PROJECT FINAL REPORT

Grant Agreement number: 305299

Project acronym: AgedBrainSYSBIO

Project title: SYSTEMS BIOLOGY OF PATHWAYS INVOLVING BRAIN AGEING

Funding Scheme: Collaborative Project

Date of latest version of Annex I against which the assessment will be made:
   5. September 2016

Period covered: from 01. January 2013 to 30. June 2017

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1.1 Executive Summary

The AgedBrainSYSBIO project provided novel results on:

I. Integration of multiple-level datasets to identify interacting protein networks including recent Late-Onset Alzheimer Disease- Genome Wide Association Studies (LOAD-GWAS) data

Integration of various data types by QURETEC gives a unique opportunity to study Alzheimer's disease through the network of complex interactions aggregated as a single data source. The BABRAHAM group set up a quantitative model of glutamate signaling pathways, one of the LOAD key pathways with modeling of FYN activation and Aβ regulation.

The EMBL-EBI group published a comprehensive review of the status of formal modeling of neurodegenerative disease related processes based on 89 separate models, curated into the BioModels database.

SIB conducted analyses of epistasis, using GWAS and whole genome sequencing data (provided by external consortia - TGen, HBRTC, and ADNI), which revealed gene-gene interactions associated with AD pathology, and with longitudinal AD-related brain imaging and cognitive performance phenotypes

HYBRIGENICS identified novel protein-protein interactions (PPI) of LOAD-GWAS gene products and INSERM characterized their subcellular neuronal location, using proximity ligation assay methods, demonstrating networks involved in synaptic function.

II. Development of novel mouse transgenic models

Novel humanized mouse models for APOE ε4 polymorphism and BAC BIN1 mouse model have been generated. With Gene Bridges GmbH, CERBM-GIE successfully engineered mouse stem cells to locate tags for mass spectrometry and super-resolution imaging in genes involved either in synapse function or in LOAD.

III. BIN1 mice display early LOAD pathologic features

BAC-BIN1 mice display an inflammatory/demyelination early phenotype and contrasted neurocognitive features before any Aβ changes. This mouse model can be instrumental to test novel therapeutic approaches of LOAD.

IV. Development of novel Drosophila models

From the identification of 19 risk loci (in addition to APOE) in which the functional genes are unknown (Lambert et al., Nature Genetics, 2013), IPL identified p130CAS (CASS4), Eph (EPHA1), Fak (PTK2B) and Rab3-GEF (MADD) as Tau toxicity modulators in Drosophila (Dourlen et al., Mol. Psychiatry. I.F. 13.2).

V. Identification of human and/or primate positive selection

TAU & INSERM identified signals of positive Darwinian selection in genes that are known to be associated with neurodegenerative human diseases and aging.

VI. A novel readout of impacted intraneuronal transport

INSERM showed that fluorescent nanodiamond tracking reveals impacted intraneuronal transport induced by brain-disease-related genetic risk factors (Haziza et al., Nat. Nanotechnology, I.F. 38.9).

VII. Analysis of human neurons derived from induced Pluripotent Stem Cells (iPSCs).

UDUS group characterized neuronal differentiation and neuronal phenotypes from neurons derived from iPSCs obtained from patients with rare LOAD mutations (CR1 or TREM2) from VIB group.

VIII. Analysis of rare variants from LOAD-GWAS patients

VIB group identified loss-of-function mutations in a LOAD-GWAS gene, ABCA7, in a resequencing study (Cuyvers et al, Lancet Neurol. 2015; I.F. 24.1).

IX. Proof of concept of drug discovery for systems medicine

Hybrigenics, ReMYND, NeuroService and INSERM generated, selected and tested intrabodies against soluble TAU and FYN. Memory performance of hTAUP.301S mice was improved with a secreted anti-TAU minibody. An anti-TAU intrabody induced a significant decrease of insoluble p-TAU AT8. An anti-FYN intrabody was able to impact selectively plasticity of hippocampal synapses in aged mice.

1.2 Summary description of project context and objectives

In spite of valuable approaches applied to get a broad understanding of genetic, epidemiologic and molecular and system-level biological principles of human aging, cognitive decline remains as one of the greatest health challenges of the old age, with nearly 50% of adults over 85 who suffer from neurodegenerative diseases such as Alzheimer's. Furthermore, drug development has not performed as expected in clinical trials, at least in part because of an insufficient mechanistic understanding at the systemic level in human.

AgedBrainSYSBIO was a timely and straightforward project based on the integration of available genetic transcriptomics, proteomics and metabolomics data, on the addition of relevant novel sets of data, on their modeling and on experimental testing in both human, mouse and drosophila.

AgedBrainSYSBIO researchers studied brain ageing, using multiple-level approaches such as systems biology based on large sets of data from ageing mammals and humans in normal situations and in pathological context such as Late-Onset Alzheimer Disease (LOAD) , protein-protein interactions, novel drosophila and mouse models based on molecular engineering, human neurons generated from cells isolated from patients with LOAD or control, engineered antibodies (intrabodies) directed against abnormal protein-protein interactions that may participate to the evolution of an abnormal ageing and whole-genome data(Genome-Wide Association Studies; GWAS) from large populations of patients with late-onset Alzheimer diseases.

We investigated themolecula pathways and interactions involved in brain ageing. We aimed to find therapeutic targets and biomarkers for late-onset Alzheimer's disease. Our scientists focused in particular on protein networks involved in the communication between nerve cells. This ambitious project integrated numerous European and national initiatives. We received the input of five small to medium-sized enterprises (SME) allowing us to get solutions for curing and preventing common age-related diseases. The links between academia and industry was the driving force of this work programme.

Our Consortium was based on the unique expertise of molecular geneticists, molecular biologists, clinicians involved in ageing diseases, computer experts and mathematicians involved in pathways modelling. A special emphasis was given to the involvement of the five SMEs as *AgedBrainSYSBIO* was expected to have potential benefit to these SMEs.

This combination of expertise was expected to give outstanding interactions to analyze and make sense of heterogeneous data sets.

Our project addressed the basis of human ageing by focusing on the identification of pathways linked to normal and pathological ageing taking advantage of literature data of LOAD-GWAS data generated by three Partners and novel data based on patient and normal person-derived human neuron transcriptomics, drosophila and mouse models.

AgedBrainSYSBIO obtained novel information from subsets of neurons and subregions of neurons in order to pinpoint the location of protein-protein interactions and get a better understanding of relevant pathways through which the ageing phenotype develops in normal and/or disease conditions. This topic addressed the basis of human ageing by defining the interactions through which the ageing phenotype develops in normal and/or disease conditions.

1.3 Main S&T results

WP1 – Identifying pathways involved in normal and pathological ageing: database, curation and modelling

Bringing together heterogeneous datasets related to Alzheimer's disease (QURETEC)
Alzheimer's disease and other dementias are the top cause for disabilities in later life and various types of experiments are performed to understand the underlying mechanisms of the disease with the aim to come up with potential druggable targets. These experiments are carried out by scientists working in different domains such as proteomics, molecular biology, clinical diagnostics, and genomics. The results of such experiments are stored in databases designed for collecting the data of similar types. However, in order to get a systematic view of the disease from these independent but complementary data sets, we need

integrated data sets. This approach generated a data collection comprised of 20 original datasets of 6 data types (GWAS, PPI, co-expression, epistasis, positive selection, expression patterns in brain regions) originating from 9 data sources. This integrated dataset, which is freely available, will allow scientists to effectively explore and analyse their own results in the broader context of Alzheimer's disease and enables to obtain a more systematic view on the disease.

Mechanistic models in neurodegenerative diseases (EMBL-EBI)
Considering the complex heterogeneity of neurodegeneration, efforts on a systems-level understanding of the disease using mathematical modelling approaches are being undertaken. We synthesized the current state of modelling in the area of neurodegeneration by integrating the major pathological processes implicated in neurodegenerative disorders. We collected 89 mechanistic models from the literature, developed over the past two decades, which describe different aspects of neurodegeneration. These models of neurodegeneration, developed at different biological scales, provide insights into the mechanisms underlying the pathogenicity involving multiple pathways. Through the systematic analysis of the existing models, the unprecedented mechanistic insights gained into the pathogenesis of the disease were analysed and objectively compared with the existing experimental / clinical knowledge.

We constructed a landscape of ND-related molecular processes using the mechanisms described in our collection of 89 models. The objectives of generating this model landscape map are to aid increasing activities in model composition in the field of neurodegeneration. Bearing in mind, the potential role of mathematical modelling in drug discovery and development, a comparative analysis was performed between the target of known drugs of neurodegenerative disorders and the processes that have mechanistic models. This study informs us about correlations, gaps and scope for mechanistic models in the clinical domain of neurodegeneration. Information on drugs for neurodegenerative disorders was obtained from ChEMBL using the Human Disease Ontology (DOID) terms. A total of 166 clinical drugs targeting 10 neurodegenerative diseases were retrieved from ChEMBL. The drug types include small molecules, proteins, enzymes and antibodies. Analyses show that mechanistic models and clinical drugs for neurodegenerative diseases have chronologically evolved in separate ways. We compared the molecular processes of neurodegeneration that have known drug targets in the corresponding pathways with those that have mechanistic models (Lloret-Villas et al, CPT Pharmacometrics Syst Pharmacol. 2017).

Model-driven predictions to be tested in vitro (Babraham Institute)
Computational model was focused on the quantitative description NR2B-NMDA receptor endocytosis to include NR2A-containing NMDA receptors. NR2A was considered as a much stable subunit of NMDA receptor and undergoes endocytosis at a slower rate than NR2B, although the endocytosis of both subunits can be facilitated by increased BIN1 expression. Tyrosine 1472 site on NR2B subunit is key phosphorylation site on NR2B subunit. Its mutation to phenylalanine (Y1472F)blocks it from phosphorylation. This mutation did not impair NR2B's binding with PSD95. However, without it, NR2B-NMDAR had a tendency to bind to AP-2 and to undergo endocytosis. Y1472F mutation near completely excluded NR2B from the surface of PSD, and it abolished the biphasic change induced by Aβ oligomers, indicating Aβ oligomers require this phosphorylation site to establish their effects. The over expression and relocation of BIN1 from extrasynaptic to synaptic site, elevated synaptic NR2B to a steady level, due to the local endocytosis and recycling of these receptors. However, without Aβ-oligomers-induced biphasic receptor quantity change, BIN1 could not increase synaptic NR2B to the levels required to induce excitotoxicity, showing that the combined effects from both BIN1 and Aβ oligomers are necessary for inducing deaths of dendritic spines. As the NR2B-Y1472F mutant mice have been developed and used in previously published experiments, we propose that this mutation can be utilised as a negative test for Aβ oligomers' effects on synaptic NR2B-NMDAR. We further recommend, combining this mutation with BIN1 over-expression to test BIN1's effects on synaptic NR2B expression.

Comparative and evolutionary analyses of pathways (TAU, INSERM)
In order to characterize the evolutionary dynamics in genes that are associated with Alzheimer's disease, we analyzed the SNPs data from Lambert et al. (2013). We first mapped the 500 most significant SNPs to their encoded human gene. As many SNPs mapped to the same genes, we removed duplications, yielding a list of 42 unique genes.

A bioinformatics pipeline to collect the human gene IDs was developed and used to query the Ensembl (Aken et al. 2017) database (version 89, accessed on 16/06/2017). An additional component was then developed and used to query the Ensembl database and search for 1:1 orthologs for each of these human genes, across 41 mammalian species. When querying the Ensembl database, only genes with transcripts whose status is "known" and "coding" were retained. Only 36 of the genes matched these criteria. In cases for which more than one transcript was available per human gene, the longest one was retained. For each of the mammalian orthologs, if more than one transcript was available, the transcript with the highest Needlemen-Wunsch (1970) score against the retained human transcript was chosen. This procedure resulted in 23 genes (sequence sets) for which at least 4 (human + at least 3) orthologs were collected. Codon multiple sequence alignments (MSAs) for each of these sets were computed using a Perl script, by first aligning the translated protein sequences using MAFFT v7.182 (Katoh and Standley 2014) and then back-translating this MSA to nucleotide-based alignment. We next searched for positive Darwinian selection in each orthologous group. Two types of tests were conducted, site test and site-branch test. Both tests were conducted using PAML version 4 (Yang 2007). Out of the 23 genes 14 were shown to evolve under a positive

WP2 - Identification of interacting protein partners in brain ageing processes including Late-onset AD

Identification of protein-protein interactions in pathways relevant for human brain ageing in drosophila and human using 60 distinct screens and their localization (HYBRIGENICS, INSERM)
More than 60 Yeast two-hybrid assays have been performed using LOAD-GWAS gene products as baits. Upon physical binding of the protein of interest (bait) to a protein fragment from the library (prey), the DNA Binding Domain (DBD) of the TF is brought in close proximity to its Activation Domain (AD). Reconstitution of the functional TF activates the transcription of the HIS3 reporter gene, which allows yeast cells to grow on a selective medium lacking histidine. The DNA of the positive clones was then sequenced and analyzed to identify the protein partners. Local interactions were directly quantified in sub-regions of neurons using proximity ligation assays. The localization of 100 interactions has been performed by combining proximity ligation assays (PLA) and Mass Spectrometry from mouse brain synaptosomes. Altogether, these two approaches allowed defining networks of proteins located in dendritic spines, that include the « PSD risk » network defined by Li et al (Li J, Wilkinson B, Clementel VA, Hou J, O'Dell TJ, Coba MP. Long-term potentiation modulates synaptic phosphorylation networks and reshapes the structure of the postsynaptic interactome. Sci Signal. Aug 9;9(440):rs8 2016).

Novel knock-in mice to be used for either super-resolution or proteomics (CERBM-GIE, GeneBridges)
The objective of this task was to develop tagged mouse models to allow local immunoprecipitation in brain synaptosomes and to visualize protein expression using super-resolution microscopy. After the establishment by the whole AgedBrainSYSBIO consortium of an initial list of 7 genes that was complemented by a second list of 13 genes in June 2014, the genetic strategy for the 20 models was designed by partner 9 (Genebridges). The TwinStrep, HA and Halo tags were selected to be used for mass spectrometry and for imaging. We also chose one new tag, namely the Malissen-Dendra2 tag, already experienced for the analysis of the immune system and developed by our collaborator B. MALISSEN (CIPHE, Marseille). All the 20 vectors were successfully generated by Genebridges.

A total of 19 knock-in lines have been generated and will be available for the community.

Local IP of cortex proteins using knock-in transgenic mice (INSERM)
The objective of this task was to develop tagged mouse models to allow local immunoprecipitation in brain synaptosomes and in specific neurons type using transgenic mice. Genebridges was in charge of design and vector construction and GIE-CERBM was in charge of all the others steps to generate these mutant models. After establishment of the lines, cortex proteins of selected generated models were purified from transgenic mice and subjected to tap tag purification and LC/LC MS/MS analysis on proteomic platform (INSERM).

Analysis of Quantitative Traits & epistasis from LOAD-GWAS patients (SIB)

SIB performed systematic epistasis analyses to uncover genetic variations that, when combined, are affecting LOAD susceptibility and progression. The analyses used data from three independent cohorts, comprising a total of 2'394 patients, provided by external institutions and consortia: TGen, HBTRC, and ADNI. Genetic data consisted of genome-wide genotypes of SNPs, as well as whole genome sequencing for a subset of 819 ADNI participants. Epistatic effects were assessed on several AD-related quantitative phenotypes: brain histopathology (Braak score, in the TGen and HBTRC cohorts), longitudinal changes in brain morphology (ventricle volume, in the ADNI cohort) and in cognitive performances (23 distinct scores, in ADNI). For each phenotype, the analysis involved assessing effects of hundreds of billion pairs of SNPs, a computational challenge that could be overcome by leveraging High-Performance computing resources and fine-grain optimization of algorithms.

These analyses resulted in the identification of large networks of genes linked by significant epistasis: respectively 599, 947 and 1'202 genes in the TGen, HBTRC, and ADNI cohorts, at the genome-wide significance threshold that was applied to region-wise adjusted p-values (1.0e-11). These sets of genes show significant overlaps: 141 genes were associated to Braak score in both of the first two cohorts, and 27 of these genes were also associated with change of ventricle volume in the third cohort. Regarding cognitive performances, the identified epistasis network comprised 735 protein-coding genes, linked through 567 interactions. Analysis of annotations assigned to protein-coding genes in these sets showed significant enrichment in genes expressed in brain and involved in neuronal differentiation, neuron adhesion, axon guidance, pre and post-synaptic structures, glutamate signalling, and regulation of synaptic transmission.

These epistasis networks provide a new layer of functional disease-relevant relationships, which may effectively guide prioritizing further in-vitro interventions. For each phenotype and cohort, the graph of epistatic relations between pairs of genomic regions (both genes and intergenic regions) was provided to QURETEC for integration in the *AgedBrainSYSBIO* database, where these results are available, at https://shiny.cs.ut.ee/AgedBrain/?project=agedbrain.

WP3 - Manipulating pathways using human iPSC-derived neurons, mouse and drosophila models

Derivation of reporter lines and patient-specific iPSCs to enable further differentiation of human neuron subtypes (UDUS)

Derivation of reporter lines and patient-specific iPSCs was performed with a special emphasis on analysis of CR1 and TREM2 mutations found in patients by VIB group. Eight EBV-transformed patient lymphoblast cell lines from partner VIB were selected for iPSC derivation and full characterization. Global transcriptome analysis of the functional interneural network derived from Control, CR1- and TREM2- patient iPSC lines identified genes expressed exclusively in the AD neural network and not control healthy individuals. KEGG pathways analyses were performed in order to get further information on deregulations of glial partners. These results demonstrated the importance of non-neuronal cells in the pathophysiology of LOAD.

Identification and analysis of rare variants from LOAD GWAS patients (VIB)

VIB extended their investigation of the AD risk gene *ABCA7* (Cuyvers et al, Lancet Neurology 2015; Van den Bossche et al, Neurology 2016) to the European Early Onset Consortium cohort. Sequencing of the *ABCA7* CDS in 928 EOAD patients and 980 control individuals revealed 17 different PTC mutations (six frameshift indels, six nonsense mutations, and five PTC-introducing splice site mutations), which were more frequent in EOAD patients (3.02%; n = 28) than controls (0.61%; n = 6) [p value = 0.0004, OR_{MH} = 5.01 (95% CI = 1.59–15.72)], confirmed an enrichment of premature termination codon (PTC) mutations in *ABCA7* in patients compared to control individuals. In line with observations in LOAD by VIB group, *ABCA7* PTC mutations in EOAD are also enriched among familial AD.

Manipulating neurons from drosophila models (IPL)

The work of IPL was based on the recent genome-wide association meta-analysis for Alzheimer's disease (AD) identified 19 risk loci (in addition to *APOE*) in which the functional genes are unknown. Using *Drosophila*, they

screened 296 constructs targeting orthologs of 54 candidate risk genes within these loci for their ability to modify Tau neurotoxicity by quantifying the size of >6000 eyes. Besides *Drosophila Amph* (ortholog of *BIN1*), which they previously implicated in Tau pathology, they identified *p130CAS* (*CASS4*), *Eph* (*EPHA1*), *Fak* (*PTK2B*) and *Rab3-GEF* (*MADD*) as Tau toxicity modulators. Of these, the focal adhesion kinase *Fak* behaved as a strong Tau toxicity suppressor in both the eye and an independent focal adhesion-related wing blister assay. Accordingly, the human Tau and PTK2B proteins biochemically interacted *in vitro* and PTK2B co-localized with hyperphosphorylated and oligomeric Tau in progressive pathological stages in the brains of AD patients and transgenic Tau mice.

Although the exact mechanism remains uncertain, it is interesting to note that three of our positive hits *Fak/PTK2B*, *p130CAS/CASS4* and *Eph/EPHA1* have previously been implicated in the focal adhesion pathway. These data indicate that *PTK2B* acts as an early marker and *in vivo* modulator of Tau toxicity.

Altogether, these data complement <u>human</u> protein-protein interaction network analysis based on Yeast two-hybrid approaches and subneuronal localization (at dendritic spines) in <u>mammalian neurons</u> done by Hybrigenics and INSERM groups, respectively.

Manipulating neurons from BAC transgenic mouse (CERBM-GIE, INSERM, GENEBRIDGES)

The first objective was to generate a novel humanized mouse model of the ApoE/Tomm40 locus. The targeting vector for APOE3 has to include 33.8 kb of human sequence that is flanked by two selection marker cassettes on each side. Overall the targeting vector raises a size of about 50kb in total. For ApoE4, we are waiting for chimera scoring. For ApoE2, chimeras were crossed and we are waiting for germ line transmission.

The second objective was to generate two new humanized models of APP. To mimic the human situation in mouse, a first model (T5463) was generated to humanize the Ab sequence. In a second model (T5462) the A673T variant wasintegrated in App in complement to the G676R, F681Y and R684H mutations (humanization of the Aβ sequence). Jonsson et al. found a coding mutation (A673T) in the APP gene that protects against Alzheimer's disease and cognitive decline in the elderly. This substitution is adjacent to the aspartyl protease β-site in APP, and results in an approximately 40% reduction of the formation of amyloidogenic peptides in vitro (Jonsson et al. A mutation in APP protects against Alzheimer's disease and age-related cognitive decline. Nature. 2012 Aug 2; 488 (7409):96-9). Following Jonsson et al. 2012 results, this variant should protect against Alzheimer's disease and age-related cognitive decline. The models will allow challenging this hypothesis and understanding the mechanism in vivo.

A novel readout based on fluorescent nanoparticles to detect changes in intra-neuronal transport has been generated. This approach was able to detect changes in a model of abnormal transport based on subtle overexpression of a kinase regulating attachment of MAPs to tubulin (Mark1).

This nanoparticle-based methodology proves sufficiently sensitive to detect these subtle changes, paving the way for developments in translational nanomedicine. The advantages of these strategies are that they can be applied to

   (i)   brain slices of transgenic mouse models
   (ii)   human neuronal cultures from iPSCs of LOAD patients

Fluorescent nanodiamond tracking reveals intraneuronal transport abnormalities induced by brain disease-related genetic risk factors.

WP4 - Proof of concept of drug discovery for systems medicine

WP4 had the following objectives: to raise intrabodies to target Tau and protein-protein interactions relevant to LOAD; to deliver and test these intrabodies in cellular systems and deliver most promising intrabodies in brain of TAUopathy or beta-amyloid models to evaluate pathology and behavioural amelioration.

The aim of this study was to develop highly selective and potent inhibitors against two selected targets: TAU/MAPT and FYN kinase. To achieve this goal, we took advantage of the Nanobody technology. Nanobodies (Nbs) or VHHs are small antigen-binding fragments derived from camelid heavy-chain-only antibodies that are devoid of light chains. They are superior to conventional antibodies in terms of stability, solubility, and

immunogenicity and much smaller than conventional antibodies (12–15 kDa *versus* 150–160 kDa) and can penetrate small clefts and cavities (see Schoonaert et al. Identification and characterization of Nanobodies targeting the EphA4 receptor. J Biol Chem. 2017 Jul 7;292(27):11452-11465. for a similar approach against EPHA4). Hybrigenics, ReMynd, NeuroService and INSERM partners were involved in this proof of concept program. Hybrigenics successfully generated sets of intrabodies against either TAU/MAPT or FYN proteins, as druggable candidatures. Generation of a minibody able to be secreted was also generated.

Important results have been generated *in vivo* for selected intrabodies and for a minibody against TAU. Partners agreed to patent this approach for further studies.

1.4. Potential impact and the main dissemination activities and exploitation of results

The AgedBrainSYSBIO program has been releasing novel datasets that will be useful to the whole European scientific community (https://shiny.cs.ut.ee/AgedBrain/?project=agedbrain)
Novel protein-protein interactions have been characterized both in fly and in human, based on Yeast two-hybrids (Y 2-H). Novel libraries for Y-2H are generated using subregions of human brain (entorhinal cortex, hippocampus), in order to characterize novel protein-protein interactions in these subregions. A set of 20 novel genetically-engineered mouse transgenic lines have been released either for characterization of brain functions or for modeling precise pathways involved in normal or pathological ageing. Furthermore, humanized models have been generated for APOE4 versus APOE3 risk locus and for BIN1 risk factor identified by LOAD-GWAS. These models are expected to be unique tools for further research in the field of synaptic function and of ageing. A comprehensive review of the status of formal mathematical modeling of neurodegenerative disease supports the identification of knowledge gaps and guides future formal model development.

The project will contribute directly to biogerontology by translating the gained knowledge to humans. As the project is dedicated to translational research: (i) from basic research to R&D in druggable targets involved in AD and (ii) from preclinical science to clinics, clinicians involved in daily care of ageing patients are part of this program. This interaction is expected to translate quickly druggable targets from bench side to patient's bed.

AgedBrainSYSBIO will contribute to improving the lives of older people. Alzheimer's disease is part of complex diseases, like hypertension or diabetes. From this complexity, it is unlikely that any one intervention will be found to delay, prevent, or cure it. So far, current approaches in treatment focus on interventions related to prevention and symptomatic treatment in order to maintain mental function, managing behavioral symptoms, and reducing or delaying the symptoms of the disease. *AgedBrainSYSBIO* has dedicated a work package (WP4) to Proof of Concept of Drug Discovery for Systems Medicine. This WP is led by the SME reMYND and includes two other SMEs, HYBRIGENICS and NeuroService. By combining small molecules and intrabody strategies, the Consortium expects to advance in the development of therapeutic strategies for the most common aged brain disease, LOAD. We are starting strategies using intrabodies against TAU and FYN using stereotaxic injections in diverse Alzheimer's Disease mouse models, including models generated by the *AgedBrainSYSBIO* consortium. Interestingly, two recent studies published in 2015 demonstrated that antibodies against cis P-tau and inhibitors of FYN impacts pathophysiological steps of Alzheimer in mouse models (Kondo et al., Nature 2015; Kaufman et al., Ann Neurol. 2015).

AgedBrainSYSBIO sustained the SME efforts towards research and innovation. Five European SMEs were highly implicated in the research and innovation activities of *AgedBrainSYSBIO*, three of them lead *AgedBrainSYSBIO* work packages (WP1, WP2 & WP4). The project will increase the portfolio of these five well-established SMEs with a direct impact on economic development and employment in Europe.

HYBRIGENICS - *AgedBrainSYSBIO* network sustained development of Hybrigenics Services activity over 3 dimensions: (1) Hybrigenics core production activity, (2) technological development around Proximity Ligation

Assays, library construction and VHH selection and (3) New and impressive POC around VHH. Subsequent direct business opportunities are under exploration.

QURETEC - *AgedBrainSYSBIO* has helped Quretec to technologically improve in two highly relevant bioinformatics fields, namely in omics integration and aging-related research. Thanks to the excellent research partners in *AgedBrainSYSBIO* project Quretec has obtained novel expertise in analysing and combining multitude of omics datasets. The company has now firsthand experience in working with e.g. brain data, aging phenotypes and epistasis data. This expertise can be potentially exploited by extending the knowhow and services in the company portfolio. With data integration being one of the burning challenges in current biomedical field, Quretec might gain new business opportunities thanks to the documented experience and expertise of successfully executed data integration in the field of neurodegenerative aging (publication submitted).

GeneBridges - Animal models, e.g. transgenic mice, in which the endogenous gene is replaced by a human disease-associated gene variant, are essential tools for biomedical research. Genomic engineering techniques based on homologous recombination allow the generation of such "humanised" mice but despite their widespread use, these techniques are still time consuming and costly because of technically difficulties. With our consortium partner ICS we were able to develop four humanized mouse models in total (one humanized ApoE3 mouse model, one putative functional SNP variant for ApoE4 and two putative functional SNP-variants for ApoE2), for studying the role of human ApoE polymorphism neurodegenerative disorders.

reMYND - The *AgedBrainSYSBIO* project led to the identification of DNA-based vectors for the delivery of novel Tau-directed minibodies with the potential to treat tauopathies including AD. This finding opens up the possibility (together with the other partners) to further develop these minibodies as novel neuroprotective therapeutics. If successful it may represent a significant commercial value for the parties involved. Moreover, the *AgedBrainSYSBIO* project led to the validation of a transgenic TAU animal model of AD for the testing of new therapeutic agents aimed to target neurotoxic extracellular TAU. The animal model is currently offered by reMYND to third parties, typically large pharma and biotech companies, as a fee-for-service to assess efficacy of novel products which now can also include products which capitalize the concept of targeting extracellular Tau.

NEUROSERVICE - Thanks to the AgedBrainSYSBIO project, NEUROSERVICE has developed 3 new assays based on the Multi-Electrode Array technique. This technique is mainly dedicated to the investigation and recording of neuronal networks and synaptic pathways. Up to now, NEUROSERVICE had developed classical Long-Term Potentiation (LTP) protocols at CA3-CA1 synapses and at medial entorhinal cortex synapse projecting onto the Dentate-Gyrus granular cells. Two new Long-Term Potentiation protocols have been developed in the hippocampus: (i) in the Dentate Gyrus, at synapses coming from the lateral entorhinal cortex and (ii) in the CA1 region, at synapses from the temporo-Amonic pathway. In addition, another Long-Term Potentiation protocol has been developed in temporal cortex slices. This is the first LTP protocol established by NEUROSERVICE in cortex slices. Since these synapses may present different pharmacological sensitivities/modulations, they will certainly be of interest for some NEUROSERVICEZ customers that would be interested in validating some targets and/or new mechanisms of action.

1.5 Partners involved and coordinator's contact details

Participant number	Participant organisation legal name	PI	0rganisation short name	Country
1 (CO)	Institut National de la santé et de la recherche clinique	Michel Simonneau	Inserm	France
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4	Centre Européen de Recherche en Biologie et Médecine	Yann Herault	CERBM-GIE	France
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7	Hybrigenics SA	Jean-Christophe Rain	Hybrigenics	France
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1.6 Project logo and public website

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