

# Association of a Novel Polymorphism of the $\beta$ 2-Chimaerin Gene (*CHN2*) With Smoking

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**Objective:** The *CHN2* gene encodes the  $\beta$ 2-chimaerin, a Rac-specific guanosine-5'-triphosphatase activating protein with an important role in the establishment of functional brain circuitry by controlling axon pruning. Genetic studies suggest that the *CHN2* gene harbors variants that contribute to addiction vulnerability and smoking behavior. To further evaluate the role of  $\beta$ 2-chimaerin in nicotine addiction, we investigated the association of 3 individual polymorphisms of the *CHN2* gene with smoking dependence.

**Methods:** Three hundred sixty-one healthy volunteers, 173 smokers (mean  $\pm$  SD age,  $60.4 \pm 1.4$  years) and 188 control subjects (mean  $\pm$  SD age,  $45.9 \pm 1.4$  years) were genotyped for 3 single-nucleotide polymorphisms in the *CHN2* gene (rs3750103, rs12112301, and rs186911567). The association of these polymorphisms with smoking habits was analyzed.

**Results:** There was no significant association of polymorphisms rs12112301 and rs3750103 with smoking. However, there was a significant difference in the frequency of the rs186911567 polymorphism between the smokers and the controls ( $P = 0.003$ ).

**Conclusions:** We report for the first time a significant association of the novel rs186911567 polymorphism of the *CHN2* gene with smoking.

**Key Words:**  $\beta$ 2-chimaerin, *CHN2*, addiction, polymorphisms, smoking (*J Invest Med* 2013;61: 1129–1131)

Tobacco addiction is a complex brain disorder that involves multiple molecular components and has a strong genetic influence.<sup>1</sup> Numerous factors contribute to the long-lasting nature of tobacco addiction, such as the structural changes induced by nicotine in neurons and its effect on synaptic plasticity.<sup>2</sup> Actin cytoskeleton drives the neuroadaptations produced by nicotine; and therefore, several genes encoding proteins involved in cytoskeleton regulation harbor allelic variants that contribute to smoking dependence.<sup>3</sup> Among these, the *CHN2* gene has been identified in genome wide association studies of nicotine dependence as a candidate for harboring variants involved in smoking addiction.<sup>4</sup>

The human *CHN2* gene maps to chromosome 7p15.3, has 13 exons and encodes the  $\beta$ 2-chimaerin, a guanosine-5'-triphosphatase (GTPase)-activating protein expressed in a variety of human tissues, with the highest expression level in the brain.<sup>5</sup>  $\beta$ 2-chimaerin selectively inactivates Rac, a small GTPase with a prominent role in the control of actin cytoskeleton dynamics.<sup>6,7</sup> Impaired Rac activation due to down-regulation of  $\beta$ 2-chimaerin has been associated with cancer progression, suggesting a role for  $\beta$ 2-chimaerin as a tumor suppressor.<sup>5,8</sup> In the nervous system, recent work in animal models has demonstrated the essential role of  $\beta$ 2-chimaerin in controlling axon pruning in the hippocampus.<sup>9</sup> In humans, the missense polymorphism (H204R) on the *CHN2* gene is associated with schizophrenia,<sup>10</sup> a disorder with a high rate of comorbidity with smoking.<sup>11</sup>

Considering the evidences for the involvement of the *CHN2* gene in tobacco addiction, we focused this study on identifying the association of individual single-nucleotide polymorphisms (SNPs) on the *CHN2* gene with smoking. We analyzed 3 polymorphisms of potential relevance for  $\beta$ 2-chimaerin function: polymorphism rs3750103 c.611A>G (p.H204R) in exon 7 previously associated with schizophrenia, polymorphism rs12112301 (IVS5+7C>T) located on intron 5 that could influence on splicing, and polymorphism rs186911567 c.366G>A (p.S122S) in exon 6, a novel synonymous polymorphism first identified in our laboratory in a screening for mutations on the *CHN2* gene in human breast cancer (unpublished data).

## MATERIALS AND METHODS

### Subjects

The 3 SNPs were genotyped in 361 white individuals from Castilla y León, Spain. One hundred seventy-three participants were current smokers (mean  $\pm$  SD age,  $60.4 \pm 1.4$  years; 74.0% males), which fulfilled the criteria for nicotine dependence dictated by the American Psychiatric Association in the *Diagnostic and Statistical Manual of Mental Disorder*, Fourth Edition<sup>12</sup> and were referred to the Tobacco Addiction Unit of the University Hospital of Salamanca, Spain. The control group consisted of 188 subjects (mean  $\pm$  SD age,  $45.9 \pm 1.4$  years; 75.0% males), which had a lifetime history of no more than 100 cigarettes. All individuals were 21 years or older (the mean age of smoking initiation in Spain is 16.9 years; information from the Spanish Department of Health). Neither smokers nor controls had any mental disorder or personal or familiar history of lung cancer. All subjects gave informed consent to participate, and the study was approved by institutional ethical committees.

### Genotyping

Genotypes of the rs12112301 and rs3750103 polymorphisms were determined using the TaqMan 5'-exonuclease allelic distribution assay (Applied Biosystems, Foster City, CA). The rs186911567 polymorphism was studied by PCR-restriction fragment length polymorphism-based analysis and

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**TABLE 1.** Genotype Frequencies of the *CHN2* Polymorphisms, rs12112301 (IVS5+7C>T), rs3750103 c.611A>G (p.H204R), and rs186911567 c.366G>A (p.S122S) in Smokers and Control Subjects

rs12112301 (IVS5+7C>T)											
	No. of Patients	Genotype			Grouped Genotypes		$\chi^2$	df	<i>P</i>	HWE Controls	
		CC	CT	TT	CC	CT/TT				$\chi^2$	<i>P</i>
Control subjects	188	151 (0.803)	34 (0.181)	3 (0.016)	0.803	0.197	0.071	1.000	0.790	0.448	0.503
Smokers	173	137 (0.792)	36 (0.208)	0 (0.000)	0.792	0.208					
rs186911567 c.366G>A (p.S122S)											
	No. of Patients	Genotype			Grouped Genotypes		$\chi^2$	df	<i>P</i>	HWE Controls	
		GG	GA	AA	GG	GA/AA				$\chi^2$	<i>P</i>
Control subjects	188	188 (1.000)	0 (0.000)	0 (0.000)	1.000	0.000			0.003*		
Smokers	173	165 (0.954)	7 (0.040)	1 (0.006)	0.954	0.046					
rs3750103 c.611A>G (p.H203R)											
	No. of Patients	Genotype			Grouped Genotypes		$\chi^2$	df	<i>P</i>	HWE Controls	
		His/His	His/Arg	Arg/Arg	His	His/Arg and Arg/Arg				$\chi^2$	<i>P</i>
Control subjects	188	164 (0.872)	24 (0.128)	0 (0.000)	0.872	0.128	0.489	1.000	0.485	0.874	0.350
Smokers	173	155 (0.896)	18 (0.104)	0 (0.000)	0.896	0.104					
*Fisher exact test											

\*Fisher exact test

digestion with *TaqI*. Primers used for the amplification were forward: 5'-TATCATTCACACTGTGCTTAT-3', and reverse: 5'-TCAGCAAATCGCACCTATAGT-3'. The amplified 433-base pair (bp) fragment was digested overnight at 65°C with *TaqI*, and the resulting fragments (298 and 135 bp for the A allele and 433 bp for the G allele) were separated on a 3% agarose gel.

### Statistical Analysis

The Hardy-Weinberg equilibrium was determined with the  $\chi^2$  test. Genotype frequencies of smokers and controls, and smokers' subgroups, were compared by means of the  $\chi^2$  test and the Fisher exact test when necessary (expected values below 5).  $P < 0.05$  was considered as significant for the differences between the genotypes. All statistical analyses were performed using the

statistical software Statistical Package for the Social Sciences version 19.0 (SPSS Inc, Chicago, IL).

### RESULTS

Table 1 shows genotype distributions of the 3 polymorphisms of the *CHN2* gene among the smokers and controls. Frequencies of the rs12112301 and rs3750103 polymorphisms were in Hardy-Weinberg equilibrium in the control group, and no significant differences were observed among the smokers and the controls. However, we found a significant difference in the frequency of the rs186911567 polymorphism between smokers and controls. 4.6% of smokers had the GA or AA genotypes while no carriers of the A allele were found in the control group ( $P = 0.003$ ). Hardy-Weinberg equilibrium was not considered

**TABLE 2.** Genotype Frequencies of the *CHN2* rs186911567 c.366G>A (p.S122S) Polymorphism in Smokers With Different Age and Smoking Behavior

	No. of Patients	Genotype			Grouped Genotypes		<i>P</i>
		GG	GA	AA	GG	GA/AA	
Age, yrs							
<65	80	79 (0.988)	1 (0.012)	0 (0.000)	0.988	0.012	0.070*
>65	93	86 (0.925)	6 (0.065)	1 (0.011)	0.925	0.075	
Cigarettes per day							
Up to 20	97	91 (0.938)	5 (0.052)	1 (0.010)	0.928	0.062	0.468*
>20	76	74 (0.974)	2 (0.026)	0 (0.000)	0.974	0.026	
Years smoking							
Up to 30	78	76 (0.974)	2 (0.026)	0 (0.000)	0.974	0.026	0.297*
>30	95	89 (0.937)	5 (0.053)	1 (0.011)	0.937	0.046	

\*Fisher exact test

for this polymorphism in the control group since only carriers of the GG genotype were found in these subjects.

To determine the factors contributing to the association of the rs186911567 polymorphism with smoking, we next analyzed this polymorphism in smokers grouped according to their age and smoking habits (Table 2). There was no significant association of this polymorphism with the daily cigarette consumption (up to or more than 20), years of smoking (up to or more than 30), or age (>65 vs <65 years). However, there was a trend toward an increased frequency of the polymorphism in older smokers ( $P = 0.07$ ). Of smokers older than 65 years, 7.5% had the GA or AA genotypes, whereas only 1.25% of smokers younger than 65 had the GA genotype; and no carriers of the AA genotype were found in this group. These data suggest that the rs186911567 polymorphism does not influence smoking quantity (estimated by the number of cigarettes smoked per day) but may influence duration of smoking.

## DISCUSSION

In this study, we evaluated for the first time the association of individual SNPs of the *CHN2* gene with smoking. Our results are in agreement with the suggested role for the *CHN2* gene in addiction. Uhl et al.<sup>4</sup> identified the *CHN2* among the genes that facilitate nicotine abstinence in smokers treated with nicotine replacement therapies or bupropion. Our study, however, identified an SNP that contributes to smoking. Thus, our data are consistent with whole-genome association studies that identify the *CHN2* gene among the 89 genes with variants that contribute to addiction vulnerability.<sup>13</sup>

The functional relevance of the synonymous rs186911567 polymorphism is currently unknown. Our laboratory first identified this polymorphism in breast cancer (variant identified in 1 of 90 breast cancer samples; unpublished data). Although the G  $\rightarrow$  A substitution at position 366 in exon 6 does not result in amino acid change, bioinformatics analysis predicts the disruption of exonic splicing enhancer motifs and loss of a binding site for the splicing factor SC35 (Human Splicing Finder).<sup>14</sup> Therefore, a theoretical option is that polymorphism rs186911567 could alter the splicing of the  $\beta$ 2-chimaerin transcript, rendering an inactive protein by skipping of exon 6 and generation of a premature stop codon. Inactivation of  $\beta$ 2-chimaerin would result in increased Rac activity and altered actin cytoskeleton remodeling, an important process for the nicotine effect on neural plasticity. Interestingly, *chn2* knockout mice show defects in axon and synaptic pruning, processes that are unbalanced in numerous mental disorders.<sup>15</sup> Of note, polymorphisms in other genes that participate in the regulation of dendritic and axonal arbors remodeling, like the *NRXN3*, have been associated with smoking and with schizophrenia.<sup>16,17</sup>

Other pathways may also contribute to the role of  $\beta$ 2-chimaerin on nicotine dependence. Recent data demonstrate that the effects of nicotine on learning and memory are in part mediated by c-Jun-N-terminal kinase (JNK) signaling in the hippocampus, which may explain the long-lasting modifications of behavior induced by nicotine that contributes to addiction.<sup>18</sup> It is plausible that  $\beta$ 2-chimaerin regulates c-Jun-N-terminal kinase (JNK) activity in hippocampal neurons, as it has been reported the expression of this protein in the hippocampus<sup>9</sup> and the role of  $\beta$ 2-chimaerin in the regulation of JNK have been already demonstrated in epithelial cells.<sup>19</sup>

In summary, our study is the first report of a novel SNP polymorphism of the *CHN2* gene that may contribute to smoking behavior. Because of the relatively small sample size, this is a preliminary study. However, our results are in line with a role of the *CHN2* gene in smoking and support the relevance of this protein in addiction. Based on these findings and the known

biological role of  $\beta$ 2-chimaerin, further studies are needed to corroborate the use of rs186911567 polymorphism as a genetic marker for smoking dependence.

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