Abstracts of the UK Molecular Epidemiology Group (MEG) Winter Meeting on The Future of Epidemiology: Biomarkers meet Populations. Newcastle University, United Kingdom. December 6, 2013

1. The Epidemiology of aristolochic acid nephropathy
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It has been 20 years since the first description of a rapidly progressive renal disease associated with the consumption of Chinese herbs containing aristolochic acid (AA) in Belgium, now termed aristolochic acid nephropathy (AAN) [1]. Molecular epidemiology studies have now also demonstrated that AA is the primary etiological agent in Balkan endemic nephropathy (BEN) and associated urothelial cancer where diet seems to be the likely route of AA exposure [2]. AA has been classified as a Group I human carcinogen by IARC and Aristolochia spp. and herbs that can be confused or substituted for Aristolochia have been banned in many countries. Unfortunately, these regulatory measures have been shown to be wholly inadequate in preventing exposure to AA, and there is growing evidence that AA exposure is causing a large unrecognized burden of disease in Asia with potentially devastating public health implications. This is in line with a more recent study showing that AA exposure contributes to the high incidence of urothelial cancer in Taiwan where medicinal use of Aristolochia plants is widespread [3]. AA-DNA adducts are established biomarkers of AA exposure that have been detected in urothelial tissue from AAN and BEN patients. Mechanistic evidence demonstrating that the urothelial tumour DNA has a distinct TP53 mutational signature consisting of AT to TA transversion mutations which is otherwise infrequent in TP53 in other cancer genomes provides a strong molecular link that AA directly contributes to the development of these urothelial tumours. This characteristic TP53 mutation pattern can also be recapitulated experimentally in mammalian cells that immortilised after AA treatment [4].

References

2. Exploiting molecular epidemiology in a move towards personalised medicine
Ben van Ommen

Modern nutrition science has seen a number of major developments over the last 10 years, but is nutrition science ready to “quantify” my personal health status and provide related personal dietary and lifestyle advice based on quantification of my own genotype X phenotype X environment interaction? A careful look at changes in healthcare points at the urgent need for both prevention and personal empowerment. Each individual, in order to properly take control of one’s own health, needs access or even better, needs to own all relevant information regarding personal health status. Apart from the above-mentioned integrative personal omics profile, other activities point in this direction. There is a push from the “medical records” front, and more interestingly, the Quantified Self crowd source movement (http://quantifiedself.com) launches all kinds of initiatives. Developments in personal sensors are exploding and the European Commission takes this very seriously with action plans on eHealth and mHealth (http://ec.europa.eu/digital-agenda/en/eHealth). NutGO, the Nutrigenomics Organisation has taken up the challenge to initiate an open access cohort where each individual provides and owns her/his own health data that both provide an empowerment for individual health optimization and, brought together, a powerful open access cohort. As a first step, the “Nutrition Researcher Cohort” (NRC) is established as a 2-year project to establish all analytical methods, standards and operation procedures, data infrastructure, ethical and privacy aspects, governance, etc.). Details are provided at www.nugo.org/nrc and one can enroll at http://nrc.dbnp.org. The NRC is a “crowd science” project, where researchers, as experts/subjests, both participate and build. Once the two year initial phase is passed, we can implement the lessons learned in a really new mix between a nutrition and health cohort and a personal healthcare setting.

3. Removing the bias – Mendelian Randomisation in epidemiology
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Mendelian randomization utilises genetic variants as instruments for environmentally modifiable potential causes of disease. The availability of multiple genetic variants related to any modifiable risk factor allows the use of multiple independent combinations of variants to generate instrumental variable (IV) estimates, and through examination of heterogeneity between these to assess the potential role of reintroduced confounding (primarily through pleiotropy). This potentially allows considerable strengthening of the causal inferences that can be drawn from Mendelian randomization studies. Complex networks of associated biological measures have been identified in aetiological studies of common complex diseases, and node-by-node investigation of these through Mendelian randomization methods shall help separate causal from epiphenomenal associations. Such networks include gene expression and epigenetic profiles (in particular methylation) data, and analogous approached to Mendelian randomization, that had been referred to as “genetical genomics” and “genetical epigenomics”, can be applied to these. The presentation will cover the above aspects of Mendelian randomization studies.
4. Sources of variation of white blood cell (WBC) DNA methylation in serial blood samples in the Breakthrough Generations Study (BGS)

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Numerous epigenome-wide association studies (EWAS) are currently being performed on WBC DNA to identify associations between DNA methylation and disease risk. Strong correlations between WBC DNA methylation and environmental, lifestyle and other risk factors have also been reported. However, it is crucial to understand the stability of these methylation markers over time to determine whether case-control studies will need serial samples or whether single samples are sufficient. We estimated the intraclass correlation coefficient (ICC) for each probe on the Illumina 450K methylation array in paired samples collected ~6 years apart from 91 participants in the Breakthrough Generations Cohort, and evaluated relationships with reproductive and behavioural exposures. Approximately 20% of probes on the 450K array are variable between individuals and stable over a six year gap (ICC > 0.50; stable variable methylated regions, VMRs). Stable-VMRs were enriched at approximately 1.2kb downstream from the transcription start site in the transition between the unmethylated promoter and methylated gene body. These stable-VMRs represent good candidates for EWAS using a single blood sample per subject. These findings provide support for further EWAS to identify biomarkers of exposure and disease risk.

5. Using epigenetics in epidemiology

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Epigenetics is predicted to become the focus of major advances in understanding the determinants and development of human disease. Epidemiological approaches can contribute to these advances by adopting strategies used in both conventional observational epidemiology and genetic epidemiology. The discipline of epidemiology aims to identify risk factors and identify targets for prediction, prevention and treatment. The epigenome is juxtaposed between the genome and the environment. There is a rapidly increasing body of evidence linking a variety of environmental and lifestyle factors to epigenetic variation. There is also an increasing recognition that epigenetic patterns are in part determined by underlying sequence variation. It is widely proposed that epigenetic variation lies on the causal pathway to disease and disease related traits. However it is equally important to note that epigenetic variation might be robustly linked to biomarkers or established risk factors that are not necessarily causally related to disease but may still serve as informative predictive tools. A range of epidemiological study designs and analytical strategies can be adopted, the choice of which depends upon whether epigenetic marks are considered simply as a biomarker of exposure, or to causally influence disease pathogenesis.

6. Using OMICS in human studies – investigations in the colorectal mucosa

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The functional unit of the colorectal mucosa is the crypt populated by stem cells at the base of each crypt. Progeny from these stem cells differentiate to produce several lineages of columnar epithelial cells which form the single cell thick barrier between the gut lumen and the rest of the body. We have undertaken a series of studies using proteomics, gene expression and epigenetics approaches to identify early molecular changes which are associated with increased neoplastic risk and which may be modified by exposures such as diet and nutritional status and by age. We have observed widespread changes in the proteome of the apparently normal mucosa in mucosal biopsies [1] and changes in the methylation of the promoters of cancer-related genes [2, 3] from those at higher bowel cancer risk. In addition, nutritional factors and age appear to modulate these epigenetic marks [4]. Challenges in this work include the multi-cellular nature of the tissue which limits mechanistic interpretation of findings and the paucity of intervention study data which inhibits the drawing of causal inferences.

References


7. Anti-neoplastic effects of non-digestible carbohydrates on Wnt signalling gene expression and crypt cell proliferation in the large bowel: a randomised-controlled dietary intervention

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Environmental factors, such as diet, modify colorectal cancer (CRC) risk and evidence exists for a protective role of dietary fibre, including non-digestible carbohydrates (NDCs) [1]. These beneficial effects are thought to result from butyrate, a short-chain fatty acid, production by colonic bacteria. Furthermore, butyrate positively modulates Wnt signalling, the central pathway involved in the regulation of cell proliferation in the large bowel, which is frequently altered in CRC [2]. This study aimed to investigate the effects of supplementing healthy participants with NDCs on Wnt signalling and its functional outcomes, notably cell
proliferation, in the bowel. We hypothesised that higher NDC intake will increase colonic butyrate concentrations and modulate Wnt signalling positively. Using a 2×2 factorial, double-blind RCT design, 75 participants were supplemented with resistant starch and/or polydextrose or placebo for 7 weeks. Rectal mucosal biopsies were collected pre- and post-intervention and used to quantify Wnt-related gene expression by quantitative PCR. CRYPT cell proliferative state (CCPS) was assessed following whole crypt microdissection of Schift’s reagent-stained biopsies [3]. The study remains blinded. We quantified expression of nine Wnt-related genes and observed a statistically significant effect of treatment group on SFRP1 expression. Post-intervention SFRP1 expression was two-fold higher in intervention group A and similar in the other 3 groups. SFRP1 is a Wnt signalling antagonist that is frequently down-regulated in CRCs. Furthermore, butyrate treatment restores SFRP1 expression in gastric cancer cells [4]. Preliminary analyses of CCPS suggest lower percentage of mitotic cells in the top half of the crypt post-intervention in group A.

References


8. Expression of the sFLT1 gene in cord blood cells is associated to maternal arsenic exposure and decreased birth weight

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Arsenic is an ubiquitous toxic metal that is present in soil, drinking water and foods such as juices and rice. Concern is growing that in utero arsenic exposure affects growth and neurodevelopment, however, the mechanisms underlying these effects are unknown. In a cohort study conducted in the Northern part of Belgium, including 183 newborns and their mothers, arsenic levels in cord blood samples ranged from below the limit of detection of 0.028 up to 14.4 µg/L. Multiple regression analyses showed that - even at this low dose range - the odds of having a small for gestational age (SGA)-baby was multiplied with 1.38 (95% CI: 1.11-1.71) for an interquartile range increase of 0.99 µg/L arsenic. SGA is a risk factor for development of obesity later in life. Concomitantly, higher levels were positively associated with changes in gene expression in cord blood cells of sFLT1, coding for the soluble form of vascular endothelial growth factor receptor. Playing a key role in the inhibition of placental angiogenesis this molecule may restrict fetal growth. Although in girls only, sFLT1 was significantly upregulated among higher arsenic exposed and among lower birth weight babies, adjusted for gestational age. Our study suggests that at low doses, in utero exposure to arsenic may lead to reduced fetal growth by inhibiting placental angiogenesis through increased expression of sFLT1. Various genes related to DNA methylation and oxidative stress also showed changed expression in relation to arsenic exposure but were not related to birth outcome parameters.

9. Exploring the potential of oxidative stress-related biomarkers of ageing in a population-based study of the very old

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It is greatly important to gain scientific insights that will help ensure the extra years of life we are gaining through increased life expectancy are as healthy, productive and enjoyable as possible. Biological measurements that can discriminate between individuals who differ in the timing, type and extent of age-related decline, known as biomarkers of ageing (BoA), will be useful to understand biological mechanisms, develop and test interventions and allow the prediction of age-related events so interventions can be implemented. In recent years, a variety of mechanistic candidate BoA have been discovered on the basis of a greatly improved understanding of the cellular and molecular biology of ageing. These include various measures of oxidative stress, which is thought to contribute causally to the ageing of organisms via its acceleration of cellular senescence. However, their reliability and validity as BoA, especially within population based cohorts are hardly established. This study focused on various oxidative stress-related measures as candidate BoA including: reactive oxygen species (ROS) production from dysfunctional mitochondria, by measuring superoxide levels, mitochondrial mass and mitochondrial membrane potential in peripheral blood mononuclear cells by flow cytometry; and also markers of lipid peroxidation, F2-isoprostanes, by measuring plasma 8-iso Prostaglandin F2α by Automated Dissociation Enhanced Lanthanide Fluorescence Immunoassay (AutoDELFIA). Evidence of experimental reliability for all measures was shown and also some evidence of construct validity for ROS production from dysfunctional mitochondria in terms of: associations with chronological age, associations with some markers of oxidative stress-induced cellular senescence and a role in an immunosenescent phenotype. However, there was no evidence of predictive validity in terms of longevity or age-related health outcomes in a population based cohort of the very old, the Newcastle 85+ study. This questions the predictive validity of these parameters as candidate BoA in the very old population.
10. 25-hydroxyvitamin D and increased risk of all-cause mortality in very old women: The Newcastle 85+ study
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Emerging epidemiological evidence indicates a curvilinear association between 25-hydroxyvitamin D [25(OH)D] and all-cause mortality [1]. We aimed to investigate the associations between low and high concentrations of baseline serum 25(OH)D and all-cause mortality in very old (85+) men and women over 6 years. Prospective mortality data from 775 participants in the Newcastle 85+ Study were analyzed for survival in relation to 25(OH)D (quartiles and clinical cut-off categories) and sex using Cox proportional hazard models. The models were fitted to the entire and restricted cohort (non-users of vitamin D-containing supplements and medication). In analysis with the entire cohort, mortality was higher in both the lowest (Q1: ≤25nmol/L) and highest (Q4: ≥63nmol/L) quartiles of 25(OH)D (Q1: HR=1.49, 95% CI [1.15-1.93], p=0.003; Q4: 1.64 [1.27-2.10], p<0.001) compared with middle quartiles (Q2+Q3), after adjustment for season of venipuncture and sociodemographic factors. The risk for Q4 (but not Q1) remained significant after further adjustment for lifestyle factors (HR=1.47, 95% CI [1.14-1.89], p=0.003), and additionally for morbidity-related variables (HR=1.36, 95% CI [1.05-1.76], p=0.02). In sex-specific analyses, the effect of 25(OH)D on mortality was seen only in women after adjustment for season and sociodemographic factors. Repeating analyses using 25(OH)D categories based on established clinical cut-offs led to the same conclusions, with the highest 25(OH)D category (≥75nmol/l) being associated with a 2.4-fold increased risk of mortality in women (restricted cohort) after adjusting for all confounders. Low (≤25nmol/L) and high (≥63nmol/L) serum 25(OH)D levels were associated with increased risk of mortality over 6 years in the very old, especially in women, including those who reported taking vitamin D-containing supplements and medication. Concentrations of 25(OH)D ≥75nmol/l were also associated with increased mortality in women not taking vitamin D-containing supplements or medication.

Reference

11. DNA methylation, cardiometabolic risk and type 2 diabetes in South Asians and Europeans
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People of South Asian origin have one of the highest rates of diabetes in the world. Genetic studies have not explained this excess risk and epigenetic processes may play a role. This study aims to identify the association between DNA methylation, type 2 diabetes and related traits and to identify ethnic differences between individuals of South Asian and European origin living in the UK. Baseline samples (n=192) from the extensively characterised population based SABRE (Southall And Brent REvisited) cohort were utilised. South Asian and European men aged between 40-69 years at baseline were included, matched for ethnicity, age, smoking status and subsequent development of diabetes and/or coronary heart disease (CHD) over 20 years of follow up. Genome-wide DNA methylation analysis was performed using the Illumina HumanMethylation450 array (HM450). Validation was undertaken in 90 SABRE samples at four candidate loci. Marked ethnic differences were observed in methylation sites throughout the genome following HM450 analysis (n=2234 at p<1.1 e-07, 439 CpG sites showed a median difference in methylation greater than 5% between ethnic groups. Of these, 44 individual loci contained at least two CpG sites with ethnic differences at p<1.1 e-07, with eleven being related to type 2 diabetes or related traits following literature review. Four loci were validated using pyrosequencing. Ethnic differences in methylation were confirmed at three loci: UCP1, UGGT1 and CAPN2. Pyrosequencing did not validate ethnic differences in a fourth locus, DUSP1. Wide-spread differences in methylation by ethnic group were observed. Pyrosequencing validated ethnic differences at three loci related to type 2 diabetes and/or related traits. Ongoing investigation of ethnic differences in these methylation sites, and predictive power for subsequent cardio metabolic disease may provide valuable novel insights into the determinants of excess disease risk in South Asians.

12. DNA methylation patterns in respiratory allergy cases: comparability of salivary vs blood
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Environmental exposures during fetal and early life stages may trigger and contribute to disease later in life such as complex diseases including allergy, neurodegenerative diseases and cancer. The biologic mechanisms underlying this “developmental origins of health and disease (DOHaD) hypothesis” are poorly understood, but alterations in epigenetically-regulated gene expression is a prominent candidate mechanism. Epigenetics defines processes and genomic marks that may result inheritable changes in gene expression without altering the genomic sequence, of which DNA methylation is the most widely studied. Longitudinal (birth) cohorts are instrumental to study the relation between early-life environmental factors and the development of complex diseases. The investigations are hampered because blood sampling in children is kept to a minimum for practical and ethical reasons. Saliva may be a good alternative as it can be easily collected from children at all ages and it is a good source for high quality DNA. The aim of the current pilot study is to investigate the comparability of DNA methylation patterns in blood mononuclear cells (MNC) versus saliva samples. Furthermore, we hypothesize that differential DNA methylation can be detected in respiratory allergy (RA) cases compared to controls. To investigate if saliva holds potential to discover DNA methylation patterns that correlate with the prevalence of RA, a case-control study design was applied: MNC and saliva samples from 5 adult RA cases and 5 controls were analysed on Illumina
Infinium Human Methylation 450K BeadChips. GenomeStudio software was used to normalize the data and identify differentially methylated regions between RA cases and controls. IPA pathway enrichment analysis was used to select relevant allergy-related genes with differential methylation. Especially genes for which multiple CpG changes are detected in proximity of the promoter will be selected for further confirmation by bisulfite pyrosequencing of promoter regions of interest.

13. Methylation smoking scores identify current smoking behaviour: smoking associated methylation patterns in South Asians and Europeans

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DNA Methylation is strongly associated with smoking status at multiple sites across the genome. Studies so far have concentrated on identifying individual smoking-associated loci, predominantly in European cohorts. This study investigated the utility of generating smoking scores based on individual’s overall DNA methylation profiles. Use of a dual-ethnic cohort comprising European and South Asian men allowed ethnic differences in smoking-associated loci to be investigated. Baseline samples (n=192) from the extensively characterised population based SABRE (Southall And Brent REvisited) cohort were utilised. Healthy Indian Asian and European men aged between 40-55 years were included, matched for ethnicity, age and smoking status. Genome-wide DNA methylation analysis was performed using the Illumina HumanMethylation450 array (HM450). Differential methylation in smokers was identified in 29 individual CpG sites at 18 unique loci. Interaction between smoking status and ethnic group was identified at the AHRR locus. Ethnic differences in DNA methylation were identified in non-smokers at two further loci, 6p21.33 and GNG12. A smoking score based on methylation profile was constructed. Current smokers were identified with 100% sensitivity and 97% specificity in Europeans and with 80% sensitivity and 95% specificity in South Asians. In conclusion, smoking score is a valuable tool for identification of current smoking behaviour which can be applied to other cohorts. Differences in ethnic groups were identified in both single CpG sites and methylation profiles (as indicated by smoking score), highlighting the need for careful interpretation of results when analysing the effects of smoking in more than one ethnic group.

14. Bone health at age 49-51 years is associated with IGF2 DNA methylation levels

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Inter-individual variation in DNA methylation is believed to play a key role in the development and progression of common complex diseases. The hypothesis that both global and gene-specific DNA methylation is associated with bone health at age 49-51 years was investigated in this study. Two markers of global DNA methylation and IGF2 promoter methylation were quantified by pyrosequencing in peripheral blood-derived DNA samples from 215, 127 and 203 individuals, respectively, from the Newcastle Thousand Families Study (NTFS). Associations between DNA methylation and contemporary DXA-based measures of bone health were analysed using linear regression models, adjusted for significant covariates. Bone mineral density (aBMD, g/cm²) of the lumbar spine demonstrated association with mean IGF2 methylation (%), which was slightly attenuated following adjustment for weight and sex (Coefficient (95% CI): 0.005 (0.001, 0.008), p=0.006). A similar pattern of effects was demonstrated between femoral aBMD and mean IGF2 methylation following adjustment for weight, although the effect sizes and significance were smaller (0.003 (0.000, 0.005), p=0.049). The combined effects of weight, sex and IGF2 methylation accounted for 18% if the total variation in spine aBMD, of which 3% was related to methylation (inferred from the regression R² value). In contrast, 31% of the variation in femoral aBMD was accounted for by weight and IGF2 methylation with ~1% of this related to methylation alone. Neither LINE-1 nor LUMA global DNA methylation was associated with spine or femoral BMD. Bone area (spine, femoral and total) and femoral neck shaft angle were not associated with any measure of DNA methylation. In conclusion, IGF2 methylation demonstrated association with aBMD in the NTFS population. However, these associations did not appear wholly independent of known covariates. A more comprehensive analysis of DNA methylation at the IGF2 locus is required in a larger dataset to elucidate a possible causal relationship.

15. Epigenetic biomarkers in the prediction and prognosis of post stroke dementia

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The risk of developing dementia greatly increases following a stroke. It remains unclear why some stroke patients lose cognitive function whilst others do not. Epigenetic processes may play a role. Fundamental questions relating to the potential causal role of epigenetic mechanisms or the utility of predictive epigenetic biomarkers in these conditions remain unanswered. Research into how post stroke dementia can be predicted and thus prevented or treated is becoming a priority in light of the increasing lifespan and proportion of the population at risk. This project aims to explore novel epigenetic biomarkers in peripheral blood DNA which may be used in the prediction of cognitive decline in later life. The project utilises samples from the extensively characterised COGFAST cohort which recruited stroke survivors three months post stroke (mean age at recruitment = 80.3 years) and followed them annually to check for cognitive decline (mean follow-up length = 7.4 years). Genome-wide DNA methylation analysis was performed on baseline DNA samples using the Illumina HumanMethylation450 array (HM450). In addition, HM450 analysis was undertaken on post mortem brain tissue from the hippocampus and dorsolateral prefrontal cortex from 30 individuals, 17 of whom experienced cognitive decline following a stroke and 13 who remained cognitively normal until death.
Differential DNA methylation was observed in relation to various exposures and co-morbidities associated with post stroke dementia in both blood and brain tissue. Loci associated with post stroke dementia in the blood of stroke survivors may be useful biomarkers in the prediction of post stroke dementia.

16. Culture independent analysis of the gut microbiota in preterm multiples
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We aimed to examine the development of the gut microbiota in preterm multiples, focusing on dysbiosis and its role necrotising enterocolitis (NEC). Stool (n=173) was collected from 12 twin pairs and 1 triplets. Breast milk (BM) residue (n=8) was also sampled from a subset of patients (n=4). Samples were analysed by PCR-DGGE and 454 pyrosequencing. Profiles from siblings were more similar compared to unrelated profiles, with diseased and triplet patients showing significantly different profiles. Diversity reduced pre-disease diagnosis with DGGE showing Enterococcus to be significantly (P=0.001) more abundant while 454 pyrosequencing revealed increasing abundance of Escherichia. BM samples grouped with stool profiles from the corresponding patient. Differences within the community of related twins may predispose infants to disease. A reduced diversity was observed pre-disease diagnosis and thus dysbiosis may be causal due to a reduction in commensal bacteria, leading to the prevalence of a pathogen.

17. Identification of genes susceptible to epigenetic change in response to maternal folate supply in acute lymphoblastic leukemia
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Evidence suggests altered folate metabolism and inadequate maternal folate intake may be associated with increased childhood acute lymphoblastic leukaemia (ALL) risk. Folate provides methyl groups for DNA methylation, the patterns of which are dramatically disrupted in ALL. Differences in maternal folate intake during pregnancy and/or altered folate metabolism may therefore affect DNA methylation, consequently influencing ALL risk. We investigated the potential aetiological role of maternal folate intake during pregnancy on ALL risk via aberrant DNA methylation by identifying genes in which methylation changes occur both in response to folate levels and in ALL. We used previously generated DNA methylation array data from a mouse model of in utero folate depletion to identify genes in which methylation is altered in response to inadequate maternal folate intake: 591 genes showed altered methylation. From the literature, data mining techniques identified 2615 differentially methylated genes in ALL. Sixty genes were common to both folate and ALL lists. We assessed DNA methylation by pyrosequencing in 20 ALL patient samples for 5 target genes (ASCL2, HTRA1, KCNA1, SH3GL3, SRD5A2) which were highly methylated in ALL samples. Methylation was then assessed in these 5 genes in a nested cohort of 148 cord blood samples from the North Cumbria Community Genetics Project and analysed in relation to maternal and infant red blood cell folate and vitamin B12 concentrations in the same individuals. Preliminary analysis suggests methylation of some target genes appears to be related to maternal folate and B12 levels. These findings demonstrate, that folate responsive changes in methylation identified in animal studies can be used determine relevant gene targets in human studies of diseases for which folate intake is an associated risk factor, and that DNA methylation may be a mechanism by which maternal folate intake (and related pathways) may influence ALL risk.

18. Factors associated with recurrence and length of survival following relapse in patients with neuroblastoma
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Despite advances in therapy for neuroblastoma, survival following disease relapse is poor. This pilot study aimed to investigate epidemiological, clinical and biological factors associated with recurrence and length of survival following relapse in neuroblastoma in the UK. Data on all cases of relapsed neuroblastoma diagnosed from 1990-2010 were identified from four UK Children’s Cancer and Leukaemia Group centres. Kaplan-Meier survival analyses were used to calculate the median overall survival (OS) time from diagnosis and post relapse overall survival (PROS) time. Log rank tests and Cox regression analysis were used to investigate factors that may influence survival. 198 cases of relapsed neuroblastoma were identified for the study, the median age at diagnosis was 2.9 years (range 0-19), 166 (87.5%) cases were high risk, 17 (10.4%) were intermediate risk (remaining 15 were stage 4 infants, 2 stage 4S, 2 stage 2, and 3 unknown). The median OS time was 22.7 months (inter-quartile range (IQR) 13.4–39.1) and median PROS time was 5.7 months (IQR 2.0–13.5). 5-year PROS was 12% (95% CI 8%–17%) and worse PROS (2.9 months, 95% CI 1.9–4.3 versus 10.3 months, 95% CI 7.5–13.5, P<0.001). The median time to relapse was significantly shorter for high risk cases compared with intermediate risk (14.3 months vs 22.1) (P<0.02). Stage 4 and MYCN amplified disease were significant in multivariable analyses (P<0.001, 0.003). This study confirms that MYCN amplified and stage 4 relapsed neuroblastoma cases have worse survival, and that the time to relapse for 80% of high risk cases was within 2 years of diagnosis. This data is important for planning future clinical trials for children with neuroblastoma.

19. Impact of non-digestible carbohydrates on putative epigenetic biomarkers of colorectal cancer risk
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Abstracts

21. The role of potential oesophageal carcinogens in DNA damage induction
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There is increasing evidence of correlation between Barrett’s Oesophagus (B.O) and bile acids present in the refluxate components. To investigate this association with respect to DNA damage induction, we utilized the in-vitro micronucleus (MN) assay to examine the correlation between MN levels in patients with BO and histological changes in these candidates compared to controls. Blood samples were collected from patients with BO with clinical details of reflux and histology. Experiments were also conducted in the immortalized human lymphoblastoid TK6 and p53 null NH32 cell lines. Initially, both TK6 and NH32 cell lines were treated with Hydrogen peroxide (H₂O₂) and hydrochloric acid (pH5, pH5.5, pH6), bile acid deoxycholic acid (DCA) and methyl methane sulphonate (MMS). Both cell lines exposed to H₂O₂ showed 2-3 fold increase in MN levels compared to the controls (P=0.002; P=0.001). Treatment with DCA resulted in increase in MN levels only in NH32 cells which leads to the assumption that oxidative stress could be the mechanism involved in the damage seen. To further explore the role of oxidative damage, these cells were analysed for catalase expression using western blotting. Results showed only slight increase in protein expression in NH32 cells exposed to DCA compared to controls. Initial studies in blood samples revealed a 5-fold increase in MN frequency in oesophageal adenocarcinoma patients compared to the un-diseased controls. Thus the Cytokinesis Block Micronucleus Assay may be used as the predictive biomarker to demonstrate the genomic alteration in blood samples of Barrett’s Oesophagus patients.

22. Effect of carrot consumption on intestinal cancer risk
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Several dietary factors have been suggested to play important roles in colorectal cancer risk. High intake of vegetables and fruits is associated with a reduced risk of the disease, attributed to the bioactive compounds they contain. Carrots contain bioactive compounds including polyphenols, carotenoids and falcarinol-type polyacetylenes with many functional characteristics (antifungal, anti-allergenic, cytotoxic, anti-platelet...
aggregation and anti-inflammatory) which may indicate cancer preventive effects. Mutations in the adenomatous polyposis coli (APC) gene are common in intestinal cancers, with loss of normal function of this gene being linked to disease induction in humans. Apcmin/+ mice carry a dominant germ line heterozygous mutation of the mouse homologue of human APC gene, leading to the development of intestinal adenomas, providing an appropriate model to investigate colorectal cancer. We hypothesised that carrot consumption in utero and during early neonatal life may affect tumorigenesis in offspring. To investigate this hypothesis, female C57Bl6/J mice were randomised to a control (RM3) or carrot enriched (RM3 supl) diet from mating with Apcmin/+ sires and throughout pregnancy and lactation. At weaning offspring were randomised to carrot and control diets, creating four in utero/post-weaning dietary regimens adhered to by both Apcmin/+ and wild-type offspring. Offspring were weighed weekly and at 15 weeks post-weaning, mice were killed and the intestinal tumour number and size were recorded, along with body and organ weights. Preliminary data suggests that carrot diets may influence tumour load in the Apcmin/+ mice, although the differences observed until now were not significant, however data collection is on-going and will be presented.

23. A ‘Nutrient-wide association study’ approach to identify dietary risk factors for endometrial cancer
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Dietary factors may explain some of the observed differences in endometrial cancer incidence rates worldwide, which are highest in industrialized countries; however, there are currently no modifiable dietary factors that have been identified for the prevention of endometrial cancer (EC). We therefore identified an agnostic approach to evaluate risk associations for intakes of 58 foods/nutrients measured using the baseline food frequency questionnaire in the European Prospective Investigation into Cancer (EPIC) study. Our analytical approach utilizes methods from genome-wide association studies and extends these to evaluate associations between dietary intake and risk of disease while accounting for multiple comparisons using the False Discovery Rate (FDR) [1,2]. In analyses of 1,303 EC cases and 299,804 non-cases, 10 foods/nutrients were associated with risk of EC (FDR < 10%); a high intake of monounsaturated fat, polyunsaturated fat, carbohydrates, potatoes and yogurt increased risk of EC, while high intake of total fat, monounsaturated fat, polyunsaturated fat, phosphorus, coffee and cheese decreased risk of EC. Using Cox proportional hazards regression to estimate hazard ratios adjusted for estradiol and estrone (ORq4-q1 =2.52, 95% CI: 0.60-3.99; P-trend<0.001), free estradiol (ORq4-q1 =0.45, 95%CI: 0.29-0.71; P-trend<0.001) and total estradiol (ORq4-q1 =0.62, 95%CI: 0.41-0.95; P-trend<0.12) were inversely associated with colorectal cancer risk. SHBG levels were positively associated with colorectal cancer (ORq4-q1 =2.31, 95%CI: 1.48-3.60; P-trend<0.001); this association was strengthened after further adjustment for estradiol and estrone (ORq4-q1 =2.52, 95%CI: 1.60-3.99; P-trend<0.001). No association was observed for progesterone (ORq4-q1 =0.96, 95%CI: 0.66-1.40; P-trend=0.92). Exogenous estrogen use was linked with reduced colorectal cancer risk, However, the role of endogenous sex hormones in colorectal cancer development is uncertain. Associations between circulating levels of estradiol, estrone, progesterone and sex hormone-binding globulin (SHBG) and colorectal cancer risk were determined in a nested case-control study of 1,203 post-menopausal women (401 cases and 802 age and ethnicity-matched controls) enrolled in the Women’s Health Initiative Clinical Trial (WHI-CT) non-intervention groups. Odds ratios (OR) and 95% confidence intervals (CI) for the association between the serologic parameters and colorectal cancer risk were estimated using conditional logistic regression models with control for colorectal cancer risk factors including fasting insulin, C-reactive protein and free insulin-like growth factor-I levels. All statistical tests were two-sided and a P-value of <0.05 was considered statistically significant. Comparing extreme quartiles, estrone (ORq4-q1 =0.47, 95%CI: 0.31-0.72; P-trend<0.001), free estradiol (ORq4-q1 =0.45, 95%CI: 0.29-0.71; P-trend<0.001) and total estradiol (ORq4-q1 =0.62, 95%CI: 0.41-0.95; P-trend<0.12) were inversely associated with colorectal cancer risk. SHBG levels were positively associated with colorectal cancer (ORq4-q1 =2.31, 95%CI: 1.48-3.60; P-trend<0.001); this association was strengthened after further adjustment for estradiol and estrone (ORq4-q1 =2.52, 95%CI: 1.60-3.99; P-trend<0.001). No association was observed for progesterone (ORq4-q1 =0.96, 95%CI: 0.66-1.40; P-trend=0.92). Exogenous estrogen levels were inversely, and SHBG levels positively, associated with colorectal cancer risk, even after control for established colorectal cancer risk factors. These results are consistent with observational and clinical trial data on exogenous estrogen use and colorectal cancer risk and support a potential estrogen-mediated protective pathway in colorectal tumorigenesis.

25. Repeat element methylation in blood and breast cancer risk
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References
A aberrant DNA methylation of repeat elements in non-cancerous tissue has been reported in cancer (1). This study set out to investigate methylation of LINE-1, SAT-α and site specific Alu regions in blood from women with the most common form of invasive (invasive ductal carcinoma - IDC) and in situ (ductal carcinoma in situ - DCIS) breast cancer and matched disease-free women. All women with cancer were studied prior to treatment and matched with disease-free controls for age, height, weight, BMI and menopausal status. Pyrosequencing of LINE-1, SAT-α, Alu-IGF2 enhancer (two CpG groupings), and Alu-IGF2 intron in blood DNA was completed for 608 subjects. Logistic regression was used to determine the effect of methylation on the risk of breast cancer and disease type. Average methylation in all subjects was 83.44% (SD=2.45) for LINE-1; 87.03% (SD=1.11) for SAT-α; 74.22% (SD=2.94) for Alu-IGF2 enhancer 1; 96.69% (SD=2.99) for Alu-IGF2 enhancer 2 and 66.58% (SD=4.43) for Alu-IGF2 intron. Increasing Alu-IGF2 enhancer 1 methylation was associated with increased risk of IDC cancer compared to controls (p=0.01) and DCIS cases (p=0.047). No other significant differences were observed. Increased Alu-IGF2 enhancer 1 methylation in blood of women with IDC breast cancer suggests that this region could play a role in tumour development. Expression of IGF2 has previously been associated with development of breast cancer (2) and the altered Alu methylation observed in this region could be involved in regulating this process. Breast cancer risk has also been related to birth weight, with repeat element and imprinting methylation also associated to birth outcomes (3). We are grateful to Breast Cancer Campaign and the Fraserburgh Moonlight Prowl for support.

References


26. Prediagnostic epigenetic markers of Non-Hodgkin lymphoma revealed through genome-wide DNA methylation analysis

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The etiology and pathogenesis of non-Hodgkin lymphoma (NHL) are largely unknown. In the context of the European EnviroGenomarkers project, we have conducted a genome-wide examination of DNA methylation in blood leukocytes prediagnostically (upto 12 years before disease diagnosis) collected from NHL cases and controls derived from the Northern Sweden Health and Disease Study (NSHDS) and EPIC-Italy, and evaluated their relationship with future risk of different subtypes of NHL as well as with biomarkers of exposure to polychlorinated hydrocarbons. Using NSHDS as a discovery cohort (173 cases, 177 controls), we found 7,017 CpG sites whose methylation was associated strongly (Bonferroni-corrected p<0.01) with risk of chronic lymphatic leukemia (CLL, 24 cases). Use of EPIC-Italy (83 cases, 82 controls) as a validation cohort for these signals confirmed that 2,195 (31%) of them associated with CLL risk with FDR<0.05. Most of the replicated signals were associated with hypomethylation in cases. Bioinformatics analysis revealed effects on numerous GO terms and pathways related to immune function, with the top pathway affecting being the EGFR1 activation pathway, known to be important in leukemogenesis. Examination of the DNA methylation profiles of the study subjects with the serum concentrations of various polychlorinated hydrocarbons revealed numerous signals associating (FDR<0.05) with exposure to dioxin-like PCBs (358 CpG sites) and, especially, hexachlorobenzene (HCB) (3,821 sites). Bioinformatics analysis of the HCB-associated signals showed effects on numerous GO terms and pathways related to immune function including, strikingly, EGFR1. These results show that CLL-specific epigenetic signals are present in blood cells many years before disease diagnosis and provide a new approach to the evaluation of the possible role of environmental chemicals in disease pathogenesis. Financial Support: European Union (Grant Agreement 226756).

27. Differential methylation related to response to etanercept in patients with rheumatoid arthritis

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The introduction of biologic drug therapies targeting specific components of the inflammatory response represents a huge advance in the treatment of rheumatoid arthritis (RA). Despite this, up to 40% of patients fail to respond well, making identification of reliable biomarkers of response an important area of research. Recent studies suggest that epigenetic control of gene expression may be important in RA; we have therefore hypothesized that differential DNA methylation patterns may provide useful biomarkers of response to biologics. To identify a DNA methylation signature indicative of response to TNF-blockade therapy in patients with RA. Patients were recruited from the Biologics in Rheumatoid Arthritis Genetics and Genomics Study Syndicate (BRAGGSS) longitudinal cohort. Patients (n=72) were selected based on having an extreme good or poor response phenotype after 3 months of treatment with etanercept. Whole blood DNA from each patient, sampled before initiation of etanercept therapy, was bisulfite converted and an epigenome wide association study was conducted using the HumanMethylation450 BeadChip (Illumina). Differentially methylated positions between responders and non responders were identified by linear regression following quantile normalisation. A technical validation of the top five most differentially methylated positions was
performed using pyrosequencing. 4 CpG sites showed differential methylation between responders and non-responders to etanercept passing a false discovery rate of 0.05 (p≤10^-7): cg04857395, cg16426293, cg03277049, cg14862806. The most differentially methylated probe mapped to LRPAP1, which is highly expressed in mononuclear cells and encodes a chaperone of LRP1, which is known to influence TGF-β activity. The BeadChip data showed very good correlation with the technical validation using pyrosequencing (correlation >0.8 for 4 of 5 probes tested). This is the first methylome wide investigation of treatment response to TNF blockade therapy in RA and, while further well powered studies are required, these preliminary data identify methylation biomarkers of response.