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Interactions of Cigarette Smoking with *NAT2* Polymorphisms Impact Rheumatoid Arthritis Risk in African Americans

Ted R. Mikuls, MD, MSPH[1,2], **Tricia LeVan, PhD**[1], **Karen A. Gould, PhD**[1], **Fang Yu, PhD**[1], **Geoffrey M. Thiele, PhD**[1,2], **Kimberly K. Bynote, BS**[1], **Doyt Conn, MD**[3], **Beth L. Jonas, MD**[4], **Leigh F. Callahan, PhD**[4], **Edwin Smith, MD**[5], **Richard Brasington, MD**[6], **Larry W. Moreland, MD**[7], **Richard Reynolds, PhD**[8], **Angelo Gaffo, MD**[8], and **S. Louis Bridges Jr, MD, PhD**[8]

[1]University of Nebraska Medical Center, Omaha, NE

[2]Omaha Veterans Affairs Medical Center, Omaha, NE

[3]Emory University, Atlanta, GA

[4]University of North Carolina, Chapel Hill, NC

[5]Medical University of South Carolina, Charleston, SC

[6]Washington University Medical Center, St. Louis, MO

[7]University of Pittsburgh Medical Center, Pittsburgh, PA

[8]University of Alabama at Birmingham, Birmingham, AL

Abstract

Objective—To examine whether polymorphisms in genes coding for drug metabolizing enzymes (DMEs) impact rheumatoid arthritis (RA) risk due to cigarette smoking in African Americans.

Methods—Smoking status was evaluated in African American RA cases and non-RA controls categorized as heavy (≥ 10 pack-years) vs. other. Individuals were genotyped for a homozygous deletion polymorphism in *glutathione S-transferase Mu-1 (GSTM1-null)* in addition to tagging single nucleotide polymorphisms (SNPs) in *N-acetyltransferase (NAT)1, NAT2*, and *epoxide hydrolase (EPXH1)*. Associations of genotypes with RA were examined using logistic regression and *gene*-smoking interactions were assessed.

Results—There were no significant associations of any *DME* genotype with RA. After adjustment for multiple comparisons, there were significant additive interactions between heavy smoking and *NAT2* SNPs *rs9987109* (P_{add} = 0.000003) and *rs1208* (P_{add} = 0.00001); attributable proportions (APs) due to interaction ranged from 0.61 to 0.67. None of the multiplicative gene-smoking interactions examined remained significant after adjustment for multiple testing in overall disease risk. There was no evidence of significant gene-smoking interactions in analyses of *GSTM1-null*, *NAT1*, or *EPXH1*. *DME* gene-smoking interactions were similar when cases were limited to anti-citrullinated protein antibody (ACPA) positive individuals.

Conclusion—Among African Americans, RA risk imposed by heavy smoking appears to be mediated in part by genetic variation in *NAT2*. While further studies are needed to elucidate mechanisms underpinning these interactions, these SNPs appear to identify African American smokers at a much higher risk for RA with relative risks that are at least two-fold higher compared to non-smokers lacking these risk alleles.

Corresponding Author and Request for Reprints: Ted R. Mikuls, MD, MSPH, Professor, Department of Medicine, University of Nebraska Medical Center and Omaha VA Medical Center, 986270 Nebraska Medical Center, Omaha, NE, USA 68198-6270; phone (402) 559-7288; fax (402) 559-6788; tmikuls@unmc.edu.

Keywords

rheumatoid arthritis; African Americans; cigarette smoking; anti-CCP antibody; drug metabolizing enzyme; N-acetyltransferase; epoxide hydrolase; glutathione S-transferase

Over the last thirty years, numerous investigations have confirmed the existence of an epidemiologic link between cigarette smoking and rheumatoid arthritis (RA) susceptibility [1–9]. The risk of RA that is attributable to smoking is most striking among those with heavier cumulative exposures, especially among individuals developing anti-citrullinated protein antibody (ACPA) positive disease [1, 10]. Among individuals of European ancestry, the attributable risk of RA due to ever smoking has been estimated to approach 1 in 6 [6]. In other words, 16% of all RA cases could be prevented in this population with the elimination of cigarette smoking as an environmental health hazard. The risk of ACPA positive RA appears to be even higher among cigarette smokers carrying *HLA-DRB1 shared epitope (SE)* containing alleles. In a recent Swedish case-control study, the attributable risk of ACPA positive RA due to heavy smoking among individuals homozygous for *HLA-DRB1 SE* approached 1 in 2 [11]. In this study, individuals homozygous for *SE* with a cumulative smoking exposure exceeding 20 pack-years were ~40-fold as likely to have ACPA positive RA compared to never smokers negative for *SE*.

In addition to being associated with disease risk in populations of European ancestry, our group recently observed strong associations of heavy cigarette smoking with RA risk in African Americans [12]. Similar to findings from populations of European ancestry, we found that heavy smoking defined as more than 10 pack-years of exposure was associated with a more than two-fold risk of RA in African Americans. In this study, we observed an attributable RA risk due to heavy smoking of 16%, similar to the attributable risk due to ever smoking found in a prior prospective cohort study of older women from Iowa [6].

In addition to the effect of *HLA-DRB1 SE*, variation in genes encoding drug metabolizing enzymes (DMEs) could also impact the effect of smoking on RA risk. This hypothesis is consistent with the growing epidemiologic literature showing that the incidence of other smoking-related illnesses is heightened in individuals inheriting polymorphisms that detrimentally impact DME function and ultimately impair xenobiotic metabolism and elimination of smoking-related toxins. For instance, lung cancer risk is higher in cigarette smokers homozygous for a deletion polymorphism in *glutathione S-transferase Mu-1 (GSTM1-null)* compared to smokers expressing functional GSTM1, a risk that appears to be most striking in Asian populations [13, 14]. Likewise, both breast [15, 16] and bladder cancer [17] risk appear to be higher among cigarette smokers inheriting haplotypes associated with reduced N-acetyltransferase (NAT)-2 function. Variation in genes encoding alternative DME's including NAT1 and epoxide hydrolase (EPXH1) have been suggested to mediate the risk of smoking related illnesses including select malignancies and chronic lung disease [18–21]. To date, potential interactions between these DME genes and smoking in RA risk have been subject to only limited study [22, 23] with prior investigations conducted exclusively in populations of European ancestry. The lack of such studies in African Americans represents a major knowledge gap, particularly since heavy smoking is strongly associated with disease risk in this population and smoking rates appear to be on the rise among African Americans [24].

In the present case-control study, we examined whether the risk of RA in African Americans attributable to cigarette smoking differs based on the presence of polymorphisms in a select number of *DME* genes. Specifically, we have examined whether there is evidence of

significant *DME gene*-smoking interactions impacting RA risk in this understudied population.

Patients and Methods

Study population

RA cases and controls were participants in the Consortium for the Longitudinal Evaluation of African-Americans with Early Rheumatoid Arthritis (CLEAR) [25–27]. All cases satisfied the American College of Rheumatology (ACR) RA classification criteria (formerly the American Rheumatism Association) [28] and all study participants self-reported African American race. This study included RA cases from CLEAR-I (early RA with longitudinal follow-up) with < 2 years disease duration from time of symptom onset and from CLEAR-II (cross-sectional) with any disease duration. As previously described, African American controls were enrolled based on age, gender, and geographic residence and were recruited predominantly based on telephone listings from individuals residing in the same mailing zip codes as those of RA cases [12]. Cases and controls were enrolled through one of five sites: the University of Alabama at Birmingham (Birmingham, AL), Emory University (Atlanta, GA), Medical University of South Carolina (Charleston, SC), the University of North Carolina (Chapel Hill, NC), and Washington University (St. Louis, MO). In prior efforts we have shown that CLEAR cases and controls share similar population structure as reflected in the magnitude of European genetic admixture, which was quantified using a panel of Ancestral Informative Markers (AIMs) [12]. The study was approved by the Institutional Review Board (IRB) at each participating center and all study subjects provided informed written consent prior to participation.

Smoking status

Smoking status (current, former, never) was ascertained at the time of enrollment, and among ever smokers, pack-years of smoking served as the measure of cumulative exposure. Never smoking was defined as having smoked fewer than 100 cigarettes in a lifetime based on common convention in epidemiological research [29]. Based on our prior observation in this cohort that the association of RA with smoking is limited to those with 10 or more pack-years of cumulative smoking exposure [12], individuals were categorized into one of two groups: 1) never smokers or < 10 pack-years vs. 2) ≥ 10 pack-years. In this prior study [12], we found no differences between the CLEAR-I and CLEAR-II cohorts in terms of RA risk due to heavy smoking. To assess for a potential healthy responder bias among controls, the rate of heavy smoking (≥ 10 pack-years) among CLEAR controls was compared with the frequency of heavy smoking observed among African American participants in the population-based Coronary Artery Risk Development in Young Adults (CARDIA) study [30]. For the purpose of this comparison, CARDIA data was used from the twenty-year follow-up examination that included results from 2,637 African American participants (56% women and a mean age of 44.6 years).

ACPA measurement

Anti-citrullinated protein antibody (ACPA) was measured using a commercially available second generation anti-cyclic citrullinated peptide (CCP) antibody ELISA (IgG, U/ml, Diastat, Axis-Shield Diagnostics Ltd., Dundee, Scotland, UK). Patients were considered to be ACPA positive with anti-CCP antibody concentrations ≥ 5 U/ml [25].

Genotyping

GSTM1 genotyping was completed as previously described [31]. In brief, primers “G2” and “G3” from a study by Brockmoller et al. [32] were used to amplify exons 3 through 5 of the

GSTM1 gene using genomic DNA that was prepared from whole blood. These primers produce a 650 bp amplified fragment in individuals carrying at least one functional *GSTM1* allele. This band is absent in *GSTM1-null* individuals because this mutation deletes exons 4 and 5. A 195 bp fragment of exon 7 of the *CYP1a1* gene was used as an internal positive control for sample quality and PCR using the primers described by Shields et al [33]. Amplified products were resolved by electrophoresis through 1% agarose gels. Genotypes were scored independently by two investigators (KAG and KKB). Based on associations of this genotype across multiple conditions [34–37], subjects were categorized as *GSTM1-null* (homozygous for deletion) vs. *GSTM1-present* (1 or 2 copies of the functional allele).

As part of the HapMap Phase II project, *NAT1, NAT2, and EPHX1* were re-sequenced from DNA obtained from 30 trios with Northern or Western European ancestry (CEPH) and 30 trios from Ibadon, Nigeria (Yoruba) [38]. A haplotype-tagging (ht) strategy using publicly available software and single nucleotide polymorphisms (SNPs) identified in the intronic sequence, approximately 6 kb of 5' genomic and 2 kb of 3' genomic, was implemented to reduce the number of SNPs analyzed and to capture the polymorphic structure of the gene [39]. The algorithm was based on polymorphic sites that exceeded a 10% minor allele frequency for CEPH trios, a 20% minor allele frequency for Yoruba trios and a within-bin linkage disequilibrium exceeding $r^2 > 0.7$. This algorithm resulted in several bins, often with multiple SNPs in each bin. A single SNP was selected for analysis from each bin. From bins containing multiple SNPs we preferentially selected polymorphisms located in the coding sequence, especially those causing amino acid changes. We also avoided SNPs located in repetitive sequences and in close proximity to other sites of variation that could interfere with the design of genotyping assays.

DNA samples were genotyped using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Sequenom Inc., San Diego, CA). Multi-plex PCR assays and associated extension reactions were designed using the SpectroDESIGNER 1.0 software (Sequenom Inc.). Primer extension products were loaded onto a 384-elements chip with a nanoliter pipetting system (Sequenom Inc.) and analyzed by a MassARRAY mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany). The resulting mass spectra were analyzed for peak identification by using SpectroTYPER RT 4.0 software (Sequenom Inc.). For genotyping quality control, Hardy-Weinberg calculations were performed to ensure that each marker was within the expected allelic population equilibrium. A total of 36 polymorphisms were chosen for genotyping. Of these 36, three SNPs *(NAT2 rs7832071, EPXH1 rs3753661, and EPXH1 rs10915884)* were not in Hardy-Weinberg equilibrium and were therefore excluded from additional consideration. There were three additional SNPs for which genotyping results were evaluable from < 80% of samples examined, all of which were subsequently excluded from the analyses (*NAT1 rs6586714, NAT1 rs8190870, and EPXH1 rs2854450*). Thus, 29 ht-SNPs and 1 homozygous deletion polymorphism (*GSTM1*) were examined. For the 29 remaining ht-SNPs, genotyping data were available for an average of 94% of CLEAR samples. In addition to the aforementioned DME polymorphisms, *HLA-DRB1* shared epitope (SE) containing alleles were genotyped as previously reported [40].

Statistical analyses

Patient characteristics were compared between RA cases and non-RA controls using two-sample t-test for continuous variables or chi-square test for categorical variables. Associations of each polymorphism with RA were examined using age- and gender-adjusted logistic regression analysis under the assumption of dominant inheritance. The relationships of each polymorphism with ACPA positive RA were examined in secondary analyses that included only RA cases with a positive ACPA antibody and all controls. Odds ratios (ORs) together with 95% confidence intervals (CIs) for RA were calculated. For each

polymorphism, we tested for both multiplicative and additive interaction with smoking status (≥ 10 pack-years vs. never/< 10 pack-years) in overall RA risk in the primary analyses and in ACPA positive RA in the secondary analyses. Multiplicative interaction was quantified by modeling the polymorphism-smoking product term in regression models. The attributable proportion due to interaction (AP) and corresponding p-value served as the primary measures of additive interaction [41] where an AP equal to 0 corresponds to no interaction and an AP of 1.0 corresponds to complete additive interaction. Confidence intervals (95% CIs) were calculated for the AP using the method of Hosmer and Lemeshow [42]. Given the exploratory nature of these analyses a Bonferroni correction was used to define the statistical threshold of significance for all analyses. With 30 polymorphisms examined, a p-value of < 0.0017 (0.05/30) was considered statistically significant. These analyses were performed using the software package SAS 9.2 (SAS Institute, Cary, NC).

With three SNPs showing at least borderline evidence of interaction, *NAT2* haplotypes were constructed using these SNPs and Haploview software [43]. Haplotype blocks were estimated using the confidence interval for R-squared. Associations of *NAT2* haplotypes (those with a frequency exceeding 10%) with ACPA positive and ACPA negative RA combined, stratified by smoking status, were examined using the Haplostat package in R software [44]. Haplotype analyses were adjusted for age and gender.

Results

Characteristics of RA cases (n = 727) and non-RA controls (n = 268) are shown in Table 1. Compared to controls, RA cases were more frequently women and, as expected, were substantially more likely to be ACPA positive. Although there was no significant difference in smoking status (never, former, or current) between cases and controls, RA cases were much more likely to report heavy cigarette smoking exposure defined as ≥ 10 pack-years of cumulative smoking compared to controls (28% vs. 18%, p = 0.001) (Table 1). Recognizing slight differences in age and gender distribution of the cohorts [12, 30], the prevalence of heavy smoking among CLEAR controls was the same as that observed among African American participants in the separate population-based CARDIA study (18%).

Minor allele frequencies (MAFs) for the *NAT1*, *NAT2* and *EPXH1* ht-SNPs, along with frequencies of the *GSTM1-null* genotype, are summarized for African American RA cases and African American controls in Table 2. The *GSTM1-null* genotype and MAFs for the tagging SNPs found in *NAT1*, *NAT2* and *EPXH1* were consistent with frequencies reported from the HapMap project in sub-Saharan Africans or African Americans (http://www.ncbi.nlm.nih.gov/SNP/index.html). Although there were borderline associations of two ht-SNPs (*EPXH1* rs2740171 and rs2234922) with RA case status in age- and gender-adjusted analysis, these differences were no longer significant following adjustment for multiple comparisons (Table 2).

Analyses were then conducted to examine DME gene-smoking interactions in RA risk. All SNPs were tested for gene-smoking interaction; however, only SNPs showing at least borderline evidence of multiplicative or additive interaction with heavy smoking in RA risk ($p < 0.05$) are shown in Table 3. There was no evidence of interaction between heavy smoking and the *GSTM1-null* genotype in RA risk (data not shown). There was a single *NAT1* tagging SNP (*rs11203942)* with borderline evidence of interaction with heavy smoking (AP = 0.49, 95% CI 0.12 to 0.87, $P_{add} = 0.009$; $P_{mult} = 0.06$). Individuals inheriting the *NAT1 rs11203942* minor allele with a history of heavy smoking were more than twice as likely to have RA (OR = 2.23; 95% CI 1.30 to 3.82) compared to individuals lacking both risk factors. There was a single *EPXH1* tagging SNP (*rs2260863)* that also showed borderline evidence of multiplicative interaction with heavy smoking in RA risk (P_{mult} =

0.03) but no evidence of additive interaction with smoking. None of the *NAT1*- or *EPXH1*-smoking interactions reached statistical significance following adjustment for multiple comparisons.

There were three *NAT2* tagging SNPs (*rs9987109, rs1208, and rs721399)* that showed evidence of interaction with heavy smoking in regard to RA risk. Of the *NAT2* SNPs shown in Table 3, the presence of the minor allele potentiated the risk associated with heavy smoking for two of the SNPs (*rs9987109* and *rs1208*) while the risk of RA due to heavy smoking was higher in the presence of the major allele for the remaining SNP (*rs721399*). After adjustment for multiple comparisons, evidence of additive interaction remained highly significant for both *NAT2 rs9987109* (AP = 0.67; 95% CI 0.39 to 0.95, P_{add} = 0.000003) and *NAT2 rs1208* (AP = 0.61; 95% CI 0.34 to 0.89, P_{add} = 0.00001). Although there were trends suggestive of multiplicative interaction between heavy smoking and both of these *NAT2* SNPs, none of multiplicative gene-smoking interactions examined remained significant after adjustment for multiple testing. The *NAT2*-smoking interactions observed were not substantially changed after further adjustment for *HLA-DRB1* SE status in multivariable models (data not shown).

For the *DME* SNPs showing evidence of interaction with heavy smoking in overall disease risk, evidence of interaction in the risk of ACPA positive RA was examined. Risk estimates based on composite polymorphism-smoking status and corresponding measures of interaction were generally similar between ACPA positive RA (Supplemental Table 1) and overall RA (Table 3). In slight contrast to overall RA, there was evidence of significant multiplicative interaction between heavy smoking and *NAT2 rs9987109* (P_{mult} = 0.0015).

Associations of *NAT2* haplotypes (with a frequency exceeding 10%) based on cumulative smoking exposure are shown in Table 4. The haplotype composed of *NAT2* minor alleles for *rs9987109* and *rs1208* and the major allele for rs721399 (CGA) was associated with an increased RA risk in heavy smokers (OR = 4.15; 95% CI=1.55 to 11.09, p = 0.005) consistent with the individual *NAT2* genotype analyses. This haplotype association was not seen in those with < 10 pack-years of total smoking exposure. No other haplotype showed a significant association with RA regardless of smoking exposure (Table 4).

Discussion

These results extend our previously reported findings from this cohort showing that cigarette smoking represents an important environmental risk factor for RA among African Americans. These findings illustrate that the association of smoking with RA disease risk in African Americans may be impacted by genes encoding enzymes involved in the metabolism of smoking-related toxins. Based on our prior observations, we have estimated the attributable risk of RA due to heavy smoking to be 16% [12], a risk that may be much higher in subgroups defined by specific DME genotypes. For instance, among African Americans carrying the *NAT2 rs1208* polymorphism, the attributable risk of RA from heavy smoking approaches 30%.

While our approach is consistent with recent reports examining gene-smoking interactions in RA, it is important to acknowledge that some uncertainty remains regarding the most appropriate manner in which to model gene-environment interactions [45]. In this study, we have examined measures of both additive and multiplicative interaction. Multiplicative interaction, often referred to as ‘statistical’ interaction, refers to the inclusion of a product term in regression analyses in order to generate an optimal ‘fit’ of the data in a given risk model. The absence of multiplicative interaction, such as in this study, does not exclude the existence of highly relevant biologic interactions [45]. The present study suggests that one

or more 'pathways' to RA development in African Americans involve the simultaneous presence of two risk factors in this case, heavy smoking and presence of a select DME gene polymorphisms.

Ours is the first study to date involving an African American population to investigate the role of *DME* gene-smoking interactions in RA susceptibility. Perhaps not surprisingly, our findings share both similarities and differences with the few studies that have been conducted in populations of European ancestry. In their prior case-control study involving 82 individuals with RA, Pawlik and colleagues found that *NAT2* alleles associated with the "slow acetylator" phenotype were ~five-fold more common in RA than in controls [23]. This study did not report smoking rates nor were results provided specific to any possible gene-smoking interactions. In contrast to our results in African Americans showing no evidence of such an interaction, prior studies in populations of European ancestry have yielded evidence of an interaction between the *GSTM1-null* genotype and smoking in RA susceptibility [22, 46]. More recently, however, investigators from the Nurses' Health Study found no evidence of a *GSTM1-null*-smoking interaction in disease risk in a population primarily of European ancestry [47]. It is possible that the *GSTM1-null* genotype, which is substantially less common in African Americans than in individuals of European ancestry, may exert a markedly varied impact across different study populations. Underscoring the potential importance that *DME* genes might play in mediating RA risk secondary to smoking, Keenan et al recently reported evidence of significant gene-environment interactions between the *GSTT1-null* genotype (a genotype not examined in the present study) and heavy smoking in their assessments of RA risk [47].

It is important to point out that rather than selecting only SNPs with known biologic function, our approach involved the use of ht-SNPs with the broader goal of capturing the overall polymorphic nature of each gene of interest. Therefore, it is quite possible (even probable in select cases) that the SNPs examined and shown to interact with heavy smoking may have little functional biologic consequence. For instance, the *rs9987109* SNP that showed a significant additive interaction with heavy smoking is found in the *NAT2* intron and is thus unlikely to exert a functional role that increases disease risk. We anticipate that the association of this particular SNP with RA observed in heavy smokers likely relates to other regions in the gene that are in close linkage disequilibrium. It is noteworthy that the *NAT2 rs1208* polymorphism is a missense polymorphism, one that is represented in many of the "slow acetylator" haplotypes that have been previously well characterized [48]. A prior Japanese case-control study showed that *NAT2* "slow acetylator" genotypes were significantly more frequent among individuals with systemic lupus erythematosus (SLE) compared to healthy controls [49]. Results from this study also suggested a possible interaction between smoking and *NAT2* with an AP of 0.50 (95% CI 0.12 to 0.88). The combination of cigarette smoking and a "slow acetylator" genotype conferred a more than 6-fold increased risk of SLE compared to the combination of never smoking and a "rapid acetylator" genotype.

These results may provide insight into the chemical compounds found in cigarette smoke that could drive heightened RA risk. Both NAT1 and NAT2 enzymes are known to be important in the metabolism of aromatic and heterocyclic carcinogens, both of which are known constituents of cigarette smoke. Moreover, NAT2 function has been implicated as a risk factor in arylamine-related carcinogenesis [50]. Cigarette smoking represents a major environmental source of arylamine exposure in humans with urine levels that are at least twice as high in smokers as non smokers [51]. Whether increased arylamine exposure in the context of reduced NAT2 function could help to explain the findings of this study remains uncertain.

In summary, we have found evidence of significant biologic interactions between heavy smoking and genetic variation in *NAT2* in RA risk among African Americans. In addition to extending and replicating these findings in other populations including an independent cohort of African Americans, further work will be necessary to better understand the precise biologic mechanisms underlying these observed gene-smoking interactions in RA risk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Enrollment characteristics of African American rheumatoid arthritis cases and African American controls*

Characteristic	Cases (n = 727)	Controls (n = 268)	P-value
Age, years	54.0 ± 12.9	52.4 ± 12.6	0.27
Female gender	608 (83.6%)	199 (74.3%)	0.001
Disease duration at baseline visit, years	6.0 ± 8.7		
ACPA positive	474 (67.7%)	11 (4.6%)	<0.001
Rheumatoid factor (RF) positive	538 (75.7%)	37 (15.2%)	<0.001
Smoking status			0.23
Never	346 (47.7%)	141 (53.0%)	
Former	180 (24.8%)	65 (24.4%)	
Current	199 (27.5%)	60 (22.6%)	
Cumulative smoking exposure			0.001
Never or < 10 pack-years	517 (72.0%)	217 (82.2%)	
≥ 10 pack-years	201 (28.0%)	47 (17.8%)	

*ACPA = anti-citrullinated protein antibody; comparisons using chi-square test for categorical variables and two-sample t-test for continuous variables

Table 2

Age- and gender-adjusted associations of drug metabolizing enzyme (DME) polymporphisms with rheumatoid arthritis risk in African Americans*.

Gene & Polymorphisms	Call rate	Minor/Major alleles	MAF - Cases	MAF - Controls	OR	95% CI	P-value
GSTM1							
GSTM1-null		----	224 (31.9)	69 (27.2)	1.29	(0.94, 1.78)	0.12
NAT1							
rs7003666	93.8	T/G	445 (34.7)	172 (33.7)	1.04	(0.77, 1.40)	0.78
rs11203942	95.6	G/C	492 (35.7)	184 (36.5)	1.04	(0.77, 1.40)	0.79
rs10888150	88.5	C/T	321 (26.8)	125 (24.9)	0.72	(0.34, 1.53)	0.39
rs7017402	96.7	A/G	390 (28.5)	165 (31.9)	0.84	(0.63, 1.12)	0.25
rs13253389	94.9	G/A	377 (28.0)	166 (32.5)	0.81	(0.60, 1.08)	0.16
rs9325827	92.0	C/T	77 (6.0)	27 (5.4)	1.08	(0.67, 1.74)	0.75
rs4921879	93.4	A/G	593 (45.1)	221 (43.7)	1.07	(0.78, 1.46)	0.67
rs17126350	96.6	G/A	312 (22.9)	127 (24.1)	0.93	(0.70, 1.25)	0.64
rs4921880	94.8	T/A	527 (40.0)	198 (38.7)	1.16	(0.86, 1.57)	0.33
rs7003890	96.3	C/T	517 (37.7)	213 (40.8)	1.04	(0.78, 1.40)	0.78
rs8190845	95.3	A/G	528 (40.6)	196 (37.8)	1.31	(0.97, 1.77)	0.08
rs15561	95.0	A/C	666 (49.3)	246 (47.1)	1.32	(0.96, 1.81)	0.09
NAT2							
rs4646246	93.7	G/A	326 (24.8)	142 (28.4)	0.80	(0.60, 1.08)	0.15
rs6984200	94.1	T/A	386 (29.2)	145 (28.7)	1.13	(0.84, 1.51)	0.42
rs9987109	94.9	C/T	360 (27.7)	169 (32.5)	0.91	(0.68, 1.22)	0.54
rs1041983	92.5	C/T	623 (47.8)	276 (54.1)	0.73	(0.51, 1.03)	0.07
rs1799930	92.9	A/G	327 (25.7)	135 (26.2)	1.16	(0.86, 1.55)	0.33
rs1208	96.4	G/A	584 (42.2)	208 (39.2)	1.31	(0.97, 1.76)	0.08
rs721399	92.8	G/A	517 (39.5)	206 (40.4)	1.17	(0.87, 1.59)	0.30
EPXH1							
rs1877724	96.2	T/C	110 (8.0)	45 (8.5)	0.85	(0.58, 1.26)	0.43
rs2671272	93.9	C/T	571 (43.0)	223 (43.6)	1.05	(0.76, 1.43)	0.78
rs1051740	87.6	C/T	290 (23.1)	110 (24.2)	0.86	(0.63, 1.16)	0.32
rs2292566	98.4	A/G	154 (10.8)	63 (12.0)	0.88	(0.62, 1.24)	0.47
rs2260863	95.0	G/C	534 (41.1)	223 (43.1)	1.01	(0.74, 1.38)	0.94

Gene & Polymorphisms	Call rate	Minor/Major alleles	MAF - Cases	MAF - Controls	OR	95% CI	P-value
rs2740168	91.1	G/A	530 (42.6)	209 (42.3)	1.01	(0.74, 1.38)	0.95
rs2740170	95.9	T/C	192 (14.5)	79 (15.4)	0.93	(0.67, 1.28)	0.64
rs2740171	92.5	A/C	504 (38.5)	173 (33.9)	1.38	(1.03, 1.86)	0.03
rs2234922	93.7	G/A	496 (38.6)	177 (35)	1.39	(1.03, 1.88)	0.03
rs1051741	97.4	T/C	195 (14.1)	67 12.9)	1.21	(0.87, 1.69)	0.26

*
GSTM1 = glutathione S-transferase Mu-1, *NAT* = N-acetyltransferase, *EPXH1* = epoxide hydrolase; MAF for *GSTM1-null* represents the frequency of homozygosity for deletion polymorphism; ORs are age-and gender-adjusted; $p < 0.0017$ considered significant after adjustments for multiple comparisons

Table 3

Associations of *NAT1, NAT2, and EPXH1* polymorphisms with RA in African Americans based on cumulative smoking history; measures of gene-smoking interaction*

Gene/polymorphism	Minor allele status	Smoking history	Cases (no.)	Controls (no.)	O.R.	95% C.I.	P-value
NAT1							
rs11203942	Absent	Never, < 10py	195 (28.6)	76 (30.6)	1.00	----	----
	Present	Never, < 10py	295 (43.3)	125 (50.4)	0.91	(0.65, 1.28)	0.58
	Absent	≥ 10py	76 (11.2)	25 (10.1)	1.22	(0.72, 2.08)	0.46
	Present	≥ 10py	115 (16.9)	22 (8.9)	2.23	(1.30, 3.82)	0.004
						P_{mult} = 0.06 AP (95% CI) = 0.49 (0.12 to 0.87) P_{add} = 0.009	
NAT2							
rs9987109	Absent	Never, < 10py	245 (38.1)	93 (36.3)	1.00	----	----
	Present	Never, < 10py	220 (34.2)	116 (45.3)	0.73	(0.52, 1.01)	0.06
	Absent	≥ 10py	85 (13.2)	31 (12.1)	1.07	(0.66, 1.73)	0.79
	Present	≥ 10py	93 (14.5)	16 (6.3)	2.42	(1.34, 4.38)	0.003
						P_{mult} = 0.003 AP (95% CI) = 0.67 (0.39 to 0.95) P_{add} = 0.000003	
rs1208	Absent	Never, < 10py	162 (23.6)	75 (28.7)	1.00	----	----
	Present	Never, < 10py	334 (48.8)	139 (53.3)	1.07	(0.76, 1.51)	0.69
	Absent	≥ 10py	60 (8.8)	26 (10.0)	1.10	(0.64, 1.90)	0.73
	Present	≥ 10py	129 (18.8)	21 (8.0)	3.03	(1.76, 5.23)	0.00006
						P_{mult} = 0.01 AP (95% CI) = 0.61 (0.34 to 0.89) P_{add} = 0.00001	
rs721399	Absent	Never, < 10py	156 (24.1)	85 (33.9)	1.00	----	----
	Present	Never, < 10py	313 (48.3)	120 (47.8)	1.45	(1.03, 2.03)	0.03
	Absent	≥ 10py	63 (9.7)	9 (3.6)	4.13	(1.94, 8.78)	0.0002
	Present	≥ 10py	116 (17.9)	37 (14.7)	1.83	(1.15, 2.91)	0.01
						P_{mult} = 0.008 AP (95% CI)= −1.50 (−3.35 to 0.35) P_{add} = 0.11	

Gene/polymorphism	Minor allele status	Smoking history	Cases (no.)	Controls (no.)	O.R.	95% C.I.	P-value
EPXH1							
rs2260863	Absent	Never, < 10py	142 (22.0)	74 (29.0)	1.00	----	----
	Present	Never, < 10py	320 (49.7)	135 (52.9)	1.23	(0.87, 1.74)	0.24
	Absent	≥ 10py	66 (10.2)	10 (3.9)	3.68	(1.77, 7.66)	0
	Present	≥ 10py	116 (18.0)	36 (14.1)	1.76	(1.09, 2.83)	0.02
						P_{mult} = 0.03 AP (95% CI) = −1.22 (−2.87 to 0.42) P_{add} = 0.15	

*NAT = N-acetyltransferase, EPXH1 = epoxide hydrolase; AP = attributable proportion due to interaction; ORs are age- and gender-adjusted

Table 4

Associations of *NAT-2* haplotypes with RA risk based on smoking exposure*

	Never and < 10 pack-years		
	Control haplotype frequency	**RA case haplotype frequency**	**O.R. (95% CI)**
TAA	26.0%	26.8%	1.00 (Referent)
TAG	27.5%	28.1%	1.17 (0.78 to 1.77), p = 0.45
CGA	30.4%	22.9%	0.83 (0.56 to 1.22), p = 0.34
	≥ 10 pack-years		
	Control haplotype frequency	**RA case haplotype frequency**	**O.R. (95% CI)**
TAA	30.0%	23.4%	1.00 (Referent)
TAG	34.9%	29.1%	1.03 (0.41 to 2.62), p = 0.94
CGA	11.3%	25.5%	4.15 (1.55 to 11.09), p = 0.005

* Single nucleotide polymorphisms comprising the haplotypes (in order) are rs9987109; rs1208; rs721399. TAA = haplotype from major alleles; ORs age- and gender-adjusted.