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Description and validation of high-throughput simultaneous genotyping and mutation scanning by high-resolution melting curve analysis

T. Nguyen[1], F. Lesueur[1], N. Forey[1], D. De Silva[2], R. Weigel[2], S. Tavtigian[1], F. Le Calvez-Kelm[1]
[1]International Agency for Research on Cancer (IARC), Lyon, France; [2] Idaho Technology Inc, Salt Lake City Utah, USA

A key step in the search for potentially deleterious genetic variants in known or candidate susceptibility genes is mutation screening of the exonic/coding sequences and splice sites of the entire gene in large subject series. Mutation scanning using high-resolution melting curve analysis has been described as an effective and sensitive method to detect sequence variations. However, the presence in a mutation screening amplicon of a common SNP may considerably complicate the interpretation of results and increase the number of samples flagged for sequencing by interfering with the clustering of melt curve groups according to melting profiles. A protocol describing simultaneous high-resolution gene scanning and genotyping has been reported. We aimed to validate the efficiency of this method in a large-scale case-control mutation study. The method is based on hybridization of unlabeled oligonucleotide probes designed to anneal to the common polymorphic site and blocked at the 3' end to prevent extension during PCR amplification. A single asymmetric PCR in presence of both LCGreen® Plus dye and unlabeled oligonucleotides probes leads to simultaneous production of probe-target and whole amplicon double-stranded DNA duplexes that can be analyzed from the same HR melting run. Common SNP genotypes are called from the probe-target melting data. Analysis of the whole amplicon melt curves (e.g., mutation scanning) is then performed separately on heterozygous and homozygous samples to distinguish curve shape differences due to other unknown variants. To validate this approach, we chose the 22nd and 36th coding exons of the ATM gene (NM_000051), both of which contain a common SNP that interferes with standard HR-melt mutation scanning. The assay was performed on 650 cases and 650 controls enrolled in an international breast cancer genetics study. The exon 22 amplicon contains a common 1bp deletion. The exon 36 amplicon is more challenging, as it contains a common SNP adjacent to an uncommon SNP. In both cases, we succeeded in identifying rare known and unknown variants while dramatically reducing the amount of sequencing required. In presence of common SNPs, combining genotyping and mutation scanning showed good accuracy for the identification of rare variants. This simple procedure greatly reduces the number of samples to sequence. Hence, high-resolution melting analysis is a rapid, efficient and cost-effective tool that can be used for high-throughput case-control mutation screening for research, as well as for molecular diagnostic and clinical purposes.

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Natural lgl-tumor supressor alleles in Drosophila: genetics and epigenetics of their stress-adaptive effects

N. Weisman[1], M. Golubovsky[2], N. Plus[3]
[1]Institute of Cytology and Genetics Siberian Division Russian Academy of Sciences, Population Genetics, Novosibirsk, Russian Federation; [2] University of California, Department of Molecular and Cell Biology, Berkeley, USA [3] Station de recherches de Pathologie comparée, St. Christol, Les-Ales, France

Drosophila tumor suppressor (TS) gene lethal (2) giant larvae (lgl) is the first case of monogenically controlled neoplasia found in animals (Gateff, 1978). The loss of lgl function leads to overgrowth of imaginal discs (benign tumor) and invasive malignant brain neuroblastoma. The Lgl protein turned out a new type of TS involved in signal transduction, asymmetric cell division and tissue architecture. lgl orthologues have been discovered in diverse species. Mutations of TS human homologue Hugl were found in about 70% of solid carcinoma. Surprisingly, our long-termed studies showed omnipresence of loss-of-function lgl alleles in heterozygotes in distant Drosophila populations. Most of natural lgl alleles appeared to be deletions. To address this population paradox we analyzed some parameters of biological adaptation of lgl loss-of-function heterozygous carriers both in normal and stress conditions. The summarized data include: (i) embryonic survival and aging dynamics in normal and soft temperature stress conditions, (ii) resistance to RNA lethal infectious DCV-virus, (iii) maternal and transgeneration effects of the one dose of lgl TS loss on the progeny survival and life span. Animals heterozygous on 15 natural lgl-alleles showed increased resistance to infectious highly virulent DCV virus. We demonstrated significant improvement in survival and aging for one dose of the TS-deficient animals namely in the temperature stress conditions. The effect was maternal and sex-dependent. Impulse thermal stress (3 hours, 29°C) applied during five successive stage of lgl/+ mothers oogenesis showed essential F1 pre-zygotic survival and longevity increase. The primary germ cells differentiation stage was epigenetically most stress sensitive. Observed stress-adaptive effects of natural widespread lgl alleles are discussed in the frame of phenomenon of possible haploadaptivity and diplo-redundancy of some vital genes. The data obtained are important for an understanding of population spreading of some risk factor mutations in humans and their health effects in environment/stress conditions. The data draw attention to the possible oogenesis-dependent transgeneration aspect of determination and expression of human mutant factors. They model pre-zygotic transgeneration epigenetic effects of TS deletion heterozygozity on aging and longevity, some multiple epidemic pathologies.

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Cis-acting genomic elements of Pas1 locus control Kras mutability in lung tumors

G. Trincucci[1], G. Manenti[1], A. Pettinicchio[1], E. Amendola[2], M. Scarfò[2], T.A. Dragani[1]
[1]Istituto Nazionale Tumori, Experimental Oncology, Milan, Italy; [2] IRGS Biogem s.c.a.r.l., Dipartimento di biologia e patologia cellulare e molecolare "L. Califano", Naples, Italy

Inbred mouse models display different susceptibilities to spontaneous or carcinogen-induced lung cancer. Genetic linkage analyses have uncovered a number of Quantitative Trait Loci (QTLs) modulating lung cancer risk and development in mice. In particular, Pas1 (Pulmonary Adenoma Susceptibility 1) locus, located in the distal region of chromosome 6, appears to play a major role in the inherited predisposition to spontaneous and chemically induced lung tumorigenesis in mouse models. Pas1 locus displays two different haplotype: a susceptible one (A/J-like) and a resistant one (C57BL/6J-like). Many reports suggested that Kras, one of the six gene mapping in Pas1 core haplotype, is a good candidate for Pas1 locus function. In order to investigate Kras role in lung tumorigenesis, we propose a new mouse model developed by replacing Kras gene with Hras transcript in a Pas1 susceptible background and crossing these mice with either a resistant (C57BL/6J) and a susceptible (A/J) strain. After lung cancer induction with urethane treatment, we observed that, in both crosses, heterozygous mice carrying the Hras-replacement gene were more prone to develop lung tumors than wide-type mice, indicating that Hras-replacement gene not only supplies Kras functions but it is also more active. Furthermore, most of the lung tumors carried a Gli61Leu substitution in Hras-replacement gene, whereas no mutations were observed in the endogenous Hras gene. Thus we suggest that Pas1 locus context is able to drive ras genes mutability. Moreover, in tumors obtained from mice carrying Hras-replacement gene, the mutation frequency affecting the wild-type Kras gene was higher when this gene was located in the susceptible (A/J) than in the resistant (C57BL/6J) Pas1 locus context (12% versus 0%, -log P=5.0). These findings indicate that cis-acting elements located in Pas1 locus are the functional components modulating Kras gene mutability and controlling susceptibility to lung tumorigenesis in mouse strains.

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Differential expression of Trefoil factor family reflects their different roles in liver fluke related Cholangiocarcinoma

K. Muenphon[1], T. Limpaiboon[1], P. Jearanaikoon[1], B. Sripa[2], V. Bhudisawadi[2]
[1]Faculty of Associated Medical Sciences Khon Kaen University, Centre for Research and Development of Medical Diagnostic Laboratories, Khonkaen, Thailand; [2] Faculty of Medicine Khon Kaen University, Liver Fluke and Cholangiocarcinoma Research Center, Khonkaen, Thailand

Background: The Trefoil factor (TFF) gene family composes of TFF1, TFF2 and TFF3. TFF plays an important role in restitution and repair of the epithelium in response to mucosal injury. However, TFF peptides are overexpressed in several human solid tumors. Prolonged inflammatory caused by parasitic infection frequently occurs in liver fluke related cholangiocarcinoma (CCA), TFF may be constitutively activated for repairing and subsequently undesirable effects during chronic inflammation may lead to tumor development and progression. This study attempted to explore TFF genes from genomic through mRNA and protein expression and clarify correlation between DNA copy number and mRNA and protein expression of TFF genes in CCA patients. The associations between TFF gene expression and clinicopathological parameters were also determined by statistical analysis.

METHODS: Tumor tissues from 110 CCA patients were used to determine DNA copy number, mRNA and protein expression of TFF genes. Correlations between DNA copy number and mRNA expression of TFFs were analyzed by linear regression. Association between mRNA and protein expression, TFF expression and clinicopathological parameters of CCA patients were evaluated by means of the Chi-square test.

RESULTS: No significant relationship between DNA copy number and mRNA expression of TFF genes was found. In normal bile ducts, TFF