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Considering a New Paradigm for Alzheimer's Disease Research

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ABSTRACT

Using Alzheimer's disease as a case study, this review argues that it might be time to consider a new paradigm in medical research and drug discovery. The existing framework is overly dependent on often unvalidated animal models, particularly transgenic mice. Translational success remains elusive and costly late-stage drug failure is common. The conventional paradigm tends to overlook species differences and assumes that animal-based findings are generally applicable to humans. Could pathways-based research using advanced human-specific models probed with new tools, including those of systems biology, take centre stage? The current transition in chemical toxicology to a 21st-century paradigm could be a model for health research, with probable medical and economic benefits.

Introduction

Total new drug approvals have continued to fall whereas the costs of producing novel medicines have grown exponentially. Despite increasing investment, 92% of all novel drugs fail in clinical trials, mainly because of unpredicted toxicity or insufficient efficacy in humans [1]. Problems in basic medical research, drug discovery and effective translation from laboratory to clinic are widely recognised. Meanwhile, in chemical toxicology a transformation is already unfolding, following a seminal report from the US National Research Council in 2007 [2]. This recommended a '21st-century paradigm' for safety testing, involving an explicit transition away from a reliance on adverse endpoints in animal tests and towards a novel framework based on understanding toxic perturbations to cellular pathways, mainly using in silico tools and human-specific cell and tissue models. The National Research Council's vision is being implemented actively worldwide, including by the US multi-agency Tox21 consortium [3] and the Environmental Protection Agency's multi-million dollar ToxCast programme [4].

A recent refinement in toxicology is the concept of adverse outcome pathways (AOPs), which are intended to provide clear mechanistic representations of critical toxic effects spanning molecular, cellular, organ, individual and population levels. AOPs have a common structure comprising exposure to the first molecular initiating event (e.g. a chemical binds to a cell receptor), intermediate steps and key events and an adverse outcome that (in toxicology) could for example be cancer, allergy or liver damage. The first validated AOP (for skin sensitisation) has now been accepted at the Organisation for Economic Cooperation and Development and several further AOPs are in draft form [5].

The transition in toxicology could provide a template for modernising the disease modelling and drug discovery paradigm. Developments in systems biology have enabled studies of human gene pathways and networks linked to disease, and expanding this concept to an AOP approach would have obvious relevance, widening consideration of disease pathways to include environmental factors at the start of the pathway and whole-person or population-level outcomes at the pathway's conclusion. Incorporating

advanced scientific tools into a research framework emphasising pathways and networks in human-specific models could offer better progress towards understanding and treating diseases than the current emphasis on animal models.

The animal model paradigm

For many decades, animal models have had key scientific and conceptual roles in health research and drug discovery, because human experimentation was unethical and impractical and in vitro models were simplistic and poorly representative of the in vivo situation. Within the traditional research paradigm, animal models remain dominant and animal data are used in a 'gate-keeper' role [6] for studying pathophysiological mechanisms, for probing novel therapeutic approaches and as preclinical models in scientific guidelines [e.g. those of the International Conference on Harmonisation.

The animal model paradigm, although widely supported [7], has also been described as 'seriously flawed' and 'not well suited' for predicting human responses in clinical trials, where failure rates are very high [8]. Some authors refer to a 'crisis of validation' for animal models in neuroscience drug discovery, leading to a high risk of developing mainly 'me-too' compounds [9]. Van Meer and colleagues describe the current approach as 'a stalemate in which animal studies, predictive or not, continue to exist with little room for innovation' [10]. They and others call for a critical assessment of the predictive value of animal studies, from which it might emerge that new technologies can be implemented that predict efficacy as well as, or better than, animal studies.

The regulatory requirement for preclinical animal data has been challenged, because much is of 'unclear relevance' to human disease [11]. Tacit assumptions about the adequacy of rodent models in disease research need to be questioned [12]; it is too often simply assumed that there are good correlations between an accepted animal model and human subjects [13]. An analysis of 76 highly cited studies on a range of animal species published in seven high-impact scientific journals found that only 37% accurately predicted human outcomes [14].

Despite sustained investment in animal models, disease-modifying therapies remain elusive for major illnesses such as Alzheimer's disease (AD) [15], stroke [16], motor neuron disease [17], Huntington's disease [18], asthma [19], sepsis [20] and inflammatory diseases [6]. The animal-model paradigm tends to discourage a critical appraisal of the differences between species and encourages a view that animal-based findings are generally applicable to humans [21]. However, evolutionary biology dictates that the species barrier cannot be overcome and significant differences between animal models and human diseases will continue to frustrate progress. For example, it is hard to envisage how animal models, limited by inter- as well as intra-species variations, could expedite the development of more personalised medicine.

Animal studies can provide useful in vivo data about selected pathologies, such as the amyloid pathway in AD research, but increasingly this research could be conducted using novel human-and disease-specific models and tools. These models and techniques are being incorporated into research in a piecemeal manner but without a serious review of the long-standing gate-keeper role of animal studies. It was the recognition that animal tests were inadequate and that advanced research tools were being insufficiently exploited in chemical toxicology that led to the transition currently progressing in that field [2].

With developments in systems biology, a systems understanding of human disease pathophysiology is moving within our reach [1]. The coming together of a crisis of confidence in animal research with the

emergence of much better human in vitro models [22] and advanced techniques for human in vivo studies creates a timely opportunity to review how a new paradigm could best incorporate these advances in a coherent research framework. AD research is examined here as a case study of the limitations of the present research framework, how a new vision for medical research might look and the potential benefits it could achieve.

Alzheimer's disease: translational failures

AD is a progressive dementia (Box 1) with classic pathologies comprising amyloid plaques in the brain, neurofibrillary tangles (NFTs) containing abnormal tau and neuronal degeneration. Symptoms include cognitive deficits, including memory disruption and impaired judgment, disorientation, confusion, behavioural changes and difficulties moving, speaking and swallowing. Ultimately fatal, AD causes suffering to patients and their families over a long period of time. The prevalence of AD worldwide is expected to triple over the next 40 years, so the need for progress is very pressing.

The five approved drugs for AD can stabilise symptoms temporarily, but do not slow disease progression. Around half of patients benefit modestly [23], but there is an urgent need for better, disease-modifying therapies as well as preventative measures. The link between cholinergic deficits and AD, first discovered through analysis of post-mortem human brain tissue in the 1970s and 1980s and then pursued with animal studies, led to the four existing cholinesterase inhibitor drugs. Since the approval of memantine (a glutamate receptor blocker) a decade ago, many novel compounds for AD have entered clinical trials, but so far none has successfully completed a Phase III trial despite encouraging preclinical results in transgenic (Tg) mice (Table 1).

There are many acknowledged reasons for failures of translational research [24]. Although numerous improvements can be made to the methodology of animal studies, including for AD [15], they remain inevitably flawed by: the 'insuperable species barrier' [13], comprising fundamental under-lying species differences between animals and humans; the dis-parities between animal models and human diseases in complexity, causation, pathophysiology and progression; the uncertain relevance to humans of the results of behavioural studies in animals [25].

The limitations of mouse models in AD research

Some AD research is conducted in dogs, primates, ageing rats and chemical- and lesion-induced rodents, and newer models include genetically modified zebrafish and the nematode worm Caenorhabditis elegans. However, by far the dominant animal models over the past 15 years have been Tg mice. Most of the Tg mouse lines are based on one or several inserted human genes relevant to the amyloid hypothesis of AD causation [7,26].

The reliability of a model is considered in terms of its validity (summarised in Box 3) [27]. Regarding face validity, some lines of Tg mice develop plaques and/or NFTs, a few show some neuronal loss and some have cognitive deficits. However, the disease dynamics differ, none of the models fully recapitulates AD and the phenotypic similarities are species-andstrain-dependent. In the case of construct validity, although sporadic AD (sAD) is dominant in humans, Tg mouse genotypes resemble the much rarer (~5% of cases in humans) familial AD (fAD). In humans, fAD is linked to mutations in the amyloid precursor protein (APP), presenilin 1 and presenilin 2 genes, none of which singly or combined leads to a full spectrum of AD pathologies in mice. None of the Tg mouse lines overexpressing APP 'by any stretch of the imagination' develops cognitive or behavioural deficits approaching those typical of AD [7]. Some Tg mice expressing human tau variants have a fuller pathology, but in humans these tau variants are not

associated with any form of AD. Triply Tg mice, generated using presenilin 1 knockin mice and micro-injecting APP and FTDP-17 (a tau mutation) transgenes, develop amyloid plaques, NFT-type lesions and deficits in spatial memory; but they reflect a composite of two distinct diseases neither of which is AD.

Few AD cell pathways are known and those studied in Tg mice, such as amyloid deposition, are considered to have generated useful mechanistic data but are limiting in terms of novel drug targets and have been poorly predictive of clinical outcomes. The development of Tg mice for model human disease pathways can only be attempted once a pathway is known to be significant in patients. Emerging human- and disease-specific models potentially offer a way out of this stalemate by enabling the discovery and detailed study of new human pathways and drug targets.

In translational science predictive validity is crucial. Studies of Tg mice have certainly contributed to an understanding of some AD pathways and more than 300 interventions have been tested as a result; however, as discussed above, none has translated into disease-modifying therapies [7,15]. Models that meet more levels of validity are clearly of higher utility and relevance than those where validity is weaker. Most animal models, including those for AD, do not fulfil sufficient validity measures and the conclusions that can be drawn from their use should be more strongly qualified [13].

Many animal models have never been evaluated systematically, for example by systematic review and meta-analysis of performance characteristics such as reproducibility, specificity, sensitivity, clinical relevance or mechanistic basis [28]. At the time of writing, no systematic reviews have been published about the predictive ability of Tg mouse models of AD. This is in stark contrast to recommendations that systematic reviews of the clinical relevance and the risk of bias of preclinical research should be conducted before the start of clinical trials [29,30]. Without systematic evidence of validity, the utility of animal models including Tg mice in AD research is unproven; and yet costly clinical trials, of potential risk to participants, are conducted primarily on the basis of these data.

The species barrier is a highly significant problem in developing valid models of human diseases. Mouse models seldom sufficiently recapitulate human disease pathways and pathologies, including in AD, because of important underlying species differences in genetics, protein pathways, metabolism, pharmacology and physiology that have accumulated since rodents and humans diverged 65–85 million years ago. Evolutionary divergence in protein functions and gene regulatory networks can complicate studies in animals, and it should not be assumed that gene function is conserved between animals and humans until functional equivalence is demonstrated [31]. In considering AD, unlike humans mice are naturally resistant to age-related amyloid pathology. Mouse and human APPs differ by 17 amino acids – three in the amyloid-b peptide (Ab) sequence. Knockin mice with APP mutations express humanised Ab but do not develop amyloid plaques or neuropathology [26], implicating fundamental differences such as a shorter lifespan or dissimilar processing of mouse APP by b-secretase. Mouse brain only has 4R tau, whereas human brain has 3R and 4R tau isoforms that are both hyperphosphorylated in AD. There are 14 amino acid differences in the N-terminal region of human and murine tau, and the difficulties in inducing NFTs in mice could be attributable to these disparities [26].

Only humans have genetic variants of the apolipoprotein E gene (APOE), a major gene associated with sAD. Human APOE*e4 has a unique domain interaction between the Arg61 and Glu255 residues, responsible for most of its associated neuropathology. The one murine APOE has Thr61 instead of Arg61, preventing the APOE*e4 domain interaction. Researching the role of APOE*e4 in mice has necessitated the generation of complex Tg lines, possibly introducing confounding issues. Presenilin 1 correlates highly with oligodendrocyte markers only in humans, not in mice [32]. The human presenilin 2

promoters are modulated differently from the murine equivalent, and presenilin mutations produce almost no plaque pathology in Tg mice.

A major known risk factor of sAD is ageing. Humans experience a dramatic increase in age-dependent repression of broad-spectrum neuronal genes compared with mice, likely to alter neural networks and result in cognitive changes. Adult neurogenesis occurs significantly in the rodent hippocampus but is much less obvious (or absent) in longer-lived species, including humans [33]. Of 49 well-known genes examined in the mouse cortex and hippocampus, over 50% showed inter-strain expression variation, with probable consequences for behaviour and drug responses [34]. Neuronal nitric oxide synthase is prominently expressed in C57B6 mice but its levels are much lower in SV129 mice. Fundamental strain- and species-dependent variations such as these cause difficulties in extrapolation from rodent data to humans. In different species, functionally equivalent receptors can have distinctive pharmacological profiles. Currently, drug candidates are frequently optimised for rodent pharmacology and the first time a novel drug is challenged by a range of human pharmacologies is often at the clinical trial stage, when failure is very costly.

In Tg mice, connections between the increase in Ab, lesions and symptoms have resisted clarification. Many mice show behavioural changes before significant plaque deposition – the reverse of the human situation. The relative abundance of Ab peptides in APP23 mice and AD patients differs, suggesting dissimilar mechanisms underlying amyloid deposition. In AD brains the unfolded protein response is upregulated; but in Tg2576 mice neither the unfolded protein response nor the cell-death pathway is induced [35]. There are also species variations in post-translational modifications of amyloid peptides and their resistance to degradation, thought to be related to differences in lifespan and physiology between mice and humans.

One of the strengths of in vivo models of AD, and a contributing feature of their construct and predictive validities, should be the opportunity to measure relevant behavioural indicators of cognitive deficits. Tg mouse models are sometimes defended as essential because learning and memory deficits are core to AD and cannot be replicated in conventional in vitro models. But functional studies with Tg mice are not predictive and it is not known how, if at all, behavioural tests reflect AD cognitive deficits [26]. Philipson et al. point out that behavioural studies with Tg mice can be relevant to understanding whether an amyloid species or a pathology has a functional effect on neurotransmission but are inadequate for predicting whether a drug alters AD symptoms [36].

Functional studies in mice are also poorly reproducible, between and even within laboratories, and test performance is influenced by the selected promoter gene, by transgene overexpression and by the choice of background strain [37]. Some strains are notably aggressive, neophobic or anxious which confounds interpretation of aspects of cognitive performance, and some perform particularly badly in the Morris water maze test of spatial memory [38]. In the mid-1990s, Tg2576 mice were reported to have a memory deficit that correlated with amyloid plaques, but this was challenged [39] and the argument was still rumbling on nine years later. Dramatically different phenotypes can arise from using the same mutations and promoters, and Tg mouse lines are subject to sensorimotor and cognitive impairments that do not replicate AD [40]. Cognitive deficiencies in Tg mice are not only age-dependent but also task- and sometimes gender-dependent, making extrapolation to humans very difficult.

Under the current research paradigm, the mainstream opinion is that animal models remain necessary and should be improved [9,13,15,36]. For example, it has been argued that disease end-points other than massive Ab deposits could be used in rodents and might have more translational relevance. Olfactory dysfunction, an early symptom in AD, could provide a marker of disease progression and drug effect in Tg

mice [41]. Such developments could yield improved predictive validity over existing Tg mouse models, but further research and validation will take time and the poor face and construct validities of mouse models, as well as the species barrier, would remain unaddressed. With the emergence of 21st-century human-specific models and tools, positioning human rather than animal pathophysiology at the centre of research efforts might be more productive [6].

Advances in human cell models and tools

Until recently, research and drug discovery in AD (as in other fields) have been hampered not only by animal models of limited utility and unproven validity but also by overly simplistic human cellular models. The standard cell models have often used cancer cell lines with many genetic changes in static, monolayer culture, which fails to replicate the architecture, cellular interactions, differentiation status and functionality of human tissue [42].

But this century has seen new advances in human cell models and applicable tools, which are expected substantially to increase the value and relevance of human cell-based research. Human induced pluripotent stem cells (hiPSC), generated by reprogramming adult human cells and differentiated in vitro, provide significant opportunities for generating unique disease-relevant, tissue- and human-specific cell models, even in genetically complex diseases such as sAD. hiPSC generated from patients with fAD and sAD have been differentiated into functional and electrophysiologically active neurons [43] with increased Ab1–40, active glycogen synthase kinase-3b and phosphorylated tau. In another study, Ab peptides accumulated in neurons and astrocytes derived from hiPSC from fAD and sAD patients, leading to endoplasmic reticulum and oxidative stress. The research further demonstrated the usefulness of patient-specific hiPSC in probing AD pathogenesis and evaluating drugs [44]. Thus, hiPSC studies are expected to provide novel insights into pathways of AD initiation and pathogenesis, the roles of different cell types, drug screening and development studies, patient-specific drug responses, prospective diagnostics [45] and the role of sAD susceptibility genes identified in genome-wide association studies (GWAS). hiPSC derived from individuals with genetic variations of interest and differentiated into neural cells can now be deep-sequenced using high-through-put, automated technology. The combined availability of patient-and disease-specific genetic material and living in vitro models could help achieve the ultimate goal of relating genotype and phenotype, for example correlating individual genetic variants with gene expression patterns, cellular disease pathways and altered functions of neural cells [46].

Another adult human source of pluripotent stem cells is the olfactory mucosa, which is easily biopsied or sampled postmortem. Post-mortem olfactory stem cells from individuals with Alzheimer's disease showed differences in APP processing and oxidative stress, and in vitro models of neurological diseases based on biopsied human olfactory stem cells offer several practical advantages over hiPSC for drug discovery [47].

So that human stem-cell-derived models can achieve their full research potential, optimisation of reprogramming techniques for somatic cells, identifying markers that predict differentiation potential, improving in vitro differentiation protocols and generating purer cell populations are some of the challenges that must be overcome [42]. Where post-mortem tissue from the same patient is available, detailed findings in hiPSC-derived cells in vitro can be directly validated. Acknowledging that animal disease models are time-consuming, costly and predict drug efficacy in humans imperfectly, the US National Institutes of Health (NIH) envisages that better target validation using human tissues and hiPSC could eliminate testing for drug efficacy in animals altogether [48]. Grskovic and colleagues also foresee hiPSC technology providing a new, human-disease-based drug discovery paradigm, in which older models (including 'nonpredictive animal models' and simplistic cell cultures) are replaced, leading to

human efficacy and toxicity data being available earlier and even moving directly ‘from in vitro clinical trials to actual clinical trials’ [49].

In vitro clinical trials would provide human efficacy (and toxicity) information at multiple dose levels and data on the heterogeneity of the patient population, minimising risks of later failures and achieving faster timeframes with lower-cost, high-throughput biological platforms [49]. A clearer understanding of genotype– phenotype relationships in disease-specific human cells is also on the horizon, potentially providing a crucial link between basic research and translation. Patient-derived hiPSC models should be able to identify hits that alter the disease phenotype, as well as having promise for target validation, lead optimisation, candidate selection, biomarker discovery and personalised medicine [50].

Tissue engineering creates robust and controllable 3D in vitro human tissue models that better replicate the in vivo spatial environment. Compared with 2D cultures, tissue engineered models have improved viability and many cellular and tissue processes are closer to the in vivo situation. The production and function of these tissue constructs can be tightly controlled with fewer confounding factors than when using living animals, and they are expected to have promising applications in disease modelling and toxicology [51].

Microfluidics technology employs microchip devices incorporating a laminar flow of culture medium, improving the transport of nutrients and waste products. They offer rapid, reproducible and sensitive platforms compatible with high-throughput processing or high-content analysis. Organ-on-a-chip devices combine microfluidics with 3D culture, aiming to reproduce key structural, functional and biochemical features of human organs in vitro. Human lung and gut are two of several models available, with brain-on-a-chip systems currently under development [22]. These technologies are another key tool to create high-quality, high-throughput in vitro models for better testing of drug efficacy and toxicity, and potentially for studying disease pathways and identifying new drug targets in human-relevant systems [22,52]. The NIH anticipates that they have ‘the potential to change paradigms of how we develop therapies, inform regulatory decision-making process and shorten clinical trials’ (http://www.ncats.nih.gov/about/faq/tissue-chip/tis- sue-chip.html). There have also been developments in electrophysiological techniques applicable to human cells in vitro, providing key functional data. Automated patch-clamping combined with microfluidic channels now yields sensitive, high-quality, high- throughput data including measurements of very fast ligand-gated ion channels and receptors in cell lines [53].

In AD research, studies of deficits in learning and memory have classically relied on mouse models [9] and in vitro alternatives are not yet validated. However, if human neural networks can be studied reliably in vitro, they could overcome a key challenge: assessing drug effects on aspects of learning and memory. A reliable tool for functional studies would improve translational success, because rodent models are poorly reproducible [37], time-consuming [36] and of unknown relevance [25]. Microelectrode array devices populated in vitro with human-stem-cell-derived neuronal cells allowed functional studies of spontaneous network activity and neuronal receptor responses over several weeks and studies of mechanisms underlying learning and memory [54]. Optogenetic techniques combined with multielectrode arrays applied to cultured neuronal networks enable studies of short-term memory mechanisms in vitro, and should provide a rapid screening tool for drugs [55]. Human cortical development to the level of functional synapses and networks has recently been achieved in vitro using hiPSC, opening the door to novel functional models resembling the in vivo cortex in circuit specificity and laminar organisation of cortical projection neurons [56]. These emerging human models certainly need further investment.

The wide range of emerging human-specific cellular models and 'next-generation' tools with which to probe them are undergoing rapid development and validation. If their potential is realised they will become game-changers for disease research and drug discovery.

Human tissue and omics analyses

Human post-mortem tissue research into AD has declined since it led to the discovery of the cholinesterase-inhibitor drugs, but that will change as better quality specimens become available and next-generation sequencing enables a rapid and unbiased characterisation of messenger RNA, proteins and metabolites associated with disease. New transcriptomics analyses needing no a priori etiological hypotheses will advance understanding of AD pathogenesis. In 2013, an elegant study integrated publicly available data from GWAS with that from human cortical transcriptional networks and human neuroimaging, to advance knowledge of key regulatory molecules and pathways involved in APOE-related risk of AD [57]. Laser-capture microdissection enables gene expression profiling of selected cortical neurons from post-mortem brain, and has revealed significant differential expression of AD-implicated genes in regions of AD brains compared with controls. In the epigenetics field, research is underway to elucidate specific genes affected by epigenetic changes and whether these implicate proteins and pathological processes relevant to AD [58].

Human pathology can also be probed by analysing cerebrospinal fluid (CSF) or blood samples from subjects at different disease stages, and linked with neuroimaging and magnetic resonance spectroscopy data. Current diagnostic guidelines for AD include three CSF biomarkers [59] and these human in vivo data are of potentially high value to understanding AD pathology, following disease progression and developing new drugs [59].

Quantitative proteomics offers comprehensive insights into disease phenotypes and pathways and advanced analytical techniques have dramatically improved in speed and precision. An analysis of cortical samples of AD and normal brains using high- resolution mass spectrometry [60] recently identified and quantified 197 proteins of significantly different abundance in AD brain samples. Mapping with bioinformatics tools revealed associations with multiple pathways and processes important in AD. Protein identification with capillary electrophoresis coupled to an electrospray ionisation time-of-flight mass spectrometer was used recently with AD brain tissue to reveal abnormal phosphorylation in nine proteins that influence cell metabolism, signal transduction, cytoskeleton integration and synaptic function [61].

Advanced human in vivo studies

Neuroimaging is one expanding approach to human in vivo research in AD and other neurological disorders. Imaging technologies are advancing rapidly in specificity and sensitivity, in spatial and temporal resolution, and in automated image analysis, impacting progress in elucidating pathways of human disease, early diagnosis, patient stratification, personalised therapies and treatment evaluation, including effects on cognitive dysfunction. Safe human in vivo studies are a key component of a new research paradigm, also providing a species-relevant link that helps inform and validate in vitro techniques.

Ultra-high-field magnetic resonance imaging (MRI) can now directly visualise cortical plaque disposition and early tissue loss in the hippocampus of patients previously only visible in postmortem tissue [62]. Multimodal magnetic resonance tools can measure and correlate structural, functional, metabolic and haemodynamic changes in the brain and have been used in several studies of AD. Magnetic resonance spectroscopy detects and quantifies in vivo metabolites that reflect the status of neuronal and glial cells, energy metabolism, inflammation and neurotransmitters. Improvements in sensitivity are expected to lead

to progress in real-time monitoring of in vivo metabolic processes in the human brain. Diffusion tensor imaging traces neural tracts and micro-structural damage in white matter in the human brain that can be correlated with omics, pathogenesis, disease progression and cognitive features of AD.

Positron emission tomography (PET) studies in patients have revealed new information about plaque distribution and some clinical trials have exploited Ab as a biomarker. In 2013, a new tau ligand was developed that enables sensitive PET imaging of tau NFTs in living patients with AD for the first time [63]. This development offers exciting possibilities for earlier AD diagnoses, better stratification of patients in clinical trials, a new marker for treatment effect and insights into the pathophysiology and progression of AD in human patients.

Human connectomics is a scientific concept emerging in response to imaging advances. The Human Connectome Project (http://www.humanconnectomeproject.org) aims to combine imaging data from hundreds of participants to create a comprehensive circuitry map of the human brain and to link this to genetics and behaviour. Connectome studies of AD have already demonstrated abnormal functional connectivity between and within hemispheres. A recent genome-wide analysis of the connectome involved 366 twins scanned with MRI and high- angular resolution diffusion imaging to trace fibre tracts through the whole brain [64]. Significant associations between gene variants and connectivity were found in some fibre tracts, and the study was also important in indicating the relative contributions of genetic and environmental factors to brain connectivity.

Genome-wide association studies

Genome-wide association studies enable the automated analysis of the entire genome. Sequencing costs have plummeted by orders of magnitude during the past ten years, whereas levels of accuracy have increased. Common single nucleotide polymorphisms and other DNA variants significantly associated with disease can be identified. For example, nine genes newly associated with sAD through GWAS were mapped onto pathways linked to immune function, cholesterol metabolism and synaptic membrane processes, offering new angles for research and therapeutic intervention [65]. As susceptibility genes are catalogued and pathways are increasingly clustered, convergent nodes will be identified that could provide targets for new treatments.

The characterisation and reconstruction of molecular interactions related to GWAS-discovered genes for five complex disorders, including AD, suggested that susceptibilities converge on com-mon molecular and biological networks [66]. In AD, unexpected significant relationships between immune function and growth factor signalling pathways were found. Other approaches are being developed to look for rarer novel variants and mutations, such as a pooled-DNA technique with next-generation, high-throughput sequencing and bioinformatics analyses, which found variants in APP and presenilins 1 and 2 that cause or increase the risk of sAD [67]. The deCODE Iceland study identified a low-frequency coding mutation in the APP gene that protects against AD, the first protective sequence variant found [68].

In contrast to genetics, the tools used to discover environmental influences in disease have hardly progressed since the mid 20th century. A new concept to address this is the exposome, envisaged as the totality of a person’s environmental exposures that can be characterised in an unbiased way by measuring all the external exposures (e.g. chemicals, drugs, radiation, infection, stress) that create internal toxins (e.g. produced by inflammation, oxidative stress, lipid peroxidation) in biological fluids, such as blood samples taken at different time points [69]. Exposome research could explore these nongenetic influences in AD, identifying early steps in an AOP for AD.

Systems biology and computational modelling

Computational interpretation, integration and modelling of different levels of human experimental data--molecular, cellular, tissue, organ, clinical and population--is a rapidly progressing approach for understanding complex, nonlinear biological processes. Current research emphasises the discovery of single drug targets for which highly selective ligands are designed. But this strategy might be inadequate when the pathology is complex, as in AD. Many effective drugs act through effects on multiple proteins; systems biology research recognises a dynamic network of cellular pathways that could provide multiple targets for treatment [70].

A systems biology approach is essential for making sense of the data explosion resulting from omics studies, and can give a clearer understanding of cellular disease pathways and progression, as well as helping to identify sensitive and early biomarkers of drug efficacy. The complexity of human illnesses--such as cancer, cardiovascular disease and AD--demands a systems-based under-standing, as does the integration of human molecular, cellular, physiological and environmental data to extend the concept of cellular pathways to the elucidation of disease AOPs (Fig. 1).

The NIH proposes developing quantitative and systems pharmacology (QSP) to advance drug discovery and development by exploiting new knowledge of human cellular and tissue networks [71]. Using QSP to combine computational and experimental methods at multiple scales from biochemistry through to population levels is expected to result in less attrition in drug development, the discovery of new uses of existing drugs and novel tools for translating cell-level discoveries to tissues and to patients.

The development of small computational models of protein signalling pathways for major diseases, including AD, has shown that small-module systems analysis can be performed rapidly to generate new ideas to guide experimental research and to suggest new therapeutic concepts [72], whereas Tg mouse research is costly, time-consuming and oriented to single targets. AlzPathway, a publicly available map of signalling pathways, will help to evaluate candidate risk genes identified by GWAS and to analyse omics data [73].

Combining pathway analysis of GWAS data with composite memory scores in patients has highlighted pathways associated with memory impairment, demonstrating the potential of this technique to elucidate key targets in humans [74]. This study also thereby provided information for starting to build an AOP for AD. Bioinformatic analysis of networks identified by global gene expression profiling in AD brains is generating data about cell-level events in AD pathogenesis. Differential co-expression correlation network analysis of APOE*e4 and AD transcriptomic changes recently identified candidate core regulatory mediators related to sAD, and a gene variant that significantly affected amyloid deposition in human brain and AD age of onset of sAD. The results suggest a molecular pathway associated with APOE*e4 that promotes sAD [57].

Novel technologies are being developed for large-scale screening of protein–protein interactions and for following specific interactions in depth. In 2012, a transcriptional atlas of the adult human brain was generated, combining extensive histology and microarray profiling of about 900 brain areas, based on an analysis of the Allen Human Brain Atlas [75]. Anatomically precise, genomewide maps of transcription patterns complement genomic sequence data and will help correlations of functional and genetic brain architecture.

Recently, data from diffusion tensor imaging and whole-brain microarray gene expression from a single individual have been analysed to identify connectivity between the hippocampi and the rest of the brain,

as well as protein–protein interactions of relevance to AD in the same fibre tracts [76]. This showed that protein–protein interaction data can be related to measurable fibre tract deterioration in AD brains. Large, collaborative, longitudinal studies combining data from multiple technologies such as MRI, PET, CSF and blood biomarkers, genetics and neuropsychology are invaluable for understanding structural and functional connectivity and cognitive outcomes in AD research [77]. Systems biology developments provide, for the first time, a way to make sense of complex systems, such as AD and brain circuitry, with their nonlinear dynamics, multiscale organisation and emergent properties.

Concluding remarks

Animal studies remain dominant in the current paradigm for health research and drug discovery, yet costly and significant translational failures are acknowledged to be unsustainable. In AD research, the focus on Tg mice continues despite their incomplete pathophysiology and unrepresentative etiologies, and a notable lack of evidence for their construct and predictive validity. If animal models are insufficiently predictive then disease and drug discovery research pursued within the traditional framework, with animal data playing a key part, will continue to disappoint. With the inevitable limitations of species variations, the quest for better animal models begins to seem outdated when a suite of advanced techniques could be applied to reliable human-specific models.

In toxicology, a structured and deliberate paradigm change is underway, moving away from animal testing and apical endpoints of toxicity towards a framework built more on advanced in vitro and in silico methods with a focus on human biology and AOPs. Thus, key aspects of the toxicology transition are the structured implementation of next-generation techniques and the shared understanding that animal use will decline, because better science is needed to make faster progress.

This review proposes that a similar change in conceptual think-ing and research practice would benefit health research, argued here in the case of AD. Health research has an advantage over toxicology because the ‘gold standard’ human model is available with the technologies to use it. As discussed in this review, data from human in vivo and modern in vitro models, integrated and interpreted using systems biology and computer modelling, will elucidate human disease AOPs that link causes and effects from external factors through cellular changes to individual and population outcomes. This will help to create the systems-based under-standing of complex diseases that so far has remained elusive, and which provides a core aspect of a new research paradigm.

A modern framework for disease modelling and drug discovery that rationally integrates important new and emerging techniques needs to be considered now. In-depth analyses of the limitations and advantages of animal models and of next-generation human-biology-based in vitro, in silico and in vivo methods could point to research funding and effort being directed away from efforts to improve animal models and towards the further development of non-animal methods. As in toxicology, the expectation would be for more cost-effective and more predictive data (than currently provided by conventional in vitro and animal studies), by reducing reliance on Tg mice, providing earlier human-relevant information and minimising late-stage drug attrition.

Considering change in a major paradigm of medical research is certainly a daunting task, but the probable advantages are substantial. For decades animal models have been seen as core to health research, but therapeutic progress has been slow, increasingly disappointing and costly. Emerging models and tools need further development and validation, but serious discussion and planning of a new framework for basic medical research and drug discovery should start now.

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Conflicts of interest

The author is a consultant to Humane Society International.

Box 1

Classic pathologies of Alzheimer's disease (AD)

Underlying the progressive cognitive deficits of AD are three classic pathologies: extracellular plaques containing amyloid peptides; intracellular neurofibrillary tangles (NFTs); and neuronal degeneration including synaptic loss.

Amyloid plaques occur mainly in the cerebral cortex and in the hippocampus, regions of the brain associated with higher cognition and memory function. The plaque cores are formed from abnormally folded amyloid-b (Ab) peptides, generated from the proteolytic actions of b- and g-secretases on the larger amyloid precursor protein (APP). Presenilins form part of the g-secretase complex which, together with b-secretase, cleaves APP.

According to the amyloid cascade hypothesis, APP processing is abnormally shifted towards Ab production. This leads to increased amounts of Ab39–42 aggregating into insoluble plaque-forming fibrils that disrupt neural function. Amyloid plaques often appear many years before people develop symptoms of AD and amyloid burden alone poorly predicts cognitive function.

Intracellular NFTs comprise hyperphosphorylated and abnormally aggregated forms of tau protein. Normal tau promotes the assembly and stability of neuronal microtubules but when hyperphosphorylated it aggregates into NFTs in neurons, leading to cell death. The deposition of tau aggregates correlates spatially and temporally with the development of dementia in AD. However, despite the obvious significance of tau in AD, no tau mutations have yet been associated with the human disease.

Synaptic and neuronal degeneration comprise the third characteristic pathology of AD. Changes in synaptic density correlate strongly with decline in cognitive ability. The neurons of the hippocampus and the association areas involved in all other cognitive functions become increasingly dysfunctional, with loss of dendritic spines and synapses. Neurotransmitter pathways start to fail, notably but not only the cholinergic system. Progressive brain atrophy, particularly in the neocortex and hippocampus, is observable in structural magnetic resonance imaging (MRI) scans.

Box 2

Common reasons for failures of translational research

- Methodological flaws and poor design in preclinical studies, leading first to systematic bias and then to inadequate data and incorrect views of efficacy.
- Critical disparities between humans and animals (and also within animal species), including pathophysiological differences, which result in unreliable outcomes.
- Disparities between animal studies and clinical trials (e.g. in co-morbidities, the use of co-medication, the stage of disease, the timing and dosing of the test treatment, and selection of outcome measures).
- Insufficient reporting of details of animals, materials and methods.
- Publication bias, which could account for a substantial part of the efficacy reported in the experimental literature for various diseases.

Box 3

Considering the validity of a human disease model

An established framework posits three types of validators: face, construct and predictive validity. Face validity addresses phenomenological similarities between a model and a human disease. Although useful in the development of a new model, face validity is less suitable for validating a model, because superficial similarities do not always have the same underlying mechanisms or pathways. Construct validity addresses the theoretical rationale, asking whether the model reflects the etiology and underlying mechanisms or pathways of the human disease, and if the outcome measures match the clinical setting. Predictive validity asks whether a model accurately predicts what happens in humans, especially the effects of therapeutic interventions. It is a key factor in translational science.

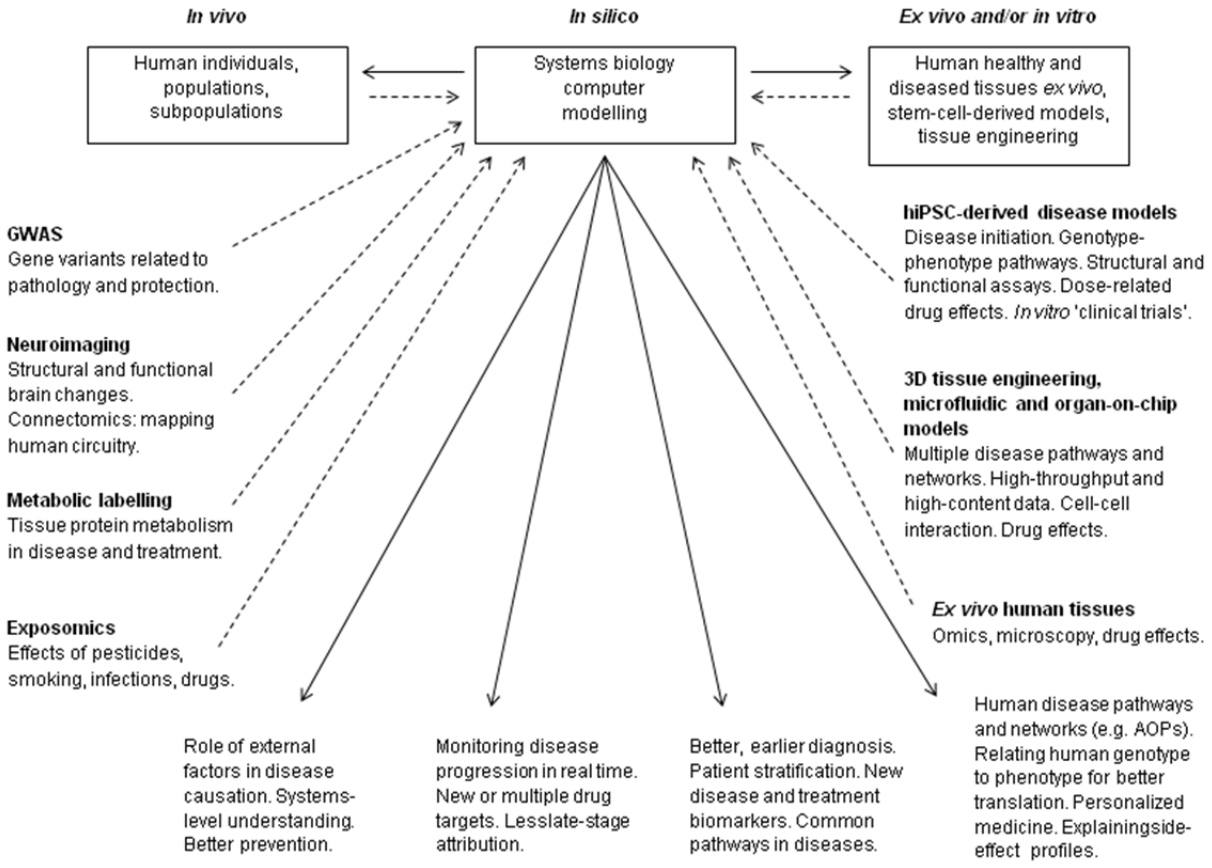

Figure 1: Likely information sources and data outputs in a new research paradigm using human-specific models to understand disease pathways. Data from 21st-century human in vivo and in vitro models, integrated and interpreted using systems biology and computer modelling, could help elucidate human disease AOPs (linking causes and effects for human diseases) from external factors through cellular changes to individual and population outcomes. This will help to create a systems-based understanding of complex diseases that so far has remained elusive. Black arrows indicate information sources, red arrows indicate data outputs. Abbreviations: AOPs, adverse outcome pathways; GWAS, genome-wide association studies; hiPSC, human induced pluripotent stem cells; omics, group including genomics, transcriptomics, proteomics and epigenomics.

Table 1: Results from Tg mouse studies compared with recent Alzheimer's disease (AD) clinical trial outcomes

Generic drug name (trademark name) and company	Proposed mechanism of action	Preclinical Tg mouse results	Clinical trial results	Refs
AN1792 Elan	Aβ1–42peptide, active immunotherapy targeting amyloid pathway.	Generated anti-Aβ antibodies that reduced plaques, neuritic dystrophy and astrogliosis in older PDAPP mice and prevented these pathologies in younger animals. No meningoencephalitis.	Phase II, terminated 2002: removed plaque but failed to affect cognitive decline. No improved survival or time to severe dementia. Several patients developed autoimmune meningoencephalitis.	[78,79]
Tramiprosate (Alzhemed™), Neurochem	An amino acid that binds to Aβ monomers to prevent plaque formation.	In TgCRND8 mice reduced brain amyloid plaque by 30% and reduced plasma Aβ levels.	Phase III: no cognitive improvement, no significant treatment effect seen. Withdrawn from development.	[80,81]
Tarenflurbil (Flurizan™), Myriad	Gamma-secretase modulator intended to reduce Aβ.	Attenuated spatial learning deficits if given early in Tg2576 mice .Older Tg2576 mice had significant decrease in plaques.	Phase III: did not slow cognitive decline or delay loss of normal daily activities. Discontinued for AD indications.	[82,83]
Semagacestat , Eli Lilly	Gamma-secretase inhibitor, intended to reduce production of Aβ plaques.	Reduced plaques and lowered Aβ in plasma, CSF and brain in a dose-dependent manner in PDAPP Tg mice.	Long-term Phase III: failure to slow disease progression and did not improve cognitive status. Patients on higher dose had significant worsening of functional ability. Trial stopped 2010.	[84,85]
Tideglusib , Noscira	Inhibitor of glycogen synthase kinase- 3, intended to reduce tau hyperphosphory lation.	In Tg mouse models, it reduced lesions including Aβ and tau deposits, gliosis and neuronal loss, and significantly improved behavioural impairments.	Phase II, 2012: company announced that primary cognitive end point and two of the secondary endpoints were not met (http://zeltia.com/actualidad.cfm?anyo=2012&semestre=2)	[86]
Rosiglitazone (Avandia®), GlaxoSmithKline	Antidiabetic drug that activates peroxisome proliferator-activated receptors.	Reduced Aβ1–42 levels without affecting amyloid deposition, and improved spatial learning and memory function in Tg2576 mice.	Phase II: modest cognitive improvement in non-*APOE*e4* subjects but decline in *APOE*e4* patients. Phase III: no significant cognitive efficacy at any dose, in any test group. Trials discontinued.	[87,88] [89]
Atorvastatin (Lipitor®), Pfizer	Statin targeting amyloid pathway.	Markedly attenuated brain Aβ deposition in PSAPP doubly Tg mice.	Large scale randomised controlled trial for mild-to-moderate AD: no benefit to cognition or global function compared with placebo.	[90,91]
Bapineuzumab , Pfizer and Janssen Al	Humanised monoclonal antibody: passive immunotherapy intended to bind and clear Aβ.	An anti-Aβ monoclonal antibody (3D6) substantially prevented and/or reduced amyloid deposits in cerebral vasculature in PDAPP mice.	Phase II: weak to nonexistent clinical benefits on cognition. High doses caused brain oedema and microbleeding in some patients. Phase II: decreases in total tau and phosphorylated tau in CSF. No clear changes in CSF Aβ. Two Phase III trials, 2012 : no clinical benefit in mild-to-moderate AD compared to placebo. Development scaled back.	[92,93] [94] [95]
Solanezumab , Eli Lilly	Humanised monoclonal antibody: passive immunotherapy intended to bind and clear soluble Aβ.	Acute and subchronic treatment of Tg mice attenuated or reversed memory deficits.	Two Phase III trials, 2012: dose-dependent increase in unbound cerebrospinal fluid Aβ1–42, but no change in cognitive decline or functional ability in mild and moderate AD. Secondary analysis suggested it slowed cognitive decline in patients with mild AD. (http://www.hcplive.com/conferences/aan-2013/Efficacy-and- Safety-of-Intravenously-Administered-Solanezumab-in-the- Treatment-of-Patients-with-Mild-to-Moderate-Alzheimers-Disease)	[96,98]
Latrepirdine (Dimebon), Pfizer and Med ivation	Oral antihistamine with proposed but unproven effect on mitochondria.	Treated TgCRND8 mice showed improved learning behavior and less accumulation of Aβ1–42 and α-synuclein (conducted after clinical trial).	Phase II results, Russian trial 2008: improvements in cognitive, global, daily function and behavior endpoints. Phase III, 2012: patients with mild-to-moderate AD showed no improvement in cognition or functional ability.	[99,100] [101]

Abbreviations: Aβ, amyloid-β; CSF, cerebrospinal fluid; PDAPP mice, (Tg) transgenic mice overexpressing mutant human amyloid precursor protein V717F.

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