**Genetic and Environmental Etiology of Nicotine Use in Sri Lankan Male Twins**

**Running title: Genetic and Environmental Etiology of Nicotine Use**

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**Abstract**

Little is known about the prevalence and etiology of tobacco use in Asian populations. This study aims to test whether the finding of substantial heritability for tobacco-related phenotypes in Western populations is generalizable to developing countries. The twin method was used to estimate the relative contribution of genetic and environmental influences on nicotine-related phenotypes. Participants were selected from the population based Sri Lankan Twin Registry. The Composite International Diagnostic Interview was administered to 1804 male individuals to assess five phenotypes: nicotine use; desire and unsuccessful attempts to quit smoking; subjective feeling of being tobacco dependent; and two DSM-IV diagnoses; nicotine dependence and nicotine withdrawal. Almost one third of the male twins were life-time smokers. The genetic results were consistent with the previously reported findings from Western and Chinese populations, in that the nicotine use traits were significantly heritable, with environmental influences being of the non-shared nature. The results derived from the Causal Contingent Common pathway model (CCC) supported previous findings that show that liabilities to regular smoking and subsequent problem smoking have both shared and specific genetic influences.

**Key words:** twin method, phenotype, nicotine use, heritability, environmental effects, CIDI, Causal Contingent Common Pathway Model (CCC)

**Introduction**

Smoking related diseases account for approximately five million deaths around the world every year, they are estimated to be the largest preventable cause of disease and premature death making it a serious public health concern (WHO, 2002). Whilst tobacco use seems to be on the decline in developed countries, rates of smoking appear to be stable or increasing in the developing world (Perera, Fonseka, Ekanayake et al., 2005; WHO, 2002). Understanding the prevalence and etiology of smoking behavior in non-Western cultures is therefore of great importance.

Smoking prevalence in US has been estimated at 31% for males and 23% for females (WHO, 2011). According to a representative sample of Sri Lanka, smoking prevalence was estimated at 38% for males and 0.1% for females (Katulanda, Wickramasinghe, Mahesh et al., 2011). This is in line with results published in 2005 where prevalence of smoking in Sri Lanka was estimated at 41% in males and 3.4% in females (Perera et al., 2005). However, whilst current rates of smoking in Sri Lanka seem to be fairly stable, they are substantially lower than those reported in the previous decade when prevalence in men was estimated at 57.9% (Mendis and Ekanayake, 1994). Smoking rates in Sri Lankan women have remained low and are significantly lower than rates in Western populations. Smoking prevalence in Sri Lanka have also been shown to vary according to income, age and education, with smoking prevalence highest in younger men with low levels of education and income (Katulanda et al., 2011). This is in line with research from Western countries where lower levels of income and education have also been associated with increased prevalence of nicotine dependence (Siahpush, Borland and Yong, 2007).

Individual differences in tobacco use have been shown to be due to a combination of genetic and environmental influences. For example, a review of 10 twin studies found that the weighted mean heritability of smoking initiation was 56% (Sullivan and Kendler, 1999). The remaining variance was explained by both shared (24%) and non-shared (20%) environmental influences. Other, more recent, studies have however reported slightly higher heritability for smoking use (e.g. Maes, Sullivan, Bulik et al., 2004; Munafo and Johnstone, 2008). For example, in one study, regular tobacco use was estimated at 80% with no influence of shared environment (Maes et al., 2004). Interestingly, in spite of the changes in smoking prevalence over time, heritability has remained relatively stable (Kendler, Gardner, Jacobson et al., 2005; Vink and Boomsma, 2011). Far less research has, however, been conducted in non-Western counties. One of the few studies to examine non-Western populations was conducted in Chinese male twins. Results showed that heritability estimates were very similar to those reported in Western populations (h2=75%, Lessov-Schlaggar, Pang, Swan et al., 2006).

The extent to which genes and environments influence tobacco use has been shown to vary according to a number of factors including sex and age. For example, several studies have reported greater genetic influences in females than males. This is supported by a meta-analysis of 17 studies in which, genetic influences on smoking initiation were found to be higher in females than males (55% in females versus 37% in males) (55% in females versus 37% in males, Li, Cheng, Ma et al., 2003). It should be noted, however, that not all studies have found evidence of sex difference (Kendler et al., 2005). Heritability estimates also appear to vary according to the age with younger samples showing greater shared environmental influences than older samples (Koopmans, van Doornen and Boomsma, 1997). However, by late adolescence, the etiological structure of smoking initiation closely resembles that of older samples (e.g. Kendler, Schmitt, Aggen et al., 2008).

Twin studies have also implicated genetic influences for nicotine dependence. For example, additive genetic influences were estimated at 67% in a review of 7 studies (Sullivan and Kendler, 1999). Interestingly, shared environment seems less important for nicotine dependence than for smoking initiation.Again, gender differences in genetic and environmental influences seem evident with studies generally showing greater genetic influences in males than females (Li et al., 2003).

Studying the genetics of any substance dependence variable requires the use of a special Causal Contingent Common Pathway model (CCC), which involves modeling two causally linked liabilities (initiation to dependence). This model applies the necessary statistical constraint to account for the fact that dependence can be assessed only in those who have actually initiated smoking and so the genetic and environmental influences on initiation can only affect dependence when initiation has occurred (Kendler, Neale, Sullivan et al., 1999).

In addition, the CCC model can assess aetiological relationship between substance initiation and progression to problem use, providing possible insight for planning successful interventions. For example, the progression from smoking initiation to regular use and to potential addiction involves conditional processes where an initial ‘gateway’ event necessarily precedes the development of a subsequent outcome (Neale, Harvey, Maes et al., 2006). Maes et al. (2004) showed that the CCC model rather than a single-liability model was favored for the relationship between smoking initiation, regular use, and nicotine dependence, suggesting that at least partially different genetic and environmental factors contributed to liabilities of these phenotypes. In this report, initiation is measured with the variable ‘nicotine use’ which closely corresponds to the phenotypes used in previous research, where a similar definition of ‘using of an average of at least seven cigarettes per week for a minimum of 4 weeks’ indexed ‘Smoking Initiation’ (Kendler et al. 1999) and ‘Regular Tobacco Use’ (Maes et al. 2004). Approximately 69% of the genetic variance in liability to Nicotine Dependence was in common with Regular Tobacco Use, while 31% are unique to Nicotine Dependence (Maes et al., 2004).

The present study is based on the Sri Lankan Twin Registry, which was established to provide a genetically informative resource for investigation of common psychopathology and health-related behaviors, including nicotine use and addiction. The main objectives were: (1) to investigate prevalence of different nicotine related phenotypes in the Sri Lankan population; and (2) to investigate the relative contribution of genetic and environmental factors to each phenotype, using a causal contingent common-pathway approach (dependence being contingent on using nicotine). We investigated standard DSM-IV diagnoses, two other nicotine-related problems (unsuccessful attempts to quit smoking and feeling tobacco dependent), as well as general nicotine use, limiting the analyses to phenotypes with higher prevalence (non-zero number of concordant affected MZ and DZ pairs).

This is one of the first reports from a non-Western population to examine the prevalence and etiology of nicotine use and problem use. Cross-cultural comparisons are informative as the environmental exposures associated with nicotine use and abuse in the Sri-Lankan cultural, geographical and economical context may be different to those in Western countries, leading to potential differences in prevalence and etiology. The results of the present study will allow us to address two major issues: (1) whether differences in prevalence of nicotine use and abuse exist between Sri Lankan and other populations; and (2) whether differences in etiology of nicotine-use behaviors exist between Sri Lankan and other populations.

**Materials and Methods**

The study received research ethics approval from the Institute of Psychiatry, King’s College London, the Ethical Review Committee, University of Sri Jayewardanepura, and the World Health Organization’s Research Ethics Committee.

*Participants and Procedure*

The study used a population-based sample in the Colombo District of Sri Lanka, an area with population of 2.2 million which includes the island’s capital, and varies from urban to semi-urban (see Siribaddana, Ball, Hewage et al., 2008 for full details of the sample). The study capitalized on the annual update of the electoral register which consists of a household census conducted by local civil servants. We added a question asking whether the householder knew of any twins, and identified 19,302 individual twins by this method. We used random numbers to sample 6,600 twins aged 16 years or older from this database. Potential participants were excluded if the individuals said they were not twins; one or both of the pair had died or gone abroad; or there were no twins at the given address. 4,387 individual twins were eligible to take part in the project on common mental disorders, of whom 4,024 (91.7%) actually participated, including 1,954 complete pairs of twins (1804 males and 2104 females in male-male, female-female, and opposite sex pairs). The analyses reported here are based on the sample of 1772 male twins after excluding 32 individuals who had a proxy interview (completed by a relative rather than by the twin). The data from the female twins are not included as only 26 females (1.2% of the sample, CI: 1.0-1.8) reported life-time smoking and prevalence of any other nicotine use phenotypes investigated in this study were rare (<1%). For genetic analyses, only males from same-sex pairs were used (N = 1258). The number of pairs with complete data for both twins varied slightly for different phenotypes: 357-359 MZ male pairs and 256-257 DZ male pairs.

During the course of the twin study, we also identified all households in Colombo in which no twins were residing. These household data were organized into the smallest administrative units (for more detail of the selection see Siribaddana et al., 2008). Having performed the twin sampling, we identified the administrative units from which each selected twin came. The non-twins were sampled from the lists specific to each unit in a ratio of 1:1 (twin pair: non-twin). Households were selected using random numbers such that each household in the administrative unit had an equal chance of selection. Letters were sent to the selected households explaining about the study and the twin registry field workers visited the house to determine how many individuals over 15 years were residing there. One research participant from each selected household was selected at random from this list. The consequence of this approach is that the non-twin sample was selected from the same geographical area as the twin sample, but no other socio-economic variables have been used to match the two groups. In the study reported here we use this non-twin sample as a comparison for prevalence data addressing the issue of generalizability from twin studies. The total non-twin sample includes 2019 participants (males and females). As with the twin sample, the prevalence of the investigated phenotypes in women was at or less than 1% (with the exception of Nicotine use: 3.5%).

*Measures and Testing procedures*

Research workers visited the twins’ homes to interview them separately. Questionnaires were administered including the World Health Organisation’s Composite International Diagnostic Interview (CIDI: WHO, 1990). This gives DSM/ICD diagnoses of mental disorders. All other substance use phenotypes operationalized and investigated in this study were derived from CIDI. The operationalization criteria for all nicotine-use categories used in this study are described in Table 1. We defined probands separately for each of the 5 phenotypes, two of which were DSM-IV diagnosed disorders (nicotine dependence and nicotine withdrawal). The remaining three categories indexed nicotine use, unsuccessful attempts to cut down or quit the use of nicotine, as well as subjective feeling of being nicotine-dependent.

*Training, supervision of research workers and quality control*

Interviewers (mostly doctors with no mental health experience) were extensively trained in the Composite International Diagnostic Interview by a CIDI approved trainer. The interviewers then trained additional field workers who had A-level or graduate level education (but no experience in healthcare) during a 14-day intensive course. The training involved interview techniques, distributing the correct information on informed consent, mental disorders and the purpose of the study in general. After performing pilot interviews there was a further 3-day training session where fieldworkers were asked to recount difficulties in the use of the measure, and one month after the start of the main data collection, a further 2-day training session was conducted in order to detect difficulties and consolidate knowledge.

During the course of the study, field workers were given weekly group supervision by the CIDI trainers. Data quality control was further established by random checks of the project managers. Where possible, a different interviewer was used for each member of the twin pair (for more details see Siribaddana et al., 2008).

***Insert Table 1***

## Analyses

*Genetic Analyses.* The twin method is based on estimating the relative genetic and shared (common) and non-shared environmental components of variance by comparing the correlation of monozygotic twins (MZ) who are genetically identical and dizygotic (DZ) twins whose genetic relatedness is on average .50. The effects of shared (common) environment are assumed to be 1.0 for both MZ and DZ twin pairs when reared together. Thus, if addiction or related behaviors are more similar within MZ twin pairs than those within DZ pairs, influences of genetic factors are suggested. Further details of the twin method and its assumptions are described elsewhere (Neale and Cardon, 1992; Plomin, Defries, McClearn et al., 2008).

For dichotomous traits we summarize the observations in contingency tables (CT) with the number of concordant affected, concordant unaffected and discordant MZ and DZ pairs. Similarity in MZ and DZ pairs can be estimated by Probandwise Concordances, which represent the risk that a co-twin of a proband is affected (Plomin et al., 2008), is the ratio of the number of probands in concordant pairs to the total number of probands. Greater MZ than DZ concordances suggest genetic influence. Tetrachoric correlations using a *liability-threshold* model are a more sophisticated measure of similarity obtained by maximum likelihood estimation (Sham, 1998). The model assumes an underlying continuous liability which follows a standard normal distribution with a mean of 0 and variance of 1. The disorder is assumed to be present in all individuals whose liability is above a certain threshold value and to be absent in all other individuals. The threshold is estimated from the population frequency of the disorder (Smith, 1974). The difference in MZ and DZ correlations as estimated from the CTs can be used to quantify genetic and environmental sources of variation in liability in the population using maximum likelihood structural equation model fitting (Mx program: Neale, Boker, Xie et al., 2006): the relative contribution of genetic (‘A’), shared environmental (‘C’), and non-shared environmental (‘E’) influences which also include measurement error (Neale & Cardon, 1992).

***Insert Figures 1a and 1b here***

*Causal Contingent Common Pathway (CCC) model.* This bivariate model applies the necessary constraint to account for the fact that ‘dependence’ can be assessed only in those who have actually initiated smoking. This is modeled by a causal path from the liability to initiation to the liability of dependence (Figure 1a). Estimating twin correlations for dependence data requires this model. Table 2 shows the contingency tables summarizing the data for smoking initiation and nicotine dependence for the MZ and DZ twin pairs. The predicted cell proportions are computed by integrating the multivariate normal distribution over different dimensions: 2 (cell 1), 3 (cells 2, 3, 4 and 7) and 4 (cells 5, 6, 8 and 9). Therefore these analyses require a user specified fit function (chi-square) which was programmed in Mx (scripts available from the authors). The thresholds, the within-person, univariate twin, and bivariate cross-trait twin correlations were estimated using Mx based on the cross tabulation of the outcomes. The genetic CCC model (Figure 1b) was fitted to same data. This model has been described in detail in previous reports (Kendler et al. 1999; Maes et al. 2004, Neale, Harvery et al. 2006). Briefly, the implied MZ and DZ cross trait cross twin correlations provide the information to estimate the A, C and E variance components of the liability to e.g. nicotine dependence that is specific for dependence and those that come from the risk factors for initiation (via the causal path *b*).

The more complicated CCC model was used rather than the proposed two-stage model (Heath, Martin, Lynskey et al., 2002), where liability to Initiation is redefined using at least 3 categories (e.g. ‘never used’, ‘late onset’ and ‘early onset’), since age of smoking onset was not systematically recorded in the sample for all smokers, only for those indicating health problems due to smoking (n=77). Not using raw data analyses (and reading definition variables per person) prohibited us to investigate the effect of covariates (e.g. age) on thresholds.

***Insert Table 2 here***

### Results

*Descriptive Statistics and Prevalence*

 The demographic characteristics of the sample are described in detail elsewhere (Siribaddana et al. 2008). The mean age in the twin sample was 33.2 years (SD = 13.2; range: 15-85). The mean age in the non-twin sample was 43.7 (SD = 16.5; range: 15-84).

Prevalence of each phenotype is presented in Table 1 for males only.The total male twin sample (N=1772) excludes families in which at least one twin failed on X8 and X9 variables in CIDI (failed mini-mental assessment and had a proxi interview, i.e. completed by a relative rather than by the twin). The prevalence from the non-twin sample are reported here only for males (N = 920) after excluding one male based on the exclusion criteria described above.

### Prevalence for life-time nicotine use in the twin sample was 32%. DSM diagnoses of either nicotine dependence or withdrawal were much lower at 4.3% and 1.0% respectively. It can be seen that, with the exception of nicotine dependence and nicotine withdrawal, the prevalence of smoking behavior phenotypes was significantly higher in the non-twin sample. This effect might be explained by the 10-year difference in average age between our twin and non-twin samples.

***Insert Table 3 here***

*Genetic Analyses*

Genetic and environmental influences on the five categories were first examined by comparing MZ and DZ probandwise concordances. As can be seen from Table 3, with the exception of nicotine withdrawal (for which concordance could not be estimated), the MZ concordance was always higher than that of the DZ pairs suggesting some genetic liability. The tetrachoric correlations, derived from concordance and prevalence data using Mx are also presented in Table 3 and show a similar pattern.

Formal model-fitting was only performed on feeling tobacco dependent and attempting to quit nicotine as the concordance rate for nicotine dependence and nicotine withdrawal were very low (no concordant pairs for nicotine withdrawal for either zygosity group, and only 1 concordant pair of DZ twins for nicotine dependence, see table 3). Both models fitted the data well.

Nicotine use was highly heritable (average A from the two models = 64%). Common environmental effects were modest (average = 15%) and not significant. Non-shared environmental influences on nicotine use were approximately 20%.

In terms of the magnitude of the relationship between nicotine use and feeling tobacco dependent, 37% of the smokers in the sample felt dependent. The phenotypic correlation between nicotine use and feeling tobacco dependent was estimated as .68 (-.42/.88), again reflecting lack of power in this sample to establish this relationship with confidence. The Beta coefficient value (b) was substantial (.66), but with confidence intervals including zero. The following estimates were derived from the model: heritability of feeling tobacco dependent .56 (CI = .01/.81), of which 30% was due to factors affecting nicotine use; common environmental factors influencing feeling tobacco dependent .04 (CI = .00/.46), of which 4% was due to factors affecting nicotine use; and non-shared environment influencing feeling tobacco dependent .40 (CI = .19/.76), of which 9% were due to factors affecting nicotine use. Overall, these results suggest that genetic and non-shared environmental factors have the strongest effect on feeling tobacco dependent, and that most of these effects are independent of the factors affecting nicotine use.

In terms of the magnitude of the relationship between nicotine use and unsuccessful quitting phenotypes, 43% of the smokers in the sample wanted or unsuccessfully tried to quit smoking. The phenotypic correlation between nicotine use and nicotine quit variables was estimated as .01 (-.94-.94) reflecting lack of power in this sample to establish this relationship with confidence. The relationship between nicotine use and nicotine quit reflecting the risk factors shared between them (b value) was modest (.15), with confidence intervals including zero, again suggesting the lack of power

 The total heritability of nicotine quit was .32 (CI = .00-.63), of which 1% were due to factors affecting nicotine use. Total common environmental factors influencing nicotine quit .01 (CI = .00-.50), of which 0% was due to factors affecting nicotine use; and non-shared environment influencing nicotine quit .68 (CI = .36-1.00), of which 2% were due to factors affecting nicotine use. Overall, these results suggest that non-shared environmental factors have the strongest effect on unsuccessful attempts to quit nicotine, and most of these effects are independent of the non-shared effects on nicotine use. The results for familial effects on unsuccessful quitting could not be disentangled due to the lack of power, but the results are suggestive of specificity of these effects to quitting.

**Discussion**

The first objective of the current study was to investigate the prevalence of several nicotine-related phenotypes in a south Asian population. The prevalence of all investigated phenotypes was very low in women with only 1.2% reporting life-time smoking. Similar trends have been observed in a previous large epidemiological studies in Sri Lanka (Katulanda et al., 2011; Perera et al., 2005). It is also consistent with WHO reports from other Asian countries, confirming that substance use is generally a masculine habit in these countries. An alternative explanation for the substantial gender differences could be that women show higher adherence to the religious guidelines, such as strong opposition to the use of any addictive substance in Buddhism and Islam. However, it is also possible that gender differences can be explained by a greater tendency for women to respond to surveys in a socially acceptable way. Whilst it is difficult to rule out this latter form of social desirability influencing the participants’ responses to some extent, the authors’ experience of Sri Lankan society leaves us unsurprised by the reported gender differences and we believe that they are more readily explained by a combination of cultural and societal factors. It will be particularly interesting to see whether the prevalence of smoking in women increases in the future, as there has been some suggestion that tobacco companies are attempting to promote smoking in among females in Sri Lanka (Katulanda et al., 2011).

The prevalence of life-time smoking in men in our study was 32.3%. This estimate is similar than those previously reported from Sri Lanka: between 38% and 41% for life-time smoking (Katulanda et al., 2011; Perera et al., 2005). However, much lower than those reported in 1994 (Mendis and Ekanayake, 1994).

In terms of problems associated with nicotine use, our results show that 43% of the smokers reported wishing to quit smoking but being unable to do so. These numbers were higher than those reported for the Chinese smokers (14-16%) and lower than those reported for the Western populations, where up to 75% report desire to quit smoking with only 15% success rate (Lessov-Schlaggar et al., 2006).

When looking at the DSM-IV diagnoses of nicotine abuse, the results of this study suggest that the prevalence of nicotine dependence and withdrawal is lower than those reported from other populations (Kendler et al., 2008), although, as discussed in the introduction, not enough data are available for direct comparisons between different populations. The prevalence of nicotine dependence and withdrawal as diagnosed using the CIDI algorithm in this study was 4.3% and 1.0% respectively (5.9 and 1.1 in the non-twin sample).

The second objective of this study was to investigate the genetic and environmental etiology of the investigated phenotypes. Although this was the largest twin study investigating multiple substance use phenotypes in a South Asian sample, the low prevalence led to a small sample size for the DSM-IV diagnostic categories and to very low concordance rates for these, only moderately familial, traits. This, in combination with the categorical nature of the data of the liability-threshold analysis made it impossible to conduct model-fitting on the diagnostic categories and reduced our power to detect significant effects for the remaining problem-use categories (Neale, Eaves and Kendler, 1994). For example, only 6 pairs of MZ twins and 1 pair of DZ twins were concordant for nicotine dependence. The low prevalence of this phenotype means that an even greater sample size is necessary to assess the genetic and environmental aetiology.

However, the overall pattern of results suggests moderate to substantial genetic and non-shared environmental influences and with little influence of shared environment (non-significant in formal model-fitting) shared environmental influence for nicotine use and associated problems. For example, the concordances (.68 for MZ vs. .51 for DZ) for nicotine use and associated genetic and environmental estimates are very similar to those obtained by Kendler et al. (1999) (.76 for MZ vs. .61 for DZ) and are consistent with those previously reported in other samples from Sweden, Australia, Holland, and USA (e.g. Kendler et al., 2008). Less research is available for nicotine dependence. Heritability of about 70% was reported from two previous studies (Kendler et al. 1999; Kendler et al. 2008) in both men and women, which is similar to that expected from concordances in this study (.41 MZ vs. .10 for DZ twins). Other associated phenotypes, such as persistent or heavy smoking, have also been found substantially heritable (Kendler et al., 1999). In the Chinese population, the heritability was 70% for current smoking and 66% for current heavy smoking, with modest influences of shared environment (Lessov-Schlaggar et al., 2006).

Overall, the relative contributions of genetic and environmental factors to different phenotypes associated with nicotine use are consistent with those from Western and Chinese populations, despite possible differences in the nature and variability of environmental influences across cultures. This is in line with research which has shown that whilst prevalence of smoking has changed significantly in the last decade, heritability estimates have not (Kendler et al., 2005; Vink and Boomsma, 2011). Indeed, our findings support the conclusion that there is no substantial relationship between heritability and prevalence of substance use and that heritability of substance use may be a relatively stable characteristic of human populations and not highly variable as a result of changing patterns of accessibility and consumption (Kendler et al., 2005). However, some indication has been suggested in the previous research that the nature of shared environmental effects may differ across cultures (Lessov-Schlaggar et al., 2006).

In terms of the relationship between the liability to nicotine use and the liabilities to contingent problem use phenotypes, our results support previous findings in that the liabilities to nicotine use and the subsequent problem use do not seem to be either completely overlapping nor completely separate, with evidence of substantial specificity of the liabilities of problem phenotypes, and in particular of non-shared environmental factors involved in unsuccessful attempts at quitting nicotine.

Taken together, the results of this study suggest that the pathways to problem nicotine use are complex, involving both genetic and socio-cultural and/or other environmental factors, some of which are also involved in smoking use (initiation). The examination of several phenotypes was motivated by the growing understanding that the existing diagnostic categories might not reflect the etiology of the disorders. Such non-etiology based diagnoses are not optimal, as reflected in continuous efforts to devise the best possible classification of addiction and substance abuse (Schuckit, Smith, Daeppen et al., 1998). Currently utilized definitions of smoking status mask a great deal of complexity (Munafo and Johnstone, 2008). For example, smoking initiation, progression to regular smoking, and to nicotine dependence are often characterized as distinct categories. However, this distinction might not be etiologically valid. This and previous studies showed that liabilities to smoking initiation, regular smoking, problem use, and nicotine dependence are correlated, but not identical. Due to the power limitations, this study was unable to investigate the relationship between the liabilities of problem nicotine use (e.g., feeling tobacco dependent) and the clinical diagnosis of nicotine dependence.

This lack of power to investigate low prevalence phenotypes was the main limitation of the present study. Despite the initial large sample size, we only had 112 concordant pairs for nicotine use. Numbers were even smaller for all other phenotypes. In a conventional twin study of nicotine dependence, a sample size of approximately 400 concordant pairs would be needed to detect genetic effects at the 5% level with 80% power given a true heritability of ~45% (Neale et al., 1994). Therefore we had limited power to determine whether familiarity of some of the investigated phenotypes is due to genetic or environmental factors (e.g. desire and unsuccessful attempts to quit nicotine).

As this study is based on the twin method, which relies on the assumption that trait-relevant environments are equal for MZ and DZ twins, this study is open to possibility of a violation of this assumption. Several previous studies directly examined the validity of the equal environment assumption, supporting its validity in twin studies that examine phenotypes under investigation in this study (Kendler et al., 1999; Lessov-Schlaggar et al., 2006; Slutske, Eisen, True et al., 2000).

The current study contributes to a continuous effort to gain understanding of the complex etiology of nicotine use phenotypes where genes, environments, and gene-environment interaction represent key elements. It is likely that many genes and environments of small effects are involved, both overlapping for multiple phenotypes and specific to each phenotype. Future work should include longitudinal descriptions to shed light on the developmental aspects of the etiology. Further cross-cultural comparisons are extremely important in terms of gaining understanding on the relationships between cross-cultural differences in prevalence and potentially related differences in etiology. Our results so far suggest that despite differences in prevalence of tobacco use between Sri Lankan and other populations there are no clear differences in etiology.

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Table 1. Clinical and Sub-clinical Categories assessed by CIDI and the number of individuals (proportion of the total sample) meeting each category for males only.

|  |  |  |  |
| --- | --- | --- | --- |
| **Smoking Behavior****Phenotypes** | **Definition and Assessment** | **Prevalence in the twin sample**  | **Prevalence in the non-twin sample** |
| 1. Nicotine Use | Daily use of nicotine for at least several weeks  | N = 572 32.3% | N = 469 51.0%\*\* |
| 2. Nicotine Dependence | DSM-IV 305.10 diagnosisA maladaptive pattern of substance use leading to clinically significant impairment or distress | N = 77 4.3% | N = 54 5.9% |
| 3. Nicotine Withdrawal | DSM-IV 292.0 diagnosis. In this study the diagnosis was based on 3 criteria (A, B, and C). Criterion D was not assessed by CIDI, and therefore is not included in the diagnosis. | N = 18 1.0% | N = 10 1.1% |
| 4. Attempt to Quit Nicotine | Wish and failure to stop nicotine use.Yes to both: “Have you more than once wanted to quit or cut down on smoking/using tobacco?” & “Have you ever tried to quit or cut down on tobacco and found you could not?” | N = 249 14.1% | N = 185 20.1%\*\* |
| 5. Feeling Tobacco Dependent | Yes to “Have you ever felt like you needed or were dependent on tobacco?” | N = 211 11.9% | N = 168 18.3%\*\* |

Note: The total male twin sample includes 1772 participants, which are all male twins after excluding families in which at least one twin failed the mini-mental assessment and had the interview completed by a relative). The total non-twin male sample includes 920 participants (after excluding one male based on the exclusion criteria described above). Prevalence rates of smoking phenotypes are given for the whole sample. \*\*p<0.01

Table 2.Observed numbers of MZ and DZ twin pairs according to ‘Smoking Initiation’ (SI) and two ‘Nicotine Dependence’ variables (unsuccessfully tried to quit smoking (QS) and feeling tobacco dependant (FTD).

|  |  |  |  |
| --- | --- | --- | --- |
| **MZ** | Twin1 | **SI=0** | **SI=1** |
| Twin2 |  |  | *ND=0* | *ND=1* |
| **SI=0** |  | 1 (**215**, *214*) | 2 (**20**, *23*) | 3 (**16**, *12*) |
| **SI=1** | *ND=0* | 4 (**22**, *27*) | 5 (**28**, *32*) | 6 (**15**, *15*) |
| *ND=1* | 7 (**11**, *6*) | 8 (**14**, *8*) | 9 (**17**, *19*) |

|  |  |  |  |
| --- | --- | --- | --- |
| **DZ** | Twin1 | **SI=0** | **SI=1** |
| Twin2 |  |  | *ND=0* | *ND=1* |
| **SI=0** |  | 1 (**145**, 145) | 2 (**20**, *18*) | 3 (**11**, *13*) |
| **SI=1** | *ND=0* | 4 (**17**, *24*) | 5 (**12**, *14*) | 6 (**8**, *8*) |
| *ND=1* | 7 (**23**, *18*) | 8 (**9**,*10*) | 9 (**9**, *6*) |

Note: MZ, monozygotic; DZ, dizygotic; SI = Smoking Initiation (0 = no, 1 = yes on ‘Nicotine Use’ variable); ND = Nicotine Dependence (0 = no, 1 = yes); Cells are numbered 1-9 in superscript; cell counts (number of pairs) are given in brackets: for QS (bold face) and for FTD (italics).

Table 3. N concordant and discordant pairs, N probands in the analyses (% of the sample), Proband-wise Concordances and Tetrachoric correlations for MZ and DZ twins for the 5 variables

|  |  |  |
| --- | --- | --- |
|  | MZ | DZ |
| 1. Nicotine Use | CP = 74DP = 70N probands = 218 (30%)**PC = .68****TC = .76 (.66-.84)** | CP = 38DP = 73N probands = 145 (28%)**PC = .51****TC = .49 (.30-.65)** |
| 2. Nicotine Dependence | CP = 6DP = 17N probands = 29 (4%)**PC = .41****TC = .74 (.48-.90)** | CP = 1DP = 18N probands = 20 (4%)**PC = .10****TC = .24 (-.30-.66)** |
| 3. Nicotine Withdrawal | CP = 0DP = 6N probands = 6 (1%)**PC = n/a****TC = n/a** | CP = 0DP = 6N probands = 6 (1%)**PC = n/a****TC = n/a** |
| 4. Attempt to Quit Nicotine | CP = 18DP = 56N probands = 92 (13%)**PC = .39****TC = .55 (.35-.72)** | CP = 10DP = 52N probands = 72 (14%)**PC = .28****TC = .32 (.06-.56)** |
| 5. Feeling Tobacco Dependent | CP = 20DP = 43N probands = 83 (12%)**PC = .48****TC = .70 (.52-.82)** | CP = 6DP = 50N probands = 62 (12%)**PC = .19****TC = .19 (-.11-.47)** |

Note: N = 1772; MZ = monozygotic twins (N of pairs with full data included in the analyses: 357-359), DZ = dizygotic twins (N of pairs with full data included in the analyses: 256-257); CP = N of concordant pairs; DP = N of discordant pairs; PC = probandwise concordance; TC = Tetrachoric correlation; N probands = N of affected individuals in the sample, reflecting the prevalence of the phenotype in the population (% of the total sample). These numbers may differ from those reported in Table 1 because only the pairs with complete data were used for these analyses.

 

Figure 1a



Figure 1b

**Figure Legends**

Note to Figure 1(a): The Phenotypic Causal-Contingent Common Pathway model for nicotine use and feeling tobacco dependent (FTD) for a pair of twins. The two liabilities (LN and LD) are linked via the causal path *b*, a necessary constrained to account for the fact that dependence can be assessed only in those who have actually initiated smoking and so the genetic and environmental influences on nicotine use can only affect dependence when initiation has occurred. The twin correlations for the liabilities to nicotine use and FTD are estimated separately for MZ and DZ pairs.

Note to Figure 1: (b) The Genetic Causal Contingent Common Pathway model for nicotine use and feeling tobacco dependent (FTD) for a pair of twins. The risk factors for the liability to nicotine use are decomposed into latent additive genetic (AN), common environmental (CN) and individual specific environmental (EN) influences. The risk factors for FTD are divided in those shared with nicotine use (via the causal effect of path *b*) and specific effects: AD, CD and ED. In accordance with the standard classical twin model, the correlations between the A factors are 1 for MZ twins and 0.5 for DZ twins, whereas the correlations between the C factors are 1 for both types of twins.