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The case-only test for gene-environment interaction is not uniformly powerful: an empirical example

Chen Wu^{1,2}, Jiang Chang², Baoshan Ma¹, Xiaoping Miao³, Yifeng Zhou⁴, Yu Liu², Yun Li⁵, Tangchun Wu³, Zhibin Hu⁶, Hongbing Shen⁶, Weihua Jia⁷, Yixin Zeng⁷, Dongxin Lin², and Peter Kraft¹

¹Program in Molecular and Genetic Epidemiology, Harvard School of Public Health, Boston, Massachusetts, USA

²State Key Laboratory of Molecular Oncology and Department of Etiology and Carcinogenesis, Cancer Institute and Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

³Key Laboratory for Environment and Health (Ministry of Education), School of Public Health, Huazhong University of Sciences and Technology, Wuhan, Hubei, China

⁴Laboratory of Cancer Molecular Genetics, Medical College of Soochow University, Suzhou, Jiangsu, China

⁵Department of Genetics, Department of Biostatistics, University of North Carolina, Chapel Hill, North Carolina, USA

⁶Department of Epidemiology and Biostatistics, Cancer Center, Nanjing Medical University, Nanjing, Jiangsu, China

⁷State Key Laboratory of Oncology in Southern China and Department of Experimental Research, Sun Yat-Sen University Cancer Center, Guangzhou, Guangdong, China

Abstract

The case-only test has been proposed as a more powerful approach to detect gene-environment (G×E) interactions. This approach assumes that the genetic and environmental factors are independent. While it is well known that Type I error rate will increase if this assumption is violated, it is less widely appreciated that gene-environment correlation can also lead to power loss. We illustrate this phenomenon by comparing the performance of the case-only test to other approaches to detect G×E interactions in a genome-wide association study of esophageal squamous carcinoma (ESCC) in Chinese populations. Some of these approaches do not use information on the correlation between exposure and genotype (standard logistic regression), while others seek to use this information in a robust fashion to boost power without increasing Type I error (two-step, empirical Bayes and cocktail methods). G×E interactions were identified involving drinking status and two regions containing genes in the alcohol metabolism pathway,

Correspondence should be addressed to D.L. (lindx72@cicams.ac.cn) and P.K. (pkraft@hsph.harvard.edu).

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4q23 and 12q24. Although the case-only test yielded the most significant tests of G×E interaction in the 4q23 region, the case-only test failed to identify significant interactions in the 12q24 region which were readily identified using other approaches. The low power of the case-only test in the 12q24 region is likely due to the strong inverse association between the SNPs in this region and drinking status. This example underscores the need to consider multiple approaches to detect gene-environment interactions, as different tests are more or less sensitive to different alternative hypotheses and violations of the gene-environment independence assumption.

INTRODUCTION

The genome-wide association study (GWAS) has emerged as a powerful and successful tool to identify common disease alleles by using high-throughput genotyping technology. It interrogates a large number of tagging single nucleotide polymorphisms (SNPs) that serve as surrogates for untested common SNPs across the genome. However, some true associations might not be detected by GWAS without accounting for environmental risk factors, because some susceptibility loci might act in an environment-responsive manner [Garcia-Closas, et al. 2005; Kilpelainen, et al. 2011; Wu, et al. 2011]. Many statistical methods have been proposed for investigation of statistical gene-environment (G×E) interactions in the context of case-control GWAS, and the relative effectiveness of these methods remains an area of active investigation [Cornelis, et al. 2012; Hsu, et al. 2012; Khoury and Wacholder 2009; Kraft, et al. 2007; Mukherjee, et al. 2012; Murcray, et al. 2011; Murcray, et al. 2009; Thomas, et al. 2012]. Throughout this paper we define "gene-environment interaction" as a departure from a multiplicative odds ratio model for the joint effect of genotype and exposure, i.e. $OR(G,E) \neq OR(G) OR(E)$. We focus on these so-called multiplicative interactions because they are widely studied but note that other definitions of statistical gene-environment interaction exist (and depending on context may be more relevant), and we note that definitions of statistical and biological interactions are quite distinct [Kraft and Hunter 2010; Siemiatycki and Thomas 1981].

The standard test for gene-environment interaction (GE), based on the coefficient of the product of the genetic and environmental exposures in a logistic regression, remains a popular method to analyze case-control data, but is known to have low power [Hein, et al. 2008; Hunter 2005]. The case-only approach (GE-CO) has been proposed as a potentially more powerful method. This test leverages the fact that under the assumption that the genetic and environmental factors are independent in the general population, a gene-environment interaction will induce an association between genotype and exposure in the cases. In the simple case of a binary exposure, the interaction odds ratio $OR_{GE} = OR(G,E)/[OR(G) OR(E)]$ is equivalent to the odds ratio from a logistic regression of exposure on genotype (holding all other risk factors constant). However, it is well known that violations of the gene-environment independence assumption can lead to increased Type I error rate for the case-only test [Albert, et al. 2001; Mukherjee, et al. 2012; Piegorsch, et al. 1994; Satten and Epstein 2004]. It is arguably less well known that the case-only test and other methods that leverage the gene-environment independence assumption can lose power when the G×E interaction effect and the correlation between genotype and exposure go in opposite directions. Several recent papers have illustrated this hypothetical situation through

simulations [Li and Conti 2009; Mukherjee, et al. 2012]. Here we provide an empirical example of the phenomenon using a case-control study of esophageal squamous-cell carcinoma (ESCC). In particular, we give a practical example where the case-only test fails to identify makers associated with ESCC risk and participating in G×E interactions although other methods could identify these markers.

A number of methods have been proposed that seek to leverage the power boost of the case-only test while retaining control of the Type I error rate when the gene-environment independence assumption is violated [Hsu, et al. 2012; Li and Conti 2009; Mukherjee, et al. 2008; Murcray, et al. 2011; Murcray, et al. 2009]. Some of these methods have been shown to lose power relative to the standard case-control analysis when the G×E odds ratio and gene-environment association go in opposite directions [Mukherjee, et al. 2012]. We also illustrate the performance of these methods in this situation using the ESCC data.

Esophageal squamous-cell carcinoma ranks as the tenth most prevalent cancer in the world, with marked regional variation and a particularly high incidence in regions of China. Molecular epidemiological studies using the candidate gene approach have established that a set of genetic variations primarily related to alcohol metabolism confer susceptibility to ESCC [Brooks, et al. 2009; Cui, et al. 2009; Hashibe, et al. 2008; Hiyama, et al. 2007]. We previously conducted a genome-wide association study of ESCC and detected two previously-identified regions in the alcohol metabolism pathway that showed strong evidence for gene-environment interaction: 12q24 harboring *ALDH2* and 4q23 harboring a cluster of seven genes encoding alcohol dehydrogenase (ADH) family (5' *ADH7-ADH1C-ADH1B-ADH1A-ADH6-ADH4-ADH5*-3') [Wu, et al. 2011; Wu, et al. 2012]. ADHs oxidize alcohol to acetaldehyde, a likely important carcinogen in the etiology of alcohol-related cancers. *ALDH2* encodes aldehyde dehydrogenase-2, which detoxifies acetaldehyde to acetate. Individuals who carry an *ALDH2**2 allele, which slows this detoxification process, typically experience an unpleasant flushing reaction to alcohol due to the buildup of acetaldehyde, and are less likely to drink regularly or heavily [Brooks, et al. 2009]. Thus, it is biologically plausible that alleles in *ADH* and *ALDH2* might be associated with an increase in the effect of alcohol intake on the risk of ESCC and, at the same time, be associated with decreased alcohol intake—consistent with the pattern previous studies have observed [Cui, et al. 2009; Lewis and Smith 2005].

In this report we illustrate the effect of analytic strategy on the ability to identify these two loci known to be involved in a gene-environment interaction.

METHODS

Genome-wide G×E interaction studies of ESCC

Details of the genome-wide association study analyzed here have been reported in previous papers [Wu, et al. 2011; Wu, et al. 2012]. Briefly, 2,031 ESCC cases and 2,044 controls were recruited from the Han Chinese population in Beijing region, China. Demographic characteristics including age, sex, smoking status and drinking status were obtained from patient's medical records. Control subjects were selected from those undergoing physical examination at primary care clinics in the Beijing region and frequency-matched for age and

sex to ESCC cases. For this study, alcohol drinking status was assessed by a detailed questionnaire. For the present analysis, individuals were classified as drinkers if they reported drinking any form of alcohol at least twice a week; otherwise, they were defined as nondrinkers. Individuals who reported smoking more than 100 cigarettes in their life or smoking tobacco in a pipe more than 100 times were defined as smokers; all others were defined as nonsmokers. At recruitment, informed consent was obtained from each subject, and the study was approved by the institutional review boards of the Chinese Academy of Medical Sciences Cancer Institute, Peking University.

Statistical tests

We compared six statistical tests to identify gene-environment interactions in case-control data (Table 1). Five of the tests use gene-environment correlation to increase power to detect gene-environment interactions, and four of these are designed to control the Type I error rate when genotype and exposure are correlated in the general population.

We applied standard logistic regression and case-only tests to markers in regions on 4q23 and 12q24 spanning the *ADH* gene cluster and *ALDH2*, respectively. For these analyses we assumed a log-additive mode of inheritance, that is, we coded genotype as a count of minor alleles. These analyses also adjusted for smoking, age and gender. Additional analyses adjusting for the top three principal components of genetic variation did not appreciably change these results [Wu, et al. 2011; Wu, et al. 2012]. For additional analyses of rs11066015 and rs3805322, the markers at 12q24 and 4q23 that showed strongest evidence for gene-environment interaction from the standard logistic regression test, we used a dominant coding for the non-reference allele (A and G, respectively) when applying all the methods listed in Table 1. We did this for two reasons. First, rs11066015 is in strong LD ($r^2=0.71$ in the CHB+JPT panel of the 1000 Genomes Project Pilot 1 data [<http://www.broadinstitute.org/mpg/snap/ldsearchpw.php>, accessed 1 November 2012]) with the *ALDH2*2* (rs671) allele, which is known to act in a dominant fashion on the conversion of acetaldehyde to acetate [Brooks, et al. 2009]. Second, we are using a formulation of the Empirical Bayes test that assumes the genotype and exposure have a binary coding [Mukherjee, et al. 2008]; to be consistent we adopted this coding for the other tests. The hybrid two-step and cocktail methods both involve a screening step where only the markers that show some evidence for marginal association with disease and/or gene-environment correlation in the full sample are tested for gene-environment interaction. These methods require the user to specify p-value thresholds to use in the screening step. We used $\alpha_A=\alpha_M=0.0001$ and $p=0.5$ for the hybrid test and a screening p-value of 0.001 for the cocktail methods (with $c=0.001$ for Cocktail 1), as recommended in previous publications [Hsu, et al. 2012; Murcray, et al. 2011].

In all analyses the E term, drinking status, was modeled as a binary trait: drinker and nondrinker. (This binary coding was chosen in part to guard against inflated type I error in tests of interaction when the main effect of environment is misspecified [Cornelis, et al. 2012; Tchetgen Tchetgen and Kraft 2011].) Analyses were conducted by using PLINK, SAS, version 9.3, and R.

RESULTS

Characteristics of 2,032 cases and 2,044 controls have been presented in previous papers [Wu, et al. 2011]. Drinking status (being a drinker) was significantly associated with risk of ESCC with an OR (95% CI) of 1.63 (1.44–1.85). Quantile-quantile plots did not suggest any large-scale systemic bias due to population stratification or differential genotyping error for the marginal or case-only tests. Using marginal logistic regression adjusted by sex, age, smoking status and drinking status, three SNPs (rs11066015, rs11066280 and rs2074356) on 12q24 and eight SNPs (rs1042026, rs3805322, rs17033, rs17028973, rs1614972, rs1614972, rs1229977, rs1789903 and rs1893883) on 4q23 were identified associated with risk of ESCC with P values from 1.23×10^{-5} and 5.07×10^{-12} [Wu, et al. 2011; Wu, et al. 2012].

We applied the standard case-control and case-only tests for gene-environment interaction to 340 SNPs in a 2 Mb window around rs11066015 at 12q24 and 304 SNPs in a 2 Mb window around rs1042026 at 4q23. Using the standard case-control test, three SNPs (rs11066015, rs11066280 and rs2074356) on 12q24 showed highly statistically significant interactions with alcohol drinking to promote ESCC risk ($P_{GE} < 10^{-16}$). Two SNPs, rs1042026 and rs3805322, on 4q23 also showed significant interactions, although P_{GE} values did not reach genome-wide significance levels ($P=0.0052$ and 0.0002 , respectively).

In contrast, the case-only test did not identify any genome-wide significant associations between SNPs at 12q24 and ESCC ($p > 0.0003$), while p -values for the case-only test of gene-environment interaction for rs2074356 and rs3805322 at 4q23 were much smaller than those from the standard case-control test of interaction ($P_{GE-CO} = 4.68 \times 10^{-6}$ and 6.78×10^{-7} , respectively). To further illustrate these discrepancies, we present detailed results on rs11066015 at 12q24 and rs3805322 at 4q23 (Table 2)

The case-only estimate of the gene-environment interaction odds ratio for carriers of the A allele at rs11066015 was 1.06 (95% CI: 0.88–1.26, $P=0.55$), as compared to 3.71 (2.84–4.86, $P < 10^{-16}$) from the standard GE test. Non-drinkers who carried the minor allele of rs11066015 had a nonsignificant 0.84-fold decrease in odds of ESCC relative to non-carriers, while drinkers who carried the minor allele had a 3.12-fold increase in odds (Table 2). At the same time, controls who carried the minor allele were less likely to drink: the odds of a carrier being a drinker were 0.28 times that of non-carriers (95% CI 0.23–0.35; $p < 10^{-16}$). (The association between rs11066015 and alcohol intake did not change appreciably after adjusting for age, gender, smoking status or the top three principal components of genetic variation [Wu, et al. 2012].) This is consistent with previous cross-sectional studies in east Asian populations that showed carriers of the minor allele of *ALDH2*2* (rs671) are more likely to experience a flushing reaction to alcohol and less likely to drink regularly [Brooks, et al. 2009].

On the other hand, the case-only estimate of the gene-environment interaction odds ratio for carriers of the G allele at rs3805322 was 1.79 (1.48–2.15; $P=6.9 \times 10^{-10}$), compared to 1.59 (1.21–2.09; $P=8.1 \times 10^{-4}$) for the standard case-control analysis. There was no evidence of association between rs3805322 and drinking status among controls, consistent with previous population-based studies of the *ADH* gene cluster [Cui, et al. 2009; Hashibe, et al. 2008].

This suggests that the discrepancy between the case-only and standard tests for rs11066015 is due to a negative correlation between the deleterious exposure and the risk allele, consistent with previous theoretical results.

Table 3 shows how this pattern of gene-environment correlation affected other tests that use gene-environment correlation when testing for gene-environment interaction. All of the recently-proposed tests that use information from both the case-only and the standard case-control test were able to detect the interaction on chromosome 12. For the chromosome 4 interaction, the case-only test yielded the strongest evidence for interaction ($p=9.6\times 10^{-10}$). The standard case-control and empirical Bayes tests failed to achieve genome-wide significance ($p=8.1\times 10^{-4}$ and $p=5.4\times 10^{-5}$, respectively). Because it uses the standard case-control test at the second stage, the hybrid test would fail to identify this interaction in a genome-wide screen. Both cocktail methods use the empirical Bayes test at the second stage for this locus, which is significant after accounting for the number of markers that make it through the initial screening steps.

DISCUSSION

Many methods have been developed to investigate G-E interactions in GWAS but none of these approaches have been shown to be consistently most effective [Mukherjee, et al. 2008; Murcray, et al. 2011]. Although the standard logistic regression test is widely used, the power of this method is limited. Taking our precious GWAS of ESCC as an example, two regions, 12q24 and 4q23, were identified to be marginally associated with risk of ESCC. In an expanded sample (10,123 cases and 10,664 controls compared to 2031 cases and 2044 controls in the original GWAS), both showed genome-wide significant interactions with drinking status. However, the standard GE interaction test failed to identify the interaction at 4q23 at genome-wide significance levels in the original GWAS. This suggests that other more powerful methods might identify interactions missed by the standard method.

The case-only test has been proposed as a powerful approach to exploit G-E independence. Although it is widely appreciated that the case-only method can have increased Type I error in the presence of gene-environment correlation, it is perhaps less widely appreciated that this method can have lower power when the GE interaction odds ratio and GE correlation are in opposite directions. This study gives a practical example of this phenomenon. We also show that other, newer methods that leverage the gene-environment independence association but retain control of the Type I error retain sufficient power to detect this strong interaction at a genome-wide significance level.

In our study, the case-only method failed to identify 3 SNPs at 12q24 that are involved in a strong gene-environment interaction. The minor alleles of these SNPs at the 12q24 region are in linkage disequilibrium with the *ALDH2*2* allele, which increases the risk of ESCC because the carriers have a decreased rate of detoxifying acetaldehyde to acetate [Hashibe, et al. 2008; Lewis and Smith 2005; McKay, et al. 2011]. However, nondrinkers carry a remarkably higher frequency of this risk allele than the drinkers among controls included in this study. The individuals with this risk allele are unable to degrade aldehyde efficiently and tend to develop malaise, flushing reaction and other uncomfortable symptoms when drinking

alcohol. These conditions make individuals with inactive ALDH2 less likely to consume alcohol [Li, et al. 2006]. Therefore, the gene-environment correlation and interaction effects for SNPs in *ALDH2* region on 12q24 are in the opposite direction and decrease the power of case-only test.

On the other hand, the case-only test identified two SNPs on 4q23 with genome-wide significant gene-environment interactions, while the p-values from the standard GE test and empirical Bayes test were five orders of magnitude larger. There was no compelling evidence in this study or previous studies that alleles in this region are associated with alcohol consumption. These results are consistent with the theoretical prediction that the case-only test will be much more powerful than the standard test when the gene-environment independence assumption holds.

Of the two-stage approaches, the cocktail methods were able to identify the interaction at 4q23, while the hybrid test was not. In certain situations (specifically, when the screening p-value [p^{screen}] is equal to the p-value from the marginal test of gene-disease association [p_M]), the cocktail method uses the empirical Bayes test at the second stage, rather than the standard logistic regression test (which the hybrid test always uses). Because the empirical Bayes test leverages evidence for gene-environment independence, it had smaller p-values than the standard interaction test at this locus.

We have given an example involving a polymorphism (*ALDH2*2*) that has a very strong and direct association with a known environmental risk factor for disease (alcohol intake). The strength of this association is arguably exceptionally large—large GWAS of behavioral "exposures" such as alcohol, caffeine and tobacco cigarette intake have not identified associations with similarly strong associations [2010; Bierut, et al. 2010; Cornelis, et al. 2011; Hindorff, et al. ; Liu, et al. 2010; Thorgeirsson, et al. 2010]—but in very large sample sizes (such as those needed to reliably identify modest interaction effect) smaller gene-environment interaction associations may produce the biases seen here. Of particular note, differences in exposure frequencies (including differences in unmeasured confounders) that are correlated with genetic ancestry may lead to pervasive gene-environment correlation, and appropriate adjustment or control for population stratification should be applied. This may be of special concern in admixed populations, such as African Americans or Latinos.

In summary, our empirical results are consistent with previous theoretical studies and suggest that multiple analytic approaches should be used when screening for gene-environment interactions, as no one method is universally powerful. Different approaches will be sensitive to different alternative hypotheses and gene-environment correlation patterns. Because they combine the strengths of several approaches while maintaining appropriate control of Type I error rate, recently developed two-step tests for gene-environment interaction like the hybrid method of Murcray et al. [Murcray, et al. 2011] and the cocktail methods of Hsu et al. [Hsu, et al. 2012] may be broadly useful. In general, the choice of analytic approach(es) will depend on the primary research question and the context of the setting [Cordell 2009; Kraft and Hunter 2010; Thomas 2010]. For example, if the study aims to characterize the association between a particular marker and exposure and disease risk, then a method that provides unbiased and precise parameter estimates will be

preferred to methods that provide an interaction test but do not provide estimates of all parameters of interest (which will typically include both main effect and interaction parameters). Several recent papers have considered the performance of many tests for gene-environment interaction across a wide range of hypothetical scenarios (strength of main and interaction effects, presence and size of gene-environment correlations) [Hsu, et al. 2012; Li and Conti 2009; Mukherjee, et al. 2008; Murcray, et al. 2011; Murcray, et al. 2009]. It remains an open question which of these hypothetical situations are most relevant in practice. We have provided an empirical, cautionary example of one of the more interesting hypothetical situations.

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Table 1

Interaction tests considered in this report

	<i>Explicitly considers gene- environment correlation?</i>	<i>Robust to population- level gene- environment correlation?*</i>	Reference
Standard case-control test	N	Y	[Cornelis, et al. 2012; Mukherjee, et al. 2012] and many others
Case-only test	Y	N	[Piegorsch, et al. 1994]
Empirical Bayes test	Y	Y	[Mukherjee, et al. 2008]
Hybrid two-step approach	Y	Y	[Murcray, et al. 2011]
Cocktail 1	Y	Y	[Hsu, et al. 2012]
Cocktail 2	Y	Y	[Hsu, et al. 2012]

* Assuming genotype and exposure are measured without error. For the effects of measurement error, see: [Garcia-Closas, et al. 1998; Lindstrom, et al. 2009].

Table 2

Association between rs11066015 (12q24) and rs3805322 (4q23) and ESCC, stratified by drinking status

12q24: rs11066015 (A)				
	Non-Carrier	Carrier		
<i>Non-drinkers</i>				
Control	615	523		
Case	513	367		
	OR _{G D=0}	0.84 (0.70, 1.00)	<i>p</i> =0.06	
<i>Drinkers</i>				
Control	728	176		
Case	650	491		
	OR _{G D=1}	3.12 (2.55, 3.82)	<i>p</i> <1×10 ⁻¹⁶	
	OR _{G×E-standard}	3.71 (2.84, 4.86)	<i>p</i> <1×10 ⁻¹⁶	
	OR _{G×E-case-only}	1.06 (0.88,1.26)	0.55	
4q23: rs3805322 (G)				
	Non-Carrier	Carrier		
<i>Non-drinkers</i>				
Control	847	291		
Case	620	260		
	OR _{G D=0}	1.22 (1.00, 1.49)	<i>p</i> =0.05	
<i>Drinkers</i>				
Control	652	252		
Case	652	489		
	OR _{G D=1}	1.94 (1.61, 2.34)	<i>p</i> <3.4×10 ⁻¹²	
	OR _{G×E-standard}	1.59 (1.21, 2.09)	<i>p</i> <8.1×10 ⁻⁴	
	OR _{G×E-case-only}	1.79 (1.48, 2.15)	<i>p</i> =9.6×10 ⁻¹⁰	

Table 3

Genome-wide significance of tests for gene-environment interaction for rs11066015 (12q24) and rs3805322 (4q23)

	Genome-wide Significant? ($\alpha=5\times 10^{-8}$)	
	rs11066015 ^a	rs3805322 ^b
Standard case-control test	Yes	no
Case-only test	no	Yes
Empirical Bayes test	Yes	no
Hybrid two-step approach	Yes	no
Cocktail 1	Yes	Yes
Cocktail 2	Yes	Yes

^aEmpirical Bayes estimate of $OR_{G\times E}=3.66$ (2.79,4.80); for the screening stage of the hybrid test, both G-E association and marginal G-D tests were significant with $p_A=6.0\times 10^{-14}<\alpha_A$ and $p_M=7.3\times 10^{-8}<\alpha_M$, and the standard test of $G\times E$ interaction at the second stage was quite significant ($p<10^{-16}$); for the cocktail methods, $p^{\text{screen}}=p_M$ for cocktail 1 and $p^{\text{screen}}=p_A$ for cocktail 2, both of these pass the first stage threshold, and the second stage tests (the Empirical Bayes test for Cocktail 1 and standard case-control test for Cocktail 2) are both very significant ($p<10^{-16}$).

^bEmpirical Bayes estimate of $OR_{G\times E}=1.70$ (1.36,2.20), $p=5.4\times 10^{-5}$; for the screening stage of the hybrid test, both G-E association and marginal G-D tests were significant with $p_A=1.1\times 10^{-9}<\alpha_A$ and $p_M=9.3\times 10^{-13}<\alpha_M$, however, the standard test of $G\times E$ interaction at the second stage did not meet the second stage threshold ($\approx 4.2\times 10^{-4}$); for the cocktail methods, $p^{\text{screen}}=p_M$ for cocktail 1 and 2, which passes the first stage threshold, and the second stage test (the Empirical Bayes test for both) meets the second stage threshold ($\approx 4.2\times 10^{-4}$).