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Homocysteine, B Vitamins, MTHFR Genotype, and Incident Age-related Macular Degeneration

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The relationship between moderately elevated plasma homocysteine levels and risk of age-related macular degeneration (AMD) remains unclear. Cross-sectional and case-control studies suggest a direct association, primarily with advanced AMD, but data from prospective studies are less supportive. Moreover, homocysteine levels may be elevated due to nutritional or genetic factors that may be more closely related to AMD risk.

We recently reported that moderately elevated plasma homocysteine was associated with a small, and statistically non-significant increased risk of incident AMD among 27,479 participants in the Women's Health Study (WHS), a completed randomized trial of aspirin, vitamin E, and beta carotene.¹ In this report, we sought to extend these findings by also considering nutritional and genetic factors associated with homocysteine metabolism. Thus, we examined the prospective relation of plasma homocysteine, dietary intake of B vitamins (folate, vitamin B₂, vitamin B₆, vitamin B₁₂) and related compounds betaine and choline, and two common variants in the methylenetetrahydrofolate reductase (MTHFR) gene, 677C>T and 1298A>C, with risk of AMD in the Women's Genome Health Study (WGHS), a genetic sub-study of the WHS. The WGHS comprises 23,294 WHS participants of European ancestry who provided a baseline blood sample and consent for blood-based analyses related to risks of incident chronic diseases. Homocysteine concentration was measured by an enzymatic assay, dietary intake was measured by food frequency questionnaire, and genotyping was performed using the genome-wide Illumina Infinium II Human HAP300 Duo "+" platform. The present analysis includes 22,773 WGHS

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participants who, at baseline, had dietary data on B vitamins and related compounds, measured homocysteine level and MTHFR genotype, and no reported diagnosis of AMD. Distributions for plasma homocysteine and nutrient intakes were log-transformed to normalize them, and we used the residual method to adjust nutrients for energy intake. AMD was ascertained through participant self-reports confirmed by medical record review. The study was approved by the institutional review board of the Brigham and Women's Hospital. This research adhered to the tenets of the Declaration of Helsinki and is registered at clinicaltrials.gov (NCT00000161).

As expected, findings for homocysteine in this sub-study were similar to those observed in the parent WHS cohort; women in the highest quartile, compared to the lowest, had a small, and statistically non-significant increased risk of total (hazard ratio [HR], 1.22, 95% confidence interval [CI], 0.90–1.66; p trend=0.17) and visually-significant AMD (20/30 or worse) (HR, 1.15, 95% CI, 0.72–1.84; p trend=0.40) in analyses adjusted for age, treatment assignment (aspirin, vitamin E, beta carotene), and other AMD risk factors.

Nutritional analyses indicated no statistically-significant relation between any of the nutrients examined and incident AMD, although findings for higher intakes of folate and vitamin B₂ were generally in the direction of protection (Table 1). The suggestive findings for folate appear broadly consistent with those of three other prospective studies, all of which report an inverse relation between some combination of high folate in the diet or blood and risk of early or late AMD (either neovascular AMD or geographic atrophy).^{2–4} For vitamin B₂, the only previous report was an observational analysis from the Age-Related Eye Disease Study 2 that indicated no significant relation between intake of vitamin B₂ from food sources only and progression to geographic atrophy.⁴

Genetic analyses indicated no association of the MTHFR 677C>T polymorphism with total or visually-significant AMD, and no association of the 1298A>C polymorphism with total AMD. However, the risk allele for 1298A>C was positively associated with risk of visually-significant AMD (CA vs. AA: HR, 1.29, 95% CI, 0.92–1.82; CC vs. AA: HR, 1.58, 95% CI, 0.96–2.60; p trend=0.04) in analyses adjusted for age, treatment assignment (aspirin, vitamin E, beta carotene), homocysteine level, and other AMD risk factors (Table 2; available at <http://www.opthalmology-retina.org>). To our knowledge, the only previous report to examine this polymorphism and AMD was a small case-control study in Austria (75 white AMD patients; 75 sex- and age-matched controls) that found no association.⁵ In other studies, the 1298A>C variant has been associated with ischemic stroke, especially in Asian populations, and thus our finding of an association of 1298A>C with AMD appears consistent with the hypothesis that AMD and stroke share similar underlying mechanisms. With respect to the 677C>T genotype, our finding of no association is consistent with two previous reports of no increased risk of AMD for those with the variant allele.

Of note, homocysteine levels were significantly associated with MTHFR genotype (1.5 μ mol/L higher homocysteine for 677 TT vs CC, p <0.0001; 0.3 μ mol/L lower homocysteine for 1298 CC vs AA, p =0.002). Because genetic variants are allocated randomly from parents, the variants are generally unrelated to other factors related to the outcome. This allows a largely unconfounded estimate of the homocysteine-AMD association with

genotype as a proxy for homocysteine level. Thus, our genetic data, especially those for the 677C>T variant which was associated with higher homocysteine levels in WGHS and has been linked consistently with elevated homocysteine in numerous other populations, appear to further support the conclusion that moderately elevated plasma homocysteine level is not likely to be an important independent risk factor for AMD in healthy women.

Finally, in exploratory analyses, we examined whether AMD risk was modified by an interaction between these nutritional and genetic factors related to homocysteine metabolism. We found no significant nutrient-gene interaction when we examined nutrient intake above or below the median and the 677C>T polymorphism. For 1298A>C, choline intake above the median, as compared to below the median, was associated with a significantly lower risk of visually-significant AMD only among those homozygous for the high-risk C allele (p interaction=0.02). Choline functions indirectly in one-carbon metabolism where it is first oxidized to betaine which then contributes a methyl group to the folate-independent remethylation of homocysteine to methionine. Of course, this finding of a possible nutrient-gene interaction in AMD could be due to chance, particularly given the multiple comparisons, and needs to be confirmed in other populations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AMD	age-related macular degeneration
HR	hazard ratio
CI	confidence interval
MTHFR	methylenetetrahydrofolate reductase
WHS	Women's Health Study
WGHS	Women's Genome Health Study

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HRs (95% CI) for the association between quartiles of dietary intake of calorie adjusted folate, vitamin B₂, vitamin B₆, vitamin B₁₂, choline and betaine and incident AMD in the Women's Genome Health Study.

Table 1

	Q1	Q2	Q3	Q4	P trend
Total AMD					
Folate intake, µg/d	<276.37	276.37–350.42	350.43–545.34	>545.34	
Model 1 *	1.00	0.60 (0.45–0.82)	0.80 (0.61–1.06)	0.77 (0.58–1.01)	0.50
Model 2 †	1.00	0.62 (0.46–0.84)	0.80 (0.60–1.07)	0.78 (0.54–1.12)	0.49
Vitamin B ₂ intake, mg/d	<1.60	1.60–2.00	2.01–3.10	>3.10	
Model 1 *	1.00	0.77 (0.58–1.02)	0.62 (0.46–0.83)	0.78 (0.59–1.02)	0.27
Model 2 †	1.00	0.77 (0.58–1.03)	0.60 (0.44–0.82)	0.70 (0.48–1.01)	0.14
Vitamin B ₆ intake, mg/d	<1.87	1.87–2.28	2.29–3.62	>3.62	
Model 1 *	1.00	0.92 (0.68–1.23)	0.94 (0.70–1.25)	0.92 (0.69–1.23)	0.72
Model 2 †	1.00	0.95 (0.70–1.29)	0.95 (0.70–1.30)	0.91 (0.62–1.33)	0.67
Vitamin B ₁₂ intake, µg/d	<4.69	4.69–6.42	6.43–10.01	>10.01	
Model 1 *	1.00	1.04 (0.78–1.39)	0.82 (0.60–1.10)	0.96 (0.72–1.27)	0.63
Model 2 †	1.00	1.03 (0.77–1.37)	0.77 (0.56–1.06)	0.90 (0.63–1.27)	0.47
Choline intake, mg/d	<294.91	294.91–329.60	329.61–366.50	>366.50	
Model 1 *	1.00	0.86 (0.65–1.15)	0.91 (0.69–1.21)	0.91 (0.69–1.20)	0.62
Model 2 †	1.00	0.84 (0.63–1.12)	0.84 (0.63–1.12)	0.90 (0.68–1.19)	0.50
Betaine intake, mg/d	<90.70	90.70–113.20	113.21–143.10	>143.10	
Model 1 *	1.00	1.13 (0.86–1.48)	1.00 (0.75–1.33)	0.88 (0.66–1.18)	0.25
Model 2 †	1.00	1.13 (0.86–1.49)	0.98 (0.74–1.31)	0.91 (0.68–1.22)	0.35
Visually-significant AMD					
Folate intake, µg/d	<276.37	276.37–350.42	350.43–545.34	>545.34	
Model 1 *	1.00	0.65 (0.40–1.05)	0.89 (0.57–1.37)	0.80 (0.51–1.24)	0.72
Model 2 †	1.00	0.70 (0.43–1.13)	0.94 (0.60–1.47)	0.76 (0.43–1.33)	0.51
Vitamin B ₂ intake, mg/d	<1.60	1.60–2.00	2.01–3.10	>3.10	

	Q1	Q2	Q3	Q4	P trend
Model 1 *	1.00	0.68 (0.43–1.08)	0.77 (0.50–1.19)	0.78 (0.51–1.20)	0.61
Model 2 †	1.00	0.72 (0.45–1.16)	0.79 (0.50–1.25)	0.65 (0.37–1.16)	0.23
Vitamin B ₆ intake, mg/d	<1.87	1.87–2.28	2.29–3.62	>3.62	
Model 1 *	1.00	0.81 (0.51–1.31)	0.92 (0.59–1.44)	0.89 (0.56–1.38)	0.83
Model 2 †	1.00	0.88 (0.55–1.43)	0.99 (0.62–1.58)	0.80 (0.44–1.43)	0.46
Vitamin B ₁₂ intake, µg/d	<4.69	4.69–6.42	6.43–10.01	>10.01	
Model 1 *	1.00	1.00 (0.63–1.60)	0.85 (0.53–1.36)	1.08 (0.70–1.66)	0.67
Model 2 †	1.00	1.00 (0.62–1.60)	0.79 (0.48–1.30)	1.02 (0.60–1.74)	0.90
Choline intake, mg/d	<294.91	294.91–329.60	329.61–366.50	>366.50	
Model 1 *	1.00	0.88 (0.56–1.38)	0.88 (0.56–1.38)	1.04 (0.68–1.59)	0.82
Model 2 †	1.00	0.87 (0.55–1.37)	0.80 (0.51–1.26)	1.00 (0.65–1.53)	0.97
Betaine intake, mg/d	<90.70	90.70–113.20	113.21–143.10	>143.10	
Model 1 *	1.00	1.59 (1.02–2.47)	1.19 (0.74–1.91)	1.35 (0.85–2.13)	0.47
Model 2 †	1.00	1.60 (1.02–2.49)	1.19 (0.74–1.94)	1.42 (0.90–2.26)	0.33

Abbreviations: HR, hazard ratio; CI, confidence interval; AMD, age-related macular degeneration.

* Adjusted for age and randomized treatment assignment (aspirin, vitamin E, beta carotene).

† Additionally adjusted for smoking (current, past, never), alcohol use (rarely/never, 1–3 drinks/month, 1–6 drinks/week, and 1 drinks/day), body mass index (continuous), postmenopausal hormone use, history of hypertension (ever diagnosis by physician or self-reported blood pressure 140/90; yes or no), history of hyperlipidemia (baseline history of cholesterol-medication use or a physician diagnosis of high cholesterol or a self-reported cholesterol of at least 240 mg/dL; yes or no), multivitamin use (current, past/never), history of eye exam in the last 2 years.