Journal of Alzheimer's Disease 47 (2015) 857–868
DOI 10.3233/JAD-150281
IOS Press

Hypothesis

A Human-Based Integrated Framework for Alzheimer's Disease Research

Francesca Pistollato*, Sarah E. Cavanaugh and P. Charukeshi Chandrasekera
Physicians Committee for Responsible Medicine, Washington, DC, USA

Accepted 21 May 2015

Abstract. Animal models of Alzheimer's disease (AD) have been extensively utilized for decades in an effort to elucidate the pathophysiological mechanisms of this disease and to test novel therapeutic approaches. However, research success has not effectively translated into therapeutic success for human patients. This translational failure is partially due to the overuse of animal models that cannot accurately recapitulate human AD etiopathogenesis or drug responses and the inadequate use of human-relevant research methods. Here, we propose how to mitigate this translational barrier by employing human-based methods to elucidate disease processes occurring at multiple levels of complexity, accounting for gene and protein expression and the impact of disease at the cellular, tissue/organ, individual, and population levels. In particular, novel human-based cellular and computational models, together with epidemiological and clinical studies, represent the ideal tools to facilitate human-relevant data acquisition, in the effort to better elucidate AD pathogenesis in a human-based setting and design more effective treatments and preventive strategies. Our analysis indicates that a paradigm shift toward human-based, rather than animal-based research is required in the face of the ever-increasing prevalence of AD in the 21st century.

Keywords: Alzheimer's disease, animal models, biomarkers, computational methods, human-based methods, risk factors, stem cells, translational gap

INTRODUCTION

Alzheimer's disease (AD) represents the most common cause of dementia, accounting for 50–75% of all dementia cases [1]. This is a devastating disease affecting every aspect of life, as AD patients progress from very mild to severe cognitive impairment, losing their memory, their relationships with their families, and their ability to perform daily functions, such as talking, eating, and walking. The number of people living with AD in the United States alone is expected to increase from 5.2 million in 2013 to 13.8 million in 2050 [2], with an estimated cost of care expected to reach more than $1 trillion per year [1, 3]. It is clear that effective preventive and therapeutic strategies are urgently needed. In this regard, the AD research community has been very active with extensive research efforts designed to elucidate disease mechanisms and to develop novel therapeutics. However, these efforts have not successfully translated into effective drugs for AD patients. To date, only three drugs have been approved for AD (donepezil, galantamine, and rivastigmine) and they can only treat the symptoms of AD. It is well known that they are effective in only a small subset of patients, and only for a limited period of time [4]. Since 1998, 101 potential AD drugs have advanced to human trials but failed [5], and with recent costly failures such as Dimebon [6], drug companies are downgrading their efforts to develop novel pharmacotherapeutics for AD [7]. Understanding and resolving the reasons for these translational failures in AD is therefore imperative. Additionally, several lines of evidence indicate that AD

*Correspondence to: Francesca Pistollato, Physicians Committee for Responsible Medicine, 5100 Wisconsin Avenue NW, Suite 400, Washington, DC 20016, USA. Tel.: +1 202 527 7381; Fax: +1 202 527 7481; E-mail: fpistollato@pcrm.org.

ISSN 1387-2877/15/$35.00

should be reinterpreted as part of a constellation of diseases, in particular, as a complex systemic/metabolic dysfunction, given the significant correlations found between AD and the metabolic syndrome [8], as well as hypometabolism, oxidative stress, and glucose-fatty acid cycle modifications [9]. Consequently, current and past research failures might also be attributed to the fact that traditional research efforts and strategies have not been taking this complexity into account. Indeed, over the last decade, AD molecular mechanisms and drug efficacies have been studied extensively in animal models—primarily in murine models, but also in nonhuman primates, rats, dogs, and other species—all in an effort to assess in animals the contributions of specific genes and proteins to the onset of AD, commonly without accounting for the multifactorial nature of the disease. These animal models consistently fail to accurately recapitulate human AD causes, complex molecular and cellular dynamics, clinical manifestations, and drug responses. This is primarily due to anatomical, biochemical, and physiological as well as genetic and epigenetic interspecies differences between animals and humans [10]. In this review, we propose a novel human-based integrated framework, accounting for multiple levels of biological complexity, which can be used to improve characterization of human AD and develop effective treatments and preventive strategies for human patients.

AD AND INADEQUACY OF TRADITIONAL AD MODELS

The two types of AD are familial AD (∼5% of all AD cases), often occurring before age 60, and late-onset AD (95% of all AD cases), occurring in people over the age of 65. Familial AD is generally characterized by the presence of mutations found in genes encoding the γ-secretase complex components, such as presenilin-1 and −2, or in the amyloid-β (A4) protein precursor (AβPP) [11]. Duplication of the AβPP gene has also been linked to autosomal dominant familial AD [12]. Late-onset AD is generally defined as 'sporadic', given the lack of specific genetic factors directly associated with the disease. Genome-wide association studies (GWAS) have shown that AD is also associated with variations of several gene loci, such as the apolipoprotein E ε4 allele (APOE ε4) [13–17]. Accumulation of amyloid-β (Aβ) plaques and neurofibrillary tangles, composed of accumulated microtubule-associated total, and phosphorylated tau (t-tau and p-tau) protein, are the two physiological hallmarks of human AD, though it is unclear what role, if any, they play in the disease [18] (Supplementary Table 1).

Over the last decade, AD research has focused on the development of animal models to mimic at least some features of human AD described above. However, while certain murine models have been able to exhibit some of the genetic traits found in early-onset familial AD [19–21], their suitability to study late-onset AD is questionable [22], as confirmed by the dramatically high number of failed clinical trials [10, 23]. Without an understanding of the genetics of AD as it occurs in humans, the genetic contribution to disease pathogenesis is virtually impossible to model in mice [24]. In addition to the use of animals, overly simplistic cellular models of AD, often utilizing cancer cell lines or nonhuman cells cultured under nonhomeostatic and nonphysiologic *in vitro* culture conditions [25] have been commonly applied, further hampering research and drug discovery in AD [10]. Altogether, this highlights the need to rethink current research strategies and improve both sensitivity and specificity of research methods that will mitigate this wide translational gap.

RETHINKING AD RESEARCH FOCUSING ON HUMAN-RELEVANT DATA ACQUISITION

Human-based cellular and tissue models, combined with epidemiological data and high-throughput readouts, can serve as the basis for a paradigm shift in AD research, facilitating human-relevant data acquisition. These tools and novel assays will allow researchers to better elucidate AD pathogenesis in a human-based setting and to design more effective treatments and preventive strategies. To integrate the vast amount of data deriving from comprehensive 'omics' studies, systems biology methods are of fundamental importance. This approach will allow researchers to describe molecular mechanisms underlying AD pathogenesis. In particular, several environmental and lifestyle risk factors, such as specific food nutrients and environmental toxicants, are known to play a pivotal role in the onset of pathologic changes underlying AD, which often appear many years before the symptomatic stages [26]. By using human-based models and novel computational methods, it is now possible to unravel the epigenetic and molecular mechanisms underlying AD pathogenesis (Fig. 1, Table 1).

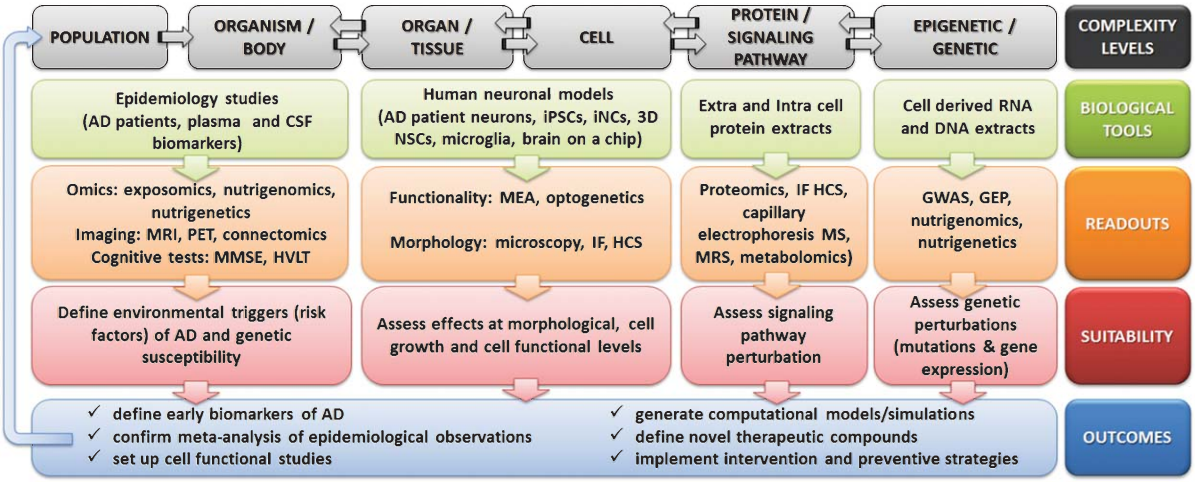

Fig. 1. Overview of the novel available tools and readouts applicable to design human-oriented AD research, accounting for multiple levels of complexity. CSF, cerebrospinal fluid; MRI, magnetic resonance imaging; PET, positron emission tomography; MMSE, Mini-Mental State Examination; HVLT, Hopkins verbal learning test; iPSCs, induced pluripotent stem cells; iNCs, induced neuronal cells; NSCs, neural stem cells; MEA, microelectrode array; IF HCS, immunofluorescence-high content screening; MS, mass spectrometry; MRS, magnetic resonance spectroscopy; GWAS, genome-wide association studies; GEP, gene expression profiling.

The population and individual levels: Epidemiological studies and novel 'omics' readouts

Epidemiological studies have been important in identifying AD-related risk factors. The primary risk factors for AD include advancing age [27–29], nutritional patterns characterized by low intake of plant-derived foods [30], together with metabolic syndrome-related dysfunctions (e.g., cardiovascular disease and diabetes) [8, 31], low socioeconomic status and a low level of educational attainment [32–34], low level of daily physical activity [35, 36], and low cognitive training [37]. Additionally, sleep disorders [38–40], known to positively correlate to early Aβ deposition [41, 42], exposure to air pollution [43], smoking [44], and the intake of metals (e.g. aluminum [45], dietary copper [46], and manganese [47]) have been described as possible risk factors. Knowledge of these risk factors may enable the discovery of early biomarkers of AD and the development of intervention strategies to ameliorate or prevent early symptoms of AD.

In particular, analysis of both AD patient-derived plasma and cerebrospinal fluid (CSF) has been proven essential to identify possible early biomarkers of AD (Table 2). The presence of chronic neuroinflammation, caused by the progressive senescence of the immune system and by a sustained secretion of adipose tissue cytokines, seems to play a major role in cardiovascular disease and neurodegeneration [48], possibly contributing to AD [49, 50]. The presence of high levels of both plasma and brain homocysteine (HCys) is correlated to AD and neurodegenerative disorders. Elevated HCys in brain microvessels may be implicated in the disruption of the blood-brain barrier [51] and induction of excitotoxicity in cells expressing glutamate receptors of the N-methyl-D-aspartate class [52]. Other biomarkers considered relevant to AD are: low levels of plasma uric acid [53, 54], high levels of serum 3,4-dihydroxybutanoic acid ($C_4H_8O_4$), docosapentaenoic acid (C22:5) [55], and hexacosanoic acid (C26:0) [56], the presence of insulin resistance and high insulin-like growth factor expression [57–59], impaired glycemic levels [60], mitochondrial damage and increased mitochondrial O-linked N-acetylglucosamine transferase activity [61], the presence of a dyslipidemic profile [31, 62–66], deregulation of plasma orexin [67–70], and high levels of the secreted heparin-binding glycoprotein YKL-40 [71, 72] (Table 2). Additionally, the CSF milieu is altered in subjects presenting AD. Analysis of the CSF revealed the deregulation of several biomarkers currently hypothesized to play a key role for AD early diagnosis, such as the $A\beta_{1\text{-}42}$, t-tau, and p-tau_{181} [73], and in particular the levels of p-tau_{181}/t-tau and p-tau_{181}/$A\beta_{1\text{-}42}$ ratios, considered good indicators of dementia severity and contributing to discrimination between mild or moderate-to-severe AD cases [74] (Table 2).

The novel concept of the 'exposome', accounting for the totality of environmental exposures from gestation

Table 1
Human-based models and methods for AD research

Complexity levels	Models/Readouts	Characteristics/Possible applications	References
Population/ individual level: data from epidemiological, clinical studies; tests on AD patients; test on AD patient samples (e.g., CSF, serum)	Ultra-high-field MRI	To assess cortical plaque disposition and early hippocampal tissue loss	[86]
	PET	To monitor Aβ plaque distribution in patients	[87]
	Connectomics (e.g., diffusion magnetic resonance tractography)	3D reconstruction of neuronal network within the human brain; allows registering variations of brain cortical surfaces in AD patients	[91–93]
	Electron microscopy	Electron microscopy images suitable to generate 3D neuron reconstruction	[94]
	Exposomics	To assess environmental exposure effects on gene expression	[75]
	Nutrigenomics	To assess nutrient effects on gene expression	[77, 78]
	Nutrigenetics	To assess genetic variants' susceptibility to specific nutrients	[85]
	Neuropsychological/cognitive tests (e.g., MMSE, HVLT)	To assess cognitive level	[95]
Tissue/cell levels: AD patient-derived tissues and cells	AD patient-derived primary cells (e.g., neurons)	Accounting for patient heterogeneity, available only postmortem, short periods of time in culture	[96]
	AD patient-derived hiPSCs and further differentiation into neurons	Accounting for patient heterogeneity, recapitulating common features of AD, applicable for drug evaluations, suitable for high-throughput assays	[97–102]
	AD patient-derived iNCs	To study late-onset AD	[98, 103]
	AD patient-derived olfactory mucosa	Easily biopsied or collected postmortem	[96]
	3D human NSCs expressing AD-related mutations	Recapitulate extracellular Aβ deposition, plaque formation, high levels of p-tau and filamentous tau in both neuronal soma and neurites. Suitable to study familial AD	[19]
	Human microglial cells	Derived from elderly patients; useful to define the role of active microglia in the inflammatory process at the onset of AD	[106]
	3D *in vitro* human tissue models, with microfluidics	To better mimic *in vivo* physiological conditions	[104]
	Brain-on-a-chip	To test drug efficacy and toxicity	[107, 108]
	Readout: MEA	To directly record neuronal culture activity on chip, for drug testing	[112]
	Readout: Optogenetics	To monitor the activities of individual neurons in living tissue	[113]
	Readout: IF-HCS	High-throughput, to assess the effects of compounds at cellular level	[96]
Signaling pathway/protein level: human AD cell-derived protein samples	Proteomics and phosphoproteomics	High-throughput, to define molecules and signaling pathways involved in APOE ε4-related late-onset AD	[114]
	Capillary electrophoresis-mass spectrometry	To assess phosphorylation of metabolic proteins, signal transduction, cytoskeleton integration and synaptic functions	[115]
	Proton MRS and metabolomics	To analyze *in vivo* metabolites of neuronal and glial cells representative of energy metabolism, level of inflammation and neurotransmitter release	[116]
	Further functional studies: reporter human AD cell cultures of identified perturbed signaling pathways	To assess perturbation of identified pathways upon exposure to compounds	[126, 127]

(*Continued*)

Table 1
(*Continued*)

Complexity levels	Models/Readouts	Characteristics/Possible applications	References
Epigenetic/genetic level: human AD cell-derived RNA and DNA samples	Laser-capture microdissection and GEP	High-throughput, to analyze subtypes of cortical neurons obtained from postmortem brain	[117, 118]
	GWAS	To identify associations between specific gene loci and AD	[13–17]
	Further functional studies: gain- or loss-of-function, RNA interference	To define the role of specific genes onset of AD	[128, 129]

MRI, magnetic resonance imaging; PET, positron emission tomography; MMSE, mini-mental state examination; HVLT, Hopkins verbal learning test; hiPSCs, human induced pluripotent stem cells; iNCs, induced neuronal cells; NSCs, neural stem cells; MEA, microelectrode array; IF-HCS, immunofluorescence-high content screening; MRS, magnetic resonance spectroscopy; GEP, gene expression profiling; GWAS, genome-wide association studies.

Table 2
Summary of biomarkers possibly useful for AD detection

Biomarkers	Localization	References
IL-6, IL-1, TNF-α, CRP	Plasma	[48]
High HCys	Plasma, brain	[51, 52]
Low uric acid	Plasma	[53, 54]
High levels of: 3,4-dihydroxybutanoic acid ($C_4H_8O_4$), docosapentaenoic acid (C22:5), hexacosanoic acid (C26:0)	Serum, plasma	[55, 56]
Insulin resistance	Plasma, brain	[57, 58]
IGF resistance	Plasma, brain	[59]
Impaired glycemia	Plasma, brain	[60]
Mitochondrial damage and high mOGT activity	Plasma, brain	[61]
Low HDL-cholesterol	Plasma, brain	[62]
High LDL-cholesterol, total cholesterol and triglycerides	Plasma, brain	[31, 63–66]
Low orexin level	Plasma, CSF, hypothalamus	[67–70]
High YKL-40 (a secreted heparin-binding glycoprotein)	Plasma, brain	[71, 72]
$A\beta_{1\text{-}42}$, t-tau, and p-tau_{181}, p-tau_{181}/t-tau and p-tau_{181}/$A\beta_{1\text{-}42}$ ratios	CSF	[73, 74]

IL-6, interleukin-6; TNF-α, tumor necrosis factor α; CRP, C-reactive protein; HCys, homocysteine; IGF, insulin growth factor; mOGT, high mitochondrial O-linked N-acetylglucosamine transferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; YKL-40, Chitinase-3-like protein 1 (or CHI3L1).

onward, is currently considered complementary to the genome in the study of disease etiology [75, 76]. In particular, among the possible triggers of the neurodegenerative process and in particular of AD, nutrients have the potential to be either prevention- or risk-related factors. The study of their effects at the gene expression level, by means of nutrigenomic analyses, is a field of broad scientific interest [77, 78]. Despite some conflicting evidences, plant-based, plant-rich diets and plant-derived bioactive nutrients appear to inhibit neuroinflammation and neurodegeneration molecular and cellular processes [79] and seem to be particularly beneficial in the management of early stages of cognitive impairment [80–84]. Some nutrigenetic studies have highlighted correlations between specific nutrient intakes and single-nucleotide polymorphisms. In this regard, it has been reported that subjects presenting the TT homozygous methylene tetrahydrofolate reductase allelic variant showed the lowest serum folate level, the highest serum HCys level and the lowest Mini-Mental State Examination (MMSE) score, as compared to all other genotypes [85]. Further studies aiming to identify the genetic susceptibility to AD and the interaction between specific genetic variations and nutrient intake might be invaluable in designing patient-tailored therapies and preventive approaches based on patient genetic makeups.

Novel and more powerful *in vivo* imaging readouts, such as ultra-high-field magnetic resonance imaging (MRI) and positron emission tomography, are currently available to diagnose AD [86, 87] and assess the effects of specific nutritional interventions [88–90]. Additionally, novel human connectomics (the production and study of connectomes) provides a three-dimensional reconstruction of neuronal networks within the human brain. In particular, diffusion magnetic resonance tractography, by combining MRI and computer-based imaging analysis, allows studying human brain anatomy through 2D and 3D images [91], and registering variations of brain cortical surfaces in AD patients [92, 93]. Additionally, electron microscopy images have been suitable

to generate 3D neuron reconstruction in publicly available electron microscopy datasets [94]. Neuropsychological/cognitive tests (e.g., MMSE and the Hopkins Verbal Learning Test [95]) can be also used to assess multiple correlations among Aβ plaque distribution, specific AD-related biomarkers in CSF and plasma, and cognitive performance (Fig. 1, Table 1).

The organ/tissue and cellular levels: Novel human stem cell models and cell function readouts

In recent years, novel cellular models cultured under conditions more closely reflecting the human physiological cellular microenvironment and accounting for patient heterogeneity have been developed. AD patient-derived primary cells, such as neurons, might represent an ideal cellular source to study AD pathogenic features. However, these cells are generally available only postmortem and are difficult to keep in culture for extended periods of time [96]. To overcome these limitations, human induced pluripotent stem cells (hiPSCs) have been recently applied [97, 98]. hiPSCs can be generated by reprogramming adult somatic cells (e.g., skin fibroblasts) into pluripotent stem cells and further differentiating them *in vitro* to obtain AD-relevant tissues [10]. hiPSCs have been generated from patients affected with either familial or late-onset AD and have been differentiated into electrophysiologically active neurons, recapitulating common features of AD, such as high level of $A\beta_{1-40}$, active glycogen synthase kinase 3β, and p-tau [99], Aβ accumulation in neuronal cells and astrocytes, and endoplasmic reticulum oxidative stress [100]. Additionally, these differentiated neurons might be useful to identify the specific roles of cellular subtypes in the pathogenesis of AD, to obtain insights into patient-specific drug responses, for prospective diagnostics [101], and to assess the susceptibility of specific AD-related genes [102]. More recently, reprogramming of AD patient-derived non-neuronal somatic cells into neuronal mature cells (iNCs) [98, 103], AD patient-derived olfactory mucosa stem cells [96], genetically modified human neural stem cells (NSCs) [19], and three-dimensional culture systems [104] have been applied. Additionally, microglial cells, known to get activated in AD [105], have been derived from elderly patients to define the role of active microglia in the inflammatory process characterizing AD [106], and can be applied in co-culture systems to complement hiPSC, iNC, and NSC cultures. Moreover, novel brain-on-a-chip systems are currently under development, ideally leading to the design of novel drugs in human-based systems [107, 108]. Despite their current limitations, such as the lack of standardized criteria to build such systems [109] and their poor suitability for long term treatments [110], in general, these models might better represent the heterogeneity of the patient population, might reduce experimental timeframes and costs, might more accurately identify human AD characteristics, might be better tools for drug development, and might be applicable for use on high-throughput platforms [111] (Fig. 1, Table 1).

A wide range of high-throughput readouts are currently available to assess etiopathological aspects of AD at tissue and cellular levels. Microelectrode array (MEA) devices have been developed in recent years, as substitutes of the more challenging and time-consuming patch clamp analyses [112]. Optogenetic techniques, used to monitor the activities of individual neurons in living tissue, together with MEA assays, antibody immunofluorescence (IF) and high content screening (HCS), could enable rapid and reproducible screening of drugs *in vitro* [96, 113] (Fig. 1, Table 1).

The signaling pathway/protein and epigenetic/genetic levels: High-throughput readouts

Several high-throughput technologies are currently available to unravel the molecular mechanisms underlying AD pathogenesis. Protein-related readouts (e.g., proteomics, antibody IF-HCS, metabolomics) will provide knowledge on the signaling pathways and protein interactions that are perturbed in AD at the translational and/or post-translational level (Fig. 1, Table 1). In particular, proteomics and phosphoproteomics have been useful to uncover signaling pathways involved in APOE ε4-related late-onset AD [114]. The use of capillary electrophoresis–mass spectrometry can help identify abnormal phosphorylation patterns of proteins implicated in cell metabolism, signal transduction, cytoskeleton integration, and synaptic function [115]. Also, analysis of *in vivo* metabolites of neuronal and glial cells, representative of energy metabolism, level of inflammation and neurotransmitter release, can be performed by using proton magnetic resonance spectroscopy [116]. Finally, genomics, epigenetics and gene expression analyses (e.g., GWAS, gene expression profiling, nutrigenomics, and nutrigenetics), will serve to identify perturbed gene expression particularly in late-onset AD [117, 118]. Late-onset AD patients often present abnormal patterns of histone acetylation and

methylation and deregulated noncoding microRNA, which may play a role in AD pathophysiology [118].

Computational models, data extrapolation, and functional studies to validate epidemiological observations

Novel computational models that are currently applied in toxicology, such as *in vitro-in vivo* extrapolation, physiologically based pharmacokinetic and pharmacodynamics modeling, suitable to define kinetics and dynamics of compound exposure and to predict chemical long term effects [119], might be applied to predict the absorption, distribution, metabolism, and excretion of chemicals, such as chlorpyrifos [120] and manganese [121], both implicated in Aβ deposition [47, 122], and also to assess the efficacy of compounds for AD treatment, such as β-secretase inhibitors [123], the bioactive phytocompound curcumin [124], and APOE ε4 inhibitors [125].

Extrapolation of data obtained from the above described high-throughput readouts and computational simulations might be useful to define early biomarkers of AD, confirm meta-analysis of epidemiological observations, and predict therapeutic efficacy (Fig. 1). In particular, functional studies might be useful to confirm specific roles of identified perturbed pathways/genes at the onset of AD [126, 127]. In this regard, reporter human cell cultures of an identified perturbed signaling pathway might be generated, in an effort to investigate at high-throughput scale possible perturbation of the identified pathway when exposed to known and unknown compounds, in relation to AD pathogenesis. The ultimate goals would be the definition of novel therapeutic compounds and the implementation of intervention strategies aimed at preventing and/or reducing the early symptoms of AD. Additional *in vitro* functional studies aimed at defining the role of specific genes in the onset of AD would include gain- or loss-of-function approaches and also RNA interference-induced gene silencing using novel human cellular models of AD [128, 129].

DISCUSSION

Following decades of extensive research, it is now clear that traditional animal and cell culture models of AD are not reliable for studying complex pathophysiological aspects of the disease or for designing effective drugs. This deep gap in translational research highlights the need for a paradigm shift in AD research, from animal *in vivo* models and suboptimal *in vitro* cell lines, toward a more reliable and reproducible human conceptual framework. Here we described an integrated human-based framework suitable for investigating cellular and molecular mechanisms underlying AD pathology, pharmacotherapeutics, and preventive strategies for human patients. In recent years, the shift toward a new human-based paradigm has been advocated extensively in toxicology and regulatory testing [130], but also in other research fields, including AD [10, 131–134]. The envisioned human-based framework will not only increase human relevance and translatability, but also contribute to the reduction and/or replacement of animals traditionally used in AD research. In this day and age where there is growing concern for the ethical justification of the use of animals in research [135], it is important to consider not only the scientific dimensions, but also the ethical cost of the use of sentient beings in AD research.

Several human stem cell models of AD have been developed, spanning from patient-derived neurons and reprogrammed cells to three-dimensional genetically modified NSCs and the more complex brain-on-a-chip. Human stem cell-based tools and high-throughput readouts, supported by epidemiology studies, represent the basis of a paradigm shift in AD research that will increase knowledge of the molecular mechanisms that are perturbed at the onset of the disease, helping define novel biomarkers for early detection, and establishing preventive and treatment strategies.

It should be noted that in the envisioned strategic framework, the use of patient-derived cellular models such as iPSCs, and the application of omics readouts—while addressing human relevance—would still constitute the lower level/scale of 'wet lab' research. Therefore, large computational approaches together with large-scale epidemiological data sets represent the essential tools required to account for higher level/scale and to establish systemic correlations among signaling pathways, epigenomic and genomic perturbations, patients heterogeneity, and lifestyle components. In this regard, a map of signaling pathways and networks that are deregulated in AD is provided by 'AlzPathway', which may help in identifying candidate genes as predictors of AD risk, in combination with other 'omics' data [136].

Importantly, the implementation of epidemiological, prevention, and intervention clinical studies required to unravel the role of risk factors currently associated with AD, will require increasing the level of current research funding (currently only 7%–9% of the total $30 billion NIH discretionary budget [137]).

Redefining the strategy by which current and future NIH research budget is allocated is of particular relevance, especially considering the major role played by lifestyle factors, such as nutrition, physical activity, and level of cognitive training in the determination of AD risk [27–30, 35–37] as well as the proven efficacy of intervention strategies aimed at preventing dementia-related symptoms [88–90].

In conclusion, combining data derived from a wide range of studies, also accounting for neuropsychological/cognitivity tests, neuroimaging, the analysis of patient-derived CSF- and plasma-related biomarkers, together with computational models and high-throughput readouts applied to patient-derived cell-based models to assess signaling pathways, post-translational, translational, and transcriptional events, represent an invaluable and more reliable strategy to better understand AD pathology, predict long-term sequelae, and develop successful treatments [10, 138]. The envisioned framework will help redefine human AD pathology and etiology according to a more holistic perspective, taking into account the numerous human-related risk factors implicated in the onset and consolidation of AD [8, 9]. Modern research must take these multifactorial aspects into account. Indeed, current and past research failures may in fact be due to (i) a failure to recognize that AD lies on the spectrum of dementia and may be more accurately considered as part of a constellation of diseases, and/or (ii) more radically, AD may be inexorably linked to aging, making it a more intractable problem with more profound implications than generally acknowledged.

The feasibility of the envisioned human-based strategy necessarily requires the combined application of several methods and readouts and, consequentially, of multiple areas of expertise and laboratory facilities. The establishment of a collaborative scenario is clearly mandatory to determine what occurs throughout the course of AD.

CONCLUSION

Human stem-cell *in vitro* models, high-throughput ('omics') readouts, computational models, together with data obtained from meta-analysis of epidemiological and interventional studies, are among the ideal tools to elucidate etiopathological aspects of AD in a human-based setting and to predict environment-elicited biological perturbations occurring in AD, accounting for multiple levels of complexity, from population/individual level down to gene level.

ACKNOWLEDGMENTS

We thank Drs. John J. Pippin and Neal D. Barnard for their constructive comments on the manuscript.

Authors' disclosures available online (http://j-alz.com/manuscript-disclosures/15-0281r1).

SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: http://dx.doi.org/10.3233/JAD-150281.

REFERENCES

[1] Alzheimer's Association (2013) 2013 Alzheimer's Disease Facts and Figures, http://www.alz.org/downloads/facts_figures_2013.pdf, Accessed March 19, 2015.
[2] Hebert LE, Weuve J, Scherr PA, Evans DA (2013) Alzheimer disease in the United States (2010-2050) estimated using the 2010 census. *Neurology* **80**, 1778-1783.
[3] Alzheimer's Disease International (2009) World Alzheimer Report 2009 http://www.alz.co.uk/research/files/WorldAlzheimerReport.pdf, Accessed March 19, 2015.
[4] Mayo Clinic, Alzheimer's: Drugs help manage symptoms, http://www.mayoclinic.org/diseases-conditions/alzheimers-disease/in-depth/alzheimers/art-20048103, Accessed March 19, 2015.
[5] PhRMA (2012) Researching Alzheimer's Medicines: Setbacks and Stepping Stones, http://www.phrma.org/sites/default/files/pdf/alzheimersetbacksreportfinal912.pdf, Accessed March 19, 2015.
[6] Bezprozvanny I (2010) The rise and fall of Dimebon. *Drug News Perspect* **23**, 518-523.
[7] Laurance J (2012) Drug giants give up on Alzheimer's cure. The Independent, http://www.independent.co.uk/life-style/health-and-families/health-news/drug-giants-give-up-on-alzheimers-cure-8153606.html, Accessed March, 19 2015.
[8] Rios JA, Cisternas P, Arrese M, Barja S, Inestrosa NC (2014) Is Alzheimer's disease related to metabolic syndrome? A Wnt signaling conundrum. *Prog Neurobiol* **121**, 125-146.
[9] Heininger K (2000) A unifying hypothesis of Alzheimer's disease. IV. Causation and sequence of events. *Rev Neurosci* **11 Spec No**, 213-328.
[10] Langley GR (2014) Considering a new paradigm for Alzheimer's disease research. *Drug Discov Today* **19**, 1114-1124.
[11] Bertram L, Tanzi RE (2008) Thirty years of Alzheimer's disease genetics: The implications of systematic meta-analyses. *Nat Rev Neurosci* **9**, 768-778.
[12] Rovelet-Lecrux A, Hannequin D, Raux G, Le Meur N, Laquerriere A, Vital A, Dumanchin C, Feuillette S, Brice A, Vercelletto M, Dubas F, Frebourg T, Campion D (2006) APP locus duplication causes autosomal dominant early-onset Alzheimer disease with cerebral amyloid angiopathy. *Nat Genet* **38**, 24-26.
[13] Chouraki V, Seshadri S (2014) Genetics of Alzheimer's disease. *Adv Genet* **87**, 245-294.
[14] Hollingworth P, Harold D, Jones L, Owen MJ, Williams J (2011) Alzheimer's disease genetics: Current knowl-

edge and future challenges. *Int J Geriatr Psychiatry* **26**, 793-802.

[15] Shibata N, Nagata T, Tagai K, Shinagawa S, Ohnuma T, Kawai E, Kasanuki K, Shimazaki H, Toda A, Tagata Y, Nakada T, Nakayama K, Yamada H,Arai H (2014) Association between the catechol-O-methyltransferase polymorphism Val158Met and Alzheimer's disease in a Japanese population. *Int J Geriatr Psychiatry*. doi: 10.1002/gps.4237

[16] Peterson D, Munger C, Crowley J, Corcoran C, Cruchaga C, Goate AM, Norton MC, Green RC, Munger RG, Breitner JC, Welsh-Bohmer KA, Lyketsos C, Tschanz J, Kauwe JS, Alzheimer's Disease Neuroimaging I (2014) Variants in PPP3R1 and MAPT are associated with more rapid functional decline in Alzheimer's disease: The Cache County Dementia Progression Study. *Alzheimers Dement* **10**, 366-371.

[17] Barberger-Gateau P, Lambert JC, Feart C, Peres K, Ritchie K, Dartigues JF, Alperovitch A (2013) From genetics to dietetics: The contribution of epidemiology to understanding Alzheimer's disease. *J Alzheimers Dis* **33** (Suppl 1), S457-S463.

[18] Ballatore C, Lee VM, Trojanowski JQ (2007) Tau-mediated neurodegeneration in Alzheimer's disease and related disorders. *Nat Rev Neurosci* **8**, 663-672.

[19] Choi SH, Kim YH, Hebisch M, Sliwinski C, Lee S, D'Avanzo C, Chen H, Hooli B, Asselin C, Muffat J, Klee JB, Zhang C, Wainger BJ, Peitz M, Kovacs DM, Woolf CJ, Wagner SL, Tanzi RE,Kim DY (2014) A three-dimensional human neural cell culture model of Alzheimer's disease. *Nature* **515**, 274-278.

[20] Tanzi RE (2012) The genetics of Alzheimer disease. *Cold Spring Harb Perspect Med* **2**, pii: a006296.

[21] Oddo S, Caccamo A, Shepherd JD, Murphy MP, Golde TE, Kayed R, Metherate R, Mattson MP, Akbari Y, LaFerla FM (2003) Triple-transgenic model of Alzheimer's disease with plaques and tangles: Intracellular Abeta and synaptic dysfunction. *Neuron* **39**, 409-421.

[22] Duyckaerts C, Potier MC, Delatour B (2008) Alzheimer disease models and human neuropathology: Similarities and differences. *Acta Neuropathol* **115**, 5-38.

[23] Cavanaugh SE, Pippin JJ, Barnard ND (2014) Animal models of Alzheimer disease: Historical pitfalls and a path forward. *ALTEX* **31**, 279-302.

[24] Livesey FJ (2012) Stem cell models of Alzheimer's disease and related neurological disorders. *Alzheimers Res Ther* **4**, 44.

[25] Hartung T (2013) Look back in anger - what clinical studies tell us about preclinical work. *ALTEX* **30**, 275-291.

[26] Drzezga A (2009) Diagnosis of Alzheimer's disease with [18F]PET in mild and asymptomatic stages. *Behav Neurol* **21**, 101-115.

[27] Chai CK (2007) The genetics of Alzheimer's disease. *Am J Alzheimers Dis Other Demen* **22**, 37-41.

[28] Gatz M, Reynolds CA, Fratiglioni L, Johansson B, Mortimer JA, Berg S, Fiske A, Pedersen NL (2006) Role of genes and environments for explaining Alzheimer disease. *Arch Gen Psychiatry* **63**, 168-174.

[29] Rossor MN, Fox NC, Freeborough PA, Harvey RJ (1996) Clinical features of sporadic and familial Alzheimer's disease. *Neurodegeneration* **5**, 393-397.

[30] Pistollato F, Battino M (2014) Role of plant-based diets in the prevention and regression of metabolic syndrome and neurodegenerative diseases. *Trends Food Sci Technol* **40**, 62-81.

[31] Rosendorff C, Beeri MS, Silverman JM (2007) Cardiovascular risk factors for Alzheimer's disease. *Am J Geriatr Cardiol* **16**, 143-149.

[32] Russ TC, Stamatakis E, Hamer M, Starr JM, Kivimaki M, Batty GD (2013) Socioeconomic status as a risk factor for dementia death: Individual participant meta-analysis of 86 508 men and women from the UK. *Br J Psychiatry* **203**, 10-17.

[33] Sattler C, Toro P, Schonknecht P, Schroder J (2012) Cognitive activity, education and socioeconomic status as preventive factors for mild cognitive impairment and Alzheimer's disease. *Psychiatry Res* **196**, 90-95.

[34] Paradise M, Cooper C, Livingston G (2009) Systematic review of the effect of education on survival in Alzheimer's disease. *Int Psychogeriatr* **21**, 25-32.

[35] Hernandez SS, Sandreschi PF, Silva FC, Arancibia BA, da Silva R, Gutierres PJ, Andrade A (2014) What are the benefits of exercise for Alzheimer's disease? A systematic review of past 10 years. *J Aging Phys Act*. doi:10.1123/japa.2014-0180

[36] Okonkwo OC, Schultz SA, Oh JM, Larson J, Edwards D, Cook D, Koscik R, Gallagher CL, Dowling NM, Carlsson CM, Bendlin BB, LaRue A, Rowley HA, Christian BT, Asthana S, Hermann BP, Johnson SC, Sager MA (2014) Physical activity attenuates age-related biomarker alterations in preclinical AD. *Neurology* **83**, 1753-1760.

[37] Gates NJ, Sachdev P (2014) Is cognitive training an effective treatment for preclinical and early Alzheimer's disease? *J Alzheimers Dis* **42**, S551-S559.

[38] Lim MM, Gerstner JR, Holtzman DM (2014) The sleep-wake cycle and Alzheimer's disease: What do we know? *Neurodegener Dis Manag* **4**, 351-362.

[39] Videnovic A, Lazar AS, Barker RA, Overeem S (2014) 'The clocks that time us'-circadian rhythms in neurodegenerative disorders. *Nat Rev Neurol* **10**, 683-693.

[40] Peter-Derex L, Yammine P, Bastuji H, Croisile B (2015) Sleep and Alzheimer's disease. *Sleep Med Rev* **19**, 29-38.

[41] Lucey BP, Bateman RJ (2014) Amyloid-beta diurnal pattern: Possible role of sleep in Alzheimer's disease pathogenesis. *Neurobiol Aging* **35**(Suppl 2), S29-S34.

[42] Ju YE, Lucey BP, Holtzman DM (2014) Sleep and Alzheimer disease pathology–a bidirectional relationship. *Nat Rev Neurol* **10**, 115-119.

[43] Moulton PV, Yang W (2012) Air pollution, oxidative stress, and Alzheimer's disease. *J Environ Public Health* **2012**, 472751.

[44] Cataldo JK, Prochaska JJ, Glantz SA (2010) Cigarette smoking is a risk factor for Alzheimer's Disease: An analysis controlling for tobacco industry affiliation. *J Alzheimers Dis* **19**, 465-480.

[45] Shcherbatykh I, Carpenter DO (2007) The role of metals in the etiology of Alzheimer's disease. *J Alzheimers Dis* **11**, 191-205.

[46] Brewer GJ (2012) Copper excess, zinc deficiency, and cognition loss in Alzheimer's disease. *Biofactors* **38**, 107-113.

[47] Tong Y, Yang H, Tian X, Wang H, Zhou T, Zhang S, Yu J, Zhang T, Fan D, Guo X, Tabira T, Kong F, Chen Z, Xiao W, Chui D (2014) High manganese, a risk for Alzheimer's disease: High manganese induces amyloid-beta related cognitive impairment. *J Alzheimers Dis* **42**, 865-878.

[48] Michaud M, Balardy L, Moulis G, Gaudin C, Peyrot C, Vellas B, Cesari M, Nourhashemi F (2013) Proinflammatory cytokines, aging, and age-related diseases. *J Am Med Dir Assoc* **14**, 877-882.

[49] Obasi CN, Cruickshanks KJ, Nondahl DM, Klein BE, Klein R, Nieto FJ, Shankar A, Fischer ME, Tsai MY, Chappell R (2012) Association of biomarkers for inflammation, endothelial dysfunction and oxidative stress with cognitive impairment. The Epidemiology of Hearing Loss Study (EHLS). *Oxid Antioxid Med Sci* **1**, 169-173.

[50] Tripathy D, Sanchez A, Yin X, Luo J, Martinez J, Grammas P (2013) Thrombin, a mediator of cerebrovascular inflammation in AD and hypoxia. *Front Aging Neurosci* **5**, 19.

[51] Kamath AF, Chauhan AK, Kisucka J, Dole VS, Loscalzo J, Handy DE, Wagner DD (2006) Elevated levels of homocysteine compromise blood-brain barrier integrity in mice. *Blood* **107**, 591-593.

[52] Boldyrev A, Bryushkova E, Mashkina A, Vladychenskaya E (2013) Why is homocysteine toxic for the nervous and immune systems? *Curr Aging Sci* **6**, 29-36.

[53] Al-khateeb E, Althaher A, Al-khateeb M, Al-Musawi H, Azzouqah O, Al-Shweiki S, Shafagoj Y (2015) Relation between uric acid and Alzheimer's disease in elderly Jordanians. *J Alzheimers Dis* **44**, 859-865.

[54] Chen X, Guo X, Huang R, Chen Y, Zheng Z, Shang H (2014) Serum uric acid levels in patients with Alzheimer's disease: A meta-analysis. *PLoS One* **9**, e94084.

[55] Mousavi M, Jonsson P, Antti H, Adolfsson R, Nordin A, Bergdahl J, Eriksson K, Moritz T, Nilsson LG, Nyberg L (2014) Serum metabolomic biomarkers of dementia. *Dement Geriatr Cogn Dis Extra* **4**, 252-262.

[56] Zarrouk A, Riedinger JM, Ahmed SH, Hammami S, Chaabane W, Debbabi M, Ben Ammou S, Rouaud O, Frih M, Lizard G, Hammami M (2015) Fatty acid profiles in demented patients: Identification of hexacosanoic acid (C26:0) as a blood lipid biomarker of dementia. *J Alzheimers Dis* **44**, 1349-1359.

[57] Ferreira ST, Clarke JR, Bomfim TR, De Felice FG (2014) Inflammation, defective insulin signaling, and neuronal dysfunction in Alzheimer's disease. *Alzheimers Dement* **10**, S76-S83.

[58] Willette AA, Johnson SC, Birdsill AC, Sager MA, Christian B, Baker LD, Craft S, Oh J, Statz E, Hermann BP, Jonaitis EM, Koscik RL, La Rue A, Asthana S, Bendlin BB (2015) Insulin resistance predicts brain amyloid deposition in late middle-aged adults. *Alzheimers Dement* **11**, 504-510 e501.

[59] de la Monte SM, Tong M, Lester-Coll N, Plater M Jr., Wands JR (2006) Therapeutic rescue of neurodegeneration in experimental type 3 diabetes: Relevance to Alzheimer's disease. *J Alzheimers Dis* **10**, 89-109.

[60] Morris JK, Vidoni ED, Honea RA, Burns JM, Alzheimer's Disease Neuroimaging I (2014) Impaired glycemia increases disease progression in mild cognitive impairment. *Neurobiol Aging* **35**, 585-589.

[61] Lozano L, Lara-Lemus R, Zenteno E, Alvarado-Vasquez N (2014) The mitochondrial O-linked N-acetylglucosamine transferase (mOGT) in the diabetic patient could be the initial trigger to develop Alzheimer disease. *Exp Gerontol* **58**, 198-202.

[62] Vitali C, Wellington CL, Calabresi L (2014) HDL and cholesterol handling in the brain. *Cardiovasc Res* **103**, 405-413.

[63] Blom K, Emmelot-Vonk MH, Koek HL (2013) The influence of vascular risk factors on cognitive decline in patients with dementia: A systematic review. *Maturitas* **76**, 113-117.

[64] Vikarunnessa S, Weiner MF, Vega GL (2013) LDL phenotype in subjects with mild cognitive impairment and Alzheimer's disease. *J Alzheimers Dis* **36**, 571-575.

[65] Reed B, Villeneuve S, Mack W, DeCarli C, Chui HC, Jagust W (2014) Associations between serum cholesterol levels and cerebral amyloidosis. *JAMA Neurol* **71**, 195-200.

[66] Warren MW, Hynan LS, Weiner MF (2012) Lipids and adipokines as risk factors for Alzheimer's disease. *J Alzheimers Dis* **29**, 151-157.

[67] Ferini-Strambi L (2014) Possible role of orexin in the pathogenesis of Alzheimer disease. *JAMA Neurol* **71**, 1478-1480.

[68] Nixon JP, Mavanji V, Butterick TA, Billington CJ, Kotz CM, Teske JA (2015) Sleep disorders, obesity, and aging: The role of orexin. *Ageing Res Rev* **20**, 63-73.

[69] Adam JA, Menheere PP, van Dielen FM, Soeters PB, Buurman WA, Greve JW (2002) Decreased plasma orexin-A levels in obese individuals. *Int J Obes Relat Metab Disord* **26**, 274-276.

[70] Fronczek R, van Geest S, Frolich M, Overeem S, Roelandse FW, Lammers GJ, Swaab DF (2012) Hypocretin (orexin) loss in Alzheimer's disease. *Neurobiol Aging* **33**, 1642-1650.

[71] Antonell A, Mansilla A, Rami L, Llado A, Iranzo A, Olives J, Balasa M, Sanchez-Valle R, Molinuevo JL (2014) Cerebrospinal fluid level of YKL-40 protein in preclinical and prodromal Alzheimer's disease. *J Alzheimers Dis* **42**, 901-908.

[72] Craig-Schapiro R, Perrin RJ, Roe CM, Xiong C, Carter D, Cairns NJ, Mintun MA, Peskind ER, Li G, Galasko DR, Clark CM, Quinn JF, D'Angelo G, Malone JP, Townsend RR, Morris JC, Fagan AM, Holtzman DM (2010) YKL-40: A novel prognostic fluid biomarker for preclinical Alzheimer's disease. *Biol Psychiatry* **68**, 903-912.

[73] Engelborghs S (2013) Clinical indications for analysis of Alzheimer's disease CSF biomarkers. *Rev Neurol (Paris)* **169**, 709-714.

[74] Huded CB, Bharath S, Chandra SR, Sivakumar PT, Varghese M, Subramanian S (2015) Supportive CSF biomarker evidence to enhance the National Institute on Aging-Alzheimer's Association criteria for diagnosis of Alzheimer's type dementia - A study from Southern India. *Asian J Psychiatr* **13**, 44-47.

[75] Rappaport SM (2012) Discovering environmental causes of disease. *J Epidemiol Community Health* **66**, 99-102.

[76] Yegambaram M, Manivannan B, Beach TG, Halden RU (2015) Role of environmental contaminants in the etiology of Alzheimer's disease: A review. *Curr Alzheimer Res* **12**, 116-146.

[77] Oboudiyat C, Glazer H, Seifan A, Greer C, Isaacson RS (2013) Alzheimer's disease. *Semin Neurol* **33**, 313-329.

[78] Nicolia V, Lucarelli M, Fuso A (2015) Environment, epigenetics and neurodegeneration: Focus on nutrition in Alzheimer's disease. *Exp Gerontol* **68**, 8-12.

[79] Virmani A, Pinto L, Binienda Z, Ali S (2013) Food, nutrigenomics, and neurodegeneration–neuroprotection by what you eat! *Mol Neurobiol* **48**, 353-362.

[80] Jicha GA, Markesbery WR (2010) Omega-3 fatty acids: Potential role in the management of early Alzheimer's disease. *Clin Interv Aging* **5**, 45-61.

[81] Bourre JM (2006) Effects of nutrients (in food) on the structure and function of the nervous system: Update on dietary requirements for brain. Part 1: Micronutrients. *J Nutr Health Aging* **10**, 377-385.

[82] Feart C, Samieri C, Alles B, Barberger-Gateau P (2013) Potential benefits of adherence to the Mediterranean diet on cognitive health. *Proc Nutr Soc* **72**, 140-152.

[83] Schaffer S, Asseburg H, Kuntz S, Muller WE, Eckert GP (2012) Effects of polyphenols on brain ageing and Alzheimer's disease: Focus on mitochondria. *Mol Neurobiol* **46**, 161-178.

[84] Joseph J, Cole G, Head E, Ingram D (2009) Nutrition, brain aging, and neurodegeneration. *J Neurosci* **29**, 12795-12801.

[85] Kageyama M, Hiraoka M, Kagawa Y (2008) Relationship between genetic polymorphism, serum folate and homocysteine in Alzheimer's disease. *Asia Pac J Public Health* **20 Suppl**, 111-117.

[86] Kerchner GA (2011) Ultra-high field 7T MRI: A new tool for studying Alzheimer's disease. *J Alzheimers Dis* **26**(Suppl 3), 91-95.

[87] Maruyama M, Shimada H, Suhara T, Shinotoh H, Ji B, Maeda J, Zhang MR, Trojanowski JQ, Lee VM, Ono M, Masamoto K, Takano H, Sahara N, Iwata N, Okamura N, Furumoto S, Kudo Y, Chang Q, Saido TC, Takashima A, Lewis J, Jang MK, Aoki I, Ito H, Higuchi M (2013) Imaging of tau pathology in a tauopathy mouse model and in Alzheimer patients compared to normal controls. *Neuron* **79**, 1094-1108.

[88] Blasko I, Hinterberger M, Kemmler G, Jungwirth S, Krampla W, Leitha T, Heinz Tragl K, Fischer P (2012) Conversion from mild cognitive impairment to dementia: Influence of folic acid and vitamin B12 use in the VITA cohort. *J Nutr Health Aging* **16**, 687-694.

[89] Mosconi L, Murray J, Tsui WH, Li Y, Davies M, Williams S, Pirraglia E, Spector N, Osorio RS, Glodzik L, McHugh P, de Leon MJ (2014) Mediterranean diet and magnetic resonance imaging-assessed brain atrophy in cognitively normal individuals at risk for Alzheimer's disease. *J Prev Alzheimers Dis* **1**, 23-32.

[90] Titova OE, Ax E, Brooks SJ, Sjogren P, Cederholm T, Kilander L, Kullberg J, Larsson EM, Johansson L, Ahlstrom H, Lind L, Schioth HB, Benedict C (2013) Mediterranean diet habits in older individuals: Associations with cognitive functioning and brain volumes. *Exp Gerontol* **48**, 1443-1448.

[91] Jbabdi S, Johansen-Berg H (2011) Tractography: Where do we go from here? *Brain Connect* **1**, 169-183.

[92] Gutman B, Leonardo C, Jahanshad N, Hibar D, Eschenburg K, Nir T, Villalon J, Thompson P (2014) Registering cortical surfaces based on whole-brain structural connectivity and continuous connectivity analysis. *Med Image Comput Comput Assist Interv* **17**, 161-168.

[93] Prescott JW, Guidon A, Doraiswamy PM, Roy Choudhury K, Liu C, Petrella JR, Alzheimer's Disease Neuroimaging I (2014) The Alzheimer structural connectome: Changes in cortical network topology with increased amyloid plaque burden. *Radiology* **273**, 175-184.

[94] Ensafi S, Shijian L, Kassim AA, Chew Lim T (2014) 3D reconstruction of neurons in electron microscopy images. *Conf Proc IEEE Eng Med Biol Soc* **2014**, 6732-6735.

[95] Folstein M (2007) Improving dementia assessment by reducing sample heterogeneity. *Int Psychogeriatr* **19**, 383-389.

[96] Mackay-Sim A (2013) Patient-derived stem cells: Pathways to drug discovery for brain diseases. *Front Cell Neurosci* **7**, 29.

[97] Saporta MA, Grskovic M, Dimos JT (2011) Induced pluripotent stem cells in the study of neurological diseases. *Stem Cell Res Ther* **2**, 37.

[98] Qiang L, Inoue K, Abeliovich A (2014) Instant neurons: Directed somatic cell reprogramming models of central nervous system disorders. *Biol Psychiatry* **75**, 945-951.

[99] Israel MA, Yuan SH, Bardy C, Reyna SM, Mu Y, Herrera C, Hefferan MP, Van Gorp S, Nazor KL, Boscolo FS, Carson CT, Laurent LC, Marsala M, Gage FH, Remes AM, Koo EH, Goldstein LS (2012) Probing sporadic and familial Alzheimer's disease using induced pluripotent stem cells. *Nature* **482**, 216-220.

[100] Kondo T, Asai M, Tsukita K, Kutoku Y, Ohsawa Y, Sunada Y, Imamura K, Egawa N, Yahata N, Okita K, Takahashi K, Asaka I, Aoi T, Watanabe A, Watanabe K, Kadoya C, Nakano R, Watanabe D, Maruyama K, Hori O, Hibino S, Choshi T, Nakahata T, Hioki H, Kaneko T, Naitoh M, Yoshikawa K, Yamawaki S, Suzuki S, Hata R, Ueno S, Seki T, Kobayashi K, Toda T, Murakami K, Irie K, Klein WL, Mori H, Asada T, Takahashi R, Iwata N, Yamanaka S, Inoue H (2013) Modeling Alzheimer's disease with iPSCs reveals stress phenotypes associated with intracellular Abeta and differential drug responsiveness. *Cell Stem Cell* **12**, 487-496.

[101] Ooi L, Sidhu K, Poljak A, Sutherland G, O'Connor MD, Sachdev P, Munch G (2013) Induced pluripotent stem cells as tools for disease modelling and drug discovery in Alzheimer's disease. *J Neural Transm* **120**, 103-111.

[102] Vaccarino FM, Stevens HE, Kocabas A, Palejev D, Szekely A, Grigorenko EL, Weissman S (2011) Induced pluripotent stem cells: A new tool to confront the challenge of neuropsychiatric disorders. *Neuropharmacology* **60**, 1355-1363.

[103] Qiang L, Fujita R, Abeliovich A (2013) Remodeling neurodegeneration: Somatic cell reprogramming-based models of adult neurological disorders. *Neuron* **78**, 957-969.

[104] de Vries RB, Leenaars M, Tra J, Huijbregtse R, Bongers E, Jansen JA, Gordijn B, Ritskes-Hoitinga M (2013) The potential of tissue engineering for developing alternatives to animal experiments: A systematic review. *J Tissue Eng Regen Med* doi: 10.1002/term.1703

[105] Schlachetzki JC, Hull M (2009) Microglial activation in Alzheimer's disease. *Curr Alzheimer Res* **6**, 554-563.

[106] Walker DG, Lue LF (2005) Investigations with cultured human microglia on pathogenic mechanisms of Alzheimer's disease and other neurodegenerative diseases. *J Neurosci Res* **81**, 412-425.

[107] Huh D, Torisawa YS, Hamilton GA, Kim HJ, Ingber DE (2012) Microengineered physiological biomimicry: Organs-on-chips. *Lab Chip* **12**, 2156-2164.

[108] Sutherland ML, Fabre KM, Tagle DA (2013) The National Institutes of Health Microphysiological Systems Program focuses on a critical challenge in the drug discovery pipeline. *Stem Cell Res Ther* **4 Suppl 1**, I1.

[109] Abaci HE, Shuler ML (2015) Human-on-a-chip design strategies and principles for physiologically based pharmacokinetics/pharmacodynamics modeling. *Integr Biol (Camb)* **7**, 383-391.

[110] Marx U, Walles H, Hoffmann S, Lindner G, Horland R, Sonntag F, Klotzbach U, Sakharov D, Tonevitsky A, Lauster R (2012) 'Human-on-a-chip' developments: A translational cutting-edge alternative to systemic safety assessment and efficiency evaluation of substances in laboratory animals and man? *Altern Lab Anim* **40**, 235-257.

[111] Grskovic M, Javaherian A, Strulovici B, Daley GQ (2011) Induced pluripotent stem cells–opportunities for disease modelling and drug discovery. *Nat Rev Drug Discov* **10**, 915-929.

[112] Pizzi R, Cino G, Gelain F, Rossetti D, Vescovi A (2007) Learning in human neural networks on microelectrode arrays. *Biosystems* **88**, 1-15.
[113] Dranias MR, Ju H, Rajaram E, VanDongen AM (2013) Short-term memory in networks of dissociated cortical neurons. *J Neurosci* **33**, 1940-1953.
[114] Rhinn H, Fujita R, Qiang L, Cheng R, Lee JH, Abeliovich A (2013) Integrative genomics identifies APOE epsilon4 effectors in Alzheimer's disease. *Nature* **500**, 45-50.
[115] Zahid S, Oellerich M, Asif AR, Ahmed N (2012) Phosphoproteome profiling of substantia nigra and cortex regions of Alzheimer's disease patients. *J Neurochem* **121**, 954-963.
[116] Graff-Radford J, Kantarci K (2013) Magnetic resonance spectroscopy in Alzheimer's disease. *Neuropsychiatr Dis Treat* **9**, 687-696.
[117] Mastroeni D, Grover A, Delvaux E, Whiteside C, Coleman PD, Rogers J (2011) Epigenetic mechanisms in Alzheimer's disease. *Neurobiol Aging* **32**, 1161-1180.
[118] Millan MJ (2014) The epigenetic dimension of Alzheimer's disease: Causal, consequence, or curiosity? *Dialogues Clin Neurosci* **16**, 373-393.
[119] Chetty M, Rose RH, Abduljalil K, Patel N, Lu G, Cain T, Jamei M, Rostami-Hodjegan A (2014) Applications of linking PBPK and PD models to predict the impact of genotypic variability, formulation differences, differences in target binding capacity and target site drug concentrations on drug responses and variability. *Front Pharmacol* **5**, 258.
[120] Poet TS, Timchalk C, Hotchkiss JA, Bartels MJ (2014) Chlorpyrifos PBPK/PD model for multiple routes of exposure. *Xenobiotica* **44**, 868-881.
[121] Santamaria AB (2008) Manganese exposure, essentiality & toxicity. *Indian J Med Res* **128**, 484-500.
[122] Salazar JG, Ribes D, Cabre M, Domingo JL, Sanchez-Santed F, Colomina MT (2011) Amyloid beta peptide levels increase in brain of AbetaPP Swedish mice after exposure to chlorpyrifos. *Curr Alzheimer Res* **8**, 732-740.
[123] Janson J, Eketjall S, Tunblad K, Jeppsson F, Von Berg S, Niva C, Radesater AC, Falting J, Visser SA (2014) Population PKPD modeling of BACE1 inhibitor-induced reduction in Abeta levels *in vivo* and correlation to *in vitro* potency in primary cortical neurons from mouse and guinea pig. *Pharm Res* **31**, 670-683.
[124] Rao PP, Mohamed T, Teckwani K, Tin G (2015) Curcumin binding to beta amyloid: A computational study. *Chem Biol Drug Des*. doi: 10.1111/cbdd.12552
[125] Huang HJ, Chen HY, Lee CC, Chen CY (2014) Computational design of apolipoprotein E4 inhibitors for Alzheimer's disease therapy from traditional Chinese medicine. *Biomed Res Int* **2014**, 452625.
[126] Jin N, Qian W, Yin X, Zhang L, Iqbal K, Grundke-Iqbal I, Gong CX, Liu F (2013) CREB regulates the expression of neuronal glucose transporter 3: A possible mechanism related to impaired brain glucose uptake in Alzheimer's disease. *Nucleic Acids Res* **41**, 3240-3256.
[127] Vukic V, Callaghan D, Walker D, Lue LF, Liu QY, Couraud PO, Romero IA, Weksler B, Stanimirovic DB, Zhang W (2009) Expression of inflammatory genes induced by beta-amyloid peptides in human brain endothelial cells and in Alzheimer's brain is mediated by the JNK-AP1 signaling pathway. *Neurobiol Dis* **34**, 95-106.
[128] Gan X, Huang S, Wu L, Wang Y, Hu G, Li G, Zhang H, Yu H, Swerdlow RH, Chen JX, Yan SS (2014) Inhibition of ERK-DLP1 signaling and mitochondrial division alleviates mitochondrial dysfunction in Alzheimer's disease cybrid cell. *Biochim Biophys Acta* **1842**, 220-231.
[129] Esposito G, Scuderi C, Lu J, Savani C, De Filippis D, Iuvone T, Steardo L Jr, Sheen V, Steardo L (2008) S100B induces tau protein hyperphosphorylation via Dickopff-1 up-regulation and disrupts the Wnt pathway in human neural stem cells. *J Cell Mol Med* **12**, 914-927.
[130] Gibb S (2008) Toxicity testing in the 21st century: A vision and a strategy. *Reprod Toxicol* **25**, 136-138.
[131] van der Worp HB, Howells DW, Sena ES, Porritt MJ, Rewell S, O'Collins V, Macleod MR (2010) Can animal models of disease reliably inform human studies? *PLoS Med* **7**, e1000245.
[132] Begley CG, Ellis LM (2012) Drug development: Raise standards for preclinical cancer research. *Nature* **483**, 531-533.
[133] Geerts H (2009) Of mice and men: Bridging the translational disconnect in CNS drug discovery. *CNS Drugs* **23**, 915-926.
[134] Mak IW, Evaniew N, Ghert M (2014) Lost in translation: Animal models and clinical trials in cancer treatment. *Am J Transl Res* **6**, 114-118.
[135] National Institutes of Health (2013) NIH to reduce significantly the use of chimpanzees in research, http://www.nih.gov/news/health/jun2013/od-26.htm, Accessed May 19, 2015.
[136] Ogishima S, Mizuno S, Kikuchi M, Miyashita A, Kuwano R, Tanaka H, Nakaya J (2013) A map of Alzheimer's disease-signaling pathways: A hope for drug target discovery. *Clin Pharmacol Ther* **93**, 399-401.
[137] Calitz C, Pollack KM, Millard C, Yach D (2015) National institutes of health funding for behavioral interventions to prevent chronic diseases. *Am J Prev Med* **48**, 462-471.
[138] Reid AT, Evans AC (2013) Structural networks in Alzheimer's disease. *Eur Neuropsychopharmacol* **23**, 63-77.