The Effect of Chromosome 9p21 Variants on Cardiovascular Disease May Be Modified by Dietary Intake

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Several genome-wide association studies have identified the chromosome 9p21 region as the most robust genetic association for cardiovascular disease (CVD) and myocardial infarction (MI). The authors state that observed variation in the effect of genetic factors in different populations may be partly attributed to varying levels of environmental exposures (eg, physical activity, smoking, and diet) and that gene-environment interaction studies may contribute to explaining some of the phenotypic variance that is not accounted for by common variants. They further state that the interaction of the 9p21 locus with environmental factors has not been extensively explored and that prior studies have been plagued by small sample sizes and limited to single environmentally homogenous populations of European origin. Therefore, they hypothesized that testing the effects of 9p21 and environmental factors related to MI in individuals of different ethnicities may increase the ability to discover gene-environment interactions.

How Was the Hypothesis Tested?

The authors conducted a large-scale multiethnic study of variants in the 9p21 region and acute MI in 8114 individuals (3820 cases and 4294 control subjects) from 5 ethnicities—European, South Asian, Chinese, Latin American, and Arab—who participated in the INTERHEART study, a global prospective case-control study of risk factors for acute nonfatal MI. Standardized dietary phenotypes were measured by a short qualitative food frequency questionnaire of 19 food items, which identified 3 dietary patterns subjectively labeled as Oriental (soy sauce, tofu, pickled foods, green leafy vegetables, eggs, and low sugar), Western (eggs, meats, fried and salty foods, sugar, nuts, and desserts), and prudent (raw vegetables, fruits, green leafy vegetables, nuts, desserts, and dairy products). They also generated a dietary risk score using a point system from the food items that included a tabulation of food items that were considered to be protective of CVD (fruits and green, leafy vegetables, other cooked vegetables, and other raw vegetables) and associated with risk (meat, salty snacks, and fried foods).

To validate findings of gene-environment interactions from INTERHEART, the authors next examined a series of population-based CVD risk factor surveys conducted every 5 years in Finland called FINRISK, where CVD was defined as coronary deaths, nonfatal MI, unstable angina, revascularization (coronary artery bypass graft or percutaneous transluminal coronary angioplasty), and ischemic stroke events. Dietary information was collected from a food frequency questionnaire consisting of up to 130 food items, and a composite score based on 3 responses to questions about fruit, vegetable, and berry intake was created similarly to the INTERHEART dietary risk score.

In INTERHEART, 4 SNPs (rs10757274, rs2383206, rs10757278, rs1333049) from the Chromosome 9p21 region were selected on the basis of previous results from genome-wide association studies for coronary heart disease/MI. For the FINRISK study, rs4977574 from the 9p21 region was selected because it is in high linkage disequilibrium with rs2383206 ($r^2=0.91$) in HapMap Europeans and was analyzed in 19,129 FINRISK participants (including 1014 incident cases of CVD). In INTERHEART, the authors tested for interactions between 9p21 SNPs and physical activity, smoking, and previously defined diet variables by including the main effects of the SNP and the environmental variable (physical activity, smoking, or diet) in addition to the interaction term in the model. For the FINRISK study, Cox proportional hazard models, adjusted for age and sex, were used for estimating the effects of 9p21 and diet groups on CVD. Interaction analyses were performed by including the main effects of the SNP and diet in addition to the interaction term in the model.
Principal Findings

Association of MI With 9p21 in INTERHEART

The authors found that the 4 9p21 SNPs were associated with MI in the combined sample that included all ethnicities, with odds ratios (ORs) ranging from 1.18–1.20 and probability values ranging from 1.85×10^-10 to 8.52×10^-7. Examination by ethnicity revealed differential effects: (1) all 4 SNPs were significantly associated in Europeans (1.17≤OR≤1.18, 0.016≤P≤0.024), South Asians (1.22≤OR≤1.27, 0.0003≤P≤0.0025), and Chinese (1.16≤OR≤1.21, 0.0017≤P≤0.011); (2) 3 were associated in Latin Americans (1.22≤OR≤1.32, 0.0066≤P≤0.029); (3) none of the SNPs demonstrated a significant association with MI in Arabs but had consistent directionality with that of the other ethnicities (1.04≤OR≤1.12), suggesting a lack of power.

Association of Lifestyle With 9p21 and MI in INTERHEART

The prudent diet was protective against MI (OR=0.81; 95% confidence interval [CI], 0.77–0.85), the Western diet pattern was associated with an increased risk of MI (OR=1.14; 95% CI, 1.09–1.19), and the Oriental diet pattern was not associated with MI (OR=0.94; 95% CI, 0.88–1.06). They next examined whether physical activity, smoking, or diet influenced the effect of chromosome 9p21 SNPs on MI in the INTERHEART study and found no significant interaction or trend with physical activity (OR=0.90; P=0.23) or smoking (OR=0.98; P=0.56). No significant interactions were found for the dietary risk score or for the Oriental diet score, whereas the SNP rs2383206 had only a nominal interaction with the Western diet score (P=0.028). However, they found significant interactions with the prudent diet score for all 4 SNPs for the outcome of MI, with the strongest effect seen for the interaction of rs2383206 (unadjusted P=0.0004, adjusted for the risk factors apoB/apoA1, waist/hip ratio, diabetes, hypertension, and smoking, P=0.018) even after correction for multiple testing for 6 tests (physical activity, smoking, dietary risk score, Western diet score, Oriental diet score, prudent diet score). A closer examination of this interaction revealed that rs2383206 was strongly associated with MI in the group with the lowest prudent diet score (first tertile: OR=1.32; 95% CI, 1.18–1.48, P=6.82×10^-7 for all individuals), whereas the effect was diminished in a stepwise fashion for the medium (second tertile: OR=1.17; 95% CI, 1.05–1.31, P=0.0049 for all individuals) and high-scoring prudent diet groups (third tertile: OR=1.023; 95% CI, 0.92–1.14, P=0.68 for all individuals). The interaction was strongest in South Asians and Latin Americans.

Validation of 9p21 and Dietary Interactions in the FINRISK Study

The authors found similar results for 9p21 (rs4977574) with CVD (HR=1.17; 95% CI, 1.075–1.275; P=0.0003) in FINRISK, and they observed a similar diet interaction. A diet high in vegetables, fruits, and berries was inversely associated with CVD (HR=0.79; 95% CI, 0.66–0.94; P=0.0076), and, similar to the findings from the INTERHEART analysis, the chromosome 9p21 SNP showed an effect of the risk allele on incident CVD among individuals with low (HR=1.22; P=3×10^-4) and medium (HR=1.35; P=4.1×10^-3) consumption of vegetables, fruits, and berries but demonstrated no effect in the high consumption group (HR=0.96, P=0.73).

Number of Alleles at 9p21 and the Effect of Dietary

The authors finally examined whether the number of alleles at a 9p21 SNP and diet patterns was associated with MI and CVD. The rs2383206 genotype and tertiles of the prudent diet score in the INTERHEART samples demonstrated that individuals with 2 copies of the risk allele and with a low prudent diet score had a ~2-fold increase in MI risk when compared with the reference group of individuals with 2 copies of the protective allele and a high prudent diet score. Strikingly, for the group with the most prudent diet, very little effect was observed for 9p21 variation. Similarly, in the FINRISK study, they found that the combination of a diet low in fruits, berries, and vegetables and 2 copies of the risk allele was associated with a 1.66-fold increase in risk for CVD (HR=1.66, P=0.003), but again no consistent effect of the 9p21 SNP was observed in the group with the most prudent diet, suggesting that the deleterious effects of the 9p21 risk genotype in both studies were abrogated by a healthy, “prudent” diet.

The authors state that despite this effort being the first large-scale study to specifically examine gene-environment interactions, the study is limited by potential recall bias in dietary intake measurement and this being the first study of its kind necessitating confirmation. Despite this, the study demonstrates the importance of studying multiple ethnic groups with rich environmental phenotyping to better understand the effect of genotype on complex disease phenotypes.

Implications

These findings further validate the increased risk of MI and CVD observed with common variation within the chromosome 9p21 region in various ethnicities. More importantly, the authors demonstrate that the risk of harboring risk alleles in the 9p21 region may potentially be reduced or even mitigated by increasing intake of raw vegetables, fruits, green, leafy vegetables, nuts, desserts, and berries. They also show that if risk alleles are present and a prudent diet is not followed, there is an approximate 60–100% increase in MI in the INTERHEART study, demonstrating that the deleterious effect of genetic influence can be multiplicative with an unhealthy lifestyle.

The authors state that the interaction of 9p21 SNPs with diet is intriguing because very little is known about how these variants influence CVD, given that these variants are not associated with known risk factors for CVD. Other studies in diabetics have suggested that the effect of the 9p21 risk allele was larger in those with poor glycemic control, which probably was mediated through diet. Despite the unknown mechanism, this study of gene-environment interaction makes a significant contribution to the evolving field of modern human genomics: the effect
of a genetic variation may be influenced in a bidirectional fashion due to environmental influences. Furthermore, for those who have risk alleles at 9p21, this study suggests that by controlling diet and lifestyle factors, the susceptibility of CVD and MI may be suppressed, giving further support to the American Heart Association’s dietary recommendations in the Special Report of Strategic Impact Goal Through 2020 and Beyond.\(^3\)

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**Disclosures**

None.
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