Keynote Lectures

From gene polymorphisms to biological system genetics – The example of atherosclerosis

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Atherosclerosis is a typical complex disorder responsible for major common diseases, especially myocardial infarction and stroke. Atherosclerosis has been explored in great details with regard to its environmental and genetic determinants. Guided by the vast knowledge accumulated on its pathophysiology, polymorphisms of more than 150 candidate have been explored in relation to atherosclerosis and its complications. The major finding may be that a surprisingly high fraction of polymorphisms affecting genes for which 'proximal' phenotypes exist, such as RNA or protein expression, quantity or function, are strongly associated with these phenotypes. Concurrently the results of a large number of studies also indicates that associations with more 'distal' clinically relevant phenotypes and disease end-points, are much weaker or absent, and often inconsistent across studies. The variable genes are component of complex biological systems (BS) or networks and because complexity increases with the number of intermediate steps and possible pathways in a network, the weaker functional consequences of genetic variability on distal than on proximal phenotypes is understandable.

BS genetics and current research strategies
The system's genetics perspective suggests that with regard to complex diseases a number of advocated research strategies may not be optimal: 1. Studies focused on single genes are largely irrelevant, 2. because sets of polymorphisms of different genes belonging to the same systems must be analyzed simultaneously, studies investigating a large number of genes are limited, unless genes are investigated in a coherent system-based way (the analysis of gene-gene interaction which is usually highly problematic is naturally incorporated in the BS approach), 3. For the same reason, whole genome approaches, either based on random SNPs or on haploblocks may miss most of the genetic variability that is relevant for complex traits, 4. Phenotypes are crucial and large sample size cannot be a substitute for inappropriate phenotypes. As a consequence, whether the currently ongoing or planned mega-studies will be of any help in understanding the genetics of complex traits is questionable, 5. Proposed phenomics strategies mainly relying on collection of large data sets followed by statistical modeling would have difficulties to incorporate the BS perspective.

A research strategy focused on BS
As an alternative to current approaches, a system's genetics research strategy follows the following steps: 1.delineate BS of interest for atherosclerosis, identify their core components and the corresponding genes, built a genotypic and phenotypic database that will be improved iteratively, 2. investigate the variability of all genes encoding the core components, and use statistics, bioinformatics and cell biology to select relevant polymorphisms, 3. Develop BS genotyping kits or chips, 4. Develop methods to quantify BS phenotypes, including systems' function, and develop high throughput assays, 5. Initiate highly focused clinical and epidemiological

studies to relate BS genotypes and phenotypes in different human populations, 6. Investigate BS within and across species to increase genotypic and phenotypic variability, an important aspect to make the modeling of the system more general and accurate, 7. Adapt and invent statistical and bioinformatic methods for modeling the genotype/phenotype space of whole BS and develop in silico models of BS incorporating the variability of their components.

Genomic instability and cancer

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Maintenance of genome stability is of vital importance for all living things and numerous DNA repair pathways that deal with different types of DNA damage have evolved. Several rare human chromosome breakage syndromes have long been known to cytogeneticists. However, only recently has it been possible to use these syndromes to define defects with respect to DNA damage response and chromosomal abnormalities in cancer cells.

Double strand breaks are a very serious form of DNA damage, which must be efficiently repaired for cell survival. DSBs are known to be caused by external agents such as ionizing radiation and chemicals but DSBs are also formed and sealed as part of normal cellular functions. DSBs can be repaired by virtually error-free homologous recombination repair and the more error-prone non-homologous-end-joining repair. Repair of DSBs is needed, at a low level, at all times. Exposure to radiation or free radicals, however, leads to a damage response, which involves increased repair activity. Many proteins involved in these pathways have been identified and several have been shown to be affected in human breakage syndromes and cancer.

The products of the ATM gene (mutated in Ataxia telangiecasia) and ATR play a key role in damage response activating a number of different pathways. Involved in these pathways are genes affected in several human syndromes. The breast cancer susceptibility genes BRCA1 and BRCA2 are also involved. All these syndromes are characterized by chromosomal abnormalities and increased cancer susceptibility. Telomere dysfunction has been shown to cause of chromosomal instability through breakage-fusion–bridge cycles in age-related epithelial carcinogenesis. Studies on genomic instability and telomere dysfunction in primary human epithelial cells will be discussed with respect to BRCA abnormalities.

DNA Adducts, genotypes and disease: Discovering causal pathways

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The application of biomarkers of exposure such as DNA adducts to molecular epidemiology studies has the capacity to increase our ability to identify causes of human cancer. Antibodies specifically recognizing particular adducts or classes of adducts can be used in highly sensitive assays such as ELISA or immunohistochemistry. We have used antibodies recognizing 4-aminobiphenyl (4-ABP)-, aflatoxin B1 (AFB1)- and polycyclic aromatic hydrocarbon (PAH)-DNA adducts to measure damage in blood and tissue samples. Genotyping for polymorphisms in carcinogen metabolism and DNA repair genes has also been carried out. In a population-based case-control study of breast cancer, PAH-DNA adducts were measured in blood mononuclear cells of over 1800 subjects. Risk was significantly elevated in those subjects with the highest quintile of adduct levels but there was no dose-response. Genotyping for SNPs in *XPD* codon 751 was also carried out. The presence of at least one variant allele was associated with increased risk and appeared limited to those with higher PAH_DNA adduct levels and current smokers. We have also carried out a DNA repair phenotyping assay using lymphoblastoid cell lines of sis-

ters discordant for breast cancer. When DNA repair capacity was quartiled based on control values, a dose response was observed with subjects with the poorest repair having a 3-fold increased risk.

In a nested case-control study hepatocellular cancer in Taiwan, albumin adducts, used as a surrogate for DNA adducts, were higher in cases and an interaction with hepatitis B virus was observed. Differences in risk were also seen in those with deletion polymorphisms in glutathione *S*-transferase M1 and T1. In addition, DNA adducts are being measured in liver tissue of cases and controls by immunohistochemistry. While results are limited by the small number of control subjects, a highly increased risk was found for those subjects with elevated levels of all three adducts measured (PAH, 4-ABP and AFB1) compared to those with low levels.

Genes and environment in cancer etiology

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The relative contribution of heritable and environmental causes to cancer etiology is an important scientific issue with many practical consequences. Cancer geneticists have emphasized the former while epidemiologists traditionally argued for the latter. The literature on heritable cancers is full of overstatements regarding their prevalence but recent data from twin and family studies may offer scientifically based estimates on the etiological apportioning of cancer causation. With the emergence of single nucleotide polymorphisms (SNPs) as versatile tools, studies on 'gene-environment interactions' have become popular, however with many embedded controversial issues. Mass publication is going on and results are reported without consideration of the functionality of SNPs or tissue of expression of the relevant genes in subgroups lacking any biological rationale. It is ironical that the historical roles of epidemiologists and geneticists appear to be completely changed: the proponents of gene-environment interactions appear to trust on the overwhelming importance of heritable factors when they act in concert with environmental factors while geneticists are raising concerns. There is a common failure to recognize that SNPs are inherited and that the related studies focus essentially on heritable effects. Analogously, nesting of an association study in familial cancers is beneficial for statistical power. The prerequisite for all gene identification and quantification studies is an assurance that the disease under study has a heritable component and, thus, it has to show familial clustering. However, many association studies take heritability for granted and fail to consider the magnitude of the familial component. An *a priori* consideration of the familial effect will be helpful in defining the study population, sample size, attractive candidate genes and limits of the expected results. For example, a disease with a small familial component is not likely to give any reasonable results in a case-control study of unselected cases; rather the sampling should be on familial cases expected to have an inherited susceptibility. As another example, a common polymorphism is unlikely to show a high risk, unless the disease has a major heritable component. These statements may seem self-evident, yet not so in much of the published literature: hardly any association study is nested in familial cases and large risks are reported for common polymorphisms in diseases showing only a moderate familial aggregation.

Cytogenetic predictors of cancer risk

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The presence of a causal relationship between chromosomal damage and cancer development was postulated since the beginning of the century,

but only recently prospective cohort studies have shown that the frequency of chromosome aberrations (CA) measured in PBL of healthy individuals is directly associated with cancer risk. The use of validated biomarkers of effect as surrogate endpoints of disease has been proposed as a possible alternative to studies based on traditional outcomes like incidence or mortality. This approach offers practical advantages in the design of population studies, including the potential to anticipate, by many years, the detection of risks for exposed populations. These advantages are counterbalanced by the fact that the association between the intermediate biomarker and the final outcome is not perfect, generating uncertainty of estimates.

In the last decade the results of three national cohort studies have shown increased risk of cancer in Nordic European countries, Italy and the Czech republic. A new independent cohort has been assembled in the framework of the CRB European project and the preliminary results of this study will be presented here. The new cohort is composed by data from 5 countries, i.e., Slovakia, Croatia, Poland, Hungary, and Lithuania, for an overall amount of 6408 subjects. Preliminary results will be presented also concerning the impact of genetic polymorphisms on the predictivity of CA of the risk of cancer, reporting the results of a case-control study nested within the ESCH cohort study. In this study, involving data from Italy, Norway, and Denmark, nearly 50 cancer cases with data on CA, Micronuclei frequency, and GSTM1/T1 will be compared with a group of controls. The implication for public health of data concerning cytogenetic endpoints validated as predictors of cancer will be discussed.

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Pooled analysis of biomarker studies

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There is a need to conduct large epidemiological studies including biological markers in order to answer questions such as the possible association between cancer and gene polymorphisms, or the presence of intermediate end points as predictors of future cancer development. Pooled analysis is a useful approach, when conclusions cannot be drawn from individual studies because of the small numbers of subjects included. With such approach, individual data from observational studies can be pooled in order to re-analyze the data. This approach gives several advantages over the classical meta-analysis of published data, mainly the possibility to perform interaction tests and sub-group analysis including dose-response curves. An ongoing-pooled analysis on metabolic gene polymorphisms and cancer is described here (GSEC study). The study started in 1997, and has collected data from over 54,000 subjects, half of which are cases, half controls. The distribution of the cases by cancer site shows that the most represented cancer is lung, followed by bladder, and breast cancer.

Several examples of analysis on rare subgroups are presented, such as lung cancer in non-smokers, metabolic gene polymorphisms and Bronco Alveolar Carcinoma of the lung, the role of genetic polymorphisms in cancer at younger ages.

Pooling data on genetic polymorphisms among control populations is necessary in order to estimate the appropriate allelic frequency in epidemiological studies The imprecise definition of allele frequencies in the population may reflect on the conduct of association studies, on assessment of the effects of multigenic mechanisms, and on the determination of genetic diversity.

Diet and cancer: Gene-nutrient interactions

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The relationship between dietary exposures and cancer outcomes has been the focus of an immense amount of research over the past few decades. There are numerous challenges facing analytical epidemiologic studies, including issues of confounding, precision of dietary instruments, and complex mixtures. Biomarkers for specific nutrients or broad groups of nutrients have helped to provide a more objective assessment in some cases, and recently there has been interest in the use of gene-nutrient interactions to shed light on the mechanisms and provide support for a biological basis to these relationships.

The gene-nutrient relationship is a bi-directional one: availability of specific compounds regulates gene expression, and conversely, much of the response of the body to these nutrients is genetically determined. Technological approaches to studying these interactions include the use of knockout mice, association studies using candidate-genes, and DNA microarrays.

Investigating associations between nutrient intake and polymorphisms occurring in putative genes in their metabolic pathways is a potential tool for clarifying the carcinogenic process. Examples in the literature include the study of genes, which contribute to endogenous antioxidant capacity, such as superoxide dismutase (MnSOD), and dietary antioxidants in breast cancer risk; as well as the interaction between folate status, and MTHFR gene mutations in determining DNA methylation. Using the 'Mendelian randomization' approach, it is possible to see if individuals stratified on the basis of their metabolic genotype exhibit differences in the effect of dietary components that mirror the effects that would be predicted by the metabolic pathways involved. The influence of glutathione S-transferase on the chemoprotective effect of isothiocyanates from cruciferous vegetables is an application of this approach.

Immunogenetics factors in chronic beryllium disease

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Exposure to beryllium in the workplace can cause beryllium sensitization and chronic beryllium disease. Sensitization to beryllium can be detected in the laboratory using a beryllium lymphocyte proliferation test. It was shown that anti-HLA antibodies could block the beryllium-specific response in the beryllium lymphocyte proliferation test, thereby implicating HLA-genes in chronic beryllium disease. A supratypic genetic marker, *HLA-DPB1[E69]*, has been shown to be strongly associated with immunologic sensitization to beryllium and chronic beryllium disease in beryllium workers. Among the 36 *HLA-DPB1* gene variants that code for E69, molecular epidemiological studies have suggested a risk hierarchy; where some variants appear to convey low to moderate risk (e.g., *HLA-DPB1*0201*, ~2-fold), some convey an intermediate risk (e.g., *HLA-DPB1*1901*, ~5-fold) and others convey high risk (e.g., *HLA-DPB1*1701*, >10-fold). Computational chemistry has been used to further investigate a potential mechanistic basis for these observations. A strong correlation has been found between the hierarchical order of risk of chronic beryllium disease associated with specific alleles and the predicted surface electrostatic potential of the corresponding isotypes. This approach has further been used to predict the binding affinities of different residues for positively charged beryllium ions in different HLA-DPB1 molecules. These findings suggest that preferential cation binding to specific HLA amino acid sequences in a putatively metal-free antigen-binding pocket might selectively alter the innate specificity of antigen recognition. In addition, it may be possible to use a computational chemistry approach to identify candidate susceptibility genes for further investigation of occupational diseases.

Exposure and oxidative stress caused by urban air pollution

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Exposure to urban air pollution has been associated with risk of lung cancer as well as aggravation and possibly development of cardiovascular and pulmonary diseases. However, little is known of the involved mechanisms and specific relevant exposure characteristics. Experimental studies indicate that inflammation, oxidative stress and DNA damage are important for the effect of air pollution particles and benzene. Animal exposure studies also indicate enhanced expression of oxidative stress response and DNA repair genes, including OGG1, as defense mechanism, which should be considered.

By means of personal monitoring and biomarkers we have attempted to characterize individual exposure, explore mechanisms and identify significant sources to air pollution particles size fractions and benzene with respect to relevant biological effects. Biomarkers included 8-oxodG (8-oxodeoxyguanosine from oxidized guanine in DNA or the nucleotide pool) in urine, strand breaks, base oxidation, 8-oxodG and bulky adducts in lymphocyte DNA, markers of oxidative stress in plasma, acute phase reactants, phenylmercapturate and trans-transmuconic acid for benzene exposure as well as genotypes of GSTs and NQO1.

Only biomarkers of oxidative stress and damage showed significant positive correlation with the individual exposure. 8-oxodG in lymphocyte DNA was correlated with exposure to $PM_{2.5}$, ultrafine particles outdoor and indoor as well as with benzene in different setting. NQO1 was a significant effect modifier of the relationship between benzene exposure and 8-oxodG level in lymphocytes. Markers of oxidative damage to lipids and protein in plasma correlated with individual $PM_{2.5}$ exposure. Seasonal variation included increased levels of bulky adducts, DNA strand breaks and 8-oxodG in lymphocytes increased significantly in the summer period

Oxidative stress may be an important mechanism of action of urban air pollution. Related biomarkers and personal monitoring may be useful tools for risk characterization and should be applied in risk groups.

Carcinogenicity of diesel engine exhaust

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Diesel engine exhaust is a major component of outdoor air pollution, and is likely to contribute substantially to its health effects.

When all risk estimates available from epidemiological studies are combined, the summary relative risk of lung cancer is 1.33 (95% confidence interval 1.24, 1.44). These results are reasonably consistent within and between groups of studies; neither selection bias nor publication bias are likely to explain completely these results, and the available evidence suggests that tobacco smoking does not act as a confounder; although the potential confounding effect of other risk factors, such as diet and socio-economic status, has not been adequately addressed.

The results of epidemiological studies of bladder cancer suggest a positive association (summary relative risk 1.29, 95% confidence interval 1.12, 1.49). However, the overall results depend on the inclusion of studies of truck drivers, there is no clear evidence of a dose-response relationship and the results of cohort studies do not suggest an increased risk. Although an increased hazard of bladder cancer among workers exposed to diesel emissions is plausible, the available evidence does not support a causal interpretation. Results on other cancers are limited and no conclusions can be drawn: an increased risk of kidney cancer has been reported in two studies, but

the overall evidence is negative; positive results have been reported in single studies for cancers of the colon, liver, pancreas and prostate, and for malignant melanoma.

Future research should focus on the development and application of biomarkers of diesel exhaust exposure and of early biological effects.

Wood dust exposure and health effects: The WOOD-RISK project

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Exposure to wood dust is associated with impaired lung function and symptoms in both upper and lower respiratory tract. These effects have been observed in association with exposure to wood dust from a wide variety of tree species, and in various occupational settings. In addition, it has long been known that risk for sino-nasal cancer is elevated among woodworkers. The relative risks are highest, in some studies extremely high, in association to hardwood dust (oak and beech in particular) and for adenocarcinoma histology. The high risks have been observed among workers in furniture and cabinet manufacturing, but clearly elevated risks also exist in other wood industries. Excess risks reported for squamous cell histology are smaller, and may also be associated with exposure to softwood dust.

Large estimated numbers of exposed workers, around 65 000 in Finland and 2.5 million in the European Union, together with the multiple health effects make occupational exposure to wood dust an important target for research. Estimates of exposure, as well as knowledge about etiology and biological disease mechanisms are all basis for risk assessment and prevention. We are currently running a EU-funded project on wood dust called WOOD-RISK, to assess prevalence, levels, and types of occupational exposure, as well as biological effect mechanisms. The presentation gives an overview of the on-going research on human sino-nasal cancer, as well as *in vitro* and *in vivo* studies on wood dust-induced pulmonary inflammation.

Quantitative SNP genotyping in the microarray format

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We have developed a microarray genotyping system for multiplex analysis of single nucleotide polymorphisms (SNPs) by DNA polymerase-assisted "minisequencing" single nucleotide primer extension with four-color fluorescence detection. The system is based on an "array of arrays" conformation to facilitate analysis of up to 200 SNPs in eighty individual samples per standard microscope slide. Owing to the high sequence specificity of the minisequencing primer extension reaction, the system allows quantitative analysis of SNP alleles in DNA and RNA. We have used it for determination of SNP allele frequencies in pooled DNA samples, for following up the success of allogenic stem cell transplantation in patients with haematological disorders, for evaluation of whole genome amplification procedures, and for detecting imbalanced expression of SNP alleles in RNA. The performance of the minisequencing system in quantitative analysis of SNPs will be described.

Human sequence variation and disease – The HapMap project

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With a finished reference sequence of the human genome in hand, we have the ability to establish a comprehensive list of sequence variants and study patterns of variation at a fine-scale across the genome. The outcome of this research will be key in our quest to characterise the genetic basis of common disease, susceptibility to pathogens, and variable response to external stimuli.

The International HapMap project, launched in October of 2002, aims to generate a haplotype map of the human genome, a tool that will facilitate the study of association to disease risks and drug responses. The project's study-design includes four population samples namely 30 trios from CEPH / Utah families (North European descent), 30 Yoruba (Nigeria) trios, 45 unrelated Japanese and 45 unrelated Chan Chinese. The consortium adopted a hierarchical mapping strategy and in the first phase of the project, a map of evenly spaced SNPs (1 per 5 kb) with minor allele frequency ≥0.05 is being generated. Assessment of local LD patterns in each population will identify both regions of strong uninterrupted LD and regions that require data from additional SNPs. The latter will be the focus of the second phase, which will generate data on circa 2.25 million additional SNPs. Work is in progress to develop optimal statistical approaches to both describe accurately the highly variable nature of LD and provide precise parameters to assess completion of the project. Data are being released regularly into the public domain via the consortium's Data Coordination Center (DCC) (http://www.hapmap.org). The May release figures data on 453,512 SNPs (CEPH panel), which fulfil map criteria.

Over the past year the HapMap project engaged in the discovery of new SNPs. Shotgun sequence data was generated across the whole genome using libraries made from DNA samples of a range of individuals. The May release of dbSNP (build 121) will have 7.9 M uniquely mapped SNPs. The accumulation of sequence data allowed to devise a filter for selecting SNPs that are likely to be common; circa 3.7M of the SNPs. Empirical data confirm that these 'double-hit' SNPs convert to working assays at a much higher rate than random SNPs. Sanger's contribution to the HapMap project includes chromosomes 1, 6, 10, 13 and 20 which account for 24% of the genome. Genotyping is carried out with the 'Golden Gate' assay (Illumina), which allows typing 1,536 SNPs in a single reaction.

In parallel, we have undertaken an in depth analysis of chromosome 20. Over 60,000 SNP assays (1 SNP per kb on average) were designed and genotyped across CEPH families as well as unrelated Caucasian, African-American, and Asian samples. We obtained circa 30,000 SNPs with m.a.f ≥ 0.04. Analysis is being carried out in collaboration with groups at the Wellcome Trust for Human Genetics (Lon Cardon), University of Southampton (Newton Morton) and University of Oxford (Peter Donnely) to assess common patterns of linkage disequilibrium, recombination and selection as well as define optimal sets of tag SNPs.

Large-scale proteomics approaches to the study of cancer

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We have finished constructing a high-throughput 2D gel electrophoresis laboratory to enable large-scale studies of disease to be carried out. In a preliminary study we have analysed over 180 images of protein expression profiles in ovarian tumours and are currently working on a 250 tumour set from patients with hereditary breast cancer and 400 tumours from sporadic patients. We will described the methodology and results and how we are using them to develop antibody based tests for blood samples for diagnosis and prognosis. We are also developing non-gel based methods

for analysing human sera for marker definition and identification. We will present preliminary results from sera from patients with malignant and benign tumours.

What does biomonitoring really tell us?

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In January, 2003, the U.S. Centers for Disease Control released the 2nd National Report on Human Exposure to Environmental Chemicals, a "report card" of biomonitoring information for 116 synthetic chemicals and their metabolites, in addition to the 27 chemicals reported on in 2001, in the blood and urine of a population- based, statistical sample of the civilian US population. The initiative by CDC will greatly enhance the information on chemicals present in blood and/or urine of the U.S. population. However, considerable research will be needed to actual interpret such biomonitoring data to address public concerns and questions such as: What do "snapshot in time" measurements tell us? What does it mean if chemicals are found in blood and not urine? What does it mean for health consequences? Clearly, measurements of chemicals in biological samples tell us some exposure has occurred, but we must have information on the kinetic behavior of these chemicals to better understand what the exposure(s) means. For a persistent chemical, the measurement may reflect integrated exposure over time, but with only one measurement, there is no way to tell whether this represents a peak, an average of ongoing exposure, or a decline. For rapidly eliminated chemicals, measurement implies ongoing exposure, but says little about history, or peak concentration. If there are multiple metabolites which are to the ones of concern that should be followed? For each chemical measured, knowledge of the appropriate dosemetric is critical to relating the measurement to a potential health outcome. Information on windows of susceptibility is also key to relating the measured level to potential effects. The potential that biomonitoring data presents will be most useful when accompanied by pharmacokinetics, dosemetrics, and effects data. (This abstract does not reflect EPA policy.)

Nickel Carcinogenesis: Epigenetics and Hypoxia Signalling

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Exposure of cells to both water soluble and Insoluble Nickel compounds is known to activate Hif-1 alpha (HIF) transcription factor. The mechanism of this effect involves the depletion of intracellular Fe which causes a general inhibition of Fe dependent enzymes such as Aconitase and Proline hydroxylase. Inhibition of Hif-dependent Proline Hydroxylase is known to stabilize HIF protein and we have previously shown that water soluble Ni can activate HIF dependent transcription by a PI3K/AKT dependent pathway. Additionally Carcinogenic Ni compounds are also known to silence genes by inducing de novo DNA methylation. We have previously shown that water soluble Ni salts decrease the Acetylation of Histone H4 in yeast and that a loss of histone acetylation and the methylation of Lysine 9 in Histone H3 is associated with Ni silenced genes. Since the antibodies to aceylated and methylated histones have improved significantly since we did our yeast studies 5 years ago we now can detect substantial global inhibition of Histone acetylation of H2A,H2B,H3 and H4 in addition to methylation of Lysine 9 of H3 induced by water soluble Ni compounds. Hypoxia also increases the Methylation of Lysine 9 of Histone H3 and results in a loss of lysine 9 acetylation. Additionally Histone 2A and 2B are increased in their ubiquitination by soluble Ni,

however the consequence of Histone ubiquitination is not know. These changes in Histone modifactions are early events involved in the Ni induced silencing of genes and may be useful early markers of Ni exposure and effect. (This work was supported by grant numbers ES00260,ES10344, and T32ES07324 from NIH/NIEHS and CA16087 from the National Cancer Institute.)

Biomarkers in clinical practice: Example from stomach cancer

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Direct diagnosis of gastric cancer does not succeed so far by available single serological biomarkers and tests. However, certain gastric diseases or conditions, *H.pylori* gastritis and atrophic gastritis in particular, precede gastric cancer in 50–80% of the cases. Strategies intended to find the subjects with these preceding conditions with serological tests followed by diagnostic endoscopy has provided promising results in early diagnosis and screening of the gastric malignancy. Subjects with premalignant conditions have an. 2-90-fold risk of gastric cancer compared to subjects with normal, healthy stomach (normal gastric mucosa: no inflammation, no atrophy, no *H. pylori*). The risk increases with increasing grade and extent of atrophic gastritis in the stomach, and is highest in those with severe atrophy in entire stomach. Assays of the levels of serum pepsinogen I (S-PGI) and gastrin-17 as well as *H.pylori* –antibodies provide a possibility of high sensitivity and specificity to diagnose the subject has gastritis, and to establish whether the gastritis is atrophic or not, and in which part of the stomach the atrophic changes are located and how extensive they are. Thus, the tests enable to identify the subjects whose risk for cancer is highest. In two Finnish cross-sectional population-based studies on "asymptomatic" subjects, gastric cancer or its early stage was found in 4–6% of the 50-65 year-old men who showed a moderate or severe atrophy of the corpus mucosa in the S-PGI test. The atrophy was diagnosed by screening more than 20.000 men and the gastroscopy was performed to 1,344 men who had a low serum level of PGI (S-PGI < 25 μg/l). Of these, 80% had *H.pylori* antibodies, and 63 out of 1344 men had gastric cancer or cancer preceding lesion (dysplasia, intramucosal neoplasia). The study demonstrated further that approximately. 70% of the identified cancers in the screening program were at early stage ("early cancers"), all of which patients could be curatively healed by surgery. As compared to findings in endoscopy and histology, the sensitivity and specificity of the low PG I level (< 25 μg/l) were 78% (95% confidence interval 75–80%) and 98% (95–100%) for advanced (moderate or severe) atrophic gastritis.

Approximately 5–10% of Finnish males at age over 50 have an advanced (moderate or severe) atrophic gastritis of the corpus which may lead, in addition to gastric cancer, to low output of intrinsic factor and consequently to malabsorption of vitamin B12. Of patients with advanced atrophic corpus gastritis, 30% have an exceptionally low (< 170 pmol/l) levels of vitamin B12 in serum, and 50% have the vitamin levels <220 pmol/l that associates with increased serum levels of homocysteine. On the basis of the available prevalence rates of atrophic gastritis in the populations and of vitamin B12 deficiency in subjects with corpus atrophy, it can be estimated that there are thousands of people in Finland (total population approximately 5 millions) who have or who are at high risk for the deficiency of vitamin B12 caused by the atrophic gastritis, in the majority of whom (80% at least) the atrophic gastritis is initiated by *H.pylori* infection, and who still have an undiagnosed ongoing *H.pylori* infection.

Cross-sectional biomarker studies in human: Urinary biomarkers of PAH exposure and oxidative stress

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Cross-sectional biomarker studies provide valuable insights of delineating the multi-step association between exposure and disease occurrence. Although the cross-sectional studies have several advantages (e.g., easy to conduct, incorporate multiple biomarkers, etc.), they also have a few limitations (e.g., selective survival, hard to understand the temporal precedence, etc.). This paper will discuss the pros and cons of cross-sectional biomarker study in environmental health research by introducing the multiple biomarkers study of urinary polycyclic aromatic hydrocarbons (PAHs) exposure and biomarkers related to oxidative stress.

We have conducted a numbers of studies investigating the use of urinary biomarkers of PAHs exposure (e.g., 1-hydroxypyrene glucuronide (1-OHPG), 1-hydroxypyrene (1-OHP), 2-naphthol) and biomarkers related to oxidative stress (e.g. urinary 8-hydroxy-deoxyguanosine (8-OHdG), malondialdehyde (MDA) and $1,N^6$-ethenodeoxyadenosine (εdA) for individuals with occupational PAH exposure (e.g., coke oven workers, painters in shipbuilding yard, workers incinerating industry wastes, etc.) and various environmental exposure (e.g., outdoor particulate, environmental tobacco smoke, the Asian Dust Events, individuals participating the fasting program, etc.)

Levels of urinary PAH metabolites, as internal dose markers of PAHs exposure were measured by synchronous fluorescence spectroscopy and HPLC with fluorescence detection. Urinary 8-OHdG were measured by ELISA, and MDA and εdA levels, as oxidative stress biomarkers were measured by immuno-affinity purification followed by HPLC-fluorescence detection.

Surveillance, Medical Screening, and Intervention

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Much of molecular epidemiologic research has focused on validating biomarkers, that is, assessing their ability to indicate accurately, exposure, effect, disease, or susceptibility. To be of use in surveillance, medical screening, or interventions, biomarkers must already be validated so that they can be used as outcomes or indicators that can serve a particular function. In surveillance, biomarkers can be used as indicators of hazard, exposure, disease, and population risk. However, to obtain rates for these measures, the population at risk will need to be assessed. In medical screening, biomarkers can serve as early indicators of disease in asymptomatic people. This allows for the identification of those who should receive diagnostic confirmation and early treatment. In intervention (which includes risk communication, risk management, and various prevention efforts), biomarkers can be used to assess the effectiveness of a prevention or control strategy as well as help determine whether the appropriate individuals are assigned to the correct intervention category. Biomarkers can be used to provide group and individual risk assessments that can be the basis for marshalling resources. Critical for using biomarkers in surveillance, medical screening, and intervention is the justification that the biomarkers can provide information not otherwise accessible by a less expensive and easier-to-obtain source of information, such as medical records, surveys, or vital statistics. The ability to use validated biomarkers in surveillance, medical screening and intervention will depend on the extent to which a strategy for evidenced–based

procedures for biomarker knowledge transfer can be developed and implemented. This will require the interaction of researchers and decision-makers to collaborate on public health and medical issues.