An investigation of maternal bonding, and polymorphisms of CHRNA4 and CHRNB2 genes in connection with smoking and smoking related psychological symptoms

Ph.D. theses

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Introduction

Smoking is the leading cause of premature death, preventable morbidity, and disability worldwide. In non-developed countries alone, more than 1 billion people are involved in smoking, and smoking is currently responsible for almost 5 million deaths a year.

The treatment of nicotine dependence (ND) as a psychiatric disorder is a major challenge for clinicians, because of the side effects or the inefficacy of the therapeutic agents, and because of frequent relapses.

The rise of the risks of depression and suicidal risk, as smoking-related comorbid symptoms, or symptoms of withdrawal itself, or as side effects of the therapeutic agents, are the most difficult symptoms to treat. Therefore, the common molecular and psychosocial background of ND and depression is an intensively researched area.

The most important psychoactive molecule absorbed by the body in the course of smoking is nicotine, which takes its effect on the central nerve system mostly through nicotinic acetylcholine receptors (nAchR).

Nicotine intensively activates the mesocortiolimbic dopamine system, i.e. the reward system of the brain that plays a significant role in the development of ND.

Specialised literature about the molecular background of the comorbid appearance of smoking and depression considers the dysfunctional mechanisms of the cholinergic system as an important common molecular background. Still, very few studies have

analysed the genes encoding subunits of α4 and β2 in connection with the symptoms of ND and depression.

It is likely that besides their common molecular background, similar psychosocial factors also have a role in the connection of ND and depression. One of those factors is the experienced maternal parenting style in childhood.

The negative effects of dysfunctional parental rearing styles have been investigated in several mental issues and psychiatric disorders.

Low maternal care was demonstrated to have a harmful effect on symptoms of depression, particularly on negative automatic thoughts, and similar results were found in the case of suicidal behaviour.

However, only a few studies have investigated the effects of maternal parenting on smoking, and results are ambiguous.

Certain studies emphasize the role of maternal love, others the role of maternal control and monitoring in the background of psychopathological issues.

In our research, we aimed at investigating the effects of maternal parenting style, and the polymorphisms of genes CHRNA4 and CHRNB2 on depressive symptoms among smokers.

Objectives

The primary aim of our research was to examine maternal bonding among smokers trying to quit, and to analyse its effect on the extent of smoking, on the severity of ND, and on mood in the context of genetic markers. Furthermore, our aim was also to identify among smokers distinctive subgroups with regards to genetic markers, whose members are vulnerable to severe nicotine withdrawal, depressive symptoms and potential side effects of certain agents.

The main objectives of our study were as follows:

1. Do the CHRNA4 gene variants show any correlation with the variance of smoking phenotype?
2. In the studied population are there any distinctly vulnerable groups with regards to emotional life, ND, nicotine withdrawal and earlier smoking habits? If so, how are these groups connected to the polymorphisms of CHRNA4 gene?
3. What is the effect of the experienced maternal bonding on depressive symptoms among smokers?
4. Is there any correlation between the studied polymorphisms of CHRNB2 gene and the severity of depressive symptoms?
5. Is there any connection between the studied polymorphisms of CHRNB2 gene and smoking quantity and the level of nicotine dependence?
6. Is there any connection between the studied polymorphisms and the level of smoking and nicotindependence?
7. How does the quality of the experienced maternal bonding relate to the development of smoking, smoking quantity and the level of ND?

Methods

Subjects

In this dissertation three studies were presented.

Our study sample consisted of 255 adult treatment-seeking smokers from pulmonary cessation centers in Budapest and regional centers. Subjects were only included in this study if all of the following criteria of regular smoking were fulfilled: above five points of FTND, above 10 ppm CO concentration in exhaled air, and at least 10 smoked cigarettes per day in the last month.

In our third study a control non-smoker group was also used, which consisted of 610 voluntary, mentally healthy medical students.

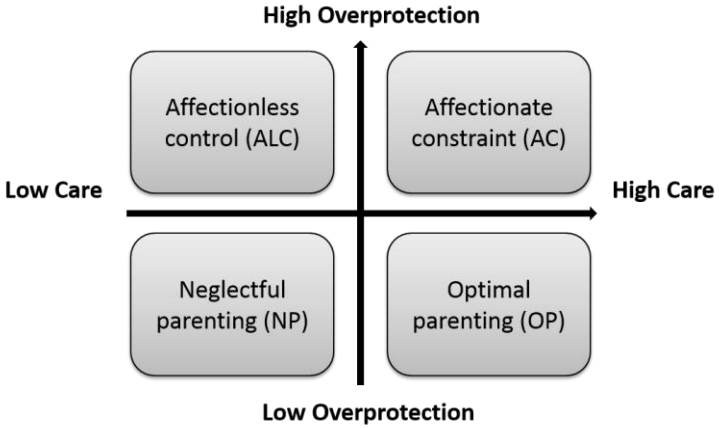
Smoking phenotype was investigated precisely; smoking habits were measured by the number of cigarettes smoked daily (*cigarettes per day, CPD), Fagerstrom Test for Nicotine Dependence (FTND)* and exhaled CO concentration. The heavy smoker subgroup *(HS)* was defined as a group of smokers with a daily consumption of over 20 cigarettes, and the light smoker subgroup *(LS)* was defined as the group of those with a daily consumption of 20 or below, based on the findings of relevant literature.

The *Minnesota Nicotine Withdrawal Scale (MNWS)* was used to assess the symptoms of withdrawal. Depressive symptoms were measured with the *Zung Self-Rating Depression Scale (ZSDS)* and with certain subscales calculated from its items: the total score of ZSDS *(ZSDS-T)*, impulsivity subscale *(ZSDS-I)*, and the item of ZSDS about suicidal ideation *(ZSDS-S)*.

The maternal version of *Parental Bonding Instrument* (*PBI*) was used for assessing maternal parenting style. The PBI is a 25-item self-rating questionnaire, which collects subjective information

retrospectively about the relationship between subjects and their parents from birth to the age of 16. The 25-item questionnaire as developed by Parker et al. consists of two dimensions: care and overprotection, which scales can be used separately and can be divided into high and low scores according to defined cut off points: high care, HC; low care, LC; high overprotection, HOP; low overprotection, LOP). The two dimensions and high-low cutoff points describe four parenting styles: optimal parenting (OP: HC, LOP); affectionate constraint (AC: HC, HOP); affectionless control (ALC: LC, HOP); and neglectful parenting (NP: LC, LOP).

The four maternal bonding styles of the PBI



Summary of the measurements and the population data of the sthree studys

	1. study	**2. study**	**3. study**	
			Smokers	**Non-smokers**
N	236	226	129	610
Males	114 (48.3%)	110 (48.7%)	61 (47.3%)	198 (32.5%)
Females	122 (51.7%)	116 (51.3%)	68 (52.7%)	412 (67.5%)
Mean ager±SD	51.2±12.9	51.5±12.7	52.4±12.8	22.4±2.1
Questionnaires used in these studies				
FTND	+	+	+	+
Exhaled CO	+	+	+	--
CPD	+	+	+	+
PBI	--	+	+	+
ZSDS	+	+	--	--
MNWS	+	--	--	--

Genotyping

DNA samples from buccal mucosa were collected from only the treatment-seeker smoker group.

The selected SNPs were genotyped with the use of Sequenom MassARRAY technology and iPLEX Gold chemistry at the Technology Center, University of Helsinki, Institute for Molecular Medicine, Finland (FIMM),

Three SNPs of the CHRNB2 gene (rs6660775, rs11264222 and rs2072660) and seven SNPs of the CHRNA4 gene were selected for genotyping (rs4522666, rs6090378, rs3787138, rs1044396, rs3787140, rs2093107, rs755203).

Statistical analysis

In descriptive statistics and in certain comparative examinations Chi-square test and Mann-Whitney U-test (SPSS 20.0 software) were used. In our first study, a two-step cluster analysis was performed. GLM and HapScore tests were used for haplotype analysis (R 2.0. software). To assess the effects of the independent variables on dependent variables, binary logistic regression, GLM and ANOVA tests were performed, depending on the type of the variables.

To avoid false positive results in testing genetic association, the Bonferroni correction was used. Tests were adjusted for age and gender, in all cases in which statistical analysis made it possible.

Results

Results of the first study

In our first study, according to the variances of the phenotype variables, significantly distinct patterns were tested among smokers. In addition, these patterns were compared to the variances of the CHRNA4 gene.

In our examination, a two-step cluster analysis was used by stepping in FTND, ZSDS and MNWS scores, CPD and exhaled CO level to the model. CO level, MNWS and ZSDS scores were in the strongest model within which three separated clusters were identified: C1, C2 and C3.

Characteristics of the 3 phenotype clusters

	MNWS (M±SD)	ZSDS (M±SD)	CO level (M±SD)	*p*-value (ANOVA)
Total sample	12.0±6.1	37.7±7.4	19.0±8.7	
C1 (n=110)	8.8±3.8	34.1±5.1	15.0±3.6	<0.001
C2 (n=47)	12.0±4.3	37.2±5.7	30.7±9.3	<0.001
C3 (n=44)	20.7±3.9	47.5±6.2	16.5±4.6	<0.001

C3 was characterized by the highest ZSDS and MNWS scores. Affective hyper-vulnerability as a crucial character of C3 is also demonstrated by the significantly higher lifetime prevalence of MDD in the C3 group than in the others (C1 =13/113, C2 = 7/48, C3 =14/48, chi-square = 7.84, df= 2; p =0.019).

Polymorphisms of the CHRNA4 gene rs3787138, rs1044396 and rs3787140 create a haploblock. Estimation of the frequency of this haploblock showed that three haplotypes had greater than 5% frequency in the sample (ATT = 48%; ACT= 38%; GCC= 9%).

FTND, CPD and ZSDS total scores were not associated with haplotypes in the total sample. The highest MNWS score was associated with the GCC (p= 0.040) and the lowest score with the ATT haplotypes (p= 0.020).

An important result of our research is that GCC haplotype, which had the highest estimated MNWS score compared to ATT and ACT haplotypes, showed significant relation to cluster C3.

Distribution of the three phenotypic clusters

	C1	**C2**	**C3**	***p*-value**
ATT	46%	46%	38%	0.013
ACT	41%	38%	31%	ns.
GCC	7%	7%	17%	ns.

The odds ratio for having the C3 phenotype was almost three times higher in subjects with GCC haplotype compared to others (OR = 2.74; p= 0.013).

Results of the second study

In the second study we investigated the effects of maternal parenting style and CHRNB2 gene on depressive symptoms among smokers.

Our results show that higher care scores were associated with lower scores of ZSDS-T (F=8.1; β= -0.20; p=0.005), ZSDS-I (F=8.1; β= -0.02; p=0.005) and ZSDS-S scores (F=14.8; β= -0.02; p<0.001). Besides, lower overprotection scores correlated with lower ZSDS-T (F=4.9; β= 0.15; p=0.028) and ZSDS-S scores (F=12.8; β= 0.02; p<0.001).

The binomial variables of PBI, care and overprotection dimensions gave similar results. HC was associated with lower ZSDS-T (p=0.005), ZSDS-I (p=0.002) and ZSDS-S scores (p<0.001) compared to LC. Moreover, with regards to the dimension of overprotection, we found that LOP was related to significantly lower ZSDS-T (p=0.020), ZSDS-S (p=0.004) and ZSDS-I scores (p=0.017) compared to the scores of HOP.

As regards the single marker association TT carriers of rs2072660 polymorphism of CHRNB2 gene had higher FTND scores compared to C carriers (p=0.012). CPD and exhaled CO level showed no connection to the studied SNPs.

No direct association was found between CHRB2 gene and depressive symptoms. However, interaction analyses of SNP rs2072660 and maternal bonding style yielded significant results on ZSDS-S. We found that individuals in the LC subgroup scored higher on ZSDS-S if they were homozygous TT of rs2072660 than C allele carriers, and individuals in HC subgroup with any genotype at a nominally significant level (TT_{HC}=1.00±0, n=7; TT_{LC}=2.00 ± 1.2, n = 4; $(CC + CT)_{HC}$ = 1.13 ± 0.4, n = 99; $(CC+CT)_{LC}$=1.35±0.6, n=88; p_{int}=0.021). Furthermore, subjects with homozygous TT for rs2072660 in the affectionless control subgroup had significantly higher ZSDS-S points compared to different genotypes or subjects in not-ALC subgroups (TT_{ALC}= 2.33±1.2, n=3; $TT_{not\text{-}ALC}$=1.00±0, n=8; $(CC+CT)_{ALC}$=1.47 ± 0.7, n=57; $(CC + CT)_{not\text{-}ALC}$ = 1.13 ± 0.4, n=126; p_{int} =0.005).

Results of the third study

In our third study we investigated the differences in maternal bonding between smokers and non-smokers, and the effect of maternal bonding on smoking variables.

Our results indicate that maternal bonding had no effect on either FTND score, or exhaled CO level.

As regards the daily consumption of smoking (CPD), CPD was significantly associated with higher care scores ($F=3.9$; $\beta= -0.16$; $p=0.050$) and lower overprotection scores ($F=5.9$; $\beta= 0.18$; $p=0.016$). In this context we also found that in the LC subgroup, the odds of being a heavy smoker were significantly higher compared to those in the HC subgroup in the total sample ($p = 0.050$, $Exp(B) = 2.2$).

Exploring the association between smoking and maternal bonding, only one maternal bonding subtype showed significant association with smoking: the neglectful parenting style, which is defined as the combination of low care and low overprotection of the mother. The odds for being a smoker were significantly higher among individuals who experienced neglectful parenting from their mothers ($Exp(B) = 32.5$, $p = 0.020$).

Conclusions

In our research, we identified a special subgroup with a new method combining cluster analysis and haplotype association test. In this special subgroup, the affective vulnerability and withdrawal symptoms themselves of the subjects were significant during the acute withdrawal period. According to our results, subjects of this subgroup carried the risk allele of the CHRNA4 gene.

Since both mood disorders and nicotine withdrawal symptoms reduce the effectiveness of smoking cessation, it would be worthwhile to assess and take into account the risk factors of the subjects in order to provide individualized treatment for cessation.

In our study, childhood experience of maternal care was a protective factor, while maternal overprotection was a risk factor of smoking-related depressive symptoms. However, no correlation was found between the quality of maternal bonding and exhaled CO level, or the severity of ND. These results suggest that the patterns of maternal bonding have an important role in the development of smoking but they have no effect on the level of ND. Maternal bonding patterns also influenced the smoking quantity in the group of moderately or highly qualified smokers.

The CHRNB2 gene showed significant relationship with the level of ND, but it had no effect on the smoking quantity or depressive symptoms. Contrary to this, the investigation of the polymorphisms of CHRNB2 gene and maternal bonding in interaction analysis, proved that these factors have a significant effect on the development of suicidal thoughts.

Our results suggest that CHRNB2 and CHRNA4 could be a shared molecular component between ND and smoking-related depression. This hypothesis is supported by the fact that the target of the varenicline – one of the most important cessation agent – is nAChRα4β2. Moreover, severe side effects of varenicline might be mood instability and suicide.

Our data implicates the possibility of developing a screen test based on genetic markers, which could allow a special treatment that does not include prescribing varenicline, but an antidepressant after psychiatric examination. In the spirit of a personalized treatment model, such a screen test could significantly improve the efficacy of cessation, which could cause better morbidity and mortality results on the long-term.

Publication list

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