86 Exposure to environmental tobacco smoke in childhood and incidence for selected cancers in adulthood: an analysis in the European prospective investigation into cancer and nutrition

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Introduction: Exposure to environmental tobacco smoke (ETS) during childhood has been linked to childhood respiratory illness and childhood cancers. Some studies claimed that exposure during childhood, when the target organs are still growing, is important for health later in life. However, observations on adulthood cancers in previous studies were not consistent. Here we explore the association between childhood ETS exposure and adulthood cancer development among never smokers in a large cohort.
Material and Methods: We examined the association between childhood ETS exposure and the most frequent cancers within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. We selected the sites that accumulated at least 500 cases by 2004: upper aero-digestive tract, stomach and esophageal, colorectal, pancreas, lung, breast, cervix, endometrial, ovary, prostate, bladder, kidney, brain, thyroid cancers, and lymphoma. The analyses were restricted to never smokers (N = 112,430). Hazard ratios (HR) and 95% confidence intervals (CI) were estimated by Cox proportional hazard model stratified by age, sex, and center. Models were adjusted for education, body mass index, physical activity, total energy intake, vegetable intake, and fruit intake. Whenever applicable, models were further adjusted for or stratified by adulthood ETS exposure, previous respiratory illness, or family history (for breast and colorectal cancers).
Results: Higher risks were observed for lung (HR = 2.87, 95% CI = 1.20–6.91) and pancreatic cancers (HR = 3.18, 95% CI = 1.30–7.76) comparing childhood ETS exposure daily for many hours to never or seldom. A higher risk for brain cancer (HR = 1.98, 95% CI = 1.06–3.71) was also observed for those exposed a few times during a week, albeit non-significant for those exposed many hours daily (N = 4, HR = 1.75, 95% CI = 0.60–5.10).
Conclusions: We observed a higher risk of adulthood lung, pancreatic, and brain cancers for those who self-reported childhood ETS exposure in never-smokers, after adjusting for well-known confounders, such as education (as a proxy for socio-economical status) or adulthood ETS exposure. Further work includes examining whether other lifestyle risk factors, such as diet and physical activity, and reproductive factors (for female cancers) would modify the observed associations.

87 NTX and VEGF in cancer patients with bone metastases treated with zoledronic acid

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Background: Patients with breast, lung, and prostate cancer frequently develop bone metastases (BM), which are responsible for high morbidity and reduced quality of life. Zoledronic acid (Zometa®, Zol), routinely used to treat patients with BM, inhibits bone resorption and has antitumour properties. It has also been reported to have an antiangiogenic effect.
Material and Methods: The study prospectively evaluated serum levels of vascular endothelial growth factor (VEGF) and cross-linked N-telopeptide of type I collagen (NTX) in 45 consecutive patients with advanced breast, lung, or prostate cancer. Patients were eligible if they were at first diagnosis of bone metastases and if they had not previously undergone bisphosphonate treatment. All patients received the standard Zol schedule of a 4 mg infusion every 28 days. Patients were monitored for about 9 months and blood samples were collected before the first infusion of Zol and every 3 months thereafter.
Results: The baseline VEGF median value was 318.9 pg/ml (interquartile [IQ] 182.4–656.1). Median value at 3 months was 345.2 pg/ml (IQ 182.8–656.1), 329.5 pg/ml (IQ 183.7–568.8) at 6 months and 395.4 pg/ml (IQ 253.2–702.9) at 9 months, none of which reached statistical difference. Interestingly, when only patients with prostate and lung cancer were considered, VEGF levels had decreased by 35% at 3 months. NTX median values significantly decreased with respect to baseline (median value 15.9, range 129–22.9 nm BCE) at 3 (10.2 nm BCE, range 7.0–12.7) and 6 months (10.3 nm BCE, range 6.8–13.3) ($p < 0.001$), but not at 9 months (12.0 nm BCE, range 11.1–14.2). Serum NTX median values at 3 months were 35% less than those of baseline values. Blood samples at 6 and 9 months showed a decrease of 39% and 26% with respect to baseline, respectively. There was no correlation between VEGF and NTX values.
Conclusions: The present prospective study focused on serum markers that are potentially associated with bone metastases. Our results show that standard monthly treatment with zoledronic acid induced a rapid and long-lasting decrease in NTX levels in the majority of our patients. Conversely, conclusions on the VEGF analysis cannot be drawn perhaps because of the small number of cases involved.

88 Genetic polymorphisms in the MMP2 and MMP9 genes decreased lung cancer risk

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Background: Lung cancer constitutes one of the leading causes of death in worldwide, approximately one million people per year, 850.000 men and 330.000 women. Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes involved in process of tumour progression due to its ability to degrade components of the extracellular matrix. However, recent studies have shown that they are involved in all stages of cancer progression not only in process of tumour invasion and metastasis, but also in cancer development processes such as proliferation, adhesion, migration, differentiation, angiogenesis, senescence, autophagy, apoptosis and evasion of the immune system. Unexpectedly, recent studies based on the generation of loss-of-function animal models have provided definitive evidence of the existence of MMPs with anti-tumour properties. These results support an emerging and paradoxical role of MMPs in tumour progression. Genetic variants in the MMP genes may influence the biological function of these enzymes and change their role in carcinogenesis and progression. We have investigated the association between three polymorphisms (−735 C/T, −1562 C/T and 5A/6A) in two human gellatinases (MMP2 and MMP9) and one human stromelysin (MMP3) and the association with development or progression of lung cancer.
Material and Methods: The CAPUA (Lung cancer in Asturias) study is a hospital-based case-control study was designed including 762 lung cancer patients and 649 controls. Genotypes were determined by PCR-RFLP. Results were analyzed using unconditional logistic regression and the Kaplan–Meier method.
Results: The *MMP2* and *MMP9* promoter polymorphisms were associated with lung cancer risk. The *MMP9* T/T genotype was associated with a statistically significant decreased risk of developing lung cancer (ORadj = 0.29; 95% CI = 0.10–0.85) while *MMP2* T/T genotype was associated with a no statistically significant decreased risk (ORadj = 0.52; 95% CI = 0.22–1.26). No association was identified between *MMP3* promoter polymorphism and lung cancer risk (ORadj = 0.98; 95% CI = 0.68–1.41). The Kaplan–Meier analysis showed that the polymorphisms in *MMP9*, *MMP2* and *MMP3* not seem to modify the overall survival.
Conclusions: This study confirms that polymorphism in MMP9 have a protector effect on lung cancer risk, which can be used as a prognostic marker in lung cancer and also lead to more effective cancer therapeutics.

89 Influence of functional genetic variants of TGFβ1/TGFβR2 pathway in prostate cancer development

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Background: The transforming growth factor beta 1 (TGFβ1)/TGFβ type 1 and 2 receptors (TGFβR1−2) signaling pathway play an important role in prostate cancer (PC) development and progression through the regulation of cell proliferation and differentiation. The functional polymorphisms TGFB1 +869T>C and TGFBR2 −875G>A, have been associated with higher circulating levels of TGFβ1 and modified TGFBR2 transcriptional activity. Different levels of these molecules can modulate cellular microenvironment influencing TGFβ1/TGFβR1−2 pathway activation, with impact in PC oncobiology. Our purpose was to investigate the role of *TGFB1 +869T>C* and *TGFBR2 −875G>A* functional polymorphisms in PC risk.
Material and Methods: We conducted a case-control study in histopathologically individuals with confirmed PC (n = 688) and benign prostate disease (n = 378). *TGFB1 +869T>C and TGFBR2 −875G>A* polymorphisms were detected by allelic discrimination using Real-Time PCR with TaqMan® SNP Genotyping Assay.
Results: Frequencies of CC/CT genotypes for *TGFB1+869T>C* polymorphism were significantly lower in PC patients compared with control group (62% and 71%, respectively), carriers of C allele had a protective effect for PC development (age adjusted Odds Ratio (aOR) = 0.68, 95% Confidence Interval

(95% CI) = 0.51–0.89, $P = 0.006$). The *TGFBR2–875G>A* polymorphism frequencies for homozygous GG and GA/AA were 63% and 37% in PC group and 62% and 38% in the control group, respectively. We found lack of statistical significant association of *TGFBR2* genetic variants with PC risk (aOR = 1.05, 95% CI = 0.80–1.38, $P = 0.731$).

Conclusions: Our results show a protective effect associated with C allele (*TGFB1+869T>C*) for PC development. Functional polymorphisms that influence cellular microenvironment may help determine individual higher risk genetic profiles, which can impact PC diagnosis and chemoprevention strategies.

90 NQO1 polymorphism, maternal exposure and the risk of infant leukemia

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Introduction: Chromosomal abnormalities associated with infant leukemias (IL) originate during fetal life and often involve rearrangements of the *MLL* gene. The finding that similar abnormalities develop in children and adults treated with inhibitors of topoisomerase II (topo II) has led to the hypothesis that maternal exposure to topo II inhibitors, such as pesticides and benzene metabolites, during pregnancy might induce infant leukemias. NAD(P)H:quinone oxidoreductase 1 (NQO1) protects cells against oxidative stress and toxic quinones. A C609T polymorphism in the *NQO1* gene destabilizes and inactivates the enzyme and it has been reported as a susceptibility factor to IL. Taken together, infant and maternal genotypes of *NQO1*, in combination with exposure, could be important in etiology of IL. The aim of this study was to explore *NQO1* polymorphism in IAL with *MLL* translocation and, also evaluate mothers' genotypes in relation to different exposures during pregnancy.

Materials and Methods: The study population comprised 332 children (ages, 0−24 months-old), being 143 IL and 189 aged-matched controls. Samples from 177 mothers, who answered an epidemiological questionnaire, were also genotyped. Cases were diagnosed according to standard classifications. *MLL* characterization was done by reverse transcription-PCR and/or by fluorescence *in situ* hybridization. The *NQO1* C609T polymorphism was evaluated by PCR-RFLP. Statistical analyses were done using the SPSS 15.0 software. The differences in the genotype distribution between patients and controls, and across mothers of cases and controls were tested by logistic regression analysis to calculate ORs and 95% confidence intervals (CIs).

Results: Fifty eight percent of infants were positive for *MLL* rearrangements. We found the following CT + TT genotypes frequencies: 48.1% for controls and 45.4% for cases, whereas 47.8% for cases' mothers and 45.9% for controls' mothers. There was no difference across cases and controls in relation to *NQO1* genotypes frequencies [OR = 1.28; CI 95%, 0.82–1.99], nor even for mothers [OR = 1.93; CI 95%, 0.61–6.11]. Children with CT or TT genotypes didn't appear to be more prone to have *MLL* translocations [OR = 0.90; CI 95%, 0.43–1.86].

Conclusion: These preliminary results didn't show any association between *NQO1* polymorphism and *MLL* rearrangements. We noticed a higher CT + TT genotype frequency in cases' mothers but it is not statistically significant. Also, we observed that mothers who are exposed to hormones and pesticides during pregnancy have a higher risk to give birth to children who later developed leukemia. We believe that further analyses increasing the sample size may be able to demonstrate an association across mother's genotypes.

91 Analysis of BRIP1 in italian male breast cancer patients

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Background: Male breast cancer (MBC) is a rare disease compared to female breast cancer (FBC). MBC shares many similarities with FBC, including genetic predisposition factors such as *BRCA1/2* mutations. The frequency of *BRCA1/2* mutations ranges between 4 and 40% for *BRCA2* and up to 10% for *BRCA1* in different MBC series, thus suggesting the contribution of additional susceptibility genes.

Several studies identify *BRIP1* (BRCA1-interacting protein 1, also known as BACH1 and FANCJ) as a moderate-penetrance breast cancer (BC) susceptibility gene, accounting for about 1% of *BRCA1/2* negative familial/early-onset BCs. *BRIP1* encodes a DEAH helicase which interacts with the BRCT domain of BRCA1 and has BRCA1-dependent DNA repair and checkpoint functions. Interestingly, there are evidences that *BRIP1* might also play a role in susceptibility of prostate cancer, a tumour which may share risk factors with MBC. However, the role of *BRIP1* in MBC susceptibility is still unknown. In this study, we aimed to assess whether *BRIP1* alterations may contribute to MBC risk in Italy.

Material and Methods: We performed a mutational screening in 70 Italian MBC cases, negative for *BRCA1/2* mutations, selected from a population-based series of 123 MBCs. The complete coding region and intron-exon boundaries of *BRIP1* were analyzed by using SSCP (Single Strand Conformation Polymorphism). Cases displaying abnormal SSCP patterns were evaluated by direct sequencing. Statistical analysis was performed using the chi-square test.

Results: No truncating mutations were found. Two previously reported variants in the BRCT binding domain (E879E and P919S), and a neutral intronic variant (IVS4−28G>A) were identified. In order to evaluate the putative influence of the *BRIP1* P919S variant on MBC risk, we carried out a population-based case-control study based on a total of 97 MBC cases and 130 healthy adult male population controls from the same area. The frequency of the rare allele in cases was 36.2%, compared to 33.5% in population controls. No statistically significant difference in the distribution of the three specific *BRIP1* P919S genotypes was observed between MBC cases and controls ($p = 0.7$).

Conclusions: Overall, our results suggest that *BRIP1* do not play a major role in MBC susceptibility in Italy. However, larger studies are needed to explore its potential role as low risk gene.

92 Role of EGFR, HER2 and PIK3CA alterations in male breast cancer

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Background: EGFR and HER2 are tyrosine kinase receptors that activate different pathways, including PIK3-Akt, involved in cell proliferation, migration and survival. Thus EGFR, HER2 and PIK3 can play a relevant role in tumourigenesis, by mediating processes involved in neoplastic progression. *EGFR, HER2* and *PIK3* are frequently alterated in breast cancer (BC). EGFR and HER2 are amplified or over-expressed in about 20−40% of BC and mutations at their kinase domains are observed in about 2−4% of BC. Mutations at helical and kinase domains of *PIK3CA* gene, coding the catalitical subunity of PIK3, are reported in 8−40% of BC.

Male BC (MBC) is a rare and less investigated disease compared with female BC (FBC). Current knowledge on MBC biology is mainly derived from FBC. MBC shares many similarities with FBC, including genetic predisposition factors.

To date, the role of *EGFR*, *HER2* and *PIK3CA* alterations in MBC is very limited. Taking into account that EGFR, HER2 and PIK3CA have both prognostic and predictive value in BC, studies on the role of these genes could have important implications in the elucidation of pathogenetic mechanisms of MBC and in the clinical management of MBC patients.

Material and Methods: This study was performed on a series of 102 MBC cases characterized for clinicopathological features and *BRCA1*/*BRCA2* germ-line mutations. We have analyzed the presence of somatic mutations, amplification and expression of *EGFR*, *HER2* and *PIK3CA* by SSCP and automatic sequencing, qRT-PCR and IHC respectively.

Results: A mutation frequency of 4% was observed for *PIK3CA.* In our series *PIK3CA* common mutation (E545K) was identified in three different cases and a novel mutation (S553X) in one case. Interestingly, all tumours harboring *PIK3CA* mutations were ER+/PR+, in agreement with data obtained in FBC. Moreover *PIK3CA* resulted amplified with a frequency of 16%. No pathogenetic mutations were identified in *EGFR* and *HER2* genes but *EGFR* resulted amplified in 17% and HER2 over-expressed in 27.8% of cases and a statistical significant association emerged between HER2 over-expression and PR− ($p = 0.022$), MIB+ ($p = 0.028$), G3 ($p = 0.001$).

Conclusions: Our data indicate that alterations of *EGFR*, *HER2* e *PIK3CA* are involved in the pathogenesis of MBC at a comparable level as in FBC. Over-expression of HER2 allows the identification of a subgroup of MBC cases with specific pathological and biological characteristics indicative of aggressive clinical behavior.

93 Are variations in Helicobacter pylori cag pathogenicity island-genes associated with neoplastic progression in gastric cancer?

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Background: Helicobacter pylori is a bacterium that colonizes the human stomach and can establish a long-term infection of the gastric mucosa. Hp infection affects over 50% of the worldwide population, with a prevalence ranging from 20% in developed countries to over 90% in developing countries. Persistent Hp infection often induces gastritis and is associated