

Genetic and Environmental Sources of Variation in Heart Rate Response to Infused Nicotine in Twins

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Abstract

The heart rate response to nicotine may be an important component of the process leading to dependence. The present study is the first to determine the extent to which genetic and environmental sources play a role in various components of the heart rate response. One hundred and ten monozygotic and 29 dizygotic twin pairs received an i.v. infusion of nicotine and cotinine over 30 min. Before, during, and for 30 min after infusion, heart rate was measured via an electronic monitor. The clearance of nicotine was determined as a measure of the rate of nicotine metabolism. Average resting heart rate before infusion was 64.7 beats per minutes (bpm), and at the termination of infusion, heart rate had increased to an average of 72.7 bpm. At 30 min after infusion, heart rate had decreased to 67.5 bpm. Age, current smoking status, body mass index, and nicotine clearance were associated significantly with heart rate levels over the full

60 min of measurement. After adjustment for several covariates, including dose of administered nicotine and rate of nicotine clearance, the variance in several characteristics of the heart rate response curve was examined for the relative contribution from genetic and environmental sources. In the total sample, as much as 30.3% of the variance in the acceleration of heart rate was due to additive genetic sources. In nonsmokers, 34.8% and 31.0% of variance in the acceleration and deceleration of heart rate, respectively, was due to genetic sources. Heart rate acceleration and deceleration may be a reflection of central nervous system responsiveness to nicotine. The contribution from genetic sources to heart rate response characteristics should be investigated further as a potential endophenotype for use in genetic studies of nicotine dependence. (Cancer Epidemiol Biomarkers Prev 2007;16(6):1057–64)

Introduction

Nicotine increases heart rate through activation of the sympathetic nervous system with release of norepinephrine and epinephrine (1, 2). On average, smoking a single cigarette following overnight abstinence from smoking results in an increase in heart rate of about 13 beats per minute (bpm; refs. 3–5). An equivalent dose of nicotine delivered i.v. results in similar cardiovascular activation (6–11). Increases in heart rate following smoking are associated with higher levels of positive subjective reactions (5).

Certain characteristics of heart rate activation, as pharmacodynamic effects of nicotine, represent candidate intermediate phenotypes that could serve as endophenotypes for genetic studies of susceptibility to becoming dependent on nicotine. This is a reasonable hypothesis given that the increase in heart rate is mediated at least in part by actions of nicotine on the central nervous system (12). For a measure, such as heart rate activation, to be accepted as an endophenotype, however, it must meet a number of criteria, including evidence of heritability (13). Previous studies indicate that heart rate

reactivity to physical and mental stressors is heritable (e.g., 30–50%; refs. 14–16), and that variation in candidate genes is associated with cardiovascular stress reactivity (17–21). However, there are no previous reports of heritability of the heart rate response to nicotine. Evidence of heritability, if present, would indicate that further work to determine molecular genetic associations with the heart rate response may be warranted. Therefore, as a first step, we sought to examine the heritability of the heart rate response following controlled exposure to nicotine.

In previous reports, we presented the methodology and results of a large twin study of nicotine metabolism (22–26). Before, during, and after the controlled infusion of deuterium-labeled nicotine and cotinine, participants' heart rate was measured at regular intervals. The goals of the present analysis were to (a) examine the heart rate response over the course of a 30-min infusion and for 30 min thereafter; (b) determine the extent to which individual difference variables, including the rate of nicotine clearance, are associated with the heart rate response; and (c) determine the extent to which the heart rate response is influenced by genetic and environmental factors.

Materials and Methods

Setting. The study involved two primary research centers: the Center for Health Sciences at SRI International (Menlo Park, CA) and the Division of Clinical Pharmacology and Experimental Therapeutics, University of California, San Francisco, based at the San Francisco General Hospital Medical

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Center. SRI International was responsible for the recruitment, screening, and scheduling of twin pairs drawn from the SRI Northern California Twin Registry (NCTR; described below) and was responsible for biometric analyses. University of California, San Francisco was responsible for conducting the nicotine/cotinine infusion procedure in the General Clinical Research Center at San Francisco General Hospital; for analytic chemistry procedures to determine nicotine, cotinine, and metabolite levels; and for quantification of pharmacokinetic variables. Additional contributions were made by the Department of Neurology, University of California, San Francisco (molecular determination of zygosity).

Participants. The NCTR was created in 1995 to fill the need for a pool of twins for genetic studies of drug metabolism. In 1996, an extensive advertising campaign was conducted in 19 newspapers, San Francisco Bay Area-wide movie theaters, and AM/FM radio stations. Within 2.5 years, this campaign resulted in the enrollment of 1,054 twins, a large enough pool from which to recruit twins to the present study. Contact was (and continues to be) maintained with twins in the NCTR via annual newsletters and birthday cards. In addition to the above-described methods, a 5-year NCTR anniversary celebration party held in July 2000 increased enrollment to 1,765 individual twins. A NCTR web site⁴ is maintained, and referrals to the NCTR by registered twins are encouraged. Currently, there are over 2,000 twins registered with the NCTR.

Inclusion Criteria. To be eligible for inclusion in this study, volunteers needed to have a twin who was also willing to participate and be between the ages of 18 and 65 years. Both members of a twin pair needed to be in good general health.

Exclusion Criteria. To minimize the effect of medical conditions and/or medication usage known to influence drug metabolism, individuals were excluded from participation if they met any of the following criteria: age <18 years or older than 65 years; weight >30% over ideal height-adjusted weight; pregnancy; the presence of any of the following conditions: use of drug metabolism-altering medications, such as anticonvulsant drugs and barbiturates; uncontrolled hypertension or diabetes; a history of heart disease as indicated by self-report or history of bypass surgery, valve replacement, use of a pacemaker, or angioplasty procedures; Raynaud's disease; chronic diseases, such as cancer, liver, and kidney diseases, or asthma, that were not stable or were not in remission for at least 1 year; migraine headaches, anemia, abnormal blood sugar levels that were not well controlled by medication, substance abuse and/or dependence (other than tobacco), psychiatric disorders that could limit study compliance or require the use of metabolism-altering psychotropic medications, positive HIV status, hepatitis B or C, history of vasovagal reactions, discomfort with venipuncture procedures, or a self-reported history of "difficult veins." Because the study procedures could be seriously confounded or could lead to adverse events for the participants by the presence of the conditions described above, a three-tiered screening procedure (telephone, in-person, and in-hospital) was employed (described more completely elsewhere; ref. 22).

All methods for recruitment, informed consent, screening, data and DNA collection, and genetic analysis were reviewed and approved by the Institutional Review Boards of SRI International and University of California, San Francisco.

Questionnaire Measures. Zygosity status was assessed by standard zygosity questionnaire items (27) and confirmed by

DNA genotyping (see below). Zygosity questions included whether, as children, the twins were "as alike as two peas in a pod"; whether parents, siblings, or teachers had trouble telling them apart; and the twins' own knowledge of their zygosity. A series of standard demographic questions were asked to determine date of birth, race/ethnicity, marital status, number of children, and educational attainment. In addition to information pertinent to the previously described exclusionary conditions, participants also provided a history of hospitalization for major medical illness or surgery. The timing of each female participant's menstrual cycle was determined following a previously published approach (28). The infusion protocol was scheduled to occur in the mid- to late-follicular phase (operationally, between the end of the menses and day 11 of the menstrual cycle). A series of questions were also asked to ascertain smoking status (never, former, or current smoking). Current smoking status and the number of cigarettes smoked were important determinants of the dose of nicotine infused (see below). Current smoking was defined as either regular or occasional current smoking. Nonsmoking was defined as former or never smoking.

In-Hospital Pharmacokinetic Study Procedures. Participants were asked to abstain from alcohol or recreational drugs for 1 week before the study and during the study. They were also asked to fast and to refrain from tobacco use from 10 p.m. the night before the exam. Two factors determined the nicotine dose level: body weight and screening plasma cotinine levels. Participants received 0.5 µg/kg/min if plasma cotinine levels were ≤50 ng/mL (levels consistent with not smoking or smoking five or fewer cigarettes per day), 1.0 µg/kg/min if plasma cotinine levels were 50 to 150 ng/mL (levels consistent with smoking 5-15 cigarettes per day), and 2.0 µg/kg/min if plasma cotinine levels were >150 ng/mL (levels consistent with smoking ≥15 cigarettes per day). Subjects with lower cotinine levels received lower doses of nicotine to prevent nicotine toxicity, typically seen when higher doses of nicotine are administered to nonsmokers. The dose was always based on the lower plasma cotinine level within a twin pair so that both twins of pairs discordant for current smoking received the same dose. The dose of cotinine was the same as that for nicotine. In-dwelling catheters were placed in each arm. The catheter in the right arm was used to deliver the stable isotope-labeled nicotine/cotinine solution, whereas the catheter in the left arm was used to obtain periodic blood samples.

Participants received an i.v. infusion of deuterium-labeled nicotine (nicotine-d₂, 3',3'-dideuteronicotine). Deuterium-labeled cotinine was also administered as part of our metabolic study, but at the doses administered, cotinine has minimal, if any, cardiovascular activity; thus, its pharmacologic effects were not considered in the present analysis. Labeled compounds are necessary for metabolic studies because individuals who use tobacco already have considerable levels of nicotine in their bodies that would make measurement of clearance of unlabeled nicotine impossible. The synthesis of this deuterium-labeled compound and its preparation for infusion have been described previously (29). During all infusions, participants underwent continuous cardiac monitoring and frequent blood pressure measurements.

Before the start of the 30-min nicotine infusion, baseline blood and urine samples were obtained from each participant. After the start of the infusion, 10 blood samples (5 mL) were obtained at the following intervals: 10, 20, 30, 45, 60, 90, 120, and 180 min and at 4 and 6 h.

Pharmacokinetic Measures. Concentrations of natural and deuterium-labeled nicotine in blood were measured by gas chromatography-mass spectrometry with the use of

⁴ <http://www.sri.com/policy/twin/>

nicotine-3',3'-d₂-N'-methyl-d₂ (nicotine-d₄) as an internal standard (30). The limit of quantitation for nicotine was 0.5 ng/mL. Concentrations of nicotine-d₂ were corrected for the presence of naturally occurring stable isotopes in nicotine from tobacco.

A standard pharmacokinetic variable was estimated from blood concentration data using model-independent methods described previously (31, 32). Total nicotine clearance was computed as:

$$CL_{\text{nic-d}_2} = \text{Dose}_{\text{nic-d}_2} / \text{AUC}_{\text{nic-d}_2}$$

where CL is clearance, AUC is area under the plasma concentration curve extrapolated to infinity, and nic-d₂ is dideuteronicotine.

Genotyping for Zygosity. Self-reported zygosity was confirmed by genotyping of highly polymorphic microsatellite markers on 11 chromosomes (D1S1612, D2S1788, D3S1764, D4S2368, D5S1501, D7S513, D8S1110, D13S1796, D14S608, D16S753, and D15S657) using the method described by Weber and May (33). Because microsatellites have a low, but detectable, somatic mutation rate, the zygosity of one twin pair could not be determined without additional marker testing. As a check on the accuracy of DNA genotyping for zygosity, a subset of DNA samples from 30 randomly selected pairs was sent to an independent laboratory (Rutgers University Cell and DNA Repository). All zygosity determinations from the first laboratory were confirmed by subsequent analysis from the independent laboratory.

Heart Rate Monitoring. Heart rate measures were taken by an automated recording machine (Critikon Dinamap 845XT) before, during, and following infusion of nicotine at consistent time points: one measure immediately before the start of the infusion; at 5, 10, 15, 20, 25, and 30 min during the infusion; and at 15 and 30 min after termination of the infusion.

Statistical Methods. Raw heart rate data were reviewed, and aberrant values were either eliminated or corrected before data analysis. The percent change in heart rate was then calculated for each individual at 5, 10, 15, 20, 25, 30, 45, and 60 min using the pre-infusion resting measure as the baseline. The average percent change in heart rate at each point for the entire sample is presented in Fig. 1.

The effect of potential covariates [age, gender, ethnicity, current smoking, body mass index (BMI; kg/m²), infused nicotine dose, nicotine clearance, and cumulative nicotine concentration in plasma over the course of the 30-min infusion] on the total time course of heart rate (0-60 min) and heart rate response phenotypes of interest was examined using the

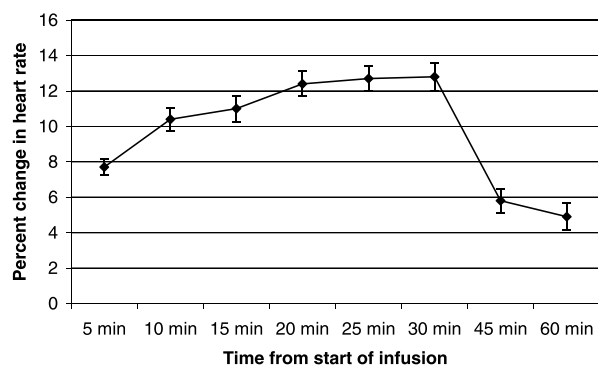


Figure 1. Percent change in heart rate during (0-30 min) and after (30-60 min) of nicotine infusion in the entire sample.

autoregressive feature of the repeated measures function of PROC MIXED (34). For the portion of the analysis that dealt with the 0- to 60-min time course, the eight heart rate measurements were treated as a repeated dependent variable. The procedure then tested for the significance of each covariate, time of measurement, and an interaction between each covariate and time of measurement while also adjusting for twinning and the cumulative nicotine concentration in plasma. For display purposes of significant associations, adjusted means for dichotomous (current smoking/nonsmoking), and continuous variables stratified at the median (age and BMI) are presented in Fig. 2A to C.

Three intermediate heart rate response characteristics were of interest to the portion of analysis focused on genetic and environmental influences. Inspection of the heart rate response curve presented in Fig. 1 revealed a curvilinear time course coincident with onset and offset of the nicotine infusion. Through the first 30 min (the duration of infusion), the heart rate response increased steadily followed by a steady decline in heart rate after the termination of the infusion. We therefore defined "acceleration" (5, 10, 15, 20, 25, and 30 min) and "deceleration" (e.g., 30, 45, and 60 min) portions of the response curve. Using linear regression, a best-fitting line through the means for percent change at each time point was created and then characterized by the slope for both the acceleration and deceleration portions of the response curve. The slope of each portion of the response curve was then examined for its association with the same covariates as described above.

Heart rate response in the first 5 min following the onset of nicotine infusion may be an indicator of sensitivity to nicotine because short-term tolerance to the effects of nicotine has not yet occurred (35). Therefore, in addition to the heart rate phenotypes mentioned above, analyses were also focused on the percent change from baseline at this single point.

The relationship between nicotine clearance and 30-min nicotine concentration in plasma and the three heart rate response phenotypes was examined by dividing the sample at the median value for both variables. The resulting two groups were then compared on each heart rate phenotype by conducting an *F* test within PROC MIXED.

The relative contribution of genetic and environmental influences on phenotypic variation in heart rate phenotypes was determined using the structural equation-modeling program Mx (36). Using standard twin methodology of twins reared together, the total phenotypic variance was decomposed into three components including additive genetic (A) factors (heritability), shared environmental (C) factors that are common to both members of a twin pair (e.g., family, school, and neighborhood), and to non-shared environmental (E) factors that are unique to each twin. The fit of this three-component model (ACE model) to the observed raw MZ and DZ twin pair data was tested using methods of maximum likelihood. Sequentially dropping each component from the model and comparing the fit of the reduced model (AE, CE, or E) to the fit of the full model (ACE) by a likelihood-ratio χ^2 difference test determined the significance of the contribution of the A and C components to the model. Genetic and environmental influence on variance of heart rate phenotypes was estimated after adjusting means for age, current smoking, BMI, ethnicity, infused nicotine dose, and nicotine clearance. The effect of the covariates was concurrently modeled. Nicotine dose and clearance were included because together they determine the blood levels of nicotine to which an individual is exposed. All Mx analyses of the heart rate phenotypes described above were repeated in nonsmokers only to further reduce possible confounding effects of current smoking on the heart rate response.

Heart Rate Response to Nicotine in Twins

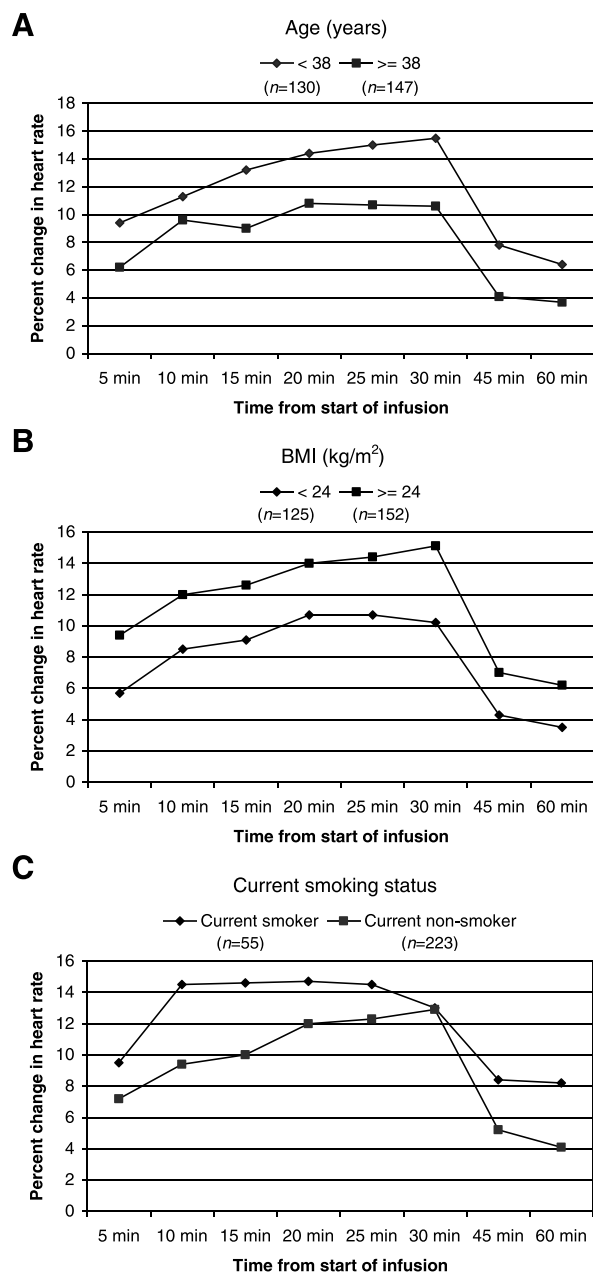


Figure 2. Adjusted percent change in heart rate from baseline (0 min), during (0-30 min), and following (30-60 min) nicotine infusion as a function of (A) age, (B) BMI, (C) and current smoking. All means adjusted for twinning and total nicotine concentration.

Because total nicotine concentration in plasma over the 30 min of infusion represents the combined effects of variance in dose administered as well as rate of clearance, the entire sequence of analysis described above was repeated using the concentration value as a covariate in place of dose and clearance.

Results

Demographic Characteristics. Characterization of the participants who did and did not participate in the present study has been reported elsewhere (22). Generally, application of the

exclusion criteria resulted in a comparatively younger, healthier study sample compared with those who were deemed ineligible. Participants ranged from 18 to 65 years of age, with a mean of 38.8 years (Table 1). The majority of these participants were women (69.8%), MZ in zygosity (79.1%), and Caucasian (76.3%). This is a well-educated sample, with more than half (52.1%) having obtained at least a Bachelor's degree. Overall, 19.8% of the sample was smoking cigarettes at the time of the study, and 24.1% were former smokers.

Heart Rate Response to Infusion. Heart rate increased from an average \pm SD of 64.7 ± 10.1 bpm before infusion to an average \pm SD of 72.7 ± 12.1 bpm at the end of the period of infusion. For the period following the termination of the infusion, from 30 to 60 min, heart rate declined, from an average \pm SD of 72.7 to 67.5 ± 10.6 bpm. At 5 min from the beginning of infusion, heart rate increased to an average of 69.3 ± 10.9 bpm, corresponding to $7.7\% \pm 10.8\%$ increase over baseline. No differences between MZ and DZ twins on raw or percent change in heart rate over the measurement points were observed. Inspection of the curve in Fig. 1 reveals a steady increase in heart rate relative to baseline through the period of infusion to a high of $12.8 \pm 13.1\%$ at 30 min. The average \pm SD slope for this portion of the curve in all participants was 0.20 ± 0.4 (Table 3). Following termination of nicotine infusion, heart rate steadily declined relative to baseline levels but was still $4.9 \pm 12.6\%$ higher than pre-infusion levels at 60 min. The average \pm SD slope during the deceleration phase was -0.25 ± 0.4 . Consistent with the observations based on the adjusted heart rate means, the repeated-measures mixed models revealed time of measurement to be highly significant ($P < 0.0001$).

Covariates in Relation to Overall Heart Rate Response Time Course (0-60 min). Group differences in heart rate response over the full time course were observed for age (<38 versus ≥ 38 years: $F_{1,2050} = 6.60$, $P < 0.02$), BMI (<24.0 versus ≥ 24.0 kg/m²: $F_{1,2049} = 11.39$, $P < 0.01$), and current smoking status (current smoker versus current nonsmoker: $F_{1,2049} = 5.66$, $P < 0.02$). These differences remained significant after adjustment for 30-min plasma nicotine concentration. No significant group differences were evident for gender (male versus female: $F_{1,2049} = 0.02$, $P < 0.90$), the dose of nicotine infused ($\leq 1,062$ versus $> 1,062$ μg : $F_{1,2049} = 3.60$, $P < 0.06$), and ethnicity (White versus non-White: $F_{1,2050} = 0.07$, $P < 0.80$).

Compared with those with a faster rate of clearance, individuals with slower weight-adjusted nicotine clearance (≤ 17.02 versus > 17.02 mL/min/kg) tended to have higher percent heart rate change scores across all time points ($F_{1,2041} = 4.77$, $P < 0.03$). However, individuals with a smaller 30-min total nicotine concentration (< 145 ng/mL \times min) did not exhibit a significant difference from those with a larger nicotine concentration (≥ 145 ng/mL \times min: $F_{1,2049} = 0.05$, $P < 0.83$).

Inspection of Fig. 2A to C reveals that younger individuals exhibited consistently higher heart rate responses than did older participants during and after nicotine infusion. Heavier participants (BMI ≥ 24 kg/m²) had consistently elevated heart rate responses compared with lighter participants. Current smokers showed consistently elevated percent change in heart rate relative to current nonsmokers.

No significant time \times covariate interactions were observed over the full time course.

Heart Rate Response Phenotypes in Relation to Covariates

Five-minute Percent Change. As summarized in Table 2, none of the covariates, including administered nicotine dose, was associated significantly with the percent change in heart rate

Table 1. Characteristics of the full sample and by zygosity

Characteristic	Full sample (<i>n</i> = 278), mean ± SD	MZ twins (<i>n</i> = 220), mean ± SD	DZ twins (<i>n</i> = 58), mean ± SD
Age (y)	38.8 ± 12.1	38.7 ± 12.1	39.0 ± 12.2
BMI (kg/m ²)	25.0 ± 4.0	24.9 ± 4.0	25.4 ± 3.9
Dose infused (μg)	1,183.1 ± 593.9	1,160.2 ± 558.0	1,269.8 ± 712.9
CL _{nic} (mL/min/kg)	17.9 ± 6.2	17.9 ± 6.2	17.7 ± 6.2
Nicotine concentration (ng/ml × min)	161.4 ± 100.4	159.6 ± 102.5	168.0 ± 92.7
Gender (% female)	69.8	68.2	75.9
Ethnicity (% White)	76.3	76.4	75.9
Current smoking (% yes)	19.8	17.3	29.3

after the first 5 min of infusion. There was a marginally significant relationship between age and early heart rate change, with younger participants having a slightly higher mean percent change compared with older participants.

Heart Rate Acceleration (0-30 min) and Deceleration (30-60 min). None of the covariates was significantly associated with the slope describing heart rate acceleration during infusion. Current smoking status was the only covariate significantly associated with the slope describing heart rate deceleration, with current nonsmokers having a more rapid decline than current smokers ($M_{\text{slope}} = -0.30$ versus -0.16).

Relationship between Metabolic Measures and Heart Rate Response Phenotypes. The clearance rate of nicotine as well as total nicotine concentration during the 30 min of infusion were not associated significantly with any of the heart rate response phenotypes examined in this analysis.

Intraclass Correlations by Zygosity for Heart Rate Phenotypes. As a prelude to formal biometric model fitting, intraclass correlations were calculated for MZ and DZ twins on the percent change in heart rate in the first 5 min, on the slopes of the acceleration and deceleration portions of the curve, and on total heart rate response. These are shown in Table 3. Overall, for the three heart rate phenotypes, there was a pattern of significant intraclass correlation among MZ but not among DZ twins suggestive of significant genetic effect.

Estimates of Genetic and Environmental Sources of Variance in the Heart Rate Phenotypes. Maximum likelihood

estimates of the additive genetic and environmental portions of variance in 5-min percent change, and the acceleration (0-30 min) and deceleration (30-60 min) portions of the heart rate response curve, are shown in Table 4. In the total sample, no significant familial effects (either genetic or shared environmental factors) were detected for the 5-min percent change in heart rate. The rate of change in the acceleration portion of the heart rate curve was influenced by modest genetic effects (28.1%), with the remaining variance largely attributable to non-shared environmental sources. Variance in the rate of change in the deceleration portion of the heart rate curve was entirely due to individual-specific environmental factors.

For heart rate acceleration, either A or C, but not both simultaneously, could be equated to zero (i.e., dropped from the model) without significant deterioration of model fit. These statistical results suggest evidence for significant familial effects on heart rate acceleration and inability to resolve the relative contribution of A versus C. However, the point estimate of the shared environmental effects for this phenotype is consistent with the observed pattern of MZ and DZ twin pair correlations shown in Table 3. Similarly, the observed MZ and DZ twin pair correlations for 5-min percent change and heart rate deceleration were consistent with the inference of some familial influences on these phenotypes. This conclusion was not confirmed by the model fitting results in the total sample.

When twin analyses were limited to nonsmokers only (lower half of Table 4), genetic and environmental estimates and model fit criteria were the same as in the total sample for the 5-min heart rate percent change phenotype. For heart rate acceleration, the balance of the relative contribution of genetic

Table 2. Summary table of associations between covariates and heart rate response phenotypes

Covariates	Percent change (0-5 min)			HR acceleration (0-30 min)			HR deceleration (30-60 min)		
	<i>F</i>	<i>df</i>	<i>P</i>	<i>F</i>	<i>df</i>	<i>P</i>	<i>F</i>	<i>df</i>	<i>P</i>
Age (y)*	3.73	1,137	<0.06	1.63	1,137	—	3.09	1,137	<0.09
BMI (kg/m ²) [†]	2.84	1,32	—	0.87	1,33	—	2.14	1,33	—
Gender [‡]	0.51	1,5	—	0.90	1,5	—	1.90	1,5	—
Ethnicity [§]	1.43	1,137	—	0.47	1,137	—	0.15	1,137	—
Current smoking	2.10	1,22	—	1.83	1,22	—	5.46	1,22	<0.03
Dose infused (μg) [¶]	1.94	1,25	—	0.02	1,26	—	1.27	1,26	—
CL _{nic} (mL/min/kg)**	0.04	1,32	—	3.30	1,33	<0.08	0.52	1,33	—
Nicotine concentration ^{††}	0.20	1,41	—	4.03	1,41	<0.06	0.52	1,41	—

NOTE: All *F* statistics were computed to test for the significance of group comparisons after adjustment for twinning. Continuous variables were dichotomized at the median of the distribution.

Abbreviations: HR, heart rate; *df*, degree of freedom.

*<38 vs. ≥38 y.

[†]<24 vs. ≥24 kg/m².

[‡]Males vs. females.

[§]Whites vs. non-Whites.

^{||}Current smokers vs. current nonsmokers.

[¶]≤1,062 vs. >1,062 μg.

**≤17.02 vs. >17.02 mL/min/kg.

^{††}<145 vs. ≥145 ng/mL × min.

Heart Rate Response to Nicotine in Twins

Table 3. Intra-class correlations for percent change in the first 5 min, heart rate acceleration (0-30 min) and deceleration (30-60 min) in MZ and DZ twin pairs following nicotine infusion

HR phenotype	MZ <i>n</i> = 110 pairs			DZ <i>n</i> = 29 pairs		
	Mean	SD	<i>r</i>	Mean	SD	<i>r</i>
Percent change (0-5 min)	7.36	10.88	0.31*	8.95	10.50	-0.14
Acceleration (0-30 min)	0.20	0.43	0.33*	0.18	0.48	0.09
Deceleration (30-60 min)	-0.23	0.45	0.22*	-0.32	0.31	-0.13

NOTE: *, *P* < 0.01.

versus shared environmental effects was shifted toward greater contribution of shared environmental factors (6.0%). However, as before, either the genetic or shared environmental components, but not both simultaneously, could be equated to zero without significant deterioration of model fit, suggesting significant familial effects and no ability to distinguish genetic from shared environmental influences. In contrast to the non-familial influences on variance in the rate of heart rate deceleration in the total sample, in nonsmokers, deceleration variance seemed to be influenced by modest familial effects, but the relative individual contribution of genetic versus shared environmental factors could not be delineated.

The pattern of results remained essentially unchanged (see Table 5) after adjusting for plasma nicotine concentration during the period of infusion instead of nicotine dose and clearance.

Discussion

In the present analysis, change in heart rate over the full time course following infusion was associated with age (negatively), BMI (positively), and current smoking status (negatively; e.g., higher in current smokers than in current nonsmokers). The results for age and BMI are consistent with previously reported results. Nicotine is well known to lead to an increase in heart rate (37, 38). Older age is associated with less heart rate reactivity to nicotine (7), whereas higher levels of BMI are associated with increased levels of cardiovascular reactivity in general (39). To our knowledge, the difference in heart rate time course between current smokers and nonsmokers has not been reported previously, although the present results may be confounded by the fact that smokers received higher doses of nicotine than nonsmokers did. The likelihood of serious

confounding, however, is diminished because (a) all genetic analyses were adjusted for smoking status; (b) neither dose infused nor nicotine concentration in plasma were associated significantly with any of the heart rate response phenotypes studied here; and (c) the estimates of familial influence on the heart rate response phenotypes remained essentially unchanged when examined in nonsmokers only.

The amount of variation in heart rate acceleration to nicotine attributable to genetic sources of variation may be as high as 34.8%, and the magnitude of the estimate of additive genetic variance is consistent with previously published estimates of genetic variance in the heart rate response to physical and mental challenge (e.g., 30-50%; refs. 14-16). Candidate genes of interest include those in the adrenergic (14-16), serotonergic (16, 20), and dopaminergic (21) pathways.

There was no evidence for a significant contribution of familial sources to individual variability in 5-min percent change in heart rate, or heart rate deceleration in the total sample following the termination of the infusion, suggesting the sole contribution of individual-specific environmental factors. However, the twin pair intra-class correlations suggested heritable effects on both these phenotypes. For heart rate deceleration, a point estimate for the genetic variable of up to 31.0% was detected in nonsmokers. However, the large confidence intervals likely due to the small sample of DZ twins precluded delineation of the relative contribution of the genetic versus shared environmental effects.

The present study was an important first step in determining whether the heart rate response to nicotine could be useful as an endophenotype in studies of the genetics of nicotine dependence. However, before heart rate response characteristics can be viewed as credible candidate endophenotypes, they will need to satisfy additional criteria, including (a) association with dependence in the population of smokers, (b)

Table 4. Proportion of phenotypic variance attributable to additive genetic (A), shared environmental (C), and non-shared environmental influences (E) for the best fitting models for three HR phenotypes during or following 30-min infusion of nicotine

HR phenotype	Source of variation		
	A (95% CI)	C (95% CI)	E (95% CI)
All participants (<i>n</i> _{pairs} , MZ = 110, DZ = 29)			
Percent change (0-5 min)	—	—	100
Acceleration (0-30 min)*	28.1 (0, 44.8)	0 (0, 0)	71.9 (55.2, 90.8)
Deceleration (30-60 min)	—	—	100
Nonsmokers only (<i>n</i> _{pairs} , MZ = 88, DZ = 17)			
Percent change (0-5 min)	—	—	100
Acceleration (0-30 min)*	25.8 (0, 50.7)	6.0 (0, 44.5)	68.2 (49.3, 90.4)
Deceleration (30-60 min)*	22.1 (0, 48.7)	9.2 (0, 47.4)	68.7 (51.3, 88.6)

NOTE: Results are shown for analyses adjusting for age, BMI, current smoking, ethnicity, *CL*_{nic}, and nicotine dose in the total sample as well as analyses limited to nonsmokers, adjusting for the remaining covariates.

Abbreviation: 95% CI, 95% confidence interval.

*Either A or C could be dropped from the model without significant worsening of model fit; dropping both A and C, however, contributed to significant worsening of model fit (*P* < 0.05).

Table 5. Proportion of phenotypic variance attributable to additive genetic (A), shared environmental (C), and non-shared environmental influences (E) for the best fitting models for three HR phenotypes during or following 30-min infusion of nicotine

HR phenotype	Source of variation		
	A (95% CI)	C (95% CI)	E (95% CI)
All participants (n_{pairs} , MZ = 110, DZ = 29)			
Percent change (0-5 min)	—	—	100
Acceleration (0-30 min)*	30.3 (0, 46.3)	0 (0, 0)	69.7 (53.7, 87.8)
Deceleration (30-60 min)	—	—	100
Nonsmokers only (n_{pairs} , MZ = 88, DZ = 17)			
Percent change (0-5 min)	—	—	100
Acceleration (0-30 min)*	34.8 (0, 52.4)	0 (0, 0)	65.2 (47.8, 86.0)
Deceleration (30-60 min)*	31.0 (0, 48.1)	0 (0, 46.2)	69.0 (51.9, 88.4)

NOTE: Results are shown for analyses adjusting for age, BMI, current smoking, ethnicity, total nicotine concentration during infusion in the total sample as well as analyses limited to nonsmokers, adjusting for the remaining covariates.

*Either A or C could be dropped from the model without significant worsening of model fit; dropping both A and C, however, contributed to significant worsening of model fit ($P < 0.05$).

presence before the onset of dependence, (c) presence in other family members who have not yet exhibited signs of dependence at a higher rate than in the general population, and (d) cosegregation with dependence in families (13). Previously, we have shown that cardiovascular reactivity in smokers who then proceeded to quit is a predictor of subsequent relapse (40), thereby indicating potential clinical relevance.

This study has several limitations. First, as noted previously, dose selection based on smoking status could have resulted in confounding of the heritability analyses. However, our approach to adjustment for covariates, including smoking status, dose of nicotine, and plasma nicotine concentration along with the reanalysis of data in nonsmoking twins, leads us to the conclusion that this is unlikely. Second, the small number of smokers and low overall levels of dependence put serious constraints on the extent to which dependence could be incorporated into the biometric genetic models. The relatively small size of the twin sample and the unbalanced ratio of MZ to DZ twins also put constraints on the extent to which analyses could be conducted with covariate strata. Third, the lack of a sufficient number of mixed-sex DZ twins also prohibits the exploration of sex moderation of the observed genetic influences. Finally, the controlled infusion protocol used here may not have strong ecological validity when generalizing to the real-world pharmacokinetic and pharmacodynamic effects of exposure to nicotine in cigarettes. It would be interesting to conduct a study of smoking topography, nicotine levels and subsequent clearance, and both objective and subjective reactions in twins to determine genetic influences in a more naturalistic context.

This was not a study of tolerance or the rate of acquisition of tolerance in smoking and nonsmoking twins. Pharmacodynamic determinants of the heart rate response to nicotine may include intrinsic response to receptor binding in the brain, general cardiovascular reactivity, and the development of tolerance. Tolerance develops rapidly to effects of nicotine on heart rate, although tolerance is incomplete. Thus, heart rate remains elevated all day in smokers compared with when not smoking, and the degree of heart rate elevation is independent of the blood concentration achieved (41). An entirely different methodology from the one employed here would be required to conduct a study of tolerance in twins (35).

Further biometric modeling of the genetic relationship between nicotine clearance and heart rate sensitivity will determine the relative contribution of common and specific genetic influences to the phenotypic relationship between the

pharmacokinetic and pharmacodynamic effect of nicotine. The inclusion of measured genotypes for genes responsible for both nicotine metabolism and heart rate sensitivity would support further investigation of the extent to which genes in these pathways operate independently of each other or in combination to influence subsequent reactions to nicotine. The focus on measured physiologic characteristics associated with exposure to nicotine (in contrast to self-report of arousal) presumes that the underlying genetic determinants of heart rate response are more specific and, therefore, more readily identifiable (13). Should it turn out to be the case that the measured heart rate response to nicotine meets the necessary and sufficient conditions to become an endophenotype of nicotine dependence, progress would be made toward improving the sensitivity and specificity of the measurement and prediction of nicotine dependence.

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