Association of metabolic gene polymorphisms with tobacco consumption in healthy controls

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ASSOCIATION OF METABOLIC GENE POLYMORPHISMS WITH TOBACCO CONSUMPTION IN HEALTHY CONTROLS

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Polymorphisms in genes that encode for metabolic enzymes have been associated with variations in enzyme activity between individuals. Such variations could be associated with differences in individual exposure to carcinogens that are metabolized by these genes. In this study, we examine the association between polymorphisms in several metabolic genes and the consumption of tobacco in a large sample of healthy individuals. The database of the International Collaborative Study on Genetic Susceptibility to Environmental Carcinogens was used. All the individuals who were controls from the case-control studies included in the data set with information on smoking habits and on genetic polymorphisms were selected (n = 20,938). Sufficient information was available on the following genes that are involved in the metabolism of tobacco smoke constituents: *CYP1A1*, *GSTM1*, *GSTT1*, *NAT2* and *GSTP1*. None of the tested genes was clearly associated with smoking behavior. Information on smoking dose, available for a subset of subjects, showed no effect of metabolic gene polymorphisms on the amount of smoking. No association between polymorphisms in the genes studied and tobacco consumption was observed; therefore, no effect of these genes on smoking behavior should be expected.

Key words: *pooled analysis; molecular epidemiology; smoking*

The association between metabolic gene polymorphisms and diseases such as cancer has been studied extensively.[1,2] Recently, attention has been drawn to the possible influence of these genetic polymorphisms on risk-related behavior of healthy persons, such as the individual consumption of tobacco. It has been suggested that the extent of tobacco smoking might be influenced by the metabolism of toxic compounds in the smoke,[3] and that the level of some tobacco constituents such as nicotine in the body for the same quantity of cigarettes smoked might depend on specific metabolic genotypes.[4] Dopamine pathways have also been implicated in smoking behavior, possibly with differences by ethnicity.[5,6] In addition, metabolic gene polymorphisms could be associated with different smoking patterns in healthy individuals by modifying the amount of toxic carcinogens available in the body given the same smoking dose. The question of whether metabolic gene polymorphisms are associated with relevant environmental exposures, such as smoking, is important for several reasons. First, it would help in understanding the mechanisms through which some metabolic gene polymorphisms are associated with increased risk of cancer of various sites,[1] and it could be of importance for smoking cessation programs.[7] Furthermore, in the case-only study, a design used for studying gene-gene and gene-environment interaction, independence is required between genetic polymorphisms and exposure.[8] Such independence has never been tested within a large data set.

We investigated the association between polymorphisms in *CYP1A1* , several *GST* s (*GSTM1* , *GSTP1* and *GSTT1*) and *NAT2* genes and smoking behavior in a large sample of individuals collected in the database of the International Collaborative Study on Genetic Susceptibility to Environmental Carcinogens (GSEC).[9]

MATERIAL AND METHODS

Study population

Control subjects were selected from the International Collaborative Study on Genetic Susceptibility to Environmental Carcinogens. This is a collaborative project that collects information from case-control studies on genetic polymorphisms and cancer. Investigators who performed case-control studies on this topic have been contacted and asked to send their original data. The design of this study is explained in detail elsewhere.[9]

Subjects for the present analysis were controls gathered from several case-control studies. Individuals that were identified as carriers of precancerous lesions, such as endometriosis or colon polyposis, were excluded from this study. Subjects without any information on at least one of the selected genes and subjects without information on tobacco consumption were excluded. Sixty-two percent of the subjects were healthy subjects while 38% were hospital controls, *i.e.* , hospital patients admitted for nonneoplastic diseases. Tobacco smoking status was defined as never, ex-, or current smokers. Subjects who smoked at least 100 cigarettes in their lifetime were defined as ever smokers. In most studies, information was available to distinguish between current and ex-smokers; the latter group comprised subjects who quit smoking at least 6 months before entering the study. Whenever possible, the average tobacco consumption was expressed as both number of cigarettes smoked per day and cumulative tobacco consumption as pack-years.

We were able to use information on the following polymorphisms: *CYP1A1* , *GSTM1* , *GSTT1* , *NAT2* and *GSTP1* . These genes were chosen because they are involved in tobacco products metabolism. The genotypic data on *NAT2* polymorphisms was divided into slow or rapid acetylator status according to the following definition: the presence of at least one *4 allele determines the status of fast acetylator. For *CYP1A1* , we considered the Msp1 RFLP allele, which is found in the *2A and *2B alleles, for *GSTP1* the Ile-Val polymorphism in codon 105. In all studies, comparable PCR-based techniques were used to determine the genotype of the subjects.

Statistical methods

Frequencies were calculated for the different genes according to smoking status. We calculated crude and adjusted odds ratios (ORs) and 95% confidence interval (CI) for each genotype according to smoking status. Data were adjusted for study, age, sex and ethnicity using logistic regression models. Breslow-Day test was used to assess heterogeneity across strata. All statistical analyses were performed using SPSS software version 10.0. Differences in mean values of cigarettes smoked per day and pack-years were adjusted by study, age, sex and ethnicity with a multivariate linear model.

TABLE I – SUMMARY OF DATA INCLUDED IN THIS STUDY

Gene	Number of studies	Number of subjects	Mean age	% of smokers with information on smoking amount
CYPlA1	39	4,447	53.25	43.5
GSTM1	70	10,719	54.50	42.7
GSTT1	44	5,993	53.37	35.5
NAT2	24	4,398	53.96	37.1
GSTP1	19	2,792	49.05	47.3

RESULTS

The population used for the analyses consisted of 20,938 persons. Of these, 15,193 were Caucasians (72.6%), 1,083 were African Americans (5.2%) and 2,430 were Asians (11.6%). The remaining subjects belonged to other ethnic groups. Table I presents a summary of the data used in the analyses. The association between smoking and the selected genes for the study population is reported in Table II. None of the polymorphisms showed any association with smoking status, although there was a weak association between *GSTM1* deletion and smoking, since subjects with the homozygous deletion were less likely to be current smokers (OR = 0.86; 95% CI = 0.80–0.98) than other subjects. Since smoking frequency and amount are different in men and women, the analysis was repeated after stratification according to gender. The only observed association was between *CYP1A1* polymorphism and ever smoking in men (OR = 0.81; 95% CI = 0.66–0.99). The data were stratified by type of controls (healthy *vs.* hospital). A positive association between *GSTT1* deletion and smoking status was present in healthy controls, but not in hospital controls. The opposite was observed for *NAT2*, where a positive association was present in hospital controls only. We also analyzed the simultaneous presence of phase 1 and phase 2 metabolic gene

TABLE II – ASSOCIATION BETWEEN SMOKING AND GENETIC POLYMORPHISMS ACCORDING TO GENDER AND TYPE OF CONTROLS

Gene and smoking status	Total number with wild-type/ number with polymorphism[a]	Adjusted OR[b]	Men, adjusted OR[c]	Women, adjusted OR[c]	Healthy controls, adjusted OR[b]	Hospital controls, adjusted OR[b]
CYP1A1						
Never smoker	1,198/535	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Ever smoker	2,025/720	0.94 (0.81–1.09)	0.81 (0.66–0.99)	1.10 (0.90–1.36)	0.89 (0.74–1.08)	1.10 (0.83–1.47)
Never smoker	910/421	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Current smoker	885/389	1.02 (0.85–1.23)	0.87 (0.68–1.11)	1.30 (0.98–1.73)	0.97 (0.78–1.21)	1.36 (0.86–2.14)
Ex-smoker	711/236	0.92 (0.74–1.14)[d]	0.78 (0.59–1.03)	1.14 (0.82–1.59)	0.83 (0.65–1.07)	1.31 (0.76–2.27)
GSTM1						
Never smoker	2,346/2,561	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Ever smoker	3,533/3,491	0.92 (0.85–1.00)	0.90 (0.81–1.01)	0.92 (0.82–1.05)	0.90 (0.81–0.99)	0.92 (0.79–1.07)
Never smoker	1,995/2,182	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Current smoker	1,798/1,749	0.86 (0.80–0.98)	0.84 (0.74–0.96)	0.99 (0.84–1.18)	0.90 (0.80–1.03)	0.85 (0.70–1.02)
Ex-smoker	1,088/1,099	0.94 (0.83–1.05)	1.01 (0.87–1.18)	0.83 (0.69–1.01)	0.97 (0.84–1.12)	0.83 (0.65–1.06)
GSTT1						
Never smoker	2,322/637	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Ever smoker	3,105/899	1.03 (0.90–1.17)	1.10 (0.93–1.31)	0.82 (0.67–1.01)	1.27 (1.06–1.52)	0.87 (0.70–1.09)
Never smoker	1,871/478	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Current smoker	1,365/375	0.99 (0.83–1.18)			1.22 (0.96–1.56)	0.87 (0.65–1.17)
Ex-smoker	1,088/298	1.07 (0.88–1.30)	1.15 (0.90–1.49)	0.92 (0.66–1.29)	1.30 (1.00–1.70)	1.00 (0.72–1.38)
GSTP1						
Never smoker	740/749	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Ever smoker	1,116/1,140	1.05 (0.90–1.24)[e]	1.18 (0.97–1.42)	0.78 (0.57–1.06)	1.08 (0.89–1.30)	0.97 (0.62–1.51)
Never smoker	700/710	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Current smoker	556/585	1.05 (0.87–1.28)	1.17 (0.94–1.46)	0.67 (0.43–1.04)	1.13 (0.90–1.42)	0.88 (0.54–1.45)
Ex-smoker	422/398	0.97 (0.78–1.20)	1.02 (0.79–1.32)	0.83 (0.56–1.24)	1.00 (0.78–1.29)	0.57 (0.29–1.11)
NAT2						
Never smoker	1,273/1,014	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Ever smoker	1,710/1,356	1.02 (0.90–1.15)	0.95 (0.81–1.12)	1.09 (0.90–1.33)	0.86 (0.74–1.01)	1.28 (0.99–1.65)
Never smoker	1,144/910	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Current smoker	951/711	0.94 (0.81–1.09)	0.92 (0.76–1.11)	0.98 (0.76–1.27)	0.76 (0.63–0.92)	1.40 (1.02–1.94)
Ex-smoker	558/461	1.07 (0.90–1.28)	0.95 (0.76–1.20)	1.33 (1.00–1.77)	0.97 (0.79–1.18)	1.33 (0.84–2.12)

[a]Numbers in stratified analyses do not add up to numbers in the overall analyses due to the exclusion of subjects without available information on sex in the stratified analyses. Wild type: med type homozygotes; polymorphism: carrier of at least one polymorphic allele; for *GSTM* and *GSTT,* wild-type as wild-type homozygotes or deletion of one allele, polymorphism is deletion of both alleles.–[b]OR are adjusted for study, gender, age and ethnicity.–[c]OR are adjusted for study, age and ethnicity.–[d]*p*-value for heterogeneity = 0.03.–[e]*p*-value for heterogeneity = 0.01.

TABLE III – EFFECT OF THE SIMULTANEOUS PRESENCE OF 2 GENE POLYMORPHISMS ON SMOKING HABITS

Gene combination and smoking status	*n*	Crude OR	Adjusted OR[1]
CYP1A1 wt and *GSTM1* carrier/*CYP1A1* heterozygotes + homozygotes and *GSTM* complete deletion			
Never smoker	1,455	1.00 (reference)	1.00 (reference)
Ever smoker	2,447	0.81 (0.68–0.98)	0.91 (0.74–1.11)
Never smoker	1,080	1.00 (reference)	1.00 (reference)
Current smoker	1,069	0.89 (0.70–1.12)	0.94 (0.73–1.20)
Ex-smoker	892	0.69 (0.54–0.89)	0.83 (0.62–1.10)
CYP1A1 wt and *GSTT* carrier/*CYP1A1* heterozygotes + homozygotes and *GSTT* complete deletion			
Never smoker	1,046	1.00 (reference)	1.00 (reference)
Ever smoker	1,519	0.61 (0.42–0.88)	0.73 (0.50–1.08)
Never smoker	689	1.00 (reference)	1.00 (reference)
Current smoker	505	0.83 (0.44–1.57)	0.86 (0.45–1.64)
Ex-smoker	597	0.88 (0.49–1.60)	0.95 (0.50–1.81)
GSTM and *GSTT* carrier/*GSTM* and *GSTT* complete deletion			
Never smoker	2,895	1.00 (reference)	1.00 (reference)
Ever smoker	3,850	0.97 (0.83–1.13)	0.98 (0.83–1.17)
Never smoker	2,293	1.00 (reference)	1.00 (reference)
Current smoker	1,677	1.04 (0.85–1.27)	0.96 (0.76–1.23)
Ex-smoker	1,311	0.98 (0.79–1.22)	1.05 (0.80–1.37)

[1]OR adjusted for study, age, sex and ethnicity.

polymorphisms acting on the pathway of smoking metabolism, for example, the variants of *CYP1A1* (heterozygous plus homozygous) and the homozygous deletion of *GSTM1*. The odds of being an ever smoker were decreased in subjects carrying both a *CYP1A1* polymorphic allele and the homozygous deletion in *GSTM1* or in *GSTT1*, although we did not find any significant association (Table III).

Information about tobacco consumption in the whole population and stratified by sex is presented in Table IV. As expected, men smoke more cigarettes per day compared to women. The mean number of pack-years was higher in subjects carrying the *GSTP1* homozygous variant than in subjects with the wild-type genotype. Men with the *NAT2* rapid acetylator genotype had a significantly higher number of pack-years than men with the slow acetylator genotype. No differences were seen in daily amount of smoking; data on number of years smoked were too scarce to allow a separate analysis.

DISCUSSION

The aim of the present study was to investigate the association between polymorphisms in a set of metabolic genes and the consumption of tobacco. The hypothesis behind this analysis is that in individuals with an increased phase 1 and a decreased phase 2 enzyme activity, as a result of genetic polymorphisms, the xenobiotic that is the substrate for those enzymes would reach a higher concentration. Such high concentration would become toxic and would therefore influence smoking habits. Studies that investigated the influence of genetic factors on smoking behavior are very scarce, with contrasting results, and have been concentrated on genes involved in nicotine metabolism.[4,5,10–14] We decided to investigate the association of genes involved in the metabolism of tobacco carcinogens (*NAT2* , *CYP1A1* , as well as several *GST* s) with smoking behavior in a large population of individuals.

Our results suggest that none of the selected genes have a strong effect on smoking behavior. Information on cumulative tobacco consumption, available for a subset of subjects, showed no association between amount of smoking and metabolic gene polymorphisms. The weak effect of the *GSTP1* Val allele, and of *NAT2* acetylator status in men only, could be due to chance finding due to multiple hypothesis testing. The lack of consistency of such associations in the 2 genders detracts from their biologic plausibility.

TABLE IV – AVERAGE AND CUMULATIVE TOBACCO CONSUMPTION IN THE WHOLE POPULATION OF SMOKERS, AND FOR MEN AND WOMEN ACCORDING TO GENOTYPE

Genotype	Total		Men		Women	
	Cigarettes/day (*n*)	Pack-years (*n*)	Cigarettes/day (*n*)	Pack-years (*n*)	Cigarettes/day (*n*)	Pack-years (*n*)
CYP1A1						
Wild type	19.67 ± 13.38 (923)	29.38 ± 26.72 (1,385)	20.92 ± 13.34 (385)	34.78 ± 27.88 (449)	13.79 ± 9.84 (131)	24.02 ± 20.60 (127)
Heterozygous	19.17 ± 3.11 (349)	28.02 ± 29.82 (453)	21.62 ± 14.03 (176)	37.92 ± 31.35 (170)	13.98 ± 9.72 (48)	30.38 ± 37.22 (53)
Homozygous	21.78 ± 14.56 (88)	34.21 ± 30.64 (100)	20.80 ± 11.34 (54)	36.29 ± 20.92 (51)	14.10 ± 11.34 (10)	32.58 ± 30.65 (12)
GSTM1						
*1/*1 or *1/*0	19.24 ± 12.52 (1,658)	38.06 ± 66.06 (2,323)	18.75 ± 11.08 (768)	48.11 ± 92.70 (935)	15.30 ± 9.06 (196)	42.80 ± 65.46 (246)
*0/*0	19.67 ± 13.13 (1,689)	38.49 ± 63.30 (2,258)	18.78 ± 11.41 (746)	45.54 ± 85.14 (852)	17.72 ± 11.56 (199)	52.27 ± 87.17 (240)
GSTT1						
*1/*1 or *1/*0	18.46 ± 11.59 (1,166)	46.65 ± 93.53 (16.05)	17.41 ± 10.73 (470)	76.73 ± 146.23 (486)	15.37 ± 9.07 (122)	81.09 ± 121.34 (143)
*0/*0	18.82 ± 11.67 (445)	37.84 ± 74.55 (528)	18.65 ± 10.63 (208)	51.26 ± 112.51 (182)	13.65 ± 7.59 (43)	58.10 ± 93.97 (41)
NAT2						
Rapid	17.71 ± 11.21 (425)	50.38 ± 97.27 (678)	15.90 ± 10.62 (184)	70.95 ± 140.00 (260)[a]	19.24 ± 10.51 (103)	68.38 ± 94.96 (94)
Slow	16.63 ± 10.78 (597)	50.31 ± 99.64 (958)	15.74 ± 9.94 (266)	67.17 ± 132.04 (417)	16.75 ± 10.92 (137)	72.60 ± 114.68 (124)
GSTP1						
Wild type	18.64 ± 10.63 (499)	26.46 ± 22.73 (629)	18.07 ± 9.72 (235)	22.83 ± 17.12 (285)	16.58 ± 8.36 (36)	25.62 ± 19.27 (56)
Heterozygous	19.07 ± 10.87 (431)	26.61 ± 24.99 (598)	18.53 ± 9.87 (221)	25.60 ± 26.33 (312)	16.50 ± 9.98 (20)	30.63 ± 26.22 (32)
Homozygous	20.78 ± 12.21 (67)	29.34 ± 25.19 (98)[b]	19.17 ± 12.71 (30)	25.90 ± 24.52 (41)	22.00 ± 9.63 (4)	23.56 ± 21.43 (9)

[a]$p = 0.008$.–[b]$p = 0.03$.

Although, to our knowledge, this is the largest study ever performed on the relationship between genetic polymorphisms and individual consumption of tobacco, some specific groups still included only a small number of individuals; this was particularly true for homozygotes for certain polymorphisms. Therefore, more refined analyses, such as a stratification for both sex and ethnicity, were not possible. The definition of smoker, as well as the amount and duration of smoking, was collected through different questionnaires, therefore some misclassification is possible. However, it is unlikely that the subjects included in this analysis misreported their smoking status on purpose. Also, they did not know their genotypes; as a result, misclassification, if present, should not be related to the genotype.

Differences in laboratory techniques used for analysis of the genotype should not be a major source of bias, since PCR-based techniques currently used to analyze the genotype have become standardized. When looking at type of controls included in the analysis, we observed some differences in the association between genotype and smoking status. Although this results should be taken with caution due to the small sample size included in the subgroup analysis, they underline the well-known differences in smoking status between healthy and hospital controls, although a previous analysis showed no differences in genotype distribution among different types of controls.[15]

It must be emphasized that several other polymorphic genes, in addition to the ones analyzed in this study, are involved in tobacco smoke metabolism. The analysis of the concomitant presence of 2 different gene polymorphisms suggests a stronger association with smoking behavior. Our results do not rule out the possibility that certain haplotypes encompassing several gene polymorphisms would be associated with tobacco smoking. In order to test such hypotheses, even larger number of subjects than the ones included in the present analysis are needed.

In summary, we observed no association between polymorphisms in the genes studied and tobacco consumption, therefore there does not appear to be any relationship of these genes and smoking behavior. If a particular polymorphism had been shown to be associated with smoking behavior, then smoking would be a confounder in studies investigating the relationship between that polymorphism and smoking-induced cancers, such as lung or bladder cancer. The data presented here, derived from a large population, suggest that no such confounding should be present for *CYP1A1* , *GST* s, or *NAT2* . The use of the case-only design for epidemiologic studies including these gene polymophisms is therefore justified, at least when studying smoking habits. Studies of association between low-penetrance genetic polymorphisms and a disease or behavior such as smoking require large populations in order to be confident of the results. The GSEC project has proven useful for population genetic analyses[15] and for testing a number of hypotheses relating specific low-penetrance gene polymorphisms to a specific cancer.[16]

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