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Associations between MICA and MICB genetic variants, protein levels, and colorectal cancer: Atherosclerosis Risk in Communities (ARIC)

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Abstract

Background: The major histocompatibility complex class I chain-related protein A (MICA) and protein B (MICB) participate in tumor immunosurveillance and may be important in colorectal cancer (CRC), but have not been examined in CRC development.

Methods: sMICA and sMICB blood levels were measured by SomaScan in Visit 2 (1990–92, baseline) and Visit 3 (1993–95) samples in cancer-free participants in the Atherosclerosis Risk in Communities (ARIC) study. We selected rs1051792, rs1063635, rs2516448, rs3763288, rs1131896, rs2596542, and rs2395029 that were located in or in the vicinity of *MICA* or *MICB* and were associated with cancer or autoimmune diseases in published studies. SNPs

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were genotyped by the Affymetrix Genome-Wide Human SNP Array. We applied linear and Cox proportional hazards regressions to examine the associations of pre-selected SNPs with sMICA and sMICB levels and CRC risk (236 CRCs, 8,609 participants) and of sMICA and sMICB levels with CRC risk (312 CRCs, 10,834 participants). In genetic analyses, estimates adjusted for ancestry markers were meta-analyzed.

Results: Rs1051792-A, rs1063635-A, rs2516448-C, rs3763288-A, rs2596542-T, and rs2395029-G were significantly associated with decreased sMICA levels. Rs2395029-G, in the vicinity of *MICA* and *MICB*, was also associated with increased sMICB levels. Rs2596542-T was significantly associated with decreased CRC risk. Lower sMICA levels were associated with lower CRC risk in males (HR=0.68, 95%CI 0.49–0.96) but not in females (p-interaction=0.08).

Conclusions: Rs2596542-T associated with lower sMICA levels was associated with decreased CRC risk. Lower sMICA levels were associated with lower CRC risk in males.

Impact: These findings support an importance of immunosurveillance in CRC.

Keywords

colorectal cancer risk; immunosurveillance

Introduction

Colorectal cancer (CRC) is the third leading cause of cancer death and the third most common cancer in men and women in the U.S (1). One of the mechanisms affecting cancer development, including CRC, is tumor immunosurveillance, which allows the host immune system to identify and clear tumor cells (2–4). Therefore, understanding the interaction between colorectal tumor cells and the immune response may inform interception strategies.

Major histocompatibility complex class I-like molecules (MICA and MICB) are transmembrane proteins that may be important in tumor immunosurveillance (5). MICA and MICB serve as major ligands for natural-killer group 2, member D (NKG2D) receptors (6,7). NKG2D, which are present on natural killer (NK) cells, can recognize MICA and MICB expressed on the surface of abnormal cells and trigger NK killing (8–10). In line with this, two studies of CRC patients from China (N=863 in the primary cohort and N=556 in the validation cohort) (11) and from the United Kingdom (N = 449) (12) found that higher MICA and MICB expression in their tumors was associated with better survival. However, a smaller study of 182 CRC tumors from GSE41258 and 65 CRC tumors from GSE29621 found that higher MICA expression was associated with worse prognosis (13).

Cancer cells may escape NK cell immune surveillance by releasing MICA and MICB from the cell surface into a soluble form (sMICA and sMICB) and can be detected in blood (14,15). Many studies have investigated whether sMICA and sMICB are linked to cancer survival; a meta-analysis of 13 studies of sMICA or sMICB examined together showed that their high levels were associated with a poorer overall survival of cancer patients, although that meta-analysis did not include individuals with CRC (9). In addition, several studies have examined associations of *MICA* and *MICB* polymorphisms with cancer risk (16–22). To our

knowledge, no studies have examined the associations of *MICA* and *MICB* polymorphisms or sMICA and sMICB levels with CRC risk.

Given the important role of MICA and MICB in tumor immunosurveillance and highly polymorphic nature of the *MICA* and *MICB* genes (9,18,23–26), we examined whether *MICA* and *MICB* single nucleotide polymorphisms (SNPs) and sMICA and sMICB blood levels were associated with CRC risk. We selected seven SNPs that were located either in or in the vicinity of *MICA* or *MICB* and were previously associated with risk of different types of cancers, including breast (16), hepatocellular (19,20,27), and cervical (21,22), or autoimmune diseases, such as rheumatoid arthritis (28), Takayasu arteritis (29), and psoriasis (30) (Supplemental Table 1). Of those SNPs, rs2516448 is approximately 7.5 kb downstream of *MICA*, and rs2395029 is approximately 50 kb downstream of *MICA* and 35 kb upstream of *MICB*. All the other pre-selected SNPs are located in *MICA* (31). Using the Atherosclerosis Risk in Communities (ARIC) prospective cohort, we examined: 1) the associations between the pre-selected SNPs and protein levels (sMICA or sMICB) (Aim 1), and 2) the associations of the pre-selected SNPs and sMICA and sMICB levels with CRC risk (Aim 2). We hypothesized that the pre-selected SNPs associated with lower levels of sMICA and sMICB were associated with a lower CRC risk.

Materials and Methods

Study population

The ARIC study (RRID:SCR_021769) is a prospective cohort initiated in 1987 (32,33). In 1987–89 (Visit 1), 15,792 volunteers aged 45–64 were recruited from four study centers -- Maryland, Minnesota, Mississippi, and North Carolina. Participants in Maryland and Minnesota were primarily White, and the recruitment in Mississippi was restricted to Black residents. The ARIC study was approved by institutional review boards at each participating center, and all study participants provided written informed consent. Thus far, nine visits have been completed (32). Additionally, ARIC participants have received follow-up telephone calls annually in 1987–2012 and semi-annually after 2012, with response rates of 90%−99% for the annual follow-up calls and 83%−90% for semi-annual follow-up calls among living participants who have not withdrawn consent to be contacted (33).

Ascertainment of CRC cases and death

Incident CRC cases were ascertained through 2015 via linkage with state cancer registries in Maryland, Minnesota, Mississippi, and North Carolina. These records were supplemented by the abstraction of medical records and hospital discharge codes (33). Deaths were identified through annual (semi-annual since 2012) follow-up telephone calls to participants or their proxies, surveillance of local hospitals, state records and linkage to the National Death Index.

Blood collection

The ARIC protocol for plasma sample collection, processing, and storage was designed to minimize the spontaneous biochemical reactions after blood collection and is consistent with the recommended practice for proteomics data analysis in epidemiological

studies (34–36). After venipuncture, blood samples were put immediately in an ice water bath and centrifuged at 15–25 °C within 10 min of venipuncture. Then, the aliquots were stored at −80 °C within 90 min of venipuncture and were never thawed before this analysis (https://sites.cscc.unc.edu/aric/sites/default/files/public/manuals/Blood_Collection_and_Processing.2_7.pdf).

Measurement of sMICA and sMICB levels and quality control

We examined sMICA and sMICB levels measured in EDTA-plasma samples collected at Visit 2 (1990–92) and Visit 3 (1993–95). Samples were analyzed using a SOMAmer (Slow Off-rate Modified Aptamers)-based capture array called SomaScan® by Somalogic, Inc. (Boulder, CO, USA) (37–40). The description of the SomaScan assay and the data normalization process have been described in published papers (36,40,41). In ARIC, the median split sample reliability coefficients were 0.93 at Visit 2 and 0.88 at Visit 3 after excluding proteins with a Bland-Altman coefficient of variation greater than 50% or a variance of less than 0.01 on the log scale, or proteins binding to mouse Fc-fusion, contaminants, or non-proteins (42). sMICA and sMICB levels were measured in relative fluorescent units (RFU) and were log2 transformed to correct for skewness.

Genotyping of *MICA* and *MICB* genetic variants

The SNPs were genotyped using the Affymetrix Genome-Wide Human SNP Array 6.0 and analyzed with the Birdseed variant-calling algorithm (43). Measured SNPs used for imputation were restricted to SNPs with minor allele frequencies >1%, call rate >95%, and Hardy–Weinberg equilibrium P-values >0.00001 (44). Individuals with cryptic relatedness, defined as an identity-by-state distance >0.8, generated using PLINK, were also excluded (45). Genotypes were then imputed on the Michigan Imputation Server to the TOPMed reference panel R1, and the resulting dosage values were used in subsequent analyses (46,47). Ancestry markers, principal components (PCs) based on the GWAS data, were generated by EIGENSTRAT (48) to reflect the population structure or genetic ancestry of the ARIC participants.

Assessment of other participants' characteristics

Other variables of interest that were collected at Visits 1–3 included demographic characteristics and CRC risk factors, namely age, sex, race, study center, cigarette smoking, height, weight, hormone replacement therapy (females only), aspirin use, and diabetes status (49). Information on smoking, use of medications and the measures of height and weight were collected at each visit. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared at each visit. The detailed procedures for assessing pack-years of smoking have been published (50). Diabetes mellitus was defined as fasting glucose ≥126 mg/dL, non-fasting glucose ≥200 mg/dL, treatment for diabetes mellitus, or self-reported physician diagnosis of diabetes. Another variable of interest was estimated glomerular filtration rate (eGFR) because it was associated with different SomaScan protein levels in a pilot ARIC study, and it was associated with sMICA and sMICB levels in our analysis. eGFR (ml/min per 1.73 m^2) at Visits 2 and 3 was calculated based on serum creatinine and Cystatin C by incorporating age and sex (51). We were also interested in the

inflammatory and immune biomarkers, C-reactive protein (CRP) and beta-2 microglobulin (B2M), that were measured in blood samples collected at Visit 2 (52,53).

Statistical analysis

The meta-analyses were performed using R statistical software, version 4.1.2, package "metafor". All the other analyses were performed using SAS version 9.4 (SAS Institute Inc, Cary, NC). In all analyses, a p-value < 0.05 was considered statistically significant, because we had pre-specified hypotheses for each biomarker and each SNP (54). Dosage values of the pre-selected SNPs were modeled as continuous variables unless stated otherwise.

We excluded participants with missing sMICA and sMICB levels at Visit 2 (N=2,550), participants with race other than White or Black (N=37), and those who reported prevalent cancer at Visit 2 (N=927), resulting in 10,834 White and Black participants without a history of cancer at Visit 2. Among those participants, 312 developed CRC through the end of 2015. For the analyses involving SNPs, we additionally excluded 1,882 participants who had missing SNPs information, resulting in 8,952 participants (253 incident CRC cases through the end of 2015). Accounting for ancestry markers in the analyses of SNPs led to an additional exclusion of 343 participants who had missing information on ancestry markers, resulting in 8,609 participants (236 incident CRC cases through the end of 2015) (Figure 1).

Demographic and lifestyle/medical characteristics at Visit 2 were examined across quartiles of Visit 2 sMICA and sMICB levels as mean (standard deviation (SD)) or percentage (%). The Pearson correlation coefficient was calculated between sMICA and sMICB levels.

For Aim 1, we used linear regression to assess associations between the pre-selected SNPs and corresponding sMICA and sMICB levels (modeled as continuous variables) at Visit 2. For Aim 2, we used Cox proportional hazards regression to calculate hazard ratios (HRs) and 95% confidence intervals (CIs) for incident CRC in relation to the pre-selected SNPs and Visit 2 sMICA and sMICB levels (modeled as continuous variables per 1 RFU decrease, and in quartiles). For each participant, person-years were determined from Visit 2 date until the diagnosis of CRC or another cancer, death, loss to follow-up, or administrative censoring on December 31, 2015, whichever occurred first. For both aims 1 and 2, we adjusted for age, sex, and joint terms for race and study center (Black participants from Mississippi; Black participants from any of the other field centers; White participants from Maryland or North Carolina; and White participants from Minnesota), education, BMI, cigarette smoking, pack-years of smoking, hormone replacement therapy (in women), aspirin use, diabetes status, and eGFR at Visit 2. We adjusted for these variables because they were associated with sMICA or sMICB levels and with CRC risk. In addition, we tested for B2M and CRP as potential confounders, but they changed the estimate by less than 2% and were not included into the model. Finally, we included both sMICA and sMICB levels simultaneously in the model when examining the association with CRC risk.

The analyses of pre-selected SNPs with corresponding sMICA and sMICB levels and risk of CRC were further adjusted for ancestry markers (two ancestry-specific PCs for White individuals and four ancestry-specific PCs for Black individuals). We used fixed effects model to meta-analyze the race-specific estimates if the estimates were not

statistically significantly different from each other (p-heterogeneity > 0.05). We used random effects model to meta-analyze the race-specific estimates if the estimates were statistically significantly different from each other (p-heterogeneity ≤ 0.05) but were in the same direction. We did not meta-analyze the race-specific estimates if the estimates were in opposite directions. For the analyses of seven pre-selected SNPs and CRC risk, we accounted for the multiple testing using the False Discovery Rate (FDR) p-values.

In both aims, we also examined whether sex modified these associations since immune response may differ by sex (55,56). We also examined whether race or diabetes modified these associations because Black individuals had a higher CRC incidence (57), and individuals with diabetes might have a higher CRC risk versus those without diabetes (58).

Exploratory and sensitivity analyses

We conducted several exploratory analyses. In the exploratory analyses of pre-selected SNPs and CRC risk, we examined if the associations between the pre-selected SNPs and CRC risk were mediated by sMICA or sMICB levels, by including sMICA or sMICB levels to the models. Further, we examined the associations of pre-selected SNPs and sMICA and sMICB levels with CRC risk stratified by the family history of CRC and by subtypes of CRC – proximal colon cancer, distal colon cancer, or rectal cancer.

We also conducted two sensitivity analyses. Because we have a relatively small number of participants who had information on both SNPs and ancestry markers (N = 8,609) compared to all participants with SNPs' information (N = 8,952), to increase the sample size, we also examined these associations without adjustment for ancestry markers in all the 8,952 participants with SNPs' data. In this sensitivity analysis, for rs1131896 and sMICA levels, we reported the estimates for White and Black participants instead of overall, because these race-specific estimates were in opposite directions. In the other sensitivity analysis, we calculated sMICA and sMICB levels as the mean of their levels at Visits 2 and 3. The mean was used to reflect the usual levels of these proteins. We repeated the main analyses using the mean of protein levels instead of protein levels at Visit 2. For this sensitivity analysis, the follow up of participants started at Visit 3.

Data availability

The data used in this study are available from the corresponding author upon reasonable request (https://sites.cscc.unc.edu/aric/pubs-policies-and-forms-pg).

Results

The study included 10,834 White and Black participants without a history of cancer at Visit 2 who were followed up for 199,821 person-years. Among them, 312 incident CRC cases occurred until 2015. Those with lower sMICA levels at Visit 2 were less likely to be White and tended to have higher eGFR and lower B2M. Those in the highest quartile of sMICA levels were more likely to have diabetes and were less likely to be current smokers and ever users of hormone replacement therapy. Those with lower Visit 2 sMICB levels were less likely to be male and White, were more likely to be current smokers, and were more likely to

have higher eGFR and lower B2M (Table 1). sMICA and sMICB levels were not correlated (r=0.01, p=0.24).

Associations between the pre-selected SNPs and corresponding sMICA and sMICB levels at Visit 2

In the main analysis that meta-analyzed the race-specific estimates, six of seven pre-selected SNPs, rs1051792-A, rs1063635-A, rs2516448-C, rs3763288-A, rs2596542-T, and rs2395029-G, were associated with lower sMICA levels (Table 2). Rs1131896-A was associated with lower sMICA levels among White participants, but with higher sMICA levels among Black participants (p-heterogeneity < 0.01) (Table 2 and Supplemental Table 2). For two other SNPs, rs2516448-C and rs3763288-A, the associations with sMICA levels were statistically different in White and Black participants (p-heterogeneity < 0.05) (Supplemental Table 2), although the direction of the associations in White and Black participants were the same. Rs2395029-G was also associated with higher sMICB levels (Table 2), and this association was significantly different across race groups (p-heterogeneity = 0.01) (Supplemental Table 2), although the associations were in the same direction. Sex did not statistically modify any associations between pre-selected SNPs and corresponding sMICA or sMICB levels, while diabetes statistically modified the associations of rs1051792 and rs2596542 with sMICA levels, but the associations were in the same direction for those with and without diabetes (Supplemental Table 2).

Associations of the pre-selected SNPs and of sMICA and sMICB levels at Visit 2 with subsequent CRC risk

In the main analysis that meta-analyzed the race-specific estimates, only rs2596542-T was significantly associated with a lower CRC risk (Table 2). Sex, race, or diabetes did not statistically modify this association (Supplemental Table 3). Another pre-selected SNP, rs1051792-A, was associated with a lower CRC risk in males but not females (p-interaction = 0.29) (Supplemental Table 3). There were no associations of sMICA and sMICB levels (modeled as continuous variables per 1 RFU decrease, or in quartiles) with CRC risk (Table 3 and Supplementary Table 4). However, lower sMICA levels appeared to be associated with a decreased CRC risk in males (HR=0.68, 95% CI 0.49–0.96) but not in females (p-interaction=0.08) (Table 3). Race and diabetes status at Visit 2 did not statistically modify the associations of sMICA and sMICB levels with CRC risk (Table 3). When both sMICA and sMICB levels were included in the same model, the HR (95% CI) for CRC risk associated with sMICA was 0.83 (0.66, 1.05) and with sMICB was 1.10 (0.87, 1.39). These HRs were the same as the results for CRC risk when sMICA and sMICB levels were examined individually.

Exploratory analyses for CRC risk

In the exploratory analysis of pre-selected SNPs and CRC risk, the associations did not change markedly (less than or equal to 10%) after additional adjustment for sMICA or sMICB levels (Supplemental Table 5). Therefore, the impact of the pre-selected SNPs on the development of CRC is mainly not through sMICA or sMICB levels. In the exploratory analysis of risk of subtypes of CRC, most of the estimates for the risk of proximal colon cancer and distal colon cancer were similar (Supplemental Table 6). The estimates for the

risk of rectal cancer appeared to be different for sMICB levels and several pre-selected SNPs, including a stronger inverse association for rs2596542 with risk of rectal cancer (HR = 0.49, 95% CI 0.26–0.93) than with risk of CRC (HR = 0.75, 95% CI 0.61–0.92), but the number of rectal cancer cases was small (≤44 across different analyses) (Supplemental Table 6). In the analysis stratified by family history of CRC, family history did not modify the associations of sMICA or sMICB levels or pre-selected SNPs with CRC risk (p-interactions = 0.37–0.97) (Supplemental Table 7).

Sensitivity analyses

In the sensitivity analysis of pre-selected SNPs with sMICA and sMICB levels without adjustment for ancestry markers, the associations between all pre-selected SNPs and corresponding sMICA and sMICB levels were similar to the associations in the main analysis (Supplemental Table 8). In the sensitivity analysis of CRC risk, without adjustment for ancestry markers, two additional SNPs, rs1051792-A and rs2395029-G, examined individually, were significantly associated with a lower CRC risk (Supplemental Table 8). The difference between the findings in the main and sensitivity analyses for rs2395029-G may be because the sensitivity analysis included both White and Black participants while Black participants were excluded in the main analysis because of a small number of Black participants with one or two effect alleles. For rs1051792-A and CRC risk, it is possible that the association was not significant in the main analysis due to a smaller sample size of 8,609 participants compared to 8,952 participants in the sensitivity analysis or just due to chance, but this should be tested in larger studies. The associations of pre-selected SNPs with the mean of sMICA and sMICB levels at Visits 2 and 3 (Supplementary Table 9) and the associations of the mean of sMICA and sMICB levels at Visits 2 and 3 with CRC risk (Supplementary Table 10) were similar to the findings in our main analysis, respectively.

Discussion

In this large prospective study of White and Black individuals, we found associations between the pre-selected SNPs, located in or in the vicinity of the *MICA* or *MICB* genes, and corresponding sMICA and sMICB levels. We found that rs2596542-T that was significantly associated with decreased sMICA levels was also significantly associated with a decreased CRC risk. Although no association was observed for sMICA or sMICB levels with CRC risk overall, lower sMICA levels were associated with a decreased risk of CRC in males but not females.

The interaction between MICA and MICB and receptor NKG2D on the NK cells is essential for the activation of NK cells against tumor cells (9,59). NKG2D can recognize MICA and MICB expressed on the tumor cell surface and trigger the activation of NK cells to target and eliminate tumor cells (8–10). In agreement with this, two previous studies reported that higher MICA and MICB expression in CRC cells was associated with a favorable outcome (11,12). One of these studies found that a lower versus higher MICA expression in 449 colorectal tumor samples was associated with worse disease-specific survival: HR =1.50, 95%CI 1.14–1.97 (12). Likewise, the second study reported better overall survival in CRC patients with higher MICB expression (N=863 in the primary cohort and N=556 in the

validation cohort): for those with higher versus lower MICB expression, HR was 0.74 (95% CI 0.59–0.92) in the primary cohort and HR was 0.70 (95% CI 0.51–0.96) in the validation cohort (11). However, cancer cells may escape the immune response involving NKG2D ligands through the release of MICA and MICB via proteolytic cleavage into circulation (59,60). Circulating sMICA and sMICB can bind to NKG2D receptor and induce its downregulation and therefore decrease immune response (5,61,62). In agreement with this mechanism, a meta-analysis of 13 studies of several cancer types combined, reported that high levels of sMICA or sMICB were associated with a poorer overall survival: HR=1.65, 95%CI 1.42–1.92 compared to the low levels (9).

In our study, lower sMICA levels were associated with a decreased CRC risk only among males. Although the exact explanation for this difference is unknown, the absence of the association in females may be explained by the stronger immune response and higher activity of NK cells in females so that NK cells can exert their cytolytic effect at any levels of sMICA and the sMICA does not impact CRC (55,56). However, the p-interaction between sex and sMICA levels was 0.08, and the possibility of the findings by chance cannot be excluded.

Rs2596542 (C/T), a well-studied polymorphism, is located in the promoter region of *MICA* (63), and may initiate and promote gene expression. In our study, we found that rs2596542-T was statistically significantly associated with lower sMICA levels in cancer-free participants at Visit 2. Our result is in agreement with other studies that observed lower sMICA levels in healthy controls with rs2596542-T (19). Also, we found that the rs2596542-T was significantly associated with a lower CRC risk, which is in line with this SNP's association with lower sMICA levels. No other studies examined this SNP and CRC risk; however, previous studies investigated the association between this SNP and hepatocellular carcinoma risk. A meta-analysis of 11 studies including two European studies, reported a positive association in Whites (CC vs. CT+TT: OR=1.56, 95% CI 1.12–2.18) (20), i.e., rs2596542-T was associated with lower hepatocellular carcinoma risk, which is consistent with our findings. Of note, in our study, the association between rs2596542-T and CRC risk was inverse in both White (HR=0.72, 95% CI 0.56–0.92) and Black (HR=0.82, 95% CI 0.55–1.18) participants.

In our study, we also examined a functional *MICA*-129 polymorphism (rs1051792) that causes a replacement of valine (Val) by methionine (Met) in the position 129 (the change from G to A) (16,64). *MICA*-129Met variant (rs1051792-A) has a higher affinity of binding *MICA* to NKG2D receptor than *MICA*-129Val (rs1051792-G) (64,65). *MICA*-129Met is more prone to shedding than *MICA*-129Val (24,25). Thus, we expected a greater shedding of *MICA*-129Met; however, in our study, those with *MICA*-129Met (rs1051792-A) had significant lower mean sMICA levels in both main and sensitivity analyses and a significant lower CRC risk in the sensitivity analysis. Although the higher shedding and lower blood levels seem contradictory, previous studies (24,66) also observed this phenomenon: a study of 552 patients infected with hepatitis B virus and 418 healthy controls found that *MICA*-129Val (rs1051792-G) was associated with higher sMICA levels in both patients and controls (19); a study of 73 patients diagnosed with oral squamous cell carcinoma and 149 healthy controls reported significantly higher sMICA levels in both cases and controls with

MICA-129Val/Val versus other genotypes (24) and another study of 91 patients diagnosed with multiple myeloma reported higher sMICA levels in patients with *MICA*-129Val/Val versus other genotypes (66). These findings were explained by intracellular retention among those with *MICA*-129Met as observed in transfected cells (67). Besides the reported association between rs1051792 and sMICA levels, rs1051792-A (*MICA*-129Met) was associated with a lower breast cancer risk in a case-control study of 192 breast cancer patients and 205 age-matched healthy controls from Tunisia (A vs. G: OR=0.61, 95% CI 0.44–0.84) (16). However, there have been no reports of studies examining this SNP and CRC risk. In our study, rs1051792-A was significantly associated with decreased CRC risk only in the analysis that did not adjust for ancestry markers (HR=0.78, 95% CI 0.63–0.96; 253 CRC cases in 8,952 participants) but not in the meta-analysis that pooled the race-specific estimates adjusted for ancestry markers (HR=0.82, 95% CI 0.66–1.01); however, the latter sample size was smaller.

The other SNP under study, rs2395029, is approximately 50 kb downstream of *MICA* and 35 kb upstream from *MICB* (31). This SNP was found to be associated with psoriasis, an autoimmune disease (30), but there were no reports of studies examining this SNP and cancer. In our study, we found that rs2395029-G was associated with higher sMICB levels, but with lower sMICA levels. HRs (95% CIs) for the association between this SNP and colorectal cancer risk were 0.43 (0.18–1.03) with and 0.36 (0.15–0.88) without adjustment for ancestry markers. Future larger studies will be needed to test our results for rs1051792 and rs2395029 with CRC risk.

Two other SNPs, rs1131896 and rs1063635, which were previously associated with hepatocellular carcinoma risk (19), were not associated with CRC risk in our study. Our results are similar to those in a previous study that did not detect different distributions of these SNPs' genotypes among gastric cancer patients and healthy controls (18). In addition, another pre-selected SNP, rs2516448, was previously associated with cervical cancer risk (21,22), and pre-selected rs3763288 was associated with rheumatoid arthritis and Takayasu arteritis (28,29). However, we did not detect any associations between these SNPs and CRC risk.

To the best of our knowledge, our study is the first to report on the associations between *MICA* and *MICB* polymorphisms with sMICA and sMICB levels measured by SomaScan assay. Only a few studies, including two studies of individuals from Europe (24,66), one study of Vietnamese (19), and a previous study (mainly White participants) conducted by our group (17), examined the associations of rs1051792, rs1131896, and rs2596542 with sMICA levels measured by other assays, such as Luminex Bead-based assay or ELISA. The associations of rs1051792 and rs2596542 with sMICA levels reported in our current study and previous studies were in the same direction (17,19,24,66). However, for rs1131896 and sMICA levels, we found that the associations were in opposite directions for White and Black participants; and the direction of association reported in the previous study of Vietnamese (19) was the same as the association in Black participants in our study. Additional studies are needed to validate our results. Our study is also the first to document associations of *MICA* SNP, rs2596542, with CRC risk. The association with rs2596542

confirmed our hypothesis that rs2596542-T associated with lower sMICA levels was also associated with a lower CRC risk.

The strengths of this population-based study include the prospective design with over 20 years of follow-up, adjudicated CRC incidence, the large community-based sample of White and Black individuals, and the availability of protein levels and genetic variants in the same study. Our study may have some limitations. First, the possibility of protein degradation during long-term storage cannot be excluded. However, the blood samples were frozen right after their collection and have never been thawed reducing the possibility of degradation. In addition, ARIC samples stored for different time showed similar performance as indicated by the similar coefficient of variation (CV) for the split samples collected at Visit 2 and Visit 5 (2011–13) (CV = 6% at Visit 2 and 7% at Visit 5). Second, SomaScan provide relative quantification instead of absolute quantification, but SomaScan measurements are able to detect very low protein levels (36,68). Third, in our study, there are no participants diagnosed before age of 50; therefore, we cannot compare associations in early-onset and late-onset CRC. Last, in our study, we have low effect allele frequencies for some pre-selected SNPs. This would limit the power for detecting association as well as effect modification.

In conclusion, rs2596542-T was associated with a lower risk of CRC in our cohort. While sMICA and sMICB levels were not associated with CRC risk overall, lower sMICA was statistically significantly associated with a decreased CRC risk in males. Further studies are warranted to validate our findings and examine these associations in larger or pooled prospective studies. These findings are important to elucidate the role of immunosurveillance in CRC development, and potentially could lead to novel interception strategies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used:

ARIC	Atherosclerosis Risk in Communities
CRC	Colorectal cancer
MICA	Major histocompatibility complex class I chain-related protein A
MICB	Major histocompatibility complex class I chain-related protein B
sMICA	Soluble-MICA
sMICB	Soluble-MICB
NKG2D	Natural-killer group 2, member D
NK	Natural killer
SNP	Single nucleotide polymorphism
BMI	Body mass index
PC	Principal component
eGFR	Estimated glomerular filtration rate
CRP	C-reactive protein
B2M	Beta-2 microglobulin
SD	Standard deviation
CI	Confidence interval
HR	Hazard ratio
RFU	Relative fluorescent unit

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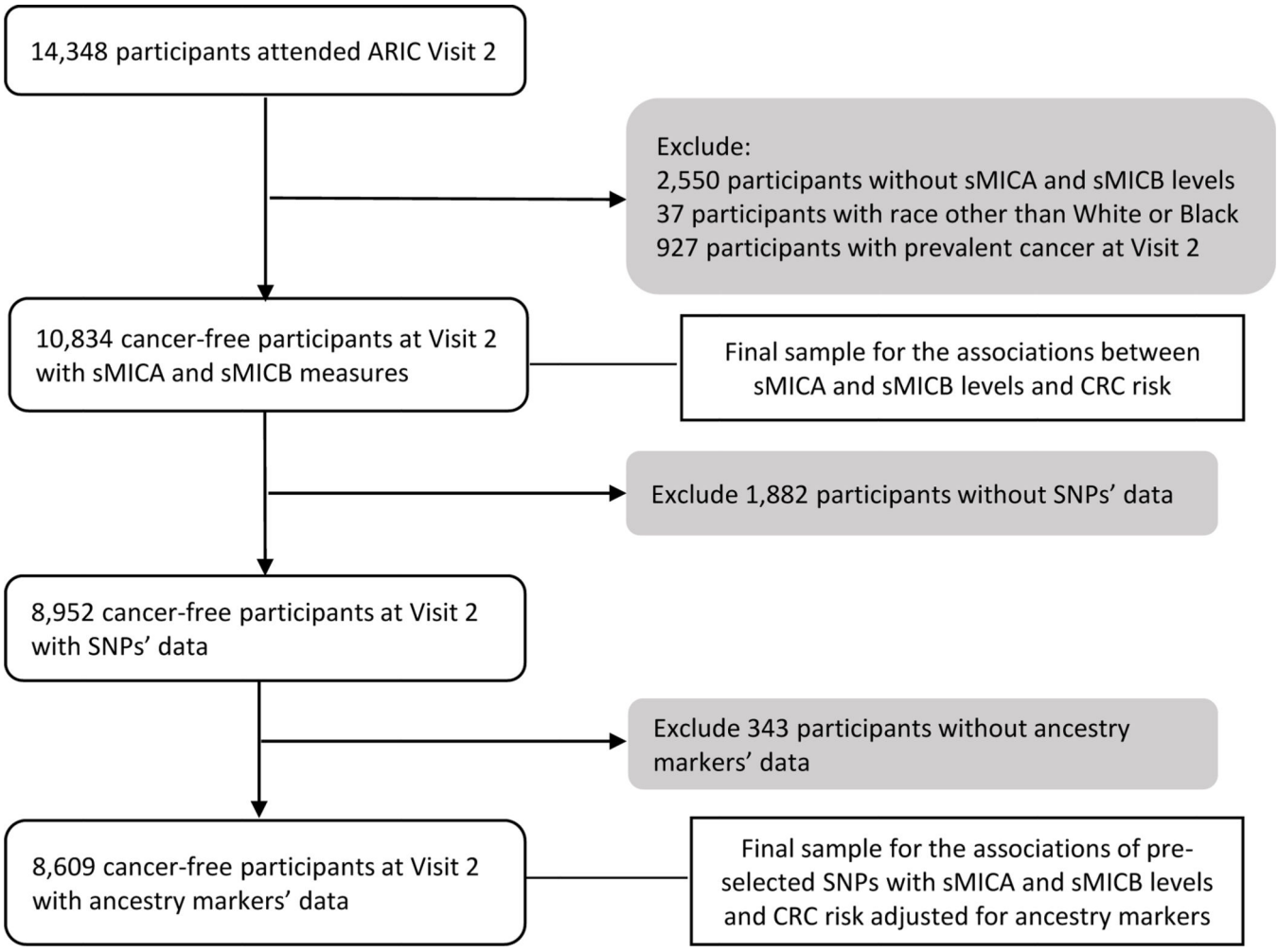

Figure 1. Study population.
The gray boxes show the participants excluded from the study.

Table 1.

Baseline characteristics across quartiles of sMICA/sMICB levels[a] of 10,834 participants in the ARIC study at Visit 2 (1990–1992)

	sMICA level quartiles					sMICB level quartiles				
	Q1 (N = 2,708)	**Q2 (N = 2,708)**	**Q3 (N = 2,711)**	**Q4 (N = 2,707)**	**p-value**	**Q1 (N =2,706)**	**Q2 (N = 2,708)**	**Q3 (N = 2,712)**	**Q4 (N = 2,708)**	**p-value**
Range of sMICA/sMICB levels	6.49 – 7.68	7.69 – 8.00	8.01 – 8.31	8.32 – 11.13		7.14 – 8.39	8.40 – 8.63	8.64 – 8.92	8.93 – 12.16	
Demographic										
Age, years (SD)	56.7 (5.7)	56.5 (5.7)	57.0 (5.8)	57.6 (5.6)	<0.0001	56.2 (5.6)	56.9 (5.6)	57.1 (5.7)	57.6 (5.8)	<0.0001
Male (%)	44.9	43.7	46.3	46.0	0.21	39.2	44.0	47.9	49.8	<0.0001
White (%)	69.0	73.6	78.6	81.5	<0.0001	61.8	74.2	82.5	84.3	<0.0001
Education (%)										
Less than high school	22.8	21.9	20.6	22.7		24.3	22.3	19.1	22.3	
High school equivalent	40.8	42.3	42.5	42.4	0.34	39.7	42.2	43.7	42.4	0.0005
Greater than high school	36.3	35.8	36.9	34.9		35.9	35.5	37.2	35.3	
Lifestyle/Medical factors										
BMI, kg/m^2 (SD)	27.9 (5.3)	27.8 (5.2)	28.0 (5.4)	28.2 (5.5)	0.01	28.3 (5.4)	28.2 (5.5)	27.6 (5.2)	27.8 (5.3)	<0.0001
Smoking status										
Current smoker	23.4	24.7	22.4	18.9		25.8	23.1	20.8	19.8	
Former smoker	37.6	37.6	37.7	38.8	<0.0001	33.3	36.8	39.6	42.0	<0.0001
Never smoker	39.0	37.7	39.9	42.4		41.0	40.1	39.7	38.3	
Pack-years of smoking among current and former smokers, pack-years (SD)	27.53 (22.26)	27.97 (22.05)	29.12 (20.98)	29.68 (23.10)	0.03	28.7 (21.8)	27.0 (20.7)	28.9 (22.3)	29.6 (23.5)	0.0101
Diabetes (%)	13.3	12.3	15.6	19.6	<0.0001	14.8	14.2	15.2	16.6	0.08
Aspirin use in the past two weeks (%)	50.3	50.0	52.3	51.3	0.29	49.1	50.9	52.7	51.3	0.06
Ever users of hormone replacement therapy among females (%)	44.5	49.1	44.6	41.1	0.0002	45.3	45.2	46.0	42.9	0.39
eGFR, mL/min/1.73 m^2 (SD)	99.2 (16.4)	99.3 (16.4)	98.2 (16.4)	96.3 (16.9)	<0.0001	99.8 (16.2)	99.2 (15.9)	98.1 (16.1)	95.8 (17.8)	<0.0001
CRP, mg/L (SD)	4.4 (8.3)	4.4 (6.8)	4.3 (6.9)	4.5 (7.9)	0.72	4.6 (7.5)	4.3 (7.5)	4.4 (7.7)	4.5 (7.1)	0.26
B2M, mg/L (SD)	1.9 (1.5)	1.9 (1.2)	2.0 (1.0)	2.1 (1.3)	<0.0001	1.9 (0.6)	1.9 (0.7)	2.0 (1.3)	2.1 (1.9)	<0.0001

[a] sMICA and sMICB levels were log2 transformed and were measured in relative fluorescent units (RFU).

Table 2.

Associations of pre-selected SNPs with Visit 2 sMICA and sMICB levels[a] and with CRC risk, ARIC (1990–2015)

SNPS	Reference/Effect allele	Association with sMICA or sMICB					Association with CRC		
		sMICA or sMICB	Race group	Regression coefficient (95% CI)[b]	Meta-analysis Regression coefficient	P-value	HR (95% CI)[a]	Meta-analysis HR	FDR P-value[c]
rs1051792	G/A	sMICA	White	−0.50 (−0.52, −0.49)	−0.51 (−0.52, −0.40)	<0.0001	0.86 (0.67, 1.11)	0.82 (0.66, 1.01)	0.13
			Black	−0.53 (−0.55, −0.50)			0.71 (0.48, 1.06)		
rs1131896[d]	G/A	sMICA	White	−0.10 (−0.12, −0.08)	--	--	1.06 (0.83, 1.37)	1.06 (0.85, 1.31)	0.71
			Black	0.23 (0.19, 0.26)			1.02 (0.68, 1.54)		
rs1063635	G/A	sMIA	White	−0.39 (−0.41, −0.38)	−0.39 (−0.40, −0.38)	<0.0001	0.93 (0.74, 1.16)	0.87 (0.72, 1.06)	0.24
			Black	−0.39 (−0.42, −0.35)			0.72 (0.49, 1.07)		
rs2516448	T/C	sMICA	White	−0.48 (−0.50, −0.47)	−0.42 (−0.54, −0.30)	<0.0001	0.90 (0.72, 1.13)	0.85 (0.70, 1.03)	0.19
			Black	−0.36 (−0.40, −0.33)			0.72 (0.49, 1.07)		
rs3763288	G/A	sMICA	White	−0.37 (−0.40, −0.33)	−0.31 (−0.46, −0.15)	<0.0001	0.93 (0.60, 1.42)	0.92 (0.61, 1.41)	0.71
			Black	−0.21 (−0.36, −0.06)			0.78 (0.10, 5.81)		
rs2596542	C/T	sMICA	White	−0.39 (−0.41, −0.37)	−0.40 (−0.41, −0.38)	<0.0001	0.72 (0.56, 0.92)	0.75 (0.61, 0.92)	0.04
			Black	−0.41 (−0.44, −0.38)			0.82 (0.55, 1.18)		
rs2395029	T/G	sMICA	White	−0.36 (−0.41, −0.31)	−0.36 (−0.41, −0.31)	<0.0001	White: 0.43 (0.18, 1.03) Black: unknown	unknown	0.13
			Black	−0.35 (−0.58, −0.12)					
		sMICB	White	0.81 (0.76, 0.86)	0.70 (0.43, 0.96)	<0.0001			
			Black	0.54 (0.33, 0.75)					

[a] sMICA and sMICB levels were measured in relative fluorescent units (RFU).

[b] Regression coefficient and HR per 1 effect allele and model adjusted for age, sex, ancestry markers, study center, education, BMI, hormone replacement therapy (in women), aspirin use, smoking status, pack-years of smoking, diabetes status, and eGFR.

[c] False discovery rate (FDR) p-value was calculated as the multiple testing of the seven pre-selected SNPs.

[d] The race-specific estimates for the association between rs1131896 and sMICA levels were not meta-analyzed because the race-specific estimates were in opposite directions.

Table 3.

Cox Regression for the associations of Visit 2 sMICA and sMICB levels (continuous variables per 1 relative fluorescent unit (RFU) decrease) with CRC risk in all participants and stratified by sex, race, or diabetes status; ARIC (1990–2015)

	N of incident CRC cases	Total person-years	HR (95% CI)[a]	
			sMICA level	sMICB level
All participants				
	312	199,821	0.83 (0.66, 1.05)	1.10 (0.87, 1.39)
Stratified by sex				
Male	161	83,933	0.68 (0.49, 0.96)	0.98 (0.72, 1.33)
Female	151	115,888	0.99 (0.71, 1.38)	1.29 (0.90, 1.85)
P-interaction			0.08	0.17
Stratified by race				
White	221	153,954	0.84 (0.64, 1.12)	1.20 (0.91, 1.58)
Black	91	45,867	0.80 (0.51, 1.25)	0.87 (0.55, 1.36)
P-interaction			0.97	0.37
Stratified by diabetes status				
Yes	59	25,983	0.79 (0.48, 1.31)	0.83 (0.53, 1.31)
No	253	173,838	0.83 (0.64, 1.08)	1.20 (0.91, 1.58)
P-interaction			0.86	0.18

[a]Model was adjusted for age, sex, joint terms for race and study center (Black participants from Mississippi; Black participants from any of the other field centers; White participants from Maryland or North Carolina; and White participants from Minnesota), education, BMI, hormone replacement therapy (in women), aspirin use, smoking, pack-years of smoking, diabetes status, and eGFR.