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Systematic and computatioanl analysis of ATM mutation screening data

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The answer to the question "what sort of sequence variation in ATM gene confers increased risk of breast cancer (BC)" was controversial.

To address this question, we pooled available ATM mutation screening data by measuring risk attributable to rare missense substitutions (MSs) in ATM gene requiring a substantially complete mutation screening of ATM in an ascertained set of 1,729 cases and 941 controls from 13 studies. We carried out a computational molecular biology-based analysis of protein truncating mutations (TMs), splice junction mutations (SJMs), and rare MSs (carrier. frequency ≥1%). To construct a protein multiple sequence alignment (PMSA) of ATM orthologs, a combination of BLAST and splice junction predictions was used to build gene models from the available genomic sequences, and then amplified by PCR from cDNA and sequenced. The PMSA used for the analyses was built by MCoffee suite, checked for anomalies, and re-aligned.

The raw scores of each in silico assessed MS was converted into a 7-stratum classifier (C65, C55, C45, C35, C25, C15, C0), which provides an ordered ranking from the most likely MSs to the least likely MSs to confer increased risk of BC. Our case-control analysis of the MSs indipendantly provided evidence that ATM is a BC susceptibility gene. A combined analysis of TMs, SJMs, and rare MSs provides stronger evidence that ATM is a BC susceptibility gene than simple consideration of TMs plus SJMs alone. There was a significant evidence of relatively high-risk both in the TMs and SJMs and in the in silico predicted highest-risk class C65 MSs but little to no risk in the C25-C0 classes of MSs. Despite the fact that all of the TMs and SJMs damage ATM function and are harmful to patient health, this cannot be said for the C35-C65 MSs, some of which confer odds ratios equivalent to TMs whereas others are probably neutral.

Based on the measured considerable risk attributed to rare MSs, we reach the conclusion that these variants as a group in ATM play an important role in BC risk. This is quite distinct from the clinical cancer genetics conclusion that any individual MS in the C35-C65 pools is actually harmful to patient health. Thus, careful analysis of MSs by measuring risk attributable to rare MSs in a susceptibility gene provides a better estimate of the case-control carrier frequencies of the mutations than an analysis that ignores them.

Risk estimates by data treatment

Class	OR	Cases	Controls
TMs+SJMs	4.20	23	3
C65 MSs	3.15	31	5
C35-C55 MSs	2.36	9	2
C15-C25 MSs	0.76	11	9
C0 MSs	1.30	78	56
No rare variant	1.0 (ref)	1577	866

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A proteomic approach for identification of plasma biomarkers of hepatocellular carcinoma in the Gambia

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Background: We are conducting a proteomic pilot case-control study, necessary for the development of a program that allows for early detection and diagnosis of Hepatocellular Carcinoma (HCC) in The Gambia.

The is a country in West Africa where chronic hepatitis B virus infection (HBV) is endemic and where there is a high dietary exposure to aflatoxin. These are two of the major risk factors for HCC, the most frequent form of primary liver cancer and a major cause of cancer death worldwide. In The Gambia, HCC is by far the most prevalent cancer form in males and the second in women.

A nationwide childhood vaccination programme initiated in The Gambia in 1986 (The Gambia Hepatitis Intervention Study, GHIS) has decreased the number of chronic HBV carriers. However, the average age at presentation of HCC is 40 years and the increased length of life and improved diagnostics has led to an increase in the number of cancer incidences in West Africa, especially among women.

Materials and methods: The pilot study consisted of nine HCC patients, five cirrhosis patients and eleven hospital-based, matched controls (the study was approved by the ethics committees of The Gambian Government/MRC laboratories and IARC, and the participants provided informed consent). HCC and cirrhosis diagnosis was based on the combination of clinical examination, ultrasonography examination and alpha-fetoprotein concentration.

Plasma samples were collected and analyzed in Dr Beretta's laboratory at Fred Hutchinson Cancer Research Center, Seattle. The plasma samples were analyzed by the in-house developed pipeline, for detection of protein expressional differences between the HCC patients/ cirrhosis patients/ hospital-based controls. This pipeline utilizes multidimensional separation of intact proteins with particular attention to the isolation of low-abundance proteins and exhaustive liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. Protein abundance was estimated for each of the identified proteins.

Results: The extensive 3-dimensional separation method developed in Dr Beretta's laboratory for separation of intact proteins according to their charge, hydrophobicity, and molecular mass, has significantly improved the detection and allowed for an even deeper coverage of the liver and plasma proteomes (1). The method has a sensitivity that allows for the detection of proteins over 9 orders of magnitude in concentration in plasma (pg/mL to mg/mL).

Conclusions: We will present and discuss the preliminary results of the protein expression profiles of the HCC patients, the cirrhosis patients and the hospital based controls of the pilot study, using the described protein separation method.

References

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High frequency of the cancer-predisposing TP53 mutation R337H in the population of south Brazil - evidence for a founder effect

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The TP53 tumor suppressor gene serves as one of the major cellular barriers against cancer development. Indeed, as many as 50% of all human tumors contain somatic p53 mutations whereas the other half maintains a wild-type TP53 gene but acquires other genetic or epigenetic alterations that compromise the p53 response. Most of the mutations within the TP53 gene are missense mutations (75%) that result in the expression of full-length mutant p53 (mutp53) proteins lacking its specific DNA-binding activity and accumulating in the nucleus of tumor cells. This is quite unique as most other tumor suppressor genes are frequently inactivated by frame shift or nonsense mutations, leading to either production of truncated proteins or complete elimination of the corresponding gene products. Among the missense mutations, approximately 80% are found in the DNA binding domain, and about 30% occur at six mutation hotspots (R175, G245, R248, R249, R273, and R282). Germline TP53 mutations predispose to Li-Fraumeni (LFS) and Li-Fraumeni-like syndromes (LFL) characterized by a wide spectrum of early-onset cancers, including in particular soft tissue sarcomas, osteosarcomas, breast carcinomas and brain tumors. Though similar mutation hotspots are observed in somatic and germline contexts, a rare germline TP53 mutation at codon 337 (R337H) has been found in a number of apparently unrelated cancer-prone families in Southern Brazil. The spectrum of tumors associated with R337H is compatible with that of LFL, with, in particular, adenocortical carcinoma in children, soft tissue sarcomas, brain tumors and breast cancer in young adults. This mutation was claimed not to result from a founder effect based on limited analysis of 5 hypervariable loci on chromosome 17p, including only one within the TP53 region. We undertook complete mutation screening of the TP53 locus, from the proximal promoter to the 3' UTR, in 48 unrelated samples in order to completely reconstruct the common haplotypes of TP53. We determined the haplotypes across the whole gene and selected 29 TagSNPs out of 182 SNPs. Using the 29 tagSNPs, we haplotyped a population sampling of 92 individuals and 10 unrelated individuals. We found that all the R337H carriers shared a single haplotype. This haplotype has a frequency of 9% in the population sampled. The probability that all our carriers share this haplotype by chance is only 2.71*10-7. These findings demonstrate that the mutation results from a founder effect.