Synopses of Research Articles

Birth Rate Increases following Improved Rural Water Supply

DOI: 10.1371/journal.pmed.0030133

Development efforts in rural Africa over the last few decades have achieved improvements in living conditions and in health. It has been argued that when such changes occur, there will be a subsequent reduction in birth rates, and experience in other parts of the developing world has tended to bear out this prediction. However, birth rates in rural Africa remain high and the population continues to grow rapidly. The situation in Ethiopia provides an illustration; spiraling population growth and slow economic growth are widely considered to be the main factors that have fuelled this country's repeated humanitarian crises.

On the basis of current trends, it is predicted that Africa's population will double in the next 50 years, but in many countries, the resources are currently not available to sustain such a level of growth, and increased human suffering may be the consequence. An important question, but one that is seldom discussed, is whether development programs in Africa fail to make sustainable improvements over the long term because they lead to unsustainable increases in population growth rate. This concern is addressed, however, in a paper by Gibson and Mace, who studied a rural development program that was intended to improve the lives of Ethiopian women. The researchers measured its impact on the health of women and children in eight villages included within the program, and also on the birth rate.

The study involved a rural area where some villages had benefited from the provision of a tapped water supply. Previously, women had to walk long distances (up to 30 km) to fetch their families' water in clay pots. The development program reduced the time they spent carrying water each day from about three hours to about 15 minutes. The researchers collected information over a four-year period, including for both villages where tapped water had been introduced and for others where it had not. In total, nearly 2,000 households were included. The nutritional status of the women and children (in terms of body mass index) was also measured. The researchers found that the availability of tapped water improved the survival of young children, although their nutritional status actually declined, and the birth rate increased. All this caused greater scarcity of resources within households.

The incremental effects of small changes to energy balance caused by development can increase strain on the household by increasing birth rates. This finding highlights the importance of continuing to improve access to contraception, especially in rural areas.

Gibson MA, Mace R (2006) An energy-saving development initiative increases birth rate and childhood malnutrition in rural Ethiopia. DOI: 10.1371/journal.pmed.0030087

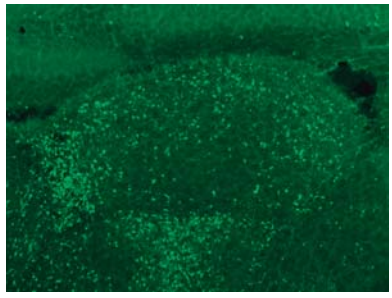
Nicotinamide: A Way to Prevent Fetal Alcohol Syndrome?

DOI: 10.1371/journal.pmed.0030147

The most common cause of nongenetic mental retardation in the Western world is fetal alcohol syndrome (FAS). About one in 1,000 United States children is born with FAS, which is caused by prenatal exposure to alcohol. Children with FAS typically have abnormal facial features and reduced growth. They also have central nervous system abnormalities that lead to impaired learning and memory skills, hyperactivity, and other behavioral problems. These neurological disabilities arise because ethanol disrupts the formation and survival of neurons in the developing brain, particularly in the last trimester of pregnancy and in the first few years of postnatal life when brain development is particularly active.

There is no cure for FAS, but it is 100% preventable. Public health officials recommend that women planning pregnancy and sexually active women who do not use effective birth control avoid alcohol—there is no safe dose of alcohol or safe time to drink it during pregnancy. Sadly, this advice is often ignored. In the US, one in 12 pregnant women admits to drinking alcohol, and one in 30 reports binge drinking (five or more drinks at one time). Given such figures, ways to prevent or attenuate the effects of alcohol on the developing brain are badly needed. Alessandro Ieraci and Daniel Herrera now report that nicotinamide (the amide form of vitamin B3) can prevent some of the deleterious effects of ethanol on developing mice brains, and suggest that nicotinamide might be suitable as a preventative therapy for FAS.

Nicotinamide is the precursor of β-nicotinamide adenine dinucleotide, which enhances the action of many enzymes and is therefore essential for cellular function. Nicotinamide and other forms of vitamin B3 have been used for many years as dietary supplements to treat and prevent pellagra, a vitamin deficiency disease. Large oral doses of nicotinamide have also been used over extended periods of time in clinical trials to treat type I diabetes and bullous pemphigoid (a chronic, autoimmune skin-blistering disease). In addition, recent animal data indicate that nicotinamide is also neuroprotective. In rat models of stroke, for example, it improves neurological outcomes by inhibiting the neuronal apoptosis caused by oxygen deprivation. In apoptosis (a highly organized form of cell death), mitochondrial breakdown releases the protein cytochrome-c, which activates enzymes known as initiator caspases. These activate effector caspases (including caspase-3), which digest other cellular substrates and kill the cell without releasing any potentially harmful molecules. Nicotinamide acts as a neuroprotectant in part by inhibiting cytochrome-c release and caspase-3 activation.



DOI: 10.1371/journal.pmed.0030147.g001

Dying neurons stained with Fluoro–Jade B after ethanol exposure

As induction of apoptosis is one known mechanism by which ethanol harms neurons, Ieraci and Herrera asked whether nicotinamide could reduce the effects of ethanol in a mouse model of FAS. Subcutaneous injection of ethanol triggers widespread neurodegeneration in seven-day-old mice—whose brain development is comparable to that of human fetuses in the third trimester of pregnancy. The researchers report that an ethanol injection sufficient to raise blood ethanol levels to those that a human fetus would be exposed to if its mother indulged in binge-like drinking activated caspase-3 and induced the release of cytochrome-c. They then show that nicotinamide injected up to eight hours after the ethanol reduced caspase-3 activation and cytochrome-c release without altering blood or brain ethanol levels.

As the "normalizing" effects of nicotinamide were strongest when it was administered zero to two hours after alcohol exposure, Ieraci and Herrera next investigated whether nicotinamide given two hours after alcohol injection could prevent ethanol-induced neuronal death. To look for early signs of neuronal injury, they stained brain sections with Fluoro-Jade B soon after exposing the mice to ethanol. Most damage occurred in brain regions particularly sensitive to ethanol at this age—namely, the anterior cingulate cortex (which is involved in cognition), the hippocampus (a region needed for learning and memory), and the thalamus (which relays messages from the outside world to other brain regions); nicotinamide treatment reduced this damage. When the researchers stained brain sections for NeuN, a marker of mature neurons, several days after ethanol exposure, they found reduced numbers of neurons (compared with control brains) in similar brain regions, and, again, nicotinamide treatment reduced ethanol's effects. Finally, the researchers used three standard behavioral assays to test whether the reduction in ethanol-induced neuronal death produced by nicotinamide affected the behavior of adult mice. They report that nicotinamide reversed the increase in hyperactivity and the decrease in fear caused by ethanol exposure, and prevented the impairment in learning and memory induced by ethanol.

In mice, then, these results show that nicotinamide can reverse the molecular, cellular, and behavioral effects of ethanol exposure on developing brains. While the beneficial effects observed were most pronounced when nicotinamide was given at the same time or shortly after alcohol exposure, the study suggests that there is a time window of a few hours during which treatment with nicotinamide might be effective. More studies are needed to determine exactly how nicotinamide protects neurons against alcohol-induced damage, but the data raise the possibility that nicotinamide treatment may provide a way to prevent some human cases of FAS.

Ieraci A, Herrera DG (2006) Nicotinamide protects against ethanol-induced apoptotic neurodegeneration in the developing mouse brain. DOI: 10.1371/journal.pmed.0030101

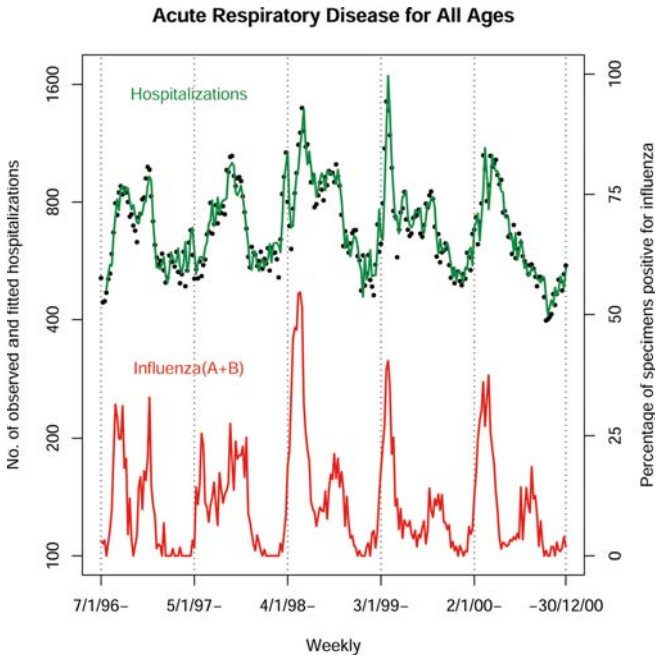
Influenza-Associated Hospitalization in a Subtropical City

DOI: 10.1371/journal.pmed.0030163

Influenza is estimated to be responsible for a million deaths worldwide every year. In developed countries, epidemics of influenza have long been known to increase hospital admissions and mortality. This impact has been measured quantitatively in many studies dating back over a century. The statistical methods used in such research have involved both "comparative" and, more recently, "regressive" techniques.

There are of course many difficulties in collecting and analyzing this kind of information in developing countries. In addition, the lack of clear seasonal patterns to epidemics in these regions prevents the use of regressive statistical methods. As a result, the impact of influenza on morbidity and hospitalization in the tropics and subtropics has been poorly quantified. However, the Hong Kong Special Administrative Region, which has a subtropical climate and a modern health-care system, presents opportunities to conduct such research. Ninety-five percent of the people admitted to hospital in Hong Kong go to public-sector hospitals, which have a central computerized system in which clinical records are kept on all patients.

In a new study, Peiris and colleagues present the results of a study in which they obtained information on patients admitted to hospitals in Hong Kong during the period 1996–2000. They developed an appropriate statistical technique that allowed them to capture influenza-associated mortality even in the absence of a predictable seasonal pattern of influenza, and also controlled for potential confounding factors as variations in temperature and humidity. The researchers found that during influenza outbreaks, hospital admissions increased, not just for respiratory diseases such as pneumonia but also for cardiovascular conditions and diabetes. The increases were most noticeable for older people. Overall, influenza was responsible for 11.6% of admissions for respiratory disease, 1.5% of admissions for stroke, 1.8% of admissions for heart attacks, and 3.5% of admissions for diabetes. These figures are comparable with what has been found in developed countries outside the tropics.



DOI: 10.1371/journal.pmed.0030163.g001

Weekly number of observed and fitted hospitalizations, and percentage of specimens positive for influenza

The finding that the influenza burden faced by Hong Kong is in fact similar to that in the United States, for example, is important, as it has usually been assumed that influenza does not have a significant impact on health outside the temperate regions. The results of this study suggest that influenza deserves to be given a higher priority than it is accorded at present in tropical and subtropical countries. The authors urge the introduction of vaccination programs for people at high risk, particularly the elderly.

Given the special circumstances of Hong Kong, some caution is of course required when extrapolating these findings to other parts of the tropics and subtropics. A wealthy subtropical city with good infrastructure is different in very many respects from low-income nations in the tropics. Most tropical countries face a massive disease burden from other medical conditions and lack the level of resources for health care that are available in Hong Kong. Nevertheless, this is clearly an important study. Its implications are discussed further in the Perspective by Viboud, Alonso, and Simonsen (DOI: 10.1371/journal.pmed.0030089). They note, in particular, the finding that hospitalization of children with influenza is apparently greater in Hong Kong than in the US, and they suggest that childhood vaccination programs may need to be considered.

Wong CM, Yang L, Chan KP, Leung G, Chan KH, et al. (2006) Influenza-associated hospitalization in a subtropical city. DOI: 10.1371/journal.pmed.0030121

Alport Syndrome: From Pathogenesis to a Potential Therapy

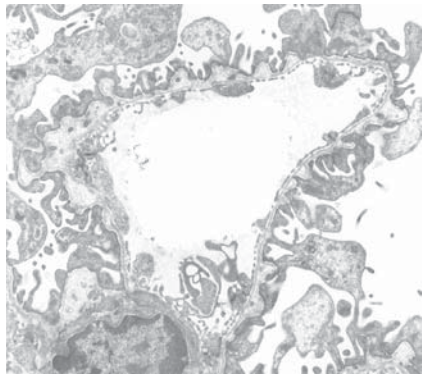
DOI: 10.1371/journal.pmed.0030154

In 1927, Cecil Alport described a family in which affected individuals developed progressive kidney failure, deafness, and sometimes eye problems. Alport syndrome, although it affects only one in 50,000 live births in the United States, is the second most commonly inherited reason for kidney failure. It is caused by mutations in the genes that encode type IV collagen, a structural component of the thin, sheet-like basement membrane that covers the glomeruli, the kidney's filtration units. The glomerular basement membrane (GBM) normally filters fluid and small molecules (but not proteins or red blood cells) from the capillaries in the glomeruli into the urine, but in Alport syndrome, the collagen scaffold of the GBM is defective and, over time, the GBM splits and thins. The first symptom of Alport syndrome is blood in the urine (hematuria), followed by proteinuria and progressive renal failure as scar tissue (fibrotic tissue) forms around the glomeruli. The syndrome has no specific treatment, but kidney transplantation is usually successful in patients with end-stage kidney failure.

There are six isoforms of type IV collagen—α1(IV) through α6(IV). In immature kidneys, the GBM contains α1(IV) and α2(IV), but these are replaced by α3(IV), α4(IV), and α5(IV) as the kidneys mature. The gene encoding α5(IV) is mutated in patients with X-linked Alport syndrome (85% of cases); mutations in the genes encoding α3(IV) or α4(IV) cause other forms of the syndrome. In all cases, the normal switch in collagen isoforms does not occur as the kidneys mature. Raghu Kalluri and colleagues have been investigating whether this failure to switch might make the GBM more susceptible to proteolytic degradation by matrix metalloproteinases (MMPs). They now report that MMPs have a dual role during disease progression in a mouse model of Alport disease, and suggest that MMP inhibition might be therapeutic during the early stages of the human disorder.

The mouse model used by Raghu Kalluri, Michael Zeisberg, and colleagues to test their ideas about the pathogenesis of Alport syndrome is the $\alpha3(IV)^{-/-}$ mouse, which lacks functional α3(IV) collagen. These mice are born normal, but by four to five weeks old, their GBMs begin to disintegrate and they develop proteinuria. By eight weeks old, fibrotic tissue has formed in the tubulointerstitial compartment of their kidneys, and the mice die by 14 weeks old from kidney failure. The researchers first examined the localization of MMP-2, MMP-3, and MMP-9 (all of which degrade GBM) in $\alpha3(IV)^{-/-}$ mice. Wild-type mice expressed low levels of these MMPs in their kidneys, but $\alpha3(IV)^{-/-}$ mice expressed increased levels of MMP-2 and MMP-3 in their glomeruli at four weeks old, and as their disease progressed, expression of all three MMPs spread to the renal tubulointerstitial compartment. Renal expression of these MMPs was also increased in patients with X-linked Alport syndrome and end-stage renal failure when compared with normal kidneys. Furthermore, GBM from humans with Alport syndrome and from $\alpha3(IV)^{-/-}$ mice was more susceptible to MMP degradation than that from normal humans or mice.

These results support the idea that increased proteolytic degradation of a defective GBM may be responsible for Alport syndrome, but renal disease still occurs in $\alpha3(IV)^{-/-}$ mice when MMP-9 is missing. Could other MMPs compensate for the loss of MMP-9? When the researchers examined renal tissue from $\alpha3(IV)^{-/-}$ mice deficient for MMP-2 and/or MMP-9, they did indeed discover compensatory upregulation of other MMPs. So the researchers then looked to see whether pharmacological agents that inhibit multiple GBM-degrading MMPs could alter disease progression in $\alpha3(IV)^{-/-}$ mice. The researchers report that giving such drugs to four-week-old mice (before proteinuria developed) delayed disease progression and increased their survival by five weeks. However, giving the same drugs to eight-week-old mice (in which there was tubulointerstitial fibrosis) shortened their lives by two to three weeks.



DOI: 10.1371/journal.pmed.0030154.g001

Transmission electron microscopy of a kidney from an $\alpha3(IV)^{-/-}$ mouse. Without treatment, glomerular basement membrane lesions and podocyte effacement cause severe proteinuria in these mice.

Based on these animal experiments, the researchers suggest that in patients with Alport syndrome, the GBM (unlike GBM in healthy individuals) is susceptible to degradation by the low levels of MMP normally present in the kidney.

Partial disruption of the GBM attracts infiltrating monocytes, which increase local MMP concentrations and accelerate GBM destruction. MMP inhibitors during this phase of the syndrome should be protective, suggest the researchers, but once the damage is sufficient to stimulate renal fibrosis, the same drugs will accelerate disease progression by inhibiting the MMPs that normally help to degrade fibrotic tissue. MMP inhibitors are already being developed for other indications and would be worth investigating as preventive drugs in Alport syndrome. But, warn Kalluri and colleagues, these drugs would be a double-edged sword, and could only be used in patients with identified genetic defects and only before the onset of proteinuria.

Zeisberg M, Khurana M, Rao VH, Cosgrove D, Rougier JP, et al. (2006) Stage-specific action of matrix metalloproteinases influences progressive hereditary kidney disease. DOI: 10.1371/journal.pmed.0030100

Antiretroviral Use in Resource-Poor Settings: Modeling Its Impact

DOI: 10.1371/journal.pmed.0030179

For many people in the developed world, a diagnosis of HIV/AIDS is no longer a death sentence. Since the introduction in the 1990s of effective antiretroviral therapy (ART)—combinations of three or four drugs that interfere with different stages of the HIV life cycle—HIV/AIDS mortality rates have dropped by 50%–70% in affluent countries. By contrast, in developing countries, at least 6 million people need immediate access to ART but fewer than 10% of them get it. In 2005, 3 million people in poor countries died from HIV/AIDS. To improve this situation, the World Health Organization (WHO), the Joint United Nations Programme on HIV/AIDS (UNAIDS), and other international bodies are working toward providing universal access to ART for all those who need it by 2010. In addition to reducing AIDS morbidity and mortality, the hope is that this strategy will also reduce HIV/AIDS prevalence, because sexual transmission of HIV is more likely if the partner who is HIV-positive has a high viral load.

Many challenges need to be overcome to achieve universal access to ART, not least of which is determining how to maximize the benefits of ART to patients and their communities in resource-poor settings. Regional differences in health-care facilities, local changes in sexual behavior in response to treatment, and many other factors can alter how ART affects both HIV transmission rates and HIV/AIDS mortality. Ideally, the best strategy for each setting would be determined through large-scale randomized trials of different approaches—for example, the time at which treatment is initiated relative to the time of infection—with HIV prevalence and HIV/AIDS–related mortality as primary endpoints. However, such trials are lengthy and costly, so researchers and policy makers are also using mathematical models to explore the impacts of different treatment and monitoring strategies. Rebecca Baggaley and colleagues now describe a new approach to modeling the impact of ART in resource-poor settings. Their model predicts that HIV epidemics in sub-Saharan Africa will not be controlled through ART alone, even if universal access is achieved. Additional prevention methods such as counseling patients and their communities about safe sex are essential. Without them, their results suggest, access to ART is likely to increase HIV/AIDS prevalence.

The researchers' deterministic model of HIV transmission incorporates ART and stratifies infection progression into four different stages (primary infection, incubation, pre-AIDS, and AIDS), each of which is associated with a different degree of infectiousness. In effect, the model is a complex flowchart through which patients move inexorably as they become infected and receive treatment—which can fail (virologic failure) or succeed (long-term viral suppression)—or from which they can withdraw. Sexual behavior and changes in sexual behavior in response to HIV/AIDS and ART is also plugged into the model—people treated with antiretrovirals often become more sexually active as they begin to feel better. Effective counseling, on the other hand, can increase safe-sex practices. To turn this flowchart into predictions of how HIV epidemics in sub-Saharan Africa might develop over time given different ART strategies and, for example, the availability of diagnostic laboratories to monitor the immune status and viral load of individuals with HIV, the researchers used published estimates of relevant parameters such as the fraction of patients that drop out at each stage of treatment and the transmission probability per sexual partnership for patients in whom ART failed.

Baggaley and her colleagues make several predictions. They suggest, for example, that unlimited ART provision initiated once patients have developed AIDS will increase the prevalence of infection (because the patients live longer and become sexually active again), a worrying result given that one aim of the universal access initiative is to reduce HIV infection rates. Furthermore, although different coverage levels in this scenario will not affect the years of life gained per person-year of treatment, increased coverage will increase the emergence and spread of drug resistance. If pre-AIDS patients are treated as well, the researchers predict that additional infections will be averted per person-year of treatment, but the effect will be small and highly dependent on how pre-AIDS patients change their sexual behavior in response to ART.

As with all modeling exercises, this new model includes many assumptions that may limit its applicability in the "real world." For example, it includes only first-line triple-therapy ART and does not allow for second-line therapy if one drug regimen fails; it does not consider the sexual behavior of people who don't know they are infected with HIV; and it does not allow for a reduction in the quality of ART programs as coverage increases, a likely problem in countries with limited resources. Nevertheless, the model's predictions sound a warning: ART is not likely to function as a direct method for transmission prevention even when coverage is high. Counseling of patients and their communities to promote safe sexual practices must accompany ART provision. Difficult decisions regarding the allocation of finite resources will have to be made as ART is rolled out in resource-poor countries, conclude the researchers, decisions that can best be made by combining mathematical modeling with data from early programs as they become available.

Baggaley RF, Garnett GP, Ferguson NM (2006) Modelling the impact of antiretroviral use in resource-poor settings. DOI: 10.1371/journal.pmed.0030124

Do Variants in the *GST* Detoxification Genes Affect the Risk of Lung Cancer?

DOI: 10.1371/journal.pmed.0030174

By the year 2020, global deaths from noncommunicable diseases are expected to increase by 77%. The projected rise is mainly due to the aging of the population and to an increase in the numbers of people exposed to tobacco. While antismoking campaigns have met with some success in developed countries, the tobacco epidemic is growing in many of the world's developing and most populous countries. Tobacco (a major risk factor not only for lung cancer but also for chronic obstructive pulmonary diseases such as emphysema) is expected to kill more people than any single disease, surpassing even the HIV epidemic.

The link between tobacco smoke and lung cancer is striking, but not all smokers get lung cancer, and not all lung cancer patients have a history of first- or second-hand exposure to tobacco smoke. Tobacco smoke contains carcinogens such as benzopyrene, which can cause mutations in the DNA of cells it comes in contact with, such as lung epithelial cells. Cytosolic enzymes such as those of the glutathione S-transferase (GST) family are part of the human body's armor against environmental carcinogens; they catalyze the detoxification of reactive electrophilic compounds like benzopyrene. Like all human genes, those encoding members of the GST family exist in multiple variant forms or alleles. Some of these alleles encode less-active or completely inactive versions of the detoxifying enzymes, and, therefore, might convey increased risk for the development of cancers with strong environmental determinants, and lung cancer in particular.

Although more than 100 studies have tested the hypotheses that particular *GST* alleles either predispose to or protect against lung cancer, the results have been inconsistent, with some studies reporting strong associations and others failing to replicate these findings. Individual association studies are notoriously prone to error, and attempts to combine results from several studies have been complicated by the fact that allele frequencies for many of the alleles differ between populations, by differences in smoking habits, and by differences in criteria for the selection of suitable control groups.

To summarize the current evidence comprehensively and attempt to resolve the controversy, Zheng Ye and colleagues undertook a large meta-analysis of 130 published studies that had examined associations between one or several of five *GST* alleles and lung cancer. The five alleles included the *GSTM1* and *GSTT1* null alleles, two missense alleles in *GSTP1*, and one intron polymorphism in *GSTM3*. (The null alleles abolish enzyme activity; the three other alleles encode enzymes with reduced activity.) A total number of 23,452 lung cancer cases and 30,397 controls were included in the meta-analysis. Ye and colleagues corresponded with the individual research groups to obtain the data in tabular form and—for a few of the studies—additional data that were not included in the original publications.

When taking all the existing evidence together, neither the I105V or A114V polymorphisms in *GSTP1* nor the *GSTM3* intron 6 polymorphism were found to be associated with increased risk of lung cancer. The two "null" polymorphisms in GSTM1 and *GSTT1* showed a weak association. However, as Ye and colleagues discuss, it is possible that the weak overall link results from bias toward publication of positive results, especially for smaller studies. This is consistent with the result that the researchers obtained when they restricted their analysis to larger studies: comparing only cases and controls from studies that had at least 500 participants resulted in no significant associations between any of the alleles and lung cancer.

While meta-analyses such as this one have their own limitations, the results reported here make it unlikely that any of the five polymorphisms convey a substantially enhanced lung cancer risk in the general population. This does not exclude the possibility that other alleles in the four genes examined here, alleles in other *GST* genes, or combinations of *GST* alleles do exert substantial influences on an individual's lung cancer risk. As the authors conclude, the chances for discovering such links in future studies are likely much higher if such studies are large and carefully designed.

Ye Z, Song H, Higgins JPT, Pharoah P, Danesh J (2006) Five glutathione S-transferase gene variants in 23,452 cases of lung cancer and 30,397 controls: Meta-analysis of 130 studies. DOI: 10.1371/journal.pmed.0030091

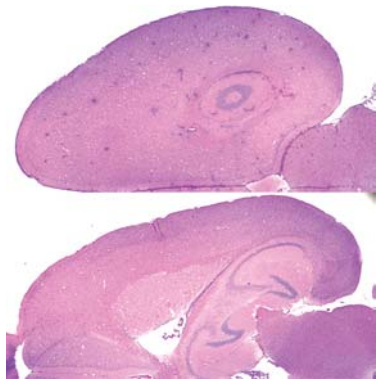
RNA Interference to Suppress Flaviviral Encephalitis

DOI: 10.1371/journal.pmed.0030139

Mosquito-borne flaviviruses such as Japanese encephalitis virus (JEV) and West Nile virus (WNV) are two of the most important examples of emerging and resurging pathogens. Currently, there are no effective drugs available to treat these infections. Moreover, infections by diverse neurotropic flaviviruses are clinically indistinguishable, which makes it important to develop broad-based therapies effective against multiple flaviviruses.

In a new study, Priti Kumar and colleagues examined whether RNA interference (RNAi)–based intervention could suppress lethal JEV and WNV encephalitis in mice. RNAi is a process in which short double-stranded RNA (dsRNA) called short interfering RNA (siRNA) inhibits gene expression in a sequence-specific manner. This process was first discovered in plant cells, and there is still speculation concerning its implications for human physiology. However, RNAi has emerged as a powerful technique for posttranscriptional gene silencing, useful in both research and, potentially, the development of new therapies.

Studies of RNAi in plants, invertebrates, and, more recently, mammalian cells have all hinted at the potential of this method. RNAi-mediated posttranscriptional gene silencing



DOI: 10.1371/journal.pmed.0030139.g001

Section from JEV-infected mouse brain obtained after treatment with control or JEV-specific siRNA

appears to have evolved to protect against invading genetic elements such as transposons and viruses. In mammals, although exposure to dsRNAs greater than 30 base pairs in length induces an antiviral interferon response that represses mRNA translation globally, shorter siRNA introduced into mammalian cells leads to specific mRNA degradation without activating the interferon response.

Previous work has shown that infection by respiratory syncytial virus (RSV) and parainfluenza virus (PIV) could be specifically prevented and inhibited by siRNAs. In an RSV mouse model, researchers used siRNA to target the P protein, a key subunit of the viral RNA–dependent RNA polymerase, and found that it strongly inhibited RSV gene expression and RSV growth in culture. They also showed that single and concurrent infections in mice could be prevented and treated by specific siRNA applied exclusively intranasally. Their findings suggested that inhaled siRNA could be a promising strategy for anti-RSV and anti-PIV therapy in humans.

Studies have also demonstrated that siRNAs expressed by a lentivirus vector could prevent and treat influenza A virus (IAV) pneumonia in mice. When siRNAs specific for conserved regions of the IAV genes (nucleoprotein, acid polymerase, and basic polymerase 1) were administered prior or subsequent to the virus challenge, there were reductions in lung virus titers, lethality, or both. Based on these results, in a recent review of siRNAs for treating influenza, Jack Bennink and Tara Palmore have suggested that siRNAs could lead to a therapeutic solution against the variability of the IAV hemagglutinin.

Kumar and colleagues from Harvard University describe promising results for the therapeutic potential of RNAi in treating viral encephalitis, both virus-specific and across species. They induced RNAi in mice, with either a lentivirally expressed short hairpin RNA or a synthetic siRNA. By targeting a species-specific sequence in cd loop coding region in domain II of the viral envelope protein for JEV or WNV, the team achieved specific protection in mice against the corresponding virus. And in addition, by targeting a sequence within the cd loop that is conserved across both viral species, they were able to protect mice against encephalitis induced by both viruses.

These results suggested that a single treatment with siRNA may be sufficient for protection against fatal encephalitis, which is encouraging from a therapeutic angle. In one set of experiments, they showed that single administration of siRNA could provide more than 60% protection even when administered 18 hours after infection. This time frame is relevant because the burst phase for JEV and WNV replication is around 18 hours—when many virus progeny are released.

However, although a single lipid–based siRNA delivery in the brain parenchyma caused some degree of lateral spread and offered protection even in an established infection, this approach is unlikely to work when the infection has spread across the brain. This observation is important for potential clinical applications since usually treatments are not given until after the appearance of clinical symptoms.

To address this limitation, the authors suggest, further studies will have to focus on developing improved methods for siRNA delivery to brain cells. And much work will need to be done to establish the safety of this approach in humans. But RNAi technology may be shifting from a laboratory tool to a realistic treatment for viral infection.

Kumar P, Lee SK, Shankar P, Manjunath N (2006) A single siRNA suppresses fatal encephalitis induced by two different flaviviruses. DOI: 10.1371/journal.pmed.0030096

GENOMOS Study Finds Weak Links between *COLIA1* Polymorphism, BMD, and Fracture Risk

DOI: 10.1371/journal.pmed.0030152

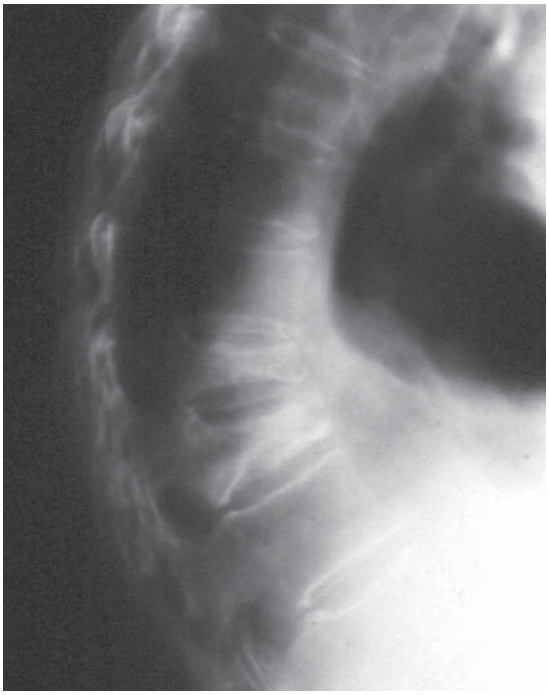
One out of every two women and one in eight men over 50 will have an osteoporosis-related fracture in their lifetime. Osteoporosis is characterized by low bone mass and structural deterioration of bone tissue, and often progresses without overt symptoms or pain until a bone breaks. Fractures occur typically in the hip, spine, and wrist. Currently, there is no accurate measure of overall bone strength. Bone mineral density (BMD) is frequently used as a proxy measure, but it can explain only a modest proportion of fracture risk.

Bone resorption and bone formation take place throughout life. Formation outpaces resorption until peak bone mass (maximum bone density and strength) is reached around age 30. From then on, bone resorption slowly begins to exceed bone formation, and the balance is further shifted toward resorption in women after menopause. Osteoporosis develops when bone resorption occurs too quickly or replacement too slowly—which happens in most individuals at a certain age and often earlier in individuals who did not reach optimal bone mass during their bone building years.

Both bone formation and resorption are under the control of genetic and environmental factors. Osteoporosis is a "complex disease," with variations in a number of different genes and in several environmental factors (such as calcium intake or alcohol consumption) thought to affect an individual's risk. A number of candidate genes have been identified, some of them through studies of rare genetic diseases affecting bone health, others through animal studies. They include genes for calciotropic hormones and their receptors, as well as bone matrix proteins.

One of them, *COLIA1*, encodes collagen 1 alpha 1, a major component of bone and cartilage. Mutations in its coding region cause osteogenesis imperfecta, a rare developmental bone disorder characterized by brittle bones, frequent fractures, and short stature. Apart from these rare mutations, *COLIA1* has a number of polymorphic sites outside the coding region, and scientists have examined associations between many of these alleles and osteoporosis. The one that has been studied most intensely is a single-nucleotide polymorphism within the promoter region at a binding site for the Sp1 transcription factor. The more common allele has a guanine nucleotide (G) at the variable position; the rarer one, a thymine (T). In vitro studies suggest that the T allele is associated with less transcript and protein produced.

Several previous studies had examined a possible association between the T allele and low bone mineral density and fractures, and a number of them had found such a link, as had three separate meta-analyses. As a consequence, some researchers have suggested that genetic testing at the population level for this polymorphism would be beneficial. Individuals who carry the T allele could be advised to get enough calcium and do weight-bearing exercises, ideally



DOI: 10.1371/journal.pmed.0030152.g001

Vertebral compression fractures in a patient with osteoporosis (Image: Stuart Ralston)

already during the bone acquisition phase in adolescence. Others have warned that the evidence that links the T allele to a higher risk for osteoporosis is not strong enough to support such action. They have pointed out some of the notorious problems with association studies in general and retrospective meta-analyses based on published studies.

The Genetic Markers for Osteoporosis (GENOMOS) project is a European Union–funded European collaborative research initiative between universities in the Netherlands, United Kingdom, Italy, Spain, Greece, Poland, and Denmark. The project, which began in 2003, currently involves around 24,000 individuals and seeks to identify genetic risk factors for osteoporosis by prospective meta-analysis. Participants have been recruited from a total of 18 European countries (most of them reside in the UK, the Netherlands, Spain, Italy, Denmark, and Poland). Data on previous and new fractures are collected, together with bone densitometry measurements, information on risk factors, and DNA analysis from blood samples.

The GENOMOS investigators, led by John Ioannidis, report now on their examination of an association between the Sp1 polymorphism in *COLIA1*, BMD, and fracture risk. Based on data from over 20,000 participants, they found a modest association between homozygosity for the T allele and lower bone mineral density at the femoral neck and the lumbar spine. The researchers also found a weak association between the T allele and vertebral fractures in women. However, T allele carriers did not have an overall increased risk of fractures.

The effects seen in this large study—GENOMOS participants reported more than five times the number of fractures than participants in all previous studies combined—were more moderate than those reported in most of the earlier studies. The Sp1 polymorphism in *COLIA1* explained only a small part of the differences in BMD and fracture risk among the GENOMOS participants. There was no association between the T allele and BMD in heterozygous carriers (and only approximately 4% of the participants were homozygous for the T allele). Regarding the fracture association, the researchers estimate that the presence of the T allele would explain at most 10% of the risk of vertebral fractures for women.

The authors conclude that "large-scale studies are needed to quantify the true effect size of genetic polymorphisms that have been implicated in the pathogenesis of complex genetic disorders." Their findings also argue against widespread genetic testing for this particular polymorphism alone. Researchers need to look at other genes (and possibly other variants in the *COLIA1* gene) and validate any findings in large studies like this one before they can predict a substantial fraction of a random individual's genetic risk for osteoporosis.

Ralston SH, Uitterlinden AG, Brandi ML, Balcells S, Langdahl BL, et al. (2006) Large-scale evidence for the effect of the *COLIA1* Sp1 polymorphism on osteoporosis outcomes: The GENOMOS study. DOI: 10.1371/journal.pmed.0030090