataract that is very different from more familiar, age-related cataracts.

Moreover, the same type of beta amyloid protein that accumulates in the lens periphery of patients with Alzheimer's disease also appears to accumulate in drunsen, which are spots in the macular area and are the early signs of macular disease. Also, the pathophysiological neurodegenerative process in the optic nerve of patients with glaucoma is identical to the process seen in the brain of patients with Alzheimer's disease and there is epidemiologic and clinical evidence that patients with glaucoma are more prone to develop Alzheimer's later in life.

In a study of the human eye, Goldstein et al. postulated that, because the human lens is vulnerable to age-dependent degenerative changes and shows progressive deposition of insoluble protein and extensive oxidative damage and because early-onset cataracts and Alzheimer's disease are typical comorbid disorders in adults with Down's syndrome and in those with familial Danish dementia, an Alzheimer's disease variant with cerebral beta amyloid amyloidosis, there may be overlap between Alzheimer's disease-associated molecular pathological findings in the lens and in the brain of people with the disease.

The authors identified amyloid plaques in the human lens and in human primary aqueous humour. They showed that concentrations of amyloid plaques in the human lens, and in primary human aqueous humour, are comparable with those in aged human cerebral cortex and cerebrospinal fluid, respectively. They also noted increased deposition of electron-dense immunoreactive aggregates within lens fibre-cell cytoplasm in the supranuclear subregion of lenses from people with Alzheimer's disease. The cytosolic localisation of lenticular plaque is important, since this peptide localises to the same cellular compartment as the highly concentrated crystallins within the lens fibre cell. These cells have limited ability to turn over protein as the lens ages. Thus, lens amyloid plaque is in a position to foster cytosolic lens protein aggregation.

These experiments, though acknowledged by the authors as being carried out in a small number of samples, appear to indicate that beta amyloid does get deposited in the eye and, in particular in the lens.

Pharmaceutical Company Interest

Alzheimer's disease is, according to some sources, now the third leading cause of death globally and, with an unprecedented patient potential, this disease area attracts a lot of pharmaceutical and biotech interest.

Over the past decade the Alzheimer's disease market has been essentially cornered by a handful of approved pharmacological agents which are either acetylcholinesterase inhibitors (AchEIs), N-methyl D-aspartate (NMDA) receptor antagonists or other non-Alzheimer's disease specific therapeutics aimed at the management of comorbidities.

The leading medications are:

- Aricept (donepezil) - Eisai Co Ltd and Pfizer Inc
- Exelon (rivastigmine) - Novartis Pharmaceuticals
- Razadyne (galantamine) - Janssen Pharmaceuticals and
- Namenda (memantine) - Forest Pharmaceuticals

However, because of the lack of selectivity of these currently prescribed drugs in addition to their known side effects which include nausea, vomiting and diarrhoea, means that several other companies have active research streams in Alzheimer's disease research.

All the large pharmaceutical companies have at least one candidate compound in their current pipelines and an expressed interest in working in the field of Alzheimer's disease because of the large unmet patient need. Moreover, there is an important raft of biotech companies looking to make strategic alliances in order to cement their position in this aggressive marketplace.

The central nervous system market is the second largest therapeutic category and is one of the fastest growing. One of the largest segments by sales is Alzheimer's disease with more than a 10% year-on-year growth forecast making Alzheimer's disease the third most expensive disease in the USA.

In addition, a recent survey by the Association of the British Pharmaceutical Industry (ABPI) demonstrated that the public wants

Measurement of amyloid plaques in the Lens

Since Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and cataracts are all feature symptoms caused or affected by protein aggregations, it may be expected that there will be some features in common among these diseases. So far, however, no epidemiological studies have showed any association between cataracts and these diseases.

Mutant presenilin and amyloid protein are found in the lens, and amyloid can aggregate in the lens, further suggesting that cataract can be associated with Down syndrome and Alzheimer's disease. In addition, in transgenic mice expressing amyloid protein, this protein aggregated in the lenses, and cataracts were more often found in these mice, indicating that cataract and Alzheimer's disease may be associated.

Since amyloid and presenilin proteins are present in the lens and brain, it is possible that Alzheimer's disease can be predicted by analysing the lens.

Amyloid Structure and Detection

Amyloid protein structure is characterized by stacked arrays of beta pleated sheets that may be soluble or insoluble. Extensive analysis of lens protein structure in fresh intact lenses and purified proteins using Raman and infrared spectroscopy has demonstrated that the proteins in normal mammalian lenses exist predominantly as beta pleated sheets.

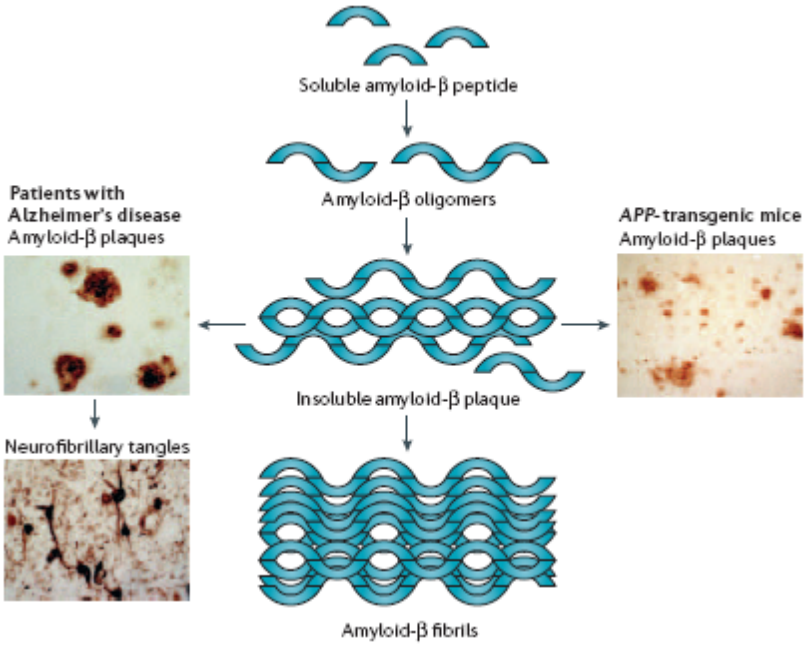

Figure 3: From Weiner HL and Frenkel D (2006) Immunology and immunotherapy of Alzheimer's disease Nature Reviews Immunology 6(5), 404-416

Beta-amyloid formation and deposition in Alzheimer's disease and in APP-transgenic mice. Soluble β-amyloid peptide polymerises to form oligomers, which fold to generate β-pleated sheet fibrils. Compact senile plaques comprised of β-amyloid fibrils are associated with pathological changes in the surrounding brain neurons, leading to their death. Recent studies have connected the β-amyloid plaques (see inset, upper left) with the formation of intracellular neurofibrillary tangles (see inset, lower left) comprised of hyperphosphorylated tau protein. A mouse model of Alzheimer's disease has been

generated in which animals overexpress a mutated form of human amyloid precursor protein (APP) in the brain. At 6–9 months of age, the APP-transgenic mice begin to develop senile plaques in the hippocampus, corpus callosum and cerebral cortex that can be stained with Congo Red (see inset, right).

Using polarising laser Raman spectroscopy on intact fresh lenses, it has been shown that lens anti-parallel beta-pleated sheets are arranged in orthogonal arrays with respect to the lens visual axis. Demonstrations of an ordered array of beta-sheet structure in lenses and the presence of well characterised amyloidogenic proteins are consistent with the formation of an amyloid protein supramolecular order in the lens.

Evidence Collection

Evidence would rely on an accurate measurement of amyloid protein or a peptide derivative thereof in the eye. The amyloid deposits are thought to appear as unusual cataracts that are distinguishable from the more common, age-related cataracts.

The only robust evidence that amyloid derivatives have been detected this way claims to have identified fragments of beta amyloid in lenses from people with and without Alzheimer's disease at concentrations comparable with those found in the brain, and amyloid in primary aqueous humour at concentrations comparable with cerebrospinal fluid. In the lenses of those individuals with Alzheimer's disease, the beta amyloid accumulates as an electron-dense deposit located exclusively in the cytoplasm of supranuclear/deep cortical lens fibre cells.

Alzheimer's Screening device as a natural progression

Although still very much at an early stage, Occuity believe that the technology being developed for screening for AGE's as a method to detect diabetes can be adapted to screen for other known markers in the eye associated with different diseases. Amyloid plaques, which many consider to be a potential marker for Alzheimer's is a perfect example of just such a marker and disease. Thus by following the development path to produce a device capable of detecting Diabetes and pre-diabetes Occuity will be proceeding along a development path that may ultimately result in a multi-function screening device with the capability of screening for many diseases that leave markers within the eye.