IL-2 and *IL-10* gene polymorphisms are associated with respiratory tract infection and may modulate the effect of vitamin E on lower respiratory tract infections in elderly nursing home residents[1–4]

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ABSTRACT

Background: Vitamin E supplementation may be a potential strategy to prevent respiratory tract infections (RIs) in the elderly. The efficacy of vitamin E supplementation may depend on individual factors including specific single nucleotide polymorphisms (SNPs) at immunoregulatory genes.

Objective: We examined whether the effect of vitamin E on RIs in the elderly was dependent on genetic backgrounds as indicated by SNPs at cytokine genes.

Design: We used data and DNA from a previous vitamin E intervention study (200 IU vitamin E or a placebo daily for 1 y) in elderly nursing home residents to examine vitamin E–gene interactions for incidence of RI. We determined the genotypes of common SNPs at *IL-1β*, *IL-2*, *IL-6*, *IL-10*, *TNF-α*, and *IFN-γ* in 500 participants. We used negative binomial regression to analyze the association between genotype and incidence of infection.

Results: The effect of vitamin E on lower RI depended on sex and the SNP at *IL-10* −819G→A ($P = 0.03$ for interaction for lower RI). Furthermore, we observed that subjects with the least prevalent genotypes at *IL-2* −330A→C ($P = 0.02$ for upper RI), *IL-10* −819G→A ($P = 0.08$ for upper RI), and *IL-10* −1082C→T ($P < 0.001$ for lower RI in men) had a lower incidence of RI independent of vitamin E supplementation.

Conclusions: Studies that evaluate the effect of vitamin E on RIs should consider both genetic factors and sex because our results suggest that both may have a significant bearing on the efficacy of vitamin E. Furthermore, common SNPs at cytokine genes may contribute to the individual risk of RIs in the elderly. This trial was registered at clinicaltrials.gov as NCT00758914. *Am J Clin Nutr* 2010;92:106–14.

INTRODUCTION

Respiratory tract infections (RIs) impose a substantial economic burden (1–3) and are a significant cause of mortality and morbidity in the United States (4). The elderly have an increased risk of infection (5), and RIs are common among elderly nursing home residents (6). The immune response plays an important role in resistance to RIs and declines with age (7–11).

Vitamin E has been shown to enhance the immune response and resistance to infection (12–15). However, not all individuals supplemented with vitamin E show improved immune responses or fewer RIs. The immunomodulatory effect of vitamin E is partially mediated via its effect on cytokine production (15–18). A vitamin E–induced reduction in influenza infection has been associated with changes in cytokine concentrations in aged mice (12). The current study focuses on functional single nucleotide polymorphisms (SNPs) at the genes for interleukin-1β (*IL-1β*), *IL-2*, *IL-6*, *IL-10*, interferon-γ (*IFN-γ*), and tumor necrosis factor-α (*TNF-α*) (19–23). Specific genetic differences at cytokine genes may account for the variable susceptibility to RIs (24) by influencing the function of immune cells or the cytokine response to pathogens. Given the public health effect of RIs and the potential use of vitamin E as a preventative measure, information on genetic factors that influence the response to vitamin E is needed. We used data from a vitamin E intervention in a nursing home population (13) to test the hypothesis that the varied effects of vitamin E on RIs [total RIs, upper RIs (URIs), and lower RIs (LRIs)] are partially due to genetic differences at cytokine genes. Furthermore, we explored the effect of sex on this interaction on the basis of previous reports that

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indicated that the functionality of some SNPs at cytokine genes may be sex dependent (25, 26).

SUBJECTS AND METHODS

Study population

Between 1998 and 2001, elderly volunteers who lived in long-term care facilities were recruited to participate in a 1-y randomized, double-blind, placebo-controlled, vitamin E intervention trial (13). Participants were randomly assigned to receive either vitamin E (200 IU *all-rac*-α-tocopherol) or placebo daily in addition to a multivitamin containing one-half of the Recommended Dietary Allowance of essential vitamins and minerals, including 4 IU vitamin E. Of the 617 participants enrolled in the study, 451 subjects completed the study. Most participants were white/non-Hispanic (93%) and women (71%) between the ages of 65 and 102 y (mean: 84.6 y). The institutional review board of Tufts–New England Medical Center approved the study protocol, and an informed consent form was provided by all participants.

Nutrient status and blood count differentials

Fasting blood samples that were collected from participants at enrollment and at the completion of the study were used to measure clinical chemistries, blood cell differentials, and plasma status for select nutrients including vitamin E as previously described (13, 15).

Infection assessment

Study nurses collected information weekly that was related to infection and included respiratory and heart rates and temperature (13). At the end of the study, data collected from the participants in each supplementation group were randomly assigned to 1 of the 2 study physicians for diagnosis of infections. Infection data from any one participant were evaluated by only one physician, except for 18 participants whose records were used to determine concurrence between physicians.

The study physicians, who were blinded to the supplementation group, evaluated data collected by the nurses from the participant examinations, interviews, and record reviews to determine the incidence and duration of the RI. Clinical definitions of RIs (13) were developed according to accepted definitions (*see* supplemental Table 1 under "Supplemental data" in the online issue) (27–29).

DNA isolation and genotyping

Five hundred participants consented to DNA analysis. DNA was isolated from blood samples with spin-prep kits according to the manufacturer's instructions (QIAamp DNA Blood Mini Kit; QIAGEN Inc, Valencia, CA). The following locations were investigated: *IL-1β* −1473G→C, *IL-1β* −511G→A, *IL-1β* 3954C→T, *IL-1β* 6054G→A, *IL-6* −174C→G, *TNF-α* −308 G→A, *IL-2* −330A→C, *IL-10* −1082C→T, *IL-10* −819G→A, *IL-10* −592G→T, and *IFN-γ* 874A→T. These SNPs were selected on the basis of previous reports of functionality in other populations, particularly associations between the genetic variants and differences in cytokine production (25, 30–36) and the role of these cytokines in protection from infection. Genotyping was performed with Taqman 5′ nuclease allelic discrimination (Assay by Design/Demand; Applied Biosystems, Foster City, CA). All genotypes were determined with Validated ABI Assays (Applied Biosystems) with the exception of *IFN-γ* 874A→T, which was determined by using primer and probe sequences described by Yu et al (37) (*see* supplemental Table 2 under "Supplemental data" in the online issue for a list of primer and probes used for genotyping).

SNPs were tested for Hardy-Weinberg equilibrium (HWE) with Utility Programs for Analysis of Genetic Linkage (J Ott, Beijing Institute of Genomics, Beijing, China). For SNPs that deviated from HWE, genotyping assays were repeated, and the HWE was recalculated in samples from our study population and in nonrelated, younger (<65 y) subjects who were enrolled in the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) Study (38). The GOLDN study population was homogenous, predominantly white, lived in the United States, and was considered a younger, ethnically similar study population.

The linkage disequilibrium between SNPs was assessed with Haploview 4.0 software (Broad Institute, Massachusetts Institute of Technology, Cambridge MA). Diplotype for the 3 *IL-10* promoter SNPs (*IL-10* −1082C→T, *IL-10* −819G→A, and *IL-10* −592G→T) was generated with HelixTree software (Golden Helix, Bozeman, MT) by using the expectation maximization algorithm. Haplotypes with a frequency of <0.05% in this population were excluded from analyses.

Statistical power calculations and analyses

Power calculations indicated that we had a ≥80% chance ($\alpha = 0.05$) of detecting significant differences in total RIs and URIs between genotypes at *IL-2* −330A→C, *IL-6* −174C→G, and *IFN-γ* 874A→T on the basis of previously published effect sizes for these SNPs (24, 39) and >61% for total RIs and URIs between genotypes at *IL-1β* −511G→A, *TNF-α* −308G→A, and *IL-10* −1082C→T, which is within the range of differences by genotype reported for other SNPs at cytokine genes (24, 39).

Statistical analyses were performed by with SAS statistical software (SAS version 9.1.2; SAS Institute Inc, Cary, NC). The distribution of continuous variables was examined and transformed by using log transformation as needed. Descriptive statistics are reported for nontransformed data. The baseline characteristics of each supplementation group were compared by using Student's *t* test for independent samples for continuous variables and the chi-square or Fisher's exact test for categorical variables. For these analyses, RIs were categorized broadly as URIs or LRIs (*see* supplemental Table 1 under "Supplemental data" in the online issue). Analyses considered associations with all RIs, LRIs, URIs, and colds. Analyses of RI incidence assumed a negative binomial distribution of infection data, with the natural logarithm of days enrolled in the study as an offset. The negative binomial model was selected over Poisson regression because the infection data were overdispersed relative to the Poisson distribution. Several models were tested to examine the effect of SNPs and vitamin E supplementation on RIs as follows.

First, 3-way statistical interactions between sex, genotype, and vitamin E supplementation were evaluated. When a 3-way

interaction reached significance, the data were divided into subgroups on the basis of sex, and the effects of vitamin E supplementation and genotype were explored

Second, if the 3-way interaction did not reach significance, statistical interactions between sex and genotype for infection were evaluated. When a statistical interaction between sex and genotype was observed, the effect of vitamin E and genotype was examined in each sex separately.

Third, the statistical interaction between genotype and vitamin E was tested. When analysis indicated a statistical interaction between genotype and vitamin E supplementation, each supplementation group was considered separately.

With the exception of the 3 *IL-10* promoter SNPs, the effect of single SNPs on infection incidence was examined in separate negative binomial models. The effect of each *IL-10* promoter SNP was assessed separately; however, the effect of the combined genotype of the 3 SNPs at *IL-10* was also examined by using estimated diplotype information for *IL-10* −1082/−819/−592. Previous reports suggested that these 3 SNPs may act in tandem to affect elicited IL-10 production, therefore, evaluation of the 3 *IL-10* SNPs together was performed to test if considering the 3 SNPs in tandem may better reflect their function. For analysis of either single SNPs or multiple SNPs (*IL-10*), individual genotypes were contrasted if the *P* value for the main effect of genotype suggested a difference in infection ($P \leq 0.05$).

Analysis examining interactions and the main effect of genotype were initially adjusted for the year of enrollment. Subsequent analyses were adjusted for other factors that might influence infection including smoking, diabetes, dementia, baseline albumin and baseline hemoglobin concentrations, chronic obstructive pulmonary diseases, age, baseline body mass index, cardiovascular disease, and hypertension. Models of LRIs and pneumonia were further adjusted for baseline zinc status. Interaction terms or main effects of genotypes did not gain significance with additional adjustments for confounding variables. Differences in infection incidence by genotype were reported as rate ratios and 95% CIs. The reference group for rate ratio calculations for *IL-2* −330A→C (20, 40), *IL-10* −819G→A (41), and *IL-10* −1082C→T (42) was assigned to the genotype group previously reported to be associated with altered cytokine production. Significance, determined at $P < 0.05$, was not adjusted for the number of statistical tests performed.

TABLE 1
Baseline characteristics of participants by supplementation group[1]

	Supplementation group	
	Vitamin E (n = 254)	Placebo (n = 246)
Age (y)	85.9 (65.4–102.2)[2]	84.3 (65.7–102.6)
Women [n (%)]	181 (71)	174 (71)
White [n (%)]	235 (93)	230 (93)
BMI (kg/m^2)	25.8 ± 5.2[3]	25.9 ± 5.1
Serum albumin (g/dL)	3.8 ± 0.3	3.77 ± 0.4
Hemoglobulin (g/dL)	12.4 ± 1.4	12.17 ± 1.4
C-reactive protein (mg/L)	8.5 ± 12.7	8.37 ± 12.6
Plasma vitamin E (μg/dL)	1153 ± 404	1166 ± 424
Serum zinc (μg/dL)	83.8 ± 25.7	84.1 ± 27.8
Total number of medications	7.5 ± 4.1	7.4 ± 4.1
Participants taking NSAIDs [n (%)]	96 (38)	85 (35)
Medical history		
Coronary artery disease [n (%)]	89 (35)	78 (32)
Congestive heart failure [n (%)]	51 (20)	52 (21)
Hypertension [n (%)]	131 (52)	135 (55)
Diabetes mellitus [n (%)][4]	42 (17)	60 (24)
Dementia		
Alzheimer disease [n (%)]	27 (11)	24 (10)
Non-Alzheimer dementia [n (%)]	101 (40)	89 (36)
Current smoker [n (%)]	14 (6)	22 (9)
COPD [n (%)]	65 (26)	56 (23)
Malignancy [n (%)]	22 (9)	27 (11)

[1] NSAIDs, nonsteroidal antiinflammatory drugs; COPD, chronic obstructive pulmonary disease.
[2] Mean; range in parentheses (all such values).
[3] Mean ± SD (all such values).
[4] Significantly different between supplementation groups, $P < 0.05$ (chi-square test).

RESULTS

Subject characteristics

Most participants in this study were white women, and the average age of the participants was 85 y old (**Table 1**). There were no significant differences in baseline characteristics between the 2 supplementation groups with the exception of diabetes mellitus, which was more common in the placebo group. Because susceptibility to infection may be affected by other factors, such as concurrent micronutrient status, diabetes, and other comorbidities; these factors were considered in our analysis. When we evaluated the relation between vitamin E and the SNPs of interest for RIs, we included factors (*see* Methods) that might influence infection rates in the statistical models. We observed that the inclusion of these factors did not affect the results.

The frequencies of genotypes at each SNP were compared with the expected frequencies that would be observed under HWE. *IL-2* −330A→C and *TNF-α* −308G→A were not in HWE in this population (**Table 2**). In some cases, deviations from HWE are due to assay failures. To ensure that the observed deviations from HWE were not due to a technical error, genotyping at these SNPs was repeated and provided the same results. When we used the same assays in the GOLDN study (38) cohort, *IL-2* −330A→C did not deviate from HWE (*A/A*: n = 31; *A/C*: n = 32, and *C/C*: n = 3; HWE: $P > 0.05$). Because these results indicated that deviations from HWE were not due to an assay failure, genotype data for *IL-2* −330A→C and *TNF-α* −308G→A were included in the analyses.

Linkage disequilibrium gives a measure of the association between alleles and can be used to assess if SNPs will be inherited together. Linkage analysis among SNPs at the same locus indicated that *IL-10* −819G→A and *IL-10* −592G→T were in significant linkage disequilibrium (R^2 = 0.99), which suggested that SNPs at these locations are likely to be inherited together. The phenotypic results for associations between infection and *IL-10* −819G→A were the same as that for *IL-10* −592G→T; therefore, only results for *IL-10* −819G→A are reported.

TABLE 2
Frequency (*n*) of single nucleotide polymorphisms (SNPs)[1]

SNP	Major allele homozygotes	Heterozygotes	Minor allele homozygotes	HWE (*P* value)
IL-2 −330A→C	264	176	60	12.08 (0.001)
IFN-γ 874A→T	161	228	108	2.57 (0.109)
IL-6 −174C→G	232	219	49	0.07 (0.797)
TNF-α −308G→A	362	117	20	6.67 (0.009)
IL-1β −1473G→C	280	187	31	0.01 (0.976)
IL-1β −511G→A	210	228	58	0.11 (0.744)
IL-1β 3954C→T	295	170	32	1.23 (0.266)
IL-1β 6054G→A	212	217	68	1.09 (0.297)
IL-10 −1082C→T	153	241	106	0.38 (0.540)
IL-10 −819G→A	278	193	29	0.35 (0.552)
IL-10 −592G→T	277	194	29	0.43 (0.514)

[1] HWE, Hardy-Weinberg equilibrium.

Single SNPs and RIs

To examine if the effect of vitamin E depended on sex and SNPs, we tested if sex, vitamin E, and each single SNP were associated with RIs. We observed a 3-way interaction between vitamin E supplementation, sex, and *IL-10* −819G→A for total RIs ($P = 0.003$) and LRIs ($P = 0.03$). The nature of the interaction was similar for total RIs and LRIs.

Because the interaction was sex dependent, we examined the effect of vitamin E supplementation and *IL-10* −819G→A within men and women separately. Analyses within men showed no difference in the incidence of total RIs or LRIs between vitamin E and placebo groups within each genotype or in incidence of infection between genotypes within each supplement group (**Tables 3** and **4**). Analyses within women indicated that, among vitamin E–supplemented subjects, subjects with the *G/G* genotype at *IL-10* −819G→A had fewer total RIs than did subjects with the *A/G* ($P = 0.003$) or *A/A* ($P = 0.08$) genotype (Table 4). Furthermore, among vitamin E–supplemented subjects, subjects with the *G/G* genotype at *IL-10* −819G→A had fewer LRIs than did those with the *A/G* ($P = 0.03$) or *A/A* ($P = 0.04$) genotype (**Table 5**). However, among subjects who received the placebo, those subjects with the *A/A* genotype at *IL-10* −819G→A had fewer total RIs than did subjects with the *G/G* genotype ($P = 0.04$) (Table 4). Subjects with the *A/A* genotype at *IL-10* −819G→A who received vitamin E had more LRIs ($P = 0.06$) and total RIs ($P = 0.03$) than did subjects with the *A/A* genotype who were given the placebo (Tables 3 and 4). However, there were no differences in total RIs or LRIs between treatments in subjects with the *A/G* or *G/G* genotypes at *IL-10* −819G→A. The small number of subjects in each subgroup limited our power to detect significant differences by either supplement group or genotype after adjustment for covariates.

There were no interactions between vitamin E supplementation and the other single SNPs for RIs. We examined if there were any associations between the SNPs and infection that were sex dependent. We observed an interaction between sex and *IL-10* −1082C→T for LRIs ($P = 0.004$). Men with the *C/C* genotype at *IL-10* −1082C→T had a lower incidence of LRIs than did men with the *C/T* or *T/T* genotype ($P < 0.01$; Table 5). Similar trends were seen for pneumonia incidence ($P = 0.006$ for interaction between *IL-10* −1082C→T and sex; $P < 0.05$ for effect of *IL-10* −1082C→T in men).

We examined the relation between each of the single SNPs and infection. We observed that *IL-2* −330A→C was associated with the incidence of all URIs and colds ($P = 0.02$ and $P = 0.04$, respectively; **Table 6**). Subjects with the *C/C* genotype at *IL-2* 330A→C had a lower incidence of URIs and colds than did subjects with *A/A* and *A/C* genotypes. Similar trends for *IL-2* −330A→C were observed for the incidence of all RIs ($P = 0.06$).

IL-10 −819G→A was associated with an incidence of common colds ($P = 0.04$; **Table 7**). Subjects with the *A/A* genotype at *IL-10* −819G→A had a lower incidence of colds than did subjects with the *G/G* and *G/A* genotypes. Similar trends were observed for *IL-10* −819G→A and the incidence of all RIs ($P = 0.09$) and URIs ($P = 0.08$). We examined *IL-2* −330A→C and *IL-10* −819G→A together and did not observe an interaction between the 2 SNPs for colds. When the 2 SNPs

TABLE 3
Effect of vitamin E on total respiratory tract infections depends on sex and *IL-10* −819G→A

	Vitamin E		Placebo	
IL-10 −819G→A	*n*	Incidence[1]	*n*	Incidence[1]
Women				
A/A	16	1.61	9	0.39[2]
A/G	68	1.61	70	1.48
G/G	99	0.99[3]	96	1.31
Men				
A/A	1	0.00	3	1.42
A/G	27	1.23	28	1.79
G/G	43	1.61	40	1.24

[1] A negative binomial distribution was assumed for regression analysis of infection incidence. Incidence is per person-year. Analysis was adjusted for the year of enrollment.

[2] Differences in the incidence of total respiratory tract infections between female subjects with the *A/A* genotype at *IL-10* −819G→A who received a placebo compared with subjects who received vitamin E ($P = 0.03$), and differences in the incidence of total respiratory infection between female subjects with the *A/A* genotype at *IL-10* −819G→A who received placebo compared with subjects with *A/G* and *G/G* genotypes ($P = 0.04$).

[3] Differences in the incidence of total respiratory tract infections between female subjects with the *G/G* genotype at *IL-10* −819G→A who received vitamin E compared with subjects with *A/G* ($P = 0.003$) and *A/A* genotypes ($P = 0.08$).

TABLE 4
Effect of vitamin E supplementation on lower respiratory tract infections may depend on both sex and *IL-10* −819G→A

	Vitamin E		Placebo	
IL-10 −819G→A	*n*	Incidence[1]	*n*	Incidence[1]
Women				
A/A	16	0.91	9	0.13[2]
A/G	68	0.68	70	0.47
G/G	99	0.38[3]	96	0.54
Men				
A/A	1	0.00	3	1.06
A/G	27	0.64	28	0.68
G/G	43	0.68	40	0.48

[1] A negative binomial distribution was assumed for regression analysis of infection incidence. Incidence is per person-year. Analysis was adjusted for the year of enrollment.

[2] Differences in the incidence of lower respiratory tract infections between female subjects with the *A/A* genotype at *IL-10* −819G→A who received a placebo compared with subjects who received vitamin E ($P = 0.06$).

[3] Significant difference in the incidence of lower respiratory tract infections between female subjects with the *G/G* genotype at *IL-10* −819G→A who received vitamin E compared with female subjects with the *A/G* genotype ($P = 0.03$) and the *A/A* genotype ($P = 0.04$).

were evaluated in the same model for colds, the main effect of each SNP remained a predictor of infection incidence ($P < 0.03$ for both SNPs). *IL-2* −330C→A and *IL-10* −819G→A were no related to the incidence of LRIs.

IL-10 −1082/−819/−592 genotypes and RIs

We examined the combined effect of the SNPs at the *IL-10* promoter (−1082C→T, −819G→A, and −592G→T) on RI incidence by using genotypes estimated with HelixTree software (Golden Helix). There were 6 different estimated *IL-10* −1082/−819/−592 promoter genotypes in this population (**Table 8**). We observed a 3-way interaction between treatment, genotype, and sex for LRIs. We also observed an association between *IL-10* −1082/−819/−592 promoter genotype and colds. However, further assessment of the data indicated that the *IL-10* −819G→A genotype was largely responsible for these associations.

DISCUSSION

Our study suggests an association between common SNPs at *IL-10* and *IL-2* genes and RIs in elderly subjects. We observed that the SNP at *IL-10* −819G→A was associated with a susceptibility to common colds, and subjects with the *A/A* genotype at *IL-10* −819G→A had a lower incidence of colds than subjects with the *G/G* and *G/A* genotypes. Furthermore, in men, *IL-10* −1082C→T was associated with incidence of LRIs, and men with the *C/C* genotype at *IL-10* −1082C→T had a lower incidence of LRIs than men with the *C/T* or *T/T* genotype. In addition, we observed that the effect of the SNP at *IL-10* −819G→A on LRIs was modified by vitamin E supplementation in women. Although the *G/G* genotype at *IL-10* −819G→A was associated with am increased incidence of LRIs in women in the placebo group, in the vitamin E group, the *G/G* genotype at *IL-10* −819G→A was associated with a decreased incidence of LRIs. In addition, women with the *A/A* genotype at *IL-10* −819G→A who received vitamin E had more LRIs than did women with the *A/A* genotype who were given the placebo. To our knowledge, this is the first report of an association between SNPs in the *IL-10* gene and RIs in elderly as well as its modification by a nutrient (ie, vitamin E) in any age group. These associations between RIs and SNPs at the *IL-10* gene may reflect the importance of IL-10 in the resolution of infections. IL-10 inhibits IL-1β, TNF-α, nitric oxide production, and proinflammatory chemokines (43) and, thereby, reduces nonspecific damage to local tissue (43) and the risk of secondary infections (44).

Previously, few studies have examined the relation between SNPs at the *IL-10* promoter and acute infections. Subjects with the *C* allele at *IL-10* −1082C→T had an increased severity of pneumococcal infections (45), including community-acquired pneumonia (45, 46), but there was no association with the risk of these infections (45, 46), severe acute respiratory syndrome (39), or the common cold (24). Patients in intensive care with the *T* allele at *IL-10* −592G→T had a lower IL-10 release and an increased mortality than did *G/T* and *G/G* subjects (47). However, in contrast to our study, most previous studies used a case-control design to compare patients admitted to hospitals with healthy control subjects (39, 45, 46), and no other studies specifically examined the elderly (39, 47). In addition, no previous studies described an interaction between *IL-10* genotypes and sex. This is particularly interesting because we observed that the relation between the *IL-10* SNPs and LRIs may be sex specific.

We observed a 3-way interaction, which suggested that the effect of vitamin E supplementation on RIs depends on both *IL-10* genotypes and sex. The interaction was similar for both total and LRIs, which indicated that LRIs may be driving the observations for total RIs. For women who were given the

TABLE 5
IL-10 −1082C→T is related to lower respiratory tract infection in men[1]

Type of infection	*IL-10* −1082C→T	*n*	Incidence[2]	RR (95% CI)	*P* for RR[3]	*P* overall[3]
All lower respiratory	*T/T*	50	0.61	3.36 (1.28, 8.83)[4]	0.01	—
	T/C	66	0.80	3.99 (1.55, 10.26)[4]	0.01	—
	C/C	26	0.20	1	—	<0.01

[1] RR, rate ratio.

[2] A negative binomial distribution was assumed for regression analysis of infection incidence. Incidence is per person-year.

[3] For analysis between men and adjusted for the natural log offset of time in the intervention study and supplementation ($\alpha = 0.05$). Adjustment for baseline zinc did not significantly alter the association.

[4] Compared with the *C/C* genotype group.

TABLE 6
IL-2 −330A→C is related to upper respiratory tract infection and cold incidence[1]

Type of infection	*IL-2* −330A→C	*n*	Incidence[2]	RR (95% CI)	*P* for RR[3]	*P* overall[3]
All respiratory	*A/A*	264	1.40	1.45 (1.05, 2.01)[4]	0.02	—
	C/A	176	1.38	1.41 (1.01, 1.97)[4]	0.04	—
	C/C	60	0.98	1	—	0.06
All upper respiratory	*A/A*	264	0.82	1.67 (1.09, 2.57)[4]	0.02	—
	C/A	176	0.87	1.78 (1.14, 2.76)[4]	0.01	—
	C/C	60	0.54	1	—	0.02
Colds	*A/A*	264	0.67	1.63 (1.04, 2.58)[4]	0.03	—
	C/A	176	0.74	1.78 (1.12, 2.84)[4]	0.01	—
	C/C	60	0.45	1	—	0.04

[1] RR, rate ratio.
[2] A negative binomial distribution was assumed for regression analysis of infection incidence. Effect was sex independent. Incidence is per person-year.
[3] Adjusted for the natural log offset of time in the intervention study, supplementation, smoking, diabetes, dementia, baseline albumin and hemoglobin concentrations, year of enrollment, chronic obstructive pulmonary disease, age, sex, baseline BMI, cardiovascular disease, and hypertension ($\alpha = 0.05$).
[4] Compared with the *C/C* genotype group.

placebo, the *A/A* genotype was associated with lower rates of infection than in women with *G/A* and *G/A* genotypes at *IL-10* −819G→A. However, in the women given vitamin E, the *G/G* group had lower rates of LRI than did women with *G/A* and *A/A* genotypes at *IL-10* −819G→A. Vitamin E–supplemented women with the *A/A* genotype (the minority genotype) at *IL-10* −819G→A had a higher incidence of total RIs and tended to have higher LRIs than did women with the *A/A* genotype at *IL-10* −819G→A who received the placebo. By contrast, vitamin E–supplemented women with other *IL-10* −819G→A genotypes did not have a different incidence of LRIs than did those who received the placebo. In summary, these results suggest that vitamin E supplementation may increase susceptibility to RIs only in women with a particular genotype.

To our knowledge, this is the first report that vitamin E may alter susceptibility to RIs in women in a genotype-dependent manner. Previous reports have shown that vitamin E intake had no overall effect on pneumonia in younger women (48) and that supplemental vitamin E did not reduce the incidence of self-reported infections in the elderly (49). Our observation that the effect of vitamin E on LRIs in women depends on *IL-10* genotypes may partly explain these previous null results and raises the potential importance of considering genetic factors when the effect of vitamin E on RIs is evaluated.

Our results build on previous reports of sex-dependent associations between *IL-10* −1082C→T and arthritis (50), hepatitis (51), and longevity (26, 52). The findings from these studies and our results could be attributed to differences in IL-10 production between men and women (53) and the effect of sex hormones (54) on IL-10 production. Future studies are needed to determine whether the interactions we observed between sex and *IL-10* genotypes for RIs are related to sex-hormone receptor binding or signaling.

Further study is needed to determine the mechanisms that drive the associations between RIs and SNPs at the *IL-10* promoter and why women with particular *IL-10* genotypes may respond differently to vitamin E. Associations between infection and the *IL-10* SNPs may have been due to differences in IL-10 production between subjects with different *IL-10* genotypes (41, 55). Vitamin E could unfavorably alter IL-10 production (56, 57) or respiratory burst (58, 59) during LRIs, particularly among people genetically predisposed to altered IL-10 production (41, 55). Future studies to determine the effect of vitamin E on IL-10 production and respiratory burst during LRIs, particularly in elderly women, will help shed light on this observed interaction between vitamin E and SNPs at the *IL-10* promoter.

In addition to the relations between SNPs at *IL-10* and infection, we also observed that a common SNP at the gene that

TABLE 7
IL-10 −819G→A is related to cold susceptibility[1]

Type of infection	*IL-10* −819G→A	*n*	Incidence[2]	RR (95% CI)	*P* for RR[3]	*P* overall[3]
All upper respiratory	*G/G*	278	0.74	1.41 (0.78, 2.55)[4]	0.25	—
	A/G	193	0.94	1.73 (0.94, 3.14)[4]	0.07	—
	A/A	29	0.5	1	—	0.08
Colds	*G/G*	278	0.63	1.90 (0.92, 3.92)[4]	0.08	—
	A/G	193	0.79	2.24 (1.08, 4.66)[4]	0.03	—
	A/A	29	0.31	1	—	0.04

[1] RR, rate ratio.
[2] A negative binomial distribution was assumed for regression analysis of infection incidence. Effect was sex independent. Incidence is per person-year.
[3] Adjusted for the natural log offset of time in the intervention study, supplementation, smoking, diabetes, dementia, baseline albumin and hemoglobin concentrations, year of enrollment, chronic obstructive pulmonary disease, age, sex, baseline BMI, cardiovascular disease, and hypertension ($\alpha = 0.05$).
[4] Compared with the *A/A* genotype group.

TABLE 8
Frequency (*n*) of *IL-10* −1082/−819/−592 genotypes

IL-10 genotype (−1082/−819/−592)	Frequency
TAT_TAT	29
TAT_TGG	75
CGG_TAT	118
CGG_CGG	105
CGG_TGG	123
TGG_TGG	49

codes for IL-2 was associated with URIs. In the current study, subjects with the *C/C* genotype at *IL-2* −330A→C had a lower incidence of all URIs and colds than did subjects with the *A/A* or *A/C* genotype. These results are notable given the limited number of studies that have examined the relation between *IL-2* −330A→C and acute RIs. A smaller study of adults reported that subjects with the *A/C* genotype at *IL-2* −330A→C had a higher incidence of self-reported common cold than did subjects with the *A/A* or *C/C* genotype (24). IL-2 regulates lymphocytes and natural killer cells (60) and SNPs that affect IL-2 could influence the immune response to infection.

Because the *C/C* genotype at *IL-2* −330A→C was previously associated with higher IL-2 production than did the *A/A* or *A/C* genotype (20, 40), we examined the relation between *IL-2* −330A→C and ex vivo IL-2 production in a subset of our study participants (n = 100). We did not observe an association between *IL-2* −330A→C and IL-2 production (data not shown). Thus, the association between *IL-2* −330A→C and URIs may not be wholly due to differences in IL-2 production, and alternate explanations should be explored.

Our observation that participants with different *IL-2* −330A→C genotypes had varied risk of RIs is particularly interesting because we observed that *IL-2* −330A→C and *TNF-α* −308G→A were not in HWE in this study. For both SNPs, the frequency of heterozygotes was lower than what would be expected under HWE. In previous studies of elderly Europeans, *IL-2* −330A→C (61, 62) and *TNF-α* −308G→A (63) did not deviate from HWE. In addition, when we tested a younger (<65 y old), predominantly white population, we observed that *IL-2* −330A→C was in HWE. In the current study, elderly nursing home residents with the *A/C* genotype at *IL-2* −330A→C had a higher incidence of URIs and colds. Together, these data suggest that there is a gradual depletion of subjects with the *A/C* genotype at *IL-2* −330A→C as the population ages, which may be related in part to greater RI risk among those with the *A/C* genotype. Future studies are needed to replicate our observation that *IL-2* −330A→C deviated from HWE in other elderly populations and to compare biological functions relevant to longevity between *IL-2* −330A→C genotypes.

In conclusion, we observed that RIs susceptibility among the elderly may be influenced by SNPs at the *IL-2* and *IL-10* promoter regions and that the efficacy of vitamin E supplementation for reducing RIs in the aged may depend on sex and *IL-10* genotypes. These observations may be used to generate further hypotheses and studies of individual susceptibility to infection and variable responses to vitamin E supplementation. Further studies are needed to verify our results and understand the mechanisms driving them, including how differences in the cause of infection may affect associations. However, our results suggest that the recommendations for vitamin E supplementation as a preventive measure against RIs should consider genetics and sex. These observations may have ramifications for public health through improved predictions of infection in the elderly and help to identify individuals who may benefit the most from taking supplemental vitamin E.

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