



Winter Meeting of the UK Molecular Epidemiology Group (in collaboration with European colleagues)

# “Linking genetic variation with exposure in the epidemiology of common disorders”

Centre for Research in Environmental Epidemiology (CREAL), Barcelona

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12<sup>th</sup> December 2008





**Winter Meeting of the UK Molecular Epidemiology Group (in collaboration with European colleagues)**

**One-day Meeting for the UK MEG  
on 12<sup>th</sup> December 2008 at the**

**PRBB Auditorium, Barcelona**

**“Linking genetic variation with exposure in the epidemiology of common disorders”**

**PROGRAMME**

08:30 - 09:00 Registration and Coffee

09:00 – 09:10 Welcome and Introduction – **Frank Martin, Manolis Kogevinas**

**MORNING SESSION (Chairs: Paolo Vineis and Jenny Chang-Claude)**

09:10 – 09:45 **John Ioannidis**, Greece. Meta-analysis in the GWA setting

09:45 – 10:20 **Paul Brennan**, France. GWAS in lung cancer: link with environmental exposures

10:20 – 10:55 **Alison Dunning**, UK. GWAS in Breast cancer. Fine Scale Mapping post-GWAS

**COFFEE BREAK**

**(Chairs: Soterios Kyrtopoulos and Tony Fletcher)**

11:15 – 11:50 **Francine Kauffmann**, France. Genes and Environment in asthma

11:50 – 12:25 **Young Investigator Oral Presentations**

- Paras B Singh. Absence of differential gene expression between UK- and India-resident human prostate tissues of candidate metabolising enzymes and oestrogen receptor
- Francesc Castro Giner. Effect of traffic related air-pollution to NO<sub>2</sub> and oxidative stress genes on asthma (ECRHS)
- Geraldine M Clarke. A comparison of case-only designs for identifying gene-environment interactions

12:25 – 12:55 MEG roundtable discussion

13:00 - 14:30 **LUNCH and Poster session**

**AFTERNOON SESSION (Chairs: Cornelia van Duijn and Thomas Meitinger)**

14:30 – 15:05 **Philippe Froguel**, UK. GWAS and obesity

15:05 – 15:40 **Xavier Estivill**, Spain. CNVs in complex disorders

15:40 – 16:15 **Manel Esteller**, Spain. Epigenetics and Human Disease

16:15 – 16:30 CLOSE OF MEETING

For further information, please see [www.creal.cat](http://www.creal.cat) or <http://www.meguk.org/>

# **SPEAKERS' ABSTRACTS**

## Meta-analysis in the GWA setting

**John P.A. Ioannidis**

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The advent of genome-wide association studies has allowed considerable progress in the identification and robust replication of common gene variants that confer susceptibility to common diseases and other phenotypes of interest. These genetic effect sizes are almost invariably moderate-to-small in magnitude and single studies, even if large, are underpowered to detect them with confidence. Meta-analysis of many genome-wide association studies improves the power to detect more associations, and to investigate the consistency or heterogeneity of these associations across diverse datasets and study populations. I will discuss the key methodological issues in the set-up, information gathering and processing, and analysis of meta-analyses of genome-wide association datasets. The efficient and unbiased use of meta-analysis methods ideally requires the existence of large collaborative consortia. Examples will be presented from the application of these methods in type 2 diabetes, osteoporosis and Parkinson's disease. I will also discuss the prospects and caveats for future application of meta-analysis methods in the genome-wide setting.

## GWAS in lung cancer: link with environmental exposures

Paul Brennan

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To identify genetic factors that modify lung cancer risk, we conducted a genome-wide association study by analysing 317,139 SNPs in 2000 lung cancer cases and 2,600 controls from 6 central European countries. We identified a locus in chromosome region 15q25 that was strongly associated with lung cancer ( $p=9 \times 10^{-10}$ ), and replicated this finding in 5 separate lung cancer studies ( $p=5 \times 10^{-20}$  overall). The association region contains several genes, including three that encode nicotinic acetylcholine receptor subunits (*CHRNA5*, *CHRNA3*, and *CHRNB4*), reinforcing interest in nicotinic acetylcholine receptors as potential disease candidates and chemopreventative targets. Similar risks were observed irrespective of smoking status or propensity to smoke tobacco.

To identify additional genes involved in susceptibility to lung cancer, we extended this study by analyzing 3,300 lung cancer cases and 4,200 controls, followed by a replication study in an additional 2,900 lung cancer cases and 5,600 controls from Europe and North America. A marker, rs402710, at 5p15.33 was detected in the genome-wide data ( $p=2 \times 10^{-7}$ ), and replicated in an independent study series ( $p=7 \times 10^{-5}$ ). A second uncorrelated SNP from this region, rs2736100, was also identified in the combined dataset ( $p=4 \times 10^{-7}$ ). The susceptibility region contains the telomerase reverse transcriptase gene, *TERT*, with a similar effect among smokers and never-smokers. Current evidence from lung cancer genome-wide studies suggest common susceptibility genes among smokers and never-smokers.

## **After GWAS – How do we find the variant(s) directly responsible for disease? Examples from breast cancer**

**Alison M. Dunning**

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**Published breast cancer GWAS have already identified more than seven new susceptibility loci, with more still anticipated. The significantly associated tag SNPs, found by the scans, mark loci (haplotype blocks) in the genome that must contain a causative variant, directly responsible for the change in cancer risk. Recognising that variant is not a trivial task: Haplotype blocks may contain several candidate causative genes, or none, and the causative variants being sought, may not look like obviously deleterious mutations. Presently, three new breast cancer loci have been mapped in detail.**

**The first big task is to re-sequence the associated haplotype blocks in sufficient individuals to generate a catalogue of all common variants in the block. For each block, SNPs that are highly correlated with the best tag SNP are considered to be candidate causative variants. Candidates may be progressively eliminated by genotyping in case-control studies from different ethnic groups (European, Asian and African ethnicities).**

**In one example – the re-sequencing of the most strongly associated breast cancer locus – a 30Kb block in intron 2 of FGFR2 revealed 117 SNPs of which, 52 had not previously been reported and 29 were highly correlated with the best tag and therefore candidate causative variants. All but eight of these could be eliminated in European and Asian samples. In another example, a “gene desert” at chromosome 8q24 has been found to contain at least five independent cancer-causing loci. One of these alters risk of breast cancer, while others are associated variously with risk of prostate, colorectal and ovarian cancers. Re-sequencing of these loci is still in progress.**

**This process of fine-scale genetic mapping will provide new insights into the architecture of the human genome and population genetics as well as revealing new biological pathways with effects on disease risk.**

## Genes and environment in asthma

Francine Kauffmann

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Asthma is a chronic disease, highly variable in its clinical expression between individuals and over the lifespan of affected patients. The increase in prevalence in the last decades has pushed research on environmental factors. Research is conducted to understand why early farming environment protects from allergy and asthma, which cleaning agents could explain the high risk encountered by hospital workers, whether outdoor air pollution could cause asthma among adults, which environmental factors increase asthma severity. The window of exposure and of expression of environmental determinants are important to consider. On the genetic side, despite replicated associations for around 30 candidate genes, the understanding of the genetic determinants of asthma is still very partial. A few genes have been evidenced by positional cloning. More recently, genome wide association has evidenced a major locus on chromosome 17, with odds ratio around 1.5. This locus, which covers genes of poorly known function *ORMLDL3* and *GSDM* appears to relate only to asthma with early age-at-onset. Candidate interactions between genes and environment are searched by considering a priori hypotheses on the known effect of environmental factors (such as antioxidant pathways when considering air pollution or smoking), the known function of genes (such as endotoxin for *CD14*, which is its receptor) or knowledge regarding a subphenotype (such as early environmental tobacco smoke for early onset asthma, for *ORMDL3/GDSM*). Larger GWA for common variants are underway in the Gabriel consortium. Till now, the understanding of genetic determinants of asthma remains low. Research may evolve towards more balanced approaches between candidate and agnostic approaches. The same could occur for environmental determinants. Interest is emerging for epigenetic aspects, study of rare variants, consideration of evolutionary origins of genetic variation. Besides large collaboration, improvement of phenotypic and environmental characterisation are needed to go further.

Supported by Ga2len



## Genome Wide Association studies of obesity and associated diabetes

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Obesity has an unexpected strong genetic basis, as shown by recent twin and family studies. Less than 5% of severe obesity is due to deleterious mutations in single genes involved in food intake regulation. Common obesity is a polygenic trait due to the effects of several genes having individual modest effect and interacting together and with the environment. Consequently, only global genomic approaches are able to unravel the genetic architecture of this disorder. Genome Wide Associations studies using high density SNP arrays have flourished since early 2007 (initiating with Sladek et al., Nature) and have provided harvests of new genes and pathways associated with higher risk for obesity and/or T2D. Case/control studies have provided replicated evidence of several gene variation contribution to T2D or obesity: most of the T2D associated new genes are involved in insulin secretion (such as the Zinc transporter 8), and most obesity associated genes such as FTO and Melanocortin Receptor 4 are modulating energy intake. Recent meta analyses of several GWA for obesity T2D have brought additional genes that will be presented. Quantitative trait GWA have a strong statistical power and have been extremely useful to elucidate the genetic regulation of fasting plasma glucose and of the body mass in general populations. They generally showed that genes increasing risk for severe obesity also modulates BMI in non obese populations. GWA have provided breakthrough in physiology providing strong bases for more efficient obesity and T2D risk assessment and for the development of more efficient treatments.

## Copy NUmber Variation in complex disorders

Xavier Estivill

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After the successful discoveries of genetic associations for common disorders using single nucleotide polymorphisms (SNPs) in genome wide association scans (GWAS), new efforts are ongoing to evaluate the contribution of structural variations to disease, mainly in the form of copy number variants (CNVs). These are mainly motivated after the identification of consistent relationships between CNVs and disease, and the recognition that there is not a unique human genome sequence at the structural level. The current knowledge reflects that few regions of the genome are free of structural rearrangements and that genes with a role in response to environment are particularly prone to contain CNVs with phenotypic consequences. In the following years many individuals will be sequenced, defining the variability of the genome at the sequence and structural levels. The characterization of regions of the genome that are variable in the orientation and order of genes and genomic segments between individuals is a major challenge, which can only be reliably tackled by high-throughput sequencing technologies and bioinformatics designs. The goal is to explore the whole set of genome diversity to extract the molecular basis of disorders that could affect any individual in the population and that is inherent to the adaptation of human groups to environmental conditions.

## Cancer Epigenomics: From Genes to MicroRNAs

Manel Esteller

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An altered pattern of epigenetic modifications is central to many common human diseases, including cancer. Many studies have explored the mosaic patterns of DNA methylation and histone modifications in cancer cells on a gene-by-gene basis, among them the seminal finding of transcriptional silencing of tumor suppressor genes by CpG island promoter hypermethylation. Epigenetic gene inactivation in transformed cells involves many "belts of silencing". We are in the process of completing the molecular dissection of the entire epigenetic machinery involved in methylation-associated silencing, such as DNA methyltransferases, methyl-CpG binding domain proteins, histone deacetylases, histone methyltransferases, histone demethylases and Polycomb proteins. The first indications are also starting to emerge about how the combination of cellular selection and targeted pathways leads to abnormal DNA methylation. In addition to classical tumor-suppressor and DNA repair genes, epigenetic gene silencing includes genes involved in premature aging and microRNAs with growth inhibitory functions. Recent technological advances are now enabling cancer epigenetics to be studied genome-wide. It is time to "upgrade" cancer epigenetics research and put together an ambitious plan to tackle the many unanswered questions in this field using genomics approaches to unravel the epigenome.

**YOUNG INVESTIGATORS'  
ABSTRACTS – oral  
presentations**

## Absence of differential gene expression between UK- and India-resident human prostate tissues of candidate metabolising enzymes and oestrogen receptor

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**Introduction and objectives** The aetiology of prostate cancer (CaP) remains obscure. However, CaP incidence in the India-resident Asian population is approximately 1/3 lower than that among UK Caucasians. Genetic makeup, dietary patterns and, metabolism of dietary and/or environmental pro-carcinogens may in part account for such differences. We set out to examine whether there might be differences in quantitative expression of candidate genes governing phase I and II metabolising enzymes, aromatase and oestrogen receptor (ER) in tissues obtained post-surgery from UK-resident Caucasian compared an India-resident Asian cohort.

**Methods** After appropriate ethical approval, fresh prostate tissue samples were collected in India (n= 14) and the UK (n= 12) from prospectively-consented patients undergoing trans-urethral resection of the prostate (TURP). These samples were stored at -85°C in RNAlater prior to analysis, by real-time RT-PCR, for inter-ethnic differences in the expression of *CYP1B1*, *COMT*, *NAT1*, *NAT2*, *SULT1A1*, aromatase (*CYP19A1*), *ERα* (*ESR1*) and *ERβ* (*ESR2*). A Pearson correlation analysis was then applied to determine any correlations between expression of the ERs and the analysed enzymes. Immunohistochemical analyses for *CYP1B1*, *ERα* and *ERβ* in the two sets of samples were also conducted.

**Results** Except for *NAT2*, there was a readily-quantifiable level of expression of all the metabolizing enzymes tested in all the tissue samples analysed. Expression of *NAT2* was only detected in prostate tissue from 7 patients. *CYP19A1*, *ERα* and *ERβ* were also expressed at readily-quantifiable levels in all of these human prostate samples. No marked differences in differential gene expression between the two study population groups were noted. However, when all the tissues examined were taken together Pearson correlation analysis revealed significant positive correlation between expression levels of *ERα* and *NAT1* ( $P < 0.0001$ ), *SULT1A1* ( $P < 0.05$ ) and *CYP19A1* ( $P < 0.0001$ ). A significant correlation was also observed between *ERβ* and *NAT1* ( $P < 0.0001$ ) and *CYP19A1* ( $P < 0.0005$ ).

**Conclusion** This study suggests that expression of xenobiotic/hormone metabolizing enzymes *per se* may not underlie risk to CaP. However, there may be a hitherto unrecognised role played by the local oestrogenic milieu in the prostate that could govern susceptibility to CaP.

**Main Reference:** John, K., Singh, P.B., Pratt, M.M., Ragavan, N., Cole, K.C., Matanhelia, S.S., Phillips, D.H., Poirier, M.C. and Martin, F.L. (2008) quantitation of phase I / II metabolizing enzyme gene expression and polycyclic aromatic hydrocarbon-DNA adduct levels in human prostate. *Prostate* (Accepted);doi: 10.1002/pros.20898.

## Effect of traffic related air-pollution to NO<sub>2</sub> and oxidative stress genes on asthma (ECRHS)

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**RATIONALE.** Traffic related air-pollution is related with asthma and this association may be modified by genetic susceptibility. **OBJECTIVES.** We examined polymorphisms potentially modifying the association between home outdoor levels of nitrogen dioxide (NO<sub>2</sub>) and asthma. **METHODS.** Adults from 14 cities of the European Community Respiratory Health Survey (ECRHS II) were included (N = 2920) for whom both DNA and outdoor NO<sub>2</sub> estimates were available. **MEASUREMENTS AND MAIN RESULTS.** Home addresses were geocoded and linked to outdoor NO<sub>2</sub> estimates, as a marker of local traffic related pollution. We examined asthma prevalence and evaluated polymorphisms in genes involved in oxidative stress pathways (*GSTM1*, *GSTT1*, *GSTP1*, *NQO1*), inflammatory (*TNFA*), immunological response (*TLR4*) and airway reactivity (*ADRB2*). A positive but non-significant association was found for a 10 µg/m<sup>3</sup> increase in home outdoor NO<sub>2</sub> and asthma (odds ratio (OR) =1.2, 95% CI 0.96-1.45); risk was higher among subjects who had not changed residence during follow-up (OR = 1.72, 95%CI 1.15 – 2.68). The association between NO<sub>2</sub> and asthma was significant for carriers of the most common genotypes of *NQO1* rs2917666 (P = 0.04), *TNFA* rs2844484 (P = 0.002) and for deletion of *GSTT1* (P = 0.01). A significant interaction was found between *NQO1* rs2917666 and NO<sub>2</sub> for asthma prevalence (P = 0.02) and new-onset of asthma (P = 0.04). **CONCLUSIONS.** Genetic polymorphisms in the *NQO1* gene is related to asthma susceptibility among persons exposed to local traffic-related air pollution. This remarks the importance of anti-oxidant pathway in the protection against the effects of air pollution on asthma. The analysis in the future of genome wide data will allow the evaluation of complete pathways involved in the response to air pollutants. The limitation will inevitable be that valid information on air-pollutants is available for a relative small number of studies.

## A Comparison of Case-only Designs for Identifying Gene-Environment Interactions

**Geraldine M. Clarke and Andrew P. Morris**

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Case-only designs have been shown to be more powerful than case-control designs in the assessment of possible interactions between genetic and environmental factors in the etiology of a disease<sup>1,2</sup>. Assuming continuously distributed exposure and categorical genotype variables we compare the power and sample size requirements of six case-only tests of association for the detection of gene-environment (GxE) interaction. We consider novel tests that use linear regression to model the exposure as the outcome and compare them to the more traditional multinomial approaches that model the genetic factor as the outcome. In each case ordinal, nominal and binary categorisations of the genetic variable are considered. Across a variety of underlying genetic models the novel approaches were roughly comparable to the traditional models but exhibited marginally greater power in all circumstances. Consequences for power are shown to be disastrous when using a test designed to detect a dominant (respectively, recessive) effect when the underlying model is recessive (respectively, dominant). Tests designed to detect a trend with increasing risk alleles perform poorly for common variants unless the main interaction effect is large. Overall, 2 degree of freedom tests using nominal categorisations of the genetic factor were most robust with just marginally less power in regions of low dominance deviation.

1. Khoury MJ, Flanders WD: Nontraditional epidemiologic approaches in the analysis of gene-environment interaction: case-control studies with no controls! *Am J Epidemiol* 1996, 144(3):207-213.
2. Piegorsch WW, Weinberg CR, Taylor JA: Non-hierarchical logistic models and case-only designs for assessing susceptibility in population-based case-control studies. *Stat Med* 1994, 13(2):153-162.

**YOUNG INVESTIGATORS'  
ABSTRACTS – poster  
presentations**



## Polymorphisms in genes within the steroid receptor superfamily modify postmenopausal breast cancer risk associated with menopausal hormone therapy

S Abbas, T Dünnebie, R Hein, C Justenhoven, U Hamann, H Brauch, YD Ko, D Flesch-Janys, J Chang-Claude and the co-authors within The MARIE-GENICA Consortium

Menopausal hormone therapy (HT) is associated with an increased breast cancer risk among postmenopausal women. However, few studies have examined individual genetic susceptibility to the effects of HT. We used two population-based case-control studies with 3,155 postmenopausal breast cancer patients and 5,496 controls to evaluate modification of breast cancer risk associated with duration of hormone use by genes coding for nuclear receptors sensing steroid hormones. Thirty-three polymorphisms in five genes (*ESR1*, *ESR2*, *PR*, *PXR*, and *AR*) including 30 single nucleotide polymorphisms and three microsatellites were genotyped by fragment analysis and MALDI-TOF MS. Breast cancer risk associated with the use of any HT was significantly modified by a *PR* polymorphism, showing a significant increase only in homozygous major allele carriers of *PR\_rs518162\_G* ( $p_{\text{interaction}}$  0.014). Stronger risk associations for both duration of any and of combined estrogen-progestagen hormones were observed for lower copy numbers of *AR\_171* repeat as compared to higher numbers (cutpoint  $\geq 22$ ) ( $p_{\text{interaction}}$  0.044 and 0.033, respectively). Odds ratios and 95% confidence intervals per 5-year-increment of combined estrogen-progestagen use were 1.29 (1.17-1.42) for  $<22/<22$  genotype as compared to 1.13 (1.04-1.23) for  $\geq 22/\geq 22$  genotype. Furthermore, the risk associated with duration of use of combined estrogen-progestagen therapy was significantly modified by two genetic polymorphisms located in *PXR*. Breast cancer risk was highest in homozygous carriers of the major *PXR\_rs1054191\_G* and the *PXR\_rs6785049\_A* allele ( $p_{\text{interaction}}$  0.048 and 0.036, respectively). Both *ESR1* and *ESR2* modified risk associated with use of estrogen monotherapy. A higher risk associated with duration of any hormones as well as estrogen monotherapy was observed in homozygous carriers of the major *ESR1\_rs910416\_T* allele as compared to non-carriers ( $p_{\text{interaction}}$  0.016 and 0.001). Only homozygous carriers of the minor *ESR2\_rs1271572\_T*, major *ESR2\_rs4986938\_G*, and minor *ESR2\_rs928554\_G* allele were at significantly increased risk after using estrogens ( $p_{\text{interaction}}$  0.024, 0.050, and 0.021). Postmenopausal breast cancer risk associated with hormone therapy is modified by genetically determined variations in genes within the steroid receptor superfamily.

## **A novel multifactor dimensionality reduction technique for high-order interaction detection in (un)related individuals**

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When searching for gene-gene or gene-environment interaction patterns, parametric regression approaches have severe limitations when there are too many independent variables in relation to the number of observed outcome events. On the other hand, when analyzing gene-gene or gene-environment interactions, adjustment for confounding variables or main effects is usually required and parametric methods might be the method of choice. Alternatively, a Multifactor Dimensionality Reduction method, MDR (Ritchie et al. 2001) can be applied. It handles the dimensionality problem in interaction analysis by pooling multi-dimensional constructs into two groups of risk: high and low.

We propose a novel multifactor dimensionality reduction strategy for interaction analysis in a genetic association analysis framework that is based on the flexible Model-Based Multifactor Dimensionality Reduction Method, MB-MDR (Calle et al. 2007). Whereas the latter was initially developed for case-control settings, it can encompass a variety of study designs. In an extension to families of any structure, referred to as FAM-MDR, we first remove familial effects so as to obtain residuals that can be treated as if independent. Second, we apply MB-MDR to the derived residuals, and interpret models obtained.

**Main Reference:** Calle ML, Urrea V, Malats N, Van Steen K. (2007) MB-MDR: Model-Based Multifactor Dimensionality Reduction for detecting interactions in high-dimensional genomic data. Technical Report n.24. Department of Systems Biology. Universitat de Vic.

# Derivation of a subtype-specific biochemical signature of endometrial carcinoma using synchrotron-based Fourier-transform infrared microspectroscopy

Jemma G. Kelly<sup>1</sup>, Maneesh N. Singh<sup>2</sup>, Helen F. Stringfellow<sup>2</sup>, Michael J. Walsh<sup>1</sup>, Katherine M. Ashton<sup>2</sup>, Mark A. Pitt<sup>2</sup>, Pierre L. Martin-Hirsch<sup>2</sup>, Francis L. Martin<sup>1</sup>

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**Introduction:** Tamoxifen, an anti-oestrogen drug used to treat oestrogen receptor (ER) positive breast cancer, is linked to an increased risk of endometrial carcinoma. Two distinct subtypes of endometrial carcinoma exist: type I (endometrioid) and type II (SP and MMMT, a rare combination of endometrial carcinoma and uterine sarcomas). Type I has a tendency to be ER positive with histology related to the normal endometrium, compared to type II which are often ER negative, exhibiting a more severe disease leading to poorer prognosis. Further risk factors of endometrial carcinoma include obesity and hypertension.

**Methods:** The aim of this study was to determine if a biochemical cell fingerprint existed for each subtype and, additionally, to establish if the tamoxifen associated endometrial carcinoma was altered, at a biochemical level, compared to that of the same non-tamoxifen associated subtype. Endometrial tissue samples were interrogated using synchrotron-based Fourier-transform infrared (FTIR) microspectroscopy to produce absorbance spectra over the mid-IR ( $4000\text{ cm}^{-1}$  -  $400\text{ cm}^{-1}$ ), and further processed in the biomolecular cell fingerprint region ( $1800\text{ cm}^{-1}$  -  $900\text{ cm}^{-1}$ ). Samples were obtained from a number of patients: non-tamoxifen associated cases reported as endometrioid ( $n = 7$ ), SP ( $n = 4$ ) or MMMT ( $n = 4$ ); tamoxifen-associated cases, endometrioid ( $n = 1$ ), SP ( $n = 3$ ) and MMMT ( $n = 4$ ); benign tissues ( $n = 3$ ) were also analysed. PCA-LDA (Principal Component Analysis-Linear Discriminant Analysis) was used to interrogate the data for inter-category variance, employing scores plots for visualisation and loadings plots to identify discriminating chemical groupings based on wavenumbers; such biochemicals would be responsible for segregation.

**Results:** Overall there exists a clear distinction between the benign samples and the endometrial carcinoma subtypes. Furthermore a difference was found within the subtypes, attributed to antecedent tamoxifen. The primary biochemicals contributing to the differences are Amide I ( $1650\text{ cm}^{-1}$ ), Amide II ( $1550\text{ cm}^{-1}$ ) and  $\nu_s\text{PO}_2^-$  ( $1080\text{ cm}^{-1}$ ).

**Conclusions:** The endometrial carcinoma subtypes were clearly segregated using PCA-LDA, from which information was extracted highlighting the main biochemical components responsible. Amide I, Amide II and  $\nu_s\text{PO}_2^-$  are the main contributors to the difference between subtypes and the effect of tamoxifen. In particular, the type I carcinomas differed from benign in the protein region, while type II carcinomas exhibited differences in the DNA/RNA region. Our conclusion is that it is possible to establish a biochemical-cell fingerprint for tamoxifen-associated and non-tamoxifen associated endometrial carcinomas, in addition to each subtype.

**Main Reference:** Kelly, J.G., Singh, M.N., Stringfellow, H.F., Walsh, M.J., Nicholson, J.M., Bahrami, F., Ashton, K.M., Pitt, M.A., Martin-Hirsch, P.L. and Martin, F.L. (2008) Derivation of a subtype-specific biochemical signature of endometrial carcinoma using synchrotron-based Fourier-transform infrared microspectroscopy. *Cancer Letters* (In press); doi:10.1016/j.canlet.2008.09.018 [Online 26 October 2008].

## $\gamma$ -Synuclein: a novel marker for endometriosis?

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**Introduction:** Endometriosis is a common, disabling disorder of women in the reproductive age group. Its aetiology remains unknown but retrograde menstruation and prolonged oestrogenic stimulation appears to play a role.  $\gamma$ -Synuclein ( $\gamma$ -syn) is an important cofactor in ligand dependent oestrogen receptor (ER) $\alpha$ -mediated transcriptional activity and elevated expression is found in oestrogen-responsive malignancies such as breast and ovarian carcinoma.

**Methods:** Tissue sets consisting of eutopic and ectopic (ovarian) endometrial tissue from patients with stage 3 or 4 endometriosis were obtained. Following RNA extraction and reverse transcription, quantitative real-time reverse transcriptase–polymerase chain reaction was performed for *ER $\beta$* , *ER $\alpha$*  and  *$\gamma$ -SYN*. Immunohistochemical analyses were also conducted.

**Results:** A 5–53-fold intra-individual increase in  *$\gamma$ -SYN* expression was demonstrated in six of nine tissue sets (a further two showed an increase that was not considered significant) when comparing ectopic and eutopic endometrium. In addition,  $\gamma$ -syn positivity was associated with endothelial cells. An elevation in *ER $\beta$*  was also noted when comparing ectopic and eutopic endometrium. This was inconsistent with regard to *ER $\alpha$* .

**Conclusions:**  *$\gamma$ -SYN* expression is up-regulated in endometriosis and may be useful as a diagnostic marker. This up-regulation may enhance the effect of oestrogen and play a part in improving the survival of implanted endometrial cells.

**Main Reference:** Singh, M.N., Stringfellow, H.F., Taylor, S.E., Ashton, K.M., Ahmad, M., Abdo, K.R., El-Agnaf, O.M.A., Martin-Hirsch, P.L. and Martin, F.L. (2008) Elevated expression of *CYP1A1* and  *$\gamma$ -SYNUCLEIN* in human ectopic (ovarian) endometriosis compared to eutopic endometrium. *Molecular Human Reproduction* (In press); doi: 10.1093/molehr/gan056 [Online 10 October 2008].



**Winter Meeting of the UK Molecular Epidemiology Group (in collaboration with European colleagues)**

**“Linking genetic variation with exposure in the epidemiology of common disorders”**

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