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Genetic variation of fifteen folate metabolic pathway associated gene loci and the risk of incident head and neck carcinoma: The Women's Genome Health Study

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Abstract

Objective—Recent studies have demonstrated the importance of folate metabolic pathway (FMP) in the pathogenesis of head and neck cancinoma (HNC). Whether the genetic variation within the FMP associated genes modulates HNC remains elusive. To date, prospective, epidemiological data on the relationship of FMP gene variation with the risk of HNC are sparse.

Methods—The association between 203 tag-SNPs (tSNPs) of 15 FMP associated genes (*CBS, BHMT, DHFR, FOLR1, FOLR2, FOLR3, MTHFR, MTR, MTRR, MTHFD1, RFC1, SHMT1, SLC19A1, TCN2, and TYMS*) and incident HNC was investigated in 23,294 Caucasian female participants of the prospective Women's Genome Health Study. All were free of known cancer at baseline. During a 15-year follow-up period, 55 participants developed a first ever HNC. Multivariable Cox regression analysis was performed to investigate the relationship between genotypes and HNC risk assuming an additive genetic model. Haplotype-block analysis was also performed.

Results—A total of 11 tSNPs within *DHFR*, *MTHFR*, *RFC1*, and *TYMS* were associated with HNC risk (all p-uncorrected <0.050). Further investigation using the haplotype-block analysis revealed an association of several prespecified haplotypes of *RFC1* with HNC risk (all p-uncorrected <0.050).

Conclusion—If corroborated in other large prospective studies, the present findings suggest that genetic variation within the folate metabolic pathway gene loci examined, in particular, the replication factor C-1 (RFC1) gene variation may influence HNC risk.

Keywords

Head and Neck; cancer; genetic epidemiology; single nucleotide polymorphisms; folate; homocysteine; Incident head and neck carcinoma; Risk factors; Polymorphisms

Introduction

Head and neck carcinoma (HNC), the fifth most common cancer worldwide, is one of the most challenging conditions in clinical oncology owing to its resistance to therapy and high capacity to re-populate during treatment, thus posing a serious public health burden (1, 2). In addition to the well-reported risk factors, including advanced age, alcohol abuse and tobacco

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use, folate deficiency --an essential (antioxidant) vitamin that is present in fruit and vegetables-- has recently been associated with an increased risk of HNC (3–7). Altered folate metabolism contributes to carcinogenesis via mechanisms that impair DNA synthesis, replication, repair and methylation (7–9). Whether the importance of folate metabolism/deficiency in the underlying pathogenesis of HNC was modulated by genetic variation within the folate metabolic pathway (FMP) associated genes remains elusive (1, 2, 10).

To date, data from large prospective, epidemiological studies on examining the relevance of FMP associated genes as potential risk markers for HNC are scarce. We, therefore, examined the potential involvement of 203 tag-single nucleotide polymorphisms (tSNPs) in fifteen FMP genes (Supplementary Data Tables I and II) with (i) baseline plasma homocysteine (hcys) levels, and (ii) incident HNC, in a large prospective cohort of 23,294 initially healthy US Caucasian middle-aged women.

Materials and methods

Study design

Details of the study design have been previously described (11). In brief, participants in the Women's Genome Health Study (WGHS) –a genetic substudy of the Women's Health Study (12, 13)– included initially healthy North American women aged 45 or older with no previous history of cardiovascular disease, cancer, or other major chronic illnesses. A baseline blood sample was collected during the enrollment phase of the Women's Health Study between 1992 and 1995. Study participants, who gave an informed consent for blood-based analyses related to risks of incident chronic diseases, were followed up for incident events that were adjudicated by an endpoints committee using standardized criteria and full medical record review (12, 13). The present investigation included 23,294 Caucasian participants of the WGHS; all were free of known cardiovascular disease and cancer at baseline. During a 15-year follow-up period, 55 cases of newly diagnosed HNC were identified. As described elsewhere, DNA extracted from the baseline WGHS blood samples underwent tSNP ($r^2 \approx 0.80$) genotyping using the genome-wide Illumina Infinium II Human HAP300 Duo "+" platform (14, 15). The Brigham and Women's Hospital Institutional Review Board for Human Subjects Research approved the study protocol.

Statistical analysis

Genotype frequencies were compared with values predicted by Hardy–Weinberg equilibrium using the chi-square test with one degree of freedom. Multivariable linear regression analysis, adjusting for age, and smoking status, was performed to assess the relationship of the tSNPs with baseline $\log_e$-transformed plasma homocysteine (lnhcys) levels. Hazard ratios (HRs) for association of each of the tSNPs with HNC were calculated separately by Cox regression analysis adjusting for age, smoking status, randomized treatment assignment, and further adjusting for baseline lnhcys levels and daily alcohol intake, assuming an additive model for genetic effects.

Haplotype estimation and inference were determined by expectation-maximization algorithm. Haplotype blocks were defined using the software Haploview v4.1. In addition, the relationship between haplotypes and HNC risk was examined by a referent (wild-homozygous) haplotype-based Cox regression analysis, adjusting for the same potential confounders/risk factors used in the single-SNP analysis. Only genes with 3 or more significant tSNPs (arbitrary cutoff) in the single-SNP analysis were further considered for a haplotype-based analysis. All analyses were carried out using SAS v9.1 package (SAS Institute Inc). A 2-tailed uncorrected p-value of 0.05 was considered a statistically significant result. Genotyping call rates were >99% per SNP.

Results

The baseline characteristics of the 23,294 initially healthy Caucasian women are shown in Table 1. Thirty-two out of the 203 SNPs evaluated were not in Hardy–Weinberg equilibrium with uncorrected p-values <0.0500 (Supplementary Data Table I). In the multivariable linear regression analysis, a total of 42 SNPs (22 *MTHFR*, 8 *MTR*, 1 *RFC1*, 4 *DHFR*, 3 *MTRR*, 1 *MTHFD1*, and 3 *TCN2*) were differentially associated with baseline lnhcys levels (p-uncorrected <0.050; Supplementary data Table III). Results from the multivariable Cox regression analysis showed evidence for differential associations of 11 SNPs (1 *MTHFR*, 7 *RFC1*, 1 *DHFR*, and 2 *TYMS*) with HNC risk (p-uncorrected <0.050; Table 2). Supplementary Data Table IV presents the nominal (uncorrected) Cox regression results for all 203 SNPs evaluated. Further adjustment for baseline lnhcys levels, and alcohol intake showed virtually identical results (data not shown). Supplementary Data Figure I presents the linkage disequilibrium (LD) pattern of the tSNPs of *RFC1* in the present sample population. The haplotype distribution (defined by Haploview v4.1) is shown in Table 3. Results from the haplotype-based analysis again showed an association of two Haploview-defined haplotypes of *RFC1* with HNC risk (p-uncorrected <0.050; Table 3), consistent with the involvement of rs10033019. All SNPs evaluated were in agreement of proportionality hazard assumption.

Discussion

To the best of our knowledge, the present prospective investigation is the first to examine the possible involvement of FMP genes in the risk of incident HNC, and we found -suggestive- evidence for an association of the genes evaluated, in particular, the replication factor C-1 (*RFC1*) gene locus.

Genetic variation within the FMP gene loci has been implicated in various forms of cancer including HNC (1). The FMP genes evaluated in the present study encode proteins for the complex one-carbon interconversion processes, which ultimately regulate and maintain genomic stability. Recent studies have demonstrated the effects of genetic variation within the FMP associated genes, in particular, *MTHFR* (*C677T*/rs1801133, *A1298C*/rs1801131, *G1793A*/rs2274976), *MTR* (*A2756G*/rs1805087), *MTRR* (*A66G*/rs1801394, *C524T*/rs1532268), *SLC19A1* (*A80G*/rs1051266) in modulating risk of HNC (reviewed by Galbiatti and coauthors (1)). However, the present study found no association of the abovementioned gene variants with HNC risk (Supplementary Data Table III). Moreover, the present study confirmed an inverse relationship of *MTR A2756G* (rs1805087) (16–19), *MTRR A66G* (rs1801394) (20) with plasma hcys levels, and a null association of *MTHFD1 G1958A* (rs2236225) with HNC (21), as previously reported.

The present investigation, moreover, suggests that the replication factor C-1 (RFC1) gene variation may play a role in the underlying pathogenesis of HNC. Replication factor C (RFC) is an important factor involved in DNA replication, repair mechanisms, post-translational methylation as well as cell proliferation. Recent experimental investigation of the subunit 1 of Arabidopsis RFC (AtRFC1) -a homologue of p140, the large subunit of human RFC1- showed that AtRFC1 mutations caused defects in embryogenesis and led to embryo and seed abortion (22), suggesting that RFC1 may play a similar role in embryogenesis in humans, and its relevance to the congenital malformations caused by folate deficiency. Furthermore, using interaction cloning, Uchiumi *et al.* showed that the large subunit of replication factor C interacts with the DNA sequence repeats of telomeres and recognizes the 5-prime-phosphate termini of double-stranded telomeric repeats, thus suggesting that replication factor C may also play a role in telomere stability or turnover (23). Telomere instability, a hallmark of tumourogenesis, is widely demonstrated to play an

important role in cancer development (24–26). Owing to the observed functional characteristics of RFC in relation to embryonic development (its relevance to congenital malformations due to folate deficiency), and telomere stability, the present findings encourage the need of further investigation into the possible involvement of replication factor C cascade, and the telomere-telomerase complex in the pathogenesis of HNC.

Strengths of the present study are the overall sample size, the prospective design and the complete long-term follow-up. Nonetheless, some potential limitations of our study require discussion. Our sample population was limited to Caucasian female healthcare professionals from the US. Thus, our results may not be generalizable to other racial/ethnic or socio-economic groups, geographical regions, or to males. Cautious interpretation of our present (uncorrected) findings should be exercised, and confirmation in other prospective studies is needed. Furthermore, pre-cancerous prediction of head and neck carcinoma based on SNPs analysis may be inadequate or low for clinical use (27).

In our study, we had the ability to detect, based on the present sample size, assuming 80% power, at an alpha of 0.05, an effect estimate of greater than 1.65 if the minor allele frequency is 0.50, and of greater than 3.50 if the minor allele frequency is 0.02, assuming a univariable-additive model. Thus, the present study may not have the power to detect a true low-to-modest risk of HNC associated with the gene variants tested.

In conclusion, if corroborated by other large, prospective investigations, the present data from a large cohort of apparently healthy Caucasian US females provide evidence for an association between the folate metabolic pathway associated gene variants tested, in particular, the replication factor C-1 gene locus and the risk of head and neck carcinoma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Baseline characteristics of the study population

Variable	N=23,294
Age, years	52.90 [48.92–59.01]
Body-mass index, kg/m^2	24.89 [22.46–28.32]
Smoking status, %	
Current	11.64
Past	37.45
Never	50.91
Aspirin use, %	49.87
Beta-carotene use, %	49.81
Vitamin-E use, %	50.08
Current hormone use, %	43.86
Homocystenine levels, μmol/L	10.48 [8.71–12.91]
Daily alcohol intake, g/day	0.86 [0–4.64]

Data are median and interquartile range for continuous, and percentages for categorical variables.

Table 2

Multivariable Cox regression analysis of incident head and neck carcinoma.

Genes	dbSNP	MA	MAF	Hazard Ratio	95%CI	*p*-uncorrected	snp
MTHFR	rs6696752	A	0.2906	0.579	0.360–0.933	*0.0246*	s4
RFC1	rs2276888	A	0.4343	0.629	0.422–0.938	*0.0230*	s43
	rs2306597	A	0.2053	0.515	0.289–0.919	*0.0247*	s46
	rs9993224	A	0.4309	0.664	0.446–0.988	*0.0437*	s47
	rs2066786	A	0.4310	0.637	0.427–0.951	*0.0273*	s48
	rs6851075	G	0.3305	0.560	0.356–0.882	*0.0124*	s58
	rs3736168	G	0.4947	1.869	1.263–2.764	*0.0017*	s59
	rs10033019	A	0.4851	1.937	1.310–2.864	*0.0009*	s60
TYMS	rs2847154	A	0.2349	0.477	0.272–0.836	*0.0097*	s116
	rs2612081	A	0.2361	0.473	0.270–0.830	*0.0090*	s119
DHFR	r6151662	A	0.0555	1.857	1.001–3.446	*0.0498*	S202

CI, confidence interval; MA, minor allele; MAF, minor allele frequency.

Adjusted for age, smoking status, and randomized treatment assignment.

Table 3

Haplotype-based Cox regression analysis of incident head and neck carcinoma.

Genes	* Haplotype-Block	HF	HR; 95%CI	*p*-uncorrected
RFC1	Block 1			
	rs2276888-rs17288757-rs2306597-rs9993224-rs2066786			
	11111 (GGGGG)	0.56242	Referent	
	21122 (AGGAA)	0.16761	0.814; 0.295–2.245	*0.6910*
	21222 (AGAAA)	0.20340	0.259; 0.079–0.849	*0.0257*
	22122 (AAGAA)	0.05630	0.080; 0.005–1.325	*0.0778*
	Block 2			
	rs13147094-rs2381375-rs3796517-rs17288033-rs2306596			
	11111 (GAAGA)	0.41702	Referent	
	11121 (GAACA)	0.10465	undetermined	--
	12212 (GGGGC)	0.34875	undetermined	--
	22112 (AGAGC)	0.11893	undetermined	--
	Block 3			
	rs6851075-rs3736168-rs10033019			
	111 (AAG)	0.18011	0.388; 0.131–1.152	*0.0881*
	121 (AGG)	0.01171	undetermined	--
	122 (AGA)	0.47692	Referent	
	211 (GAG)	0.32258	0.217; 0.083–0.568	*0.0019*

Only haplotypes with frequency greater than 1% are shown.

1 denotes the major allele, 2 the minor allele.

CI, confidence interval; HF, haplotype frequency.

Adjusted for age, smoking status, and randomized treatment assignment.

* as defined by Haploview v4.1