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DIADEM

Early Diagnosis of Alzheimer's Disease and Related Dementia

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Contractors: VERUM - Stiftung für Verhalten und Umwelt, München, Germany | Universität Zürich, Switzerland | Georg-August-Universität Göttingen, Germany | Ludwigs-Maximilians-Universität München, Germany | VIB - Vlaams Instituut voor Biotechnologie, Leuven, Belgium | Fundacion para la Investigacion Medica Aplicada, Pamplona, Spain | EleGene GmbH, Martinsried, Germany | Evotec NeuroSciences GmbH, Hamburg, Germany | Universidade de Aveiro, Portugal | Fondazione Cavalieri Ottolenghi Scientific Institute, Orbassano, Italy | National and Kapodistrian University Athens, Greece | Karolinska Institutet, Huddinge, Sweden | University College London, United Kingdom | Ruprecht-Karls-Universität Heidelberg, Germany

OBJECTIVES: The work packages of this multi-institutional research programme were designed to generate knowledge of a better understanding of the molecular mechanisms associated with the pathophysiology of Alzheimer's disease (AD) and related dementia. Basic molecular neurobiology was integrated into a clinical research programme in a two-way orientation: Molecules identified in the basic research studies were to be tested in clinical settings for their pathophysiological relevance in the diagnosis and therapy of dementia, and clinically identified molecules with a role in genetics or as a disease marker were to be analysed in basic research study protocols for their neurobiological functions. This combined approach should make it be possible to identify novel genes and proteins associated with dementia, and to validate all candidate markers that result from the pre-clinical studies for their potential as a biomarker of dementing diseases. The combination of basic and clinical research with powerful developments in high-throughput screening and chip technology and combinatorial chemistry should lead to products that are useful in the diagnosis and treatment, and, thus, in the prevention of dementia. To achieve the goal of this integrated basic, clinical and biotechnological research programme two biotech companies with access to extensive technology have been included in the consortium. It is their task to develop a highly parallel assay device that can test simultaneously many biomarkers for dementia and drugs for disease prevention.

RESEARCH AND RESULTS: In work package "animal models" the goal was to identify mechanisms that reduce brain amyloid formation, to characterise the role of alpha-synuclein in neurodegeneration associated with dementia, to analyse the role of presenilins in neurons involved in memory, and to determine the neuronal phenotypes of expressing human neprilysin, insulin degrading enzyme, PS1, PS2, BACE-1 and alpha-synuclein in experimental animal models. To detect pathological processes at early stages of neurodegeneration, experiments were carried out to test whether the disease related genes and gene products influence the expression of known and novel disease markers by applying DNA array technology. To provide insights into the roles of these genes in intact brains, additional experiments were conducted in genetically modified mice, C. elegans and Drosophila. Among the substantial advances, these stand out: (1) Neuronal expression of neprolysin reduced amyloid brain pathology from developing in APP transgenic mice but neprolysin up-regulation did not remove amyloid plaques once they were established. (2) The transmembrane domain but not the cytoplasmic tail of nicastrin is required to form an active gamma-secretase complex. (3) *C. elegans* and saccharomyces yeast are powerful systems for conducting genome-wide high-throughput screens to detect drugs that interact with proteins that are relevant to aging and neurodegeneration. (4) BACE1 and BACE2 knockout mice were both normal and fertile, although there was 50% infant mortality in BACE1 mice. (5) Transgenic mice that over-expressed both alpha-synuclein and tau had age-dependent memory impairments with a 5 times increase in amyloid and neurofibrillar tangle formation and 40% increase in neuronal loss in the

entorhinal cortex. (6) *Drosophila* that overexpress transgenic cDNAs for human BACE1 and APP display amyloid deposits in the eye and wing morphologic abnormalities that can be reversed by secretase inhibitors.

The work package "cellular pathogenesis" was to elucidate the interactions underlying the synthesis, post-translational processing, degradation, and pathological accumulation of proteins associated with neuro-degeneration and dementia using cultured cells and model organisms. Special emphasis was placed on APP proteolysis, factors that are involved in prion protein infectivity, and the links between Abeta, tau, alpha-synuclein and PrP. Another goal was to determine which of the genes/gene products can be used as disease markers and which can be targets for drug treatment. Some of the highlights are: (1) TAP or myc-tagged murine PrP rescued the lethality in mice expressing n-terminally truncated PrP. (2) Lithium treatment decreased Abeta peptide secretion from primary chicken neuronal cells but specifically increased intracellular Abeta1-38. (3) There are six individual proteolytically active gamma-secretase complexes. (4) The phosphory-lation state of specific amino acids in the intracellular domain of APP is crucial to determining APP processing and metabolism. (5) Lowering cholesterol in membrane lipid rafts increases APP beta cleavage, implying that treatment to decrease cholesterol may actually worsen AD by increasing amyloid production. A few goals had to be dropped due to insurmountable technical problems, such as low sensitivity of the microtiter format binding Abeta assay based on PrPB binding. Another few remained incomplete due to their challenging complexity.

The work package "biological and genetic markers" was to identify and characterise genetic and protein markers for the early and differential diagnosis of dementias. New candidate markers were screened in samples of DNA, serum and CSF from well documented cases of AD and related dementias collected from collaborating centres specialising in the diagnosis and clinical care of dementia. Genetic risk factors were identified by genome-wide screening, linkage and association methods. Candidate protein markers were assayed in body fluids of patients in order to determine diagnostic sensitivity and specificity. In many instances, state-of-the-art neuroimaging data were generated to complement the clinical information. Genetic analyses were carried out: a case-control association study identified five new candidate risk genes for sporadic AD, a genome-wide scan in selected AD families was started, and there is progress in understanding the pathophysiology of the arctic APP mutation. CSF candidates as diagnostic or surrogate markers were identified, and work started to optimise their measurement on quantitative high throughput technological platforms. Especially noteworthy advances include: (1) Strong collaboration among four contractors to collect new families and to store and analyse a growing number of genetic samples. (2) From an expanding set of DNA samples of histologically-confirmed patients and control subjects several cholesterol-related genes that are associated with AD have been identified. (3) Quantitative MRI volumetric measures of brain atrophy correlate highly with clinical measures of cognitive decline. (4) Active immunization with pre-aggregated synthetic Abeta42 exerts positive effects on cognition.

The detection of new candidate risk genes for developing AD by screening families with familial AD and the determination of the relation between proteins found in the biological samples and the clinical aspects of specific dementing illnesses were still in progress at the end of the project.

BENEFITS: Considering the enormous burden for society caused by AD and related dementia, the DIADEM project has a number of social, medical and economic implications. It will have a major medical impact since the findings of the DIADEM project may create the basis for a totally new approach in diagnosis and in treatment of these diseases by developing drugs which effectively arrest, prevent or reverse the progress of the illness or by replacing genes of abnormal composition, that are responsible for the breakout of the diseases, through gene therapy. It will have, too, an economic impact, since the results which led until now to the application of three patents offer a great chance to the contributing industry. Contrary to all expectations, it has proven a far more difficult goal to achieve the identification of other genes for late onset AD besides ApoE than was anticipated and to go from promising genetic linkage results to reproducible and clear genetic associations. Despite this difficulty, investigators in the DIADEM consortium conducting research on some of the most fundamental aspects of AD and related dementia were remarkably successful with their work, even if they failed to complete the development of a "DIADEM diagnostic chip". Instead, they refined existing biochemical methods and introduced new techniques that will enable chip development in the future.

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