

INTERFACE:

GENES AND THE ENVIRONMENT

CENTER FOR ENVIRONMENTAL GENETICS UNIVERSITY OF CINCINNATI Winter 1995

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Genetic Dissection of Complex Traits: Susceptibility to Environmental Agents

All things are implicated with one another, and there is hardly anything unconnected with any other thing. For they have been coordinated, and they combine to form the same universe.

— Marcus Aurelius, "*Meditations*" [160-181 A.D.]

Long before genetics was established as a scientific discipline, fundamental questions on the nature of heredity in both plants and animals have engaged the human mind. For example, why do offspring share more of the physical characteristics of their parents than they do with unrelated members of their species? Why do members within one species share more traits than they do with members of another species?

Although traits—such as height, eye color and blood pressure—have been observed to run in families, the genes or combination of genes (*genotype*) that underlie these observable characteristics (*phenotype*) remain unknown in most cases. It would be very surprising if the observed differences in susceptibility to environmental agents in humans are not likewise determined by variation in their genetic backgrounds. This review summarizes the theory and practice of methods of genetic analysis that will likely play a key role in identifying genes that confer susceptibility or resistance to environmental agents.

Simple and Complex Patterns of Inheritance

Genes are the fundamental units of heredity, and all the biological traits shown by an organism are governed in some manner by its genetic "blueprint." Over the course of evolution, variant forms (*alleles*) of the same gene can arise and, in many cases, different alleles confer differences

in the phenotype of the organism. Since each gene occurs on a chromosome pair, two alleles (the same or different) are responsible for the expression of any one gene.

In producing a phenotype, this genetic "blueprint" may be modified in varying degrees by the environment in which the genes are expressed. Studies in model organisms such as the fruit fly (*Drosophila melanogaster*) have shown that gene action can be modified by extrinsic factors such as temperature and chemicals, as well as intrinsic factors such as the action of other genes (*genetic modifiers*). The relationship between genotype and phenotype, can therefore be expressed as follows:
Genotype + Environment = Phenotype

In his classic experiments on the genetics of the pea plant (*Pisum sativum*) conducted in the mid-nineteenth century, **Gregor Mendel**, an Austrian monk, chose (either by accident or design) to study the segregation of phenotypes, each of which was controlled by a single genetic locus. The alleles at each locus were inherited in either a dominant or recessive manner, and not significantly influenced by environmental factors. Consequently, the observation of precise mathematical ratios was possible in the phenotypes of the progeny in each breeding experiment. Phenotypes that show such easily interpretable patterns of inheritance are called "*simple*," or Mendelian, traits and are generally governed by a *single genetic locus*.

In contrast, a phenotype may display a "*complex*," or non-Mendelian, pattern of inheritance. Its segregation may be neither dominant nor recessive, and a simple correspondence between genotype and phenotype does not exist. This might be observed when the same genotype gives different phenotypes, or when different genotypes give the same phenotype (*phenocopy*). Complex patterns of inheritance can result because many genes contribute to the phenotype in a quantitative manner (for example, three genes *A*, *B* and *C* might contribute 20%, 30% and 50%, respectively, to the phenotype). Importantly, complex patterns of inheritance can also be caused by a single gene, if it is significantly influenced by its environment. The challenge in the emerging and fast-paced field of *molecular ecogenetics* (gene-environment interactions) is to identify the genetic differences that underlie differential susceptibility to environmental agents.

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The Elements of Genetic Dissection

There are four approaches to the genetic dissection of phenotypes (*superbly reviewed by* Lander and Schork, 1994). These approaches consist of (1) linkage analysis, (2) allele-sharing methods, (3) association studies, and (4) controlled genetic crosses in experimental animals. Whereas the first three approaches are directly applicable to humans, the fourth is predicated on the assumption that the chosen animal model will mimic the genotype/phenotype relationship in humans.

(1) Linkage analysis

The single biggest challenge in finding the genes that cause heritable differences is the problem of scale. The nucleus of a single human sperm or egg contains approximately 3 billion base pairs of DNA (*genome equivalent*) and is estimated to contain approximately 100,000 genes. In principle, a single nucleotide substitution in a gene is sufficient to cause alterations in the function of the gene and, therefore, is capable of resulting in the altered phenotype.

Linkage analysis now makes it possible to identify a gene responsible for a specific disease without any prior knowledge of the function of that gene, other than that the disease is heritable in Mendelian fashion. This method is best suited for analyzing traits that follow a clear model (dominant or recessive) of inheritance. Linkage analysis is based on the logic that two randomly chosen segments of DNA (*markers*) will segregate together in the members of successive generations of a family, only if they are physically located close to one other on the same chromosome (*linked*). It follows that, if one of these DNA segments carries a disease gene, it is possible to find the genetic location of the disease, simply by testing many randomly chosen DNA markers until one that segregates with the disorder is found (as shown in **Figure 1**).

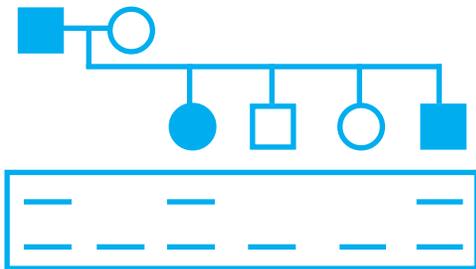


Fig. 1 Use of polymorphic DNA markers to establish genetic linkage with an inherited disorder. A pedigree showing the affected (*shaded*) and unaffected (*open*) individuals is one of the first steps in carrying out linkage analysis. By convention, males are represented by *squares* and females by *circles*. When DNA samples from the members of a family affected by the disorder are analyzed with a polymorphic marker, the pattern inheritance of alleles (A_1 and A_2) can be used to establish linkage with the disease. In this example, the disease phenotype always segregates with the A_2 allele.

The development of such DNA markers is based on slight variations, or *polymorphisms*, in the DNA of all human beings. These variations occur very frequently in the human genome, and these polymorphisms make it possible to track the inheritance pattern of a specific gene in families and, by extension, in the population at large.

The most useful polymorphic markers to date are based on individual differences in repeated dinucleotides and trinucleotides called *Short Tandem Repeats*, or STRs, that are widely prevalent in mammalian genomes.

The statistical measurement used to establish whether a randomly chosen marker is actually linked to a disease gene is called the *LOD (Logarithm Odds) score*. By convention, the LOD score is considered “significant” if it has a value of 3 or greater. Because the LOD score is a logarithmic function, a value of 3 implies that the likelihood of “true” linkage is 10^3 , or a thousand, times more likely to be true than the observation of linkage by chance alone.

On finding a marker that shows a LOD score of 3 or more, various techniques are used to obtain a stretch of overlapping DNA clones extending in both directions from the linked marker. A *genetic map* is thus constructed by positioning markers in the region containing the disease gene. This map, constructed on the basis of the LOD scores for each marker, provides information on the placement of markers, with regard to one another, as well as with regard to the approximate distances between them. It is important to note that only the first step in linkage analysis (*i.e.* finding a marker that is linked to the disease) is truly “random.” Once a linked marker is found, all subsequent markers in the vicinity of the disease gene are obtained in a systematic march towards the disease gene. The disease gene is eventually identified—on the basis of finding sequence differences between affected and unaffected individuals. Because the entire process is based solely on knowing the position of known DNA markers that segregate with the disease, this technique is called “*positional cloning*.”

Positional cloning has been used successfully to locate the genes causing hereditary diseases such as Duchenne muscular dystrophy, cystic fibrosis, neurofibromatosis type 1, myotonic dystrophy and Huntington’s disease.

The availability of relatively well-characterized families, having multiple members who are affected by the disease being studied, is equally important. A key consideration in the collection of families is that they are unequivocally shown to be “*homogeneous*” (*i.e.* affected by the same disease entity), because the inadvertent inclusion of families having similar symptoms with some other disease entity, generally would result in unsuccessful analysis. The establishment of *strict clinical criteria defining the disorder*—is therefore a crucial requirement for linkage analysis. The same would hold for environmental toxicology or occupational medicine: any incorrect definition, or assessment, of exposure to a toxic agent (phenotype) might result in unsuccessful linkage analysis.

(2) Allele-sharing (non-parametric) methods

Allele-sharing methods are based on the demonstration that a gene (or chromosomal region containing the gene) does not segregate in a random manner. The objective of this method is to demonstrate that affected relatives inherit identical copies of the region (*identity by descent*, or *IBD*) more often than expected by chance alone. Unlike linkage analysis, allele-sharing methods are not dependent on the specification of a parameter, or “*model*,” for segregation of the trait. Because of this,

allele-sharing methods are feasible—even when phenotype, genetic heterogeneity, and incomplete penetrance (not everybody who inherits a mutant allele is affected) are seen in individuals with the trait.

The most significant recent success of this method in the analysis of complex traits has been in a study of hypertension, which implicated the *angiotensinogen* gene on chromosome 1 in the pathogenesis of essential hypertension (Jeunemaitre *et al.*, 1992). In a comprehensive molecular genetic study of essential hypertension, an excess of shared angiotensinogen alleles among hypertensive siblings was observed. Based on these data, it was concluded that mutations in the angiotensinogen gene could be predisposing factors for increased blood pressure in at least 3% to 6% of “early-onset hypertension” patients. It has also been found that a specific variant of the angiotensinogen gene is associated with increased blood pressure and increased plasma levels of angiotensinogen. Taken together, these findings strongly suggest that a blood pressure regulatory locus exists at, or near, the angiotensinogen locus on chromosome 1.

A systematic scan of polymorphic markers evenly spaced throughout the human genome is now feasible—due to the availability of large numbers of highly polymorphic microsatellite PCR markers from the Human Genome Project (which administratively also includes the Mouse Genome Project). Such “*genome scans*” have recently implicated the *HLA* locus on chromosome 11 in the pathogenesis of type 1 diabetes in humans, in which approximately 250 well-dispersed markers (roughly ten per chromosome) were used in a cohort of 98 affected sib-pairs (Davies *et al.*, 1994).

(3) Association studies

This method is not directed at tracking the segregation patterns of alleles within related individuals (pedigrees or affected sib-pairs). Association studies simply consist of comparing the frequency with which a given allele occurs in a population of *unrelated affected* individuals, when compared with a population of *unrelated unaffected* individuals. Association studies were mainly used to implicate the *HLA* complex in ankylosing spondylitis, where it was shown that one particular allele (*HLA-B27*) is present in 90% of the affected population, and only in 9% of an unaffected control population.

The interpretation of positive associations

observed in the analysis of complex traits is complicated by the fact that such associations can arise even if the suspected allele (allele *S*) does not cause the trait. This is possible if the allele is in *linkage disequilibrium* with the “real” disease-causing allele (allele *D*). This error in interpretation could occur if the disease allele arose in a small “*founder*” population (bottleneck in breeding), which also happened to have the allele *S* on the same chromosome. This often means that this association might be seen between the trait and different alleles of the same gene in unrelated populations. A final caution in the interpretation of association studies is based on the fact that positive associations are sometimes seen as a result of using genetically heterogeneous, or mixed, populations. For example, assume a trait is seen more frequently in population A than in population B. Two groups from population B are compared, one showing the trait (affected) and the other not showing the trait (unaffected). If an allele *S* is observed at higher frequency in the affected group, it might simply be due to the fact that this group has greater genetic “*admixture*” with population A, and might have nothing whatsoever to do with causing the trait. Due to these caveats, therefore, association studies are not well suited to large heterogeneous populations.

(4) Controlled genetic crosses using animal models

The single biggest difficulty in the genetic dissection of complex traits in humans arises from the fact that any analysis is limited to the genetic material that is available. The ability to arrange experimental crosses is therefore the crucial advantage in the genetic dissection of animal models such as the mouse or the fruitfly. The logic of such efforts is that once the gene(s) causing the trait are found in animals, these can be used as probes to isolate similar genes from human DNA using molecular biological methods. The method is sometimes offset by the fact that the trait in the animal model might not be related physiologically to the trait in humans.

The main attraction of animal models lies in their usefulness for the dissection of *quantitative trait loci* (QTL) mapping. The arrangement of specific breeding protocols, in which animals are inbred until they are genetically homogenous, reduces the genetic “*noise*” that makes analysis of quantitative traits difficult in humans. Genome-wide QTL analyses (experimentally the same as the genome scans described in linkage analysis) have been used to study epilepsy in mice and hypertension in rats. The most useful application of QTL mapping, however, is likely to be in the identification of genes that “modify” single gene traits. For example, if the targeted “knockout”

Reader Response

Do you wish to contribute an article or news to an upcoming issue of the Interface newsletter? CEG members and others, we would like to know about any awards, publications, invited seminars, and other accomplishments. Letters to the editor are most welcome. Please fill in the information and submit text to address below.

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of a specific gene results in a different phenotype in mice having different genetic backgrounds, then it is possible to dissect out the gene(s) that modify the expression of the phenotype. Once the different genes influencing the expression of a trait are identified, sophisticated animal models—in which combinations of these genes can be introduced into mice with the same genetic background—can be used to study complex biochemical and physiological pathways.

Can DNA-based diagnostics help identify human populations at risk?

With all of the concerns inherent to the ethics of genetic testing for susceptibility to environmental agents, there is the feeling that—sooner or later—this information will be useful in the prevention and/or intervention of human environmental diseases. Many professional societies have issued statements urging caution, but at the same time commercial genetic testing laboratories are banking on the huge market that would be represented by such universal screening.

Suggested Further Reading

Davies JL, Kawaguchi Y, Bennett ST *et al.*, 1994, A genome-wide search for human type-1 diabetes susceptibility genes. *Nature* **371**, 130-136
Jeunemaitre X, Soubrier F, Kotelevtsev YV, *et al.*, 1992, Molecular basis of human hypertension: role of angiotensinogen. *Cell* **71**, 169-180
Lander E, Schork NJ, 1994, Genetic dissection of complex traits. *Science* **265**, 2037-2048

—Contributed by Anil G. Menon

CEG Members in the News

Eula Binham will be attending the 1995 Annual Assembly of the Rural Coalition at the Navajo National headquarters (Window Rock, Arizona), June 1995. The theme for the Assembly will be “one People, One Planet: Replanting Community on the Land.”

Iain Cartwright was invited to Japan in March 1995 by the Science and Technology Agency (STA) as part of ongoing research collaboration with Japanese colleagues at the National Institute of Radiological Sciences. While there, he delivered a series of seminar presentations at the National Institute of Radiological Sciences, the National Institute of Genetics, and Kyoto University.

Kathleen Dixon has been invited to serve as a member of the National Toxicology Program (NTP) Board of Scientific Counselors' ad hoc working group. This ad hoc working group will review the criteria for selecting substances nominated for listing in the Biennial Report on Carcinogens (BRC) in an open, public meeting (Washington, D.S.), April 1995.

Sohaib Khan attended a National Cancer Institute/ Gynecological Oncology Group (GOG) research retreat (Virginia), April 1995. About 50 basic scientists and oncologists were invited to develop a 5-year plan for translational research in GOG.

LETTERS

RESPONSES TO VARIOUS QUESTIONS

Q I have just received the third issue of your NewsLetter, INTERFACE, and I am quite curious as to how my name was added to your mailing list and wondered if you could please clue me in.

I am also wondering how much information you have received about our situation and whether our site is being studied in any fashion by any of your CEG members. I am the president and spokesperson for the citizen's group involved with the ---, Ohio, Superfund Site, which has actively fought for almost 12 years now to obtain a safe and permanent cleanup of this extremely toxic site, as well as to obtain the truth regarding the years of past exposures via the air, soil and water.

In addition, I would appreciate a copy of the November 4, 1994, talk by your CEG guest speaker Wolfgang Hüber on the subject of the plasticizer di(2-ethylhexyl)-phthalate (DEHP). DEHP has been found here frequently in water samples, as well as in actual barrel samples found onsite. Our cancer rate is reportedly 8-9 times higher than the national average.

A Your name was given to us by the National Institute of Environmental Health Sciences (NIEHS, Research Triangle Park, NC) as part of a list of “lay” and “semi-lay” groups all over the nation interested in the environment. We do not know any details about your “situation,” nor is any CEG member studying in any fashion your site.

I'd like to answer your query about Wolfgang Hüber, who is in Rolf Shulte-Hermann's Institute of Tumorbiology and Cancer Research (Vienna, Austria). I understand that Dr. Hüber is currently on sabbatical in the Department of Gastroenterology at Vanderbilt University (Nashville, TN). Perhaps you could call him there. Although Dr. Hüber gave a seminar in our department last November, there were no handouts or summaries distributed with his presentation. If you have further questions or comments, please do not hesitate to contact me.

Q Although I was not aware of issue #1 or #2 or your NewsLetter, I just received issue #3 and am very impressed. I want to be sure my name is on the mailing list for future issues! And is it possible for me to receive the previous issues as well? Your write-up on “Environmental estrogens” was incredibly crisp, timely and lucid. I learned a lot without having to go to the literature to try to find out the latest on this fast-moving field. Keep up the good work! Your NewsLetter is really helpful to the community!

A Thanks for the compliments.

Q Your article about “Estrogens and the environment” was very informative, but I know there is a lot of talk about estrogens that we ingest every day in our food. Could you comment on this?

A Thank you for finding this summary informative. Actually, I have already asked an expert in the field of “plant estrogens (phyto-estrogens) ;to contribute an article to a future issue of our NewsLetter.

George Leikauf received the Kenneth Morgareidge Award from the International Life Sciences Institute (ILSI), (Hannover, Germany), February 1995. The award is given to a toxicologist in recognition of a significant contribution to the broad field of toxicology. It is presented in conjunction with ILSI-sponsored international symposia on inhalation toxicology.

Grace Lemasters received an award from the Univeristy of Cincinnati to attend the Bryn Mawr Summer Institute for Women in Higher Education Administration, June 1995. She also has been invited to be a chapter editor, "Female reproductive health" for the International Labor Organization Encyclopedia of Occupational Health (Geneva, Switzerland).

John Loper was recently awarded a 5-year renewal of the Univeristy of Cincinnati NIEHS Superfund Basic Research Program, entitled "Microbial Detoxication/Degradation of Hazardous Wastes," for which he is Program Director. This program is currently in its 7th year of funding. Principal investigators of basic research in the NIEHS-SBR Program include **K. Dixon, J. Loper, M.W. Tabor** and **D. Warshawsky**, all of whom are investigators in the CEG. The Technology Resources Core was established recently under the University of Cincinnati NIEHS Superfund Basic Research Program Center and is now fully operational. John C. Loper and M. Wilson Tabor are Core Principal and Co-Investigators, respectively. This Core provides the interactive mechanism whereby results of basic scientific investigations in the Center are applied to current industrial problems having environmental and potential human health impacts.

Dan Nebert was an invited speaker at the Keystone Symposium on Molecular Toxicology (Copper Mountain, Colorado), January 1995. He was also an invited speaker at two symposia during the Annual Meeting of the American Association for Cancer Research (Toronto, Canada), March 1995: "Mechanistic Basis of Ethnic Differences in Cancer Risk," organized by the Minority Issues Community; and "Contribution of Environmental Factors to Cancer." Both symposia had been organized and chaired by Kenneth Olden, director of NIEHS.

Steven Potter will lecture on the topic "Hox genes and pattern formation" at Oak Ridge Research Laboratories (Oak Ridge, Tennessee), April 1995, and for the American Thoracic Society (Seattle, Washington), May 1995.

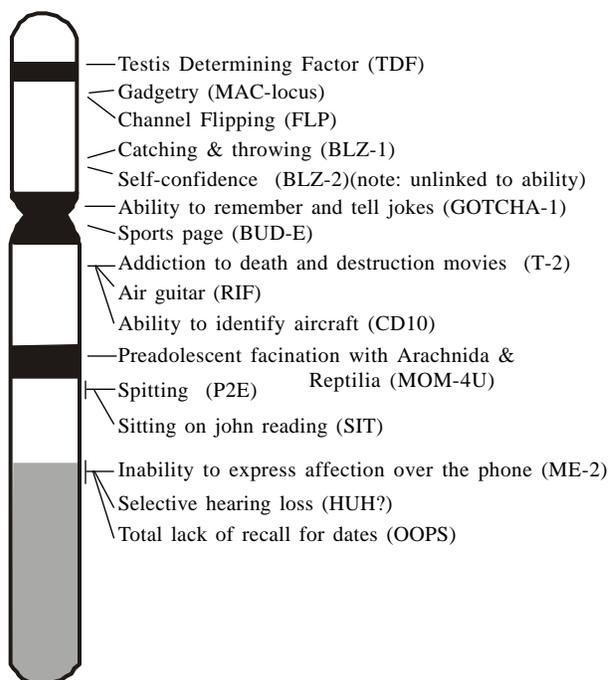
Alvaro Puga has been invited to chair the Continuing Education Course on Molecular Biology for Toxicologists at the 7th International Congress of Toxicology (IUTOX)(Seattle, Washington), July 1995.

Wilson Tabor recently was named the 1995 Distinguished Scientist by the Engineers & Scientists of Cincinnati Society. He was also the recipient of the William and Martha Defriece Award, the oldest and most prestigious honor given to an alumnus of Emory and Henry College for distinguished service to humanity.

Glenn Talaska has been invited to give three talks this spring: "Development of non-invasive biomarkers for carcinogen-DNA adduct analysis in occupationally

SCIENCE LITE

Mapping Genes on the Y Chromosome



The Y chromosome, found in Rodney Dangerfield and all other men, don't get no respect. Many geneticists have dismissed this stunted scrap of DNA as a genetic junkpile - mostly filler. But when recent research suggested that the Y may carry scores of genes after all, no one was less surprised than geneticist Jand Gitschier of the University of California, San Francisco. In fact, she had already come up with her own map of the Y, shown at left, which combines cutting-edge genetics with classic seat-of-the pants behavioral empiricism. Shown by Human Genome Project head Francis Collins at a recent meeting, the map is the careful product of years of observation and the contributions of many colleagues. Not all males will display all traits, of course, and expression is variable. For example Gitschier notes that air guitar in men over 50 is usually expressed as air violin.

- Not yet mapped, but on Y chromosome:
- Aggressiveness and competitiveness in every sport (AGGrr)
- Always leaves toilet seat up (tsIUP)
- Homophobia (HOMOφ)
- Nosepicking in public (NPP)
- When removing socks from feet, always leaves socks inside out (sox10)
- Always spitting for no apparent reason (SPTT)

exposed workers,” at the Conference on Risk Assessment Issues for Sensitive Human Populations, USEPA, ORD, ATSDR Wright-Patterson Airforce Base (Dayton, Ohio), April 1995; “Carcinogen-DNA adduct markers of human exposure to polycyclic aromatic hydrocarbons,” EPA Congress on Biomarkers of Exposure, Mickey Leland Center, M.D. Anderson Medical Center (Houston, Texas), April 1995; and “Carcinogen-DNA adducts as biomarkers of occupational chemical carcinogen exposure,” at a Roundtable on Biomonitoring for Chemical Carcinogens, American Industrial Hygiene Conference and Exposition (Kansas City, Missouri), May 1995.

Charles Vorhees will present a talk entitled, “Methamphetamine-induced developmental neurotoxicity,” at the Albert Einstein College of Medicine (New York, New York), March 1995. He will also lecture on, “Cross-species extrapolation of developmental neurotoxicity data,” at Abbott Laboratories (Chicago, Illinois), spring 1995.

Upcoming Meeting

June 19 & 20, 1995, The Ritz-Carlton at Tysons Corner, McLean, Virginia. Cambridge Healthtech Institute’s Strategies for the Design and Management of CLINICAN TRIALS. Organized and Sponsored by : Cambridge Healthtech Institute, 1000 Winter Street, Suite 3700, Waltham, MA 02154.
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INTERFACE

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RECENT CEG-SPONSORED SPEAKERS

NOVEMBER 9, 1994

Scott W. Burchiel, PhD

Assistant Dean for Graduate Studies and Research
Professor of Pharmacology, Toxicology and Immunology
The University of New Mexico College of Pharmacy
Albuquerque, New Mexico

“Alterations in Ca²⁺-dependent signaling pathways in lymphoid and non-lymphoid cells by PAHs: potential applications to immunotoxicity and human breast cancer”

FEBRUARY 22, 1995

Muin J. Khoury, MD, PhD

Deputy Chief, Birth Defects and Genetic Diseases Branch
Division of Birth Defects and Developmental Disabilities
Center for Environmental Health and Injury Control
Center for Disease Control
Atlanta, Georgia

“Epidemiologic approaches to gene-environment interactions”

APRIL 10 & 11, 1995

John Cairns, DM

Emeritus Professor, Harvard School of Public Health
Clinical Trial Service Unit
Harkness Building
Radcliffe Infirmary
Oxford OX2 6HE, England

“Control of the human environment and the prevention of untimely death,”

“The causes of spontaneous mutation and their role in human cancer”

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