

## Roche Diagnostics Award Lecture

### Molecular profiling of tumours

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Molecular diagnostics is a rapidly advancing field in which insights into disease mechanisms are being elucidated using new gene-based biomarkers. Until recently, diagnostic and prognostic assessment of diseased tissues and tumours relied heavily on indirect indicators that only permitted general classifications into broad histological or morphologic subtypes and did not take into account the alterations at the level of the individual gene. Global analysis of gene expression and DNA copy number changes using microarrays, now allows for the simultaneous interrogation of the expression and patterns of genomic imbalance of thousands of genes in a high-throughput fashion, and offers unprecedented opportunities to obtain molecular signatures of the state of activity of diseased cells and patient samples. It has been shown over the last twenty years that recurrent chromosomal rearrangements are strongly associated with amplification of oncogenes, acquisition of drug resistance and loss of tumour suppressor gene function in tumours.

In this presentation it will be shown that by performing microarray profiling in combination with advanced molecular cytogenetic analysis it is possible to characterize tumours at extremely high resolution, and to obtain molecular signatures associated with clinically relevant findings. As an example of this approach we are applying whole genome analytical methods at the DNA and RNA levels in serous epithelial ovarian cancer (SEOC) in the context of clinical parameters such as tumour stage, CA125 levels, treatment response and survival. In the first phase of this study high-resolution microarray comparative genomic hybridization (CGH) was used to identify genomic regions associated with chemotherapy resistance in epithelial ovarian cancer. The analysis has confirmed the presence of copy number alterations at specific chromosomal regions in 26 tumours. Gains at 1q, 3q, 8q, 12p and 20q as well as losses at 1p, 4q, 6q, 8p, 9q, 13q, 16q, 17p, 18q, were consistent with previously reported recurrent genomic imbalance detected by metaphase CGH. A systematic bioinformatics analysis using the high resolution CGH array datasets has further allowed us to identify small sub-genomic regions that discriminate drug resistance as determined by the kinetics of serum CA125 levels following chemotherapy. These regions include recurrent losses at 1p36.33 (2.2Mb) and 6q24.3-25.2 (18.4Mb) with gains being

detected at 1q42-44 (25.6Mb) and 13q12.2-13.1 (6.3Mb). Expression profiles were obtained in a companion parallel study and findings concerning novel pathways previously implicated in cis-platinum adduct repair will be reported.

Microarray analysis will provide invaluable information on the disease pathology, progression, resistance to treatment and response to cellular microenvironments, and ultimately result in improved early diagnosis and innovative therapeutic approaches for cancer and other types of human disease.

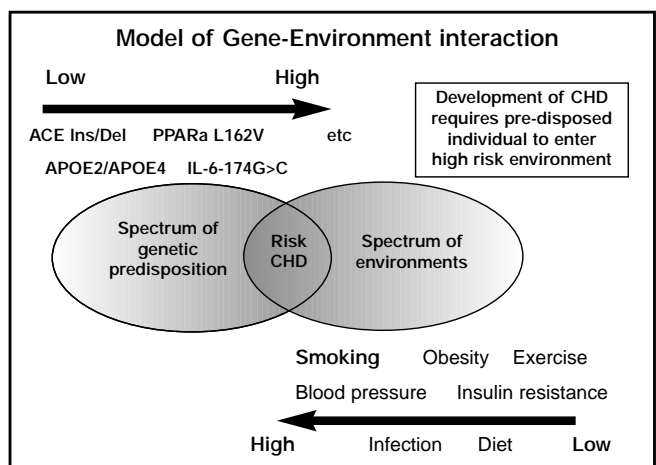
## Flynn Lecture

### Environmental modification of genotype effects: insights into atherosclerosis pathology

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Both genetic and environmental factors influence coronary heart disease (CHD), thus studies of CHD risk are often confounded by interactive and additive effects of genes and by modifications of genetic effects by environmental factors. Gene:environment interaction implies that, in combination, the impact of the genotype and the environmental factor under study is more than the additive effects of each factor alone. From a mechanistic viewpoint, interaction suggests that at the molecular level the effect or by-product of the environmental insult modifies the molecular function of the product of the gene under observation. Key environmental factors of importance at this time are obesity (which contributes to) diabetes, hypertension, dietary factors (e.g. saturated fat intake, folate) exercise and smoking. Examples of gene interactions with all these have been reported but examples of smoking and exercise will be discussed.



### Cross-sectional associations: APOE and smoking

Of the candidate genes involved in the determination of lipid levels and CHD risk, apoE is probably the most comprehensively studied. Variation in the APOE gene, coding for the three common isoforms E2, E3, E4, is known to have a strong and consistent influence on plasma lipid levels and on risk of CHD. The interaction of apoE and smoking on CHD risk was examined in the UK-based prospective Northwick Park Heart Study.<sup>1</sup> Subjects were middle-aged men, free of CAD at baseline. APOE genotype was associated with the expected effects on plasma cholesterol and apoB levels (both  $p < 0.0001$ ). During 18,836 person years of surveillance of 2258 men, 136 CHD events had occurred. The effect of smoking alone on CHD risk was 1.94 (95%CI 1.25-3.01) in agreement with previous studies which find smoking to double CHD risk. Compared to all genotype never smokers, where the hazard ratio was set at 1.00, in men who smoked, those with the genotype e3/e3 had a hazard ratio of 1.68 (95%CI 1.01-2.83) compared to 1.18 (95%CI 0.46-3.03) for e2 carriers and 3.17 (95%CI 1.82-5.51) in e4 carriers. The interaction between smoking status and APOE genotype on CHD risk was significant ( $p = 0.007$ ). Interestingly, in the non-smokers carrying the E4 allele risk was 0.84 (0.40-1.75), supporting the benefit in CAD risk reduction of smoking cessation.

In these men the increased risk associated with smoking and carrying an e4 allele was independent of BMI, blood pressure, lipid levels and markers of inflammation. Thus although the mechanisms for these cholesterol-independent effects on arterial wall thickening are unclear, it appears that APOE genotype influences CAD independently of its effects on fasting plasma lipid levels. The interaction of e4 and smoking suggests that smoking exacerbates this. The most likely mechanism to explain the e4:smoking interaction on CHD risk appears to be through a direct effect on LDL oxidation. Several studies using recombinant apoE have demonstrated that the protection against oxidation *in vitro* is E2 > E3 > E4, due probably to the fact that E2 has two free SH-groups, E3 has one and E4 none. The differential oxidation of apoE isoforms has now been confirmed *in vitro* with, as expected, E4 being more susceptible than E3 which in turn is more susceptible than E2, to oxidation.<sup>2</sup>

### Stressing the genotype analyses: the ACE gene and exercise

The human angiotensin converting enzyme (ACE) gene contains a length polymorphism consisting of the presence (insertion, I) or absence (deletion, D) of a 287 base pair "ALU" repeat sequence in intron 16, with the D allele being associated with higher ACE levels than the I allele in plasma and in tissues.<sup>3</sup> We have carried out several studies to examine the relationship between this polymorphism and cardiovascular health, and have

examined the hypothesis that if renin-angiotensin systems regulate LV growth, individuals of DD genotype might show a greater hypertrophic response than those of II genotype.<sup>4,5</sup> The basic strategy is to genotype a large number of subjects, for example a group of healthy men from a population-based sample, and then recruit for "stress" investigations only those subjects homozygous for the rare allele matched (for age or other relevant confounders) with an equal number of common allele homozygotes. As many of these "stress" tests used are time-consuming for volunteers and investigator, as well as requiring costly assays for the measures, obtaining these measures on a whole population-based sample of several hundred subjects (in order to include the necessary number of rare homozygotes for adequate power) may be prohibitively expensive.

The strategy used involved screening over 1200 male military recruits to select only subjects homozygous for the I or D allele for accurate LV mass determination by Magnetic Resonance Imaging (MRI). LV dimensions and mass were compared at the start and end of a 10-week physical training period. LV mass increased with training by 8.4 g overall ( $p < 0.0001$ ), but with DD men showing roughly 3-fold greater growth than II men. ( $p < 0.001$ ). When indexed to lean body mass, LV growth in II subjects was essentially negligible whilst remaining in DD subjects (-0.022 vs. +0.131 g/kg respectively;  $p = 0.0009$ ). Although the precise molecular mechanism of this effect remains to be elucidated it clearly demonstrates the importance of the ACE-renin-angiotensin system in determining LV dimensions in situations of high cardiac demand, which may also be important in pathology such as hypertension and heart failure.<sup>6</sup> The use of these "stress-the-genotype" approaches to explore gene-environment interactions are likely to be the key to understanding the causes determining both CAD and other multi-factorial disorders.

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### References

- 1 Humphries S E, Talmud P J, Hawe E, Bolla M, Day I N, Miller G J: Apolipoprotein E4 and coronary heart disease in middle-aged men who smoke: a prospective study. *Lancet* 2001; **358**: 115-119
- 2 Jolivald C, Leininger-Muller B, Bertrand P, Herber R, Christen Y, Siest G: Differential oxidation of apolipoprotein E isoforms and interaction with phospholipids. *Free Radic Biol Med* 2000; **28**: 129-140
- 3 Rigat B, Hubert C, Alhenc-Gelas F *et al.* An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Invest* 1990; **86**(4): 1343-6
- 4 Montgomery H, Clarkson P, Dollery C M *et al.* Association of angiotensin-converting enzyme gene I/D polymorphism with change in left ventricular mass in response to physical training. *Circulation* 1997; **96**(3): 741-747
- 5 Myerson S G, Montgomery H E, Whittingham M *et al.* Left ventricular hypertrophy with exercise and the angiotensin converting enzyme gene I/D polymorphism: a randomised controlled trial with losartan. *Circulation* 2001; **103**: 226-230
- 6 Dzau V J. Circulating vs local renin-angiotensin system in cardiovascular homeostasis. *Circulation* 1988; **77**(Suppl 1): 4-13

## AACC/ACB Transatlantic Lecture

### Natriuretic peptides

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Abstract not supplied

## Professors Prize Lecture

### Sugar and spice and all things nice

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Having so far had a career where my research interests have been less theme based and more what seemed interesting to pursue, it has resulted in an enjoyable but somewhat eclectic mix of study. However, rather than this little boy's research being made of frogs and snails and puppy-dogs' tails, it has been more akin to sugar and spice and all that's nice.

#### Sugar

It is expected that the prevalence of diabetes will continue to rise in most societies. Studies in the last decade have confirmed the importance of maintaining good glycaemic control so as to help prevent the complications associated with the disease, and the use of markers such as glycated haemoglobin have become the cornerstone of treatment management. The same decade has seen a gradual reduction in the methodological issues surrounding HbA1c but has highlighted the fundamental problems associated with the test. The challenge for the next decade does not now seem to be one of gaining clinician acceptance of the test, but rather ensuring they do not become over reliant on it.

Methodological issues also continue to surround other measures of blood glucose assessment, such as blood glucose monitoring. As near patient testing becomes more widespread, these issues may become increasingly important.

#### Spice

Cinnamon

The main reason for the epidemic of diabetes is due to rising insulin resistance in the population secondary to increasing obesity. The spice cinnamon seems to reduce insulin resistance, and other alternatives to traditional pharmacological intervention, such as the use of phytoestrogens, are being sought in an attempt to reduce the future burden of type 2 diabetes and polycystic ovarian syndrome. However, the precise mechanisms by

which rising insulin resistance develops are still poorly understood.

Accurately assessing insulin resistance routinely as part of the metabolic syndrome has also proved difficult, and its marked variability within the same individual raises further questions as to its aetiology.

#### Sage

The herb sage was used in the Middle Ages as a treatment for thyroid disease. Now, as then, thyroid dysfunction can present with numerous non-specific symptoms. It now appears that thyroid hormones can affect the blood concentrations of many small proteins and so may indirectly cause some of the features of thyroid disease, as well as cause problems in measuring these proteins in patients not already known to have a thyroid problem.

#### All things NICE

Improving accessibility to a wider breadth of evidence-based laboratory testing, reducing turnaround times for samples and providing improved IT links for results is generally regarded as a benefit to both clinicians and patients. However, we are also now in a demand based, target based, guideline based and litigious healthcare culture where the threshold for sample testing seems lower than ever before, the range of staff requesting tests is widening, and the results returned by laboratories is to clinicians with less time to ponder their significance or to keep pace with new test developments.

Although most research continues to aim for cutting edge advancement, there may also be a need to ensure that as much study is devoted to ensuring the most appropriate use of our existing services. In response, a whole new field of research is developing which is designed to ensure the best way of communicating new academic findings into routine practice.

## SAS Lecture and Thermo Electron Award formerly the Konelab Lecture

### New biochemical markers of bone turnover in osteoporosis

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Increasingly specific biochemical markers for bone remodelling have been identified in recent years. At present, the most sensitive markers for bone formation are serum osteocalcin (OC), bone alkaline phosphatase (bone ALP), and procollagen type I N-terminal propeptide (PINP). Immunological assays for deoxypyridinoline (DPD) in urine and for C-terminal and N-terminal type I collagen peptides (CTX and NTX, respectively) in serum

or urine are currently the most sensitive resorption markers.<sup>1</sup>

In osteoporosis, the main cause for concern is the increase in the risk of fractures. An important issue is whether combined use of bone markers and bone mineral density (BMD) measurements improves the accuracy of fracture risk evaluation. Several prospective studies have shown that an increased bone resorption evaluated by urinary free DPD, urinary or serum CTX was associated with increased risk of the hip, spine and non-vertebral fractures independently of BMD.<sup>2</sup> The use of bone markers in individual patients may be appropriate in some situations, especially in women who are not detected at risk by BMD measurements. In the OFELY study including 668 postmenopausal women followed prospectively over 9 years, we found that among the 115 incident fractures, 54 (47%) actually occurred in non-osteoporotic women. Among these women, the combination of bone markers and history of previous fracture was highly predictive of fracture and allowed the detection of 59% of women with incident fracture. In the FIT trial, we found that higher baseline levels of bone turnover was associated with a greater efficacy of alendronate to reduce fracture risk in non-osteoporotic women, suggesting that identification of women with no osteoporosis but high bone turnover would be relevant for treatment decision with antiresorptive therapy. Thus, bone markers may be used in the assessment of fracture risk in selected cases in which BMD and clinical risk factors are not enough to take a treatment decision.

In patients given bone resorption inhibitors, such as oestrogens, selective oestrogen response modifiers, or bisphosphonates, the changes in BMD are small as compared to the long-term reproducibility of this parameter. In addition, it has been shown that BMD changes account for a small part of the efficacy of treatment on fracture risk.<sup>3</sup> It was found that the short term changes (within 3 to 6 months) of OC and bone ALP with raloxifene treatment were associated with the subsequent risk of vertebral fractures in a large subgroup of osteoporotic women enrolled in the MORE study, while changes in hip BMD were not predictive.<sup>4</sup> In the VERT studies with risedronate it has been shown that changes of urinary CTX and NTX after 3 to 6 months predicted the risk of subsequent incident vertebral fractures after both 1 and 3 years, these changes explaining 50 to 70% of the effect of risedronate on fracture risk.<sup>5</sup> A significant association between changes of bone ALP and vertebral, hip and non spine fracture was also found in women treated with alendronate participating in the FIT trial. Recent studies have shown that anabolic

treatments including intermittent PTH, produce a marked and rapid (within a month) increase of markers of bone formation followed by a delayed increase in bone resorption markers.<sup>6</sup> In this situation, bone formation markers, especially serum PINP, appear the most promising to monitor efficacy of PTH, although this will need to be confirmed in studies using incident fracture as an endpoint.

Advances in our knowledge of bone matrix biochemistry, most notably of post-translational modifications in type I collagen, may allow identification of biochemical markers that reflect changes in the material property of bone, which is an important determinant of bone strength. In postmenopausal women of the OFELY study, we found that changes of the degree of isomerization of type I collagen as reflected by the urinary ratio of native (a) to isomerised (b) CTX was associated with fracture risk independently of BMD and of bone turnover.<sup>7</sup> *In vitro* experiments have also demonstrated that changes in the extent of post-translational modifications of type I collagen (e.g. intermolecular crosslinking such as pyridinoline and pentosidine and CTX isomerization) play a role in determining the mechanical competence of cortical bone, independently of BMD. Further studies in osteoporosis should explore the changes in these biochemical parameters of bone matrix in response to treatment, as they may represent a key component of bone quality.

#### References

- 1 Garnero P, Delmas P D Investigation of Bone: Bone Turnover in Rheumatology, 3rd edition, Hochberg M C, Silman A J, Smolen J S, Weinblatt M E, Weisman M H (eds), Harcourt Health Sciences Ltd (London, UK) volume 2, 2043-2057
- 2 Garnero P. Markers of Bone Turnover for the Prediction of Fracture Risk. 2000. *Osteoporos Int*, 11, suppl. 6: S55-S65
- 3 Cummings S R, Karpf D B, Harris F, Genant H K, Ensrud K, La Croix A Z, Black D M. Improvement in spine density and reduction in risk of vertebral fractures during treatment with antiresorptive drugs. *Am J Med* 2002; 112: 281-289
- 4 Bjarnason N H, Christiansen C, Sarkar S, Mitlak B, Knickerbocker R, Delmas P, Cummings S, for the MORE Study Group. 6 months changes in biochemical markers predict 3-year response in vertebral fracture rate in postmenopausal, osteoporotic women : results from the MORE study. *Osteoporos Int* 2001; 12: 922-930
- 5 Eastell R, Barton I, Hannon R A, Chines A, Garnero P, Delmas P D. Relationship of Early Changes in Bone Resorption to the Reduction in Fracture Risk with Risedronate. *J Bone Miner Res* 18(6): 1051-6
- 6 Black D M, Greenspan S L, Ensrud K E, Palermo L, McGowan J A, Lang T F, Garnero P, Bouxsein M L, Bilezikian J P, Rosen C J. The Effects of Parathyroid Hormone and Alendronate Alone or in Combination in Postmenopausal Osteoporosis. *New Engl J Med* 349(13): 1207-1215
- 7 Garnero P, Cloos P, Sornay-Rendu E, Qvist P, Delmas P D. Type I Collagen Racemization and Isomerization and the Risk of Fracture in Postmenopausal Women: The Ofely Study. *J Bone Miner Res* 2002; 17(5): 826-833

## ACB Foundation Award Lecture

### Androgens: a little of what you fancy does you good!?

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A large number of people regularly take a whole variety of substances in the hope of improving their health, lengthening their life, reducing the ravages of ageing or improving their performance either on the beach, in the bed or at other competitive sports. Often there is little or no scientific evidence that these products actually provide the anticipated benefits and probably many, if not most, have a very powerful placebo effect. Similarly athletes take supplements and androgens to enhance performance. Mark McGwire attributed his success in 1998 at setting a new record of scoring the most home runs in a season to taking androstenedione, and there have been several high profile cases of athletes who have tested positive for androgens. In the case of androgens there is now evidence that they do significantly increase muscle size and improve performance. In addition, many studies have shown that intense exercise results in lowered testosterone concentrations. Therefore perhaps athletes do require androgens to 'top-up' endogenous testosterone levels. The question is at what cost? There are reports that anabolic steroids may lead to liver disease, hepatomas and cardiomyopathy with heart failure. In fact the number of such incidences are low and perhaps the publicity surrounding the deaths of some body builders and athletes has led to an inflated idea as to the extent of side effects. Surprisingly androgen abuse by top body builders has very little effect on their biochemical and haematological profiles.

If athletes require androgens to 'top-up' endogenous levels how much more the needs of elderly men who show reduced free testosterone concentrations as total testosterone concentrations fall and SHBG concentrations increase with ageing. This is a group that has a

high incidence of low libido and erectile dysfunction. Therefore additional androgen may improve the quality of life in this group of patients. Some clinicians argue that elderly men with sexual dysfunction should be treated even though the total testosterone is in the normal range. Are the benefits and clinical use of androgen therapy being overlooked because of the bad publicity surrounding androgen abuse or is replacement therapy also an abuse?

Although ingestion of pharmacological amounts of androgens may cause side effects such as rage, psychoses, polycythaemia, gynaecomastia, prostate cancer and impotence, more modest administration may have real beneficial effects. The androgen that has been investigated most in this respect is DHEA. This androgen is readily available in the US as a dietary supplement. It is of particular interest to the elderly since old age is associated with very low levels of both DHEA and its sulphate. There are reports of enhanced cognitive function and improved immune characteristics. Replacement of DHEA in the hypoadrenal patient may be justified if studies suggesting it leads to better quality of life are confirmed in larger investigations that those to date. The aim of the fit healthy older person who takes DHEA is to increase their endogenous levels to those found in individuals aged 20-30 years. Perhaps there is a very good reason why DHEA levels fall in old age. GH is another hormone where the secretion is lower in old age. It has been suggested that low GH actually increases longevity whereas increased GH in old age increases the likelihood of cancer. Perhaps the same holds for DHEA.

Androgen administration will continue to attract much attention and research over the coming years. There are very few good studies using large numbers of subjects and there is confusion over what is physiological and what is pharmacological, and how *in vitro* studies equate to what happens *in vivo*. There is a need for biochemists, clinicians and those in the athletic world to come together and address the many questions that exist around androgen administration.