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INTERFACE:
GENES AND THE ENVIRONMENT

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Correlating a Trait (phenotype) with a Gene(s) (genotype): How Will "SNPs and DNA Chips" Help Us?

Around 1860, Gregor Mendel showed an association between a ***trait*** (red vs white flower color, green vs yellow seed pod, or smooth vs wrinkled seed pod) and ***segregation*** (how the trait is passed to the next generation). In the first decade of this century, Sir Archibald Garrod showed similar correlations in humans between various "inborn errors of metabolism" diseases (a trait) and segregation. With the discovery of DNA in the 'fifties and chromosomal mapping and banding techniques in the 'sixties, came the first instances of relating a trait (*e.g.* ABO blood types) to a particular region of DNA (*e.g.* ***HLA*** histocompatibility locus on human chromosome 6). In this article we describe the latest explosions in the field of correlating phenotype with genotype.

IN THIS ISSUE

The Genome Can Be Considered Linear

The ***Human Genome Project***, which was launched in October 1990, has undoubtedly contributed a great deal to these exploding advances in the field, because different types of "useful DNA markers" (little flags--unique to 1% or more in a population of individuals--located throughout the genome) are being discovered rapidly, making it easier and easier to find a particular gene. Our current methods of associating a particular trait with a region of DNA stem from the classical mathematical equations for estimating the location of a particle in a fluid ("brownian movement," which is 3-dimensional) and the location of a person during random motion ("the drunkard's walk," which is 2-dimensional). The current method, originally conceived in the 'eighties by David Botstein and Eric Lander and now available as computer software programs, is simpler than the above-mentioned examples in that it is 1-dimensional (***Figure 1***). Just as one might consider DNA, or a chromosome, linear--one can also consider the entire genome of any species linear, simply by placing all the chromosomes end-to-end.

Lod Score

Now that the "genes" are all lined up in a linear array, how do we associate a trait to this "line?" Obviously, the more DNA markers we have on this line, the more finely-tuned we are able to pinpoint a trait to a particular DNA region. Screening a human population for a correlation between a trait and each of (for example) 350 DNA markers, what is the likelihood that the trait will be correlated with one or more DNA markers, versus the likelihood of a

random match by chance alone? This is what is computed in a "log odds" (***lod score***), which can establish whether a randomly chosen DNA marker is actually linked to a particular trait. The **L**ikelihood of two (or more) loci remaining together when chromosomes are recombined (following union of the sperm and ovum) is represented by the recombination fraction, Θ ; this is written L(Θ). The closer the two loci are to one another, the smaller Θ is. The likelihood ratio, L (Θ) / L (½) measures whether the recombination fraction is equal to Θ (<½ means linkage), as opposed to being equal to ½ (*i.e.* no linkage). Hence, the equation

$$Lod\ score = \log_{10} \frac{L(\Theta)}{L(\frac{1}{2})} = \frac{likelihood\ of\ "true"\ linkage}{likelihood\ of\ chance\ alone}$$

If the likelihood of true linkage is 1,000 times greater than that by chance alone, the $\log_{10}$ of the ratio 1,000 is 3.0, and this is considered a "significant" lod score. ***Figure 1*** shows an imaginary trait having three genes throughout the genome with ***lod scores greater than 3.0*** (how to "set" the baseline, and how to interpret how many "significant" major and modifier genes are found to be correlated with a trait, is obviously complex and beyond the scope of this article).

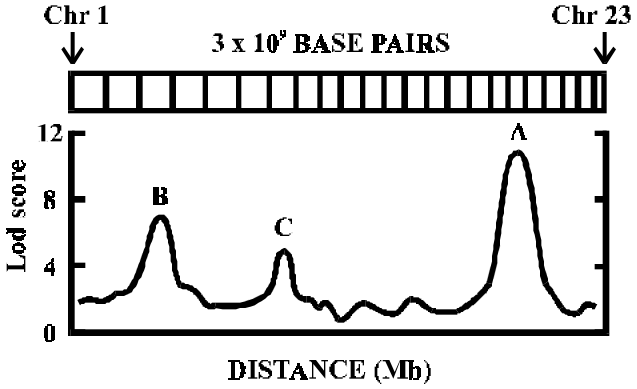

Figure 1. How to correlate phenotype with genotype. Human (or any other species) chromosomes are placed end-to-end, making the 3 billion bases (of human DNA) as if it were a straight line. Following a "genomic screen," ***lod scores*** of a trait are computed as a function of this straight line. In this imaginary example, primary gene ***A*** has a lod score of about 11, secondary gene ***B*** about 7, and secondary gene ***C*** about 5 (localized to chromosomes 18, 3, and 7, respectively). Chr, chromosome. Mb, megabases (linear distance of 1 million base pairs of DNA).

Various DNA Markers for Detecting Heterogeneity in Human Populations

Following the development of DNA-DNA hybridization analysis by Earl Southern in 1975 (*i.e.* the "Southern blot"), restriction fragment length polymorphisms (***RFLPs***) between two individuals, due to restriction endonuclease "sites" usually 4 to 6 bases in length, were described in the late 'seventies. A second class of RFLPs, in which the restriction fragment length variability is caused by a "variable number of tandem repeats" (***VNTRs***, also called "minisatellites"), was described in the mid 'eighties. A more useful subclass of VNTR polymorphism, in which the repeat unit consists of only two base pairs (called dinucleotide repeats, or "***microsatellites***"), was discovered in the late 'eighties and shown to be easily scored by the polymerase chain reaction (PCR). These microsatellites show a large variability in length per DNA locus, are distributed randomly throughout the genome, and are present in probably ***several thousand copies*** per genome. DNA microsatellite marker methodology is the most common and most successfully used technique today for linkage analysis (correlating phenotype with genotype via lod scores).

Other established methods for detecting variation between two DNA samples, in chronological order of discovery, include**:** (of course) DNA sequencing, denaturing gradient gel electrophoresis (DGGE), ribonuclease cleavage, single-strand conformation polymorphism (SSCP) analysis, heteroduplex analysis (HA), and chemical cleavage of mismatch (CCM). Within the past 24 months, new DNA scanning detection methods include**:** an *E.coli* mismatch repair enzymes plate-based assay, mass spectrometry, *T4* endonuclease *VII* cleavage method, denaturing high performance liquid chromatography (DHPLC), and a radioactive multi-photon detection system [*cf.* ***Nature Biotechnology*** **16:** 33-39 (Jan '98) for further details].

Single Nucleotide Polymorphisms (SNPs)

Microsatellite scoring procedures are less than perfect because **[i]** they are labor-intensive and **[ii]** there are only several thousand sites per genome--meaning that small numbers of affected individuals, or studies having small family size, often make it impossible to localize and assign a "particular gene(s)" to a particular trait (*e.g.* red hair, dyslexia, high blood pressure, enhanced sensitivity to cadmium toxicity, increased risk of breast cancer under 50 years of age, etc.).

SNP (pronounced "snip") fever has now hit The Human Genome Project. What is a SNP? When the entire human genome is sequenced, large stretches of

DNA, or entire chromosomes, are derived from a single individual. When multiple individuals are screened and a different base pair is present at a particular location 1% or more of the time, this is called a single nucleotide polymorphism (***Figure 2***).

Individual #1:
5'-C A C C G T A G A T**A** C G G C A A A C T A-3'
Individual #2:
5'-C A C C G T A G A T**T** C G G C A A A C T A-3'

Figure 2. Example of a SNP. DNA bases (A=adenine, C=cytosine, G=guanine, T=thymine) from an imaginary stretch of DNA are identical between two individuals except for the A in individual #1 changed to a T in individual #2. If these two bases have frequencies of >1% in the human population, this is a SNP**;** if the A and T frequencies at this position are 20-40%, this would be a particularly "useful" SNP in linkage analysis studies.

Contrary to several thousand VNTRs in the human genome, there are expected to be ***between 6 million and 30 million*** SNPs at which variation can occur**;** this means approximately every 100 to 500 base pairs (bp) in human DNA might be polymorphic. Talk about fine-tuning and high sensitivity for correlating a trait with a genotype! SNPs are estimated to occur at frequencies of 1% in coding exonic regions (*i.e.* that part of a gene encoding the protein) and 10% in noncoding regions (5' and 3' flanking, introns, and intragene spacer regions). Let's say a "typical gene" (there is no such thing, but let's just call it "the average gene!") might span 8 kilobases (kb), have a coding region of 1,500 bp, and a noncoding segment of 1,000 bp (***Figure 3***). And let's say we'd like to determine all SNPs—inside this gene and maybe 3 kb upstream and 1 kb downstream. With introns of 5.5 kb, noncoding exonic DNA of 1 kb, 3 kb of 5' flanking and 1 kb of 3' flanking sequences, this gives us a total of 10,500 bp and 10% of this = 1,050 SNPs expected. With a coding region of 1,500 bp, 1% of this = 15. Hence, this "average" gene and nearby regulatory regions would have an estimated 1,065 SNPs spanning 12 kb**;** if 10% of these are "useful" SNPs, this typical gene would contribute as many as 100 SNPs to human genome screening studies (with an estimated total number of 80,000 human genes, you are thus able to see how one can arrive at the "6 million to 30 million estimate of SNPs in the genome"). Sufficiently fine mapping of a particular gene responsible for a trait, however, would probably require only about 300,000 scorable SNPs throughout the genome (*i.e.* one marker per 10,000 bp).

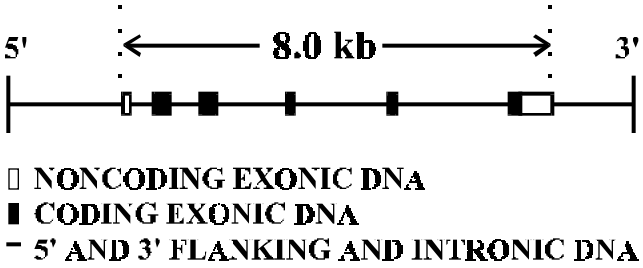

Figure 3. Illustration of the "average gene" discussed in the text. The transcript spans 8 kb and the 5' and 3' regulatory regions comprise 3 and 1 kb, respectively. The *line* represents linear DNA upstream (5') and downstream (3') of the transcript, as well as the introns in the transcript that are spliced out when the mature messenger RNA (mRNA) is ready to be translated into protein on ribosomes in the cytoplasm. The *solid boxes* depict portions of the exons that encode the protein. The *open boxes* represent portions of the exons that do not code for protein.

Many SNPs will be found to segregate together (*i.e.* in "linkage disequilibrium"). For example, a SNP 3,670 bp upstream from the 5' end of the gene, a SNP in exon 1, and a SNP in intron 4--might be shown to always go hand-in-hand. Such data are already being used in anthropological studies to estimate tribal migrations and divergence of ethnic groups. By the rapid-throughput sequencing and re-sequencing of innumerable genes, such data will be used in the near future for correlating genotype with phenotype.

Leaders and advisors to the Human Genome Project fear that private industry might discover and patent "useful SNPs" before the government and academia can characterize them for nonprofit medical and scientific research. For more on "SNP fever," see ***Science* 278:** 2046-2048 (1997). An NIH-wide SNP-related Request for Applications (RFA) is being released in January, 1998.

DNA Microchips

Established methods for scoring SNPs include single nucleotide primer extension (SnuPE) and the 5' nuclease assay. Whereas the latter appears to be too expensive for high-throughput screening, SnuPE might become the method of choice. SnuPE involves the single-base extension of an immobilized primer, in which the added base corresponds to the SNP (each of the four bases with a different fluorescent label allows scoring of the SNP on the basis of different colors).

DNA chip technology is generating as much fever as SNPs! The success of using DNA chips has already been shown for scoring SNPs in the HIV (AIDS virus) genome, the ***p53*** oncogene, the ***CFTR***

cystic fibrosis gene, and the ***BRCA1*** breast cancer gene.

What is a "DNA chip?" Basically, microarrays of DNA nucleotides are placed in columns, perpendicular to a glass (or other) support about the size of a postage stamp. The linear array of nucleotides (such as that for either individual in ***Figure 2***) is synthesized for a particular gene. Then, target probes--having all four bases (A,C,G,T) varied at each nucleotide position in the gene--are used. These fluorescently labeled PCR products are hybridized to the oligonucleotide arrays, and sequence-specific signals are detected by scanning confocal microscopy and analyzed automatically. Similar to the computer microchip industry, these DNA micro-arrays can be placed very close to one another such that, on a "high-density 160K chip," there can be 20 kb (4 oligos/base/both strands) representing two dozen or more genes. The "mismatch detection" is read by a "DNA chip reader," illustrated in ***Figure 4***.

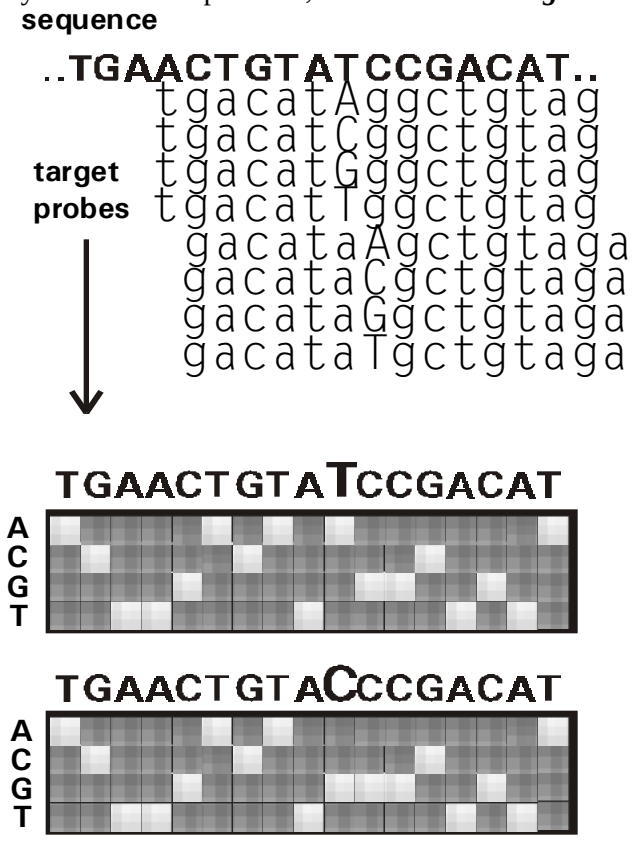

Figure 4. Illustration of how a DNA chip can detect mismatches. The "wild-type" (normal) nucleotide sequence (17 bp) in a gene is shown in capital letters at *top*. Microarrays below this sequence show that nucleotide #10 (T, thymine) is allowed to hybridize possibly to A, C, G or T. Because T hybridizes to A, this is scored in the nucleotide #10 position in the wild-type *upper box*. Replacement of T with a C at nucleotide position #10 in the variant sequence (*lower box*) is detected, because C hybridizes to G, and this is scored by the DNA chip reader as a "mismatch," or SNP. Modified from Affymetrix.

The technology enables rapid re-sequencing, and scoring of DNA variants as predictable differences in the hybridization pattern. Presently, the cost of making each DNA chip is very expensive, however, and the use of each chip for hybridization to no more than one or a few individual DNA samples (due to residual contamination by the previous sample), are two major drawbacks. As these problems become solved by numerous companies that are interested in DNA chip technology, the result will be similar to what is happening in the computer field**:** with each passing month, one can buy "more computer" for less money. No doubt the cost of DNA chip analysis (per DNA sample examined) will decrease markedly, and DNA chip technology is likely to become the method of choice in evaluating SNPs within the next several years.

Since its inception in 1991, our Center for Environmental Genetics (CEG) at the University of Cincinnati has been interested in correlating phenotype with genotype. This field has now been termed "functional genomics" [***Genomics*** **45:** 244-249 (1997)]. We and others have already begun to tackle problems in "environmental genetics-related" functional genomics, using these above-described techniques.

----***Contributed by Daniel W. Nebert***

LETTERS TO THE EDITOR

RESPONSES/COMMENTS TO VARIOUS QUESTIONS

COMMENT **For several years there has been a major concern that "environmental estrogens" might be causing endocrine disruption in alligators and perhaps other species (*cf.* issue #3 of *Interface*). While some argued that the levels of environmental estrogens are sufficient for causing these defects in animals (and possibly humans), others argued that these concentrations are far too miniscule for cause and concern (issue #5). Then came the 1996 *Science* paper by McLachlan and coworkers that environmental chemicals in complex mixtures were found in a yeast 2-hybrid system to be synergistic, thereby eliciting a 160- to 1600-fold more potent "estrogenic" effect (reported in issue #8). A**

number of laboratories have been unable to corroborate these data (discussed in issue #10). Now, in the 25 July 1997 issue of *Science* (pp 462-463), McLachlan laid this issue to rest by withdrawing his laboratory's previous (1996) report on synergism.

We are now back to where we started. Environmental estrogens might be capable of endocrine disruption in certain species under certain conditions at sufficiently high concentrations, while many still argue that the levels of dietary and industrial estrogens are far too low to contribute to human breast cancer or male reproductive problems.

Q **The reports of Minnesota school children finding a lot of deformed frogs in August, 1995, led to a flurry of surveys over the past 2 years in which the rate of "frog malformations" ranged between 8% and 67% in the lakes and ponds of Quebec, Vermont and Minnesota. Could this be a warning of the deterioration of our environment? More specifically, might this be due to the so-called"environmental estrogens?"**

A *The exact cause of these malformations remains to be determined. The three currently prevailing theories include:* ***(i)*** *a parasite,* ***(ii)*** *an environmental chemical, and* ***(iii)*** *ultraviolet irradiation. Encysted trematodes (parasitic flatworms that burrow into the limbs of amphibians) have been shown to cause limb bud anomalies similar to those found in these frogs; sporadic, normally-occurring peaks in populations of pond snails (the primary host of trematodes) might explain this apparent increase in deformed frogs. Environmental retinoid-like chemicals, such as the insecticide Methoprene widely used to kill mosquitoes, have also been suggested as the cause of the frog limb deformities. Ultraviolet B (UVB) light, known to be reaching the Earth's surface because of the thinning of the ozone layer, has recently been shown to be able to penetrate the "murkiness" of the average North American pond and cause developmental changes in amphibians. Yet, many insist that these increases in frog malformations are simply the ebb and flow of what has been observed over the past 250 years [****Science*** **278:** *2051-2051 (1997)].*

Q **There was such a clamor over the cloning of the first sheep, named Dolly. Now, this same laboratory group has cloned "Polly" and "Molly," and there seems to be no further excitement. What's the difference between the first and second reports of cloning these sheep?**

A *On 19 December 1997 [****Science*** **278:** *2130-2133], this same Scottish laboratory announced the birth of several lambs containing the human coagulation factor IX (****FIX****) gene, which is essential in blood coagulation and is defective in human hemophilia B. However, these lambs were the result of cloning from fetal cells (which gives much higher rates of successful nuclear transfers than cloning from an adult cell, as was what most likely happened in the case of Dolly--described in detail in issue #10). Cloning constructs carrying the human****FIX*** *gene and the selectable marker neomycin-resistance (****neo****) gene were transfected into sheep fetal skin fibroblasts. Then, nuclei from sheep mature egg cells were removed, and the engineered fetal fibroblasts (which had been starved of nutrients) were fused by electric shock. This electrical pulse was also capable of activating the "developmental program" in these ova, as they had reported earlier with the Dolly cloning experiments. Six transgenic lambs were liveborn: three carried the functional human* ***FIX*** *gene and the* ***neo*** *gene, whereas the other three (produced from the uncloned population) carried the* ***neo*** *marker gene only.*

These ***FIX*** *gene-containing sheep are now producing the human factor IX in their milk, which will provide an alternative source--at much lower cost (and free of the potential infectious risks associated with products derived from human blood)--for the treatment of this type of hemophilia. Another study [****Nature Biotechnol*** **15:** *971-979 (15 Oct 1997)] reports the similar production of human factor VIII (defective in hemophilia type A) in the milk of* ***transgenic pigs****! As discussed in issue #10 of* ***Interface****, the benefits of these cloning experiments in sheep and other agricultural animals to humankind will far, far outweigh all the fuss and furor over the ethical and policy issues of human cloning and the "5-year voluntary moratorium on cloning humans" that has been urged by President Clinton and the U.S. Congress.*

COMMENT **During the 47th annual meeting of the American Society of Human Genetics this autumn (Baltimore, Maryland), genetic privacy and discrimination--in the context of existing and future practices of genetic testing--was thoroughly discussed. A recent poll showed that 85% of Americans surveyed were "afraid of genetic discrimination" in the work place, by employers, etc. Five percent ($6.9 million this year) of U.S. federal funding for the Human Genome Project is set aside for special programs designed to anticipate, analyze and address the ethical, legal and social implications (ELSI) of our rapidly accumulating knowledge about human genetics. This issue was also extensively covered in *Interface* issue #10.**

In a study of 177 patients with adenomatous polyposis coli (APC; an autosomal dominant disorder that causes cancer unless the colon is removed), about four out of every five physicians were not able to understand the significance of the genetic test for APC or educate their patients in terms of genetic counseling [*New Eng J Med* 336: 823-827 (1997)].

"Genome Watch"

Some enjoy following "Baywatch" on television. This NewsLetter enjoys watching the accomplishments of the completions of each sequence of an entire genome! Whereas the human and mouse genomes are expected to be completed by 2005, what has happened so far? The following ***fourteen*** genomes of living organisms have already been completely sequenced**:**

1977 -- bacteriophage Φ x174 (5,386 bp)
1982 -- bacteriophage λ (48,502 bp)
1995 -- *Haemophilus influenzae* (first nonviral genome) (1,830,137 bp)
Mycoplasma genitalium (580,070 bp) (470 genes=minimal number to support life)

since

1996-- *Mycoplasma pneumoniae* (0.8 Mb)
Borrelia burgdorferi (1.3 Mb)
Methanococcus jannaschii (first archaebacterium sequenced) (1.7 Mb)
Helicobacter pylori (cause of stomach cancer) (1.7 Mb)
Methanobacterium thermoauto trophicum (1.8 Mb)
Archaeoglobus fulgidus (2.2 Mb)
Synechocystis sp. (3.6 Mb)
Bacillus subtilis (4.2 Mb)
Escherichia coli (4.6 Mb)
Saccharomyces cerevisiae (baker's yeast; first eukaryote; 6,034 tentative genes) (12.1 Mb)

bp, base pairs of DNA

Mb, million base pairs (human genome = ~3 billion base pairs)

As of this autumn, therefore, various genome projects have completed the sequencing of genomes from ***two*** bacteriophage (viruses), ***eight*** eubacteria, ***three*** archaebacteria, and ***one*** eukaryote. Using DNA microarray technology (described in the lead article of this issue of ***Interface***), D. Lockhart and coworkers at Affymetrix (Santa Clara, California) have now demonstrated that it is possible to "knock out" each yeast gene, or combinations of genes, in order to determine the function of that gene [***Nature Biotechnol* 15:** 1359-1367 (1997)]. Undoubtedly, such experiments will ultimately be carried out in mice (although, of course, much more difficult than such experiments in yeast). The speed at which genome sequencing and functional genomics projects are moving, however, continues to be breathtaking!

An X-linked Dominant Trait with Male Sparing

There are more than 300 traits of ***X-linked recessive*** modes of inheritance, afflicting between 8% and 10% of the male population. Because males (XY) have only a single X chromosome, they are affected (*e.g.* color blindness, hemophilia), whereas females (XX) are spared. ***X-linked dominant*** inheritance affects females disproportionately because of male lethality**;** such diseases include focal dermal hypoplasia, oral-facial-digital syndrome type I, and epilepsy with bilateral periventricular heterotopias. Ryan *et al.* [***Nature Genet* 17:** 92-95 (1997)] have shown that defects in the "epilepsy and mental retardation limited to females" (***EFMR***) gene on the X chromosome is inherited dominantly and results in male sparing rather than male lethality! Although some difference in the balance of sex steroids might explain this unusual finding, Ryan and coworkers speculate that in ***(-/+)*** females the disease might be caused by the absence of wild-type function in half of the cells (a result of the ***EFMR*** gene being subject to X-inactivation) whereas in ***(-/Y)*** males the X-chromosomal defect is complemented by a functional homologous gene on the Y chromosome. Further work will be needed to uncover the mechanism of this unusual inheritance pattern.

Patent Rights for Breast Cancer Gene Tests in Court

As described in numerous past issues of ***Interface***, Myriad Genetics of Salt Lake City, Utah [working with groups at the National Institues of Health (NIH) and the University of Utah] was the first to identify the ***BRCA1*** gene (7 Oct 1994), and they filed a patent on the gene and "several harmful mutations." OncorMed Inc. of Gaithersburg, Maryland, analyzed DNA from five normal persons (families with no history of breast or ovarian cancer) and patented a "consensus sequence" of the ***BRCA1*** gene (a number of nucleotides were different from Myriad's original ***BRCA1*** sequence) and identified seven harmless (nonfunctional) genetic variants. OncorMed was granted its patent sooner than Myriad. OncorMed then sued Myriad this November. Two weeks later, Myriad countersued OncorMed--accusing OncorMed of violating Myriad's new patent and seeking an injunction to stop OncorMed's genetic tests. It seems amazing that two patents would be issued on slightly different sequences of the same gene!

This is just the beginning. The London-based Cancer Research Campaign Technology and its partner Duke University in North Carolina (CRCT/Duke) and Myriad Genetics have both filed patents for ***BRCA2*** gene breast cancer tests. It is expected to take at least 2 years for a decision by the U.S. Patent and Trademark Office.

SCIENCE LITE

Newswire: - Houston,TX:Investigation Yields Clues to Collision

Following the docking mishap collision with the Russian space station, there has been an intensive investigation by both the Soviet and U.S. Space Agencies. After weeks of inquiry, finger-pointing and political jockeying, it appears that the cause of the mysterious accident, which placed the space station and its residents in serious jeopardy, has finally been determined.

In terse statements at a recent press conference, Russian and U.S. Space Agency spokespersons said Thursday, "We have concluded joint investigations concerning this potentially tragic accident, and each agency has independently arrived at the same conclusion regarding the cause of this incident." Everything points primarily to one problem, which we believe can be solved (as is done in American automobiles) by printing the following statement on all outside mirrors and visual devices used in future dockings**:** "***OBJECTS IN MIR ARE CLOSER THAN THEY APPEAR***."

Genetic Differences in Response to "Fen-Phen" Therapy?

The 28 August 1997 report that 24 patients, who were being given fenfluramine-phentermine ("fen-phen") for weight control, developed "unusual cardiac valvular morphology" [Connolly *et al*., ***N Engl J Med*** **337:** 581-588 (1997)] was a "shot heard 'round the world." It now appears that the incidence of some heart valve damage might be as high as 30% in those taking this combination of appetite suppressants. Why wasn't this problem picked up during animal studies and early clinical trials? Are some people affected more easily than others? And, most intriguingly, what is the mechanism causing this serious side effect?

First, there is very likely to be ***interspecies differences***--such that what happens in humans might not happen in mice, rats, rabbits or monkeys. The best example from the past is thalidomide.

Second, whether fen-phen toxicity occurs of course will be, in part, dependent on the dosage and length of treatment. But, might there be an underlying genetic predisposition to cardiac valve disease? Dexfenfluramine has been shown [***Br J Clin Pharmacol*** **41:** 311-317 (1996)] to be metabolized by CYP2D6 (see discussion in issue #11 about genetic differences in CYP2D6-mediated metabolism of ***Prozac***$^{(R)}$). This means that the 6% to 10% of human populations that are ***CYP2D6 "poor metabolizers"*** (PM phenotype) would have more trouble in clearing fenfluramine than the remaining 90% to 94% of the population who are "extensive metabolizers" (EM trait).

Finally, Connolly and coworkers noted that the valvular damage has histopathological features "identical to those seen in carcinoid heart disease," and suggested an effect of fen-phen on ***serotonin pathways***. It is fascinating that changes in biogenic amine levels have long been known to be associated with cell type-specific proliferation [reviewed in Nebert, ***Mol Endocrinol*** **5:** 1203-1214 (1991); ***Biochem Pharmacol*** **47:** 25-37 (1994)]. ***Isoproterenol*** has been used for decades to study subcellular processes during stimulation of maxillary gland cell culture growth. ***Reserpine*** stimulates adrenal medulla cell proliferation and causes adrenal medullary tumors in male rats--suggesting that cell proliferation might be a neurologic response to catecholamine depletion. Might latent neurotropic viruses also be activated by fen-phen?

The bottom line is that we probably should be careful in disturbing ***biogenic amine pathways***, just as we should be careful about perturbing our steroidogenesis pathways. A case in point that comes to mind is dehydroepiandrosterone (DHEA), which is a known peroxisome proliferator and hepatic tumor promoter [***Carcinogenesis*** **16:** 2893-2898 (1995); ***J Endocrinol*** **150:** S129-S147 (1996)], but has been touted as everything from an appetite suppressant and tumor suppressant [***Nutr Cancer*** **3:** 46-53 (1981)] to an anti-aging drug [***J Am Geriat Soc*** **45**: 1395-1401; 1402-1403 (1997)].

Observations by a Biologist

Modes of development in starfish on the temperate east coast of Australia

Although different species of the starfish of the genera *Patiriella* and *Asterina* range in size from less than 2 cm to more than 20 cm, the most intriguing differences lie in how they reproduce. Some produce tiny eggs that develop first into free-swimming larvae before being transformed into tiny stars that resemble their adult shape. Others produce larger yolky eggs that develop directly into starfish with no intervening larval phase. Some adults hold their embryos in broods in the gonad where siblings fight and cannibalize one another for the right to survive, whereas other adults disperse their eggs randomly into the sea. Hart and coworkers [***Evolution* 51:** 1848-1861 (1997)] used phylogenetic methods to reconstruct how all of this developmental diversity has evolved in eight *Patiriella* and four *Asterina* species, realizing that these species are very closely related to one another.

It had been postulated that it is more difficult for complex larval structures to be gained, than to be lost, during evolution. Others had previously reasoned that feeding larvae need more complicated signal transduction pathways to catch and digest food. Hence, ***divergent evolution*** of the loss of food-catching structures to give rise to nonfeeding larvae is more likely to occur than the ***convergent evolution*** from nonfeeding larvae to feeding larvae. It had also been expected that larval and adult characteristics might commonly evolve in stages--such as loss of larval feeding, followed by loss of dispersal into the sea, followed by gain of parental brood protection and viviparity (producing living young from within the body).

Surprisingly, Hart and coworkers found that there is no incremental progression from one developmental type to another. Moreover, they discovered that evolution of nonfeeding larvae has occurred ***at least three times independently*** within the single *Patiriella* genus and that all species having nonfeeding larvae are not even closely related. Even more unexpectedly, they determined that there have been at least 16 major shifts in developmental mode during the past 5 to 10 million years! This speed of developmental change might explain the lack of intermediates. For comparison of evolutionary time, it was about 80 million years ago that the "mammalian radiation" spawned everything from mice, rats and rabbits to chimpanzees and humans, and about 17 million years that rats and mice diverged from one another.

What does this have to do with genes and the environment? These studies further underscore the ***diversity*** with which developmental systems evolve--within particular genera and in certain environments. There is no linearity, and there are no "standardized" rules, during evolution!

Electromagnetic Fields (EMFs) Revisited

In issue #10 of ***Interface***, we reported the finding of a National Research Council (NRC) Panel that "no adverse effects on cells or animals were found at EMF levels measured in house electrical wiring or in houses under power lines." Last July, a large unequivocal study was published, arriving at the same conclusion [***N Engl J Med* 337:** 1-7 (1997)], and, in the same issue (pp 45-46), a strong editorial by E.W. Campion concluded that "18 years of research have produced considerable ***paranoia***, but little insight and no prevention. It is time to stop wasting our research resources" and redirect this money to ... discover the true causes of leukemia. No less than seven laboratories, in letters-to-the-editor [***N Engl J Med* 337:** 1471-1474 (13 Nov 1997)] objected to these strong conclusions, insisting that--although most findings were not statistically significant--some "trends in the expected direction" (*i.e.* EMFs having a slight effect on leukemia) should be further explored with research money!

It is time to begin questioning the value of ***xenophobia*** (fear of strangers? or foreign chemicals), ***chemophobia*** (fear of environmental chemicals), and "***EMFs-ophobia***" (fear of power lines). The media (newspapers, magazines and television/radio) no doubt contribute to these fears generated in the average citizen. How many more research dollars are needed to study EMFs and leukemia? When is enough enough?

CEG Members in the News

Sohaib Khan was appointed to the editorial board of ***Endocrinology*** for a term of 4 years beginning January, 1998.

Dan Nebert was an invited speaker at the Satellite symposium "Microsomes, Drug Oxidation and Clinical Pharmacology," in conjunction with the 2nd Congress of the European Association for Clinical Pharmacology and Therapeutics, Berlin, Germany (October 1997); he was also an invited Honorary Guest Speaker at the International Dioxin symposium (October 1997, Seoul, South Korea).

Jun Ma was an invited speaker and participant at the International Workshop on "Advanced Medicinal Biotechnology" (October 1997, Beijing, China) where he presented a seminar entited ***"Approaches for studying protein-protein interactions in vivo."*** He also was invited to speak to the Department of Biology at Peking University delivering a seminar entitled ***"Gene regulation and development in Drosophila"*** (October 1997).

Nicholas Schork presented invited lectures at Harvard University, Departments of Medicine, and at the School of Public Health in September 1997 (Boston, Massachusetts) which focused on ***"Methods and new directions in analyzing compex genetic traits."*** He also presented this topic to the Jackson Laboratory, October 1997 (Bar Harbor, Maine) and to the Stratagene Corporation, November 1997 (La Jolla, California).

Judy Jarrell and **Howard Shertzer** developed and taught a mini-course in conjunction with Hughes High School for the Health Professions (October and November 1997, Cincinnati, Ohio). The 8-week session taught senior students about human genetic diversity and the health risks associated with exposure to environmental and occupational chemicals. Graduate students from the Department of Environmental Health also discussed diverse methods to achieve individual goals in higher education and careers.

Rakesh Shukla is a coinvestigator in a genetic susceptibility study in stroke patients.

Nancy Steinberg-Warren has completed two issues of ***Start Healthy Times***, a newsletter published and disseminated to 1500 health care providers regarding preconceptional health in women. In addition, she published a women's booklet-type calendar for 1998 entitled "Beginning with You: Start Healthy" which has been disseminated to 2000 women of low socioeconomic and educational levels, and to pregnant/parenting teens in the Greater Cincinnati Area. She also presented a 1-day workshop for nurses, social workers, dietitians and teachers 4 December 1997, in which several speakers focused their attention on preconception education.

David Warshawsky presented a seminary entitled ***"Comparative metabolic and oncogenic activation of dibenzo-[c,g]carbazole and dibenzo[a,j]acridine"*** at the 16th International Symposium on Polycyclic Aromatic Compounds (November 1997, Charlotte, North Carolina)

Genetic Differences in the Risk of Asthma

We all know someone who is more affected by (or, more "resistant" to) an inversion layer in our city on a day when air pollution is particularly bad. Whether it's a cough, runny nose or tear-filled eyes, some people are much more affected than others--by the ***same level*** of pollutants in the air. The haze has been particularly bad in Southeast Asia (Malaysia, Indonesia) this autumn, when out-of-control fires have made visibility so limited that airplane and ship accidents have occurred as the result of this El Niño-elicited weather pattern (a prolonged drought lasting from summer until the rains in mid-November, leading to excessive smoke from uncontrollable forest fires). Risk of asthma obviously is a combination of genetic predisposition and the levels of exposure to environmental air pollutants (as discussed in issue #6 of ***Interface***).

Two laboratories (including George Leikauf's from this Center) have now completed "genomic scans" on susceptible vs resistant strains of inbred mice [***Nature Genet* 17:** 471-4, 475-8 (1997)]--similar to what is described in the lead article of this issue of our NewsLetter. By such "quantitative trait locus" (QTL) mapping, genes associated with resistance to ozone-induced inflammation and/or death were localized to mouse chromosomes 11 and 17, and a third (modifier?) gene was suggested on chromosome 13. It won't be too long before the genes responsible for the complex disease of asthma (definitely a "multiplex phenotype") are identified and characterized!

CEG - SPONSORED SPEAKERS

Muin J. Khoury, MD, PhD
Centers for Disease Control and Prevention
Atlanta, Georgia
6 October 1997 **"Translating advances in human genetics into public health action: a CDC strategic plan."**

Wendell W. Weber, MD, PhD
Professor, Department of Pharmacology
University of Michigan Medical School, Ann Arbor, Michigan
21 October 1997 ***"Pharmacogenetics: a changing scene."***
22 October 1997 ***"Mouse acetylation polymorphisms: Androgen regulation and promoter characterization."***

Nicholas Schork, PhD
Associate Professor, Department of Epidemiology and Statistics,
Case Western Reserve University, Cleveland, Ohio
13 November 1997 ***"Novel approaches for investigating complex traits in humans: a two village study in China,"*** and ***"Statistical methods for assessing complex traits."***

Genetic Differences in Fear?

Every parent with more than one child knows that the threat of punishment (fear response) varies widely among children. Could something as complicated as "conditioned fear" be "genetic," and--even if it were--how would scientists go about proving it? Using the same type of genome scanning discussed in the lead article of this issue of ***Interface***, two independent laboratories (in Boulder, Colorado, and in Albany, New York) used quantitative trait locus (QTL) analysis and genetically different mouse inbred strains for mapping the trait "contextual conditioning" (freezing behavior in response to fear) to chromosome 1. Several other chromosomes had loci with lod scores of greater than 3.0--suggesting secondary (modifier) genes [***Nature Genet* 17:** 331-334; 335-338 (1997)].

Recombinant DNA Answer to Obesity!

A single intramuscular injection of a recombinant ***adeno-associated virus*** (rAAV) vector encoding the mouse ***leptin*** gene in genetically obese (***ob/ob***) mice was shown to prevent obesity and diabetes in this mouse line [***Proc Natl Acad Sci USA* 94:** 13921-13926 (1997)]. If this work by Chiron Corporation (Emeryville, California) can be corroborated and extended to humans, the feasibility of using rAAV-based vectors for the treatment of chronic genetic disorders such as obesity holds great promise!.

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