Table 1 | Human prion diseases

Disease	Cause	Comments
Sporadic CJD	Unknown	Worldwide incidence: one or two cases per million individuals per year
Genetic prion disease	Mutations of *PRNP* gene	Various phenotypic expressions: genetic CJD, Gerstmann–Sträussler–Scheinker syndrome, fatal familial insomnia
Iatrogenic CJD	Accidental transmission	Neurosurgery, cadaver-derived hormones, dura mater grafts
Variant CJD	Human dietary products contaminated by bovine spongiform encephalopathy	Three secondary cases via blood transfusion

Abbreviation: CJD, Creutzfeldt–Jakob disease.

for <1% of sCJD cases. The atypical nature of the cases is also reflected in the illness duration: the mean duration of nine of the sCJD cases was 12.2 months, and a further two cases were alive at over 17 months. The mean duration of sCJD in most countries is around 4.5 months, and correlates with various independent factors, including *PRNP* genotype and prion protein type.[7] The atypical nature of the group studied by Lodi *et al.* calls into question the generalizability of their conclusions.

A substantially larger and more disease-type-representative study of MRI was published shortly after the Lodi *et al.* study. Zerr *at al.* evaluated MRI findings in 436 patients with sCJD and 141 controls.[4] Using pre-specified criteria based on caudate–putamen and cerebral cortical signal changes on fluid-attenuated inversion recovery and DWI sequences, the researchers found positive MRI results in 83% of cases. The proportions of *PRNP* 129 genotypes in this study—62.7% MM, 18.6% MV and 18.6% VV—were more representative of the sCJD population as a whole than were those in the Lodi *et al.* study. On the basis of their results, Zerr *et al.* propose a modification of the current clinical diagnostic criteria to include these MRI abnormalities as supportive findings.[4]

Any treatments that are developed for prion disease are likely to be most effective early in the disease process, necessitating prompt diagnosis. An understanding of the timing of emergence of MRI abnormalities in relation to the disease course is, therefore, important. In the Lodi *et al.* study, MRI was undertaken at a mean of 8 months into the disease (median 6 months), partly reflecting the atypically long durations of their cases. In the Zerr *et al.* study, MRI was undertaken at a median of 2.7 months of illness, but this is still over halfway into the average sCJD illness. MRI might, therefore, not provide diagnostic support in the very early disease phase, when treatment is likely to be most useful. Naturally, in a clinical context, MRI is undertaken only when appreciable symptomatic brain illness emerges, making it difficult to discover whether useful MRI changes are occurring at an earlier disease stage.

Cerebral imaging in prion disease has come a long way over the past few years. Initially, imaging was largely a matter of excluding other possible diagnoses. One especially fortunate finding was that vCJD is associated with a striking MRI abnormality in the form of the 'pulvinar sign',[2] and an important role for MRI in the diagnosis of the more common sCJD is now emerging. Underlying this progress is the development of more-powerful MRI machines and new MRI sequences (such as DWI). These developments inevitably bring problems, such as the necessity to compare data from equivalent MRI machines and questions of standardization of methods.

Lodi *et al.* reported unique findings with regard to ^{1}H-MRS, an MRI technique that can be performed relatively easily and quickly in modern MRI departments. ^{1}H-MRS allows measurement of *N*-acetylasparate (a neuronal marker) and myoinositol (a glial marker). Previous study of these markers in sCJD was largely confined to individual case reports, whereas Lodi *et al.* studied a series of consecutively presenting suspect cases. Their results should prompt further studies to determine the diagnostic utility of ^{1}H-MRS and its role relative to more-standard MRI studies.

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ALZHEIMER DISEASE

New light on an old *CLU*

Lars Bertram and Rudolph E. Tanzi

Genome-wide association studies (GWAS) have uncovered over two dozen candidate Alzheimer disease susceptibility genes; however, the results of these studies showed limited overlap. Two independently performed GWAS involving cohorts from Europe and the US have now identified three additional putative Alzheimer disease genes that show modest but remarkably consistent effects across data sets.

Alzheimer disease (AD) is a fatal neurodegenerative disorder characterized neuropathologically by the extracellular accumulation of amyloid-β (Aβ) plaques, and the intracellular accumulation of hyperphosphorylated tau protein in the

form of neurofibrillary tangles. AD is highly heritable, but its genetic architecture is complex, making genetic analysis difficult. 11 genome-wide association studies (GWAS) have been published, and have identified over two dozen novel AD candidate loci.[1] Until recently, however, little or no overlap was observed between the results of different studies. Two large-scale GWAS conducted by Lambert *et al.* and Harold *et al.* have now pinpointed three novel putative susceptibility genes for AD—*CLU*, *CR1* and *PICALM*—which exert modest effects on AD risk, but show strong correlation with AD risk when all available data are combined. Notably, the most prominent result in both studies was the association between AD risk and the clusterin-encoding *CLU* gene, which is located on the short arm of chromosome 8. Owing to its functional relationship with apolipoprotein E (*APOE*), *CLU* (also known as apolipoprotein J) was previously studied as a candidate gene in AD. These earlier studies, which probed for a potential association between *CLU* and AD risk, however, lacked power due to insufficient sample sizes and other methodological issues, precluding earlier recognition of this locus as an AD risk gene.

> “...*CLU*, *CR1* and *PICALM* were identified as risk factors for AD...”

Numerous studies utilizing conventional genetic linkage and positional cloning experiments have revealed that mutations in the genes encoding amyloid precursor protein, presenilin 1 and presenilin 2 can lead to altered production of Aβ, which is sufficient to cause rare, early-onset (~50 years of age) familial forms of AD. The vast majority of AD cases, however, are of later onset (>65 years of age), and this latter form of AD is widely believed to be influenced by an array of low-penetrance, common risk alleles. These alleles probably affect a variety of pathways, many of which are believed to be involved in the production, aggregation and removal of Aβ. Although the total number of AD risk genes (and their precise identity) remains unclear, good evidence exists to suggest that, in combination, these genes have a substantial impact on disease predisposition and age of onset. In the search for these late-onset AD genetic risk factors, hundreds of loci have been assessed in well over 1,200 genetic association studies. A common misconception in the field is that, overall, these studies have not yielded any consistent results besides the discovery of an association between the *APOE* ε4 allele and AD. In fact, over 30 different loci have been identified that seem, on systematic meta-analysis of the available data, to be associated with AD risk (for an up-to-date overview, see the ‘AlzGene’ database[4]). The novel genes highlighted in the recent studies by Lambert *et al.* and Harold *et al.* could, therefore, be thought merely to extend this list by three additional loci.[2,3] Several characteristics, however, set these new studies apart from most other previous AD genetic studies. As a result, *CLU*, *CR1* and *PICALM* are placed high on the current ‘Top Results’ list on AlzGene, which ranks AD genetic studies according to a combination of criteria, including effect size, *P* value, degree of heterogeneity, and assessment for reporting biases.[4]

First, and most importantly, *CLU*, *CR1* and *PICALM* were identified as risk factors for AD by means of a genome-wide association approach. GWAS simultaneously probe hundreds of thousands of genetic markers, in an essentially unbiased and hypothesis-free manner. The fact that both novel GWAS independently found *CLU* to be associated with increased AD risk is particularly interesting, given the vast number of genetic markers that were assessed. Second, the overarching theme of previous genetic association studies in complex diseases has been the lack of independent replication. In view of the probable small effect sizes exerted by any one of these loci, the inconsistencies might be at least partially explained by a lack of power due to insufficient sample size of the individual studies. Lambert *et al.* and Harold *et al.* overcame this limitation by combining large data sets for both their initial genome-wide analyses and the follow-up genotyping. The two-stage GWAS conducted by Lambert and colleagues involved nearly 15,000 participants from Europe, and the study conducted by Harold *et al.* included a similar number of individuals (>16,000) from Europe and the US. In addition, both studies have already provided independent replication for each other’s main findings, although only *CLU* showed genome-wide significant association within each study individually. Third, additional independent replication of the new GWAS results for two of the three novel loci, *CR1* and *PICALM*, was actually already (and unknowingly) provided by previously published AD GWAS.[5–7] Last, when all the available genetic evidence is classified using recently proposed guidelines for the cumulative assessment of genetic association data, all three loci show strong epidemiological credibility, a characteristic shared by only half a dozen other loci on the AlzGene ‘Top Results’ list.

CLU has long been considered to be a candidate gene for AD and, accordingly, a wealth of functional data has accrued over the past 15 years. On the basis of these data, the leading hypothesis proposes that clusterin, a 75 kDa chaperone protein that is expressed at high levels in the brain, binds to and promotes the clearance of Aβ, a function potentially shared with *APOE*. *APOE* is believed to aid the export of Aβ out of the brain into plasma; clusterin, however, is also believed to aid the re-entry of Aβ back into the brain. Excessive re-entry would be predicted to lead to enhanced Aβ levels in the brain, effectively impairing its clearance.[8]

The potential roles of *CR1* and *PICALM* in AD are less well established. *CR1* encodes complement component (3b/4b) receptor 1, the main receptor of complement C3b protein. Like clusterin, C3b binds Aβ and could promote its clearance.[9] *PICALM* encodes phosphatidylinositol-binding clathrin assembly protein, a protein that is involved in VAMP2 trafficking—a process involved in synaptic neurotransmitter release.[10] Neurotransmitter release is compromised early in the brains of individuals with AD, and Harold *et al.* speculate that the association between AD risk and *PICALM* might be linked to the production of Aβ via clathrin-mediated endocytosis.[3] These potential molecular mechanisms all require further investigation; however, the consistency of the novel genetic results identified by Lambert *et al.* and Harold *et al.* clearly warrant intensive genetic fine mapping and resequencing to identify the actual pathogenic variants.

> “...only *CLU* showed genome-wide significant association within each study...”

In conclusion, the two recently published GWAS seem to have pinpointed at least one—and potentially more—bona fide AD risk genes. It must be pointed out, however, that the effects of these genes on AD risk are

quite modest. Indeed, these effects are more than an order of magnitude lower than the effect of the *APOE* ε*4* allele on AD risk. Further studies will be needed to clarify the functional basis of these associations. Only after this objective is accomplished can we be sure that we have identified novel AD risk genes. Identification of novel AD risk genes is necessary if we are to improve our capacity for early prediction and, hopefully, prevention of this devastating disease.

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Competing interests
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PAIN

Noninvasive functional neurosurgery using ultrasound

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Ultrasound-based technologies are emerging as promising noninvasive approaches to treat brain disorders. Researchers in Switzerland have shown that chronic pain can be alleviated through thermal ablation of thalamic tissue by high-intensity focused ultrasound.

Burgeoning neurotechnologies are providing clinicians with the means to meet one of the greatest challenges in modern medicine—the effective treatment of brain disorders. The neurotechnology community is faced with the task of developing medical interventions that balance the level of necessary invasiveness with therapeutic efficacy. Deep brain stimulation, for example, has been shown to be remarkably effective in treating neurological disorders such as Parkinson disease, but it necessitates the surgical implantation of electrodes and microcontroller devices. Transcranial magnetic stimulation is noninvasive, but is hampered by poor spatial resolution and brain penetration power, and its robustness in treating psychiatric disorders such as depression remains hotly debated. Gamma knife radiosurgery is another noninvasive approach that has been used to treat various brain diseases, but concerns have been raised regarding the adverse effects of its ionizing radiation on tissues surrounding the treatment zones. More than eight decades of basic science and clinical research efforts have culminated in the emergence of ultrasound-based technologies that could provide new and improved noninvasive standards of care in neurological and psychiatric medicine. A phase I trial conducted by Ernst Martin and colleagues in Zurich, Switzerland has illustrated the efficacy of one such approach in patients with chronic pain.[1]

Ultrasound is routinely used for many types of diagnostic imaging, but has been largely overlooked in favor of other therapeutic modalities for the clinical management of CNS disorders. In 1929, Edmund Harvey Newton first demonstrated that ultrasound has an effect on neural tissues by showing that it was capable of stimulating nerve and muscle.[2] Several decades later, William Fry and colleagues demonstrated the production of functional lesions in the mammalian brain through ultrasound, in a technique that they later termed 'neurosonicsurgery'.[3] By the late 1950s, Fry and his colleagues were implementing high-intensity ultrasound to treat patients with movement disorders.[5,6] Despite some preliminary success, ultrasound as a neurotherapeutic tool was mostly discounted by the medical community at the time, since the treatment procedures required major craniotomy to enable focusing of ultrasound through the skull.

Technological breakthroughs over the past few years have made it possible to transmit and focus ultrasound through the intact human skull. To enable noninvasive treatment of brain tissues with ultrasound, Kullervo Hynynen and colleagues have refined an image-guided technique known as MRI-guided focused ultrasound (MRgFUS), which is presently used to thermally ablate uterine fibroids by means of high-intensity focused ultrasound (HIFU).[7,8] In the general MRgFUS approach to treating brain tissues with ultrasound, a CT scan is first used to generate information about the density of skull bone. Algorithms are then used to predict the location of ultrasound beams by correcting for distortions and aberrations produced by the transcranial transmission of ultrasound. To maximize the targeting accuracy, ultrasound transducers are arranged in a hemispheric phased array that transmits ultrasound to targeted brain regions, while MRI-based thermometry continuously provides feedback of ultrasound beam location and focusing in a closed-loop system.

The findings from a phase I clinical study published in *Annals of Neurology* represent a major step forward in bringing ultrasound-based treatments of brain diseases to fruition. Martin and colleagues used transcranial MRI-guided HIFU (tcMRgHIFU) to perform lesions of the central lateral thalamic nuclei in nine patients with chronic neuropathic pain.[1] These interventions relied on the capacity of

CORRECTION

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In the January 2010 issue of *Nature Reviews Neurology*, an incorrect reference was cited. Reference 4 should have been: Bertram, L., McQueen, M. B., Mullin, K., Blacker, D. & Tanzi, R. E. Systematic meta-analyses of Alzheimer disease genetic association studies: the AlzGene database. *Nat. Genet*. **39**, 17–23 (2007).