71 Rare, evolutionary unlikely missense substitutions in CHEK2 confer increased risk of breast cancer

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Background: The *CHEK2* gene (cell cycle checkpoint kinase 2) is a breast cancer susceptibility gene. It has been reported that the *CHEK2* 1100delC allele confers a 2−3 fold increased risk of breast cancer. Other rare variants (of frequency <1%) have been identified but classical association tests used in epidemiological studies are underpowered to reliably assess the risk attributable to this type of variants.
Material and Methods: We mutation screened the entire coding sequence of the gene and flanking boundaries in 1,331 breast cancer cases and 1,123 controls recruited through the population-based Breast Cancer Family Registries (BCFR) from Ontario, Northern California and Australia. Mutation screening data from 95 Canadian familial breast cancer cases and 95 controls were also included in the analyses. Using an *in silico* missense substitution analysis that provides a ranking of missense substitutions from evolutionarily most likely to least likely (Align-GVGD), we carried out analyses of protein truncating mutations (TM), splice junction mutations (SJM), and rare missense variants (rMS). Our analysis strategy of case-control mutation screening data has been successfully applied to the breast cancer susceptibility gene *ATM* to demonstrate that a subset of rMS confers intermediate risk of the disease.
Results: As already demonstrated by many others, we observed that *CHEK2* truncating variants (TM) are significantly associated with an increased risk of breast cancer. In addition, using a log-linear trend test across non-carriers and carriers of the rMS stratified in 7 grades with Align-GVGD program, we also found significant association of rMS with breast cancer risk.
Conclusion: Those *CHEK2* data reinforced our conclusion from similar work performed on the *ATM* gene where we showed that a comparison between the graded distributions of missense substitutions in cases *versus* controls can complement analyses of truncating variants to help identify susceptibility genes, and that this approach will help interpret the data emerging from new sequencing technologies.

72 Black and green tea-drinking and prostate cancer risk: a case-control study in Singapore

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Background: The incidence of Prostate cancer in Singapore has been rising; especially from 1990s, and is now the third most common cancer among Singapore males, with a world age-standardized incidence rate (ASR) of 22.0 per 100,000 per years from 2002 to 2006. Recently, there are reports about the possible opposing effects of black and green tea-drinking and prostate cancer risk. We aim to evaluate the role of black and green tea-drinking as independent risk factors for prostate cancer in Singapore.
Material and Methods: This case-control study is being conducted in one of Singapore's tertiary hospitals, where the Urology department sees approximately 60% of all prostate cancer patients who consult at public hospitals. The cases were Singaporean residents, age 50 to 85 years old, with histologically confirmed diagnosis of prostate cancer. Controls were hospital inpatients from participating wards, without prostate cancer or history of other cancers, in the same age group criteria. Participants were asked in a face-to-face interview about their tea consumption using a structured questionnaire. The risk of prostate cancer was assessed using multivariate logistic regression.
Results: Our study population consisted of 240 cases and 268 controls. The characteristics of cases and controls are fairly comparable in terms of ethnicity and age. Green tea and black tea consumption were analyzed separately. Among the cases, 75.7% were black tea drinkers compared to 72.3% of the controls. The adjusted odds ratio [ethnicity, age & BMI], OR 1.55 (95% CI = 0.93–2.59) showed no significant association between black tea drinkers and prostate cancer. 56.4% of the cases were green tea drinkers compared to 52.8% of the controls. The adjusted odds ratio [ethnicity, age & BMI], OR 1.45 (95% CI = 0.93–2.27) showed no significant association between green tea drinkers and prostate cancer.
Conclusion: Our results show that there is no association between black or green tea consumption and prostate cancer risk. This finding may be influenced by the small sample size of the study. We are in the process of conducting a population based case-control study of prostate cancer in Singapore.

73 Inherited invasive lobular breast carcinomas without diffuse gastric cancer as a special phenotype of CDH-1 germ-line mutation

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Background: Germ-line mutations of the CDH1 gene have been extensively studied in hereditary DGC (diffuse gastric cancer) families. We report a large family harboring a deleterious CDH1 germ-line mutation with predominant ILBC (invasive lobular breast cancer) cases but without pathological confirmed DGC cases. Considering the up-date literature, this is the first strong hint of the involvement of germ-line CDH1 mutations in inherited ILBC.
Material and Methods: The family is of Caucasian origin, with 5 women affected by breast cancers, 3 of them are histological confirmed ILBCs, including 2 bilateral ILBCs. There is no DGC diagnosis in the history of the family. Complete screening of CDH1, BRCA1 and BRCA2 genes was performed on 7 cases. Expression of E-cadherin was evaluated on available paraffin preserved tumour samples using standard immunohistochemical techniques. Key words "CDH1, germ-line mutation, lobular breast cancer, diffuse gastric cancer" were employed for searching literatures in Pubmed. and Google scholar. Cases with pathologically confirmed LBC and DGC reported in the literatures were identified for comparison.
Results: No BRCA1 and BRCA2 mutations were found in the family. A CDH1 Q95X germ-line mutation was found in all the 3 living women with ILBC and in a 71-year-old healthy male member. The mutation was also present in 2 obligate carriers, one of whom developed pancreatic cancer at the age of 56, the other die of accident. Immunohistochemistry showed that tumour cell in all the samples were negative for E-cadherin expression, while adjacent normal breast tissue was positive. The literatures reported one synchronous and four metachronous ILBC and DGC cases, all from HDGC families; the lapse from the diagnosis of ILBC to DGC was 3−9 years, with ILBC always occurring first in metachronous cases. Two of the three living ILBC cases in the present family were diagnosed more than 10 years ago. In each previously reported family, the CDH1 germ-line mutation was confirmed in only one case of confirmed ILBC, which may be due by chance and precludes any firm conclusion about its role in the inherited ILBC.
Conclusions: Many biological results suggest CDH1 mutations are key events in the ILBC genesis. We report the first case confirming the involvement of CDH1 germ-line mutation in inherited ILBC without DGC cases, that therefore may be considered to be a special phenotype of CDH1 germ-line mutation. The recommended systematic prophylactic gastrectomy in CDH1 mutations carriers should therefore be discussed according to the cancer family histories.

74 Genotoxicity biomarkers in occupational exposure to formaldehyde in pathology anatomy laboratories

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The International Agency for Research on Cancer (IARC) classifies formaldehyde (FA) as carcinogenic to humans (group 1), on the basis of sufficient evidence both in humans and in experimental animals. Manifold *in vitro* studies clearly indicated that FA is genotoxic, inducing various genotoxic effects in proliferating cultured mammalian cells.
The aim of this study was to compare 56 workers exposed to FA with 85 non-exposed controls using the multi-endpoint cytokinesis-blocked micronucleus assay (CBMN). This method allow to identify micronucleus (MN), nucleoplasmic bridges (NPB) and nuclear buds (NBUD) in peripheral blood lymphocytes isolated from blood samples collected by venipuncture and stained with Mäy-Grunwald Giemsa. Buccal cells were also collected with an endobrush and MN were measured after Feulgen technique.
Statistically significant differences (Mann–Whitney test, $p < 0.002$) were observed between subjects exposed and non-exposed to FA, namely in the mean and mean standard error of MN in lymphocytes (3.96 ± 0.525 *vs* 0.81 ± 0.172), in NPB (3.04 ± 0.523 *vs* 0.18 ± 0.056), in NBUD (0.98 ± 0.273 *vs* 0.07 ± 0.028) and MN in buccal mucosa (0.96 ± 0.277 *vs* 0.16 ± 0.058), respectively.
All the biomarkers in study showed statistical significant association with FA exposure therefore we can conclude that is chemical agent is a risk factor in subjects exposed occupationally to FA in pathology anatomy laboratories. The nuclear abnormalities found in lymphocytes can be explained by the fact of FA escape from the local of first contact, such as the mouth, direct contact place, where MN was found too.