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Effect of Glutathione S-Transferase T1, M1, P1 and Heme Oxygenase-1 Polymorphisms Interactions with Heavy Smoking on the Risk of Rheumatoid Arthritis

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Abstract

Objective—Glutathione S-transferase (GST) and heme oxygenase-1 (HMOX1) genes encode enzymes that detoxify carcinogens and protect against oxidative stress. We studied gene-smoking interactions on rheumatoid arthritis (RA) susceptibility.

Methods—549 matched Caucasian RA cases and controls were selected from the Nurses' Health Study. Genotyping by TaqMan and BioTrove identified GSTM1, GSTT1 homozygous deletions (null) and GSTP1 (rs1695), HMOX1 (rs2071746) alleles, respectively. We studied gene-smoking interactions on the risk of all RA and serologic RA phenotypes in separate logistic models adjusted for covariates. We assessed multiplicative interactions with product terms in the logistic models and additive interactions with the attributable proportion due to interaction (AP). For replication, we repeated significant analyses in an independent case-control sample from the Epidemiologic Investigation of RA.

Results—For all RA risk, we observed: multiplicative (p=0.05) and additive (AP=0.53, p=0.0005) interactions between GSTT1-null and smoking and multiplicative interaction (p=0.05) between HMOX1 and smoking. For seropositive RA risk, we found multiplicative (p=0.01) and additive (AP=0.63, p<0.0001) interactions between GSTT1-null and smoking and additive interaction (AP=0.41, p=0.03) between HMOX1 and smoking. After correction for multiple comparisons, additive interactions for GSTT1-null and smoking remained significant. GSTM1-null and GSTP1 did not show significant interactions. No associations were seen with seronegative RA. In replication analyses, we observed significant multiplicative (p=0.04) and

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additive (AP=0.32, p=0.02) interactions between GSTT1-null and smoking for ACPA-positive RA risk.

Conclusion—We observed significant gene-environment interactions between GSTT1-null and heavy smoking on RA risk. Future studies are needed to assess the impact of these interactions on RA prediction.

Exposure to certain environmental factors within genetically predisposed individuals is thought to be an underlying cause of the development of rheumatoid arthritis (RA), a complex autoimmune disease affecting approximately 1% of the adult population (1). Epidemiological research has suggested cigarette smoking as a strong environmental risk factor for RA (2-5). Evidence of dose effects for both smoking and the gene-environmental effects of smoking have been demonstrated within the Nurses' Health Study (2,6). Genetic variants associated with increased risk of RA within the Human Leukocyte Antigen (HLA) complex have been known for decades (7,8) and numerous studies have shown strong gene-environment interactions between HLA and smoking (3,6,9-12).

Glutathione S-transferase (GST) genes are a widely expressed supergene family encoding biotransforming enzymes that catalyze the conjugation of glutathione and are involved in the detoxification of cytotoxic carcinogens and metabolites. GST substrates found in cigarette smoke include α- and β-unsaturated carbonyls, polycyclic aromatic hydrocarbons and reactive oxygen species (ROS), which may lead to cellular damage through oxidative stress. Variations in these genes reduce glutathione conjugation and therefore may increase susceptibility to the harmful effects of carcinogen exposure and oxidative stress (13-19).

Polymorphisms within the GST *Mu* (GSTM1), *Theta* (GSTT1) and *Pi* (GSTP1) classes have been identified. Individuals with homozygous deletions at the GSTM1 and GSTT1 loci (GSTM1-null and GSTT1-null) have no functional enzymatic activity (13-19). In GSTP1, an A to G single nucleotide polymorphism (SNP) may result in reduced enzymatic activity for *AG* and *GG* genotypes when compared to *AA* (18,20,21).

Numerous studies have examined the possible associations between GST polymorphisms and disease risk or disease phenotype determination. It has been hypothesized that GST variations are associated with susceptibility in various cancers (22,23), most notably lung cancer (17-19). Previous studies have shown relationships between GSTT1-null or GSTM1 polymorphisms and RA risk and severity, but have reported inconsistent interactions between the GSTs and smoking (3,24-27). Significant GST-smoking interactions have been discovered (3,16,17,22), suggesting that underlying environmental exposures play an important role in the effect of GST genotypes.

Heme Oxygenase-1 (HMOX1), the inducible form of heme oxygenase, catabolizes heme groups into biliverdin, free iron, and carbon monoxide. HMOX1 has been shown to have anti-oxidant, anti-inflammatory and cytoprotective properties and to be up-regulated under the presence of nicotine (28-32). An A to T SNP that may affect the level of an HMOX1 production response has been identified; subjects with *TA* or *TT* genotypes have lower HMOX1 expression when compared to those with the *AA* genotype (31). Previous research has studied the associations between HMOX1 and several diseases (31,32), including lung cancer (33). Recent studies have identified a role for HMOX1 enzymes in RA biology (34) and associations between HMOX1 polymorphisms and RA susceptibility and severity (35,36).

Our study focuses on three genetic polymorphisms within the GST *Mu* (GSTM1-null), *Theta* (GSTT1-null) and *Pi* (rs1695) classes and one polymorphism within the HMOX1 gene promoter (rs2071716). We hypothesized that significant gene-environment interactions exist

between these genetic variations and heavy smoking because of the failure to detoxify cigarette smoking and studied the effect of these interactions on the risk of all RA and serologic RA phenotypes. We further hypothesized that interactions would be stronger for seropositive RA.

Patients and Methods

Study Sample

The Nurses' Health Study (NHS), established in 1976, is a prospective cohort consisting of 121,700 female nurses aged 30-55 years. During 1989-1990, blood samples for future studies were obtained from 32,826 (27%) NHS participants aged 43-70 years. An additional 33,040 (27%) provided buccal cell samples. The Nurses' Health Study II (NHS2), established in 1989, is a similar prospective cohort of 116,609 female nurses aged 25-42 years. During 1996-1999, blood samples for future studies were obtained from 29,611 (25%) participants aged 32-52. For both NHS and NHS2, demographic and exposure characteristics of those patients who provided blood were similar to those of the overall cohorts (37). We combine samples from both NHS and NHS2 and refer to the combined cohort as ‘NHS’.

All women in the NHS cohort completed initial questionnaires. Subsequent biennial questionnaires were used to update disease diagnoses, exposures and other covariates of interest. Self-reports of RA status were confirmed through a two-stage process of screening for the presence of RA symptoms on a connective tissue disease screening questionnaire (38) followed by medical record review for the American College of Rheumatology (ACR) classification criteria for RA (39) and as previously described (6). We determined seropositive RA status primarily by chart review (for rheumatoid factor) and in some cases by direct assay (for anti-CCP2) (6). Each confirmed RA case was matched to one healthy female control by cohort, year of birth, race/ethnicity, menopausal status, and postmenopausal hormone use.

Our initial nested case-control dataset from NHS consisted of 585 RA cases and 585 matched controls. We restricted our analysis to self-reported Caucasian matched pairs with available DNA in order to minimize potential population stratification, resulting in a sample of 549 RA cases and 549 matched controls for analyses on all RA risk. In analyses on seropositive RA risk, we further refined this sample to include 325 seropositive RA cases and all 549 controls. For seronegative RA risk, the sample included 224 RA seronegative cases and all 549 controls. All aspects of the study were approved by the Partners' HealthCare Institutional Review Board.

Covariate Information

Reproductive covariates including parity, duration of breastfeeding, age at menarche, menopausal status and postmenopausal hormone use were chosen based on previously identified associations with RA risk in NHS (40) and selected from the questionnaire cycle prior to date of RA diagnosis for cases or index date for controls. Lifetime history of smoking was collected at baseline and data concerning current smoking and the number of cigarettes smoked per day were updated via biennial questionnaires. Pack-years of smoking (number of packs per day × number of years smoked) was computed from the last questionnaire completed prior to RA diagnosis or index date. We focused on smoking as a dichotomous variable denoting ≤10 pack-years versus >10 pack-years, based on previous epidemiological data demonstrating an increased risk at >10 pack-years in this cohort (2). We herein refer to those with >10 pack-years of smoking as ‘heavy smokers’. We also examined smoking dichotomized as never versus ever smoking.

Genotyping

Genotyping for GSTP1 and HMOX1 alleles was conducted using the BioTrove multiplex SNP genotyping assay. For GSTP1 (rs1695) and HMOX1 (rs2071746) SNPs, we obtained allelic information on individual samples. For the GSTM1 and GSTT1 deletions, we used a TaqMan-based quantitative real-time PCR similar to that described by Covault *et al* (41), but obtained information on homozygous deletions or homozygous present genotypes only. We included 126 blinded quality control (QC) samples in each assay and the concordance rate in QC samples that yielded a non-missing call was 100%.

Statistical Methods

We calculated means with standard deviations for continuous covariates and frequencies with percentages for categorical covariates, stratifying by case-control status. Chi-square statistics and T-tests were used to compare covariate frequency distributions and means between cases and controls. We studied both additive and multiplicative interactions between GST or HMOX1 polymorphisms and heavy smoking on the risk of all RA or serologic RA phenotypes. GSTT1 and GSTM1 were dichotomized as having no deletions or homozygous deletions (GSTT1-null and GSTM1-null, respectively) for analysis. GSTP1 and HMOX1 SNPs were assessed using a dominant model, with subjects classified as having any (1 or 2) or no risk alleles. Additionally, we examined interactions between the GST and HMOX1 polymorphisms and smoking classified as never or ever smoking.

Within each of our a priori hypotheses, namely that the specific GST or HMOX1 polymorphisms would interact with smoking, we examined the association with heavy smoking and ever smoking for all RA, seropositive RA, and seronegative RA, totaling 6 analyses under each hypothesis. We assessed false positives due to multiple comparisons in two ways: (1) using the conservative Bonferonni correction with a corrected p-value of 0.05/6 (=0.008) for significance; (2) replicating significant results ($p<0.05$) in a large independent cohort from the Epidemiologic Investigation of RA.

For all RA, we assessed these associations using a conditional logistic regression model, controlling for matching factors and adjusting for age at menarche, regularity of menses, parity, breastfeeding, menopausal status and postmenopausal hormone use. For the risks of seronegative and seropositive RA, we used unconditional logistic regression models, adjusted for matching factors and reproductive covariates, within the serologically defined subsets described above. Odds ratios were interpreted as estimates of relative risks since the study was population based and the outcome is rare. All analyses were conducted using SAS version 9.1 [SAS Institute Cary, NC].

Assessing Interaction

The assessment of additive interaction was based on disease rates connected to the “pie model” introduced by Rothman (42). To test for this type of interaction, we followed the methods discussed by Lundberg (43) and Andersson (44) and calculated the attributable proportion due to interaction (AP), as described previously (6). Ninety-five percent confidence intervals were calculated using the methods described by Hosmer and Lemeshow (45). We assessed multiplicative interaction by including a product term (Gene × Smoking) in the regression model. After correction for multiple comparisons, a p-value <0.008 or a p-value <0.05 in replication analyses was considered evidence for a significant interaction on the additive or multiplicative scale, respectively.

Stratified Analyses

If we observed a $p<0.05$ for any interaction on the risk of RA, we performed a stratified analysis within the specific all RA, seropositive RA, or seronegative RA subset in which the

effect was observed. We stratified our sample into subsets based on the presence or absence of the significant genetic risk factor to test whether the effect of smoking would be stronger among those with genetic polymorphisms. We then examined the relationship between smoking and RA risk in logistic regression models adjusting for matching factors and covariates within these strata.

Replication Sample

We conducted replication analyses within the Epidemiologic Investigation of RA (EIRA), a population based case-control study on incident RA in Sweden established between May 1996 and December 2006, described in detail elsewhere (10). A case was defined as a person in the population who for the first time received a diagnosis of RA according to the ACR 1987 criteria for the classification of RA. Eighty-five percent of the cases had their symptoms for less than 1 year. For each potential case, a control was randomly selected from the population, taking into consideration the subject's age, sex, and residential area. In total, 1771 cases and 1107 controls were available for analysis. For subset analyses we defined cases based on anti-citrullinated peptide antibody (ACPA) status, with 1123 ACPA-positive cases and 648 ACPA-negative cases compared to all 1107 controls.

Information on GSTT1 deletions in EIRA was obtained using the TaqMan-based quantitative real-time PCR similar to that described by Covault *et al* (41), obtaining information on homozygous deletions or homozygous present genotypes only, as in the NHS samples. HMOX1 genotyping was performed by imputing the SNP (rs2071746) in EIRA with MACH (46) based on the Phase II HapMap data (average posterior probability for the most likely genotype for this imputation was 0.91). All aspects of the EIRA study were approved by the Karolinska Institutet Institutional Review Board.

For gene-environment interactions observed in NHS at the $p<0.05$ level, we replicated the analyses in EIRA using unconditional logistic regression models controlling for age, sex, and residential area for all phenotypes of RA (all RA, ACPA-positive RA, ACPA-negative RA).

Results

Patient Characteristics

The characteristics and genotype frequencies for the NHS sample are presented in Table 1. Mean age at RA diagnosis was 56.9 ± 10.3 years and 325 (59.2%) were seropositive. Two hundred and forty-eight (46.0%) cases were heavy smokers, compared to 187 (34.4%) of controls ($p<0.0001$). No significant differences were seen in the distributions of the GSTP1, HMOX1 alleles or GSTT1, GSTM1 deletions between cases and controls.

Descriptive statistics for our replication sample from EIRA are presented in Table 1. Mean age at diagnosis was 51.4 ± 12.5 years and 1123 (63.4%) were ACPA-positive. Eight hundred and three (45.3%) cases were heavy smokers compared to 377 (34.1%) of controls ($p<0.0001$). No significant differences were seen in the distributions of GSTT1-null deletions or HMOX1 alleles between cases and controls.

We observed no significant main effects of GST or HMOX1 polymorphisms on the risk of all RA or serologic RA phenotypes in either sample, using logistic models adjusted for matching factors, reproductive covariates, and pack-years of smoking (data not shown).

Gene-Environment Interaction Results

The results of analyses testing for additive and multiplicative gene-environment interactions between GST or HMOX1 polymorphisms and heavy smoking are presented in Table 2. For the risk of all RA, we found a 2.47 times increased odds (95% CI: 1.48- 4.12) for heavy smokers with GSTT1-null compared to never or light smokers with GSTT1 present. We observed multiplicative (p=0.05) and significant additive interactions (AP (95% CI) = 0.53 (0.23-0.82), p=0.0005) between GSTT1-null and heavy smoking. For heavy smokers with any HMOX1 alleles, we observed a 1.85 times increased odds for all RA (95% CI: 1.29-2.65) compared to never or light smokers with no HMOX1 alleles. There was multiplicative (p=0.05), but no additive interaction (AP (95% CI) = 0.29 (-0.06-0.63), p=0.10) between HMOX1 and heavy smoking.

For the risk of seropositive RA, we found multiplicative (p=0.01) and strong additive interactions (AP (95% CI) = 0.62 (0.35-0.89), p<0.0001) between GSTT1-null and heavy smoking. We observed additive interaction between HMOX1 and heavy smoking (AP (95% CI) = 0.41 (0.04-0.78), p=0.03), but no multiplicative interaction (p=0.06).

After comparing our results to the Bonferonni adjusted p-value threshold of 0.008, only the additive interactions between GSTT1-null and heavy smoking for all RA (p=0.0005) and seropositive RA (p<0.0001) remained significant. The multiplicative interaction between GSTT1-null and heavy smoking for seropositive RA was borderline significant (p=0.01). No significant gene-environment interactions were seen between GSTM1-null or GSTP1 and heavy smoking for all or seropositive RA risk or between any of the GST or HMOX1 polymorphisms and heavy smoking for seronegative RA risk.

The results of interaction analyses between the GST and HMOX1 polymorphisms and ever smoking are presented in Table 3. We observed additive interaction between GSTT1-null and ever smoking on the risk of all RA (AP (95% CI) = 0.44 (0.06-0.83), p=0.02) and seropositive RA (AP (95% CI) = 0.53 (0.15-0.91), p=0.01), but not multiplicative interaction. For the risk of seronegative RA, we observed multiplicative (p=0.04) interaction between GSTM1-null and ever smoking. However, these interactions were no longer significant after adjusting for multiple comparisons. We observed no other significant interactions between the genetic polymorphisms and ever smoking for RA risk.

Stratified Analyses

Results from analyses stratified by GSTT1 and HMOX1, the two genotypes with p<0.05 for interactions with heavy smoking in our primary analyses above, are presented in Table 4. For all RA, comparing heavy smokers to never or light smokers, we observed a 3.10 times increased risk (95% CI: 1.65-5.89) for GSTT1-null individuals. This association was stronger for seropositive RA, where we observed a 4.25 times increased risk (95% CI: 2.04-8.83) for GSTT1-null individuals. Among individuals with HMOX1 risk alleles, for all RA we observed a 1.90 times increased risk (95% CI: 1.39-2.60) comparing heavy smokers to never or light smokers. Again, this association was stronger for seropositive RA; we found a 2.26 times increased risk (95% CI: 1.58-3.24) for heavy smokers with HMOX1 risk alleles.

Analyses stratified by GSTT1, which showed interactions with ever smoking (p=0.02), are presented in Table 4. For all RA, comparing ever smokers to never smokers, we observed 1.95 times increase risk (95% CI: 1.06-3.59) among GSTT1-null individuals. This association was stronger for seropositive RA, where we observed a 2.39 times increased risk (95% CI: 1.14-5.00) for GSTT1-null individuals. Although GSTM1 showed a significant interaction with ever smoking on the risk of seronegative RA, we observed only non-significant results in stratified analyses.

Replication Analyses

Gene-environment interaction analyses in NHS identified significant associations with heavy smoking for both GSTT1-null and HMOX1 risk alleles on the risk of all RA and seropositive RA. The results of our replication analyses in EIRA are presented in Table 5. For the risk of ACPA-positive RA, we observed an odds ratio of 2.64 (95% CI: 1.82-3.81) for heavy smokers with the GSTT1-null polymorphism compared to never or light smokers with GSTT1 present. We observed significant multiplicative (p=0.04) and strong additive interactions (AP (95% CI) = 0.32 (0.04-0.60), p=0.02) between GSTT1-null and heavy smoking. No significant interactions were observed between GSTT1-null and heavy smoking on the risk of all RA or ACPA-negative RA. We found no evidence of significant interactions between HMOX1 risk alleles and heavy smoking on the risk of all RA or serologic RA phenotypes. In EIRA, we tested for GSTT1 and HMOX1 interactions with ever smoking, and observed no significant interactions (Table 6).

Results from analyses stratified by the presence or absence of the GSTT1 deletion in EIRA are presented in Table 4. As in the NHS, the strongest association was seen for ACPA-positive RA, where we observed a 3.48 times increased risk (95% CI: 2.09-5.78) for heavy smoking among GSTT1-null individuals.

Discussion

Interest in the associations between glutathione S-transferase and heme oxygenase-1 genes and RA risk stems from the role of the genes in the detoxification of carcinogens in cigarette smoke and protection against oxidative stress caused by ROS. This nested case-control study demonstrated gene-environment interactions for both GSTT1-null and HMOX1 risk alleles with smoking of greater than 10 pack-years in the NHS, although only the interactions between GSTT1-null and heavy smoking remained significant after correction for multiple comparisons. These interactions were strongest for seropositive RA risk. Interaction between GSTT1-null and heavy smoking for seropositive RA risk was replicated in an independent Swedish case-control sample. No significant interactions were seen between GSTM1-null or GSTP1 alleles and heavy smoking. We found no significant relationships with seronegative RA risk.

To the best of our knowledge, this is the first study to examine and identify a convincing interaction between the GSTT1-null polymorphism and heavy smoking on the risk of developing seropositive RA, with replication in an independent cohort. A previous study by Bohanec Grabar *et al*, looking at only RA cases, identified a significant interaction between GSTT1-null and smoking on the phenotype RA disease activity, observing an OR for high disease activity of 8.64 (95% CI: 2.00-37.43) for GSTT1-null smokers compared to GSTT1-present smokers (24). We found that the interaction between GSTT1-null and heavy smoking led to an increased risk for developing RA, with stronger effects for seropositive RA. We did not study the phenotype of RA disease activity. Our study found that among subjects with the GSTT1-null polymorphism, heavy smokers are at a 3.1 times increased risk for all RA compared to never or light smokers. This increase in risk is even greater for seropositive RA, where we observed a 4.3 times increased risk among the GSTT1-null subjects. A similar association was seen in our replication analysis, where we observed a 3.5 times increased risk of ACPA-positive RA for heavy smoking among GSTT1-null individuals. When we examined the effect of GSTT1-null among groups defined by ≤10, 10-20, >20 pack-years, to determine whether this effect varies for even higher levels of smoking, we found similar results for the 10-20 and >20 groups (data not shown). Our observation of modest interactions between GSTT1-null and ever smoking in NHS but no significant interaction in EIRA supports the importance of considering the dose effect of smoking in analysis of interactions (2,6).

The interaction between GSTT1-null and heavy smoking on RA risk may be due to the lack of enzymatic activity associated with the GSTT1-null polymorphism, which may decrease the detoxification of certain cytotoxic carcinogens and metabolites found in cigarette smoke and subsequently increase the harmful effects of heavy smoking, a proven risk factor for RA (2-5). These results establish the importance of considering genetic background when studying the effect of environmental risk factors.

Despite the similarities between GST genes, we did not observe significant interactions between GSTM1-null or GSTP1 and heavy smoking on RA risk, as we did with GSTT1-null. These differing results may be due to the differences in catalytic activity between the GST classes. GST-*Theta* has a lower affinity towards glutathione conjugates, leading to less product inhibition and thus higher catalytic efficiency, and has about a 10 times higher enzymatic activity rate when compared to GST-*Mu and GST-Pi* classes (13,19,47). There are also established differences in specific substrates for GSTT1, GSTM1 and GSTP1 and their roles in detoxification and toxification (13-16).

Previous studies in RA have reported inconsistent interactions between GSTM1 polymorphisms and smoking (3,24,26). Mattey *et al* found that GSTM1-null ever smokers were at higher risk for more severe disease outcome, but Bohanec Grabar *et al* found no significant interaction between GSTM1 and ever smoking on RA disease activity (24,26). Criswell *et al* observed a significant interaction between GSTM1 and exposure to tobacco smoke on all RA risk that suggests smoking is a stronger risk factor among GSTM1-present individuals (OR=2.10 (95% CI: 1.13-3.89)) (3). After correction for multiple comparisons, we found no significant associations with GSTM1 for RA risk.

To the best of our knowledge, this is the first study to examine possible interactions between HMOX1 and heavy smoking on RA risk. We observed interactions between HMOX1 and heavy smoking for both all RA and seropositive RA in NHS. Our results suggest that among individuals carrying at least one HMOX1 risk allele, heavy smoking was associated with a 1.9 times increased risk of all RA and a 2.3 times increased risk of seropositive RA, compared to never or light smokers. However, this was not significant after correction for multiple comparisons and we did not observe significant associations in the replication analyses in EIRA. More research is needed to determine the validity of this association.

A number of studies have shown gene-environment interactions between HLA and smoking in seropositive RA (3,6,9-12). Thus, it is important to consider the impact that HLA may have on our observed results. We examined possible gene-gene interactions between HLA and our polymorphisms of interest and found no significant associations (data not shown).

Limitations of this study include the inability to determine heterozygous deletions for GSTM1 and GSTT1 polymorphisms and the lack of cyclic citrullinated peptide (CCP) status in NHS. The lack of CCP-status is due to the absence of plasma samples in almost half of our RA cases and the reality that many cases were diagnosed prior to the widespread use of this test. However, rheumatoid factor (RF) status was available from medical record reviews, and other gene-environment interactions are similar for RF and CCP phenotypes in RA (9,10). Our rates of seropositive (CCP+ and/or RF+) RA (59%) in this study are similar to those reported by a large US registry study recruiting patients from rheumatology practices across the US (48). Also, we only have data on incident RA and cannot study disease severity phenotypes.

The NHS cohorts are comprised primarily of middle to older aged Caucasian women with high education levels. This lack of diversity in the population may raise concerns about the generalizability of our results and these interactions should be studied in other cohorts. However, the restriction of our genetic analyses to Caucasian women limits the potential for

population stratification and thus may also be viewed as a strength. While limiting our analyses to self-reported Caucasian ancestry does not remove all concerns about population stratification, prior research has examined the potential for this bias extensively within the NHS and found no evidence of significant population stratification (49,50).

Other strengths of this study include the prospective nature of the information collected, including detailed smoking information, and the access to a large, independent case-control sample for replication. Our sample size makes this one of the largest studies examining the effects of GST and HMOX1 genes on RA risk. Despite this large sample, the power to detect a significant interaction is still limited. Based on observed ORs for gene (OR=1.1) and heavy smoking (OR=1.8), we had at most 24% power in NHS and 40% power in EIRA of detecting a significant GxE interaction of 1.5. This should be considered when interpreting negative results.

In summary, we observed significant multiplicative and strong additive interactions between the GSTT1-null polymorphism and heavy smoking, with the strongest associations seen in seropositive RA risk. In replication analyses, we found that the GSTT1-null and heavy smoking interaction observed for seropositive RA risk replicates for ACPA-positive RA risk. This suggests that we have found a truly novel association with seropositive RA risk. Although we found significant interactions between HMOX1 risk alleles and heavy smoking for RA risk in the NHS, these results did not replicate. No significant interactions were seen for the risk of seronegative RA, supporting the hypothesis that different risk factors and pathways exist for seropositive and seronegative RA phenotypes. Our results add new evidence to the hypothesis that gene-environment interactions play a significant role in the complex etiology of RA. Additional research is needed to examine the validity of these interactions and to study potential biologic pathways involved. Future studies should focus on how to incorporate these findings, and other previously identified risk factors, into larger models that can be used for RA prediction.

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Table 1

Characteristics and Genotype Frequencies of RA Cases and Matched Controls in Nurses' Health Study and Epidemiologic Investigation of RA

	NHS (549 cases/549 controls)		EIRA (1771 cases/1107controls)	
	RA Cases	**Controls**	**RA Cases**	**Controls**
Characteristics				
Age at match, mean (SD)	55.4 (±8.0)	55.4 (±8.0)	51.5 (±12.3)	52.9 (±11.5)
Ever cigarette smokers	335 (61.7%)	304 (55.5%)	1175 (66.7%)	672 (61.0%)
Heavy Smokers (>10 pack-years) (%)	248 (46.0%)	187 (34.4%)	797 (45.3%)	376 (34.2%)
Parous, (%)	505 (93.2%)	513 (94.3%)	-	-
Breastfed ≥ 12 months total (%)‡	78 (14.4%)	103 (18.9%)	-	-
Age at menarche < 12 years	160 (29.1%)	152 (27.7%)	-	-
Irregular menstrual cycles (%)	88 (16.0%)	70 (12.8%)	-	-
Body mass index, mean (SD) (kg/m2)	25.9 (±4.9)	25.9 (±5.0)	25.3 (±4.3)	25.8 (±7.0)
RA Features				
Mean age at diagnosis, (SD)	56.9 (±10.3)	-	51.4 (±12.5)	-
Seropositive (%)	325 (59.2%)	-	1123 (63.4%)	-
Rheumatoid nodules, (%)	71 (12.9%)	-	-	-
Radiographic changes, (%)	161 (29.3%)	-	-	-
Gene Frequencies				
GSTT1				
present	434 (81.3%)	445 (82.9%)	1481 (83.6%)	878 (79.3%)
null	100 (18.7%)	92 (17.1%)	252 (14.2%)	150 (13.6%)
GSTM1				
present	278 (52.2%)	247 (47.2%)	-	-
null	255 (47.8%)	276 (52.8%)	-	-
GSTP1				
AA	231 (44.0%)	218 (41.1%)	-	-
AG	219 (41.7%)	245 (46.1%)	-	-
GG	75 (14.3%)	68 (12.8%)	-	-
HMOX1				
AA	168 (31.5%)	164 (31.5%)	433 (27.9%)	239 (25.7%)
AT	258 (48.4%)	255 (49.0%)	759 (49.0%)	475 (51.0%)
TT	107 (20.1%)	101 (19.4%)	358 (23.1%)	217 (23.3%)
HLA-SE				
no SE	265 (48.8%)	337 (62.3%)	432 (26.0%)	467 (47.7%)
any SE	278 (51.2%)	204 (37.7%)	1230 (74.0%)	512 (52.3%)

NHS: Nurses' Health Study, EIRA: Epidemiologic Investigation of RA

‡Calculated among parous women in NHS

Table 2

Gene-Environment Interactions between GSTs and HMOX1 and Heavy Smoking* in the Nurses' Health Study

Polymorphism and Smoking Status		Cases / Controls	OR (95%CI)	AP (95% CI)**	Additive p	Multiplicative p
			All RA†			
GSTT1	**Smoking**					
Present	≤10 pack-years	240/285	1.00 (ref)	0.53 (0.23-0.82)	0.0005	0.05
Present	>10 pack-years	186/157	1.34 (1.01-1.77)			
Null	≤10 pack-years	44/63	0.83 (0.54-1.28)			
Null	>10 pack-years	55/27	2.47 (1.48-4.12)			
GSTM1	**Smoking**					
Present	≤10 pack-years	148/165	1.00 (ref)	-0.30 (-0.86-0.27)	0.31	0.67
Present	>10 pack-years	127/81	1.76 (1.23-2.52)			
Null	≤10 pack-years	138/176	0.93 (0.68-1.26)			
Null	>10 pack-years	111/97	1.30 (0.91-1.86)			
GSTP1	**Smoking**					
AA	≤10 pack-years	108/140	1.00 (ref)	-0.36 (-0.96-0.24)	0.24	0.18
AA	>10 pack-years	121/75	1.81 (1.25-2.62)			
AG/GG	≤10 pack-years	170/206	0.96 (0.70-1.30)			
AA/GG	>10 pack-years	118/105	1.30 (0.91-1.85)			
HMOX1	**Smoking**					
AA	≤10 pack-years	88/99	1.00 (ref)	0.29 (-0.06-0.63)	0.10	0.05
AA	>10 pack-years	75/65	1.39 (0.92-2.11)			
AT/TT	≤10 pack-years	194/245	0.93 (0.67-1.28)			
AT/TT	>10 pack-years	166/106	1.85 (1.29-2.65)			
			Seropositive RA‡			
GSTT1	**Smoking**					

Polymorphism and Smoking Status		Cases / Controls	OR (95%CI)	AP (95% CI)**	Additive p	Multiplicative p
Present	≤10 pack-years	137/285	1.00 (ref)	0.62 (0.35-0.89)	<0.0001	0.01
Present	>10 pack-years	111/157	1.35 (0.98-1.85)			
Null	≤10 pack-years	21/63	0.68 (0.40-1.16)			
Null	>10 pack-years	38/27	2.70 (1.58-4.61)			
GSTM1	**Smoking**					
Present	≤10 pack-years	89/165	1.00 (ref)	-0.13 (-0.72-0.45)	0.66	0.96
Present	>10 pack-years	78/81	1.74 (1.17-2.58)			
Null	≤10 pack-years	72/176	0.80 (0.55-1.15)			
Null	>10 pack-years	70/97	1.36 (0.91-2.02)			
GSTP1	**Smoking**					
AA	≤10 pack-years	59/140	1.00 (ref)	-0.36 (-1.01-0.29)	0.28	0.16
AA	>10 pack-years	74/75	1.99 (1.30-3.03)			
AG/GG	≤10 pack-years	98/206	1.00 (0.69-1.44)			
AA/GG	>10 pack-years	75/105	1.46 (0.97-2.19)			
HMOX1	**Smoking**					
AA	≤10 pack-years	52/99	1.00 (ref)	0.41 (0.04-0.78)	0.03	0.06
AA	>10 pack-years	44/65	1.29 (0.79-2.09)			
AT/TT	≤10 pack-years	104/245	0.79 (0.55-1.15)			
AT/TT	>10 pack-years	107/106	1.82 (1.22-2.72)			
			Seronegative RA‡			
GSTT1	**Smoking**					
Present	≤10 pack-years	103/285	1.00 (ref)	0.25 (-0.34-0.84)	0.40	0.51
Present	>10 pack-years	75/157	1.28 (0.90-1.83)			
Null	≤10 pack-years	23/63	1.04 (0.61-1.76)			
Null	>10 pack-years	17/27	1.76 (0.92-3.38)			

Polymorphism and Smoking Status		Cases / Controls	OR (95%CI)	AP (95% CI)**	Additive p	Multiplicative p
GSTM1	**Smoking**					
Present	≤10 pack-years	59/165	1.00 (ref)	-0.56 (-1.43-0.30)	0.20	0.17
Present	>10 pack-years	49/81	1.73 (1.09-2.72)			
Null	≤10 pack-years	66/176	1.10 (0.74-1.64)			
Null	>10 pack-years	41/97	1.17 (0.73-1.86)			
GSTP1	**Smoking**					
AA	≤10 pack-years	49/140	1.00 (ref)	-0.44 (-1.28-0.41)	0.31	0.25
AA	>10 pack-years	47/75	1.59 (0.99-2.55)			
AG/GG	≤10 pack-years	72/206	0.91 (0.61-1.37)			
AA/GG	>10 pack-years	43/105	1.05 (0.66-1.66)			
HMOX1	**Smoking**					
AA	≤10 pack-years	36/99	1.00 (ref)	0.04 (-0.51-0.60)	0.88	0.75
AA	>10 pack-years	31/65	1.45 (0.83-2.51)			
AT/TT	≤10 pack-years	90/245	1.13 (0.74-1.71)			
AT/TT	>10 pack-years	59/106	1.64 (1.03-2.63)			

*Heavy Smoking defined as greater than 10 pack-years of smoking

**Attributable Proportion due to Interaction (AP) calculated as ($RR_{G+,E+} - RR_{G+,E-} - RR_{G-,E+} + 1$) / $RR_{G+,E+}$ and AP= 0 if there is no interaction

†OR from conditional logistic regression model controlled for matching factors and adjusted for age at menarche, regularity of menses, parity, and breastfeeding.

‡OR from unconditional logistic regression model adjusted for matching factors, age at menarche, regularity of menses, parity, and breastfeeding

Table 3

Gene-Environment Interactions between GSTs and HMOX1 and Ever Smoking in the Nurses' Health Study

Polymorphism and Smoking Status		Cases / Controls	OR (95%CI)	AP (95% CI)**	Additive p	Multiplicative p
			All RA†			
GSTT1	**Smoking**					
Present	Never	173/191	1.00 (ref)	0.44 (0.06-0.83)	0.02	0.14
Present	Ever	260/253	1.05 (0.80-1.38)			
Null	Never	32/46	0.77 (0.46-1.30)			
Null	Ever	64/46	1.48 (0.96-2.29)			
GSTM1	**Smoking**					
Present	Never	102/113	1.00 (ref)	-0.48 (-1.06-0.10)	0.10	0.22
Present	Ever	176/134	1.48 (1.05-2.08)			
Null	Never	104/117	1.06 (0.74-1.54)			
Null	Ever	146/159	1.04 (0.73-1.47)			
GSTP1	**Smoking**					
AA	Never	77/97	1.00 (ref)	-0.23 (-0.77-0.31)	0.41	0.26
AA	Ever	152/121	1.28 (0.89-1.84)			
AG/GG	Never	124/140	0.96 (0.67-1.38)			
AA/GG	Ever	166/173	1.01 (0.71-1.44)			
HMOX1	**Smoking**					
AA	Never	63/71	1.00 (ref)	0.04 (-0.37-0.45)	0.84	0.56
AA	Ever	102/92	1.35 (0.90-2.03)			
AT/TT	Never	139/168	1.02 (0.71-1.46)			
AT/TT	Ever	223/188	1.43 (1.00-2.05)			
			Seropositive RA‡			
GSTT1	**Smoking**					

Polymorphism and Smoking Status		Cases / Controls	OR (95%CI)	AP (95% CI)**	Additive p	Multiplicative p
Present	Never	97/191	1.00 (ref)	0.53 (0.15-0.91)	0.01	0.08
Present	Ever	154/253	1.06 (0.78-1.44)			
Null	Never	16/46	0.66 (0.35-1.22)			
Null	Ever	40/46	1.53 (0.94-2.48)			
GSTM1	**Smoking**					
Present	Never	63/113	1.00 (ref)	-0.21 (-0.82-0.40)	0.51	0.61
Present	Ever	105/134	1.36 (0.93-1.99)			
Null	Never	53/117	0.85 (0.55-1.31)			
Null	Ever	89/159	1.01 (0.68-1.48)			
GSTP1	**Smoking**					
AA	Never	44/97	1.00 (ref)	-0.11 (-0.69-0.46)	0.70	0.36
AA	Ever	88/121	1.27 (0.84-1.92)			
AG/GG	Never	70/140	0.91 (0.59-1.39)			
AA/GG	Ever	103/173	1.05 (0.71-1.57)			
HMOX1	**Smoking**					
AA	Never	40/71	1.00 (ref)	0.37 (-0.08-0.81)	0.10	0.14
AA	Ever	55/92	1.05 (0.66-1.68)			
AT/TT	Never	72/168	0.74 (0.48-1.14)			
AT/TT	Ever	140/188	1.25 (0.84-1.85)			
			Seronegative RA‡			
GSTT1	**Smoking**					
Present	Never	76/191	1.00 (ref)	0.24 (-0.38-0.86)	0.45	0.46
Present	Ever	106/253	1.06 (0.74-1.50)			
Null	Never	16/46	0.95 (0.51-1.79)			
Null	Ever	24/46	1.33 (0.76-2.34)			
GSTM1	**Smoking**					

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Polymorphism and Smoking Status		Cases / Controls	OR (95%CI)	AP (95% CI)**	Additive p	Multiplicative p
Present	Never	39/113	1.00 (ref)	-0.87 (-1.78-0.03)	0.06	0.04
Present	Ever	71/134	1.60 (1.02-2.50)			
Null	Never	51/117	1.38 (0.86-2.23)			
Null	Ever	57/159	1.06 (0.67-1.68)			
GSTP1	**Smoking**					
AA	Never	33/97	1.00 (ref)	-0.49 (-1.29-0.32)	0.23	0.15
AA	Ever	64/121	1.36 (0.85-2.17)			
AG/GG	Never	54/140	1.03 (0.64-1.66)			
AA/GG	Ever	63/173	0.94 (0.59-1.48)			
HMOX1	**Smoking**					
AA	Never	23/71	1.00 (ref)	-0.50 (-1.18-0.19)	0.15	0.31
AA	Ever	47/92	1.90 (1.09-3.31)			
AT/TT	Never	67/168	1.52 (0.91-2.54)			
AT/TT	Ever	83/188	1.61 (0.98-2.66)			

** Attributable Proportion due to Interaction (AP) calculated as ($RR_{G+,E+} - RR_{G+,E-} - RR_{G-,E+} + 1$) / $RR_{G+,E+}$ and AP= 0 if there is no interaction

† OR from conditional logistic regression model controlled for matching factors and adjusted for age at menarche, regularity of menses, parity, and breastfeeding

‡ OR from unconditional logistic regression model adjusted for matching factors, age at menarche, regularity of menses, parity, and breastfeeding

Table 4

Stratified Associations for Genotypes with Significant Interactions† in the Nurses' Health Study and the Epidemiologic Investigation of RA

	NHS					
	All RA (n=549 cases/549 controls)			Seropositive RA (n=325 cases/549 controls)		
Genetic Factor	**ca/co**	**OR (95% CI)***	**p**	**ca/co**	**OR(95% CI)***	**p**
GSTT1-null						
≤10 pack-years	44/63	1.00 (ref)		21/63	1.00 (ref)	
>10 pack-years	55/27	3.10 (1.65-5.89)	0.0004	38/27	4.25 (2.04-8.83)	0.0001
GSTT1-present						
≤10 pack-years	240/285	1.00 (ref)		137/285	1.00 (ref)	
>10 pack-years	186/157	1.36 (1.02-1.79)	0.03	111/157	1.41 (1.02-1.96)	0.04
GSTT1-null						
Never smoking	32/46	1.00 (ref)		16/46	1.00 (ref)	
Ever smoking	64/46	1.95 (1.06-3.58)	0.03	40/46	2.39 (1.14-5.00)	0.02
GSTT1-present						
Never smoking	173/191	1.00 (ref)		97/191	1.00 (ref)	
Ever smoking	260/253	1.09 (0.83-1.43)	0.55	154/253	1.16 (0.84-1.60)	0.37
HMOX1-AT/TT						
≤10 pack-years	194/245	1.00 (ref)		104/245	1.00 (ref)	
>10 pack-years	166/106	1.90 (1.39-2.60)	<0.0001	107/106	2.26 (1.58-3.24)	<0.0001
HMOX1-AA						
≤10 pack-years	88/99	1.00 (ref)		52/99	1.00 (ref)	
>10 pack-years	75/65	1.24 (0.79-1.95)	0.35	44/65	1.22 (0.72-2.08)	0.46

	EIRA					
	All RA (n=1771 cases/1107controls)			ACPA-positive RA (n=1123 cases/1107 controls)		
Genetic Factor	**ca/co**	**OR (95% CI)****	**p**	**ca/co**	**OR(95% CI)****	**p**
GSTT1-null						

	NHS					
	All RA (n=549 cases/549 controls)			Seropositive RA (n=325 cases/549 controls)		
Genetic Factor	**ca/co**	**OR (95% CI)***	**p**	**ca/co**	**OR(95% CI)***	**p**
≤10 pack-years	131/101	1.00 (ref)		70/101	1.00 (ref)	
>10 pack-years	121/49	2.40 (1.52-3.78)	0.0002	94/49	3.48 (2.09-5.78)	<0.0001
GSTT1-present						
≤10 pack-years	821/568	1.00 (ref)		485/568	1.00 (ref)	
>10 pack-years	660/310	1.61 (1.35-1.93)	<0.0001	450/310	1.96 (1.60-2.39)	<0.0001

† Stratified analyses performed for significant interactions with p-value <0.05 from interaction analyses presented in Tables 2, 3, 5 and 6

* All ORs are from models adjusted for adjusted for matching factors, age at menarche, regularity of menses, parity, and breastfeeding

** OR from unconditional logistic regression model adjusted for age, gender, and geographic region of Sweden

Table 5

Replication Analyses for Gene-Environment interactions in the Epidemiologic Investigation of RA

Polymorphism and Smoking Status		Cases / Controls	OR (95%CI)	AP (95% CI)*	Additive p	Multiplicative p
			All RA†			
GSTT1	**Smoking**					
Present	≤10 pack-years	821/568	1.00 (ref)	0.19 (-0.14-0.51)	0.25	0.21
Present	>10 pack-years	660/310	1.66 (1.40-1.97)			
Null	≤10 pack-years	131/101	0.90 (0.68-1.19)			
Null	>10 pack-years	121/49	1.92 (1.36-2.73)			
HMOX1	**Smoking**					
AA	≤10 pack-years	265/169	1.00 (ref)	0.17 (-0.27-0.60)	0.46	0.27
AA	>10 pack-years	189/82	1.37 (0.90-2.10)			
AT/TT	≤10 pack-years	628/474	0.73 (0.55-0.99)			
AT/TT	>10 pack-years	541/264	1.32 (0.96-1.83)			
			ACPA-positive RA†			
GSTT1	**Smoking**					
Present	≤10 pack-years	485/568	1.00 (ref)	0.32 (0.04-0.60)	0.02	0.04
Present	>10 pack-years	450/310	1.99 (1.65-2.40)			
Null	≤10 pack-years	70/101	0.80 (0.58-1.11)			
Null	>10 pack-years	94/49	2.64 (1.82-3.81)			
HMOX1	**Smoking**					
AA	≤10 pack-years	143/169	1.00 (ref)	0.19 (-0.25-0.64)	0.40	0.19
AA	>10 pack-years	124/82	1.72 (1.06-2.79)			
AT/TT	≤10 pack-years	338/474	0.70 (0.50-1.00)			
AT/TT	>10 pack-years	375/264	1.76 (1.21-2.56)			
			ACPA-negative RA†			

Polymorphism and Smoking Status		Cases / Controls	OR (95%CI)	AP (95% CI)*	Additive p	Multiplicative p
GSTT1	**Smoking**					
Present	≤10 pack-years	336/568	1.00 (ref)	-0.26 (-1.00-0.48)	0.49	0.56
Present	>10 pack-years	210/310	1.23 (0.98-1.53)			
Null	≤10 pack-years	61/101	1.02 (0.72-1.43)			
Null	>10 pack-years	27/49	0.98 (0.60-1.62)			
HMOX1	**Smoking**					
AA	≤10 pack-years	122/169	1.00 (ref)	0.08 (-0.55-0.71)	0.80	0.72
AA	>10 pack-years	65/82	1.09 (0.65-1.80)			
AT/TT	≤10 pack-years	290/474	0.77 (0.55-1.09)			
AT/TT	>10 pack-years	166/264	0.93 (0.63-1.37)			

*Attributable Proportion due to Interaction (AP) calculated as ($RR_{G+,E+} - RR_{G+,E-} - RR_{G-,E+} + 1$) / $RR_{G+,E+}$ and AP= 0 if there is no interaction

†OR from unconditional logistic regression model adjusted for age, gender, and geographic region of Sweden

Table 6

Gene-Environment Interactions between GSTs and HMOX1 and Ever Smoking in the Epidemiologic investigation of RA

Polymorphism and Smoking Status		Cases / Controls	OR (95%CI)	AP (95% CI)**	Additive p	Multiplicative p
			All RA†			
GSTT1	**Smoking**					
Present	Never	476/345	1.00 (ref)	-0.11 (-0.56-0.34)	0.66	0.62
Present	Ever	940/529	1.29 (1.08-1.53)			
Null	Never	75/51	1.07 (0.73-1.56)			
Null	Ever	163/97	1.22 (0.91-1.62)			
HMOX1	**Smoking**					
AA	Never	142/99	1.00 (ref)	-0.14 (-0.51-0.22)	0.44	0.54
AA	Ever	289/139	1.45 (1.05-2.01)			
AT/TT	Never	363/264	0.96 (0.71-1.30)			
AT/TT	Ever	749/424	1.23 (0.93-1.63)			
			ACPA-positive RA†			
GSTT1	**Smoking**					
Present	Never	261/345	1.00 (ref)	0.08 (-0.30-0.46)	0.66	0.67
Present	Ever	625/529	1.56 (1.28-1.90)			
Null	Never	35/51	0.91 (0.57-1.44)			
Null	Ever	117/97	1.59 (1.17-2.18)			
HMOX1	**Smoking**					
AA	Never	70/99	1.00 (ref)	-0.21 (-0.58-0.16)	0.27	0.33
AA	Ever	196/139	1.99 (1.37-2.90)			
AT/TT	Never	198/264	1.06 (0.74-1.52)			
AT/TT	Ever	510/424	1.70 (1.22-2.37)			
			ACPA-negative RA†			

Polymorphism and Smoking Status		Cases / Controls	OR (95%CI)	AP (95% CI)**	Additive p	Multiplicative p
GSTT1	**Smoking**					
Present	Never	215/345	1.00 (ref)	-0.60 (-1.52-0.33)	0.21	0.13
Present	Ever	315/529	0.96 (0.77-1.19)			
Null	Never	40/51	1.26 (0.80-1.97)			
Null	Ever	46/97	0.76 (0.52-1.12)			
HMOX1	**Smoking**					
AA	Never	72/99	1.00 (ref)	-0.01 (-0.57-0.56)	0.98	0.93
AA	Ever	93/139	0.92 (0.62-1.37)			
AT/TT	Never	165/264	0.86 (0.60-1.23)			
AT/TT	Ever	239/424	0.78 (0.55-1.09)			

** Attributable Proportion due to Interaction (AP) calculated as (RRG+,E+ - RRG+,E- - RRG-,E++ 1) / RRG+,E+ and AP= 0 if there is no interaction

† OR from unconditional logistic regression model adjusted for age, gender, and geographic region of Sweden