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Laboratory Measurements of MICA genetic variants and s-MICA

Version 2

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ABSTRACT

1. Study design

A population-based case-control study of the exocrine pancreas was conducted using incident cases between 20 and 93 years old (mean age = 66 years), diagnosed between 1994 and 1998 in Minnesota. Pancreatic cancer cases (n=163) were ascertained from hospitals, and controls (n=542) were randomly selected from the general population and frequency matched to cases by age (within 5 years), sex and race. All participants were asked in person about demographics, cigarette smoking, physical activity, dietary and alcohol intake, medical history, and the type of treatment for cases.

2. S-MICA measurement

Blood was collected in 9-ml aliquots collected at the time of interview, and stored at -70C. After excluding participants for whom insufficient or no blood samples were available, a total of 163 cases and 542 controls were available for the current analysis (n=705).

S-MICA plasma levels were assessed in the Cytokine Reference Laboratory (UMN) using the Luminex method MICA enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, MN). This method uses a premixed Multi-Analyte Kit and s-MICA-specific antibodies were pre-coated onto magnetic microparticles. The kits were stored at -8C until use. S-MICA measurement were conducted following the manufacturer's instructions (R&D Systems, Minneapolis, MN), available at (https://www.rndsystems.com/products/human-magnetic-luminex-assay_lxsahm#product-details).

The detection limit for MICA was set at 2 pg/ml, and all results greater than this value were considered positive. We detected s-MICA plasma levels in 85% of our participants: 20 of our 163 cases and 83 of our 542 controls had a MICA plasma level lower than 2 pg/ml.

To evaluate laboratory precision and reproducibility, we inserted 3% duplicates blinded to laboratory personnel (CV% range: 0.77 – 18.9). All samples were assayed without the knowledge of case-control status.

3. Genotyping

This project involved amplifying exons 2-5 of the MICA gene from 705 samples (163 cases, 542 controls), either individually or in a multiplex PCR. These amplicons were then indexed (by sample) and sequenced as a pool. Sequencing was performed in the UMN Genomic Center using DNA previously isolated from peripheral blood lymphocytes and stored at -70C.

Pilot phase:

A small number of samples (8) were amplified for each exon individually (32 total reactions) and using a multiplexed PCR (8 reactions), then indexed, pooled, and sequenced using ~1/8 of a 2x300 bp MiSeq lane. We compared the relative coverage of each exon from either the individual or multiplexed reactions.

Production phase:

In the production phase, all 705 samples were indexed and sequenced in a single 2x300 bp MiSeq lane providing ample read depth (~15,000,000 reads total, or an average of ~ 4,500 reads per amplicon per sample), even accounting for imperfect sample balance in the sequencing pool and reasonable abundance differences between multiplexed amplicons.

To genotype exons 2-4 in the extracellular regions of MICA, genomic DNA region (1.9KB), were amplified by PCR and genotyped by high-resolution sequence-based typing using six nested primers. Each exon was individually sequenced on both DNA strands. Relevant alleles

have been characterized in the IMGT/HLA Sequence Database, www.ebi.ac.uk/imgt/hla/.

Samples were amplified for each exon individually, using a multiplexed PCR approach, then indexed, pooled, and sequenced using a 2x300 bp MiSeq lane using the Illumina MiSeq Personal Sequencing platform in the University of Minnesota Genomic Center. The resulting reads were aligned to the hg19 reference genome using BWA-MEM (BWA version: 0.7.10-r789) and processed with GATK (GATK version: 3.3-0-g37228af) for base quality score recalibration and indel realignment. Variants outside the STR region were genotyped using the GATK HaplotypeCaller.

Genotypes in the transmembrane region (exon 5) containing the tandem repeat were assigned by counting the number of times sequences from known alleles (A4-A10, and A5.1) were seen in reads overlapping the STR region. No sequencing errors were tolerated, and on average 89% of all reads were mapped directly to a known allele without sequencing error. An allele was considered for genotyping if its sequence represented >20% of all allele counts. Samples with a single passing allele were called homozygous and samples with two passing alleles were called heterozygous. Samples with more than two candidate alleles above the 20% threshold were excluded. Four distinct alleles (A4, A5, A6, A9) corresponding to 4, 5, 6 and 9 GCT repeats, respectively, and the A5.1 allele with an additional insertion of a G base were identified.

EXTERNAL LINK

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

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