Looking for the trigger

The Environmental Genome Project to uncover the interactions of genes and the environment in disease

At first glance, Gulf War syndrome (GWS) and attention deficit hyperactivity disorder (ADHD) have nothing in common. GWS is a neurological disorder suffered by some veterans of the first Gulf War in 1991, probably caused by a combination of vaccines, exposure to burning oil fumes and chemical toxins that were released into the air after the US Air Force destroyed Iraqi chemical weapons facilities. ADHD is a behavioural disorder seen in young children that is characterized by hyperactivity and concentration difficulties. Carolee Barlow and her colleagues from The Salk Institute (La Jolla, CA, USA) think they have found the link—a variant allele of the neuropathy target esterase (*NTE*) gene that is present in the two sets of patients. The enzyme has a role in neural development, and is a known target for organophosphates, on which many pesticides and chemical weapons are based. “*NTE* mutation in mice results in high sensitivity to organophosphates and subtle neurological and behavioural changes,” said Barlow. These variations in the *NTE* gene produce slightly different forms of the enzyme, which may give rise to the seemingly different disease types. “GWS appears to be a case of delayed toxicity, whereas ADHD may result from early, low-dose exposure to organophosphate pesticides,” Barlow said.

To further stimulate research into such disorders, the US National Institute for Environmental Health Sciences (NIEHS), based in Research Triangle Park, NC, USA, initiated the Environmental Genome Project (EGP) in late 1997. The EGP is an extension of the Human Genome Project, and capitalizes on recent advances in DNA sequencing, genomics, epidemiology and population-based studies to elucidate the role of gene–environment interactions in human diseases and the genetic basis of susceptibility to environmental factors, explained Samuel Wilson, Deputy Director of the NIEHS. With a budget

of ~US \$3 million per year, plus US \$12 million for sequencing, the project supports about 100 research projects in biostatistics, bioinformatics, functional analysis, mouse genetics, epidemiology and technology development. Acknowledging the profound ethical, legal and social implications of these projects, the goal of the EGP is to advise patients and policy makers on appropriate measures to reduce potentially detrimental effects of the environment—including diet and lifestyle—on health.

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Scientists have long known that genes and the environment have equally important roles in many diseases. All organisms have evolved metabolic pathways that minimize the biological effects of toxins, but genetic variations in these pathways may give some individuals an advantage or a disadvantage in the detoxification process. An individual's genetic makeup thus influences his or her risk of developing a disease as a result of exposure to toxins, and also explains why some individuals and ethnic groups have higher mortality rates from diseases, such as cancer, hypertension and diabetes. “The relationship between genes and the environment can be compared to a loaded gun and its trigger,” explained Wilson and his co-author, Kenneth Olden, Director of the NIEHS, in *Nature Reviews Genetics* (1, 149; 2000). “A loaded gun by itself causes no harm; it is only when the trigger is pulled that the potential for harm is released. Genetic susceptibility creates an analogous situation, where the loaded gun is one or a combination of susceptibility genes (alleles) and the trigger is an

environmental exposure.” Knowing whether or not one's genetic ‘gun’ is loaded can help one to avoid pulling the trigger. The EGP's first goal, therefore, is the identification of genetic factors that make up the ‘gun’. To this end, the project has collected samples from more than 90 individuals, representing diverse ethnic groups in the US population, to serve as a bank for re-sequencing genes that are responsive to environmental effects. The NIEHS community initially identified 554 of these genes, which are involved in such diverse events as the cell cycle, signalling, apoptosis, cell division, metabolism, DNA repair, homeostasis and gene expression. This April, the EGP announced the completion of its first phase, the re-sequencing of 204 candidate genes (<http://egp.gs.washington.edu>).

“The big news is that we have discovered over 13,000 new single-nucleotide polymorphisms [SNPs] and about 600 cSNPs [coding-region SNPs] for population-based studies—many more than we thought,” said Leslie Reinlib, Program Manager of NIEHS' extramural research. Of particular interest is the use of newly discovered cSNPs as novel research tools, according to Wilson. “Compared with currently available databases that overlap with the EGP's, we've got a big increase in SNP density, and a big increase in the number of validated SNPs,” he said. But the key difference is EGP's selection of candidate genes. “Because the targeted genes are fairly well-characterized, and in most cases have a pre-established significant probability of playing a role in disease susceptibility, the likelihood that these SNPs will be of use in understanding exposure–disease relationships is much greater,” Wilson explained.

Also surprising for the researchers was the discovery that many individual SNPs are linked in blocks, or haplotypes. These haplotypes are useful in genotyping and for interpretation because this enables several

SNPs to be tracked as a single unit. "We are making a mini-haplotype map for genes that are conserved over evolution, and bringing meaning into some non-coding regions," said Debbie Nickerson, project leader of the NIEHS SNP-sequencing programme and Associate Professor of Genomic Sciences at the University of Washington (Seattle, WA, USA).

Another key area of the EGP is the Comparative Mouse Genomics Centers Consortium (CMGCC). Since 2000, scientists at five centres have been developing transgenic and knockout mouse models on the basis of human SNPs that are involved in cell-cycle regulation and DNA repair. The first genes selected included the *CHK2* checkpoint gene, which has a role in breast cancer, and the mismatch repair gene cyclin D1, which is associated with an increased risk of colon cancer. Peter Stambrook, Professor of Cell Biology at the University of Cincinnati (OH, USA), and Chairman of the CMGCC steering committee, said they hope to have cloned mice by the end of next year, which will be accessible to researchers for testing against radiation and toxins.

The first rounds of EGP grants are already bearing fruit. Projects in the USA, Poland and the Czech Republic, conducted by Frederica Perera and colleagues at Columbia University (New York, NY, USA), have identified polymorphisms that relate prenatal exposure to polycyclic aromatic hydrocarbons with lower birth rate and smaller head circumference. They have also linked prenatal exposure to the pesticide chlorpyrifos in African Americans and Dominicans to decreased birth rate and birth length. A project in Los Angeles (CA, USA), conducted by Stephanie London of the NIEHS, has examined polymorphisms in pathways relevant to oxidative stress and DNA repair in lung cancer. Freja Kamel of the NIEHS is investigating cumulative lifetime exposure to lead and other neurotoxins, such as mercury, solvents and cigarette smoke, and the associated risk of developing amyotrophic lateral sclerosis (ALS). She found a link with smoking and, surprisingly, with occupational exposure to lead, but not with chronic lifetime exposure. She thinks that this may indicate that ALS patients metabolize lead differently, and now plans to study polymorphisms in the gene encoding ALAD (δ -aminolevulinic acid dehydratase), as this enzyme seems to affect lead kinetics in the blood.

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EGP grant recipient Clement Furlong, of the University of Washington (Seattle, WA, USA), has shown that polymorphisms in the *PON1* (paraoxonase 1) gene result in increased sensitivity to certain insecticides and nerve agents and an increased risk of cardiovascular disease. Another collaboration between NIEHS and the Institut National de la Santé et de la Recherche Médicale (Villejuif, France) found that polymorphisms in the tumour necrosis factor- α and lymphotoxin A genes and a high exposure to coal dust in miners were associated with coal worker's pneumoconiosis, an inflammatory lung disease. Chronic exposure to coal dust results in overproduction of oxygen radicals that cause airway inflammation. "Not only are the polymorphisms important in this disease, so is the level of exposure to the coal dust," said Steven Kleeberger, a study leader and Chief of the Laboratory of Pulmonary Pathobiology at NIEHS. Previous studies examined polymorphisms, but did not quantify exposure, which is crucial in gene-environment interaction studies, he said. Similarly, Steven Liggett, from the University of Cincinnati, found 12 polymorphisms that are directly related to the efficacy of albuterol treatment in asthma patients. A screening assay for these SNPs would allow physicians to prescribe this drug more rapidly and efficiently. Martyn Smith, of the National Foundation for Cancer Research's Center for Genomics and Nutrition at the University of California, Berkeley (CA, USA) identified two mutations in a series of SNPs that reduce the risk of adult lymphocytic leukaemia (ALL). He found that individuals with two mutated copies of the *MTHFR* (methylene tetrahydrofolate reductase) gene, which is involved in folic acid metabolism, are up to five times less likely to develop leukaemia than those without the mutation. The mutated *MTHFR* gene directs more folic acid to DNA synthesis, which reduces the chance of damage in dividing cells. Smith estimates that 85% of Americans do not carry the 'good' polymorphism, but by consuming more folic acid the risk of developing ALL may be

reduced. Smith also investigates the link between susceptibility to benzene toxicity and various forms of leukaemia, and showed that certain chromosomal translocations have a role in the development of leukaemia and lymphomas early in the disease process—sometimes before birth—and can be detected at the molecular level a decade before the manifestation of disease.

Epidemiologist Patricia Buffler, from the School of Public Health at the University of California, Berkeley, has investigated parental occupations, exposure to pesticides, herbicides and infectious diseases early in life, immunizations, and polymorphisms, that are associated with various cancers. She found that the use of pest-control services and over-the-counter pesticides is associated with an increased risk of childhood leukaemias. Her work has implications particularly for pregnant women, as the results show that the crucial window for pesticide exposure in children with a higher risk for ALL begins as early as *in utero*.

Because many of these studies deal with occupational health and genetic differences among ethnic groups, EGP also supports programmes that deal with the manifold ethical, legal and social ramifications, as well as educational programmes. "We must ensure that the data get back to and benefit the people we are studying," said Shobha Srinivasan, Director of the EGP's programme for research into ethical, legal and social implications (ELSI). The EGP's research must ultimately benefit its subjects, she said, especially when a population is living in an environmentally hazardous situation, and must at the same time protect individuals' legal rights to privacy and confidentiality. ELSI's concerns about informed consent, genetic privacy and confidentiality, disclosure of research results and implications for non-participants were laid out by two of the original leaders of the EGP, Richard Sharp, the EGP's former leading bioethicist, from

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Baylor College of Medicine's Center for Health Ethics and Policy in Houston (TX, USA), and Carl Barrett, former head of the EGP, now at the US National Cancer Institute (Bethesda, MD, USA). They noted that the project presents unique and unprecedented challenges, perhaps most important of which is the question of personal responsibility. If certain polymorphisms are known to confer greater susceptibility to and higher risk of disease, the choice of lifestyle and occupation becomes increasingly important not only for the individual, but also for society, as it eventually has to pay the medical bill. Will society test its citizens for genetic susceptibilities and keep track of lifestyle and occupation, or will it allow its citizens free choice, even in the light of known health risks?

But, first and foremost, it is important to educate people so that they are able to understand the implications and are eventually prepared to make such choices. Paul Spicer, of the University of Colorado's Health Sciences Center (Denver, CO, USA), is working with Native Americans, with whom genetic research has long been fraught with difficulties, due to their beliefs about the sanctity of the body and an understandable fear of exploitation. Spicer started his project by articulating the problems and possibilities arising from genetic knowledge about the population, and, by consulting members of urban and rural Native American communities, he hopes to formulate guidelines with which to conduct research and deliver genetic health services in these communities. His work is equally important for forging partnerships between inner-city, minority and rural populations, and local universities and community health institutions. These studies should eventually benefit everyone—and especially communities in locations that bear a disproportionate burden of environmental pollution. As the EGP's work and other research in the area of molecular medicine is providing more information, such partnerships may even become important in helping to educate people about the significance of their genetic makeup and to deal with potential problems, such as workplace discrimination or insurance bias.

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Science and sustainability

More research is paramount for finding sustainable solutions for agriculture in developing countries. But politics has an equally important role

At the 1992 Earth Summit in Rio de Janeiro, Brazil, sustainable development emerged as a new paradigm for integrating economic growth, social development and environmental protection. Last year, the United Nations World Summit in Johannesburg, South Africa, once again brought this topic to the world's attention by reviewing the Rio agenda and proposing further implementation of sustainable development worldwide. The United Nations Secretary General, Kofi Annan, identified five main topics—water, energy, health, agriculture and biodiversity—that need to be tackled to preserve the planet's natural resources for the coming generations, and to improve the quality of life for the current human population.

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But, first and foremost, agricultural production needs to increase to produce sufficient food for the human population without further destroying already dwindling ecosystems and biodiversity. This problem was the special focus of the meeting entitled 'The contribution of life sciences and biotechnology towards sustainable agriculture for developing countries' that the European Union (EU) organized in Brussels in January this year. The participants at the two-day meeting discussed the progress already made in developing countries, and how this can be improved further through scientific advances and by applying new technologies.

The challenge facing agriculture is massive and well documented. It is clear that current methods of food production, in both the developing as well as the developed world, are neither sufficient nor sustainable.

More than 800 million people are chronically undernourished, and to meet the demands of a burgeoning human population, which is expected to hit the 8 billion mark in 2025, grain production will have to increase by 40%. Equally worrying, more than 10% of the world's current agricultural activities are based on unsustainable water resources. Overuse and over-irrigation are leading to further desertification and loss of arable land, with the result that many areas can no longer sustain any form of agriculture. At present, 11% of the world's land surface is used in crop production, and any increase in this activity in Africa, Asia and Latin America poses a grave threat to worldwide biodiversity. Industrialized agriculture in the developed world is equally as far from being sustainable, with its heavy reliance on fertilizers, pesticides and herbicides that create environmental problems and cause further loss of biodiversity. Philippe Busquin, the European Commissioner for Research, emphasized at the Brussels meeting that there is an immediate need to find scientific solutions to secure sustainable agriculture in the future. Ismael Serageldin, now the Director of the Biblioteca Alexandrina, Egypt, and former Vice President of the World Bank, also underlined the role of research in sustainable agriculture, pointing out that high-yield crops have helped to reduce the amount of land used for cultivation by 300 million hectares each year, an area equivalent to those of the USA, Canada and Brazil combined. But he stressed that the advances made through biotechnology have so far made an impact mainly on industrialized countries, and have had only a marginal effect on developing countries.

A general definition of 'sustainable', in agricultural terms, would be an adequate level of high-quality food production in the countries that are, at present, unable to feed their own populations. In many African countries, the producers and the consumers are often the same people, and any advance towards such a goal would