Complex Mixtures in a Complicated World

Environmental exposures to toxic or cancer-causing agents are almost never the result of a single, isolated toxicant. On the other hand, ironically, almost all federally funded research is designed to study one or another specific, individual environmental pollutant, rather than mixtures of two or more compounds. In the world of every day living, we are always confronted with a complex mixture of chemical entities—in numbers ranging from a few (such as may occur in an occupational exposure) to several thousand (e.g. cigarette smoke). Toxicity may occur in hours (acute), days-weeks (sub-acute), or months-years (chronic). On the other hand, for cancer to occur, the required exposures may take 20 or 30 years. The biological consequences of exposure to these mixtures are unpredictable. Although the health effects of individual chemicals may be known, the toxicity of environmental mixtures is largely unappreciated and unexplored. The long-range goal of complex-mixtures research is to develop an understanding of the mechanisms underlying the adverse health effects (toxicity and malignancy) resulting from such exposures.

In this article, we describe briefly the toxic waste dump sites in the U.S. and then focus in more detail on several of the more common contaminants.

Toxic Waste Dump Sites

How much toxic waste is generated in the U.S. each year? In 1973, the U.S. Environmental Protection Agency (EPA) estimated annual U.S. toxic waste production at 10 million tons, or roughly 100 pounds for each person in the country. In 1989, the American Chemical Society estimated the annual U.S. toxic waste production to be much higher: somewhere between 580 million tons and 2.9 billion tons. Today’s estimates are even higher, and obviously this is a serious environmental concern. The vast majority of hazardous waste is dumped in the ground (pits, ponds, lagoons, landfills) or into deep injection wells, where the waste is pumped under pressure into the ground. This means there is always the possibility (even the likelihood) that what goes into the ground will come back to haunt us—by way of our drinking water or eating the contaminated plants and animals in our food chain.
In 1980 Congress passed the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) [Public Law 96-510], which is commonly known as Superfund. University of Cincinnati’s Superfund program is detailed in issue #27 of Interface. This law requires the EPA to identify all contaminated sites, establish priorities for cleaning them up, and then clean them up. Many people assume that, because Congress passed a law to control this problem, it must be under control; they would be disappointed. EPA has listed more than 1,200 sites as official Superfund sites at this point, but they are aware of 32,000 additional sites that contain chemical wastes.

Unfortunately, there is evidence that even EPA’s list of 32,000 sites merely scratches the surface of the problem. The Office of Technology Assessment (OTA) gathered information from many government agencies and concluded that the number of chemically-contaminated sites is much larger than even the EPA’s list of 32,000. OTA said that, if you include military sites, mine wastes, leaking underground storage tanks, pesticide-contaminated sites, non-military federal properties, radioactive release sites, underground injection wells, municipal gas facilities, and wood-preserving plants—there are probably upwards of 500,000 chemically-contaminated sites in the U.S.

In a book published more than a decade ago the National Academy of Sciences concluded, from the few sites where human health has been studied, that “there is good evidence” that people have been harmed by chemical contamination—including birth defects, nervous system damage, thyroid disease, infertility, damage to the immune system, skin disorders, kidney and liver diseases, blood disorders, and cancer. There is little doubt that hundreds of toxic chemicals reside at each of these sites, but it is not certain to what extent most people have been exposed and thus directly affected. Therefore, no one really knows the extent of harm to public health today. The Academy also pointed out that half the U.S. population, and 95% of all rural populations, rely on groundwater as the main source of drinking water (—Peter Montague, at http://www.rachel.org/bulletin/).

Table 1 is a recent list compiled by the Agency for Toxic Substances & Disease Registry and the U.S. Environmental Protection Agency (ATSDR/EPA) of the “top 20 most hazardous substances found as co-contaminants in more than 40% of the National Priority List of Superfund sites”, listed in order of abundance (http://www.atsdr.cdc.gov/ccx3.html). Within this list, however, there is some redundancy: benzo[a]pyrene (BaP), benzo[b]fluoranthene, and dibenzo[a,h]anthracene are three discrete polycyclic aromatic hydrocarbons (PAHs); Aroclor 1254 and Aroclor 1260 are mixtures of polychlorinated biphenyls (PCBs). Interestingly, five metals have made the “top-20” list (arsenic, lead, mercury, cadmium and chromium). Interface previously has featured Leading Articles on the topics of: dioxin and BaP binding to the aromatic hydrocarbon receptor (AHR), which induce enzymes that metabolize PAHs (issue #2); arsenic (issue #13); PCBs (issue #14); multiple chemical sensitivity syndrome, which can result from exposures to complex mixtures (issue #22); and the Superfund Basic Research Science Program at Cincinnati (issue #27).

Table 1. ATSDR/EPA’s List of the Top Twenty Most Hazardous Substances

<table>
<thead>
<tr>
<th>Number</th>
<th>Substance</th>
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<tbody>
<tr>
<td>1</td>
<td>Arsenic</td>
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<tr>
<td>2</td>
<td>Lead</td>
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<tr>
<td>3</td>
<td>Mercury</td>
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<tr>
<td>4</td>
<td>Vinyl chloride</td>
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<tr>
<td>5</td>
<td>Polychlorinated biphenyls</td>
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<tr>
<td>6</td>
<td>Benzene</td>
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<tr>
<td>7</td>
<td>Cadmium</td>
</tr>
<tr>
<td>8</td>
<td>Polycyclic aromatic hydrocarbons</td>
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<tr>
<td>9</td>
<td>Benzo[a]pyrene</td>
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<tr>
<td>10</td>
<td>Benzo[b]fluoranthene</td>
</tr>
<tr>
<td>11</td>
<td>Chloroform</td>
</tr>
<tr>
<td>12</td>
<td>DDT (p,p’-)</td>
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<tr>
<td>13</td>
<td>Aroclor 1254</td>
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<tr>
<td>14</td>
<td>Aroclor 1260</td>
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<tr>
<td>15</td>
<td>Dibenzo[a,h]anthracene</td>
</tr>
<tr>
<td>16</td>
<td>Trichloroethylene</td>
</tr>
<tr>
<td>17</td>
<td>Chromium (hexavalent)</td>
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<tr>
<td>18</td>
<td>Dieldrin</td>
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<tr>
<td>19</td>
<td>Phosphorus, white</td>
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<tr>
<td>20</td>
<td>Chlordane</td>
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Combinations of carcinogenic PAHs and metals are almost always co-contaminants of hazardous waste sites. These toxicants are released together from everyday sources—such as fossil fuel combustion or municipal waste incineration. Of more than 9,000 Superfund sites where PAHs are major contaminants, at least 50% also have the carcinogenic metals arsenic or chromium as co-contaminants (EPA Superfund Report, 2003).

One of the current problems in applied toxicology is the lack of methods to deal with the health effects of these chemical mixtures. Data are scarce, and this scarcity underscores the urgent need to evaluate critically the assumptions used for determining human risk from exposures to complex toxicant mixtures and the need for more research to arrive at an understanding of the health effects of such exposures. For those dioxins, PCBs and other halogenated aromatic hydrocarbons that undergo virtually no metabolism, their effects of combined exposures have been proposed to be based on the relative potency of the amounts of the individual compounds (“dioxin equivalents”). For PAHs, which are readily metabolized, their effects of combined exposures have been proposed to be based on their relative amounts and carcinogenic potencies. These approaches, while an important step forward, are limited to mixtures of similar compounds. These approaches are of little use when dealing with complex mixtures of multiple components, because, as is becoming increasingly evident from the available literature, combined exposures generate substance-specific changes in gene expression that cannot be attributed to a single mode-of-action. For example, one particular compound (e.g. dioxin) can cause: [a] DNA damage (genotoxicity) and mutations due to the formation of reactive oxygen species (ROS; a form of oxidative stress); [b] tumor promotion by way of a receptor-mediated cell proliferation; [c] endocrine disruption; and [d] the induction of enzymes important in both metabolic activation and detoxication of PAHs. One particular heavy metal (i.e. cadmium) can cause: [a] mutations by way of inhibition of DNA mismatch repair; [b] toxicity in specific cell types due to excessive (unwanted) uptake of the metal; [c] endocrine disruption, including infertility; and [d] the induction of enzymes important in the cell’s oxidative stress response. Would the effects of dioxin plus cadmium be additive or synergistic? Or would some effects cancel out the potency of other effects?

**Arsenic**

Arsenic is an established human carcinogen, causing cancer of the skin and lung; it is most commonly found in the environment as arsenate (AsO$_4^{3-}$; i.e. As$^{5+}$), although arsenite (As$^{3+}$) is the most toxic (and the most likely carcinogenic form in humans). Arsenic consistently fails to show carcinogenic effects in rodent models—unless used at very high doses. Early observations that arsenic was co-mutagenic with UV light have led to the concept that arsenic carcinogenicity results from the activation of genes that enhance the effect of some other “primary” (genotoxic) carcinogen such as UV light. Using gene expression microarrays, arsenite has now been shown to be a potent deregulator of gene expression, particularly of genes involved in the cell’s oxidative stress response. Arsenite is also known to bind to, and oxidize, sulfhydryl groups (which commonly then form disulfide bridges), thereby possibly disrupting many proteins important to critical life processes.

As$^{3+}$ is metabolized by an arsenic methyltransferase (encoded by the $AS3MT$ gene on human chromosome 10q24.33), to form monomethyl- and dimethyl-arsenic (organometals). In fact, whereas the human, mouse and rat exhibit $AS3MT$ activity encoded by an active gene, the chimpanzee has a truncated $AS3MT$ gene and shows no $AS3MT$ activity [Toxicol Appl Pharmacol 2005; 204: 164].

At the time of our review on arsenic in issue #13 of *Interface*, the predominant theory was that arsenic methylation was principally a detoxication pathway. More recently, however, several lines of experimental and epidemiological evidence suggest the opposite. Inorganic arsenate was found to induce a rapid burst of oxidative stress in mammalian cells—as a result of the two 1-electron reductive steps of As$^{5+}$ to As$^{3+}$, followed by the addition of at least one methyl group to As$^{3+}$ [Annu Rev Pharmacol Toxicol 1997; 37: 397]; hence, some of the adverse health effects associated with chronic exposure to inorganic arsenic may be mediated by
these methylated metabolites. Glutathione, the most abundant antioxidant in the cell (Interface issue #5), increases the amount of monomethyl- and dimethyl-arsenic formation but suppresses the formation of trimethylarsine oxide [Chem Res Toxicol 2004; 17:1621]. Moreover, as is true of other carcinogenic agents that are sometimes used in treating malignancy, arsenic trioxide is known to help patients with acute promyelocytic leukemia and several other types of cancer [Hematology 2005; 10:205].

**PAHs**

Certain PAHs, as well as certain PCBs and dioxins, bind to the aryl hydrocarbon receptor (AHR). The activated AHR then moves to the nucleus where it binds with its partner, the Ah receptor nuclear translocator (ARNT). The AHR/ARNT complex then interacts with DNA motifs—called Ah receptor response elements (AHREs)—which reside in the regulatory domains of target genes. The end result is the up- or down-regulation of various PAH-responsive genes, such as the cytochromes P450 CYP1A1 and CYP1B1, and the NAD(P)H:quinone oxidoreductase NQO1. The CYP1A1, CYP1B1 and NQO1 enzymes are all involved in the detoxication and metabolic activation of many of the PAH and PCB inducers. Arsenic has been reported to modify AHR-dependent induction of gene expression and BaP toxicity. Arsenic also appears to have a synergistic interaction with BaP on lung tumorigenesis and to function as a co-carcinogen.

**Chromium**

For more than 100 years, chromium has been known to be a human carcinogen. Inhalation and ingestion are the two most common routes-of-entry of chromium—with the nasal passages, lung, gastrointestinal tract and liver being the tissues most often affected. Dermal exposure and urinary excretion also make the skin and the kidneys important targets for chromium. The most common form of chromium in the environment is the water-soluble salt called chromate (CrO₄²⁻).

Epidemiologically, the greatest risk of chromium-caused cancer involves exposure to Cr⁶⁺ compounds, such as chromate. Although Cr³⁺ is the most prevalent form in the cell, the vast majority of the evidence indicates that exposure to Cr³⁺ does not cause tumors in animals. Cr⁶⁺ enters the cell via the sulfate anion transporter system (principally SLC26A2) and is reduced to intermediate oxidation states, such as Cr⁵⁺ and Cr⁴⁺ in the process of forming the stable Cr³⁺ form. Chromium is genotoxic and induces DNA-protein cross-links by binding to several reactive amino acids—including cysteine, tyrosine and histidine—and linking these to the DNA phosphate backbone. Chromium also causes DNA double-strand breaks, which might be involved in inhibition of transcription. Chromium also forms nuclear foci containing phosphorylated histone H2A.X (a marker of DNA damage) similar to the H2A.X foci induced by ionizing radiation.

Chromium blocks the expression of several inducible genes, without affecting the expression of “housekeeping genes” such as ACTB (β-actin) or ALB (albumin), leading to the hypothesis that the chromatin structure of inducible regulatory regions (perhaps by virtue of having a more “open” configuration) may offer a better target for Cr-binding than the “more closed” chromatin of noninducible regulatory regions. An alternative hypothesis is that the transcription machinery in many inducible genes interacts with the nuclear matrix, and chromium-induced DNA-protein cross-links within this matrix may effectively block the function of inducible gene expression. Generation of free radicals from the reduction of Cr⁶⁺ to Cr⁵⁺ and Cr⁴⁺ also may activate NF-κB, a transcription factor involved in cell growth and programmed cell death. Thus, the intermediary chromium oxidation states may be critical factors in the effect of chromium on gene expression.

Which enzyme(s) in the cell is(are) pivotal in reducing As⁵⁺ to As³⁺ in two 1-electron steps, or in reducing Cr⁶⁺ to Cr³⁺, in three 1-electron steps? This information is not yet known.

Just like arsenic, chromium also causes oxidative stress, and there is some evidence that oxidative stress suppresses the PAH-mediated induction of enzymes via the AHR (the system discussed above). For example, H₂O₂ (hydrogen peroxide) has been shown to inhibit CYP1A1
inducibility, although this effect may be mediated by way of transcription factors other than the AHR.

How can we study the biological effects of complex mixtures?

Much work is needed on the study of two or more environmental agents on the cell, the tissue, and the intact animal. As described in this overview, multiple effects by each agent might act additively, or be synergistic or inhibitory, on various critical life processes in ways that are virtually impossible to predict. These effects will confound any attempts at human risk assessment concerning materials present in toxic waste dump sites. New experimental paradigms—that depart from the classical concept that the effect of combined exposures may be predicted from the additive effects of each of the components—are needed. Combined exposures generate substance-specific changes in gene expression that cannot be attributed to a single mechanism, or to a combination of single mechanisms.

—contributed by Dan Nebert and Alvaro Puga

Latest in Genetics and Genomics,...

Tidbits of interest from the first 6 months of 2005 with the Human Genome Project (HGP), and related genetics/genomics news, provided chronologically:

Jan 2005 The sequencing of a second Drosophila (fruit fly) species, D. pseudoobscura, has now been completed [Genome Res 2005; 15: 1]. By comparing this genome with that of D. melanogaster, new genes and conserved regulatory regions can be identified in both flies.

A “haplotype” refers to the pattern of DNA variant sites (relative to one another) along one chromosome. A child’s haplotype always differs from that of either parent because of crossovers during meiosis (development of the egg and sperm). Looking at more than 20,000 individual recombination events in spermatocytes from men aged 17 to 87, the mean number was 48.0 (i.e. slightly more than 2 events per chromosome) with a range of 21 to 65; remarkable variations were seen among the 11 individuals, but donor age did not contribute to this variation [Hum Genet 2005; 116: 172].

Focusing on the mitochondrial cytochrome c oxidase subunit I (MT-COI) gene, the Doug Wallace lab found 11-12% of all prostate cancer patients harbored nonsynonymous mutations (ones that alter amino acids). One such mutation (8993T>G) was introduced by cybrid (cytosol, including mitochondrial) transfer into the PC3 prostate cancer cell line; injected into mice, these prostate tumors grew 7 times larger than the wild-type (8993T/T) cybrids, and the mutants generated significantly more oxidative stress [PNAS 2005; 102: 719]. Again, as described also in the last issue of Interface, we must not overlook the mitochondrial genome when studying human complex diseases.

Feb 2005 The enzyme NAD(P)H:quinone oxidoreductase-1 (NQO1) binds and stabilizes ornithine decarboxylase (ODC), especially under conditions of oxidative stress. NQO1 was shown to play a role in regulating ubiquitin-independent degradation of ODC by the 20S proteasomes [Mol Cell 2005; 17: 645]—again underscoring the prediction long ago [Mol Endocrinol 1991; 5: 1203] that probably all so-called “drug-metabolizing enzymes”
possess one or more critical-life functions, in addition to drug metabolism.

Mar 2005 A multi-strain (13 lab inbred strains & 12 wild-derived strains) high-resolution haplotype map for the 99-Mb mouse chromosome 16 (~70,000 SNP markers/single nucleotide polymorphism) was constructed [Genome Res 2005; 15: 241]. Laboratory mice, at least, have only diverged over the past 100 years. Historic recombination, intra-subspecies variations, and inter-subspecies variants were discovered to have all contributed to the formation of only three distinctive genetic signatures.!

Increased RAS expression occurs in many lung cancers. A new means to regulate RAS was discovered by way of the let-7 gene in the worm, Caenorhabditis elegans. This gene (encoding a microRNA) stops cells in the worm from continuing to divide [Cell 2005; 120: 635]. Following the worm studies, it was found clinically that MIRNLET7 expression is lower in lung tumors than in normal lung tissue, whereas RAS expression is significantly higher in lung tumors—providing a possible mechanism for this MIRNLET7 miRNA in human cancer.

The major histocompatibility complex (MHC) is associated with autoimmune, inflammatory and infectious diseases. A high-resolution (1 SNP per 1.9 kb) linkage-disequilibrium (LD) map of a 4.46-Mb fragment containing the MHC is now reported [Am J Hum Genet 2005; 76: 634]; the SNP data, haplotype blocks, and tag SNPs are all entered into (an accessible) multidimensional Web-based database, called GLOVAR.

Apr 2005 Large-scale copy-number variations or polymorphisms (LCVs, CNPs) occur, on average, at least 11 times in each of us (as discussed in the last issue of Interface), but they are difficult to detect. Now comes an array-based comparative genomic hybridization (array CGH) that offers a resolution at least two orders of magnitude higher than that previously reported for LCV detection [Am J Hum Genet 2005; 76: 750].

May 2005 A 15-year search for the gene underlying the very rare Roberts syndrome (physical manifestations often include cleft lip and palate and shortened limbs, similar to that seen in babies whose mothers have taken thalidomide during pregnancy) came to a successful conclusion [Nat Genet 2005; 37: 468]. The study of affected and unaffected subjects from 15 families from Colombia, Japan, Turkey and Italy led to a region on chromosome 8; then comparative genomics (alignment of genes from human, chimpanzee, mouse, rat, chicken, and zebrafish) confirmed defects in the ESCO2 gene (involved in cell cohesion).!

Burkitt lymphoma (BL) is a cancer of B lymphocytes; there is a mouse model that mimics BL. Disabling the ornithine decarboxylase (Odc) gene, genetically or with a drug (difluoromethylornithine), impairs the oncogene MYC's ability to stimulate uncontrolled cell division [Cancer Cell 2005; 7: 433]. Because MYC genes are activated in ~70% of all human cancers, think of ODC as a possible drug target. A drug that disrupts ODC might slow down or prevent the onset of certain cancers of many different types.

Jun 2005 The Allan-Herndon-Dudley syndrome was among the first X-linked mental retardation syndromes, described in 1944. Studies of six large families determined that all affected individuals show mutations in their monocarboxylate transporter-8 (SLC16A2) gene [Am J Hum Genet 2005; 77: 41]. One essential function of the SLC16A2 protein appears to be the transport of triiodothyronine into neurons; of course, normal thyroid function in the brain during embryo development is needed to prevent mental retardation.

Mutations in another solute carrier gene (SLC19A3) are responsible for biotin-responsive basal ganglia disease [Am J Hum Genet 2005; 77: 16]. This disease includes brain damage, joint and swallowing problems, and neurological impairments; all patients diagnosed to date are of Saudi, Syrian or Yemeni ancestry and all have consanguineous (blood-relative) parents. Biotin, contained in most foods we eat, is a cofactor and is as important as the essential vitamins for proper growth and nutrition.

If the human genome has about the same number of genes as that of the tiny mustard plant (A. thaliana) and several thousand genes less than that of rice, what makes us unique? Engineering a human mobile genetic LINE-1 element (with the green fluorescent protein GFP reporter gene) into the intact mouse [Nature 2005; 435: 903], every time this “jumping gene” moved to another cell, the affected cell started glowing green; the same happened in rat neuronal precursor cells in culture. These transposable L1 elements comprise 17% of our genomic DNA and are suspected of function-
ing in germ cells. Now we are wondering if these repetitive DNA elements might also be important in complex human brain (memory, socio-behavioral) functions...!

Another study arrived at the same conclusion [Science 2005; 308: 1630], looking at a polymorphic microsatellite (repetitive DNA, showing lots of differences between individual animals) in the 5’ region of the prairie vole vasopressin-1a receptor (AVPR1A) gene. Different lengths of this repetitive DNA were associated with individual differences in both receptor distribution patterns and socio-behavioral traits of these voles.

The sequenced genomes at or near completion (by the Human Genome Project; HGP) include: the human, mouse, rat, chicken, dog, honey bee, ten fruit flies, sea urchin, two puffer fish, two sea squirts, two roundworms, more than a dozen fungi, bakers and fission yeast, and the bacterium, *Escherichia coli*. Several hundred other genomes (of viruses, bacteria, two mosquitoes, zebra finch) have been sequenced by companies or other consortia. Genomes in the HGP pipeline now include: macaque, orangutan, cow, duck-billed platypus, red flour beetle, domestic cat, several additional species of fungi, and a partridge in a pear tree. The National Human Genome Research Institute (NHGRI) also has plans to sequence nine more mammals (some seem weird, but they all have particular evolutionary significance): the 13-lined ground squirrel, the megabat, microbat, tree shrew, bushbaby, hyrax, pangolin, sloth, and Northern white-cheeked gibbon.

Several new “genetic screens” are being reported, which is a rapid way to find a particular subset of DNA within an entire genome. One study describes screening for candidate tumor suppressor genes [Cell 2005; 121: 837], a second study identifies a suppressor of the RAS oncogene activity and tumorigenicity [Cell 2005; 121: 849], and another study describes a way to identify estrogen-responsive genes [J Biol Chem 2005; 280: 21497].

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**Biotechnology,**...

Tidbits during the first half of 2005, concerning genetically-modified (GM) plants, biotechnology, and related topics:

**Jan 2005** The statin drugs act by inhibiting the rate of conversion of acetate molecules into cholesterol by blocking 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductase, the rate-limiting step in cholesterol biosynthesis. At first it was simply thought that statins help heart disease because less cholesterol means less atheroma (plaques in arteries). Atherosclerosis is now recognized to have a prominent inflammatory component, and statins appear to inhibit inflammatory processes directly. Consequently, patients with multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, and even some neurodegenerative diseases are benefitting from statin therapy [N Engl J Med 2005; 352: 73].

The AmpliChip CYP450 Test, now cleared by the U.S. Food & Drug Administration (FDA), is able to analyze some of the patient’s CYP2D6 and CYP2C19 genotypes. This information may be useful to some patients, because the CYP2D6 and CYP2C19 enzymes together handle more than 50% of all commonly prescribed drugs. Drawbacks to the test, however, are that unknown genotypes (and the uncommon genotypes) will not be detected by this DNA test [Eur J Pharmacol 2004; 500: 267].

**Feb 2005** The human CYP1A1 and CYP1A2 genes are oriented head-to-head, 23.3 kb apart, on Chr 15q24.1; a bacterial artificial chromosome (BAC) spanning these two genes, with 60-80 kb on either 3’ side, was engineered into mouse lines having the absence of the mouse equivalent Cyp1a1 or Cyp1a2 gene [Hum Mutat 2005; 25: 196]. The basal and dioxin-inducible CYP1A1 and CYP1A2 activities in various mouse tissues paralleled what happens in the intact human. Since these enzymes are induced by polycyclic aromatic hydrocarbons and dioxin, and they metabolize many environmental carcinogens, these mouse lines should be valuable for human risk assessment because such studies in humans are not ethically possible.

**Mar 2005** Acetaminophen (Tylenol(R)) overdose causes toxicity in liver and elsewhere. Using a liver-specific knockout of the NADPH-P450...
oxido-reductase gene [Mol Pharmacol 2005; 67: 623], it was found that Por(-/-) knockout mice exhibit less toxicity than wild-type mice in the lung, kidney and lateral nasal glands—leading the XinXin Ding lab to conclude that this toxicity is the result of liver-mediated acetaminophen metabolites; on the other hand, nasal mucosa toxicity was the same in liver POR-null and wild-type mice, indicating this is independent of liver-generated metabolites.

Aryl hydrocarbon receptor (Ahr) knockout mice were first generated 10 years ago [Science 1995; 268: 722] and known to be born with livers about half normal size. Finally, the reason for this phenotype has been experimentally proven [Mol Pharmacol 2005; 714]. The signal remains unexplained [PNAS 2005; 102: 3857]. Here is a great example of disrupting a gene, and the phenotype of the resulting mouse is unexpected and leads us into new, exciting directions.

Apr 2005 The discovery of specific patterns of gene expression, linked to multiple-drug resistance of leukemic cells, is giving crucial information into why standard therapies fail to cure about 20% of children with acute lymphoblastic leukemia (ALL), while curing the remaining 80%. Empirical analyses of “patterns” on DNA chips from ALL patients’ leukemic (white blood) cells are providing predictions of outcome, as well as choice of chemotherapy [Cancer Cell 2005; 7: 375]. For example, the team identified 139 genes that are closely linked to asparaginase resistance but vincristine sensitivity.

The LXR senses cholesterol levels (described above). Surprisingly, Lxrβ(-/-) knockout mice not only are unable to store fat, but, without LXRβ, their cholesterol concentrations build up to excessive levels, and this somehow becomes a signal to burn fat [Cell Metab 2005; 1: 231]. The signal remains unknown, but LXR appears to be “the master regulator of the master regulator.”

May 2005 Why does selenium cause prostate cancer cells to self-destruct? Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a cytotoxic agent that preferentially induces apoptosis (programmed cell death) in a variety of human cancers; however, some tumor cells remain resistant to TRAIL. Adding methylseleninic acid (MSA) to TRAIL, it was found (in several types of prostate cancer cell cultures) that the combination was much better (than either alone) at killing prostate cancer cells [Oncogene 2005; May 16 Epub]—suggesting a new strategy for treating prostate cancer patients.

The Icelandic company deCODE Genetics has reported success in a Phase IIa study with heart attack participants being treated with a new drug (DG031) or placebo [J Am Med Assn 2005; 293: 2245]. Of 268 heart attack patients screened, 191 were carriers of “at-risk” variants in the 5-lipoxygenase-activating protein (FLAP) gene (87%) or the leukotriene A4 hydrolase (LTA4H) gene (13%). DG031, an inhibitor of FLAP, improved several parameters in the leukotriene pathway—indicating that the drug is now ready for Phase III clinical trials. This study shows how human genetics can point the way to potentially powerful new medicine.

A vesicular stomatitis virus (VSV), having an RNA genome, has been found to target and kill glioblastoma [J Virol 2005; 79: 6005]. Since this is a deadly type of brain tumor (in adults and children) that is resistant to any current medical or surgical treatment, finding this virus could be a very significant advance in the field.

Jun 2005 Baker’s yeast (Saccharomyces cerevisiae) is the only yeast species able to produce ethanol (EtOH). S. cerevisiae has two alcohol dehydrogenase genes, ADH1 and ADH2, which probably arose from a gene duplication event of an ancestral (“ADH1”) gene. The ADH1 enzyme was designed to make (but not consume) EtOH. ADH1 and ADH2 differ by 24 (out of 348) amino acids; ADH1 allows EtOH to accumulate, while ADH2 consumes the EtOH. Making all possible combinations of what ADH1 might have looked like ~80 million years ago (before ADH1 and ADH2 formed from it and then diverged) [Nat Genet 2005; 37: 630], it was determined that the ancient yeast did not accumulate EtOH. So, when the ADH1 gene first appeared (at the time of the emergence of mam-
mals, fruit flies and fleshy fruits, and extinction of the dinosaurs), S. cerevisiae for the first time developed the capacity to accumulate EtOH—long before humans began to use EtOH for preserving foods and enjoying happy hours.

Fluoride is commonly added to drinking water so that we all might grow up with fewer cavities in our teeth. Ameloblasts are the cells in the gum responsible for dental enamel formation. Now we find out that fluoride induces a type of serious stress, called endoplasmic reticulum stress, in these ameloblasts [J Biol Chem 2005; 280: 23194].

Bacillus thuringiensis (Bt) crystal-protein genes encode insecticidal ∆-endotoxins that are widely used for the development of insect-resistant crops. An alternative transgenic strategy, which involves engineering plants with a fusion protein (combining the ∆-endotoxin Cry1Ac with the galactose-binding domain of the nontoxic ricin B-chain); this was shown to increase the potential resistance to a wider range of insects, including important pests not normally susceptible to Bt toxins [PNAS 2005; 102: 7812]. The potential impact of such fusion genes should lead to crop improvement, longer sustained resistance, and greater biosafety.

Ethical, Legal and Social Issues, ...

ELSI tidbits from the first 6 months of 2005:

Feb 2005 Gennaisance Pharmaceuticals, Inc. (Connecticut) is marketing its proprietary FAMILION™ Test, designed to detect mutations responsible for causing familial long-QT syndrome and the Brugada syndrome—two diseases in which patents have an increased genetic risk for arrhythmias of the heart. This patented test looks for several mutations in four channel genes. According to Online Mendelian Inheritance in Man (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM), however, there are at least fifteen genes responsible for long-QT syndrome. Therefore, this test might help some patients, but physicians must be aware of the limitations of such a test (sorry, but this is a recurrent theme in this NewsLetter).

Mar 2005 Gennaisance Pharmaceuticals also has received a “notice of allowance” for a patent, jointly owned with Duke University, that claims to test for a common genetic variant in the CYP3A4 gene that can predict whether or not an individual is a poor metabolizer of certain drugs. A mutation in the GSTM1 gene, which affects metabolism, is also mentioned in the patent. It is true that CYP3A4 does participate in the metabolism of more than 50% of all commonly prescribed drugs [Drug Metab Rev 2005; 37: 327]. There are dozens of reasons, however, as to why this patent would barely scratch the surface of an individual’s complete drug-metabolism profile [Eur J Biochem 2004; 500: 267].

Apr 2005 Psychological factors strongly influence the course of one’s coronary artery disease (CAD). There is an emerging field of behavioral cardiology, which is based on the understanding that psychosocial and behavioral risk factors for CAD are not only highly interrelated, but also require a sophisticated health-care delivery system to optimize their effectiveness [J Am Coll Cardiol 2005; 45: 637].

May 2005 In a follow-up to “the use of population categories in forensic genetics” article (from Nat Genet, Nov 04), which we highlighted in the last issue of Interface, there is an interchange of opinions about the accuracy and emphasis on various aspects of that article [Nat Genet 2005; 37: 449]. Hmmm...
Evolutionarily Speaking, ...

What follows is a synopsis of some of the more interesting things that have happened during the first 6 months of 2005 with the Human Genome Project (HGP), and evolutionarily-related news, provided chronologically:

**Jan 2005** By comparing genomic regions in human/mouse, human/rat and dog, cow, chicken, opossum and zebrafish, **976 human microRNA (miRNA) genes** were identified and found to be highly conserved (in sequence and position near protein-coding genes) between all species [Cell 2005; 120: 21]. Although only recently discovered, these miRNA genes are likely to be present at significantly higher numbers than previously thought. And well over one-third of all human protein-coding genes appear to be miRNA targets [Cell 2005; 120: 14].

By comparing the chicken genome with mammalian genomes [Genome Res 2005; 15: 98], the likely number of interchromosomal rearrangements between the mammalian ancestor and chicken—during at least 420 million years of evolution—is not that much more than the number between human and mouse, during the past ~75 million years. No one has a reasonable hypothesis as to why.

Transcribed genomic DNA leads to RNA containing both exons and introns; the spliceosome (in the cytoplasm) then cuts out the introns, giving rise to mRNA that usually contains only the exons. From studying 684 groups of orthologous (same in each species) genes in seven fully-sequenced eukaryotic genomes (fly, mosquito, baker’s yeast, human, fission yeast, mustard plant, and parasite), the data support a reverse-transcriptase-mediated model of intron loss rather than intron gain [PNAS 2005; 102: 713].

**Feb 2005** Looking at a refined physical map of chromosome 17q21.31 [Nat Genet 2005; 37: 129], a research team from deCODE Genetics (Iceland) has uncovered a **900-kb inversion polymorphism** which has been diverging for as long as the past 3 million years. The inversion is rare in Africans, almost nonexistent in East Asians, yet found at a 20% frequency in Europeans; carrier females have more children and higher recombination rates than noncarriers, suggesting positive selection.

An “improved” whole-genome shotgun sequence assembly for the genomes of indica and japonica rice has been completed [PLoS Biol 2005; 3: e38]. The total estimated gene count is between 38,000 and 40,000. The rice genome quite definitely has **more genes** than the human genome. Ongoing gene duplications provide a never-ending source of material for “making new genes” and are major contributors to the differences between members of the grass family. Rice is also the winner so far, for having the most cytochrome P450 (CYP) genes: 452 functional genes! [http://drnelson.utmem.edu/CytochromeP450.html]

**Mar 2005** In fungi, proline has been shown to be a scavenger of reactive oxygen species (ROS), and this could be a mechanism responsible for the ability of symbiotic fungi to protect plants against abiotic and biotic stress [PNAS 2005; 102: 3459 & 3175]. This effect by proline should be explored to see if animals also show this. Another unusual (and underappreciated) antioxidant, uric acid, comes to mind [Med Hypotheses 2004; 62: 173].

**Apr 2005** Conducting a systematic approach to extracting biologically meaningful information from the massive MEDLINE database, a bioinformatics study of more than **200,000 genes** evolutionarily conserved across **92 species** led to more than **2,700 significant associations** and more than **28,000 encoded proteins** [PLoS Biol 2005; 3: e166]. Examples of unexpected relationships are shown. Given the weekly increase in the number of genomes sequenced and new MEDLINE entries, this cross-referencing method should provide a valuable tool in helping researchers stay up-to-date.

**May 2005** Human and chimpanzee differ by a little more than 1% of their DNA sequence, so what genes are different that make humans so unique? By comparing **13,731 genes** from humans to their chimp equivalents [PLoS Biol 2005; 3: e202], the strongest evidence for positive selection was found in genes related to immune defense, sensory perception, spermatogenesis, and a “surprising number” of tumor-suppressor and apoptosis (programmed cell death) genes. There is a lot of speculation, but no firm answers, as to what any of this means.

About 14,000 years ago (more than 100,000 years after our modern forebears first spread out from Africa), descendants of archaic humans crossed the Bering land-bridge from Siberia to North America. Using statistical approaches that require DNA analysis and computer simulations, it was determined...
that the New World was colonized by a small population having an effective size of only ~70 individuals; their ancestral Asian population (from which they migrated) was estimated at ~9,000 individuals [PLoS Biol 2005; 3: e227]. Kimura’s theory of neutral evolution argues that most polymorphisms (in both DNA and protein sequence) have minor or no selective effect, but rather are governed by random processes (neutral drift). In a Herculean study of 44 million bp and 17,000 bp SNPs from 876 short fragments of the genomes of 96 Arabidopsis thaliana (tiny mustard) plants from all over the world [PLoS Biol 2005; 3: e231], the patterns did not fit the standard neutral model of evolution; instead, selective pressures predominated in plants from each geographic region.

Jun 2005 How do adorable little children turn into impossible, surly teenagers? When the DHR4 gene (encoding an orphan nuclear receptor) is disrupted in the fruit fly, normal maturation from larva to pupa does not occur. It is concluded that DHR4 coordinates growth and maturation in Drosophila, by mediating endocrine responses to the successful completion of larval development [Cell 2005; 121: 773]. It is unlikely the same gene operates in humans, but it’s a starting point (in what to look for).

In animals and plants, hundreds or thousands of microRNAs (miRNAs) are small (approximately 22-nucleotide) RNAs that serve important regulatory roles in development and gene expression—typically by forming imperfect duplexes with target messenger RNAs. Now miRNAs encoded by simian virus 40 (SV40) have been identified, which enable the virus to evade and destroy human immune cells. Thus, viral evolution has pirated, or taken advantage of, the human miRNA pathway to generate effectors that enhance the probability of successful infection [Nature 2005; 435: 682].

Methods for identifying species by using short DNA stretches from a common gene, known as “DNA barcodes”, have been proposed and initiated to facilitate biodiversity studies, identify juveniles, determine gender, and enhance forensic analyses. The cytochrome c oxidase subunit-1 (MT-CO1) gene, which has been found to be widely applicable in animal barcoding, is not appropriate for most species of plants because of a much slower rate of MT-CO1 gene evolution in higher plants than in animals. The nuclear internal transcribed spacer region and the plastid trnH-psbA intergenic spacer has been proposed as potentially usable DNA regions for applying barcoding to flowering plants [PNAS 2005; 102: 8369]. This sequence was then tested in a total of 99 species, 80 genera, and 53 families of plants—and shown to have the potential to discriminate among the largest number of plant species (of any sequence so far found) for barcoding purposes.

web-cytes

Find all the protein-name jokes you could possibly want to know http://www.chm.bris.ac.uk/sillymolecules/sillymols3.htm

Here is a great site site for your K-12 students, called DNA from the beginning. It contains animation, video, old photographs etc. of early work on inheritance http://www.dnaftb.org/dnaftb/

“Q”

Quote of the month

If you find yourself in a hole.........stop digging. Will Rogers, 1879-1935
**Gene-Environment Tidbits of Interest**

**Jan 2005** Telomeres are the “caps” on the end of chromosomes. Higher oxidative stress, lower telomerase activity, and shorter telomere length—known determinants of cell senescence (aging) and longevity. Mothers caring for a chronically ill child had telomeres as short as those of mothers a decade older with a healthy child; moreover, the telomeres were shortened in direct proportion to the stressful care-giving period [PNAS 2004; 101: 17312].

Glycosyltransferases are encoded by genes of the UGT superfamily [Pharmacogenetics 1997; 7: 255], are probably present in all animals and plants. Now it turns out that a UGT is responsible for the red color in petals of the red daisy, *Bellis perennis* [J Biol Chem 2005; 280: 899]. The UGT provides a water-soluble glucuronide of the cyanidin pigment, which is necessary to compartmentalize this pigment in the petal. This finding underscores the fact [Mol Endocrinol 1991; 5: 1203] that probably all so-called “drug-metabolizing enzymes” possess one or more critical-life functions, in addition to drug metabolism (which evolved much more recently).

Genotyping 904 SNPs from 55 ADME (drug absorption, distribution, metabolism, excretion) genes in European and Japanese populations [Nat Genet 2005; 17: 84], a set of tag SNPs was identified that represent the common variations in these genes. The study points out that rare variation is not amenable to these strategies (meaning that prediction of drug therapy will not work for everyone).

**Feb 2005** The G-protein-coupled receptor (GPR) superfamily comprises more than 800 genes in the human genome, of which ~375 are neither olfactory nor taste receptors. It has been estimated that more than half are involved in drug reception. Human GPRs can be classified into five families: A (rhodopsin), B (secretin), C (glutamate), adhesion, and Frizzled/Smoother/Taste2 [J Biol Chem 2005; 280: 5129].

In frog-egg nuclear-transfer experiments, substantial overexpression of donor cell type-specific genes (both spatially and temporally) was observed in the wrong cell types in some nuclear-transplant embryos [PNAS 2005; 102: 1957]. This led to data showing that epigenetic memory is somehow established in differentiating somatic cells—in genes that are in a transcriptionally active state. (“Epigenetics” refers to regulatory effects on inheritance and gene expression that are not controlled by classical Mendelian genetics.)

**Mar 2005** High-throughput DNA resequencing [Cancer Cell 2005; 7: 387] identified a recurrent somatic mutation (Val617Phe) in the JAK2 gene of granulocyte DNA samples of 121 out of 164 polycythemia vera patients (some showed duplication of the mutant allele), of 37 out of 115 essential thrombocythemia patients, and 16 out of 46 myeloid-metaplasia-with-myelofibrosis patients. Compared with these types of leukemias, this mutation was not seen in 269 normal individuals. The JAK2-Phe variant encodes an active tyrosine kinase.

It has long been suspected that some correlation exists between bacteria found in gingival disease (gums of the mouth) and increased risk of cardiovascular plaques and stroke. An interesting association has now been found [Arterioscler Thromb Vase Biol 2005; 25: e17].

**Apr 2005** In half the women at high risk for developing breast cancer, there is a specific alteration in their breast cells—indicating something has occurred and malignancy may be imminent. Researchers have found that the retinoic acid receptor-β2 (RARB2) gene is “silenced” (by DNA methylation) in 69% of women with early-stage breast cancer and 50% of women at high risk for the disease [Cancer Epidemiol Biomarkers Prev 2005; 14: 790]. Does some environmental agent cause this silencing to occur in people having genetic susceptibility?

Prusiner received the 1997 Nobel Prize in Medicine for his “prion hypothesis” that abnormal proteins are the cause of transmissible spongiform encephalopathy (TSE) (e.g. mad-cow disease, Creutzfeldt-Jakob disease, scrapie, kuru, and chronic wasting disease (the result of people eating infected brains of animals or humans). Devising a method called “protein misfolding cyclic amplification” (PMCA), this vastly accelerated the activity of a small number of prions, so that injected hamsters began showing symptoms of TSE in 4 mo [Cell 2005; 121: 195]. This should facilitate the development of a desperately-needed blood test for prions—for
the successful surveillance of contaminated blood. Tobacco’s harmful effects on the lung can be passed down through generations, from grandmother to grandchild, even when the child’s mother appears unaffected [Chest 2005; 127: 1232]. The study included 908 children recruited in grades 4, 7 and 10; children whose mothers smoked while pregnant were 1 1/2 times, whereas children whose grandmothers—but not mothers—smoked were almost 2 times, as likely to develop asthma! Grandmothers—but not mothers—smoked were almost 2 times, as likely to develop asthma.!

As discussed in the Leading Article of issue #25 of Interface, it would be advantageous to develop transgenic fish that would detect and quantify aquatic pollutants. DNA damage in gill biopsies is another promising means to assess the effects of contaminants on marine life [Environ Health Perspect 2005; 112: 511]. English sole were collected from the Puget Sound, WA—one group from the industrialized Duwamish River in Seattle, the other from the relatively clean Quartermaster Harbor. Differences in DNA damage, related to the degree of water pollution, could be discerned, using Fourier-transform-infrared (FT-IR) spectral analysis.

Discovery of a mouse enzyme that destroys cartilage in inflammatory arthritis [Nature 2005; 434: 648] could offer a new target for designing drugs to treat this condition, i.e. why not block that enzyme? The enzyme is encoded by the gene ADAMTS5. It remains to be seen if the same enzyme in mouse can be extrapolated to humans with severe arthritis.

May 2005

In examining 29 previous studies, it was found that the dose of cigarette smoke to an active smoker is at least 100 times the dose delivered to a passive smoker. Risk of coronary heart disease (CAD) in smokers is 80% greater than that in the nonsmoker; interestingly, the risk of CAD in passive smokers is 30% greater than nonsmokers [Circulation 2005; 111: 2684]. The authors conclude that passive smoking has relatively a much greater effect on the cardiovascular system than would be expected.

Huntingtin (HTT) is a protein involved in Huntington disease (HD), and causes aggregation of nuclear and cytoplasmic inclusion bodies—even in yeast. Fifty-two loss-of-function mutations in yeast genes were found [Nat Genet 2005; 37: 526], which enhance the toxicity caused by an HTT fragment. From a further genome-wide suppressor screen, among the most potent was kynurenine 3-monoxygenase (Bna4 in yeast; KMO gene in human). Since this enzyme has been linked to oxidative stress, and HD is suspected to be made worse by oxidant stressors in the brain, this yeast screen has led to the discovery of a potential therapeutic target for this debilitating disease.

If a smell is specific, it will stimulate the Fos gene in individual neurons across the mouse brain. Using a Fos-reporter-gene assay and a wide range of odorants—including apple, skunk, floral, fishy, urine, vanilla, musk, woody, garlic and chocolate—it was found that very small subsets of neurons (responding to each smell) are sparsely distributed over a relatively large area [PNAS 2005; 102: 7724]. This is in contrast to the olfactory bulb, where signals from different receptors are segregated. “Just like letters of the alphabet are used in different combinations to form different words, the odorant receptors are used in different combinations to detect different smells and encode their unique identities”.

Low cortisol levels have been observed in patients with different stress-related disorders—such as chronic fatigue syndrome, fibromyalgia, and post-traumatic stress disorder (PTSD). PTSD in the mother can affect the cortisol levels of infants (or even adults) who had been in utero (in the womb) at the time of the stress to the mother. Thirty-eight mothers directly exposed to the World Trade Center collapse on 11 Sept 01 during pregnancy were identified; the mothers collected salivary cortisol samples, from themselves and their 1-year-old babies, at awakening, and again at bedtime. Statistically significantly lower cortisol levels were observed in both the mothers (P = 0.030) and babies of moms (P = 0.008) who had had PTSD (in response to 9/11), compared with mothers who did not develop PTSD and their babies [J Clin Endocrinol Metab 2005; May 3 Epub]. The effect was greatest in babies whose mothers had been exposed during the third trimester.

Jun 2005

One way of treating cancer cells (which have both copies of one of their suppressor genes turned off) is to turn these genes back on (reactivate them)—by drug-induced DNA methylation. This is what the chemotherapeutic drugs 5-azacytidine and 5-aza-deoxycytidine (5-aza-CdR) are designed to do. It had been believed that 5-aza-CdR must first be incorporated into the cell’s DNA before it can work, but instead it works by triggering a series of chemical reactions (proteasomal pathway) that degrade the DNA methylase quickly [Mol Cell Biol 2005; 25: 4727]. In patients taking medica-
Obesity findings are more likely that "causing obesity, the explanation for this fascinating 2 cans consumed per day". Rather than diet soda drinking is also linked to obesity drinkers Hispanic Caucasians aged 25 to 64, for". How does a majority of the genetic changes in the virus allow it to evade the body’s immune system during infection? Two distinct mechanisms have now been discovered [J Exp Med 2005; 201: 1741]. Looking at 1,550 Mexican-American and non-Hispanic Caucasians aged 25 to 64, for regular soft-drink drinkers versus “diet pop” soft-drink drinkers, the risk of becoming overweight or obese was 47% versus 57%, respectively—for “more than 2 cans consumed per day”. Rather than diet soda causing obesity, the explanation for this fascinating finding is more likely that “something linked to diet soda drinking” is also linked to obesity [Int J Obesity 2004; 28: 933 & presented at 65th Annu Am Diabetes Assn Mtg, Abstract 1058-P]. Perhaps it is the philosophy of many people: “Because I’m having a diet soda, now I can eat more food”.

Alternatively, diet soda may elicit some unknown signal to our eating (satiety) center—about which we understand very little.

Cardiac patients who have implanted cardioverter defibrillators (ICDs) had an increased incidence of irregular and very dangerous heart arrhythmias when exposed to short-term high pollution levels in the Boston metropolitan area over a 3-year period [Environ Health Perspect 2005; 113: 670]. What is the factor in the polluted air? The strongest association of arrhythmias with sulfate suggests a link with the stationary fossil-fuel combustion sources.

Vinclozolin (a fungicide commonly used in vineyards) and methoxychlor (a pesticide that has replaced DDT) are endocrine disruptors—chemicals that interfere with the normal functioning of reproductive hormones. Pregnant rats exposed to these disruptors produce male offspring with lowered fertility; the male offspring of these males also showed lowered fertility. And this went on, for the 3rd and 4th generation, as well! This study [Science 2005; 308: 1466] suggests that some disorders that we have, ... might have begun with defects (epige-

Hepatitis C is the leading cause of liver disease in the U.S., causing an estimated 10,000 to 20,000 deaths each year. Hepatitis C is transmitted when blood and possibly other body fluids of an infected person enter another person (illicit drug use, exposures in health-care settings, sometimes through sexual contact, or from mother to baby during birth). How does this happen? The virus is now been discovered [J Exp Med 2005; 214: 8579]. This is of particular interest because dioxin (discussed in many past issues of Interface) also interacts with the WNT/β-catenin signaling pathway, and dioxin in the pregnant rodent is well known to cause in utero toxicity and miscarriages of embryos and fetuses.

Activation of the receptors for leukemia inhibitory factor (LIF) and interleukin-11 (IL-11) is essential for embryo attachment and decidua (lining of uterus during pregnancy) formation in mice; both receptors induce activation of the STAT family of signal transducers via the JAK/STAT signaling pathway. It was found that successful implantation is dependent on phosphorylation and activation of STAT3 in the uterine endometrium before implantation [PNAS 2005; 102: 8585]. This finding suggests a possible drug target—a nonsteroidal approach—for the development of contraceptive treatment.

Observations by a Biologist

Male Sexual Orientation

Samples of 456 males from 146 families having two or more “gay” brothers were genotyped with 403 microsatellite markers at 10-cM intervals (centiMorgan, a defined distance along a chromosome) across the entire genome [Hum Genet 2005; 116: 272]. This is the first such “full-genome” scan looking for human genetic loci associated with sexual orientation in men.

There is some evidence to suggest that maternal effects might act on autosomes (i.e. non-sex chromosomes) instead of, or in addition to, the sex chromosomes in influencing the trait of sexual
orientation; for this reason, Mustanski and coworkers calculated maximum likelihood estimation (mlod) scores separately—for maternal, paternal, and combined transmission of the “gay male” trait. The highest two mlod scores were 3.45 at chromosome (Chr) 7q36 and 1.96 at Chr 8p12—both having equal maternal and paternal contributions. A third suggestive effect was found at Chr 10q26 with an mlod score of 1.81 and no paternal contribution. This lab had previously found an association of the gay trait with Chr Xq28 and, comparing the previous set of data with the current set of data, the mlod scores were 6.47 and 1.99, respectively.

Human homosexuality is likely to represent a combination of susceptibility genes and the environment (“environmental factors” in mammals could include in utero effects on the developing embryo or fetus). Do these human genotype-phenotype association studies have any evolutionary counterpart to genes of other animals? To date, the only animal that has been extensively studied in this regard is the fruit fly, Drosophila melanogaster, in which male-courtship studies have been carried out over the past three decades.

The fruitless (Fru) gene encodes a set of male-specific transcription factors (FruMs) that establish the potential for courtship in the male fly. FruMs proteins are expressed in ~2% of central nervous system (CNS) neurons; at least one subset of these proteins coordinates the component behaviours of courtship. Many studies have now shown that FruM proteins specify the CNS substrates of male courtship, and defects in the Fru gene lead to abnormal male courtship behavior [see Cell 2005; 121: 785 & 795 & refs. therein]. Male inhibitory pheromones (which can be considered as an “environmental factor”) are normally used to repress male-male interaction [Curr Biol 2005; 15: 194; J Exp Biol 2005; 208: 891].

Extension of these fly studies to a laboratory animal, or correlation of the human chromosomal locations described above with a similar fly gene, would be the next logical step in studies of this kind. Until then, there is a lot of work yet to be done.

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Pilot Project Recipients 2005

Year 14 Pilot Projects have been awarded. CONGRATULATIONS! to the awardees!

M. Kathryn Brown, Ph.D., Department of Environmental Health. “Extreme makeover of women’s health education: A feasibility study of outreach to African-American women in beauty salons”

Timothy P. Dalton, Ph.D., Department of Environmental Health. “GSH biosynthesis genes and risk of heart disease”

Kim N. Dietrich, Ph.D., Department of Environmental Health. “Lead and criminal behavior: Genes and environment”

Mary Beth Genter, Ph.D., Department of Environmental Health. “CYP2F proteins in respiratory tract toxic responses”

Sue Heffelfinger, M.D., Ph.D., Department of Pathology & Laboratory Medicine. “Polygenic effects of obesity in mammary carcinogenesis: A new model”

Jessica G. Woo, Ph.D., Department of Cell Biology, Neurobiology and Anatomy. “Genetic susceptibility to bisphenol A (BPA) and bis-phenol A diglycidyl ether (BADGE) and influences on metabolic phenotypes during pregnancy”

Floyd R. Sallee, M.D., Ph.D., Department of Psychiatry. “Genetics of attention-deficit hyperactivity disorder (ADHD) using extreme discordant phenotype methodology”

If lawyers are disbarred and clergymen defrocked, doesn’t it follow that electricians can be delighted, musicians denoted, cowboys deranged, models deposed, tree surgeons debarked, and dry cleaners depressed?

😊😄😁😆😢😭
COMMENT   In the Leading Article of issue #23 of Interface, we described how effects in the uterus during pregnancy might affect the baby when it becomes an adult. An interesting study along the same lines [Circ Res 2005; 96: 12] suggests that babies of slim mothers with lower body fat stores might adapt to the supply of nutrients (from the mother) and lower the amount of blood flowing to the liver. This “liver-sparing” adaptation does not occur in babies whose mothers are eating an unbalanced diet and have greater liver blood flow; these babies, later in life, might be less able to cope with a high-fat “Western” diet, thereby having increased risk of heart disease, obesity and diabetes.

Q   In the Leading Article of issue #26 of Interface (“Cigarette Smoking: the Good(?), the Bad, and the Ugly”, you noted that smoking is sometimes associated with less risk of a disease. As a thoracic oncology (lung tumor) surgeon, I must state emphatically that cigarette smoking is never good for you! It should be emphasized that we now know a lifelong male cigarette smoker dies on average 13 years sooner, and a lifelong female cigarette smoker dies on average 14 years sooner, compared with a lifelong nonsmoker (who has also not been exposed to second-hand smoke).

A   Yes, in that article we had noted that smoking is sometimes helpful for loosening sputum as well as associated with less risk for such diseases as: ulcerative colitis, Parkinson disease, schizophrenia, uterine fibroids, endometriosis, & certain types of ulcers. We were not advocating cigarette smoking, however, but rather that these observations might provide some inroads into understanding the mechanisms-of-action or modes-of-action of these disorders. [Note that we had included a (?) next to “Good” in the Title..!]

Q   Something on the TV news described that Viagra might actually protect the heart from damage. Do you know what this is all about?

A   This story is limited to only a very small percentage of our population. Doxorubicin (DOX) is used in the treatment of breast cancer, leukemia and sarcomas; one of its unfortunate side-effects (in a few people; and no one knows why) is irreversible heart damage (progressive cardiomyopathy) — due to DOX causing mitochondrial ATP-dependent potassium channels to open, which results in programmed cell death of myocytes (heart cells). Giving Viagra (sildenafil citrate) one hour before administering DOX prevented heart damage in a chronic mouse model of DOX-induced cardiomyopathy [Circulation 2004; 431: 393]. How Viagra neutralizes this potassium defect is still not clear. Whether these data can be translated to humans is also not yet clear.

Q   My mother had a very difficult time in the hospital because of a bacterial infection that just would not give up. Her doctor said that “bacteria mutate to become resistant to antibiotics — in much the same way that tumor cells mutate to become resistant to chemotherapeutic drugs”. Is this true?

A   This is mostly true. WHY bacteria mutate, or WHY tumor cells mutate, however, are the interesting questions. According to a recent study of quinolone antibiotics [PLoS Biol 2005; 3: e221], antibiotics that cause DNA breaks or DNA damage set off a bacterium’s emergency repair mechanism, called the SOS response. Similarly, tumors commonly exhibit increased levels of oxidative stress, leading to DNA breaks and DNA damage. Thus, developing novel therapeutic agents that
target a particular step in the SOS pathway, or in the oxidative stress pathway in cancer cells, may prove a promising strategy for controlling the spread of the antibiotic-resistant superbugs and the tumors, respectively.

Q Is this issue (#28) really the latest? It is dated "Summer/Fall 2004" and this is now at the beginning of September, 2005.

A Yes. Unfortunately, we sometimes get too busy with too many other things. However, you will be happy to hear that issue #29 (Winter/Spring 2005) will appear next week, so, once again, we have caught up.

A2A Thanks for your quick reply. I know how that can be. I just wanted to make sure I was reading the latest one.

Human Variation, Disease, Migration and Evolution, ...

Tidbits on these topics from the first half of 2005:

Jan 2005 A patient is described [N Engl J Med 2004; 351: 2827] in whom opioid intoxication occurred, after being given small doses of codeine, a CYP2D6 substrate. It was determined by enzyme assays and genotyping that the patient has three or more functional CYP2D6 genes, thereby giving him the “ultra-metabolizer” (UM) phenotype.

As discussed in previous issues of Interface, enhancers (DNA motifs that regulate a gene) can be located at large distances from the gene being controlled (sometimes 200 kb or more than 1 Mb) and sometimes can reside inside an unrelated neighboring gene. Examples are given in this recent review [Am J Hum Genet 2005; 76: 8].

There are relatively few diseases for which epidemiological criteria (based on migrant studies and risk to individual admixture proportions) support genetic explanations for ethnic variation in risk; however, these diseases are major in terms of morbidity and mortality—diabetes type-2, hypertension, obesity, coronary artery disease, and prostate cancer. As noted in the last issue (“Human Variation”, Sept) and in this review [Am J Hum Genet 2005; 76: 1], most of the technical problems in admixture mapping have now been solved and, where panels of markers informative for ancestry are available, this analysis can now localize genes that contribute to ethnic variation in any measurable trait.

If there are ~11 million SNPs having frequencies of 1% or more on this planet, it would be desirable to decrease this number to perhaps 500,000 “tag” SNPs that represent all haplotypes in the world; this is the goal of the HapMap (HapMap) Consortium. Ahmadi and coworkers [Nat Genet 2005; 37: 84] therefore genotyped 904 SNPs (across 2,123 kb of genomic DNA) from 55 genes related to absorption, distribution, metabolism and excretion of drugs. This approach worked for some, but not other, individuals in the Caucasian and Japanese populations studied.

Feb 2005 In the past two issues of Interface, we have discussed the less-than-perfect association of gefitinib therapy being successful in non-small-cell lung cancer (NSCLC) patients whose tumors have a mutation in the tyrosine kinase domain of the EGFR gene. Another patient is now reported [N Engl J Med 2005; 352: 786] who had a relapse after 2 years of remission during gefitinib treatment; in addition to the one mutation conferring gefitinib-sensitivity, it seems that a second mutation then developed (in the tumor)—which conferred gefitinib-resistance. Cancer cells can be sneaky. Here’s another example of now-you-see-it-now-you-don’t (NYSINYD). An association of a mutation in the SUMO4 gene with type-1 diabetes was reported [Nat Genet 2004; 36: 837], but this finding has now been refuted in other ethnic populations [Nat Genet 2005; 37: 110].

Admixture mapping, which could lead to identification of genes contributing to complex diseases, has been discussed in recent issues of Interface. Carrying out a total genome-wide scan on 1,340 African-American patients with hypertension [Nat Genet 2005; 37: 177], chromosomal regions 6q24 and 21q21 suggested a strong likelihood of one or more genes related to the (high blood pressure) trait.
Individuals having a particular mutation in the KLF6 gene face a ~50% increased risk for developing prostate cancer. This mutation leads to alternative splicing, and the variant proteins SV1 and SV2 antagonize wild-type KLF6 function, leading to decreased p21 expression and increased cell growth [Cancer Res 2005; 65: 1213]. This up-regulation in prostate tumor tissue is a novel mechanism of self-encoded tumor suppressor gene inactivation, linking a relatively common SNP to both regulation of alternative splicing and increased risk for a major human cancer.

HapMap would like to find a single haplotype map—at least, for large ethnically-distinct population groups—so that perhaps ~500,000 tag SNPs might represent all ~11 million SNPs having 1% or greater frequency on this planet. Many reasons are given [Am J Hum Genet 2005; 76: 681], however, as to why this scenario is extremely unlikely.

Stuttering is a common and sometimes severe communication disorder, of unknown primary etiology, but must represent a combination of susceptibility genes and environmental factors. Studying 44 Pakistani highly-inbred families, a genome-wide linkage scan focused on affected individuals and their parents [Am J Hum Genet 2005; 76: 647]. Some evidence of linkage of the quantitative trait was found on chromosomes 1, 5, 7, and 12—with 12q showing a gene with perhaps the largest effect in this sample.

The human CYP1A1 and CYP1A2 genes are oriented head-to-head, 23.3 kb apart, on Chr 15q24.1; re-sequencing DNA from African, East Asian, Caucasian, Oceanian, and Amerindian subjects, 85 SNPs across a 40-kb region were discovered, 49 of which were not in any SNP database [Hum Mutat 2005; 25: 196]. A recombination hot spot was found between the two genes (indicating that they sometimes are passed independently from one generation to the next). The same (a recombination hotspot) was found (last issue of Interface) between exons 1 and 2 in the UGT1A1 gene [Clin Pharmacol Ther 2004; 75: 501], underscoring the importance of re-sequencing from the five major geographically-isolated human subgroups across all introns and a lot of 5’ flanking sequence (SNP-discovery) before determining ethnic differences in allelic frequencies (SNP-typing).

Mar 2005 In a survey of 211 community-dwelling Medicare-managed enrollees over age 65 (taking one or more potentially contraindicated drugs) and a random sample of 195 similar enrollees not taking such drugs, Rask and coworkers [Am J Manag Care 2005; 11: 145] found 134 adverse drug events (ADEs) in one-fourth of the people in just the past 6 months! There is available a “medication-appropriateness algorithm”, using pharmacy-claims data, but still this was not able to identify a subgroup of enrollees who are at higher risk of experiencing an ADE from these (commonly prescribed) medications.

Epidemiological evidence shows that, although thousands of individuals might live on soil heavily contaminated with cadmium (Cd), only a few will develop lung cancer or kidney disease, presumably related to the heavy metal exposure. Are there susceptibility genes for Cd disease? It has been known since the early 1970s that some inbred strains of mice develop testicular necrosis, while other strains do not, when mice are challenged with a small dose of Cd. A success story—in positional cloning and identification of the responsible gene—is now reported [PNAS 2005; 102: 3401]. The gene responsible for Cd-induced testicular damage is Slc39a8, encoding the ZIP8 metal transporter; ZIP8 is one of 14 members of this ZIP family, almost none of which have been studied in any detail.

Apr 2005 Studying 955 unrelated individuals of local ancestry from nine rural Scotland regions and the urban center of Edinburgh, as well as 96 unrelated subjects from the general U.K. population, there were greater levels of linkage disequilibrium (LD) in rural, as compared with urban, individuals [Am J Hum Genet 2005; 76: 763]. In the design of any association study, statistical power can be lost by not taking these distinct subpopulations into account.

Jun 2005 To thin the blood, many patients receive warfarin, which exhibits a wide variation among patients in drug response. Variants in the gene encoding vitamin K epoxide reductase complex-1 (VKORC1) may affect the response to warfarin. Five major haplotypes were found in African-American, European-American, and Asian-American populations. In human liver samples, ethnic differences in VKORC1 mRNA expression and the haplotype frequencies were found [N Engl J Med 2005; 352: 2285]. VKORC1 haplotypes thus can be used to stratify patients into low-, intermediate-, and high-dose warfarin groups and may explain differences in dose requirements among patients of different ancestries.
1:00-1:20 Dan Nebert
Using the extreme discordant phenotype (EDP) method in clinical genomics association studies

1:25-1:45 Randy Sallee
Pharmacogenomics of attention deficit hyperactivity disorder (ADHD): update on atomoxetine

1:50-2:00 coffee break

2:00-2:20 Sander Vinks
Risperidone pharmacokinetics and pharmacogenetics in children with pervasive developmental disorder

2:25-2:45 Sanjeev Pathak
Safety of antidepressants: a pediatric perspective
“Study-Section Think”
(Contributions by Study Section Members in Recent Years about the Study Sections on Which They Sit)

- “Although I have been assigned Primary Reviewer on this grant, I really don’t know anything about this field (but I cannot admit this to anyone). I do have the authority to triage this grant, however, and this is what I will do. I will find some sentence in the grant and raise ‘serious doubts’ or ‘grave concerns’. Or I will comment that the project ‘sounds too risky’, which will justify its being triaged.”

- “This new R01 grant is really outstanding, and—several years ago—we’d simply give it a score low enough to be funded on its first try. Now, however, there are many first and second revisions that take priority over these new applications. So, let’s give it a score outside the funding range and ask for a revision (this will delay the PI for 8 mo). When the First Revision is submitted, we might also give it a score closer to funding, but still outside the funding range and ask for a second revision (this will delay the PI for another 8 mo).” Consequently, from the time of proposing a novel, really cutting-edge grant application to the time it is actually funded—will often take 2 years—at which time, of course, the idea is no longer novel or particularly creative.!

[We welcome contributions from our colleagues about Study Section, past and present, which we will share in future Interface issues.]

Making a “living” is not the same as making a “life.”

A conclusion is the place one got tired of thinking!
CEG-Related Seminars

Timothy Buckley  
Bloomberg School of Public Health  
Johns Hopkins University, Baltimore, MD.  
“Asessing worker dermal knowledge, attitude, and behavior to evaluate exposure and develop intervention.”  
January 12, 2005

Kent Anger  
Center for Research on Occupational & Environmental Toxicology, Oregon Health Sciences University, Portland, OR  
“Methods for assessing neurobehavioral effects in workers”  
February 2, 2005

R. Hays Bell  
Director, Health Safety and Environment  
Eastman Kodak Company, Rochester NY  
“Managing a corporate health, safety and environment program in a global economy.”  
February 16, 2005

Yehia Zakaria  
Department of Occupational Medicine  
Cairo University, Cairo, Egypt  
“Occupational pulmonary diseases in Egypt”  
March 2, 2005

Wade H. Powell  
Department of Biology, Kenyon College, Gambier, OH  
“Mechanisms of dioxin insensitivity in developing frogs”  
March 30, 2005

Katia Del Rio  
Department of Zoology, Miami University, Oxford, OH  
“Mechanisms of retina regeneration”  
April 6, 2005

Panagiotis A. Tsonis  
Department of Biology, University of Dayton, OH  
“Molecular models for cataract research”  
April 20, 2005

Sue McDowell  
Biology Department, Ball State University, Muncie, IN  
“Calcium regulation by phosphoinositide 3-kinase (PI3K) in cardiac myocytes and endothelial cells”  
May 11, 2005

Colin R. Jefcoate  
Department of Pharmacology, University of Wisconsin Medical School, Madison, WI  
“Growth factors and TCDD synergistically regulate the differentiation of multipotential C3H10T1/2 cells—a genomic approach”  
May 18, 2005

Jo Anne Powell-Coffman  
Department of Zoology, Iowa State University, Ames, IA  
“Roles of AHR-1 and AHA-1, the C. elegans aryl hydrocarbon receptor complex, during neuronal development”  
May 25, 2005

Susan Hoffman  
Department of Zoology, Miami University, Oxford, OH  
“Evolution of the cytochrome P450 superfamily in mammals”  
June 1, 2005
A "jiffy" is an actual unit of time
1/100th of a second

1. The duration of one tick of the computer’s system clock. Often one AC cycle time (1/60 second in the US and Canada, 1/50 most other places), but more recently 1/100 sec has become common.

2. Confusingly, the term is sometimes also used for a 1-millisecond wall time interval. Even more confusingly, physicists semi-jokingly use “jiffy” to mean the time required for light to travel one foot in a vacuum, which turns out to be close to one *nanosecond*.

e.g. “The swapper runs every 6 jiffies”

CEG Members in the News

George Leikauf gave an invited lecture at World DNA Day in Dalian, China (Apr 2005) "Genes and the Environment: Acute lung injury". He also presented a lecture during the closing ceremony.

Grace LeMasters’s work was chosen as the Editors’ Choice by The Journal of Allergy and Clinical Immunology (2005;116:279-84) for findings from The Cincinnati Childhood Allergy and Air Pollution Study (CCAAPS) where she and co-workers showed that living within 100 meters of stop-and-go bus and truck traffic was associated with infant wheezing at seven months of age. Grace is the principle investigator of this NIEHS-funded study.

Dan Nebert was the 2005 Winner of the Society of Toxicology’s Distinguished Lifetime Toxicology Scholar Award, which was presented during Opening Ceremonies of the Annual SOT Meeting, New Orleans, Louisiana (Mar 05). The previous two winners of this annual award include Henry Pitot (Madison) and Gerald Wogan (Boston)—both members of the National Academy of Sciences.

An article in UC’s The New Record (Feb 05) applauded University researchers for finding a gene responsible for heavy metal toxicity. The discovery of the gene responsible for cadmium poisoning, according to Dan Nebert, is a significant breakthrough. Co-authors of the study include Tim Dalton, Lei He, Bin Wang, Marian Miller, Li Jin, Xiaoping Chang and C. Stuart Baxter, all of UC’s Department of Environmental Health, and Keith Stringer, of UC’s Department of Pathology and Laboratory Medicine.

GENERICA:
Features of the North American landscape that are exactly the same no matter where one is: fast food joints, strip malls, and subdivisions.