Behavior Genetics, Vol. 35, No. 4, July 2005 (© 2005)
DOI: 10.1007/s10519-004-1327-8

Heritability of Smoking Initiation and Nicotine Dependence

Jacqueline M. Vink,[1,2] Gonneke Willemsen,[1] and Dorret I. Boomsma[1]

Received 1 May 2004—Final 10 Nov. 2004

In contrast to other aspects of smoking behavior, little attention has been paid to the genetics of nicotine dependence. In this paper, three models (single liability dimension, independent liability dimension and combined model) have been applied to data on smoking initiation and nicotine dependence (n = 1572 Dutch twin pairs, mean age 30.5). A combined model best described the data. This model postulates a smoking initiation dimension and a nicotine dependence dimension, which are not independent. For both males and females, individual differences in smoking initiation were explained by genetic (44%), shared environmental (51%) and unique environmental (5%) influences. The nicotine dependence dimension was influenced only by genetic (75%) and unique environmental (25%) factors. The substantial impact of genetic factors on nicotine dependence emphasizes the need for further research to localize and identify specific genes and pathways involved in nicotine dependence.

KEY WORDS: Fagerström test for nicotine dependence (FTND); genetics; nicotine dependence; smoking initiation; twins; two-stage model.

INTRODUCTION

Several, possibly associated, dimensions of smoking behavior may be distinguished, e.g., smoking initiation, number of cigarettes smoked per day and nicotine dependence (Mayhew *et al.*, 2000) and each dimension potentially is characterized by a distinct genetic architecture. Li *et al.* (2003a) selected six behavior genetic studies of smoking initiation in adults for a meta-analysis. Parameter estimates for heritability (h^2), shared environmental influences (c^2) and unique environmental influences (e^2) were 0.37, 0.49 and 0.17 for male adults and 0.55, 0.24 and 0.16 for female adults, respectively. Sullivan and Kendler (1999) reported somewhat higher heritability estimates based on a review of 10 published papers. They calculated the weighted means of 10 studies of smoking initiation for h^2, c^2 and e^2 to be 0.56, 0.24 and 0.20.

Li *et al.* (2003a) also performed a meta-analysis for smoking persistence (including studies of persistence, quantity, dependence and regular use) and found that the parameters h^2, c^2 and e^2 for smoking persistence were 0.59, 0.08 and 0.37 for male adults, and 0.46, 0.28 and 0.24 for female adults. In the study of Sullivan and Kendler (1999), the weighted mean heritability for proxy measures of nicotine dependence was 0.67, the weighted mean shared environmental influence was 0.02 and the weighted mean individual-specific influences was 0.31. Most studies included in the meta-analyses used proxy measures of nicotine dependence, e.g., quantity, and employed a two-stage model for smoking initiation and persistence. A few studies did not use a two-stage model but excluded the non-smokers. Proxy measures for nicotine dependence can only be assessed in individuals who have initiated smoking. Not every person who initiates smoking becomes nicotine-dependent, and not every individual who never initiated smoking can be assumed to score zero on the dependence dimension. For genetic research,

[1] Department of Biological Psychology, Vrije Universiteit, Amsterdam, The Netherlands.
[2] To whom correspondence should be addressed at Department of Biological Psychology, Vrije Universiteit, Van de Boechorststraat 1, 1081 BT Amsterdam, The Netherlands. Tel.: ++31-20-444-8787. Fax: +31-20-4448832. E-mail: jm.vink@psy.vu.nl.

it is important to investigate whether smoking initiation and nicotine dependence are part of the same continuum or whether they represent two independent dimensions.

As far as the authors know, only one study has addressed the heritability of nicotine dependence using the Fagerström score in a two-stage model (Kendler *et al.*, 1999; this study was also included in the meta-analyses described above). Kendler *et al.* (1999) investigated the relationship between smoking initiation and nicotine dependence by using a model that estimates a path between the liability to smoking initiation and the liability to nicotine dependence, given smoking initiation. While the majority of genetic risk factors for nicotine dependence were shared with smoking initiation, a distinct set of familial factors solely influenced the risk for nicotine dependence. Genetic factors contributed to a total of 72% of the variance in liability to nicotine dependence and the remaining variance was explained by unique environmental factors. This study was performed in women only, and although the sex differences in the genetic architecture of smoking seem small (Madden *et al.*, 1999; Vink *et al.*, 2003), it is unknown if the results generalize to men.

In studies of the heritability of smoking initiation and quantity smoked in adolescent twins of the Netherlands Twin Register, a comparable but different approach to analyze these kinds of data was used (Koopmans *et al.*, 1999; Vink *et al.*, 2004a). Three different models for the relationship between the genetic and environmental causes of initiation and quantity were evaluated. The single liability model assumes that the same genetic and environmental risk factors influence initiation and quantity, while the independent liability model assumes independent initiation and quantity dimensions each determined by separate genetic and environmental risk factors. The combined model includes assumptions of both single and independent liability models. It postulates separate initiation and quantity dimensions, but there are two routes to being a non-smoker: an individual can be a non-smoker due to genetic and/or environmental factors that influence smoking initiation or because the individual is low on nicotine dependence. For smoking initiation and quantity smoked, the combined model was the best fitting model. Koopmans *et al.*, (1999) reported that 39% of the total variance in smoking initiation was explained by genetic influences and 86% of the total variance in quantity (number of cigarettes per day) was explained by genetic factors.

In the present paper, the three different models described by Koopmans *et al.*, (1999) are applied to data on smoking initiation and nicotine dependence as assessed by the Fagerström test for nicotine dependence (FTND) in a Dutch twin sample aged 30.5 years (SD = 11.9). After identification of the correct liability model, the relative contribution of genetic and environmental factors to initiation and nicotine dependence is estimated.

METHODS

Measures

This study is part of an ongoing twin/family study on health-related behavior of the Netherlands Twin Register (NTR) that assesses families with adolescent and young adult twins every two to three years since 1991 (Boomsma *et al.*, 2002).

Smoking initiation

Data on smoking behavior were collected longitudinally (1991, 1993, 1995, 1997, 2000) and 61% of the twins participated more than once. Subjects were classified as non-smokers when they reported they never smoked or when they tried smoking a few times. The answers to all surveys were taken into account so that an individual who reported to have smoked regularly in one of the surveys, was classified as ever smoker. If answers of different surveys were contradictory, the variable was set to missing. The group of non-smokers includes the never smokers and the individuals who tried smoking a few times but never reported regular smoking. In the 2000 survey, only the subjects who classified themselves as smokers and ex-smokers proceeded to complete the FTND. Subjects who classified themselves as non-smokers (including subjects who reported they tried smoking a few times) did not fill out the FTND.

We examined the longitudinal data of subjects who reported they tried smoking a few times in one of the surveys (n = 3317 subjects). For 2023 of the 3317 subjects, data were available for the next survey; 23% reported to be a smoker two years later and were therefore classified as smokers. The rest of the subjects reported again to have tried smoking a few times (41%) or reported to be non-smokers (31%) 2 years later; both groups were classified as non-smokers.

Nicotine dependence

To measure the degree of nicotine dependence, the FTND was used (Heatherton *et al*., 1991). The FTND consists of 6 items and produces a score ranging from 0 to 10 with higher scores indicating more nicotine dependence. The FTND includes items like "How soon after you wake up do you smoke your first cigarette?" and "Do you find it difficult to refrain from smoking in places where it is forbidden?". For both smokers and ex-smokers, internal consistency of the FTND is reasonably high with Cronbach's alpha ranging from 0.65 to 0.71 and test–retest correlations (Pearson–Lawley correction) range from 0.70 to 0.91 (Vink *et al*., 2005). Smokers completed the FTND on their current situation while ex-smokers completed the FTND on the period they smoked the heaviest. FTND data were collected in the 2000 survey, in a telephone interview in 2001, and in a study of the genetics of nicotine dependence. If subjects participated more than once, the highest FTND score was used for the analysis.

Subjects

The surveys of 1991, 1993, 1995, 1997 and 2000 contained items on health, personality and lifestyle (e.g. smoking behavior). For this paper, the data from the 2000 survey (which included the FTND for the first time) were used. Completed questionnaires were returned by 4610 twins/triplets. In 2001, additional data on smoking and FTND were obtained by telephone interviews (n = 56 twins/triplets). FTND-data collected with questionnaires completed by participants in a study of the genetics of nicotine dependence were also included (n = 426 twins/triplets). In total, 4672 twins participated at least once. Smoking data were missing for 24 persons, and for 208 additional persons known to have initiated smoking (from other surveys), FTND data were not available. The remaining 4440 persons were classified as non-smokers, low-dependent (highest FTND score 0–2), moderately dependent (highest FTND score 3–5) and highly dependent (highest FTND score $\geq$ 6). Data were included only when smoking data (smokers or ex-smokers) of both twins were available, so analyses were performed using the smoking data of 1572 twin pairs.

Zygosity was based on questionnaire data, or when available, on DNA typing. For 29.8% of the same-sex twin pairs, information on their zygosity was available based on DNA polymorphisms. Agreement between zygosity based on questionnaire data and zygosity based on DNA results was 96%. The sample consisted of 238 monozygotic male (MZM) twin pairs, 125 dizygotic male (DZM) twin pairs, 630 monozygotic female (MZF) twin pairs, 288 dizygotic female (DZF) twin pairs and 291 dizygotic opposite sex (DOS) twin pairs who had complete data on smoking and zygosity. The mean age was 30.5 years (SD 11.9).

Genetic Analysis

To investigate the inheritance of smoking behavior, both smoking initiation and nicotine dependence were considered to have an underlying, continuous liability. The variation of the liability is both genetic and environmental in origin (Falconer and Mackay, 1996). Thresholds divide this normal liability distribution into discrete categories. We considered three models for the relationship between smoking initiation and nicotine dependence (Fig. 1).

Single Liability Dimension Model (SLD)

The single liability dimension (SLD) model postulates that the liability to smoking behavior is unidimensional and is normally distributed. Under this model, the same genetic and environmental factors predispose to smoking initiation and to nicotine dependence. The liability distribution is divided by thresholds into discrete categories which correspond to the observed categories. The probability that an individual falls in one of the four categories is given by y_1, y_2, y_3 and y_4 and can be calculated by integrating a standardized normal distribution between the corresponding threshold values. The model predicts that the co-twins of nicotine-dependent participants are more likely to be nicotine-dependent than the co-twins of non-smokers.

Independent Liability Dimension (ILD) Model

The independent liability dimension model assumes two independent liability dimensions for smoking initiation and nicotine dependence. The initiation dimension determines the probability that an individual initiates smoking (y_1) or never starts smoking (y_2). Individuals who fall below the threshold are predicted to be smokers. The nicotine dependence dimension determines whether an individual becomes highly dependent (x_1), medium to low-dependent (x_2) or very low-dependent (x_3). Taking smoking initiation into account, the

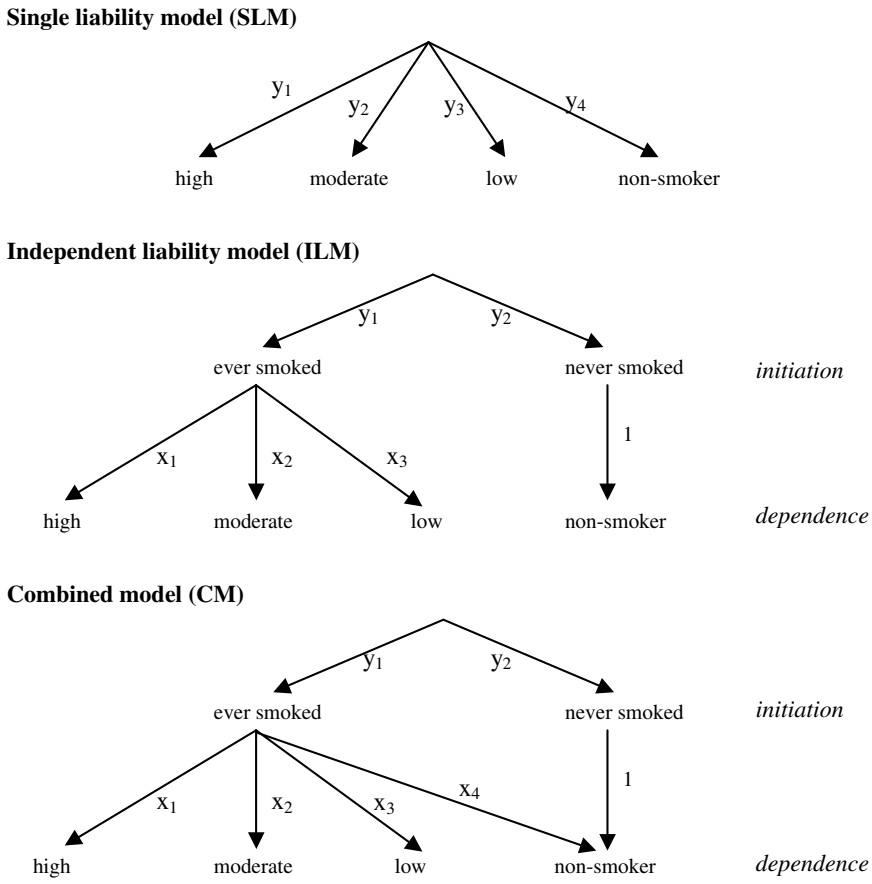

Fig. 1. Schematic representation of the single liability model, the independent liability model and the combined model for smoking initiation and nicotine dependence; $x_.$ and y are the probabilities that an individual falls in one of the categories.

probabilities that an individual becomes highly dependent, moderately dependent or low-dependent are y_1x_1, y_1x_2 and y_1x_3, respectively. The probability that an individual remains a non-smoker is y_2. The ILD model predicts that the co-twin of a twin who is non-smoker is more likely to abstain from smoking. Also, if the co-twin initiated smoking while the twin is a non-smoker, the co-twin will not, on average, be less nicotine-dependent than the co-twin of a nicotine-dependent twin.

Combined Model (CM)

The combined model includes features of both the SLD and the ILD models. Like the ILD, it postulates the existence of separate initiation and dependence dimensions, but there are two different routes to being a non-smoker: an individual can be a non-smoker due to genetic and/or environmental factors that influence the smoking initiation dimension, or because the individual is low on the nicotine dependence dimension. Thus, under this model the co-twin of a twin who initiated smoking is more likely to become a non-smoker than under the ILD model.

Model Fitting

Smoking behavior in the first twin was cross-classified with smoking behavior in the second twin, resulting in 4×4 contingency tables for each zygosity group; monozygotic males (MZM), dizygotic males (DZM), monozygotic females (MZF), dizygotic females (DZF) and dizygotic opposite sex twins (DOS), (see also Table II). The three models were fitted to the five contingency tables by methods of maximum likelihood with the structural equation modeling package Mx (Neale *et al.*, 1999). The thresholds were allowed to be different for males and females.

Table I. Predicted Probabilities for a Twin Pair under the Single Liability Dimension (SLD), the Independent Liability Dimension (ILD) and the Combined Model (CM)

		Twin 2 →			
Twin 1↓	Model	FTND ≥ 6	FTND 3–5	FTND 0–2	Non-smoker
FTND ≥ 6	SLD	y_{11}	y_{12}	y_{13}	y_{14}
	ILD	$y_{11}x_{11}$	$y_{11}x_{12}$	$y_{11}x_{13}$	$y_{12}x_{1.}$
	CM	$y_{11}x_{11}$	$y_{11}x_{12}$	$y_{11}x_{13}$	$y_{12}x_{1.} + y_{12}x_{14}$
FTND 3–5	SLD	y_{21}	y_{22}	y_{23}	y_{24}
	ILD	$y_{11}x_{21}$	$y_{11}x_{22}$	$y_{11}x_{23}$	$y_{12}x_{2.}$
	CM	$y_{11}x_{21}$	$y_{11}x_{22}$	$y_{11}x_{23}$	$y_{12}x_{2.} + y_{11}x_{24}$
FTND 0–2	SLD	y_{31}	y_{32}	y_{33}	y_{34}
	ILD	$y_{11}x_{31}$	$y_{11}x_{32}$	$y_{11}x_{33}$	$y_{12}x_{3.}$
	CM	$y_{11}x_{31}$	$y_{11}x_{32}$	$y_{11}x_{33}$	$y_{12}x_{3.} + y_{11}x_{34}$
Non-smoker	SLD	y_{41}	y_{42}	y_{43}	y_{44}
	ILD	$y_{21}x_{.1}$	$y_{21}x_{.2}$	$y_{21}x_{.3}$	y_{22}
	CM	$y_{11}x_{41}$	$y_{11}x_{42}$	$y_{11}x_{43}$	$y_{22} + y_{11}x_{44} + y_{21}x_{.4} + y_{12}x_{4.}$

Under the SLD model, y_{jk} = the probability that a twin pair falls in the j,k-th category of smoking behavior. For example, y_{11} is the probability that both twin 1 and twin 2 fall in the first category (FTND ≥ 6). Under the CM and ILD model, y_{jk} = the probability that a twin pair falls in the j,k-th category of the initiation dimension; x_{jk} = the probability that a twin pair falls in the j,k-th category of the nicotine dependence dimension; x_j = the probability that the first twin falls in the j-th category of the nicotine dependence dimension; $x_{.k}$ = the probability that the second twin falls in the k-th category of the nicotine dependence dimension.

Under the SLD model one twin correlation for each zygosity group (5) and three thresholds for males and three thresholds for females were estimated, giving in total 11 parameters to be estimated. Under the ILD model, separate twin correlations for the initiation and dependence dimensions were estimated for each zygosity group. For the initiation dimension one threshold for males and one for females was estimated. There was no "non-smoker" category for the dependence dimension, leaving two thresholds for males and two for females to be estimated. This means that 16 parameters were estimated under the ILD model. Using the CM model, the same parameters were estimated as in the ILD model, except for the thresholds: in the dependence dimension, three thresholds for males and three for females were estimated because non-smokers were also included in the dependence dimension. So for the CM model, 18 parameters were estimated.

The predicted probabilities for a twin pair under the three models are presented in Table I. Under the SLD model, y_{11} denotes the probability that both twins are highly nicotine-dependent, y_{12} denotes the probability that the first twin is highly dependent and the second twin is moderately dependent on nicotine, and so on. Under the CM and ILD model, y_{11} denotes the probability that twins both initiated smoking, y_{22} denotes the probability that both twins did not initiate smoking, and y_{12} and y_{21} denote the probabilities that twins are discordant for smoking initiation. The conditional probabilities that both twins are highly dependent on nicotine, the first twin is highly dependent and the second twin is moderately dependent is represented by x_{11}, x_{12} etc. Under the CM model, there are two routes to "non-smoker". For example, $y_{11}x_{24}$ gives the probability that both twins are smokers on the initiation dimension (y_{11}) and the first twin is moderately dependent on nicotine, while the second twin is a non-smoker on the dependence dimension, $y_{12}x_{1.}$ gives the probability that the first twin is a smoker on the initiation dimension while the second twin is a non-smoker on the initiation dimension (y_{12}), and the first twin is highly dependent on nicotine ($x_{1.}$).

The three models were fitted to the data, estimating separate polychoric correlations for each zygosity group. The goodness-of-fit of nested models was assessed with the likelihood-ratio statistic, this statistic which is distributed as chi-square.

Genetic Models

The three models were fitted to the data, and for the model that gave the best description of the data, the twin correlations in liability were expressed as a function of genetic and environmental factors (Neale

Table II. Twin Concordances for Nicotine Dependence for Each Zygosity Group

	Twin 2 →									
	Males					Females				
Twin 1↓	FTND ≥ 6	FTND 3–5	FTND 0–2	Non -smoker	%	FTND ≥ 6	FTND 3–5	FTND 0–2	Non-smoker	%
MZ										
FTND ≥ 6	1	8	2	2	5.5	14	9	5	3	4.9
FTND 3–5	3	14	10	4	13.0	10	35	19	9	11.6
FTND 0–2	2	6	33	9	21.0	2	18	81	40	22.4
Non-smoker	0	3	13	128	60.5	2	9	39	335	61.1
%	2.5	13.0	24.4	60.1	n = 238	4.4	11.3	22.9	61.4	n = 630
DZ										
FTND ≥ 6	2	3	1	3	7.2	7	3	5	3	6.2
FTND 3–5	2	5	4	4	12.0	2	10	17	14	14.9
FTND 0–2	3	3	17	8	24.8	4	6	28	19	19.8
Non-smoker	0	4	14	52	56.0	4	12	31	123	59.0
%	5.6	12.0	28.8	53.6	n = 125	5.9	10.8	28.1	55.2	n = 288
DOS, Males										
FTND ≥ 6						2	3	8	6	6.5
FTND 3–5						3	13	19	16	17.5
FTND 0–2						1	11	21	33	22.7
Non-smoker						8	6	23	118	53.3
%						4.8	11.3	24.4	59.5	n = 291

FTND = score on Fagerström test for nicotine dependence (both for smokers and ex-smokers); MZ = monozygotic; DZ = dizygotic (same-sex); DOS = dizygotic opposite sex.

and Cardon, 1992). For both the initiation and the nicotine dependence dimensions, three factors were considered, i.e. additive genetic (A), shared environmental (C) and unique environmental factors (E). Under the full model, both additive genetic and shared environmental factors contribute to resemblances between twins. Sex-differences were tested by allowing the magnitude of the genetic and environmental effects to be different for males and females and by allowing the correlation between the genetic factors in opposite-sex twins to be less than unity. For all models, different thresholds were estimated for males and females, allowing for differences in the prevalence of smoking between males and females.

RESULTS

Table II shows concordance rates and the proportions of non-smokers, low-dependent, moderate-dependent and high-dependent individuals for the first and second twin in each zygosity group. Concordance is higher in MZ twins than in DZ twins.

The three different models (SLD, ILD and CM) were fitted to the data. Table III shows the goodness-of-fit for each liability model. The ILD fitted the data somewhat better than the SLD model, but the combined model gave the best description of the data. Therefore, the combined model was used when further investigating the genetic and environmental influences on nicotine dependence.

Under the combined model, an individual can be a non-smoker due to genetic and/or environmental factors that influence the smoking initiation dimension, or because the individual is low on the nicotine dependence dimension. The predicted marginal probabilities for smoking under the full combined model are represented in Fig. 2. The full model allows for sex-differences. Under the full model, the

Table III. Goodness-of-Fit of the Single Liability Dimension (SLD), the Independent Liability Dimension (ILD) and the Combined Model (CM) to the Data on Smoking

	df	χ^2	p	AIC
SLD	64	132.40	< 0.001	4.40
ILD	59	87.87	0.009	−30.13
CM	57	61.90	0.306	−52.10

df = degrees of freedom; AIC = χ^2−2df, this is a measure of the parsimony of the model, a lower AIC indicates a more parsimonious model.

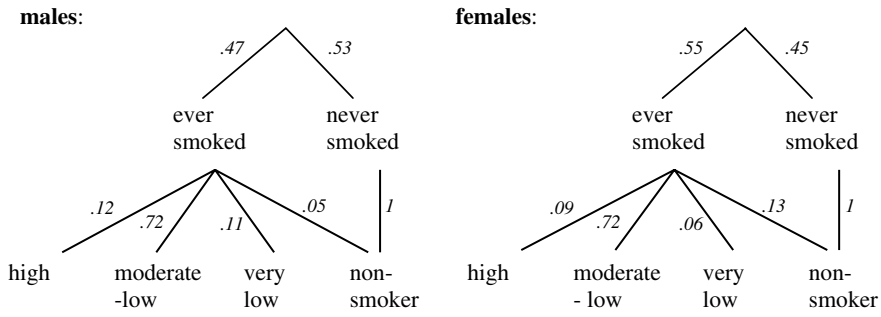

Fig. 2. Estimated probabilities under the full combined model in males and females.

Table IV. Polychoric Twin Correlations with 95% Confidence Intervals for the Initiation and Dependence Dimensions under the Full Combined Model

	Initiation		Nicotine dependence	
	R	95% CI	*R*	95% CI
MZM	0.96	0.83–1.00	0.61	0.23–0.83
DZM	0.75	0.40–0.98	0.50	0.23–0.80
MZF	0.94	0.81–0.99	0.80	0.67–0.89
DZF	0.75	0.26–1.00	0.48	0.06–0.77
DOS	0.70	0.38–1.00	0.32	−0.16–0.67

probability of being highly dependent is 6% for males (0.12 * 0.47) and 5% for females (0.09 * 0.55). The probability of being moderately dependent is 34% for males (0.72 * 0.47) and 40% for women (0.72 * 0.55). The probability of being low-dependent is 5% for males (0.11 * 0.47) and 3% for females (0.13 * 0.55). The probability of being a non-smoker on the dependence dimension while smoking is initiated is 2.3% in males (0.05 * 0.47) and 7.1% in females (0.13 * 0.55). The probability that smoking is not initiated is 53% for males and 45% for females.

Table IV shows the polychoric correlations for each zygosity group for the initiation and dependence dimension under the full combined model. For smoking initiation, the correlations between MZ twins were somewhat higher than the correlations between DZ twins suggesting both genetic and shared environmental influences on smoking initiation. For the dependence dimension, the difference between the correlations in MZ pairs and the correlations in DZ pairs is somewhat larger for females than for males, suggesting that genetic factors may be more important for females.

Table V. Model Fitting Results for a Combined Model with Smoking Initiation and Nicotine Dependence (best fitting model is given in boldface)

	Initiation	Nicotine dependence	χ^2	df	*p*	AIC
1.	full	full	61.89	57	0.306	−52.11
2.	ACE	full	61.90	60	0.408	−58.10
3.	AE	full	77.22	61	0.079	−44.78
4.	CE	full	72.29	61	0.153	−49.71
5.	full	ACE	66.62	60	0.290	−53.38
6.	full	AE	65.76	61	0.284	−54.24
7.	full	CE	82.71	61	0.034	−37.29
8.	**ACE**	**AE**	**67.84**	**64**	**0.348**	**−60.16**

Full = full model with sex-dependent effects; ACE = full model without sex differences; AE = additive genetic model; CE = shared environmental model; AIC = χ^2−2df, this is a measure of the parsimony of the model, a lower value of AIC indicates a more parsimonious model.

A series of genetic and familial models were fitted to the initiation dimension and to the nicotine dependence dimension under the combined model. The full ACE model permitted sex-differences by allowing the magnitude of the genetic and environmental effects to differ for males and females and by allowing the correlation between the genetic factors in opposite-sex twins to be less than one. The results are shown in Table V. Constraining A, C and E in the full model to be equal for both sexes in the initiation dimension did not alter the fit of the model (Model 2). Removing additive genetic factors or shared environmental factors from the initiation dimension gave a significant reduction in the goodness of fit (Models 3 and 4, respectively). The initiation dimension was best described by an ACE model without sex-differences in the variance components (Model 2). For the nicotine dependence dimension, the full model could be reduced to an AE model without sex-differences (Model 6). Overall, the best fitting model was an ACE model without sex-differences for the initiation dimension and an AE model without sex-differences for the nicotine dependence dimension (Model 8). Individual differences in smoking initiation were explained by genetic influences (0.44), by shared environmental factors (0.51) and by unique environmental factors (0.05). The nicotine dependence dimension was largely influenced by genetic factors (0.75) and the remaining variance was explained by unique environmental factors (0.25).

DISCUSSION

In this study, we investigated the relation of smoking initiation and nicotine dependence and estimated the heritabilities of these traits. The relationship between smoking initiation and nicotine dependence was explored through three different threshold models. The single liability model (SLM) was rejected indicating there is not one underlying continuum of liability to smoking initiation and dependence. The independent liability model (ILM) also fitted the data poorly indicating that the smoking initiation dimension and the nicotine dependence dimension are not independent. The combined model (CM) was the best fitting model. Under this model, there are two routes to non-smoking: an individual can be a non-smoker due to genetic and/or environmental factors that influence the initiation dimension or because that individual is low on the nicotine dependence dimension. Under the full combined model, only a small proportion of the male twins were non-smokers due to the genetic and environmental risk factors which influence the initiation dimension; for the female twins this proportion was somewhat higher. Sex-differences were also tested in the genetic models which were fitted both to the initiation dimension and to the nicotine dimension and proved to be non-significant. The sources of variation that were investigated were additive genetic variation (A), shared environmental influences (C) and a unique environmental influence (E). Variation in the initiation dimension was best described by an ACE model, while variation in the nicotine dependence dimension could be described with an AE model.

For smoking initiation, 44% of the variation could be explained by genetic factors, 51% by shared environmental factors, and 5% by unique environmental factors in both males and females. Those heritability estimates are in line with the results of the meta-analysis of Li *et al.* (2003a) and the review paper of Sullivan and Kendler (1999). For nicotine dependence, 75% of the variation was explained by genetic factors. The remaining variance was explained by unique environmental factors. Those findings closely resemble those of Kendler *et al.* (1999) who reported, in a sample of female twins, that genetic factors contributed to a total of 72% of the variance in liability to nicotine dependence. Other studies have estimated the heritability of nicotine dependence but did not use a two-stage model. True *et al.* (1999) fitted a bivariate model to data on nicotine dependence and alcohol dependence. Under this model, the heritability for nicotine dependence was 60% (True *et al.*, 1999). McGue *et al.*, (2000) used a univariate threshold model and reported a heritability of 44% (95% CI: 3–87%) and a shared environmental influence of 37% (95% CI: 0–71%) for nicotine dependence. Both studies included the never-smokers in the analysis. We explored different threshold models (single liability, independent liability and combined model) because it has been hypothesized that an incorrect definition of the phenotype could possibly lead to biased estimates of the genetic and environmental factors (Heath and Martin, 1993; Heath *et al.*, 2002). If the same genetic and environmental factors determine whether or not a person initiates smoking and how dependent a person becomes, then exclusion of non-smokers could lead to truncation of the distribution and as a consequence to biased estimates of the heritability. On the other hand, if the determinants of smoking initiation are independent of the determinants of nicotine depen-

dence, then inclusion of non-smokers in the analyses of dependence may confound two traits with different modes of inheritance (Heath and Martin, 1993; Heath *et al*., 2002). In the present study, the CM model provided the best fit to the data. For the CM model, the estimates for the heritability of nicotine dependence was 75% (when using an AE model without sex-differences). When this AE model without sex-differences was fitted using the alternative models, the heritability estimate was 71% for the ILM (non-smokers are not included when analyzing nicotine dependence), and 80% for the SLM (non-smokers score zero on nicotine dependence). Thus, the heritability estimates did not differ much between the three models.

Several measures, like smoking persistence or quantity, have been used used as proxies for nicotine dependence. In a larger, partly overlapping sample, we have found for the maximum number of cigarettes per day that the parameters h^2, c^2 and e^2 were 51%, 30% and 18%, respectively (Vink *et al*., 2004a) which is in line with the results of the meta-analyses for smoking persistence of Li *et al*. (2003a). It seems that heritability estimates for maximum number of cigarettes are slightly lower than heritability estimates for nicotine dependence, which can be regarded as the more "extreme" phenotype.

A limitation of the present study is that the data from incomplete twin pairs were excluded from the analyses. It is possible that selection bias plays a role; those individuals who are most nicotine-dependent may have refused to participate. We compared smoking behavior in complete and incomplete twins and found a somewhat higher percentage current smokers and ever smokers in the incomplete twins compared to the complete twin pairs, but these differences were small and not significant (Vink *et al*., 2004b). It is therefore unlikely that the exclusion of incomplete twin pairs significantly influenced the results.

When defining smoking initiation, the group of non-smokers included not only the subjects who reported they never smoked but also the subjects (14.5% of the total sample) who reported they tried smoking a few times. To explore the influence of this group on the results, we performed the analyses for the CM without the subjects who reported they tried smoking a few times. We again found that the best fitting model was an ACE model without sex-differences for smoking initiation and an AE model without sex-differences for nicotine dependence. The estimates for the best fitting model were only slightly different; for smoking initiation the heritability was 54%, the c^2 was 44% and e^2 was 2%. For nicotine dependence the heritability was 70% and e^2 was 30%. The 95% confidence intervals showed that the estimates were not significantly different from the estimates of the analyses where persons who tried smoking were included in the non-smoking group.

The existence of two separate dimensions for smoking initiation and nicotine dependence which are not independent of each other is supported by our recent linkage study in another, partly overlapping, sample. On chromosomes 6 and 14, LOD-scores of 3.0 and 1.7, respectively were found for smoking initiation. We obtained a LOD-score of 2.0 on chromosome 3 for quantity (number of cigarettes per day). Interestingly, an overlapping peak on chromosome 10 was found for both smoking initiation (LOD-score 1.9) and quantity (LOD-score 2.3; Vink *et al*., 2004a). The linkage analyses for initiation and quantity were performed separately and not in a two-stage model. The variable for quantity was considered as a continuous variable and the never-smokers were excluded. Several other genome scans have reported regions that could be involved in different dimensions of smoking behavior (varying from smoking initiation to nicotine dependence) (Bergen *et al*., 1999; Bierut *et al*., 2004; Duggirala *et al*., 1999; Li *et al*., 2003b; Saccone *et al*., 2003; Straub *et al*., 1999; Sullivan *et al*., 2004). Further research is needed to localize and identify the specific genes involved in both the smoking initiation dimension and the nicotine dependence dimension.

ACKNOWLEDGMENTS

This study was funded by Grants 985-10-002 (NWO) and 3100-0038-16333 (NIDA/ZONMW).

REFERENCES

Bergen, A. W., Korczak, J. F., Wieissbecker, K. A., and Goldstein, A. M. (1999). A genome-wide search for loci contributing to smoking and alcoholism. *Genet. Epidemiol.* **17**(S1): S55–S60.

Bierut, L. J., Rice, J. P., Goate, A., Hinrichs, A. L., Saccone, N. L., Foroud, T., Edenberg, H. J., Cloninger, C. R., Begleiter, H., Conneally, P. M., Crowe, R. R., Hesselbrock, V., Li, T. K., Nurnberger, J. I. j., Porjesz, B., Schuckit, M. A., and Reich, T. (2004). A genomic scan for habitual smoking in families of alcholics: common and specific genetic factors in substance dependence. *Am. J. Med. Genet.* **124**(A): 19–27.

Boomsma, D. I., Vink, J. M., Beijsterveldt, C. E. Mv., Geus, E. J. C.de, Beem, A. L., Mulder, E. J. C. M., Riese, H., Willemsen,

A. H. M., Bartels, M., Bergvan den, M., Derks, E. M., Graaff, S. C., Kupper, H. M., Polderman, J. C., Rietveld, M. J. H., Stubbe, J. H., Knol, L. I., Stroet, T., and Baal, G. C. M. (2002). Netherlands Twin Register: a focus on longitudinal research. *Twin Res.* **5**(5): 401–406.

Duggirala, R., Almasy, L., and Blangero, J. (1999). Smoking behavior is under the influence of a major quantitative trait locus on human chromosome 5q. *Genet. Epidemiol.* **17**(S1): S139–S144.

Falconer, D. S., and Mackay, T. F. C. (1996). *Quantitative genetics.* Essex: Longman Group Ltd.

Heath, A. C., and Martin, N. C. (1993). Genetic models for the natural history of smoking: evidence for a genetic influence on smoking persistence. *Addict. Behav.* **18**:19–34.

Heatherton, T. F., Kozlowski, L. T., Frecker, R. C., and Fagerström, K. O. (1991). The Fagerström Test for Nicotine Dependence: a revision of the Fagerström Tolerance Questionnaire. *Br. J. Addict.* **86**:1119–1127.

Kendler, K. S., Neale, M. C., Sullivan, P., Corey, L. A., Gardner, C. O., and Prescott, C. A. (1999). A population-based twin study in women of smoking initiation and nicotine dependence. *Psychol. Med.* **29**:299–308.

Koopmans, J. R., Slutske, W., Heath, A. C., Neale, M. C., and Boomsma, D. I. (1999). The genetics of smoking initiation and quantity smoked in Dutch adolescent and young adult twins. *Behav. Genet.* **29**(6): 383–393.

Li, M. D., Cheng, R., Ma, J. Z., and Swan, G. E. (2003a). A meta-analysis of estimated and environmental effects on smoking behavior in male and female adult twins. *Addiction* **98**(1): 23–31.

Li, M. D., Ma, J. Z., Cheng, R., Dupont, R. T., Williams, N. J., Crews, K. M., Payne, T. J., and Elston, R. C. (2003b). A genome-wide scan to identify loci for smoking rate in the Framingham Heart Study population. *BMC Genetics* **4**(Suppl 1): S103 .

Madden, P. A. F., Heath, A. C., Pedersen, N. L., Kaprio, J., Koskenvuo, M. J., and Martin, N. G. (1999). The genetics of smoking persistence in men and women: a multicultural study. *Behav. Genet.* **29**(6): 423–444.

Mayhew, K. P., Flay, B. R., and Mott, J. A. (2000). Stages in the development of adolescent smoking. *Drug Alcohol Depend.* **59**(S1): S61–S81.

McGue, M., Elkins, I., and Iacono, W. G. (2000). Genetic and environmental influences on adolescent substance use and abuse. *Am. J. Med. Genet.* **96**:671–677.

Neale, M. C., Boker, S. M., Xie, G., and Maes, H. H. (1999). *Mx: Statistical Modeling*. Richmond, VA. 23298, VCU Box 900126.

Neale, M. C., and Cardon, L. (1992). *Methodology for genetic studies of twins and families.* Dordrecht, The Netherlands: Kluwer Academic Publishers.

Saccone, N. L., Neuman, R. J., Saccone, S. F., and Rice, J. P. (2003). Genetic analysis of maximum cigarette-use phenotypes. *BMC Genetics* **4**(Suppl 1): S105 .

Straub, R. E., Sullivan, P. F., Ma, Y., Myakishev, M. V., Harris-Kerr, C., Wormley, B., Kadami, B., Sadek, H., Silverman, M. A., Webb, B. T., Neale, M. C., Bulik, C. M., Joyce, P. R., and Kendler, K. S. (1999). Susceptibility genes for nicotine dependence: a genome scan and follow up in an independent sample suggest that regions on chromosomes 2, 4, 10, 16, 17 and 18 merit further study. *Mol. Psychiatry* **4**:129–144.

Sullivan, P. F., and Kendler, K. S. (1999). The genetic epidemiology of smoking. *Nicotine Tobacco Res.* **1**:S51–S57.

Sullivan, P. F., Neale, B., Oord, E.van den, Miles, M. F., Neale, M. C., Bulik, C. M., Joyce, P. R., Straub, R. E., and Kendler, K. S. (2004). Candidate genes for nicotine dependence via linkage, epistasis, and bioinformatics. *Am. J. Med. Genet. Part B (Neuropsychiatric Genetics)* **126B**:23–36.

True, W. R., Xian, H., Scherrer, J. F., Madden, P. A. F., Bucholz, K. K., Heath, A. C., Eisen, S. A., Lyons, M. J., Goldberg, J., and Tsuang, M. (1999). Common genetic vulnerability for nicotine and alcohol dependence in men. *Arch. Gen. Psychiatry* **56**:655–661.

Vink, J. M., Beem, A. L., Posthuma, D., Neale, M. C., Willemsen, G., Kendler, K. S., Slagboom, P. E., and Boomsma, D. I. (2004a). Linkage analysis of smoking initiation and quantity in Dutch sibling pairs. *Pharmacogenomics J.* **4**:274–282.

Vink, J. M., Willemsen, G., Beem, A. L., and Boomsma, D. I. (2005). The Fagerström Test for Nicotine Dependence in a Dutch sample of daily smokers and ex-smokers. *Addict. Behav.* **30**:575–579.

Vink, J. M., Willemsen, G., and Boomsma, D. I. (2003). The association of current smoking behavior with the smoking behavior of parents, siblings, friends and spouses. *Addiction* **98**:923–931.

Vink, J. M., Willemsen, G., Stubbe, J. H., Middeldorp, C. M., Ligthart, R. S. L., Baas, K. D., Dirkzwager, H. J. C., Geus, E. J. C.de, and Boomsma, D. I. (2004b). Estimating non-response bias in family studies: application to mental health and lifestyle. *Eur. J. Epidemiol.* **19**:623–630.

Edited by Richard Rose