

Cholecystokinin (CCK) Gene as a Possible Risk Factor for Smoking: A Replication in Two Independent Samples

David E. Comings,* Shijuan Wu,* Nancy Gonzalez,* William G. Iacono,† Matt McGue,† Warren W. Peters,‡ and James P. MacMurray§

*Department of Medical Genetics, Beckman Research Institute, City of Hope, Duarte, California 91010; †Department of Psychology, University of Minnesota, Minnesota; ‡Center for Health Promotion, Loma Linda University, Loma Linda, California; and §Department of Psychiatry, Loma Linda University, Loma Linda, California

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Background. CCK is a satiety neuropeptide. Animal studies have shown that both acute and chronic exposure to nicotine results in weight loss which is associated with an increase in hypothalamic CCK and that CCK antagonists ameliorate symptoms of nicotine withdrawal. A major detriment to smoking cessation, especially in women, is the fear of gaining weight. These observations suggested that genetic variants in the CCK gene might be a possible risk factor for smoking.

Methods. To test this hypothesis we examined the association of the C-45T promoter polymorphism in the Sp1 binding region of the CCK gene with smoking and BMI in two independent groups of subjects.

Results. Group 1 consisted of 191 Caucasian women participating in an obesity study. The T allele was present in 15% of women who had never smoked, 20% of ex-smokers, and 58% of current smokers, $P \leq 0.0014$. The T allele was present in 26.8% of ever-smokers (ex-smokers + current smokers). There was no association with BMI. Group 2 consisted of 725 parents of twins from the Minnesota Twin and Family Study of substance abuse. Logistic regression analysis showed that a diagnosis of nicotine dependence was significantly associated with the T allele ($P \leq 0.002$) and with gender (males > females) ($P \leq 0.001$), but not with BMI ($P \leq 0.68$). The T allele was present in 15.9% of parents who had never smoked and 24.7% of ever-smokers, very similar to the results for group 1.

Interpretation. These results are consistent with a role of the CCK gene as a risk factor for smoking.

Key Words: tobacco; nicotine; CCK; smoking cessation; satiety; obesity.

Despite knowledge of the potential consequences, after a decade of decreases in the percentage of the United States population that smokes, the decline has leveled off at about 25% (1–3). Approximately half of these smokers are women, and women find it particularly difficult to stop smoking due to fears of gaining weight (4–6). The identification of the mechanism by which nicotine influences appetite could lead to the identification of more effective smoking cessation programs. In this regard, CCK regulates weight by producing a feeling of satiety (7) and animal studies show that both acute and chronic exposure to nicotine results in increased plasma CCK levels and weight loss (8–10). This weight loss is associated with a decrease in food intake and an increase in metabolism, as well as decreases in plasma glucose and insulin levels (11,12).

Cholecystokinin (CCK), one of the most abundant neuropeptides in the brain, plays a role in a wide range of behaviors in addition to feeding, including learning, memory, anxiety, pain, drug dependence, and withdrawal (11,13,14). Rasmussen *et al.* (15) reported that a CCK antagonist significantly decreased the symptoms of nicotine withdrawal in animals.

The identification of a C-45T polymorphism in the Sp1-binding *cis*-element of the CCK gene (16) has allowed the investigation of role of CCK variants in

various human behaviors. Harada *et al.* (16) reported a significant increase in the frequency of the T allele in Japanese alcoholics compared to controls, but this was not replicated in a Japanese in a study by Ishiguro *et al.* (17). Fujii *et al.* (18) reported a potential association between C-45T polymorphism and Parkinson's disease and vulnerability to hallucinations in patients treated with L-DOPA. Studies of a C-36T mutation also in the Sp1-binding region have suggested a role of the *CCK* gene in panic disorder in some (19). Based on the above observations we hypothesized that the T allele of the C-45T polymorphism of the *CCK* gene might be associated with smoking and/or obesity, and might provide insights into the role of smoking in weight control.

METHODS

Subjects

Group 1. For a study of the genetics of obesity, we advertised for women who were overweight and willing to participate in a study of weight control. There were 191 women in the study with an average age of 54.5 (SD = 6.28). Some of those who agreed to participate were able to bring a nonobese female friend of comparable age, and socioeconomic and ethnic background as a control. All subjects in this study were non-Hispanic Caucasians. The mean BMI of the total group was 35.74 (SD 9.25). The mean BMI of the 151 obese subjects was 38.97 (SD 7.48) and of the 40 non-or less-obese subjects was 23.54 (SD 2.78). Questions were also asked about whether the subjects had ever smoked, smoked but stopped, or were currently smoking. All of the current smokers had previously attempted to stop smoking. There was no significant difference in the prevalence of smoking in the obese versus the control group. The study was approved by the respective IRBs of both the Beckman Research Institute where the genetic studies were performed and Loma Linda University where the clinical sample was obtained.

Group 2. The second study sample consisted of 725 non-Hispanic Caucasian parents of twins recruited by the Minnesota Twin Family Study. The subjects had an age range of 28–63 years, with a mean of 42.3 years. They were 40% males and 60% females. In addition to providing a blood sample for genetic studies, the subjects were administered the Structured Clinical Interview for DSM-III-R (SCID-R) (20). The presence of nicotine and other

drug dependence was based on lifetime diagnoses. This identified 138 male and 261 female controls with no substance abuse including alcohol, nicotine, or other drug dependence, and 151 males and 175 females with nicotine dependence. This subset was used in the study. Weight and height were available on all subjects allowing for the determination of BMI—weight in kg/ (height in cm) (2). Since we were interested in the interrelationship of four variables, gender, age, BMI, nicotine dependence, and *CCK* genotype, to avoid the loss of power that would have occurred from examining multiple two-way comparisons, we compared all four variables simultaneously using logistic regression analysis. The males were scored as 1 and the females as 2. Those without nicotine dependence were scored as 1 and those with nicotine dependence as 2. The BMI ranged from 18 to 57 with a mean of 27.6 (SD 5.74) and was used as a quantitative trait. The *CCK* genotypes were scored as CC = 1, CT or and TT = 2. There were only 9 individuals with the TT genotype. Nicotine dependence was the dependent variable and the remaining three factors were the independent variables. The study was approved by the respective IRBs of both the Beckman Research Institute where the genetic studies were performed and the University of Minnesota where the clinical sample was obtained.

Genotyping

The C-45T polymorphism of the *CCK* gene (16) was used and we utilized the PCR conditions described in this reference.

Statistics

In group 1, the association of the *CCK* genotypes with smoking status was assessed by Pearson χ^2 analysis. ANOVA, using BMI as the dependent variable, and *CCK* genotype as the independent variable was used to determine if there was an association between these two variables. In group 2, logistic regression analysis was used with nicotine dependence as the dependent variable (control = 0, nicotine dependence = 1) and age, BMI, gender, and *CCK* genotype as the independent variables. All statistical tests used the SPSS statistical package (SPSS, Inc, Chicago, IL).

RESULTS

Group 1. As shown in Fig. 1, 15% of those who never smoked carried the T allele. This increased

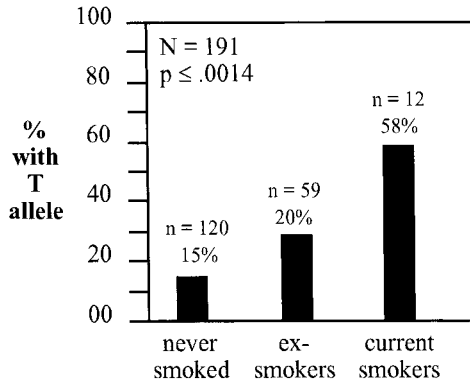


FIG. 1. Relationship of the C-45T polymorphism of the *CCK* gene with smoking status in women.

to 20% for those who were smokers but had stopped smoking, and 58% for those who had attempted to stop smoking but were still current smokers, $\chi^2 = 13.16$, $df = 2$, $P \leq 0.0014$. The T allele was present in 26.8% of those who ever-smoked (ex-smokers + smokers), $\chi^2 = 3.95$, $df = 1$, $P \leq 0.05$. When ex-smokers were compared to current smokers, $\chi^2 = 7.3$, $P \leq 0.0067$. We also examined the possible association between BMI and the C-45T polymorphism. For the total group the BMI for those with the CC genotype was 35.7 (SD 9.46) while the BMI for those with the CT genotype was 35.8, $F = 0.0024$, $P = 0.96$. Finally, we compared the mean BMI of ex-smokers to current smokers. For the ex-smokers it was 36.38 (SD 9.43) and for current smokers it was 33.75 (SD 7.58), $F = 0.81$, $P = 0.36$.

Group 2. The P values based on logistic regression analysis were sex ($P \leq 0.0001$), age ($P \leq 0.009$), CCK ($P \leq 0.007$), and BMI ($P \leq 0.57$). For all four factors, $P \leq 0.0001$. The correlation coefficient for gender (male = 1, females = 2) was -0.12 , indicating males contributed more than females, and -0.07 for age. Again, BMI was not significant ($r = 0.0000$, $P = 0.37$). To allow a better comparison with group 1, a post hoc χ^2 analysis was performed. For the total group, 15.9% of those without a current diagnosis of nicotine dependence carried the T allele, compared with 24.7% of those with a diagnosis of nicotine dependence, $\chi^2 = 8.8$, $df = 1$, $P \leq 0.003$. In group 2 the diagnosis was for a lifetime diagnosis of nicotine dependence and no data were available to distinguish ex-smokers from current smokers. The genotypes in both samples were in Hardy-Weinberg equilibrium.

DISCUSSION

CCK is a satiety neuropeptide (7) and CCK agonists result in a decrease in food intake in animals and humans (11). There are two CCK receptors, A and B. The A receptors are associated with satiety and pancreatic enzyme secretion, including insulin, and potentiation of dopamine-mediated behaviors (21,11). The B receptors are associated with anxiety, symptoms of withdrawal from drugs of abuse including nicotine, pain, and inhibition of dopamine-mediated behaviors (14). Studies with CCK agonists indicate that a target site of action is in the periphery and an intact vagus nerve is required for CCK to reduce food intake (11). However, central sites such as the paraventricular nucleus of the hypothalamus and the nucleus tractus solitarius contribute to the pathway that mediates the effect of peripherally administered CCK on appetite (11,22–24). In this regard, it is of interest that exposure to cigarette smoke also produces increases in CCK levels in the paraventricular hypothalamic region (24). These studies suggest that the stimulation of CCK production in the paraventricular hypothalamic region by nicotinic cholinergic receptors may be the mechanism by which smoking contributes to weight control.

To examine the potential interaction between alleles of the *CCK* gene and smoking we have examined the C-45T promoter polymorphism in the Sp1-binding region of the *CCK* gene. Although we are not aware of any prior studies of the potential role of the *CCK* gene in obesity, Funakoshi *et al.* (25) reported an association between a polymorphism of the promoter region of the CCKA receptor and percentage body fat and insulin and leptin levels in Japanese subjects. Hansen *et al.* (26) examined the function of the effect of the C-36 T variant on transcription of the human *CCK* gene. They demonstrated that substitution of the C-36 residue caused a slight reduction of Sp1 and Sp3 binding, but this had no effect on transcription *in vivo* and no difference in the response to physiological stimuli was observed. We are not aware of any comparable studies of the C-45T polymorphism that also resides in the Sp1-binding region. These studies suggest that neither the C-36T nor the C-45T variants have a direct effect on function of the CCK gene. However, we have suggested elsewhere (27) that that by an effect on gene function, the micro- and minisatellite polymorphisms associated with virtually all genes play a major role in polygenic inheritance. As a result of the great

variability of these short tandem repeat polymorphisms and the long distances over which they exert their effect (28), all genes are likely to be present in a range of hypo- and hyperfunctional variants (29). As a result any common polymorphism, whether it has a direct local effect on gene function or not, is likely to divide the gene into groups of varying function. Thus, the fact that it was a common polymorphism, and by occurring in the promoter region of the CCK could have a direct effect on gene function, we chose to use the C-45T variant.

Figure 1 shows there was a significant association between the C-45T polymorphism and smoking in sample 1 with a progressive increase in percentage with the T allele across the three smoking groups, $P \leq 0.0014$. Despite their demographic differences, there was a remarkable degree of agreement between sample 1 and sample 2. Thus, the percentage of T allele carriers for never smokers was 15.0% for sample 1 and 15.9% for sample 2. For ever-smokers it was 26.8% for sample 1 and 24.7% for sample 2. Since all the reported studies of the CCK C-45T polymorphism have been in Japanese we have no prior Caucasian samples for comparison of allele frequencies. However, the virtually identical frequency of the T allele for non-smokers and smokers in our two independence samples suggests these are reproducible results.

The low prevalence of current smokers (6.3%), in group 1 compared to approximately 25% in the general population, is of interest. This may have been a result of the fact that these studies were carried out at Loma Linda University and the majority of the volunteers were Adventists, who are very health conscious. Such a group would be more likely to have a low prevalence of current smokers than the general population.

For both samples, these results suggest an association between the CCK gene and smoking status. Of interest, although CCK is viewed as a satiety neuropeptide both studies showed no association with BMI. Based on the literature reviewed above, our initial hypothesis was that the CCK gene might be associated with smoking since many individuals, especially women, report the use of cigarettes as a method of appetite and weight control. Figure 2 summarizes the effects of smoking and food on CCK and their possible interaction. Our results suggest that the role of the CCK gene in satiety may account for its association with smoking, but this did not translate into an association with BMI.

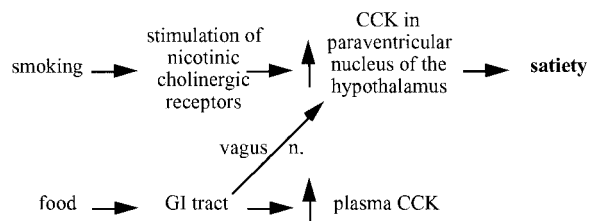


FIG. 2. Possible model of the relationship between smoking and weight control.

Twin studies of smoking have shown that while genes contribute to approximately 50% of smoking initiation, they contribute to 70% of smoking persistence or resistance to smoking cessation (30,31). This suggests that genetic factors play a greater role in an inability to stop smoking than to start smoking. We propose that the present results are consistent with a role of genetic variants of the CCK gene as a risk factor for smoking, and may play role in resistance to smoking cessation. There was a non-significant trend for the BMI to be lower (33.7) in current smokers in sample 1 than in ex-smokers (36.4). While the present results are consistent with the CCK gene as a risk factor in smoking, the degree to which this is secondary to the known effect of smoking on weight control is not clear since we found no association of the CCK gene with BMI. Further studies of this potential association are recommended. The findings do suggest that CCK-acting agents might be useful aids for smoking cessation.

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