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Scriptie

Titel Scriptie: *	The combined and individual influence of the *CHRNa5* gene, mental wellbeing, age, profession and education level on nicotine dependence.
Taal Scriptie: *	Engels
Samenvatting:	**Background** Cigarette smoking has become a worldwide health problem. When tobacco use turns into nicotine dependence it causes users to keep smoking cigarettes despite serious risks to health, withdrawal symptoms when an attempt to quit is made, as well as a high probability of fallback. Several factors are known to contribute to nicotine dependence, such as genes, socioeconomic status and mental wellbeing. The aim of this study is to unravel the effects of the *CHRNa5* gene, mental wellbeing and socioeconomic status are analyzed to see if we can replicate the known effect these have on nicotine dependence and to see how these factors work together. **Method** This study was performed with individual health profiles of 1393 current smokers available including socioeconomic information, SCL-90 score used to measure mental wellbeing, age and, of a smaller group, *CHRNa5.* **Results** Individual effects were found for *CHRNa5*, mental wellbeing, education and age. Logistic regression

	showed that *CHRNa5* and mental wellbeing were the largest contributors; age and socioeconomic status were no longer significant. **Conclusion** *CHRNa5* and mental wellbeing are unrelated and have an individual influence on nicotine dependence. The exact mechanisms of these two factors is a subject for future studies on this subject.
Trefwoorden:	Nicotine dependence; *CHRNa5*; mental wellbeing; socioeconomic status; age
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* = Verplicht in te vullen velden

Master Thesis

September 2011

The combined and individual influence of the *CHRNα5* gene, mental wellbeing, age, profession and education level on nicotine dependence.

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Abstract

Background Cigarette smoking has become a worldwide health problem. When tobacco use turns into nicotine dependence it causes users to keep smoking cigarettes despite serious risks to health, withdrawal symptoms when an attempt to quit is made, as well as a high probability of fallback. Several factors are known to contribute to nicotine dependence, such as genes, socioeconomic status and mental wellbeing. The aim of this study is to unravel the effects of the *CHRNα5* gene, mental wellbeing and socioeconomic status are analyzed to see if we can replicate the known effect these have on nicotine dependence and to see how these factors work together.

Method This study was performed with individual health profiles of 1393 current smokers available including socioeconomic information, SCL-90 score used to measure mental wellbeing, age and, of a smaller group, *CHRNα5.*

Results Individual effects were found for *CHRNα5*, mental wellbeing, education and age. Logistic regression showed that *CHRNα5* and mental wellbeing were the largest contributors; age and socioeconomic status were no longer significant.

Conclusion *CHRNα5* and mental wellbeing are unrelated and have an individual influence on nicotine dependence. The exact mechanisms of these two factors is a subject for future studies on this subject.

Introduction

Nicotine dependence

Ever since the link between tobacco smoking and lung cancer was identified in the 1950s, research to the dangers of smoking kept increasing, and let us to the current knowledge that cigarette smoking is the number one preventable cause of death and disability in developed countries (Edwards, 2004). Even with these facts in mind, around 1.2 billion people worldwide smoke, and it is estimated that 8.8% of deaths are caused by tobacco use (World Health Organisation, 2002).

When tobacco use turns into nicotine dependence it causes users to keep smoking cigarettes despite serious risks to their health, withdrawal symptoms when an attempt to quit is made, as well as a high probability of fallback (Piasecki, Piper & Baker, 2010). The Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) defines substance abuse as

a pattern of substance use that causes significant limitation in everyday life and/or suffering including three or more of seven criteria:

1 Tolerance (marked increase in amount; marked decrease in effect)
2 Characteristic withdrawal symptoms; substance taken to relieve withdrawal
3 Substance takes in larger amounts and for longer period than intended
4 Persistent desire or repeated unsuccessful attempt to quit
5 Much time/activity to obtain, use, recover
6 Important social, occupational, or recreational activities given up or reduced
7 Use continues despite knowledge of adverse consequences (e.g. failure to fulfil role obligation, use when physically hazardous)

Even though the DSM-IV criteria for nicotine dependence are still widely used in tobacco research, its usability and definition has been under a lot of debate (Caraballo, Novak & Asman, 2009). The definition and criteria the DSM-IV uses for nicotine dependence are identical to those used for other substance abuse disorders. The criteria for substance dependence were based on observations regarding alcohol and opioid dependence, which differs from nicotine dependence in that there is an added social and behavioural harm, which does not exist in nicotine dependence. Consequently, many of the criteria the DSM-IV mentions are not applicable to tobacco users and are therefore rarely used in research on nicotine dependence (Hughes, Baker, Breslau, Covey & Shiffman, 2011). In addition, the criteria set by DSM-IV have less validity in predicting nicotine dependence than other measures used in research, such as cigarettes smoked per day (CPD) or time to the first cigarette after waking up in the morning. Berrettini et al (2008) performed an analysis to create a clearer picture of the relationship between CPD and the criteria set by the DSM-IV for nicotine dependence. With this analysis, they discovered that a CPD of 25 cigarettes or more per day gave a specificity of 90%, meaning that of people smoking 25 cigarettes a day or more 90% were defined as nicotine dependent by the DSM-IV, making it a reliable cut-off for separating light smokers from nicotine dependent smokers.

In the search to develop a better understanding and find a possible solution for the worldwide problem smoking has become, studies on smoking behaviour have suggested several factors playing a role in nicotine dependence, including genetic factors, mental wellbeing and socioeconomic status.

Genetic factors

Family, adoption and twin studies have indicated that genes influence both smoking initiation and maintenance, with the genetic factors being more prominent for nicotine dependence (Sullivan & Kendler, 1999). The heritability of nicotine dependence is substantial, with estimations ranging from 44 to 60% (Lessov et al., 2004). When smoked, nicotine binds to nicotinic acetylcholine receptors, which are then decoded by specific genes (Greenbaum, & Lerer, 2009). On the quest for these genetic contributors, many candidate genes have been researched. Saccone et al. (2007) did an extensive study targeting 348 candidate genes with 3713 single-nucleotide polymorphism (SNPs), including the gene families known to be involved in the biological pathways of general dependence: nicotinic receptors, dopaminergic receptors and GABA receptors. One gene-cluster found on chromosome six, was discovered as being most influential. This gene-cluster includes cholinergic receptor, nicotinic, beta 3 (*CHRNβ3*), cholinergic receptor, nicotinic, alpha 3 (*CHRNα3*) and cholinergic receptor, nicotinic, alpha 5 (*CHRNα5*). Of these three, SNP rs16969968 in *CHRNα5* struck as the most interesting, with people with the AA genotype, as opposed to people with other genotypes, being nearly twice as likely to have symptoms of nicotine dependence. Conlon and Bewick (2011) found that Women with the AA genotype were even 5.5 times more likely to be heavy smokers as opposed to women with the GG genotype. Further support is found by Berrettini et al. (2008). In their study, *CHRNα3* was found to be the most obvious candidate gene, but they describe the possibility that allele(s) that influence nicotine dependence may lie within *CHRNα3, CHRNα5* or both. In addition, Weiss et al. (2008) found the *CHRNβ3, CHRNα4* and *CHRNα5* cluster to be related to nicotine dependence when the subject had started smoking before the age of sixteen, but found no relation when the subject had started smoking after the age of sixteen.

Socioeconomic status

Socioeconomic status (SES) is most frequently measured by education level, income and occupation, and has a clear relationship with poor health, morbidity and mortality (Adler & Newman, 2002; Siegrist & Marmot, 2004). SES has also been linked with smoking behaviour; those with low SES are more likely to smoke cigarettes, to smoke for longer periods of time, have more trouble to quit smoking and are more likely to suffer from smoking-related diseases (Harwoord, Salsberry, Ferketich & Wewers, 2007; Businelle et al. 2010). Cigarette smokers are also more likely to be poor and have a low education (Healton &

Nelson, 2004). An interesting study on the relationship between these three different aspects of SES and smoking was performed by Barbeau, Krieger & Soobader (2004). Using data of a National Health Survey they analyzed current smoking, attempts to quit smoking and former smoking in people of different social positions, clustered in different occupational classes, income, education, race/ethnicity and gender. A difference was found when looking at current smoking behaviour. Compared to an estimated 26% smokers in the total population, prevalence rates of current smoking among whites with less than a high school degree were 33.9% to 57%, among those with lower rated occupations 37.3% to 38.9%, and among those falling in the lowest income group 41.8%. These numbers give a clear picture of the distribution of smoking in different socioeconomic groups. Marie, Fergusson & Boden (2010) found that SES is not just related to smoking behaviour, but to nicotine dependence as well. They performed a longitudinal study on a New Zealand birth cohort, and found that Maori had significantly higher rates of nicotine dependence than non-Maori. However, this effect disappeared when they controlled for SES.

Mental wellbeing

Previous studies have found that smoking is highly associated with numerous mental disorders, including depression, alcoholism and schizophrenia. In addition, depression is associated with being less likely to quit smoking (Glassman, 1993). This association may be a two-way causality. It is often suggested that people with mental disorders use cigarette smoking as a form of self-medication, mostly for alleviating depressive symptoms, since the psychoactive effects of nicotine help elevate mood (Glass, 1990). On the other hand, some studies found evidence to support the fact that cigarette smoking contributes to the development of mental disorders, anxiety disorders in particular, because of the presumed anxiogenic effects of nicotine (Johnson et al. 2000; West & Hajek, 1997).

Lasser and colleagues (2000) performed a population-based prevalence study on smoking behaviour and patterns in current and lifetime smokers with history of mental illness divided in none, past problems, and mental illness in the past month. Both the group that had mental illnesses in the past and the group that had mental illnesses in the past month smoked significantly more (current and lifetime) than the group which never had mental illnesses. The same trend is also found among nicotine dependent people (John, Meyer, Rumpf & Hapke, 2004), and it has been shown that at least 50% of nicotine dependent smokers meet criteria for one or more other mental disorders (Schmitz, Kruse, & Kugler, 2003). They concluded that

the onset of psychiatric illnesses might be facilitated by nicotine dependence, and the persistence of this dependence might be reinforced by psychiatric illnesses. It has also been shown that this relationship does not only apply to psychiatric disorders. Negative affect, compared to positive affect, was also found to predict nicotine dependence (McChargue, Cohen, & Werth Cook, 2004).

The present study

We know *CHRNα5* is strongly related to nicotine dependence. However, it remains unclear how other factors, such as SES and mental wellbeing, which are known to have an effect on nicotine dependence, coincide with the effect of *CHRNα5*. The aim of this study is to unravel the effects of the previously described three factors on nicotine dependence in a normal population. To our knowledge, no previous studies have combined these three factors together. We hypothesize that *CHRNα5* acts independently, mental wellbeing and SES, however, are known to influence each other (Lorent et al. 2003) because different SES groups have different access to mental health resources, different knowledge on mental health and different lifestyles (Link & Phelan, 1995). Our research question is whether *CHRNα5*, SES and mental wellbeing are independent or if they act in concert and which of these factors proves to be most predicting.

Method

Participants

Participants are part of the Utrecht Health Project (UHP; Grobbee ea 2005), an ongoing population-based follow-up study embedded in primary care in a newly developed residential area in the Netherlands (Leidsche Rijn, Utrecht). Starting in 2000, all new inhabitants were invited by their general practitioner to participate in the UHP, forming a dynamic 'general population' cohort. When Informed Consent was obtained, general assessments (medical history, socio-demographical characteristics, biometrical and physiological measurements including SCL90, smoking, DNA) were obtained forming an individual health profile (IHP). A group of 7257 subjects participated and submitted a blood sample for genetic analysis. The first 2,400 participants with four Dutch ancestors, available IHP data, and sufficient available DNA, were genotyped for candidate genes, including *CHRNα5*. Relatives were not included. For this study, the original group of 7257 people was

reduced to 1393, selecting only those who currently smoke one cigarette per day or more. Of this group of 1393 participants who smoke, genes of 462 people were available. To test the combined influence of the factors, we used data of 334 current smokers of whom all data on the four factors was available. Of these current smokers 289 smoke 1-24 cigarettes per day and form the light smokers group, the remaining 45 smoke 25 or more cigarettes per day and form the nicotine dependent group.

Instruments

Nicotine dependence was measured by number of cigarettes currently smoked per day and categorised into groups of light smokers and nicotine dependents using the cut-off of 25 cigarettes or more per day (Berretti et al. 2008). Current smoking was assessed in the IHP questionnaire.

To measure participants' mental wellbeing, the Dutch translation of the Symptom Check-List 90-R (SCL-90-R; Arrindell & Ettema, 2005) was used; a widely administered psychological status symptom inventory, originally created by Derogatis, Lipman & Cavi (1973). The total score of the SCL-90-R represents the overall level of psychological and physical dysfunction. The total scores were categorised on an ordinal 7 point scale from 1 (*very low*) to 7 (*very high*) on the basis of norm scores from the general population (SCL-90-R manual; Arrindell & Ettema, 2005).

Participants' socioeconomic status was derived from questions in the IHP. Participants were asked for their highest level of education, their current profession or last profession if unemployed as well as current salary of all family members combined.

Genotyping

SNP rs16969968 (*CHRNα5*) was genotyped using a Taqman® SNP Genotyping assay on a 7900HT Real-Time PCR System according to the manufacturer's protocol (Applied Biosystems, Foster City. assay order number: C__26000428_20 and C__11592758_10).

Procedures

After moving to the newly developed district 'Leidsche Rijn' in Utrecht, the new inhabitants had to subscribe to a new healthcare centre and general practitioner. At this first visit, inhabitants were asked to take part in a general health survey (LRGP). In this survey, they filled out a list of questionnaires, ranging from general information to environmental,

medical and psychological questions, including the SCL-90-R and questions about their socioeconomic status. They were also asked to submit a blood sample for genetic analyses.

Data analysis

Missing values affect the power of statistical analyses if participants with incomplete data are excluded. Furthermore, complete-case analysis will give biased results because non-response to a particular question is usually not completely at random (van der Heijden, 2006). Therefore, missing values were substituted using multiple imputation based on linear regression under the assumption that these values depend on observed values (missing at random).

Chi-square analyses were performed to test if *CHRNα5*, mental wellbeing and socioeconomic status are related to nicotine dependence. To correct for demographic differences in smoking behavior, a chi-square analysis is performed to test for differences in sex, and a t-test for age. All significant or trend factors were then combined in correlation analyses to see if they were associated with each other, for these analyses, a two-tailed Spearman's correlation was used. To measure the combined influence of these factors, logistic regression analyses were performed, where *CHRNα5* was analyzed using the three different alleles separately. All analyses were performed using SPSS 15.0.1.

Results

Table 1 provides the characteristics of the groups of light smokers (<25 CPD) and nicotine dependents on *CHRNα5*, mental wellbeing, socioeconomic status, age and sex.

Table 1 Age, sex, *CHRNα5*, mental wellbeing and socioeconomic status for light smokers and nicotine dependents.

	< 25 CPD N (%)	≥ 25 CPD N (%)	Sig
Age (M, sd)	38,91 (11.333)	42,24 (10.946)	*p*<.001
Sex			*p*=.180
- Male	563 (46.6%)	96 (51.9%)	
- Female	645 (53.4%)	89 (48,1%)	
CHRNα5			*p*= .031
- AA	39 (9.8%)	10 (18,2%)	
- AG	178 (44.5%)	29 (52,7%)	
- GG	183 (45.8%)	16 (29,1%)	
Mental wellbeing			*p*<.001
- very low	63 (7.4%)	9 (6.0%)	
- low	116 (13.6%)	16 (10.6%)	
- below average	365 (42.7%)	47 (31.1%)	
- average	90 (10.5 %)	16 (10.6%)	
- above average	60 (7.0%)	8 (5.3%)	
- high	119 (13.9%)	38 (25.2%)	
- very high	42 (4.9%)	17 (11.3%)	
SES education			*p*= .003
- high	281 (23.3%)	31 (16.8%)	
- medium	575 (47.6%)	78 (42.2%)	
- low	352 (29.1%)	76 (41.1%)	
SES profession			*p*= .065
- paid work	929 (78.0%)	133 (71.9%)	
- no paid work	262 (22.0%)	52 (28.1%)	
SES income			*p*= .132
- high	795 (65.8%)	111 (60.0%)	
- medium	186 (15.4%)	39 (21.1%)	
- low	229 (18.8%)	35 (18.9%)	

Abbreviations: SES is Socioeconomic Status

Light smokers have an average age of 38.91 (sd=11.333). In contrast, nicotine dependents have an average age of 42.24 (sd=10.946). This difference is significant; t(1390)=-3.741, p<.001, nicotine dependents are on average older than light smokers.

Of the 1393 current smokers, 47.3% was male, 52.7% female (Table 1). No significant effect was found, $\chi^2(1, N=1393) = 1.798$, p=.206.

Of the light smokers, 54.3% is either carrier of the AG-allele or the AA-allele. Of the nicotine dependent group, 70.9% was A-carrier. The difference in proportions is significant, $\chi^2(2, N=455) = 6.980$, p=.031. Proportions are also shown in figure 1.

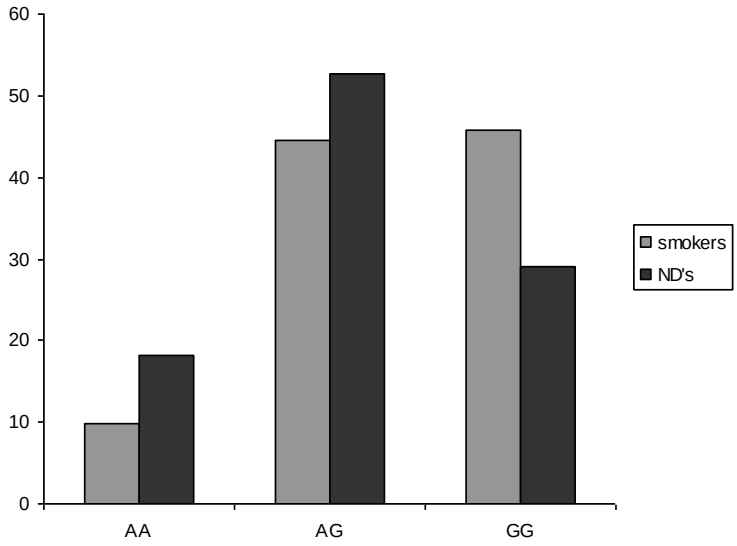

Figure 1. Distributions (%) of different genotypes in light smokers and nicotine dependents.

The difference in SCL-90 scores were differently distributed over light smokers and nicotine dependents, which was significant, $\chi^2(1, N=1006) = 23.559$, p <.001 (Table 1). This means that nicotine dependents score higher on the SCL-90 compared to light smokers. Proportions are also shown in figure 2.

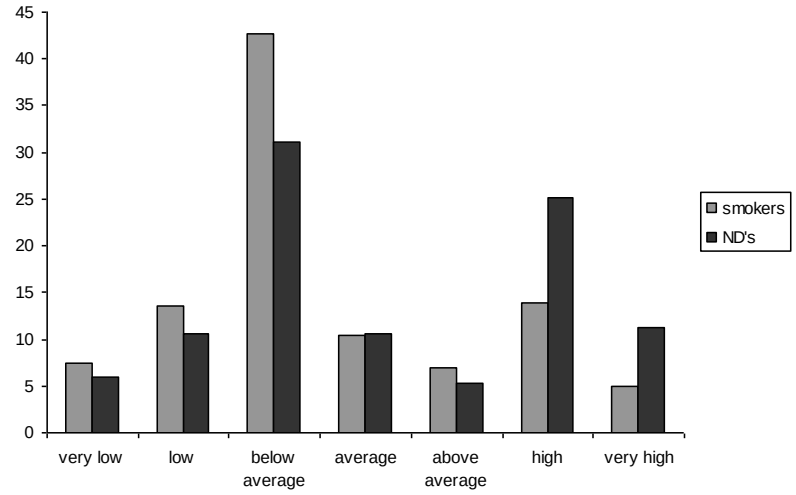

Figure 2. Distributions of SCL-90 norm scores in light smokers and nicotine dependents.

The difference in proportions when we look at education is significant, $\chi^2(2, N=1393) = 11.489$, $p=.003$, meaning that the education level of nicotine dependents is significantly lower than the education level of light smokers. When we look at profession, a trend is visible, yet no significant effect is found, $\chi^2(1,N= 1376) = 3.394$, $p=.065$. Looking at the third factor of SES, income, we found no effect, $\chi^2(2, N=1393) = 4.043$, $p=.132$ (Table 1). Proportions of level of education for the groups of smokers are also shown in figure 3.

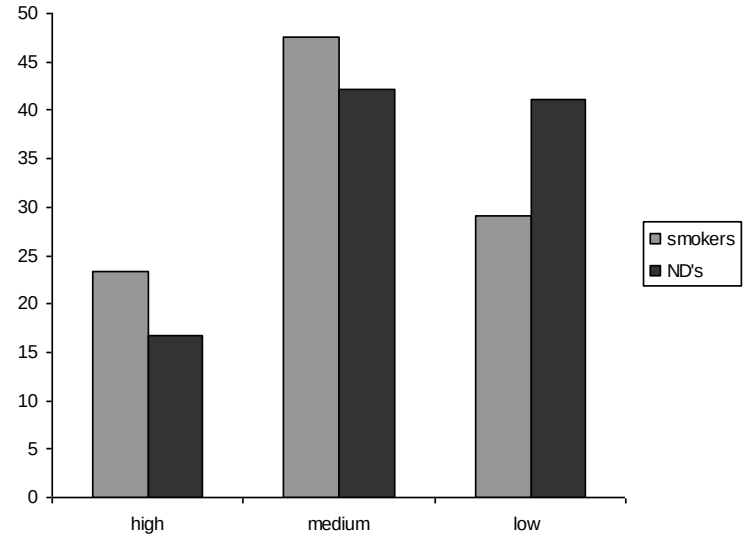

Figure 3. Distributions of education level in light smokers and nicotine dependents.

Relations among significant and trend factors

The four significant factors from the univariate analyses, i.e. mental wellbeing, *CHRNα5*, education level, and age were then combined in different bivariate analyses, adding profession, which was not significant but showed a trend. They were analyzed in pairs to see how these influence each other. Table 2 provides the correlations between *CHRNα5*, mental wellbeing, education, profession and age.

Table 2. Correlations between *CHRNα5*, mental wellbeing, education, profession and age

	CHRNα5 Spearman's ρ (sig.)	M. wellbeing Spearman's ρ (sig.)	Education Spearman's ρ (sig.)	Profession Spearman's ρ (sig.)	Age Spearman's ρ (sig.)
CHRNα5		-.007	.009	-.018	-.022
		(*p*=.800)	(*p*=.656)	(*p*=.384)	(*p*=.299)
M. wellbeing			-.154	.179	.065
			(*p*<.001)	(*p*<.001)	(*p*<.001)
Education				-.306	-.300
				(*p*<.001)	(*p*<.001)
Profession					.344
					(*p*<.001)

No significant correlations were found when *CHRNα5* was combined with either mental wellbeing, education, profession or age. Other combinations, not including *CHRNα5*, were all significant (Table 2).

Relation between CHRNα5 and other factors

The four significant factors from the univariate analyses, i.e. mental wellbeing, *CHRNα5*, education level and age, as well as profession, which showed a trend in univariate analysis, were then combined in a regression analysis to test for independent effects of the factors (Table 3).

Table 3. Regression coefficients of five factors on nicotine dependence.

	Wald chi-square (df)	Sig
Education	4.933 (2)	*p*= .085
Mental wellbeing	23.646 (6)	*p*= .001
CHRNα5	11.421 (2)	*p*= .003
Age	46.294 (50)	*p*= .623
Profession	2.179 (6)	*p*= .140

When all five factors were included in the model, the overall fit is $\chi^2(61, N=331) = 80{,}110$ $p=.051$. Both mental wellbeing and *CHRNα5* remained significant, meaning they contribute to the ability to predict nicotine dependence. Education, profession and age were no longer significant and do not add to the ability to predict nicotine dependence.

Table 4. Regression coefficients of two factors on nicotine dependence.

	Wald chi-square (df)	Sig
Mental wellbeing	23.029 (6)	*p*< .001
CHRNα5	7.764 (2)	*p*= .021

When education and age were removed from the model, the overall fit is $\chi^2(8, N=334) = 29.318$, $p<.001$, mental wellbeing and *CHRNα5* remain significant (Table 4). This result suggests that both factors contribute independently to the ability to predict nicotine dependence.

Discussion

This study is, to our knowledge, the first to combine several important risk factors for nicotine dependence. Mental wellbeing and *CHRNα5* were both significant when all factors were combined in logistic regression, meaning they were independently associated with nicotine dependence. This also means both have their own way of influencing smoking behaviour, which we also found when we compared mental wellbeing of the three different genotypes in a separate analysis; no significant effect was found here, meaning there is no relation between *CHRNα5* and mental wellbeing. We found that the effect of profession, education and age was lost once mental wellbeing and *CHRNα5* were added. Meaning that the

individual effect education and age have on smoking behaviour and the trend that was found when profession was analyzed, could be explained by either mental wellbeing, *CHRNα5* or a combination of both.

In performing correlation analyses of all factors that proved to be influential to smoking and nicotine dependence, we found that *CHRNα5* was unrelated to any of the other factors, meaning that *CHRNα5* is not related or influenced by any of the other factors used in this study, and can be seen as a separate influence on smoking and nicotine dependence. Every combination of mental wellbeing, education, profession and age were significantly correlated, meaning these factors all influence each other.

We were also able to confirm existing studies in finding individual effects of *CHRNα5*, mental wellbeing, education level and age. (Berrettini et al., 2008; Saccone et al., 2007; Marie, Fergusson & Boden, 2010; McChargue, Cohen & Werth Cook, 2004). One of the factors studied was the known risk-gene *CHRNα5*. In the present study, this effect was also found in that there was a significant difference between light smokers and nicotine dependents. People with either the AA or AG allele were more likely to be nicotine dependent than people with the GG allele. The difference with existing studies is that they found the AA-allele in particular to be the risk allele (Saccone et al. 2007), in the present study, being a carrier (AG) gave as much chance of being a nicotine dependent smoker as having the risk allele AA. Analyses on the individual effects of the known factor mental wellbeing showed us that low mental wellbeing corresponds to high-risk smoking behaviours i.e. nicotine dependence. This is congruent with previous studies on the effects of mental wellbeing on nicotine dependence (McChargue, Cohen, & Werth Cook, 2004). The risk of a low socioeconomic status has been partially confirmed in the current study. One of the three factors that form socioeconomic status, education, was found to be a significant predicting factor in nicotine dependence. A trend can be seen for profession, but not a significant difference. For income, no effect was found. Another difference with other studies is that the current study focuses on nicotine dependence compared to normal smoking, whereas other studies tend to focus on smoking in general. More study is probably necessary on the effect this gene has on smoking behaviour to get a clearer picture.

How exactly genes and mental wellbeing influence smoking and nicotine dependence cannot be derived from the results of the current study. It did, however, became evident that mental wellbeing and *CHRNα5* both contribute to predicting nicotine dependence, meaning they both have their own influence on nicotine dependence. This might suggest that there is

both a biological (*CHRNα5*) as a more psychological (mental wellbeing) risk factor for smoking. In the current study, the effect of mental wellbeing was slightly stronger than the effect of *CHRNα5*. Further studies could focus on this point, and maybe find out how both factors work precisely. Analyses could be made using more detailed information about people's smoking habits, such as the age at which one starts smoking and self-report data on craving. Further studies could also include neurobiological research on nicotine receptors in the brain, or they could focus on linking this neurobiological research to finding like the current study.

Several limitations must be kept in mind when interpreting the results of this study. First, this study is limited to a group of middleclass Dutch people from the same neighbourhood, meaning it could lack the diversity to make the results applicable to the general population and thus limiting the generalizability. Therefore, future research should include a broader range of people, with a greater variety in race, social status and income. Still, the analysis was performed using a large group, increasing its reliability. Second, there still is no consensus on how best to measure nicotine dependence. The test which is currently the most widely used to measure nicotine dependence is the Fagerström Test for Nicotine Dependence (FTND) (Heatherton, Kozlowski, Frecker & Fagerström, 1991). Two of the six items the FTND, i.e. number of cigarettes smoked per day and time to the first cigarette after waking up in the morning, are likely to be the two most important items in defining nicotine dependence (Kozlowski, Porter, Orleans, Pope & Heatherton, 1994). A combination of these two items has been called the Heavy Smoking Index (HSI) since its validity was proven using biological measures by Heatherton, Kozlowski, Frecker, Rickert, and Robinson (1989). In case researchers want to incorporate questions about smoking but are unable to include all six question of the FTND Leon, and colleagues (2003) explored options of shortening the FTND to include only number of cigarettes smoked per day and time to the first cigarette after waking up in the morning, or to shorten it to the mere statistic of number of cigarettes smoked per day. They found that number of cigarettes per day was overall comparable to using all 6 questions of the FTND, but that it had a low sensitivity. In contrast, time to first cigarette in the morning had very high sensitivity but a quite low specificity. The combination of the two (HSI) turned out to be a good predictor for the overall FTND score and might be best to use in future research. This could mean that the cut-off point used in the current study of 25 cigarettes per day used might not be the best way to measure nicotine dependence. Before a consensus has been achieved, it might give insight to reproduce the current study but change

the way in which nicotine dependence is measured. This could be done by using one of the available questionnaires, such as the Fragerström test for nicotine dependence. This questionnaire was unavailable in this study, which led to the decision to use the cut-off point of 25 cigarettes per day.

Concluding

The *CHRNα5* gene and mental wellbeing are associated with nicotine dependence and seem independent of each other. Level of education and age are related with ND in univariate models but no longer when studied together with mental wellbeing and *CHRNα5*. Individual effects we found for the *CHRNα5* gene is that carriers of either the AA or AG allele were more likely to be nicotine dependents than carriers of the GG allele. People with lower SCL-90 score, meaning lower mental wellbeing, were also more likely to be nicotine dependent. We also found that lower education level and higher age were associated with nicotine dependence.

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