APOPIS
Abnormal Proteins in the Pathogenesis of Neurodegenerative Disorders

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PRESENTATION AT THE END OF THE PROJECT IN DECEMBER 2006

The APOPIS project
Prof. Dr. med. Franz Adlkofer, VERUM - Stiftung für Verhalten und Umwelt, München

The APOPIS Consortium consists of 38 European research institutions experienced in the area of neuroscience. It was brought together and coordinated by the VERUM Foundation, a non-profit organisation in Munich, Germany. In line with the expectations of the EU-Commission which supported the research project together with the Swiss government with Euro 11.5 Mio the goal was several-fold, i.e. to combine the still scattered European resources, to foster the integration of high-profile European research talents, to establish synergies by co-operation and, thus, to increase European competitiveness in this area of research.

For the last three years the APOPIS Consortium attempted to get insight into the mechanisms of neurodegeneration, to improve the clinical diagnosis of neurodegenerative disorders, and to develop prevention strategies and, eventually, effective treatment. The neurodegenerative diseases such as Alzheimer disease, Parkinson disease, Huntington disease, motor neuron and prion diseases are amongst the most debilitating illnesses. This is especially true for Alzheimer which is responsible by far for most of the cases with dementia. At present, there live about 5.5 million Alzheimer patients in Europe the number of which will double every 20 years due to the constantly increasing lifespan of people, should prevention and therapy remain inaccessible. This will put an enormous strain on both social and healthcare budgets all over Europe, and in the light of the decreasing ratio of working to retired populations the financial dilemma may even become dramatic. There is currently no treatment available that can halt or prevent – let alone reverse – the resulting nerve cell degeneration, mostly due to the fact that the underlying causes of these diseases are only poorly understood.
Generation and turnover of abnormal protein aggregates

Prof. Bart de Strooper, Flanders Interuniversity Institute for Biotechnology, Leuven

Human neurodegenerative diseases are incurable disorders caused by the gradual loss of nerve cells (neurons) in the brain. Depending on the part of the brain that is affected, the disease is characterized by movement problems, like Parkinson disease, or by memory loss and ultimate dementia, like Alzheimer, Huntington and prion diseases. Microscopic analysis of post-mortem brains from patients affected by different neurodegenerative disorders revealed in each case the presence of protein aggregates, also known as 'amyloid' deposits, in specific brain regions. Proteins are the building blocks of life and as such perform different functions in the cell. Despite intense research, relatively little is known on why a harmless and functional protein, under certain pathological conditions form insoluble aggregates that deposit in the brain and cause neuronal death.

In the APOPIS Consortium we studied in detail the process of conversion of soluble proteins into insoluble aggregates and could define the basic rules that govern this crucial process. We demonstrated that the driving force for aggregation and amyloid formation is centred in a small region of the protein. Further, we developed a computer algorithm -the amyloid pattern- to identify such 'amyloidogenic' regions in disease-associated proteins. Interestingly, taking the 'amyloidogenic' region of one protein (a piece of ~6 amino acids) and fusing it to a normal protein not associated with disease is sufficient to convert it into an amyloid-prone protein that is toxic for cells. We next hypothesized that if the force driving amyloid formation is centred in a small portion of a protein, then targeting this region with small compounds might be sufficient to prevent protein aggregation and toxicity. Indeed, we demonstrated this by using compounds that we specifically designed to bind the amyloidogenic regions in individual proteins. Such anti-aggregation compounds constitute a promising novel therapy.

For the study of any human disorder it is crucial to have animal models that reproduce (some) aspects of the human disease and are therefore suitable to test the effectiveness of novel therapeutic strategies. In the Consortium we generated novel animal models that carry a human disease-associated gene and suffer from the characteristic protein aggregation and neurodegeneration. These include small animals with a short generation time (they get adult and age in few days) like the soil worm Caenorhabditis elegans and the fly Drosophila melanogaster, as well as mice that develop a disease resembling more the human condition. Promising therapies developed in the Consortium were tested in these models.

Genes and their function in neurodegenerative diseases

Prof. Christian Haass, Ludwig-Maximilians Universität, München

Most of the neurodegenerative conditions exist as sporadic and familiar forms. Whereas the cause of disease in sporadic cases is not clear, familiar cases are caused by mutations in specific genes, and therefore usually several members in a family develop the disease. Analysis of affected families led to the identification of the gene implicated, and knowing the gene has contributed to our understanding of the underlying pathogenic process. However, in most cases we know relatively little about the function of the disease-linked gene in the normal brain and on how the mutation causes the disease.

The APOPIS Consortium has greatly contributed to the identification of novel genes and gene mutations associated with neurodegenerative conditions. Just to mention one example, we identified the long-sought gene responsible for a subtype of frontotemporal dementia. Little is known at present on the function of this gene - Progranulin - and further investigation will hopefully reveal new aspects of the disease and potential novel therapeutic targets.

To understand the function of disease-associated genes both in health and disease, various animal models were generated that include soil worms (Caenorhabditis elegans), flies (Drosophila melanogaster), Zebrafish and mice. These models either carry the human mutated gene ('knock-in' models) or have the animal counterpart of the human gene specifically ablated ('knock-out' models). Detailed anatomical and behavioural analysis of these animal models provided important clues about the mechanism of disease and about the function of the gene under physiological and pathological conditions.

An additional strategy that we exploited to understand the function of specific genes linked to disease was to identify the binding partners of the encoded protein. To carry out their specific task in the cell, proteins need to interact with several other proteins, and identifying those partners can provide clues on the metabolic pathways where they participate and therefore on their cellular function. We carried out different approaches to pinpoint binding partners of a number of disease-associated proteins and we also determined whether and how specific mutations affected those interactions. An example is the beta-secretase, the function of which has been within the APOPIS Consortium clarified. This enzyme is one of the most important proteins in Alzheimer
disease and as such one it is a most promising target for future therapeutic approaches. The
data that we obtained from animal models combined with those from protein interactors have
substantially contributed to our understanding of the function of genes involved in neurodegenerative
disorders. And the more we know about these genes, the higher the possibilities for therapeutic intervention.
Thus, by knowing in detail what the disease-linked protein does in the nerve cell, in which cellular location,
with which partners it has to interact to carry out its specific function and how mutations affect these
processes offer different steps where specific compounds could act to prevent or correct the disease.

**Improving clinical detection of neurodegenerative diseases**

*Prof. Christoph Hock, University of Zurich*

In most neurodegenerative diseases diagnosis is made once symptoms are well established and therefore
when substantial brain damage has already occurred. There is therefore an urgent need for early diagnosis to
be able to treat the diseases before brain damage is irreversible. There is also a need for good 'biological
markers', substances that can be easily detected in patients and whose presence indicates a particular disease
state.

In the APOPIS Consortium we invested significant efforts to identify very early changes that occur in these
diseases, i.e. before symptoms commence. One major tool that we used to detect such changes is brain
imaging, which provides a non-invasive and reproducible method to analyse the structure and function of the
brain of living patients. We focussed on young healthy subjects that are at high risk of developing a
neurodegenerative condition latter in life because of a positive family history. For Alzheimer disease for
example, we were able to detect changes in the brain that occur decades before the disease is manifested. We
studied a family in which the age for the clinical manifestation of Alzheimer disease is ~50 years. Brain imaging
analysis of a 20 years-old individual from this family who carries the mutation revealed increased brain activity
when compared with subjects with the same age that do not carry the mutation. This increased brain activity
probably reflects a compensatory effort to overcome neuronal dysfunction caused by first pathological
changes.

Because brain imaging can be performed in the same patient at different time points as the disease progresses,
we were able to identify specific patterns of brain alterations characteristic of some diseases. This represents a
major advance, since some neurodegenerative disorders present similar or overlapping clinical symptoms. The
brain imaging tools developed in APOPIS should then help clinicians make a more accurate diagnosis and
therefore administer more adequate treatments.

APOPIS efforts were also centred in the identification of biomarkers; specific molecules present in the body
fluids of patients (like blood or cerebral spinal fluid that are easily accessible) but not in healthy individuals or in
patients affected by a different neurodegenerative condition. Thus for example by comparing the protein
profile of CSF taken from healthy and diseased individuals we were able to identify 6 novel biomarker
candidates that are either increased or decreased in samples from AD patients.

In conclusion, in the APOPIS Consortium we achieved important advances in various brain imaging techniques
and in biological markers that should aid in early and accurate diagnose and allow monitoring disease
progression. Moreover, these can be used in clinical trials to assess the efficacy of treatments, to evaluate the
capacity of a drug to slow down or prevent disease onset.

**Therapy of neurodegenerative diseases**

*Prof. Roger M. Nitsch, University of Zurich*

There is a huge medical need for causal treatments of neurodegenerative conditions. Available symptomatic
treatments can at best delay disease progression and temporarily ameliorate some of the clinical signs. Thus,
considerable efforts in the APOPIS Consortium focused in the development of novel therapeutic approaches for
the treatment and eventual prevention of neurodegenerative diseases.

Because many neurodegenerative disorders have in common the presence of abnormal protein aggregates in
the brain, prevention of aggregate formation was a common therapeutic objective. We developed assays that
allowed us to test thousands of compounds for their anti-aggregation capacity. Positive candidates identified in
these screenings were subsequently tested in cellular assays to identify those with the ability to reduce not
only protein aggregation but also toxicity. Interestingly, some candidates were effective against more than one
amyloidogenic protein, i.e. against huntingtin and Abeta that accumulate in the brains of Huntington and
Alzheimer disease patients, respectively. The most promising candidates were then evaluated in small animal
models and shown to be effective in preventing aggregate-induced neurodegeneration. These compounds with therapeutic potential against a range of neurodegenerative disorders are currently being optimized to improve their brain penetration upon intravenous or intra-peritoneal injection. Additional promising anti-aggregation compounds developed in the Consortium are those described by Prof. de Strooper. These compounds (indeed D-peptides) were specifically 'designed' to bind to the amyloidogenic portion of a protein as identified with the amyloid pattern mentioned above. They were active in cell cultures and are currently tested in animal models. A rather revolutionizing approach to treat neurodegenerative diseases emerged a few years ago and consists in vaccinating patients with the same protein that accumulates in their brains. The idea is to induce an immune reaction that eventually leads to the clearance of the deposits. Immunotherapy was first tested as an approach to treat Alzheimer disease, but the clinical trial had to be stopped due to brain inflammation in ~6% of the vaccinated patients. APOPIS members participated in this trial and were the first to show that despite the unwanted side effects there was a clear beneficial effect of immunotherapy. In the APOPIS Consortium we developed an alternative immunotherapy approach that should reduce or abolish the negative side effects observed in the initial clinical trial.

In conclusion, in the APOPIS Consortium we have developed novel anti-aggregation compounds (small chemical compounds and biologicals) that have promising therapeutic potential and guarantee further investigation.

**Genes affecting Parkinson disease**

*Prof. Ralf Baumeister, Albert-Ludwigs-Universität Freiburg*

Parkinson disease (PD) is the most common movement disorder and the second most common neurodegenerative disease. It affects about 1% of the population over 65 years of age. The main hallmark of this devastating disease is the selective loss of neurons producing the neurotransmitter dopamine. The APOPIS Consortium includes some of the leading European research laboratories studying the molecular mechanisms underlying the Parkinson pathology. APOPIS researchers contributed to understanding the role of several genes involved in the disease — all of them have just recently been identified. In a study published this year in “The New England Journal of Medicine”, they could for example show for the first time that a particular mutation in the gene LRRK2, responsible for a familial form of Parkinson, may be important in some human populations in North Africa, while it is rarely observed throughout the world. These results will allow diagnosing Parkinson by genetic analysis and offering genetic counseling, in particular in these high-risk communities.

APOPIS researchers established several laboratory models to analyse the role of LRRK2 and other genes affecting Parkinson. They could demonstrate that the majority of these genes are involved in the stress management of cells, and can interact with signals that may also regulate cell death and neuronal aging. Their results suggest that the defects causing Parkinson may interfere with an important genetic program whose normal biological function is to control the survival and lifespan of cells.

Among other animal models, research in APOPIS firmly established the only 1 mm long nematode worm *Caenorhabditis elegans*, a prime model for senescence and aging studies, as an important animal for studying Parkinson mechanisms and for testing drugs. Using a technique similar to that invented by the recipients of this year’s Nobel Prize in medicine, Craig Mello and Andrew Fire, a high-throughput test was developed to find target genes for the development of anti-Parkinson’s medication. With the same goal in mind, APOPIS mouse researchers also succeeded in identifying a receptor protein called RET whose function is critical for the survival of those neurons affected by Parkinson.

Three years of APOPIS research have considerably improved the understanding of the various factors contributing to the onset of Parkinson disease. Based on APOPIS research results, several chemical compounds have now been synthesized whose effects are currently being tested in the contributing laboratories.

**PUBLICATIONS** (team leader in bold):

**2004**


2005


Springer W, Hoppe T, Schmidt E, Baumeister R (2005) A Caenorhabditis elegans Parkin mutant with altered solubility couples a-synuclein...


2006


terminal intracytosolic fragment. Hum Mutat 27(9):888-96.


2007


2008


2009


2010


