## Practice of Epidemiology

# A Likelihood Ratio Test for Gene-Environment Interaction Based on the Trend Effect of Genotype Under an Additive Risk Model Using the Gene-Environment Independence Assumption

Matthieu de Rochemonteix, Valerio Napolioni, Nilotpal Sanyal, Michaël E. Belloy, Neil E. Caporaso, Maria T. Landi, Michael D. Greicius, Nilanjan Chatterjee, and Summer S. Han*

* Correspondence to Dr. Summer S. Han, Quantitative Sciences Unit, Department of Medicine, Stanford University School of Medicine, 1701 Page Mill Road, Palo Alto, CA 94304 (e-mail: summer.han@stanford.edu).

Several statistical methods have been proposed for testing gene-environment (G-E) interactions under additive risk models using data from genome-wide association studies. However, these approaches have strong assumptions from underlying genetic models, such as dominant or recessive effects that are known to be less robust when the true genetic model is unknown. We aimed to develop a robust trend test employing a likelihood ratio test for detecting G-E interaction under an additive risk model, while incorporating the G-E independence assumption to increase power. We used a constrained likelihood to impose 2 sets of constraints for: 1) the linear trend effect of genotype and 2) the additive joint effects of gene and environment. To incorporate the G-E independence assumption, a retrospective likelihood was used versus a standard prospective likelihood. Numerical investigation suggests that the proposed tests are more powerful than tests assuming dominant, recessive, or general models under various parameter settings and under both likelihoods. Incorporation of the independence assumption enhances efficiency by 2.5-fold. We applied the proposed methods to examine the gene-smoking interaction for lung cancer and gene–apolipoprotein E ε4 interaction for Alzheimer disease, which identified 2 interactions between apolipoprotein E ε4 and loci membrane-spanning 4-domains subfamily A (*MS4A*) and bridging integrator 1 (*BIN1*) genes at genome-wide significance that were replicated using independent data.

additive risk model; Alzheimer disease; case-control design; gene–APOE ε4 interaction; gene-environment independence; gene-environment interaction; gene-smoking interaction; GWAS

Abbreviations: APOE, apolipoprotein E; BIN1, bridging integrator 1; G-E, gene-environment; GWAS, genome-wide association study; LOAD, late-onset Alzheimer disease; LRT, likelihood ratio test; LRT-P, prospective likelihood ratio test; LRT-R, retrospective likelihood ratio test; MOR, marginal odds ratio; RERI, relative excess risk due to interaction; SNP, single nucleotide polymorphism.

A gene-environment interaction is defined as the joint effect of genetic and environmental factors that cannot be explained by their separate marginal effects (1). Statistically, an interaction is defined as a departure from the underlying disease risk model, which depends on the selection of proper scale for measuring the presence of interactions (2). While multiplicative interaction based on a logit model is more commonly used for case-control studies, additive interaction has been shown to be more relevant for evaluating prevention or intervention strategies in public health decision-making (3–5). Additive interaction measures the departure from an additive risk model, which assumes that gene and environment act additively on the risk of the disease itself (6, 7). It has been shown that conceptual models for biological interactions translate to the presence of interaction on the additive scale and not necessarily the multiplicative scale (8). Recently, several studies reported G × G or G × E interaction findings applying additive interaction tests for various complex diseases (9–11).

A number of methods have been proposed for detecting additive interactions between risk factors for case-control studies (12–16), including the methods for estimating the

confidence intervals of the relative excess risk due to inter-action (RERI), which measures the magnitude of additive interaction [17–19]. A likelihood ratio test (LRT) has been developed for additive interaction based on a set of con-straints to ensure the additive joint effects of gene and environment, which also incorporates the gene-environment (G-E) independence assumption to increase power [13]. Another recent study extended this method and applied an empirical Bayes-type approach to exploit the G-E indepen-dence assumption in a data-adaptive way [14]. However, the main limitation of these methods is their strong assumptions from the underlying genetic model, such as dominant or recessive effects that are known to be less robust when the true genetic model is unknown. A more general genetic model that treats genotype as a categorical variable (i.e., allowing 2 separate parameters for the main effect of geno-type) has also been proposed [13], but it has been shown to have reduced power due to increased degrees of freedom (df). It is also known that most common variants associated with complex diseases from genome-wide association study (GWAS) have been identified under an additive genetic model [20], where the effect of genotype is linear (i.e., the trend effect of genotype), rather than the effect being under a dominant or recessive model. Studies have shown that association tests based on the trend effect of genotype are more robust compared with dominant or recessive model–based tests under varying true genetic models [21], which have been widely used in analyzing the main effects of common variants in most recent GWAS [22, 23].

In this study, we developed a new statistical test for detect-ing additive interaction that incorporates the trend effect of genotype by extending the previously developed LRT [13]. We use a constrained likelihood approach to impose 2 sets of constraints for: 1) the trend effect of genotype and 2) the additive joint effect of gene and environment, using the parameter estimates obtained from a saturated logit model. To incorporate the G-E independence assumption, a retrospective likelihood is used. We conducted a simulation study with varying genetic models and varying magnitudes of interaction. We applied the proposed method to examine the gene-smoking interaction for lung cancer and both gene–apolipoprotein E (*APOE*) ε4 and gene-sex interactions for late-onset Alzheimer disease (LOAD). We implemented this method in the freely available R (R Foundation for Statistical Computing, Vienna, Austria) package, CGEN (https://www.bioconductor.org/packages/release/bioc/html/CGEN.html).

## METHODS

### Additive interaction under a dominant or recessive genetic model

For subject $i$, $G_i$ is a binary genetic factor, where $G_i = 1$ if the subject has at least 1 copy of the variant of interest in a single nucleotide polymorphism (SNP) and $G_i = 0$ other-wise for a dominant genetic model; $G_i = 1$ if the subject has 2 copies of the variant in a SNP and $G_i = 0$ otherwise for a recessive genetic model. $E_i$ is an environmental risk factor that is a categorical variable, and $D_i$ is the disease status ($D_i = 1$ if one has a disease and $D_i = 0$ otherwise). Without

loss of generality, we assume a binary $E_i$ in this setting. An additive risk model assumes that $G_i$ and $E_i$ act additively on the risk of the disease itself without any (nonidentity) link function: $P(D_i = 1|G_i, E_i) = b_0 + b_G G_i + b_E E_i$. A departure from this model is called an additive interaction, which can be tested using the null hypothesis of $H_0 : b_{GE} = 0$ in the following model:

$$R_i = P(D_i = 1|G_i, E_i) = b_0 + b_G G_i + b_E E_i + b_{GE} G_i E_i.$$

Suppose $R_{ge} = P(D = 1|G = g, E = e)$ for $g, e = 0, 1$. The null hypothesis, $H_0 : b_{GE} = 0$, can be expressed as $R_{11} - R_{01} = R_{10} - R_{00}$, which implies that the risk difference associated with $G_i$ is constant across different levels of $E_i$. By dividing this equation by the reference risk $R_{00}(RR_{ij} = R_{ij}/R_{00})$, we obtain the relative risk relationship for the null: $RR_{11} - RR_{01} = RR_{10} - 1$. Assuming a rare disease, we can approximate a relative risk by an odds ratio, and hence we obtain $H_0 : OR_{11} - OR_{01} = OR_{10} - 1$. This implies that additive interaction can be tested using the following null hypothesis:

$$H_0 : \exp(\beta_G + \beta_E + \beta_{GE})$$
$$- \exp(\beta_G) - \exp(\beta_E) + 1 = 0, \qquad (1)$$

where the parameters are estimated using the saturated logit model,

$$\text{logit}\{\Pr(D_i = 1|G_i, E_i)\}$$
$$= \beta_0 + \beta_G G_i + \beta_E E_i + \beta_{GE} G_i E_i. \qquad (2)$$

Additive interaction can be tested using a likelihood ratio test using the null hypothesis in equation 1 against the alternative using the model in equation 2, which gives a 1-df test. The magnitude of additive interaction can be measured by RERI, which is defined as: $RERI_G = \exp(\beta_G + \beta_E + \beta_{GE}) - \exp(\beta_G) - \exp(\beta_E) + 1$. This additive interaction LRT can be generalized to $G_i$ as a 3-level categorical variable ($G_i = 0, 1, 2$) and multilevel $E_i$ ($E_i = e_1, e_2, \ldots, e_k$), which has been derived in a previous study [13] and implemented in CGEN [24]. This general model–based test has larger df (df = 2k versus df = $k$ for a dominant or recessive model–based test).

### Additive interaction under the trend effect of genotype (additive genetic model)

To extend the method described in the previous section to an approach based on the trend effect (linear effect) of genotype, we consider the following saturated additive model for a 3-category genetic factor, $G_i$ ($G_i = 0, 1, 2$):

$$R_i = P(D_i = 1|G_i, E_i)$$
$$= b_0 + b_{G_1} G_{1i} + b_{G_2} G_{2i} + b_E E_i$$
$$+ b_{G_1E} G_{1i} E_i + b_{G_2E} G_{2i} E_i,$$

Where $G_{1i}$ and $G_{2i}$ are dummy variables indicating whether subject $i$ has 1 or 2 copies of the variant in a given SNP. Any covariates can also be included in the model without

**Table 1.**  Disease Risk and Odds Ratio for Gene (G = 0,1,2) and Environment (E = 0,1) Based on the Saturated Additive Risk Model and the Saturated Logistic Regression Model, Respectively

| G | Disease Risk | | Odds Ratio | |
|---|---|---|---|---|
| | $E = 0$ | $E = 1$ | $E = 0$ | $E = 1$ |
| $G = 0$ | $R_{00}(= b_0)$ | $R_{01}(= b_0 + b_E)$ | $OR_{00}(= 1)$ | $OR_{01}(= \exp(\beta_E))$ |
| $G = 1$ | $R_{10}(= b_0 + b_{G_1})$ | $R_{11}(= b_0 + b_{G_1} + b_E + b_{G_1E})$ | $OR_{10}(= \exp(\beta_{G_1}))$ | $OR_{11}(= \exp(\beta_{G_1} + \beta_E + \beta_{G_1,E}))$ |
| $G = 2$ | $R_{20}(= b_0 + b_{G_2})$ | $R_{21}(= b_0 + b_{G_2} + b_E + b_{G_2E})$ | $OR_{20}(= \exp(\beta_{G_2}))$ | $OR_{21}(= \exp(\beta_{G_2} + \beta_E + \beta_{G_2,E}))$ |

Abbreviations: E, environmental factor; G, genetic factor; OR, odds ratio; R, risk.

affecting the derivations that are shown in this section. The corresponding risk and odds ratio for each G and E are shown in Table 1. The trend effect of $G$ on the disease risk (or linear effect of $G$) can be expressed as:

$$R_{20} - R_{10} = R_{10} - R_{00} \text{ (for } E = 0) \text{ and} \quad (3)$$

$$R_{21} - R_{11} = R_{11} - R_{01} \text{ (for } E = 1). \quad (4)$$

The null hypothesis of no additive interaction, $H_0 : b_{G_1,E} = b_{G_2,E} = 0$, can equivalently be expressed as:

$$H_0 : R_{11} - R_{01} = R_{10} - R_{00} \quad (5)$$

and

$$R_{21} - R_{11} = R_{20} - R_{10}. \quad (6)$$

These equations can be rewritten in terms of relative risks by dividing them by the baseline risk, $R_{00}$. These relative risks can be approximated using odds ratios under a rare disease assumption. We use the following saturated logit model to obtain the odds ratio for each combination of levels of $G$ and $E$:

$$\text{logit} \{\Pr (D_i = 1 | G_i, E_i)\}$$
$$= \beta_0 + \beta_{G_1} G_{1i} + \beta_{G_2} G_{2i} + \beta_E E_i$$
$$+ \beta_{G_1E} G_{1i} E_i + \beta_{G_2E} G_{2i} E_i, \quad (7)$$

which are shown in the last 2 columns in Table 1. Using these odds ratios, the trend effect relationships in equation 3 and equation 4 are expressed as:

$$\beta_{G_2} = \log(2 \exp(\beta_{G_1}) - 1)$$
$$(\Longleftrightarrow \exp(\beta_{G_2}) - \exp(\beta_{G_1}) = \exp(\beta_{G_1}) - 1) \quad (3b)$$

and

$$\beta_{G_2,E} = \log \left( \frac{2 \exp (\beta_{G_1} + \beta_{G_1,E}) - 1}{2 \exp (\beta_{G_1}) - 1} \right)$$
$$= \log \left( \frac{2 \exp (\beta_{G_1} + \beta_{G_1,E}) - 1}{\exp (\beta_{G_2})} \right). \quad (4b)$$

We note that the "usual" additive coding of the genotype for the trend effect for multiplicative interaction (i.e., coding $G$ as 0, 1, or 2 depending on the number of a minor allele) is different from the trend effect of genotype for additive interaction (shown in equations 3b and 4b) due to the different link functions used for these models (logit versus identity). Similarly, the null hypothesis in equation 5 and equation 6 is expressed as:

$$H_0 : \beta_{G_1,E} = \log \left( \frac{\exp (\beta_{G_1}) + \exp (\beta_E) - 1}{\exp (\beta_{G_1} + \beta_E)} \right)$$
$$(\Longleftrightarrow RERI_{G=1} = 0) \quad (5b)$$

and

$$\beta_{G_2,E} = \log \left( \frac{\exp (\beta_{G_2}) + \exp (\beta_E) - 1}{\exp (\beta_{G_2} + \beta_E)} \right)$$
$$(\Longleftrightarrow RERI_{G=2} = 0). \quad (6b)$$

Based on simple algebra, we show that the equations in equation 5b and equation 6b are identical when the trend effect relation equations in equation 3b and equation 4b hold. Therefore, under the trend effect of genotype, the null hypothesis for testing additive interaction is equation 5b.

**Constrained LRT for additive interaction under the trend effect of genotype**

In conducting an LRT, we first fit the saturated logit model shown in equation 7 with the 2 trend effect–related constraints shown in equation 3b and equation 4b (i.e., the full model). This model includes the following free parameters to be estimated by the maximum likelihood method: $\beta_0, \beta_{G_1}, \beta_E,$ and $\beta_{G_1,E}$. In the second step, we further impose the additional null hypothesis related constraint from equation 5b to fit the null model, which has 3 free parameters: $\beta_0, \beta_{G_1},$ and $\beta_E$. We conduct an LRT by comparing the full model with the null model, which gives a 1-df test. For fitting these models, we use a "prospective likelihood" that is commonly used for case-control data: $L = \prod_i \Pr(D_i = d | G_i, E_i) = \prod_i \{R_i^{d_i}(1 - R_i)^{1-d_i}\}$ (for $d_i = 0,1$), and henceforth we refer to the corresponding test as LRT-P.

## Extension to incorporate the G-E independence assumption

For case-control analysis, an assumption of G-E independence in the underlying population can be used to enhance the power of G-E tests (13, 25, 26). It has been shown that a retrospective likelihood for case-control data can help exploit the G-E independence assumption to obtain efficient parameter estimates of a general logistic model (25). More recently, a study showed how the retrospective likelihood framework can enable the G–E independence assumption to enhance the power of additive interaction tests (13).

To incorporate the G-E independence assumption in our method, we used the profile likelihood-based approach developed by Chatterjee and Carroll (25) to develop a retrospective LRT for additive interaction under the trend effect of genotype, henceforth denoted LRT-R. The profile likelihood is given by:

$$L = \prod_i \Pr(D_i = d, G_i = g | E_i = e, S_i = s, R = 1),$$

where $R$ indicates whether or not a subject is included in the case-control design, and $S_i$ is a stratifying variable (such as ethnicity or principle components of population stratification).

## Simulation study

We conducted a simulation study to evaluate the type I error rates and power of the proposed additive interaction tests under the trend effect of genotype and compared the power with those under the existing dominant, recessive, and general model–based additive interaction tests. For the power simulation, we considered the 4 different scenarios where the true disease risk model is under: 1) the trend effect of genotype (i.e., additive genetic model), 2) the dominant model, 3) the recessive model, and 4) the general model (i.e., a departure from the dominant, recessive, and trend effect models). In each scenario, we varied the magnitude of additive interaction (i.e. $RERI_{G=1}$) from 0.8 to 1 and 1.2. We assumed that $G$ and $E$ are independently distributed in the underlying population. The minor allele frequency of $G$ was set as 0.3, and the prevalence for $E$ was set as $\Pr(E_i = 1) = 0.2$. We also assumed the disease was rare, so that disease-free subjects approximately represented the underlying population. The disease prevalence was assumed to be 0.01. We fixed the marginal odds ratio (MOR) for the gene (MOR(G))—that is, the disease odds ratio for $G = 1$ (vs. $G = 0$) if environment is ignored in the analysis—at 1.25, reflecting the modest strength of genetic association typically observed in GWAS, and varied the MOR for environment (MOR(E)) from 2.5 to 3.5. The saturated logit model in equation 7 was used to simulate data. We chose the parameter values for $\beta_0, \beta_{G_1}, \beta_{G_2}, \beta_E, \beta_{G_1,E},$ and $\beta_{G_2,E}$ in the logistic model so that MOR(G), MOR(E), and RERI are fixed at given values (see Table 2). To compare the power of the proposed trend effect–based LRT-R versus LRT-P, we used the parameters under the truth of the trend effect model (the first 6 rows in Table 2). In each simulation, we generated $G$ and $E$ data for 5,000 cases and 5,000 controls. A significance level of $\alpha = 1.00 \times 10^{-7}$ was used for power

calculation with 1,000 replicates, and 10,000 replicates were used for assessing type I error.

## Data description for lung cancer and smoking

We applied the proposed method to examine the gene × smoking interaction for lung cancer, where smoking is a known risk factor. We used National Cancer Institute GWAS data that included 5,739 cases and 5,848 controls from 4 studies (27). This data included 14 SNPs that were identified from previous GWAS ($P < 5 \times 10^{-8}$), conducted in either White (28–31) or Asian populations (32–34) listed in the National Human Genome Research Institute GWAS catalog (https://www.ebi.ac.uk/gwas/). The genetic regions for this data include 15q25.1, which is known to interact with smoking under an additive model (16) and is associated with smoking behaviors (35–41). Therefore, we applied only LRT-P to the SNPs in this region. The goal of this analysis was to compare the results using the proposed tests with those using the existing additive interaction tests based on dominant or general models. For each SNP, we applied both LRT-P and LRT-R (except 15q25.1), and the model adjusted for age, gender, and study, where the study variable was used as a stratification variable ($S$) for LRT-R.

## Data description for LOAD and gene × sex and gene × gene interactions

We also applied the proposed methods to the GWAS data for LOAD to examine gene × sex interaction and gene × APOE ε4 gene interaction. The ε4 allele of the apolipoprotein E gene (*APOE*, on chromosome 19q13.32, Online Mendelian Inheritance in Man (OMIM)∗107741) is the strongest genetic risk factor for LOAD (42). Individuals carrying 2 copies of the *APOE* ε4 allele have more than a 10-fold increased risk of LOAD (43). It is reported that women have a higher risk of LOAD than men (44). Our goal was to examine interactions between SNP × *APOE* ε4 and SNP × sex by applying the proposed methods. For the discovery phase, we used GWAS data that includes 8,861 cases and 7,613 controls who are of Northwestern European ancestry, collected from 18 different studies. These data were made available by the National Institute on Aging Genetics of Alzheimer's Disease Data Storage Site and similar LOAD repositories (Web Appendix 1; Web Tables 1–2) (45). The GWAS data included 89,936 SNPs across the genome, which were pruned from approximately 5 million SNPs using the following procedure: 1) we ranked SNPs by the significance of the main effect for SNP-LOAD risk association; 2) starting from the top ranked SNP, we evaluated pairwise linkage disequilibrium between the given SNP and each other SNP to remove the SNPs that were in high linkage disequilibrium ($r^2 > 0.9$); and 3) we removed SNPs with a minor allele frequency below 5% (45). This procedure was used to reduce the computational burden of the analysis through the removal of highly correlated SNPs but retain the SNPs that were relevant to LOAD risk (45). The pruning was conducted based on PriorityPruner, v.0.1.4 (http://prioritypruner.sourceforge.net/). For the validation phase, we used an independent data set obtained from the second

**Table 2.**   Twenty-Four Sets of Parameter Values Used to Simulate Gene, Environment, and Disease Indicator Using the Logistic Regression Model in Equation 7

| MOR and Parameter | Trend Model | | | Dominant Model | | | Recessive Model | | | General Model | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | RERI[a] | | | RERI[a] | | | RERI[b] | | | RERI[a] | | |
| | 0.8 | 1 | 1.2 | 0.8 | 1 | 1.2 | 0.8 | 1 | 1.2 | 0.8 | 1 | 1.2 |
| MOR(E) = 2.5, MOR(G) = 1.25 | | | | | | | | | | | | |
| $\exp(\beta_0)$ | 0.008 | 0.008 | 0.008 | 0.008 | 0.008 | 0.008 | 0.008 | 0.008 | 0.008 | 0.008 | 0.008 | 0.008 |
| $\exp(\beta_{G1})$ | 1.160 | 1.108 | 1.064 | 1.160 | 1.114 | 1.070 | 1.000 | 1.000 | 1.000 | 1.160 | 1.111 | 1.067 |
| $\exp(\beta_{G2})$ | 1.320 | 1.216 | 1.128 | 1.160 | 1.114 | 1.070 | 1.172 | 1.132 | 1.093 | 1.237 | 1.164 | 1.099 |
| $\exp(\beta_E)$ | 2.200 | 2.040 | 1.880 | 2.260 | 2.120 | 2.030 | 2.490 | 2.477 | 2.440 | 2.230 | 2.080 | 1.954 |
| $\exp(\beta_{G1E})$ | 1.238 | 1.393 | 1.572 | 1.228 | 1.369 | 1.519 | 1.000 | 1.000 | 1.000 | 1.233 | 1.381 | 1.545 |
| $\exp(\beta_{G2E})$ | 1.419 | 1.716 | 2.079 | 1.228 | 1.369 | 1.519 | 1.186 | 1.287 | 1.400 | 1.320 | 1.533 | 1.777 |
| MOR(E) = 3, MOR(G) = 1.25 | | | | | | | | | | | | |
| $\exp(\beta_0)$ | 0.007 | 0.007 | 0.007 | 0.006 | 0.006 | 0.006 | 0.007 | 0.007 | 0.007 | 0.007 | 0.007 | 0.007 |
| $\exp(\beta_{G1})$ | 1.186 | 1.140 | 1.099 | 1.189 | 1.143 | 1.095 | 1.000 | 1.000 | 1.000 | 1.187 | 1.141 | 1.097 |
| $\exp(\beta_{G2})$ | 1.372 | 1.280 | 1.198 | 1.189 | 1.143 | 1.095 | 1.199 | 1.157 | 1.117 | 1.277 | 1.210 | 1.145 |
| $\exp(\beta_E)$ | 2.790 | 2.630 | 2.450 | 2.840 | 2.700 | 2.520 | 3.000 | 2.980 | 2.960 | 2.815 | 2.665 | 2.485 |
| $\exp(\beta_{G1E})$ | 1.141 | 1.257 | 1.392 | 1.134 | 1.245 | 1.383 | 1.000 | 1.000 | 1.000 | 1.138 | 1.251 | 1.387 |
| $\exp(\beta_{G2E})$ | 1.244 | 1.459 | 1.720 | 1.134 | 1.245 | 1.383 | 1.112 | 1.200 | 1.294 | 1.188 | 1.348 | 1.542 |

Abbreviations: E, environmental factor; G, genetic factor; MOR, marginal odds ratio; RERI, relative excess risk due to interaction.

[a] RERI denotes $RERI_{G=1}$ for trend, dominant, and general model; the values for $RERI_{G=2}$ for the general model are: 1.175, 1.45, and 1.76, with corresponding $RERI_{G=1}$ values of 0.8, 1, and 1.2, respectively.

[b] RERI denotes $RERI_{G=2}$ for recessive model.

wave of National Institute on Aging—Alzheimer's Disease Centers GWAS (ADC4–7) (Web Appendix 1), which included 1,907 cases and 1,677 controls who were of European ancestry, and comprised the top 2 SNPs that showed significance ($P < 5 \times 10^{-8}$) in our discovery phase (show below). The *APOE* ε4 variable was coded as 1 for mutation carriers versus 0 for noncarriers, as commonly used in the literature (46, 47). This gene is located on chromosome 19q, and hence the SNPs in this gene (and neighboring SNPs) might violate the assumption for G-E (in this case G-G) independence. Therefore, we excluded this region for LRT-R-based SNP × *APOE* ε4 analysis. The model adjusted for age, sex, and study, and we used the study variable as a stratification variable for LRT-R.

## RESULTS

### Simulation study

The results in Table 3 show that the proposed methods for additive interaction have correct type I error rates for both LRT-P and LRT-R. The power simulation results under the prospective likelihood are shown in Figure 1, where the first column shows the power of the 4 additive interaction tests applied to the data generated under an additive genetic

model. The results demonstrate that the proposed trend effect–based interaction test has a larger power compared with the existing (dominant, recessive, and general model–based) tests across different magnitudes of additive interaction (RERI). When data were simulated under the truth of the dominant model (the second column in Figure 1), the additive interaction test assuming the dominant model was most powerful, as expected, and the general model–based and the proposed trend effect–based tests showed comparable power. For data simulated under the model that departs from the dominant and the trend effect models (the third column in Figure 1), the proposed test showed a larger power than the dominant model–based test and had a comparable power to the general model–based test.

We also compared the power of the proposed trend effect–based additive test under the prospective versus retrospective likelihood that assumes the G-E independence assumption. The results in Figure 2 show that the trend effect–based interaction test under the retrospective likelihood provides a larger power than the one under the prospective likelihood across different RERIs. The third column in Figure 2 shows that the retrospective likelihood-based approach has 2.4–2.5 times increased efficiency compared with the prospective likelihood approach, by exploiting the G-E independence assumption. For comparison, the simulation results using

**Table 3.**   Type 1 Error Rates of the Additive Interaction Tests Based on the Trend Effect of the Genotype, Dominant Model, Recessive Model, and General Model

| α | LRT-R | | | | LRT-P | | | |
|---|---|---|---|---|---|---|---|---|
| | Trend Test | Dominant Test | Recessive Test | General Test | Trend Test | Dominant Test | Recessive Test | General Test |
| 0.1000 | 0.0987 | 0.1007 | 0.1018 | 0.1018 | 0.0987 | 0.0991 | 0.0987 | 0.0997 |
| 0.0500 | 0.0466 | 0.0497 | 0.0534 | 0.0507 | 0.0497 | 0.0510 | 0.0508 | 0.0494 |
| 0.0100 | 0.0109 | 0.0090 | 0.0112 | 0.0093 | 0.0076 | 0.0092 | 0.0087 | 0.0097 |
| 0.0050 | 0.0050 | 0.0047 | 0.0054 | 0.0044 | 0.0034 | 0.0046 | 0.0047 | 0.0051 |
| 0.0010 | 0.0009 | 0.0009 | 0.0010 | 0.0010 | 0.0009 | 0.0012 | 0.0012 | 0.0011 |

Abbreviations: LRT-P, prospective likelihood ratio test; LRT-R, retrospective likelihood ratio test.

the multiplicative interaction tests (trend, dominant, recessive, and general) under both likelihoods are shown in Web Figures 1–2. These showed consistent results compared with the additive interaction tests (Figure 1 and Figure 2), where the trend effect–based test showed robustness across different underlying genetic models, and the approach under the retrospective likelihood increased efficiency compared with the prospective likelihood.

### Application to the lung cancer and smoking data

The results of the SNP × smoking interaction analysis for lung cancer are shown in Table 4. Among the 14 SNPs we examined, 3 SNPs (rs8034191 and rs8042374 in 15q25.1 and rs31489 in 5p15.33) showed statistical significance ($\alpha$ = 0.0006 based on Bonferroni correction). Web Table 3 displays the 3 × 2 odds ratio tables and RERI estimates for these SNPs. The topmost significant interaction was with rs8034191 ($P = 5.55 \times 10^{-16}$ using the trend effect–based test; RERI = 2.16, 95% CI: 1.54, 2.78), which confirmed the previously reported additive interaction between this SNP and smoking (16). For this SNP, the proposed trend effect–based test showed improved significance ($P = 5.55 \times 10^{-16}$) compared with the dominant ($P = 1.13 \times 10^{-14}$) or general model–based tests ($P = 7.88 \times 10^{-15}$). Increase of risk induced by the C allele of this SNP is significantly larger among ever smokers versus never smokers. Another SNP that reached statistical significance was rs31489 at the cleft lip and palate transmembrane protein 1-like protein gene (*CLPTM1L*) at 5p15.33, which showed improved significance for trend effect–based tests compared with the dominant or general model–based tests, with highest significance observed for the trend effect–based test under the retrospective likelihood ($P = 4.11 \times 10^{-4}$; RERI = −0.75, 95% CI: −1.20, −0.28).

### Application to the LOAD GWAS data

Web Table 4 shows the top 30 most significant findings from the SNP × *APOE* ε4 interaction analysis (discovery

phase) and is sorted by the significance of the additive interaction test based on the trend effect of genotype under the retrospective likelihood approach. Applying the genome-wide significance level ($\alpha = 5 \times 10^{-8}$), 2 SNPs were statistically significant across the different tests conducted (see Table 5 for odds ratios and RERI estimates), the proposed trend effect–based additive interaction test showing the most significant *P* values for both of them. The topmost significant SNP × *APOE* ε4 interaction was found with rs6733839 on 2q14.3 (additive interaction trend test $P = 5.56 \times 10^{-14}$; RERI = 1.11, 95% CI: 0.788, 1.422), where the absolute increase of risk induced by the T allele was significantly larger among *APOE* ε4 carriers versus noncarriers. To examine this region further, we pulled out all the SNP data in the region harboring rs6733839 (+/− 200kb) and conducted the same set of tests. The zoom-in Manhattan plot in Web Figure 3A shows a peak around the SNP rs6733839, where a neighboring SNP rs4663105 that shows stronger significance ($P = 4.77 \times 10^{-15}$) is highly correlated with rs6733839 ($r^2 = 0.94$). The second-most significant interaction was identified with rs1582763 ($P = 5.32 \times 10^{-9}$; RERI = −0.64, 95% CI: −0.852, −0.426) in 11q12.2, an intergenic variant near membrane-spanning 4-domains subfamily A member 4A (*MS4A4A*). The zoom-in plot for this SNP, shown in Web Figure 3B, shows a peak around rs1582763. The results of SNP × sex interaction did not show any significant findings ($P < 5 \times 10^{-8}$) (see Web Figure 4). We conducted a validation study using an independent data set to evaluate the top 2 findings at rs6733839 and rs1582763. The result is shown in Table 5, where both interaction findings were replicated using this independent data set, showing consistent directions of RERIs with improved significance when data were pooled for discovery and validation.

### DISCUSSION

In this study, we developed a robust LRT for detecting G × E interaction based on the trend effect of genotype under an additive risk model that incorporates the G-E independence assumption. We used a constrained likelihood approach to impose 2 sets of constraints, the linear trend
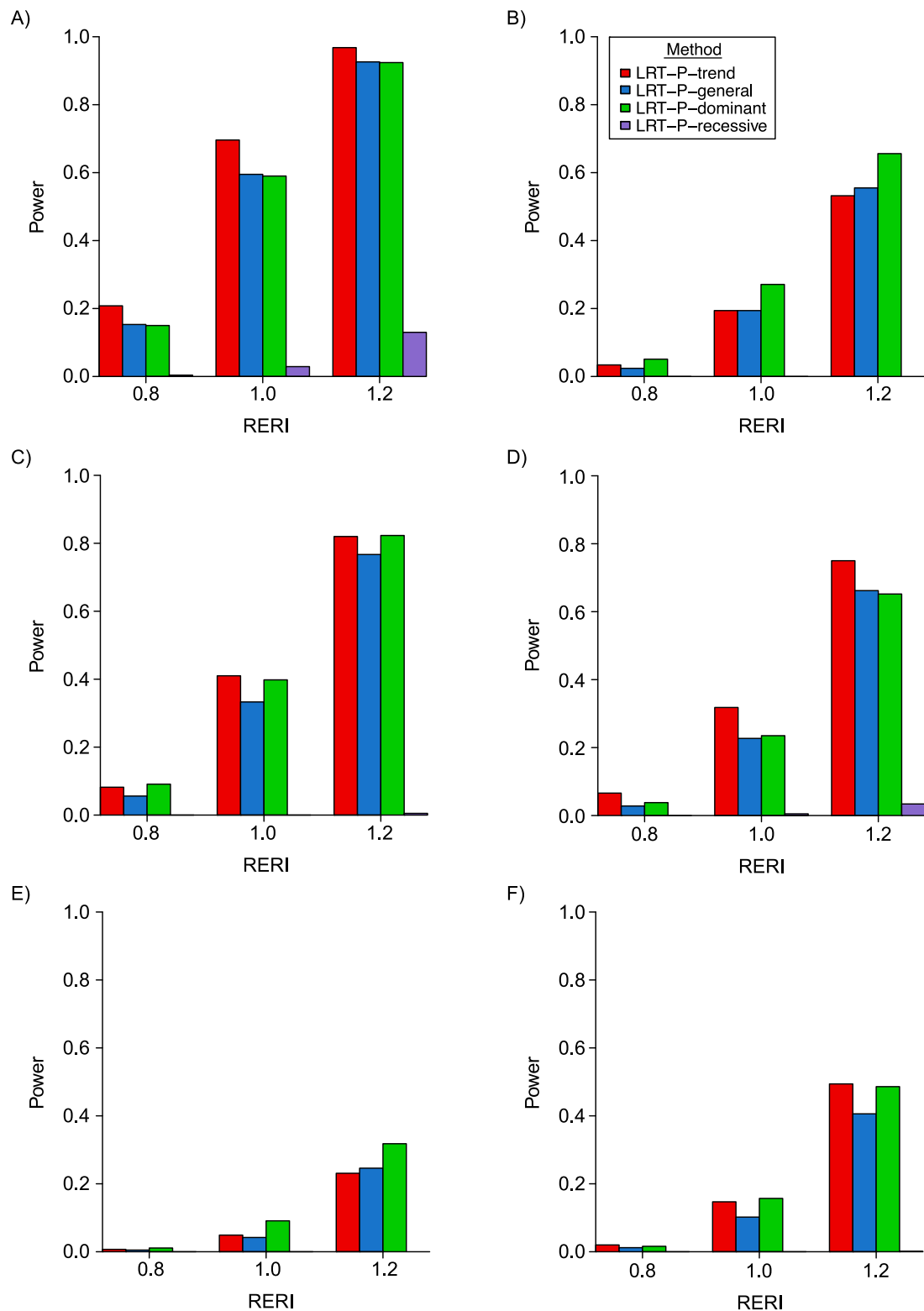
**Figure 1.** The results for power simulation of additive interaction tests based on a prospective likelihood for data generated under trend model and marginal odds ratio (MOR)(E) = 2.5 (A), dominant model and MOR(E) = 2.5 (B), general model and MOR(E) = 2.5 (C), trend model and MOR(E) = 3 (D), dominant model and MOR(E) = 3 (E), general model and MOR(E) = 3 (F). Significance level of $\alpha = 1 \times 10^{-7}$ was used. 1,000 replicated data sets were simulated for 5,000 cases and 5,000 controls. For each set of simulation, we applied the following 4 additive interaction tests based on a prospective likelihood: the likelihood ratio test (LRT) under the trend effect of genotype (prospective LRT, LRT-P-trend), a general model (LRT-P-general), a dominant model (LRT-P-dominant), and a recessive model (LRT-P-recessive). RERI, relative excess risk due to interaction.
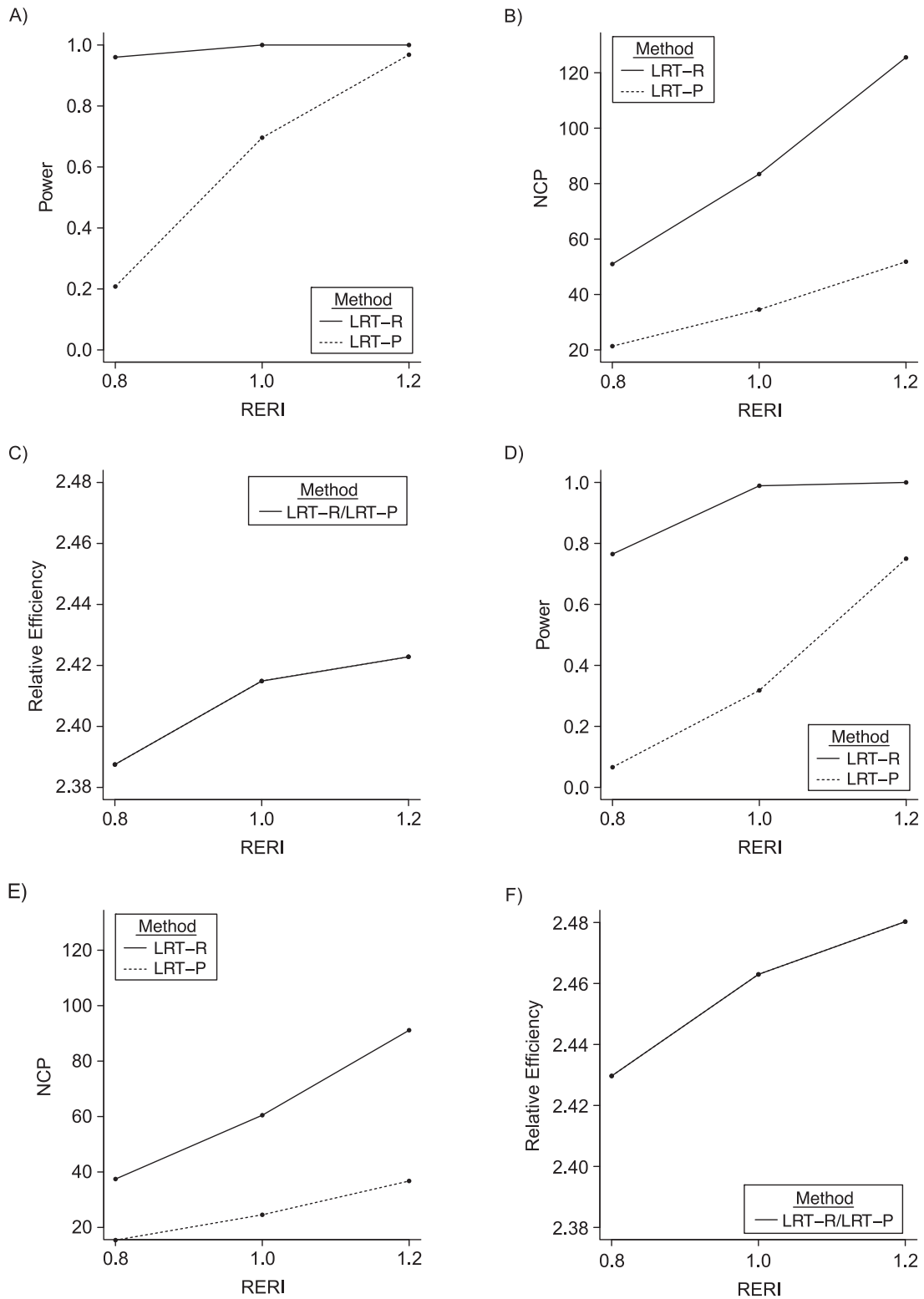
**Figure 2.** Comparison of the power of the trend effect–based additive interaction tests for the retrospective likelihood ratio test (LRT-R) versus prospective likelihood ratio test (LRT-P) for data generated with marginal odds ratio (E) (MOR(E)) = 2.5 (A) and MOR(E) = 3 (D). The noncentrality parameter (NCP) for each LRT, for MOR(E) = 2.5 (B) and MOR(E) = 3 (E), was estimated to compare the performances of the tests regardless of significance levels. The relative efficiency of LRT-R with regard to LRT-P, for MOR(E) = 2.5 (C) and MOR(E) = 3 (F), was estimated by taking the ratio of the NCP of LRT-R to the NCP of LRT-P. RERI, relative excess risk due to interaction.

**Table 4.** *P* Value of Lung Cancer Data Analysis[a] Using a Set of Additive Interaction Tests Based on the Trend Effect of the Genotype, Dominant Model, and General Model

| SNP | Chromosomal Region | LRT-R[b] | | | LRT-P | | |
|---|---|---|---|---|---|---|---|
| | | **Trend** | **Dominant** | **General** | **Trend[c]** | **Dominant** | **General** |
| rs8034191 | 15q25.1[d] | N/A | N/A | N/A | $5.55 \times 10^{-16e}$ | $1.13 \times 10^{-14e}$ | $7.88 \times 10^{-15e}$ |
| rs8042374 | 15q25.1[d] | N/A | N/A | N/A | $2.77 \times 10^{-12e}$ | $1.37 \times 10^{-11e}$ | $1.49 \times 10^{-11e}$ |
| rs3117582 | 6p21.33 | $4.07 \times 10^{-3}$ | $1.28 \times 10^{-2}$ | $3.24 \times 10^{-3}$ | $3.08 \times 10^{-3}$ | $1.46 \times 10^{-2}$ | $1.23 \times 10^{-3}$ |
| rs31489 | 5p15.33 | $4.11 \times 10^{-4e}$ | $1.18 \times 10^{-2}$ | $1.67 \times 10^{-3}$ | $1.84 \times 10^{-2}$ | $6.39 \times 10^{-2}$ | $5.73 \times 10^{-2}$ |
| rs2395185 | 6p21.32 | $1.81 \times 10^{-2}$ | $3.10 \times 10^{-2}$ | $2.88 \times 10^{-2}$ | $4.02 \times 10^{-2}$ | $3.75 \times 10^{-2}$ | $1.10 \times 10^{-1}$ |
| rs4324798 | 6p22.1 | $2.76 \times 10^{-1}$ | $4.76 \times 10^{-1}$ | $2.63 \times 10^{-2}$ | $6.12 \times 10^{-2}$ | $1.39 \times 10^{-1}$ | $3.98 \times 10^{-2}$ |
| rs4975616 | 5p15.33 | $3.77 \times 10^{-3}$ | $5.76 \times 10^{-3}$ | $1.76 \times 10^{-2}$ | $7.11 \times 10^{-2}$ | $4.02 \times 10^{-2}$ | $1.19 \times 10^{-1}$ |
| rs401681 | 5p15.33 | $1.94 \times 10^{-3}$ | $4.98 \times 10^{-2}$ | $3.11 \times 10^{-3}$ | $7.16 \times 10^{-2}$ | $1.89 \times 10^{-1}$ | $1.77 \times 10^{-1}$ |
| rs2736100 | 5p15.33 | $5.17 \times 10^{-2}$ | $8.98 \times 10^{-2}$ | $1.27 \times 10^{-1}$ | $2.56 \times 10^{-1}$ | $5.22 \times 10^{-1}$ | $4.79 \times 10^{-1}$ |
| rs7216064 | 17q24.2 | $5.46 \times 10^{-1}$ | $7.12 \times 10^{-1}$ | $8.10 \times 10^{-1}$ | $3.16 \times 10^{-1}$ | $4.82 \times 10^{-1}$ | $4.27 \times 10^{-1}$ |
| rs3817963 | 6p21.32 | $1.19 \times 10^{-1}$ | $1.98 \times 10^{-1}$ | $2.94 \times 10^{-1}$ | $3.65 \times 10^{-1}$ | $5.16 \times 10^{-1}$ | $5.86 \times 10^{-1}$ |
| rs9387478 | 6q22.1 | $9.68 \times 10^{-1}$ | $5.29 \times 10^{-1}$ | $6.10 \times 10^{-1}$ | $4.29 \times 10^{-1}$ | $4.69 \times 10^{-1}$ | $7.20 \times 10^{-1}$ |
| rs10937405 | 3q28 | $9.84 \times 10^{-1}$ | $8.74 \times 10^{-1}$ | $9.64 \times 10^{-1}$ | $8.92 \times 10^{-1}$ | $6.10 \times 10^{-1}$ | $5.06 \times 10^{-1}$ |
| rs753955 | 13q12.12 | $7.68 \times 10^{-1}$ | $9.06 \times 10^{-1}$ | $9.58 \times 10^{-1}$ | $9.64 \times 10^{-1}$ | $8.36 \times 10^{-1}$ | $8.82 \times 10^{-1}$ |

Abbreviations: LRT-P, prospective likelihood ratio test; LRT-R, retrospective likelihood ratio test; N/A, not applicable; SNP, single nucleotide polymorphism.

[a] Using genome-wide association study data from the National Cancer Institute (Landi et al. (27)).

[b] Retrospective likelihood analysis assumes G-E independence.

[c] The rows are sorted by the *P* value of LRT-P.

[d] The SNPs in 15q25.1 are known to be associated with smoking (E) and hence not tested using the retrospective likelihood due to violation of G-E independence.

[e] Statistically significant *P* values; $\alpha = 0.0006$ ( $= 0.05/(14 \times 6)$) was applied for statistical significance.

effect of genotype and the additive joint effect of gene and environment based on a saturated logit model. Our simulation study demonstrated that the proposed test is robust across different underlying genetic models, showing increased power compared with alternative methods based on the dominant, recessive, or general models. The proposed trend-based interaction test using the retrospective likelihood showed approximately 2.5-fold increased efficiency compared with the method based on the standard prospective likelihood when the G-E independence assumption holds.

Application of the proposed method to the GWAS data for LOAD yielded 2 significant interactions at genome-wide significance between rs1582763 and *APOE* ε4 and rs6733839 and *APOE* ε4, which were replicated using independent data. Notably, both of these SNPs were previously identified to be associated with LOAD risk using GWAS data (48, 49). rs1582763 is located in the membrane-spanning 4-domains subfamily A (*MS4A*) gene cluster (closest to *MS4A* member 4A (*MS4A4A*), on chromosome 11q12.2, Online Mendelian Inheritance in Man *606547), and rs6733839 is located near/in the bridging integrator 1 gene (*BIN1*, on chromosome 2q14.3, Online Mendelian Inheritance in Man *601248). The A allele of rs1582763 was found to be associated with a decreased risk of LOAD ($P = 4.72 \times 10^{-9}$) using proxy-phenotype analysis of GWAS with subjects with parental LOAD status (48), and

further *MS4A4A* was implicated in AD family history-based GWAS (50), LOAD GWAS (45), and sporadic AD GWAS (51). rs6733839, located near *BIN1*, was associated with LOAD risk (49–51) (odds ratio = 1.22; $P = 6.9 \times 10^{-44}$ in Lambert et al. (49)), and *BIN1* was further implicated in LOAD GWAS (45, 52) and in sporadic AD GWAS (51). While a recent study based on 53,711 subjects (46) showed that both *MS4A* and *BIN1* had potentially differential associations with LOAD risk among *APOE* ε4 carriers versus noncarriers, none attained significant SNP × *APOE* ε4 (multiplicative) interactions ($P = 0.27$ and $P = 0.87$ for *MS4A* and *BIN1* respectively). On the other hand, our analysis detected additive interactions at both of these loci at genome-wide significance. While we also examined multiplicative interactions (data not shown) for the top 30 SNPs obtained from SNP × *APOE* ε4 analysis, none of them, including rs1582763 and rs6733839, showed statistical significance using $\alpha = 5 \times 10^{-8}$ or $\alpha = 1 \times 10^{-5}$ (using both retrospective and prospective likelihoods). This implies that either a multiplicative risk model holds well overall for explaining the joint effect of each SNP and *APOE* ε4 for LOAD, or a multiplicative test is underpowered to detect true SNP × *APOE* ε4 interactions due to potentially smaller interaction effect sizes (versus RERIs that can be detected using additive interaction). We further note that besides the top 2 SNPs, 2 other SNPs,

**Table 5.**   Odds Ratio for Each Genotype and Apolipoprotein E ε4 Allele Status Under the Trend Effect of Genotype for the Top 2 Single-Nucleotide Polymorphisms That Exceed the Genome-Wide Association Study Significance Level ($P < 5 \times 10^{-8}$) for Alzheimer Disease Data Under the Retrospective Likelihood[a]

| Data Source and SNP | No. | Chromosome | Gene | Genotype | APOE ε4 Carrier | | RERI | 95% CI | P for Interaction |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | No | Yes | | | |
| Discovery data | 16,474 | | | | | | | | |
| rs6733839 | | 2 | *BIN1* | CC | 1.00 | 5.04 | | | |
| | | | | CT | 1.26 | 6.41 | 1.11 | (0.788, 1.422) | $5.56 \times 10^{-14}$ |
| | | | | TT | 1.53 | 7.78 | | | |
| rs1582763 | | 11 | *MS4A4A* | GG | 1.00 | 5.19 | | | |
| | | | | GA | 0.88 | 4.43 | −0.64 | (−0.852, −0.426) | $5.32 \times 10^{-9}$ |
| | | | | AA | 0.76 | 3.68 | | | |
| Validation data | 3,857 | | | | | | | | |
| rs6733839 | | 2 | *BIN1* | CC | 1.00 | 3.39 | | | |
| | | | | CT | 1.19 | 4.02 | 0.44 | (0.011, 0.87) | 0.036 |
| | | | | TT | 1.38 | 4.65 | | | |
| rs1582763 | | 11 | *MS4A4A* | GG | 1.00 | 3.25 | | | |
| | | | | GA | 0.78 | 2.67 | −0.36 | (−0.637, −0.078) | 0.011 |
| | | | | AA | 0.55 | 2.08 | | | |
| Pooled data | 20,331 | | | | | | | | |
| rs6733839 | | 2 | *BIN1* | CC | 1.00 | 4.56 | | | |
| | | | | CT | 1.25 | 5.74 | 0.93 | (0.676, 1.187) | $1.17 \times 10^{-14}$ |
| | | | | TT | 1.50 | 6.92 | | | |
| rs1582763 | | 11 | *MS4A4A* | GG | 1.00 | 4.64 | | | |
| | | | | GA | 0.86 | 3.93 | −0.57 | (−0.743, −0.401) | $5.97E \times 10^{-11}$ |
| | | | | AA | 0.72 | 3.22 | | | |

Note: Abbreviations: APOE, apolipoprotein E; BIN1, bridging integrator 1; CI, confidence interval; MS4A4A, membrane-spanning 4-domains subfamily A member 4A; RERI, relative excess risk due to interaction; SNP, single nucleotide polymorphism.

[a] The results under the prospective likelihood are shown in Web Table 5. Data made available by National Institute on Aging Genetics of Alzheimer's Disease Data Storage Site and similar LOAD repositories (Web Appendix 1; Web Tables 1–2) (45).

rs1752684 and rs12444073, retained statistical significance using a suggestive threshold ($\alpha = 1 \times 10^{-5}$) (Web Table 4). Specifically, zinc finger protein 423 (*ZNF423*, where rs12444073 is located) has recently been shown to be associated with LOAD risk (gene-level $P = 2.1 \times 10^{-6}$) (53).

To the best of our knowledge, our study presents the first method that incorporates the trend effect of genotype for testing additive interaction using both retrospective and prospective likelihoods. Previously, an LRT has been proposed for testing additive interaction that assumes either binary genetic data (dominant or recessive models) or general model, which has been applied to various complex diseases including bladder cancer and breast cancer (9–11). However, the major limitation of this method was strong assumptions from genetic models. Application of the proposed trend effect–based method that overcomes this limitation has led to reproducible interaction findings for LOAD. Last, our new method is implemented in the freely available R package, CGEN (24), which can facilitate its wide application among researchers in molecular epidemiology.

Despite these strengths, our study has limitations. While the proposed test based on the retrospective likelihood is known to increase power when the assumption of G-E (or G-G) independence holds, it should be used with caution because violation of the assumption can produce a bias (54). To handle this issue, an empirical Bayes-type shrinkage estimator was previously developed for examining multiplicative interaction (55). This method employed a weighted average of the retrospective and prospective likelihood-based estimators for multiplicative interaction, yielding an acceptable trade-off between bias and efficiency. A recent work proposed a similar approach for additive interaction, assuming a dominant or recessive model (14). Currently, an extension of our proposed trend effect–based method is under way to incorporate an empirical Bayes-type shrinkage estimator.

To conclude, we have developed a test for detecting additive G-E interaction based on the trend effect of genotype and extended it to exploit the independence between gene and environment. Our simulation study shows that the proposed method is robust under varying genetic models and improves power when the assumption on G-E independence is held. Future work is needed to relax this strong assumption to be more data-adaptive.

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