News in brief

Targets and mechanisms

Endogenous antibiotic against bacterial infection

Researchers have shown that an endogenous peptide acts as an antibiotic in mammalian cells [1]. The scientists, from the University of California, San Diego (UCSD) School of Medicine and the Veterans Affairs (VA) San Diego Healthcare System (San Diego, CA, USA), conducted research in mice and experimental culture systems, focussing on peptides called cathelicidins (caths), which are found in various mammalian tissues, including the skin, lungs, intestines and circulating white blood cells.

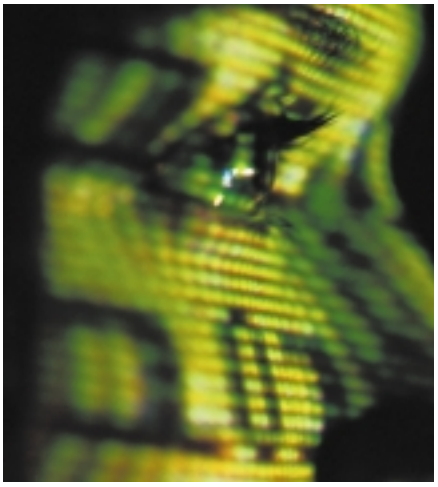
Richard Gallo, senior author of the study, UCSD Associate Professor of Medicine and Pediatrics, and chief of the dermatology section at the VA, said 'Although we've suspected for nearly 20 years that certain anti-microbial peptides contribute to the immune system's first response in fighting infection, we've never had proof of the precise mechanism in living animals until now.'

The research centred on the cath protein in mice, and used two approaches: (1) genetic engineering to produce mice without caths, and (2) genetic selection for bacteria with altered cath resistance. When cath-deficient mice were infected with group A *Streptococcus* (GAS) – chosen because of its high sensitivity to cath anti-microbial action – they developed rapidly growing lesions similar to human infection. To test the second part of the experiment, a mutant of GAS that was resistant to caths *in vitro* was used to infect normal mice, resulting in large lesions like those seen in cath-deficient mice.

The study's first author, Victor Nizet, UCSD Assistant Professor of Pediatrics and an infectious disease specialist, said, 'Overuse of pharmaceutical antibiotics often leads to bacterial resistance. The natural antibiotic we studied, however, has continued to be effective in killing bacteria for tens of thousands of years. With further study of its properties and the bacterial genes that determine sensitivity or resistance, we hope to gain insight into why and how some bacteria develop resistance to antibiotics while others do not.'

1 Nizet, V. *et al.* (2001) Innate antimicrobial peptide protects the skin from invasive bacterial infection. *Nature* 414, 454–457

A trillion computers in a 100 μl droplet!



A nanocomputer consisting of trillions of computers in a miniscule water droplet has been developed by scientists at the Weizmann Institute of Science (Rehovot, Israel) [2]. Devices that convert information from one form into another are called automata. Ehud Shapiro and colleagues have used biological molecules to build a programmable, two-state, two-symbol, finite automaton. Until now, computing devices operating autonomously on the molecular scale have been rarely reported.

The computer's input and output software is made up of DNA molecules and the hardware comprises two naturally occurring DNA-manipulating enzymes, Fok I and ligase. When the hardware and software are mixed in solution, the molecules create an output from the input molecule; thus, forming a simple mathematical computing machine.

The nanocomputer can be programmed by choosing different subsets of input molecules and could be used, for example, to determine whether, in an input molecule consisting of a list of 0s and 1s, the 0s precede all the 1s. A total of 735 different programs can be created by varying the input software molecules.

Shapiro explains the idea behind their research, 'The living cell contains incredible molecular machines that manipulate information-encoding molecules such as DNA and RNA in ways that are fundamentally very similar to computation. Since we don't know how to effectively modify these machines or create new ones yet, the trick is to find naturally existing machines that, when combined, can be steered to actually compute.'

The nanocomputer uses the four bases of the genetic code to encode the input data and the program rules that comprise the software. The sticky end (created by a single-strand overhang in the DNA molecule) of the input molecules encodes the current symbol and the current state of the computation, whereas the sticky end of the software molecule is designed to detect a particular state–symbol combination. Thus, a two-state, two-symbol automaton has four such combinations. For each of these, the computer has two possible next moves – to change to the next state or remain in its current state. The way in which the computer executes its next move is facilitated by the DNA-manipulating enzymes. The resulting molecule, the output, can then be analyzed by gel electrophoresis.

This nanocomputer is too simple to have immediate use, but is a starting point for future computers that can operate within the body and have unique therapeutic functions.

2 Beneson, Y. *et al.* (2001) Programmable and autonomous computing machine made of biomolecules. *Nature* 414, 430–434

Atomic bomb for cancer cells

An innovative anti-cancer weapon has been developed that fires atomic fragments from molecular-sized generators at tumour cells [3]. Researchers at the Memorial Sloan-Kettering Cancer Center (MSKCC; New York, NY, USA) have found a way to capture a single radioactive actinium atom (^{225}Ac) inside a molecular cage to form a nanogenerator. This is attached to an internalizing monoclonal antibody that targets the nanogenerator to the tumour cell.

In vitro studies showed that the alpha-emitting isotope delivered by the construct killed leukaemia, lymphoma, breast, ovarian, neuroblastoma and prostate cancer cells at picocurie levels. Moreover, a

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significant proportion of mice with solid prostate carcinoma or disseminated human lymphoma had prolonged survival and tumour regression after a single, nanocurie dose of the nanogenerator construct, with no associated toxicity. David Scheinberg, senior author of the study, says his team hopes to start clinical trials of the nanogenerator, probably against leukaemia, in 2002. They believe that it might be possible to use nanogenerators to target a wide variety of cancers.

3 McDevitt, M.R. *et al.* (2001) Tumour therapy with targeted atomic nanogenerators. *Science* 294, 1537–1540

A good night's sleep...

The first molecular pathway involved in the rejuvenating effects of sleep has been identified by researchers at the University of Pennsylvania (Philadelphia, PA, USA) [4]. The finding could undercover the basic mechanisms of how sleep is controlled, thereby helping those with changing sleeping schedules and those with sleep disorders.

Using sleep-deprived *Drosophila melanogaster* (fruit fly), researchers found that the duration of rest and rest rebound (the need to sleep after long periods of wakefulness) was inversely related to the level of cAMP signalling and cAMP response element binding protein (CREB) activity.

The findings also indicate that cAMP signalling and CREB activity are not related to a circadian role for CREB activity in triggering the onset of rest. By contrast, it is probable that CREB serves as a restorative function of rest that permits sustained wakefulness.

CREB is evolutionarily conserved in species from flies to humans, and has an important role to play in learning in *D. melanogaster*. The new work could strengthen links between rest and the consolidation of memory.

4 Hendricks, J.C. *et al.* (2001) A non-circadian role for cAMP signaling and CREB activity in *Drosophila* rest homeostasis. *Nat. Neurosci.* 4, 1108–1115

Clinical trials

Arrested development of type 1 diabetes

Scientists at the Hadassah-Hebrew University Medical School (Jerusalem, Israel) and the Weizmann Institute of Science (Rehovot, Israel) have shown that a small peptide fragment (p277) can halt the progression of insulin-dependent type 1 diabetes [15]. The experimental drug DiaPep277 (developed by Peptor, Rehovot, Israel) is a synthetic version of p277, which itself is derived from hsp60, a 60 kDa heat shock protein; these are produced when the body is stressed or shocked and function as molecular chaperones. DiaPep277 was shown, in Phase II clinical trials, to arrest the progression of type 1 diabetes, prevent destruction of insulin-producing pancreatic cells and reduce the need for injected insulin in newly diagnosed patients.

In type 1 diabetes, the immune system attacks and destroys the β cells of the pancreas, which leaves the pancreas unable to produce insulin. Late-stage complications of type 1 diabetes include heart disease, stroke, high blood pressure, blindness, kidney disease, nervous system damage and pregnancy complications, leaving sufferers with a lifespan that is 15 years shorter than average.

The research was performed in a 10-month randomised, double-blind study of 35 men recently diagnosed with type 1 diabetes. Eighteen patients received injections of DiaPep277 at one-month and six-months into the study; the remainder received placebo. Those that received DiaPep277 showed a halt or delay in the attack or destruction of the β cells by the immune system. The mechanism of this improvement was traced back to changes in the immune lymphocytes, or T cells. No significant side effects of DiaPep277 were found.

Dana Elias, Vice President of R&D at Peptor, said 'Our research has shown that it is possible to modulate the immune system and prevent or stop it from attacking the insulin-producing cells. DiaPep277 holds the promise of becoming a breakthrough therapy for those already diagnosed with autoimmune diabetes, and perhaps a preventive treatment for those at high risk for the disease.'

15 Raz, I. *et al.* (2001) β-Cell function in new-onset type 1 diabetes and immunomodulation with a heat-shock protein peptide (DiaPep277): a randomised, double-blind, Phase II trial. *Lancet* 358, 1749–1753

Cystic fibrosis: a new theory

Scientists have proposed a new theory for the infections that are found in cases of cystic fibrosis [5]. The researchers, at the University of Michigan Medical School (Ann Arbor, MI, USA) and the University of New Mexico Health Sciences Centre (Albuquerque, NM, USA), showed that the gene which is mutated in cystic fibrosis (CF) can alter the internal chemistry of lung cells, making them more susceptible to certain pathogenic bacteria.

'This research proposes a novel explanation for why lung infections are so persistent in CF,' said Christopher Taylor at the National Institute of Allergy and Infectious Diseases (NIAID), which funded the study.

Cystic fibrosis causes chronic respiratory infections and sufferers ultimately die from a build-up of *Pseudomonas aeruginosa*, which rarely causes disease in healthy humans. The CF gene, known since 1989, encodes for the CF transmembrane conductance regulator (CFTR), which regulates ion conductance and is essential for the proper functioning of cells. However, it was not known how an ion imbalance could make the lungs an attractive site for bacterial colonisation. Defective CFTR leads to the undersialylation of plasma membrane glycoconjugates, which promote lung pathology and bacterial colonisation. The researchers showed, by imaging with lumenally exposed pH-sensitive green fluorescent protein, that dysfunctional CFTR causes excessive acidification of the trans-Golgi network in CF lung epithelial cells. The features were corrected by incubating CF respiratory epithelial cells with weak bases.

If these findings are further substantiated, the researchers see the potential for a new approach to treatment. 'We already have ion pump inhibitors and antacids for treating heartburn', says Vojo Deretic (University of New Mexico). 'If we can design similar compounds to go to the lungs, we might have a simple solution to greatly improve the health of CF patients.'

5 Poschet, J.F. *et al.* (2001) Molecular basis for defective glycosylation and *Pseudomonas* pathogenesis in cystic fibrosis lung. *Proc. Natl. Acad. Sci. U. S. A.* 98, 13972–13977

T cell molecules linked to ischaemia

A subset of T cells has been implicated in the pathogenesis of ischaemia in acute renal failure (ARF) [6]. ARF is associated with an overall mortality rate of 50% and, despite developments in dialysis treatment, mortality rates have not improved over the past 30 years. In addition, ischaemic ARF greatly reduces the success of kidney transplants.

Mice with induced ischaemia and kidney failure bred without $CD4^+$ cells (or helper T cells) were found to be protected from damage by ~40%. When normal $CD4^+$ cells were reintroduced into the mice, the amount of ischaemic damage increased, confirming that the $CD4^+$ cells somehow mediate the damage.

Investigating further, the researchers found that ischaemic damage could be caused by certain molecules on the surface of the $CD4^+$ cells. Adoptive transfers of cells that lacked either CD28 or the ability to produce IFN-γ, were inadequate to restore injury phenotype, suggesting that CD28 and IFN-γ molecules contribute to the pathogenesis of the ischaemia.

The study of T cells in ARF is a novel approach, and could use developments in T cell biology to devise novel ARF therapies. The finding could also apply to all areas of ischaemia, including that caused by strokes and heart attacks.

6 Burne, M.J. *et al.* (2001) Identification of the $CD4^+$ T cell as a major pathogenic factor in ischemic acute renal failure. *J. Clin. Invest.* 108, 1283–1290

Structure of cellular motor uncovered

Researchers have determined the crystal structure of a complex called Arp2/3 [7], which is involved in the movement of cells. The complex is one of the largest asymmetrical protein structures to be determined by X-ray crystallography at high resolution.

The Arp2/3 complex is an assembly of seven proteins, which initiate actin polymerization in eukaryotic cells. Actin polymerization is thought to push the cell forward, enabling movement. The complex is inactive until stimulated by WASp/Scar proteins, which require activation by chemotactic signalling pathways that guide the direction of cellular movement. Using highly purified crystal structures of bovine Arp2/3, researchers at Yale University (New Haven, CT, USA) and the Salk Institute (La Jolla, CA, USA) determined the complete structure of the complex.

The structure provides insight into the overall shape of the complex and the arrangement of the seven subunits. It could also help to explain how the complex is activated; researchers now predict that WASp/Scar proteins activate the Arp2/3 complex by bringing Arp2 into proximity with Arp3, thereby enabling nucleation of pre-existing filaments of the actin-related proteins.

Although more work is needed to characterize the activation of the complex and the mediation of filament branching, the new structure provides a foundation for detailed analysis of these mechanisms and studies of actin filament dynamics in cells.

7 Robinson, R.C. *et al.* (2001) Crystal structure of Arp2/3 complex. *Science* 294, 1679–1684

IFN-α implicated in paediatric lupus

Abnormal secretion of interferon-α (IFN-α) has been linked to the paediatric form of the autoimmune disease, systemic lupus erythematosus (SLE) [8]. The finding could lead to better gene therapy for the disease.

SLE is a disease of the immune system that causes damage to the kidneys, skin, heart and other organs, and can be fatal without early treatment. As dendritic cells are important in regulating immunity and tolerance, it was thought that the disease might be characterized by alterations in these cells.

The study used blood samples taken from 70 SLE patients of 7–18 years old with a similar number of age-matched controls. Circulating IFN-α in the SLE patients induces monocytes to differentiate into dendritic cells, which capture foreign agents such as apoptotic cells and nucleosomes in SLE blood. Subsequent presentation of these autoantigens to $CD4^+$ cells could initiate the expansion of autoreactive T cells, followed by differentiation of autoantibody-producing B cells. It is thought that these autoantibodies could form complexes with circulating nucleosomes, thus sustaining IFN-α production.

Blocking the abnormal production of IFN-α in patients with SLE could therefore represent a potential target for therapeutic intervention.

8 Blanco, P. *et al.* (2001) Induction of dendritic cell differentiation by IFN-α in systemic lupus erythematosus. *Science* 294, 1540–1543

X marks the spot

Recent research has uncovered new clues about how mental retardation develops in individuals with fragile X syndrome [9,10]. The condition is caused by an inherited loss of fragile X mental retardation protein (FMRP), with symptoms ranging from mild-to-moderate cognitive and behavioural deficits to subtle facial malformations.

The disease is characterized by long and spindly dendritic spines, which are required for communication with other neurons. The new research suggests that FMRP could regulate the production of specific proteins that bind to mRNA molecules, which carry genetic information from the nucleus to ribosomes. This function is essential for learning and memory. Specific FMRP binding sites in a population of mRNAs was found to be abnormally regulated in fragile X patients.

The proteins that are coded by these mRNAs could underlie the problems found in these patients. Most of the mRNAs identified were those that are involved in some aspect of synaptic biology, where information is exchanged between the axon of one neuron and the dendrite of a second neuron.

'It is possible that FMRP is responsible for shuttling certain proteins out to the individual dendritic spines of neurons, and/or subsequently activating them at the appropriate time during development as well as during adult memory function,' said Jennifer Darnell, a Research Assistant Professor at the Rockefeller University (New York, NY, USA). The discovery could provide new ways to treat the disease by manipulation of the individual mRNAs or the resulting proteins responsible for the various symptoms of the disease.

9 Darnell, J.C. *et al.* (2001) Fragile X mental retardation protein targets G quartet mRNAs important for neuronal function. *Cell* 107, 489–499

10 Brown, V. *et al.* (2001) Microarray identification of FMRP-associated brain mRNAs and altered mRNA translational profiles in fragile X syndrome. *Cell* 107, 477–487

Gene profiling predicts treatment outcome in lung cancer

Two reports [11,12] have brought new order to the classification of lung cancer based on differential gene expression in tumours. Clinicians could use this information to decide which treatment is likely to be successful and to design better lung cancer drugs.

Compared with lung tumours such as small cell, squamous cell or large cell carcinomas, the prognosis of patients with other tumour types, such as adenocarcinoma, is hard to predict. This is because the response to standard chemotherapy regimes varies greatly between patients. Both groups have used DNA microarray technology to examine the global RNA expression in normal and lung tumour samples. The use of microarrays has been previously documented in the search for patterns of gene expression in other cancers [13,14].

In the first report [11], researchers at Stanford University Medical Center (Stanford, CA, USA) determined the global gene expression profile of 67 lung tumours from 56 patients using a cDNA microarray technique. In the majority of cases, tissue that was assigned to a category by morphological assessment (using light microscopy) was also assigned to the same genetic group.

The second paper [12], by scientists at the Dana-Farber Cancer Institute (Harvard Medical School, Boston, MA, USA), reports the analysis of mRNA expression levels in 186 lung tumour samples, including 139 adenocarcinomas, using oligonucleotide arrays of 12,600 transcript sequences. By studying clusters of gene expression, the group was able to classify tumours according to their expression of neuroendocrine genes and type II pneumocyte genes. When correlated with patient outcomes, it was observed that patients with adenocarcinomas expressing higher levels of neuroendocrine genes had a less favourable prognosis.

Analysis of gene expression data, coupled with the knowledge of the patients' outcome after chemotherapy, can provide vital prognostic and drug sensitivity information for clinicians. The potential of tumour expression profiling is further emphasized by that fact that primary tumours can be distinguished from metastases of extra-pulmonary origin. Moreover, it is likely that future drug trials will test a drug's effect against tumours with different genetics subtypes.

11 Garber, M.E. *et al.* (2001) Diversity of gene expression in adenocarcinoma of the lung. *Proc. Natl. Acad. Sci. U. S. A.* 98, 13784–13789

12 Bhattacharjee, A. *et al.* (2001) Classification of human lung carcinomas by mRNA expression profiling reveals distinct adenocarcinoma subclasses. *Proc. Natl. Acad. Sci. U. S. A.* 98, 13790–13795

13 Perou, C.M. *et al.* (2000) Molecular portraits of human breast tumours. *Nature* 406, 747–752

14 Sorlie, T. *et al.* (2001) Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc. Natl. Acad. Sci. U. S. A.* 98, 10869–10874

HIV gene therapy

Research performed at the Women's and Children's Hospital in Adelaide (Australia) has shown that modified HIV type-1 can safely be used to transfer therapeutic genes into human cells without transferring the disease.

Senior researcher, Don Anson, said 'Viruses are very good at transferring their genetic material into cells, so it is logical to use them to carry any therapeutic genes we want to get into those same cells… HIV-1 is particularly suited to this purpose because…it transfers genetic material to cells that are not growing and not all viruses can do this – and it is these non-growing cells that we need to target with therapeutic genes.' He continued that, as a result of the modification, the transferred viral genes did not cause infection of the cells that would result in AIDS.

The group in Adelaide has developed a method to increase the safety of transferring genes using HIV-1 without causing infection. 'We are now able to modify HIV's genetic material so those viral proteins essential for it to reproduce and cause disease in our cells are removed,' said Anson.

Miscellaneous

Branding a growing issue for pharma companies

Strong corporate brands are being jeopardised by the internal politics associated with large mergers, claimed a report by Corporate Edge (London, UK) recently. In a poll of city analysts, 80% said that they thought stronger brands were being sacrificed in at least half of mergers or acquisitions in the pharmaceutical sector because of internal politics, namely because it is the smaller commercial partner. While this was seen as a general problem with mergers, participants used the Pfizer/Warner Lambert case an example of how the pharmaceutical sector is particularly bad.

'[Consumerization] may only just be starting to take effect, but its potential is enormous,' said Jonathan Hall, Director of Corporate Edge. If customer loyalty to a certain drug allows it to sell well, even for a short period after it loses its patent, 'this will have a huge impact on its bottom line,' he said.

Sixty percent of analysts also said that they thought brand innovation was an important factor. One in five replied that corporate identity and clarity of brand strategy were vital. All agreed that the share price of a pharmaceutical company is harmed when a name change does not work.

First comprehensive depression centre

The first comprehensive centre devoted to the treatment, research and education in depression will be established at the University of Michigan Health System (MI, USA). Pending approval by the University of Michigan Regents, the centre will enable expansion of the university's activities in this field and house a network of more than 100 scientists, physicians and other support staff.

'We hope to lead the way in accelerating the pace of neuroscience research in depression,' said John Greden, Executive Director of the centre, Chair of the Department of Psychiatry, and Rachel Upjohn Professor of Psychiatry and Clinical Neuroscience at the University of Michigan Medical School (Ann Arbor, MI, USA). 'What better time to launch a comprehensive centre to catalyze the momentum that we have?' he said.

The centre will aim to characterize the genetic and neurochemical basis of the disease as well as its relationship with other mental and physical health problems. It will also test new medications and treatment interventions and aim to offer a continuous care service designed to support long-term recovery.

New foundation to combat diabetes in developing countries

Novo Nordisk (Bagsværd, Denmark) is to establish an international foundation, the World Diabetes Foundation (WDF), to support projects that improve diabetes care in developing countries. The company will then donate approximately DKK 500 million (about € 67 million) over the next ten years to the foundation, which will be part of the company's larger LEAD (Leadership in Education and Access to Diabetes Care) initiative.

'It is extremely important that one of the leading companies in diabetes care has shown the way with real action,' said Professor Sir George Alberti, President of the Royal College of Physicians (London, UK) and the International Diabetes Foundation (Brussels, Belgium). The initiative 'tackles the issues of diabetes care in the developing countries in a complete fashion, [and] we hope many others will follow.'

The donation is subject to shareholder approval in March 2002, but is not expected to affect financial results for 2001.

European biotech well placed to see out economic storm

Financial prudence has left 60% of European biotechnology firms with more than four years' worth of funds at the end of 2000, and 27% with between one and four years' reserves, concludes a recent Ernst & Young (Brussels, Belgium) report entitled *Back to Basics*.

Biotechnology markets performed poorly in the first six months of 2001. Share prices of platform-based European companies fell by over 30% since flotation in 2000 as the Human Genome Project failed to deliver on its initial promise. Meanwhile, public biotechnology company valuations in Germany were down by an average of 45%. In the UK, public biotechnology companies were insulated from this downturn by maturing product pipelines (130 products compared with the nearest rival Denmark, with 30 products) but stocks still fell by 10% over the first six months of 2001.

Keeping a tight rein on expenditure was advised in the biotechnology sector due to the high cash burn rate in the industry. Companies are to emulate their US counterparts when offering themselves publicly. Glenn Crocker, Head of Biotechnology at Ernst & Young and co-author of the report advises that the burgeoning number of IPO-ripe biotechnology companies should wait until the next IPO window as this will enable them to maximise fundraising opportunities. US biotechnology companies that floated during the peak of the IPO window in February–April 2000 raised approximately € 160 million on average, compared with the € 80–90 million by European firms a few months later. 'Pre-IPO companies in Europe must ensure that all elements of the fundraising process are in place now so that when investor sentiment starts to change, they…are ready,' said Glenn Crocker.

The first six months of 2001 also saw very large private equity rounds, with investors placing higher sums on a limited number of 'high-quality' bets. During the period, the European industry raised more than € 670 million, threefold the amount raised in private equity during the same period in 2000. There was also a surge in the formation of new companies in the UK, though whether this would be replicated in Europe was unknown.

High prevalence of memory loss

Mild cognitive impairment and memory loss has been shown to affect nearly one in four Americans over the age of 65 years and is a major risk factor for later development of Alzheimer's disease [16]. Of the 2212 community-dwelling people residing in Indianapolis (IN, USA) that were assessed, 23% had mild cognitive impairment. This figure was dependent on age, with 19.2% of 65–74-year-olds shown to have cognitive impairment, increasing to 38.0% for those over 85 years.

Of the 105 people that were diagnosed with cognitive impairment, 66 were re-examined after 18 months. Of those, 26% were diagnosed with Alzheimer's disease or another type of dementia. A further 50% continued with a diagnosis of cognitive impairment, while the remaining 24% returned to normal cognitive functioning.

The most frequent cause of cognitive impairment was medically unexplained memory loss (12.5% prevalence), followed by medical illness-associated cognitive impairment (4.0%), stroke (3.6%) and alcohol abuse (1.5%).

The lead author of the report, Frederick Unverzagt, Assistant Professor of Psychiatry at Indiana University School of Medicine (Fesler Hall Indianapolis, IN, USA), called for more research into the risk factors associated with developing cognitive impairment. He also suggested that one possibility might be to use Alzheimer's therapies at this early stage.

16 Unverzagt, F.W. *et al.* (2001) Prevalence of cognitive impairment: Data from the Indianapolis Study of Health and Aging. *Neurology* 57, 1655–1662

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