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INTERFACE: GENES AND THE ENVIRONMENT

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Marshall W. Anderson, PhD, Director **Daniel W. Nebert, MD, Editor** Marian L. Miller, PhD, Editorial Assistant

Endometriosis and Dioxin-- Is There a Cause-and-Effect?

The "***endometrium***" is the normal lining of a woman's uterus, which develops during the first 3 weeks of each menstrual cycle and then is discarded if no pregnancy has occurred. A normal pregnancy involves implantation of the fertilized egg in the endometrium. ***Endometriosis*** is a disease that occurs when endometrial tissue grows and proliferates outside the uterus. The ***ectopic*** presence of endometrial tissue (outside-the-uterus) can often be found in the ovary, urinary bladder, intestine, and pelvic ***peritoneum*** (lining of abdominal cavity). Endometriosis can result in infertility, chronic pelvic pain, ***dyspareunia*** (pain during intercourse), ***menometrorrhagia*** (intermittent uterine bleeding at abnormal times of the month), and ***dysmenorrhea*** (difficult menstruation).

IN THIS ISSUE

Possible Link between Environmental Chemicals and Risk of Endometriosis

The prevalence of endometriosis in the general population is estimated at 10% in women of reproductive age; this means about 6.6 million women in the United States alone. The positive diagnosis of endometriosis can only be made by ***laparoscopy*** (surgically opening the abdominal cavity, or looking through the abdominal wall with fiber-optic instrumentation) and, for this reason, the actual prevalence in the general population could be much higher—perhaps as high as 18% to 25%. In certain populations, the incidence of endometriosis has indeed been found to be considerably higher; for example, one Belgian study found that 60-80 % of women who experienced infertility or pelvic pain also had endometriosis. Interestingly, the prevalence of endometriosis in Belgium was suggested to coincide with elevated concentrations of environmental contamination from polyhalogenated aromatic hydrocarbons (PHAHs). This suggestion was offered, after elevated polychlorinated biphenyl (PCB) levels had been found in women suffering from endometriosis [the subject of PCB toxicity was covered in issue #14 of the ***Interface***]. In Israel, a more recent study of 44 infertile women with endometriosis showed elevated blood levels of another environmental contaminant—2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, or dioxin).

What Is "Dioxin?"

Dioxin is one of a family of PHAHs, and a related organochlorine to PCBs. In the past, dioxin was produced as a contaminant during the commercial synthesis of an ingredient (***hexachlorophene***) in antiseptic soaps, and synthesis of the defoliant "***Agent Orange***" (2,4,5-trichlorophenoxy acetic acid) used heavily as an herbicide during the Vietnam War—but also still used today as a weed-killer along interstate highways, especially in the Southern

half of the United States. Dioxin continues to be produced as a by-product of numerous industrial combustion processes.

Dioxin exposure results in a broad range of toxic responses—including ***chloracne*** (overgrowth of cells in sebaceous, or sweat, glands), ***hyperkeratosis*** (overgrowth of the top layer of skin cells), ***edema*** (swelling of soft tissues), ***hyperplasia*** of ***epithelial*** tissue (*e.g.* overgrowth of liver cells, or cells lining the stomach), ***thymic atrophy*** (wasting away of the thymus gland), ***immune dysfunction*** (fewer white cells to fight infection), ***hepatotoxicity*** (toxic effects to the liver), ***embryotoxicity*** and/or ***teratogenicity*** (birth defects, early abortion, stillbirths), and ***reproductive toxicity*** (*e.g.* infertility). Dioxin is known to up-regulate several dozen genes—including the cytochromes P450 1A1, 1B1 and 1A2, and at least one quinone reductase, aldehyde dehydrogenase, UDP glucuronosyltransferase and glutathione S-transferase**;** growth regulatory genes involved in inflammation and differentiation [*e.g.* plasminogen activator inhibitor-2 (*PAI2*) and interleukin-1β (*IL1B*)**;** and various hormone receptor pathways [*e.g.* estrogen receptor, progesterone receptor, androgen receptor, prolactin receptor, and epidermal growth factor receptor (*ESR1, ESR2, PGR, AR, PRLR, EGFR*)]. Dioxin mediates its effects by way of binding to the aryl hydrocarbon receptor (AHR). Following activation of the AHR, the ***receptor-ligand complex*** is translocated to the nucleus, binds as a heterodimer [with the Ah receptor nuclear translocator (ARNT)] to a specific DNA motif in regulatory regions that control gene activity, and activates transcription of various genes [this subject was addressed in issue #2].

Animal Studies Support Clinical Studies Suggesting a Link between Dioxin and Endometriosis

In terms of reproductive toxicity, exposure of various laboratory animal species to dioxin and other PHAHs has long been known to result in reduced fertility, decreased litter size, lowered uterine weight, and altered ovarian function. The mechanism(s) by which dioxin causes various reproductive toxicity is(are) unknown. Furthermore, there are few data with regard to understanding dioxin's effects on the female reproductive tract.

It has only been during the past decade that epidemiological research had suggested a possible link between dioxin exposure and prevalence of endometriosis in certain populations of women. As a result of these findings, a number of animal studies have been done to investigate further this apparent correlation between dioxin exposure and the development and/or proliferation of endometrial tissue.

A key animal study—linking dioxin exposure to the development of endometriosis—was the research by Rier *et al.* (1993) in which Rhesus monkeys were chronically exposed to dioxin for 4 years. Ten years later, following the dioxin exposure, the prevalence of endometriosis was determined in the monkey colony. This study was therefore initiated in the late 'seventies! Twenty-four female monkeys had been chronically exposed to either 5 ppt (parts per trillion) or 25 ppt dioxin, daily, in their food. At the completion of the study, 43% (5 ppt) and 71% (25 ppt) of the monkeys were diagnosed as having endometriosis, when compared with controls in an untreated group. However, 33% of the untreated monkeys in this colony exhibited endometriosis and, genetically, these monkeys are random-bred (meaning that there is little experimental control over each individual's genetic make-up).

What Are the Possible Causes of Endometriosis?

Although the etiology of endometriosis is currently unknown, proposed mechanisms include**: [a]** retrograde menstruation (up through the Fallopian tubes and into the peritoneal cavity), **[b]** immune dysfunction, **[c]** endocrine disruption/hormone mimicry, and **[d]** a genetic basis. The ***retrograde menstruation*** theory, although the most widely accepted, fails to explain the development of endometriosis in the majority of cases. Thus, the other mechanisms have been receiving more and more attention lately.

The involvement of the ***immune system*** has been suggested by findings of alterations in cell-mediated immunity, and a reduction in serum complement factors (proteins that fight infections), in endometriosis patients. It is also well documented that endometriotic lesions are estrogen-dependent**;** thus, a role for perturbation of the ***endocrine system*** in the development of endometriosis is feasible. Interestingly, environmental contaminants such as PHAHs, including dioxin, are known to cause immunosuppression in humans and laboratory animals and to disrupt endocrine systems in laboratory animals. Thus, it is not unreasonable to postulate that PHAHs might affect the development and potentiation of endometriosis through either immunosuppression, or an endocrine disruptive mechanism, or both.

There is also evidence to suggest a ***genetic component*** involved in the development of endometriosis. Studies have shown a higher prevalence of the

disease among sisters of affected women, as compared with that in the general population. Moreover, the age of onset of pain symptoms in identical-twin sisters who invariably both come down with the disease—almost always occurs within the same year of age. Racial differences have also been noted**;** Asian women have the highest prevalence of endometriosis, whereas women of African origin have the lowest.

Endometriosis Is a Complex Trait

The mode of inheritance of endometriosis is unknown, but the disease is thought to be a "multiplex phenotype," similar to diabetes or asthma, in which two or more (perhaps more than a dozen) genes are involved ["multiplex phenotype" was discussed in issue #4]. Interaction of these genes with an environmental component such as PHAHs (including PCBs and dioxin), therefore, seems quite likely. An intensive investigation, known as the OXEGENE study, is underway. This research involves the Oxford Endometriosis Group (centered in Oxford, United Kingdom), and includes workers at 52 centers around the world. The primary aim of the OXEGENE study is to identify ***human susceptibility genes*** involved in the development of endometriosis.

Possible Role of Glutathione S-Transferase

One gene that appears to contribute to the susceptibility of endometriosis is the gene for glutathione S-transferase M1 (*GSTM1*). The GSTM1 protein serves as both a detoxification enzyme and—more relevant to the hormonal problem of endometriosis—as an intracellular binding protein for hormones and drugs. Two functionally active alleles of the *GSTM1* gene (*GSTM1*1* and *GSTM1*2*, earlier termed **A* and **B*), and one null allele in which the gene is deleted (*GSTM1*0*) have been described so far. Curiously, the *GSTM1*1/*2* genotype has been reported as having the highest GSTM1 enzymic activity, whereas both the *GSTM1*1/*1* and *GSTM1*2/*2* homozygotes exhibit decreased GSTM1 enzyme activity. The prevalence of *GSTM1*0/*0* homozygotes in certain ethnic populations can run as high as 40-50%. The GSTT1 enzyme is another candidate that should be examined in endometriosis patients, because the incidence of *GSTT1*0/*0* homozygotes in human populations is also quite significant.

Two different studies have investigated the frequency of the *GSTM1*0/*0* genotype in patients with endometriosis. The first study was conducted in Slavic populations from the north-western and central-eastern regions of European Russia. The proportion of patients with endometriosis and the *GSTM1*0/*0* genotype was 81%, as compared to 38.8% and 67.5% in the general population (north-western and central-eastern regions, respectively). The second study was carried out in a French population. The *GSTM1*0/*0* genotype was identified in 86% of patients, as compared to 45.8% in a non-patient population. The *GSTM1*1/*1* or *GSTM1*1/*0* genotypes in the French were significantly more frequent in the healthy group, as compared with that in the endometriosis group (29.2% versus 6.1%, respectively). On the other hand, the incidence of the *GSTM1*2/*2* or *GSTM1*2/*0* genotypes was relatively equal between the two groups. No patient was identified with a *GSTM1*1/*2* genotype. These two studies thus indicate that the *GSTM1* gene (and, hence, GSTM1 enzyme) deficiency could potentially predispose women to endometriosis.

In addition to its function as an intracellular protein for binding sex hormones, GSTM1 has been shown to have a high specificity for detoxifying a number of PHAH reactive metabolites. Therefore, individuals with the *GSTM1*0/*0* genotype could be at increased risk for toxicity caused by various PHAHs as a result of an enzymatic defect in detoxification by GSTM1. The *GSTM1*0/*0* genotype has also been shown to be an individual risk factor for urinary bladder cancer in workers exposed to various PHAHs in the coal, iron, and steel industries. In addition, studies have shown that cigarette smokers have an enhanced risk of bladder cancer, when their risk profile includes the *GSTM1*0/*0* genotype.

Summary

In conclusion, the *GSTM1*0/*0* genotype appears to predispose women to developing endometriosis. In addition, increased levels of dioxin have been found in women suffering from endometriosis. The *GSTM1*0/*0* genotype has also been implicated in the aforementioned occupational and lifestyle (cigarette smoking) studies**;** however, in these cases the subjects were all men, and reproductive parameters were not studied. It is noteworthy that ***cellular proliferation*** is an important characteristic of both endometriosis and cancer. It seems obvious that genes involved in ***angiogenesis*** (growth of blood vessels, normally during embryonic development, abnormally during tumorigenesis) should also be pursued for their possible role in endometriosis. Further studies determining exposure levels of a variety of PHAHs in addition to dioxin, screening women with the disease *versus* those without endometriosis and looking at their *GSTM1* and *GSTT1* genotypes, should be important in our understanding the etiology of this disease. Presently, it seems quite clear that endometriosis is an excellent example of a polygenic disease with an environmental exposure component.

———***Contributed by Amy L. Roe, PhD, and Daniel W. Nebert, MD***

READ *INTERFACE*:online

The current issue #17, and previous issues # 13-16, can be viewed online as PDF files, through the WWW site. This online version is virtually identical to the printed version and you can download ***INTERFACE*:online** at http://www.med.uc.edu/ceg/ceg,html, then access our Community Outreach and Education Program page. Drop us an email and we will notify you through a *LISTSERVE* when new ***INTERFACE*** newsletters are published. If you are already on our mailing list and wish to be switched to the *LISTSERVE* please email us with your request (millermn@email.uc.edu).

Endocrine Disrupters– Fact or Fiction?

In a 4-year investigation, members of a panel put together by the U.S. National Research Council, part of the National Academy of Sciences complex, have concluded that "insufficient evidence exists to provide an accurate assessment of the risks to public health posed by the chemicals known as endocrine disrupters" **[*Nature* 400:** 607,1999]. Because of the incredibly low ambient doses found in the environment, the doses were "far too small to trigger a conventional toxic response." The current data also "do not support (or refute) the widely-propagated theory that the overall human sperm counts worldwide have been falling." The panel of course recommends more research to test the endocrine disrupter hypothesis. This (unfortunate) lack of an unequivocal conclusion by scientists is a big problem discussed on page 12 in this issue ["Does the Public Trust Science?"].

Mutations in Our DNA?

The frequency of mutations in the human genome has been estimated to be approximately one per billion nucleotides per year [***Proc Natl Acad Sci USA* 83:** 389, 1986]. With 3 billion nucleotides per haploid genome and 25 years per generation, this would be (on average) 75 mutations per haploid genome per generation, or 75 mutations from male germ cells over 25 years (***haploid*** means "one of each chromosome pair"). If our DNA receives about 10,000 oxidative "hits" per cell per day (discussed in the "***Letters to the Editor***" page 9), this would be more than 91 million "hits" per 25 years. Only 75 mutations do not get properly repaired, out of 91 million "hits?" The bottom line is that our DNA repair systems are incredibly efficient at detecting damage and correctly fixing it up!

deCODE, Revisited

In several of our previous issues, we have described the ongoing saga in Iceland where a law was passed that permits the granting of an exclusive license to a single company to do genetic and other research on the whole population database of the nation. State-controlled banks have now purchased almost half of the U.S. venture capitalists' original investment in deCODE, which increases the Icelanders' concern about close ties between deCODE and the government. Loop holes in the law exist**;** the consequences have been "lack of informed consent, lack of traditional ethics control, and lack of freedom to withdraw information entered into the database" [***Nature* 400:** 707, 1999]. Furthermore, more than 11,000 Icelanders have already chosen to get out of the database, and many doctors have promised patients not to send information about them to the national database. The bottom line is that, what naively seemed so feasible 3 years ago, is not working out that way!

Latest on SNPs

The excitement about single-nucleotide polymorphisms (SNPs) was discussed in the leading article of our issue #12. Two major papers [***Nature Genet* 22:** 231 & 239, 1999)], which studied SNPs in and around about 200 different human genes, now conclude that the most common type of SNPs ("coding SNPs," or ***cSNPs***, which cause an amino acid change) may not be the most informative. Most SNPs are not likely to have a direct impact on their protein products, because they are in the estimated 95% that fall outside the coding area (perigenic, or ***pSNPs***), or because they behave in a silent way influencing expression of the same protein that a cSNP codes for.

Ten large pharmaceutical companies, a handful of academic laboratories, and the Wellcome Trust philanthropy of Britain formed a nonprofit alliance called The SNP Consortium (TSC) in April. They plan to create a SNP archive of about 300,000 SNPs throughout the human genome by mid-2001. Such genome-wide screens for SNPs will probably not find as many informative SNPs, however, as the hardcore approach (cited above) of straightforwardly resequencing candidate genes in as many patients as possible.

CEG Members in the News

Tatiana Foroud presented three invited talks: the first entitled ***"Search for new osteoporosis-related genes through genome scanning in human families"*** was given at the AIMM/ASBMR John Haddad Young Investigators' Meeting (April 1999, Snowmass CO); the second, ***"QTL analysis in outbred populations"*** was delivered to the Genetic Workshop at the University of Colorado Health Science Center (April 1999, Denver CO); and the third, ***"Genetics of alcoholism, biological vulnerability to alcoholism and drug addiction"*** was delivered to the Federation of American Societies for Experimental Biology Summer Research Conference (August 1999, Copper Mountain CO).

George Leikauf presented invited talks on ***"Genetic susceptibility of mice to particulate matter"*** at a Mini-symposium, American Thoracic Society International Conference, (April 1999, San Diego CA), and on ***"Pathogenetics of particulate matter"*** for the Health Effects Institute Annual Meeting, (May 1999, San Diego CA). He also was invited to speak on ***"Quantitative trait loci analysis and acute lung injury"*** for the Genetics and Physiology of Airway Hyperresponsiveness session of the American Thoracic Society Workshop (May 1999, Cambridge MA). At Harvard School of Public Health he spoke on ***"Genetic determinants of acute lung injury"*** (July 1999, Boston MA). He co-chaired a session on "Use of genetically altered and susceptible mice in understanding environmental and occupational exposures," at the American Thoracic Society International Conference (April 1999, San Diego CA).

Dan Nebert was an invited speaker at**:** the National Institutes of Health/U.S. Food and Drug Administration (NIH/FDA) Conference on "Biomarkers and Surrogate Endpoints: Clinical Research and Applications," (April 1999, Bethesda Maryland)**;** the Second International Human Gene Nomenclature Workshop (INW2) (May 1999, Cambridge England)**;** the 7th International *Biochemical Pharmacology* Symposium, "Redox-Controlled Gene Regulation in Environmental Toxicity and Cancer" (June 1999, Oxford England)**;** and Invited Keynote Speaker at the Annual Meeting of the Japanese Society of Toxicology (July 1999, Sapporo Japan). In June 1999 he received the University of Cincinnati **George Rieveschl Jr Award for Distinguished Scientific Research**, "in recognition of a lifetime career of highly successful, innovative, and distinguished scientific research in the field of environmental genetics, toxicology, evolution and gene nomenclature."

Grace Lemasters was recently appointed to the NIEHS National Toxicology Program Board of Scientific Counselors until June 30, 2002.

Frank McCormack was a visiting professor in the Department of Anesthesiology at the University of Alabama (June 1999, Birmingham) where he presented an invited lecture entitled ***"Structure and function of surfactant protein A."***

Alvaro Puga presented talks on his recent work on the molecular mechanisms of dioxin action at the following places during a tour (June-July) of several European research institutions: Institute of Toxicology, University of Mainz, (Germany); Finnish Institute of Occupational Health (Helsinki, Finland); Institute of Environmental Medicine, Karolinska Institute (Stockholm, Sweden); and Central Toxicology Laboratory, Astra/Zeneca Pharmaceuticals (Alderley Park, Cheshire, UK).

Nancy Steinberg-Warren was elected to the American Board of Genetic Counseling 1/2000-12/2005, and presented invited talks on ***"Folic acid: Genetic and environmental interactions***" to 100 nursing faculty attending the Genetics Summer Institute (June 1999, Cincinnati OH) and ***"Pedigrees"*** which was presented at a Workshop for 40 nursing faculty attending Genetics Summer Institute (June 1999, Cincinnati OH).

Glenn Talaska was elected to the Biological Exposure Indices (BEI) Committee of the American Conference of Governmental and Industrial Hygienists (ACGIH). This committee makes recommendations about markers and their levels for work place exposures. Glenn has also been named to the Organizing Committee for the Fifth International Symposium on Biological Monitoring which will be held in September 2000 (Banff, Alberta, Canada).

David Warshawsky was elected this spring as a Fellow of the Graduate School, University of Cincinnati. He was also appointed to the Editorial board for *Antioxidants and Redox Signaling*.

Eat Your (rice) Cereal!

There have been some recent debates about the benefits and liabilities of genetically modified (GM) crops. Plant molecular biologist Ingo Potrykus (Swiss Federal institute of Technology, Zürich), however, has clearly produced a winner. His group [*Science* **285:** 994 (1999)] has genetically engineered rice to make ***β-carotene*** (by a sequence of four genes encoding enzymes in the vitamin A pathway in daffodil!) and to double the ***iron*** content in the rice grains! To achieve this latter feat, they**: [a]** cloned in a gene that codes for a metallothionein-like protein which helps iron absorption in the human digestive system, **[b]** cloned in a ferritin gene to promote iron storage in the rice kernel, and **[c]** disrupted the rice gene making phytate which normally blocks most iron from being absorbed by the human digestive tract. Use of this GM rice in third-world countries should help poor populations who have vitamin A deficiency and anemia.

SCIENCE LITE

"Twas the night before 2K"

'Twas the night before 2K, and everyone in the house
Awaited "the year end" as each sat by his mouse.
New chips were placed in their computers with care,
In hopes the Y2K bug would not show up there.
Some folks were nestled snug under their beds,
While visions of "bedlam" danced in their heads.
And Ma with her PC, and I with my Mac,
Had logged on the Net, and kicked back with a snack.
When over the server there arose such a chatter,
I sprang from my chair to see what was the matter.
Connections were down, so I flew like a flash,
Off to my bank and withdrew all my cash.
Then, what to my wondering eyes should I see,
But my poor old Mac, looking sick as can be.
The hack of all hackers was acting so smug,
I knew in an instant 'twas the Y2K Bug.
His image downloaded and in no time at all,
He whistled and shouted "Let all systems fall."
Go Intel! Go Gateway! Now, HP! Yahoo!
Out Compaq! Out Dell! Out Pentium II!"
All processors big and all processors small,
Now crash away! Crash away! Crash away all!"
As I drew in my breath and was turning around,
Out through my mouse he came with a bound.
He was covered in bytes from his head to his toe,
And the screen of my PC showed nothing but snow.
I saw the great pack he had slung on his back,
Was a sackful of viruses all primed for attack.
His drives how they whirred! his circuits how bright!
As midnight approached, prophets proved to be right.
He had a broad bus and a round little drive,
And his sack filled with chaos was virtually alive.
He was chubby and plump, perpetually grinning,
I laughed when I saw him, then,
My hard drive stopped spinning.
A wink of his eye and a twist of his head,
Soon gave me to know the sick feeling of dread.
He spoke not a word, but went straight to his work,
He switched all the circuits, then turned with a jerk.
With control-alt-delete and a quick little wink,
All things electronic soon went on the blink!
Then he zoomed from my system,
To the next folks online,
Where he caused much disruption, as was his design.
I heard him exclaim with a loud chilling cry,
"Happy Y2K to all, and kiss your PC's good-bye."

.....modified significantly from author unknown.

Frogs "Dying Like Flies"

In several of our recent issues, we described the birth defects and anomalies found in frogs in the Great Lakes Region, and several (environmental and genetic) causes had been postulated. Another phenomenon, not necessarily related, is that there have been massive frog die-offs occurring around the world [***Science* 284:** 728, 1999]. The latest convincing explanation comes from Queensland, Australia**:** a virulent amphibian parasitic ***chytrid fungus*** has been shown to be the pathogen responsible for killing off more than a dozen frog species in that area. It now appears likely that this chytrid is the cause of catastrophic die-offs in Panama, Costa Rica, and the U.S. Since an occasional frog survives chytrid infection, some argue that this pathogen cannot be the only cause. But, what about genes predisposing to pathogen resistance in some individual frogs, just as we have the occasional human resistant to HIV? Possibilities for the chytrid success in mass die-offs of frog populations include two competing ideas**: [a]** that the potent chytrid infection has emerged because these particular ecological niches had never before been exposed, or **[b]** that an environmental cofactor(s)—such as increased UV light, or climate change—has magnified the chytrid's potency.

Double Your Pleasure,...

Milton Gallardo (Universidad Austral de Chile) in 1990 accidentally discovered that the red viscach rat (*Tympanoctomys barrerae*) had 51 pairs of chromosomes. Other rat species have 26 pairs (***diploid***). Gallardo's group has now found the viscach rat has twice as much DNA per cell. Although some amphibians and fish (*e.g.* trout) carry four copies of chromosomes (called ***tetraploidy***), this is the first mammal established to do so. However, the viscach rat has only one pair of the sex (X,Y) chromosomes—which is why the species has 51 instead of 52 pairs. In all likelihood, for mammals to have tetraploid sex chromosomes means lethality [***Science* 285:** 195, 1999].

Air pollution is a mist-demeanor.

Observations by a Biologist

Why Don't Snakes Have Legs?

In tetrapods (animals having two front legs and two hind legs), the skeletal vertebrae vary in shape—according to their position along the anterior-posterior axis. Homeobox (*HOX*) gene expression is limited to well-defined subregions of the trunk of tetrapods. During the past decade, developmental biologists have identified genes that control a limb's growth (from trunk to tip, and from front to back) in these subregions—as a ***forelimb*** from the shoulder or as a ***hindlimb*** from the hip. Almost all the genes are the same ones—used in both arm and leg formation. In the past year, three genes have been discovered to be more specific**:** *Tbx5* occurs in wings and arms, while *Pitx1* and *Tbx4* occur in legs. There is a rare clinical defect, ***Holt-Oram syndrome***, in which there are severely shortened arms (and heart problems), traced to a defective *TBX5* gene. Knocking out the leg-specific *Pitx1* gene in mice leads to animals with all four extremities "looking like forelimbs," whereas expressing *Pitx1* in the wings of developing chicks leads to "wings looking a lot like legs." The *Tbx4* gene seems to go hand-in-hand (so to speak), because it is "turned on" whenever the *Pitx1* gene is active. These three genes, therefore, influence "forelimbness" or "hindlimbness" by changing a cell's response to similar growth factors.

So, why don't snakes have limbs? In the python, the axial skeleton consists of hundreds of similar vertebrae**;** forelimbs are absent, but the hindlimbs are vestigial. *Hox* gene expression domains are expanded along the snake's axial skeleton, causing hindlimb buds to be initiated, but apical-ridge and polarizing region signaling by Sonic hedgehog (SHH) pathways do not become activated. By application of fibroblast growth factor, or by recombination with chick apical ridge, Cohn and Tickle (University of Reading, United Kingdom) were able to rescue this pathway—leading to pythons having more than 200 pairs of hindlegs! Early in the evolution of snakes, these authors theorize that the failure to activate the SHH signaling pathways during normal snake development might stem from changes in *Hox* gene expression [***Nature* 399:** 474, 1999].

MicroArrays, Beware!

At the Human Genome Organization/ European Union (HUGO/EU) Workshop on DNA Arrays (May 1999, Tartu Estonia), critical issues such as standardization of array data and presentation, as well as reproducibility and validation, were discussed. Small differences in probe sequences or target preparation can cause large differences in what superficially appears to be very similar experiments ["probe" = immobilized nucleic acid tethered on the surface; "target" = the free nucleic acid being examined]. Participants at the workshop concluded that it should become imperative for suppliers and users of the different technologies to come up with ***ways of normalization*** that will allow cross-referencing and cross-validation. For example, if two *Arabidopsis thaliana* clones in dilution series were included on the array with each individual spotting device worldwide, this would take into account variation between the individual devices [***Nature Genet* 22:** 211, 1999].

Congratulations to Pilot Project Recipients for 1999

Zalfa Abdel-Malek, PhD "Differential responses of human melanocytes with different constitutive melanin content to ultraviolet A exposure"

Nira Ben-Jonathan PhD "Xenoestrogens and neuroendocrine abnormalities"

Iain Cartwright, PhD "A genetic component to arsenic susceptibility"

Michael Carvan, PhD "Development of mutant zebrafish lines with enhanced resistance to alcohol-induced developmental toxicity: The role of oxidative stress"

Kathleen Dixon, PhD "Investigation of the mechanism of arsenic-induced carcinogenesis in a yeast model system"

George Leikauf, PhD "Genetic determinants of chronic obstructive pulmonary disease"

Daniel Woo, MD "Genetics of hypertension in intracerebral hemorrhage"

LETTERS TO THE EDITOR

RESPONSES/COMMENTS TO VARIOUS QUESTIONS

Q **I live in a small community of about 700 people. I am reticent to name my community for fear that I might spark some bad press or something. Our neighboring community (within 5 miles) is a "company town," so to speak, and the company is quite large with international business ties. To my knowledge, the company has a few toxic waste dump sites, but nothing nuclear. Otherwise, our community is largely agrarian. In the last few months, at least ten persons have been diagnosed with cancer: for example, a 38-year-old has breast cancer, and her sister was diagnosed with ovarian cancer; a 50-year-old with leukemia died within 2 months of diagnosis; a 50-year-old with colon cancer has a 7-year-old grandson with a rare bone cancer; a 43-year-old nonsmoker with lung has metastases to the brain; a 40-year-old with an immune deficiency (non-HIV) disorder had a 13-lb. spleen removed; and a 40-year-old had an enlarged thymus ("the size of a grapefruit") removed. Does this sound at all like "too much within a 6-month period" for a community of 700 people? Should we be concerned that we have a serious environmental hazard?**

A *Thank you for your interesting email. The only "scientific explanation" (and you might call it a "cop-out," because it essentially says we really don't know what's going on) for these kinds of anecdotal stories that appear from time to time—is that these events are called "clusters." Some form of disease, or multiple diseases or cancers, occur in a cluster (in this case, a community of 700 people). It is somewhat similar to tossing a coin and unexpectedly having it land "heads" 20 times in a row (although you'd expect one "heads" out of every two tosses). Or rolling five "sevens" in a row during a crap game (chances to do this are about one in 10,000). Or in hitting a jackpot in Las Vegas. Observing a cluster of medical problems in a small community and trying to correlate the illness with a particular environmental exposure are extremely difficult, or close to impossible, to prove in a court of law. The only scientific way to do this would be to unequivocally demonstrate that a particular chemical (in the blood, urine or fatty tissue)—at a certain concentration or higher—is always associated with a specific disease. This would confirm an exposure dose-effect; the other factor in the equation, however, is the* ***genetic make-up of each person!*** *Interindividual differences as to how we each might respond differently to the same dose of the same chemical (the theme of our CEG)—will make scientific studies even more difficult to show unequivocally that a particular chemical (in the blood, urine or fatty tissue)—at a certain concentration or higher—might always be associated with a specific disease.*

Q **On the television evening news, I learned that tap-water tests had been done on several cities (one of which is very close to where I live), and the results of these tests can be found at www.foodnews.org/atrazine.html So, I looked at that web site, and it scares me. What is your opinion of these data, and what do you know about atrazine?**

A *Thank you for pointing out this web site to me. As a representative member of the Society of Toxicology (SOT), I recently wrote a letter to Carol Browner (Head, U.S. Environmental Protection Agency, EPA), in support of the SOT's concern that the Environmental Working Group (EWG) is doing more harm than good—with its "Alarmist" tactics. As we should tell our children, "Not everything on the Internet is good, and not everything on the Internet is correct, or truthful." This web site is a good example of that.*

*Unfortunately, the EWG is encouraging "**chemophobia**" (fear of chemicals). Let's put this into perspective by using an example offered by Bruce Ames (Berkley CA). More than a thousand chemicals have been identified in roasted coffee; more than half of those tested (19 of 26) cause cancer in mice or rats (of course, at large doses). There are more "natural" cancer-causing chemicals by weight in a single cup of coffee than all potentially cancer-causing synthetic pesticide residues in the average U.S. diet for one year (and there are still more than a thousand chemicals in roasted coffee that have not yet been tested in rodent cancer assays)!*

Atrazine is a selective herbicide with a remarkably low toxicity index in humans; the inhalation hazard—in workers who synthesize this chemical—is very low, and no apparent skin irritation has been seen (which is often observed in workers exposed to a myriad of occupational

chemicals). The LD_{50} (dose causing 50% of animals to die) in mice is 1.75 and in rats is 3.08 ***grams*** *per kilogram. This would be the equivalent of the average-sized man eating between a quarter and a half pound of this chemical—although we cannot extrapolate from rodents to humans because we appear to be more resistant to atrazine than rodents. On that web site they state "Lifetime cancer risk as a multiple of the legal standard = 12.77" and "Years of age at which person exceeds the legal lifetime cancer risk = 1.2" This is utter nonsense, because* ***[a]*** *atrazine has not been shown to be cancer-causing in any laboratory animal, to my knowledge, and* ***[b]*** *the chemical is readily degraded by enzymes in the body, and excreted quickly, so that there would not be any cumulative effects. Finally, atrazine is clearly not considered to be an established human carcinogen at this time.*

Q **I just read a short article ["What's killing clones?"** ***U.S. News & World Report,*** **24 May 1999] that states "Many clones are dying while still young, due to genetic defects." One possible explanation they offered is "genetic imprinting." The article says that maternal and paternal genes are both necessary to "ensure that neither predominates in the offspring." About half of all cloned sheep and cows harbor serious abnormalities, including peculiar defects in the heart, lungs and other organs, often before birth. Some of these defects are similar to those in which imprinting has gone awry (*** ***e.g.*** **Beckwith-Wiedemann syndrome,** ***BWS*** **gene region on Chr 11p15.5). If the donor cell comes from a healthy individual and, thus, is the product of genetic imprinting, it should have the best genes of the mother and of the father. Why, then, if you "clone" this individual, would genetic imprinting be the "culprit" in producing an individual with genetic abnormalities?**

A *Admittedly, the process of micro-injecting a nucleus isolated from one cell into an enucleated ovum, or other cell type, is technically less than perfect [in some of the previous* ***Interface*** *issues, we have described the success rates of "achieving a viable cloned animal" as anywhere from one in eight to one in several hundred]—simply due to the mechanical manipulation of such cells in a culture dish. The phenomenon known as "imprinting" (which genes to turn "on," which to turn "off," at each critical point during embryogenesis and fetal development) is still only vaguely understood, so—whereas "defects in imprinting" might be a reasonable hypothesis—currently there are no solid experimental data either to support or reject that hypothesis.*

COMMENT **As described in detail in our NewsLetter issue #10, Dolly was a sheep cloned from the nucleus of mammary epithelial cells from a 6-year-old sheep. Three years later, the ages of this and two other cloned sheep were examined using the "telomere-degradation assay." As we and all animals age, it is now known that the chromosome ends (telomeres) become shortened at a rate that is dependent on the animal's age. Consequently, "3-year-old" Dolly was found to look more like a 9-year-old than a 3-year-old sheep. Similar findings were seen in the other two cloned sheep [*** ***Nature*** **399: 316, 1999].**

To this editor, these data are not at all surprising and would have to be taken into account if anyone ever plans to clone a human. The number of oxidative "hits" to DNA per cell per day is estimated to be about 100,000 in the rat and perhaps 10,000 in the human [* ***Proc Natl Acad Sci USA*** **92: 5258, 1995]. Although almost all of these DNA lesions are repaired daily, there is the natural accumulation over a lifetime of increasing numbers of mutations in one's DNA. It would therefore be much more reasonable, for example, to clone a 2-month-old or 6-year-old child than to clone Einstein at the age of 70. In other words, a cloned individual will inherit not only the genes but, in many respects, the age of the cells or animals from which the clone is derived.**

COMMENT **Cloned goats appear to have advantages over both cloned sheep and cows: goats are more abundant milk producers and don't take as long as cows to mature. Researchers at Genzyme Transgenics (Framingham, MA) cloned their first goat last October [reported in the May 99 issue of** ***Nature Biotechnol*** **] to produce human antithrombin III, a protein used to prevent damaging blood clots after strokes or heart attacks. Nexia Biotechnologies (Montreal, Canada) has cloned four rapidly-maturing dwarf goats to produce a protein that orb-weaving spiders use in making drag-line silk (the protein has incredible strength and is biodegradable). Both proteins are secreted in milk, because the enhancer/promoter that has been engineered to drive the gene in each case specifically "turns on" gene expression in excreted milk.**

Genetic Background Is Important

As described in some of our earlier issues, the importance of "genetic background" has become increasingly realized—especially from transgenic and knockout mouse studies. A gene knockout in one inbred mouse strain might cause a phenotype of death in utero, whereas the same gene knockout in another inbred mouse strain might be lethal only after 1 month post partum. Two recent examples underscore this concept.

The mouse mismatch repair gene, *Msh2*, and the p53 gene that plays a role in genomic instability, *Trp53*, were studied for effects of their combined deficiencies. Females having the *Msh2(-/-)/Trp53(-/-)* double-knockout in a C57BL/6J (B6) and 129/Ola (129) genetic background died at gestational day 9.5, while males died of thymic lymphomas on average 73 days following birth. From another laboratory, in contrast, females having the *Msh2(-/-)/Trp53(-/-)* double-knockout in a BALB/c + B6 + 129 genetic background remained viable throughout gestation, and both females and males died of thymic lymphomas on average 65 days following birth. At least one modifier gene has been postulated to account for the female- and strain-specific embryonic lethality [***Mamm Genome* 10:** 1020, 1999].

While studying "***environmental endocrine disrupters***," the effects of estrogen dosage on testes weight and spermatogenesis were highly variable, depending on which inbred mouse strain was used. More than **16-fold differences** in susceptibility to disruption of juvenile male reproductive development were found between the most sensitive (C57BL/6J) and most resistant (CD-1) strain [*Science* **285:** 1259, 1999]! These findings are especially relevant to studies by the U.S. Environmental Protection Agency (EPA) in Atlanta, because CD-1 has been their "mouse of choice" for endocrine disrupter studies. And, of course, if ***this*** much variability in response to estrogens is seen between mouse strains, what will we expect to see between different humans in any population study? An additional point worth noting is the phytoestrogen content in rodent diets [***Environ Health Perspect* 107:** A182, 1999], which can affect the results even further!

Genomically Speaking, ...

Human genome race is on. As we move from spring to summer of 1999, the U.S. National Human Genome Research Insitute (NHGRI) has accepted the challenge of J. Craig Venter (Celera Genomics; Rockville, Maryland) to complete "a working model of the human genome by spring of 2000," a "rough draft" by the end of 2001, and to polish it up into a "highly accurate complete version" by 2003. In March three major centers (Washington University, St. Louis; Baylor College of Medicine, Houston; and the Whitehead Institute for Biomedical Research, Cambridge, Massachusetts) altogether were awarded $81.6 million to do high-volume sequencing over the next 10 months to complete the job. The ABI 377XL-96 slab gel machine outperforms the ABI 3700 capillary machine for sequencing, by about 200 bases per run [***Science* 283:** 1867, 1999].

Horizontal gene transfer. It has become increasingly appreciated that genes from one kingdom or phylum are constantly being "captured" by another. Eubacteria genomes clearly contain thermophile (archaebacterial) genes **[*Nature* 399:** 323, 1999]. A plant might pick up a gene from an insect feeding on the plant. *Mycobacterium tuberculosis*, which infects and causes tuberculosis in humans, has taken on at least eight human genes. Why? Clearly, if the genes help the bacteria fight off host defenses, such "gene capture" is advantageous to the microbe. Needless to say, for those designing phylogenetic "trees of life" with the aid of computer programs in order to better understand evolution, genes that have been horizontally transferred are messing things up [***Science* 284:** 1305, 1999].

The tiny acoel worm. Looking at DNA from 18 acoel species from around the world, a group from the University of Barcelona (Spain) concludes that the acoel alone is a living relic of the transition between radially symmetrical animals (*e.g.* jellyfish) and more complex bilateral organisms such as arthropods, mollusks and vertebrates. Acoel biology may therefore offer clues as to which traits evolved first in evolutionary history [***Science* 283:** 1823, 1999].

The rice genome. An international rice genome project, which includes the Japanese government's Rice Genome Sequencing Project, had announced in 1998 that they planned to complete the sequencing by 2008. J. Craig Venter (Celera Genomics) now proposes to sequence the entire 430-megabase genome in just 6 weeks. Although some researchers are skeptical, this pronouncement has thrown the entire project into chaos. Celera plans to make the data available to companies for $30 million on a 5-year contract [***Nature* 398**: 545, 1999].

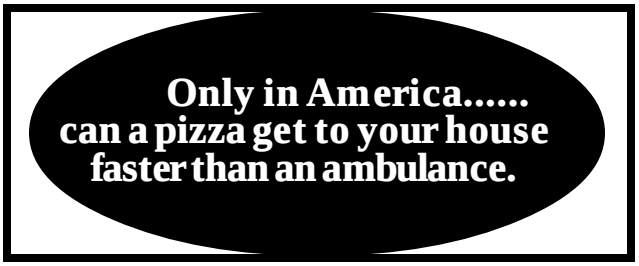
Want to learn more about human genetics? The NHGRI, the American Medical Association, and the American Nurses Association have recently banded together to form the National Coalition for Health Professional Education in Genetics (NCHPEG). This is a national effort (***http://www.nchpeg.org***) to promote professional education and access to information about the latest advances in human genetics.

Do You Really Wanna Know?

In our ***Interface*** issue #10 about ethical problems, the question was posed**:** if a genetic test is now available to tell for sure whether you will develop a particular serious disease, would you want to know? Would you opt for taking that test? Prior to the development of a genetic test for Huntington disease (HD), a 1979 study of persons at risk for the disease indicated that about 75% would want to know. Interestingly, now that DNA testing for HD is available and reliable, only about 25% of persons at risk are actually agreeing to take the test.

But, what about the emotional responses of persons undergoing genetic testing for HD? In a survey of 100 centers in 21 countries on 4,527 individuals who had received predictive genetic testing for HD [***Am J Hum Genet* 64:** 1293, 1999], forty-four (0.97%) had a catastrophic event**:** five successful suicides, 21 suicide attempts, and 18 hospitalizations for psychiatric reasons. This dilemma was described by T.D. Bird (Seattle) as the proverbial "canary in the coal mine." If the catastrophic event rate (the canary losing consciousness) is too high, then genetic counselors and family members (the coal miners) might consider abandoning presymptomatic HD testing (the mine).

In previous issues of this NewsLetter we've discussed the *BRCA1* dilemma, which has some similarities. For women who have mutations in the *BRCA1* gene, they have about a 50% chance of getting breast cancer by age 50 and 85% by age 70 [***Lancet* 343:** 692, 1994]. Combining this penetrance with the prevalent ***6174delT*** mutation in *BRCA1* among Ashkenazi Jews, about 19% of all women diagnosed with breast cancer by age 50, and about 9% of individuals diagnosed by age 70, have been estimated to carry this mutation. Up to 40% of all Ashkenazi Jewish breast cancer patients under the age of 50 years had originally been predicted by the Breast Cancer Linkage Consortium (BCLC) to be carriers of the known founder mutations in either *BRCA1* or *BRCA2*—thus providing a good rationale for broad testing. A number of other studies have now come up with considerably lower frequencies [reviewed in ***Am J Hum Genet* 64:** 943, 1999], however, suggesting that the original estimates by the BCLC were too high. Hence, the "genetic burden" caused by *BRCA1* and *BRCA2* mutations among patients diagnosed with breast cancer before age 70 years (*i.e*. the difference between genetic and nongenetic breast cancer) is only 6% of this population. Thus, the rationale for genetic testing among Ashkenazi Jews has become less apparent**:** screening out those 9% carriers among all Jewish breast cancer patients will leave 91% testing negative—including quite a few with a significant history of breast cancer. What to do? What are the psychological side effects of leaving patients uncertain about their genetic risk? The ethical issues of genetic testing continue to be very problematic!



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INTERFACE:
Genes and the Environment
Center for Environmental Genetics
University of Cincinnati
Cincinnati, Ohio 45267-0056
Fax: 513-558-0925 and 513-821-4664
E-mail: dan.nebert@uc.edu

Does the Public Trust Science?

In a recent European survey, when asked whom they trusted most to "tell the truth about genetically modified crops," 26% named environmental organizations. Only 6% named universities, 4% named national public figures, and 1% named industry. Why such dwindling credibility of scientific institutions? Answers ranged from the recent disaster in England over bovine spongiform encephalopathy ("mad cow disease" affecting humans permanently, if they ate contaminated beef), to the possibility of cloning a human, to chlorofluorocarbons (CFCs) and the ozone hole in the 'eighties, and various debates about global warming, toxic substances in our food and drinking water, and even cigarette smoking. The role of corporate scientists in each of these cases has not been exemplary, especially in the last decade, and the stuffy behavior of academic and industrial scientists—demanding "absolute scientific proof before we can justify preventive policy action"—simply turns off the average layperson.

It should be obvious that all "scientific knowledge" has been acquired and, therefore, is a mixture of "reality" and our perception of that reality. Pablo Jensen provided an interesting analogy [***Nature*** **399:** 406, 1999]**:** "... science summarizes reality as much as a football score sums up 2 hours of emotions, missed opportunities, and referees' mistakes. Any fan knows that the score does not exhaust the game, it only allows us to build a league table."

There are no clear answers to many of the global scientific debates, because **[a]** we lack the knowledge, **[b]** arbitration between different answers is beyond scientific competence, **[c]** the line between fundamental science and applied technology appears to be particularly thin (especially in present-day molecular biology) where corporate funding is often the driving force behind research, and **[d]** the explosive increase in available data and specialization makes it more likely that scientific knowledge and perception can appear fragmented [***Nature*** **400:** 499, 1999].

How to remedy this problem? First, the scientist—especially in companies, but also in academia and government—must adopt a strong ***code of ethical conduct***, not unlike the Hippocratic oath pledged by physicians. Second, the public at all levels must be ***educated*** to understand science. From preschoolers to college graduates to legislative officials, everyone must try hard to keep up with the explosive advances in molecular biology and genetics and with statistical analyses of these data. Even breezy overviews, such as those presented in this NewsLetter, are designed to help close this gap! Similar light-hearted overviews should appear every day in all local newspapers. Only after the grade school and high school teacher understands and enjoys science, and this knowledge and excitement can be transferred to every student, will the scientist begin to gain trust in the public's perception.