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Nicotine Addiction Development: From Epidemiology to Genetic Factors

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ABSTRACT

Background: Nicotine addiction is a complex and multifactorial disease affecting the central nervous system and consists of a set of characteristic symptoms and signs. **Objective:** The objective of this study was to provide an overview on smoking and the complexity of dependency, with special emphasis on the involvement of genetic factors, including neurexin and nicotinic cholinergic receptor genes. **Methods:** The following two aspects are discussed in the present article: (i) epidemiology in Mexico; and (ii) a review of the published literature on genetic association studies using the National Center for Biotechnology Information (NCBI) database of the USA as a search tool. The search key words were: nicotine, smoking, dependence, genetic, tobacco, neurobiology and GWAS. The publication period of the reviewed articles was January 2005 to July 2015. **Results:** There are numerous studies that provide evidence of the involvement of a genetic component that contributes to the risk of developing nicotine addiction, but the multifactorial nature of addiction requires coordinated research from multiple disciplines. **Conclusion:** Research is needed on the factors associated with genetic risk for nicotine addiction and their interaction with environmental factors. (REV INVES CLIN. 2015;67:333-43)

Key words: Nicotine addiction. Neurobiology. Genetic association studies. Genome-wide association study. GWAS. SNP.

INTRODUCTION

The World Health Organization (WHO) Framework Convention for Tobacco Control (FCTC) recognizes the damage caused by tobacco consumption and the need to prevent it since six million people die annually of this cause, including 600,000 nonsmokers[1]. There is an increase in the rates of consumption, particularly among adolescents and women, as well as a decrease in the age of initiation. It is estimated that by the year 2020, more than 10 million people will die from cardiovascular diseases, chronic obstructive pulmonary

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disease (COPD) and lung cancer caused by tobacco consumption. Half of the deaths will occur during the productive years, with an individual loss of 10-20 years of life. In 2030, 7/10 deaths associated with consumption will occur in developing countries[2-4]. Adolescents constitute a group considered to be at high risk. More than 60% of smokers start consuming tobacco at 13 years of age, and more than 90% of smokers start consuming tobacco before reaching 20 years[5]. Data from the 2011 National Survey of Addictions in Mexico (ENA for its Spanish initials) report a 21.7% prevalence of active cigarette smoking in the population aged 12-65 years (17.3 million Mexicans; 31.4% men and 12.6% women)[6]. On average, daily smokers start consuming at 20.4 years (men, 20 years; women, 21.7 years), and smoke 6.5 cigarettes a day (men, 6.8; women, 5.6). Among the major reasons for starting to smoke are curiosity (56.2%) and living with smokers (34.5%). The time it takes a smoker to light the first cigarette of the day is one of the most important indicators of addiction, and 11.4% of active smokers in Mexico smoke their first cigarette within the first 30 minutes after waking. Of the smokers in Mexico, 58.4% have tried to quit[6]. Data from 2011 estimate that active smokers spend on average 397.4 pesos (32 US$) on cigarettes a month. The treatment to quit smoking is successful in only 35%, reported as the percentage of abstinence after one year[7].

In Mexico, 5.8% of the population comprises adolescents between 13 and 15 years. Of them, 42.8% tried a cigarette at some point in their life (43.2% men and 42% women)[8]. Among these young smokers, there are no conscious personal reasons to initiate smoking, and it is accepted as an almost inevitable experience of adolescent growth[9]. In Mexico, total healthcare costs associated with smoking-related diseases were estimated to be 75,200 million pesos (65 million US$) in 2008[10]. The Mexican Social Security Institute in 2004 spent 4.3% of total operating costs on diseases related to tobacco consumption[11]. This information is useful for implementing preventive strategies in the home and raising awareness among smokers.

COMPONENTS OF TOBACCO

Cigarettes are the most studied and consumed products in Mexico[12]. In addition to nicotine, cigarettes contain more than 4,000 substances in combustion smoke, which are divided into gaseous and solid phases. The gas phase components include carbon dioxide, acetone, acetonitrile, ammonia, methane, propane, pyridine, and propionaldehyde. The solid phase components include nicotine, aniline, benzopyrene, naphthalene, phenol, pyrene, and quinolone. There are minor quantitative variations secondary to the cigarette's characteristics, including type of filter and use of fertilizer. Tobacco combustion causes two gas streams: the primary stream, which smokers inhale into their lungs; and the secondary stream, which is inhaled as second-hand smoke. Absorption of components depends on pH and solubility. Thus, more soluble components are absorbed in the upper airway, while less soluble ones are absorbed at the alveolar level. Most commercial cigarettes contain 10 mg or more of nicotine of which between 1 and 2 mg/cigarette is inhaled. Nicotine is primarily present in cigarette smoke in the form of acid salts, which are absorbed in the lungs[13].

NICOTINE DEPENDENCE

In general, the initial decision to consume a drug is voluntary, but it becomes a disease when the individual's ability to exercise self-control becomes extremely limited since addiction is considered a brain disease that modifies its structure and function[14]. Nicotine dependence is caused by consumption of the alkaloid that participates in brain processes and disrupts emotional and motivational processes. A mechanism called "tolerance" develops, i.e., the need to increase the dose to achieve the desired effect. A significant characteristic of dependence is a complex group of withdrawal symptoms, which occurs after discontinuation of use. Common withdrawal symptoms include depression, insomnia, irritability, anxiety, difficulty concentrating, restlessness, decreased heart rate, and increased appetite. The association of substance abuse with a range of environmental or emotional stimuli and their effects creates psychological dependence[15,16]. Most smokers are dependent on nicotine, but there are individuals who do not meet the criteria to be considered dependent[17,18]. The number of cigarettes that an individual smokes per day is a good indicator for assessing the degree of dependence[19,20] as well as the urge to smoke ("craving"), which appears after withdrawal and is responsible for compulsive use, both difficulties associated with withdrawal, and high rates of relapse after treatment[21]. The tool most commonly used worldwide

Table 1. Alpha and beta subunits of the nicotinic cholinergic receptors found in humans; chromosomal localization and products encoding each subunit[24]

Subunits		Gene encoding	Chr	bp	Transcripts
alpha	1	*CHRNA1*	2	16,880	5
	2	*CHRNA2*	8	20,121	8
	3	*CHRNA3*	15	28,243	5
	4	*CHRNA4*	20	35,088	4
	5	*CHRNA5*	15	29,749	4
	6	*CHRNA6*	8	43,772	3
	7	*CHRNA7*	15	14,2031	6
	9	*CHRNA9*	4	19,888	1
	10	*CHRNA10*	11	5,797	3
beta	1	*CHRNB1*	17	12,646	6
	2	*CHRNB2*	1	12,245	1
	3	*CHRNB3*	8	40,031	2
	4	*CHRNB4*	15	96,167	4

Chr: chromosome; bp: base pairs.

for assessing nicotine dependence is the Fagerström test for nicotine dependence due to its accuracy, reproducibility, and ease of use[22]. The Fagerström test comprises six items and allows classification of dependence regardless of psychiatric disorders associated with the smoker, especially depression and anxiety. However, the American Psychiatric Association, in the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) establishes psychological criteria for evaluating depression and anxiety[23].

NEUROBIOLOGY OF NICOTINE ADDICTION

Nicotine interacts in the brain by binding to nicotinic cholinergic receptors (nAChR), causing an increase in dopamine in the *nucleus accumbens* (Nac). This molecular mechanism is responsible for reinforcing addiction and a constant search for the drug.

The nAChR are part of a family of 13 subunits encoded by different genes[24] (Table 1) that combine to form a pentameric ligand-gated ion channel whose biological neurotransmitter is acetylcholine[25]. The Nicotinic Acetylcholine Receptors Subcommittee of the International Union of Basic and Clinical Pharmacology Committee on Receptor Nomenclature and Drug Classification (NC-IUPHAR) identifies subunits with a Greek letter (a or b) followed by an Arabic number[26]. Subunits that are not assembled or coupled with calnexin are rapidly degraded via the proteasome[27]. In lipid rafts of the cell membrane, nAChR are translocated to form neuronal synapses[28]. Each subunit has the following general structure:

– an integral membrane protein with its extracellular amino terminal domain containing a domain of 13 amino acids bound by two cysteine residues that form a disulfide bridge called the cys domain, which is characteristic of these receptors;

– four transmembrane segments (called TM1-TM4) where the amino acid sequence of the TM2 segment determines the type of ion and conductive properties of the pore[29];

– a cytoplasmic domain of variable size between the third and fourth transmembrane segments, which contains a unique amino acid sequence for each subunit;

– and a small extracellular carboxyl terminus.

The amino terminal domain has a beta sheet structure that is involved in assembly of the pentameric complex, and the transmembrane segments are alpha helices that should be aligned to form a hydrophilic ion pore[30]. Maintaining density of nAChR in the synapse is important for regulating effective communication between neurons, and significant variation of these receptors and the

recycling mechanism affects brain plasticity[31]. Through electron microscopy and electrophysiology techniques, the structure and transition states of the receptor in its various conformations have been discovered as follows: closed (in the absence of ligand), open (in the presence of ligand), and desensitized (where there is a high affinity for ligand)[32]. For each state, rearrangements have been observed according to the presence or absence of agonist where cys and aromatic amino acid domains are involved in forming the union at the binding site[33,34].

DOPAMINERGIC SYSTEM AND ADDICTION

The dopaminergic system in the central nervous system (CNS) is the main neurobiological structure involved in the phenomenon of drug addiction. This system comprises different pathways and nuclei. Of these pathways, the mesolimbic pathway has been related to processes of nicotine addiction. The mesolimbic pathway has cell bodies in the ventral tegmental area (VTA) and spreads its axons towards the Nac. In the presence of alkaloid, dopamine flow increases in the ventromedial Nac, and this increase in dopamine stimulates brain circuit activity that regulates feelings of pleasure and satisfaction. The increase in dopamine also exerts a stimulatory effect[35]. In parallel to dopaminergic hyperactivity, release of serotonin in the acute phase of nicotine consumption has been associated with the reward phenomenon in the Nac. Prolonged exposure desensitizes the GABAergic system involved in reward and sensitization of dopaminergic projections, thus producing the behavior of compulsive use of nicotine[36]. The glutamatergic system, whose mechanism of action is the dopaminergic pathway, plays an important role through excitatory amino acids in the addiction process upon activation of N-methyl-D-aspartate (NMDA) receptors by nicotine, thereby maintaining this activation and prolonged potentiation of afferent dopaminergic pathways. In addition, there is a decrease in the amplitude of action of the cholinergic system, which is of great significance in cognitive and affective functions. When nicotine reaches the VTA acetylcholine receptors, which are expressed via gamma-aminobutyric acid (GABA), they increase inhibitory charge and are desensitized rapidly, thereby decreasing the negative modular effect on dopaminergic neurons, ultimately increasing their excitatory effect[37].

During chronic nicotine consumption, sensitization of mesolimbic dopamine release occurs, a significant fact in the establishment of chronic dependence and withdrawal symptoms after cessation of use. In experimental animals and in postmortem studies on the brains of addicted persons, chronic consumption has been detected to be accompanied by decreasing numbers of cellular receptors that respond to the drug (after internalization) in neurons of the mesolimbic pathways and an increase of transcription factors (CREB, FRA1, and delta-Fosb) known to be involved in synaptic remodeling. Increased dopaminergic activity in the VTA is manifested by increased glutamatergic markers and expression of tyrosine hydroxylase (TH). In Nac, an increase in the expression of cyclic AMP (cAMP), protein kinase A (PKA), and delta-Fosb is detected. Neurotrophic factors released in the VTA in response to glutamate could be involved in permanent synaptic changes. Such factors include basic fibroblast growth factor, brain-derived neurotrophic factor, neurotrophin-3, and glial-derived neurotrophic factor. Neurotrophic factors mediate permanent changes in the VTA neuronal circuit such as changes in neuronal size, development of glial filaments, and atrophy of neurofilaments[37,38].

As a result of environmental factors surrounding the body, the brain processes information and transmits signals via neuronal synapses. Such information must be conducted in an efficient, dynamic, and regulated fashion. Neurexin 1 is among cell adhesion molecules that are responsible for stabilizing and originating synaptic GABAergic and glutamatergic communication. Neurexin 1 is a type 1 membrane protein that is expressed in the embryonic stage and can be found in long (a-NRXN) and short (b-NRXN) forms. The former is expressed in a higher proportion (69%) in presynaptic membranes and less (31%) in postsynaptic membranes, and most b-NRXN1 (96%) is found in presynaptic membranes[39]. Both forms contain different extracellular sequences in the amino terminus, but are identical in the transmembrane region and the carboxyl terminus. The extracellular region of the alpha form has six laminin/neurexin/sex (LNS) hormone-binding globulin domains and three interspersed epidermal growth factor (EGF) domains. The short form has only one LNS domain. The long form has five alternative splice sites located adjacent to the LNS domain, while the short form has only two[40], resulting in hundreds of isoforms[41]. For neurexin to perform its biological function, it specifically binds to neuroligins, which is a calcium-dependent interaction. In addition, neurexin is regulated by a variety of molecular and biological mechanisms

that include alternative splicing, glycosylation, and oligomerization[42]. Neurexin is also involved in exocytosis of synaptic vesicles and in brain plasticity[43].

GENETIC STUDIES ON NICOTINE ADDICTION

There are two approaches for finding genes and genetic variants involved in common diseases and traits, such as nicotine addiction: (i) linkage studies, which are based on the study of inheritance of genetic markers and phenotypes within families of affected individuals (for example, twin studies indicate that the genetic component for cigarette consumption contributes up to 35%)[44]; and (ii) genetic association studies based on the identification of significant differences in the frequency of genetic variants between study groups that generally consist of a case group (smokers with some degree of dependency) and a control group (nonsmokers). Knowledge of the variants in these groups leads to identification of genetic factors associated with risk or protection. These studies are challenged by the fact that complex diseases in relation to their genetic bases have a more problematic scenario than monogenic diseases since they include simultaneous participation of several genes and genetic variants (genetic and allelic heterogeneity, respectively). In addition, one particular genetic variant is not sufficient or necessary for the disease, and there is also the influence of interactions between genotypes and the environment[45]. Of the many newly identified genes, there is no indication of their involvement in the biology of the disease. In addition, even associations with areas of the genome of unknown function have been described. These results have been achieved largely because genome-wide association studies (GWAS) examine from hundreds of thousands to millions of single nucleotide polymorphisms (SNP) across the whole genome with strategies that provide a unique opportunity for hypothesis-free genome exploration[46].

POLYMORPHISMS ASSOCIATED WITH ADDICTION TO NICOTINE IDENTIFIED BY FUNCTIONAL GENE STRATEGY

Genetic studies of the dopamine receptor D4 (DRD4) have focused on a variable number tandem repeat (VNTR) polymorphism. The biological significance of such genetic variation translates to changes in the length of the receptor. The 48-base VNTR resides in exon 3, which encodes the third intracellular domain of the protein. Short alleles, called S (consisting of four repeats), have less signaling efficiency compared to long alleles, called L (consisting of seven repeats). In a study of African American smokers and nonsmokers, Shields, et al. found a significant association between a high risk of smoking and individuals carrying the long allele (L). However, in the same study using a Caucasian population, this association is not reproduced[47]. In a study of a Caucasian population with nicotine addiction, Perkins, et al. associated the presence of the long allele (nine repeats) in the *SLC6A3* gene (dopamine transporter) with an increase in the number of cigarettes smoked and state of anxiety[48]. In 2009, Sieminska, et al. found that individuals in a Polish population who did not carry the long allele of *SLC6A3* have a higher risk of smoking before 20 years of age compared to individuals carrying the long allele[49]. Likewise, they associated the allele with the amount of cigarettes smoked per day as subjects carrying the long allele could spend more time without consuming a cigarette compared to those with the short allele.

The serotonergic system is involved in nicotine consumption, and the serotonin transporter (5-HTT) maintains the concentration of serotonin in the nerve synapse. The gene encoding this protein is a study candidate for addiction because it is related to anxiety and depression. There are studies that associate the promoter region of the gene with transcriptional efficiency. In this region, there are two alleles as follows: a 44-bp insertion (L allele) or a deletion (S allele). The S allele has been associated with decreased transcriptional efficiency compared to the L allele. Chu, et al. found a higher frequency of L/L and S/L genotypes in Chinese male smokers compared to the group of nonsmokers. In addition, such genotypes were associated with the level of cigarette consumption and nicotine dependence[50]. In contrast, Sieminska, et al. did not find an association between the L allele and smoking status. The differences start from the distribution of allele frequencies when comparing their population with the Japanese one studied by Ishikawa, which emphasizes different genetic distribution by ethnicity. It is also important to note that behavior patterns of the smoking habit and the ability to quit are influenced by other genes related to addiction, personality traits, and anxiety state[51].

SINGLE NUCLEOTIDE POLYMORPHISMS IN GENES RELATED TO NICOTINE ADDICTION IDENTIFIED BY GENOME-WIDE ASSOCIATION STUDIES

Gene regions have been characterized with SNPs associated with tobacco consumption that are related to components of the dopaminergic pathway or metabolism of neurotransmitters. Such is the case for neurexin 1 (*NRXN1*) and neurexin 3 (*NRXN3*). The family of neurexin genes encodes a group of cell surface proteins that are expressed mainly in neurons and are necessary for the normal release of neurotransmitters. Neurexin is an important factor in the genesis of the synapse. The *VPS13A* gene is another candidate for susceptibility to tobacco addiction as variations in this gene cause progressive neurodegeneration[52].

Liu, et al. published a GWAS meta-analysis that identified the presence of genetic markers located in an area near the *BDNF* gene associated with tobacco consumption[53]. This gene encodes proteins of the neurotrophin family that regulate plasticity and survival of dopaminergic and cholinergic neurons. It is plausible that variations in *BDNF* may alter the rewarding effects of nicotine through modulation of dopamine reward circuits. Regarding cessation of tobacco, the *DBH* gene in chromosome 9 (in particular rs3025343) is associated with the condition of ex-smokers, and the protein encoding this gene is involved in dopamine metabolism[54]. Thorgeirsson, et al. published in 2010 a GWAS meta-analysis where, in addition to nAChR, they associate genes that code for enzymes that metabolize nicotine with tobacco addiction[55].

GENETIC APPROACH TO NICOTINIC CHOLINERGIC RECEPTORS IN NICOTINE DEPENDENCE

Several twin studies indicate that there is high heritability of nicotine dependence, which varies according to the population studied[56]. Genes coding for brain nAChR are candidates in nicotine addiction. The high-affinity receptor is formed by $\alpha4\beta2$ subunits, which may have the $(\alpha4)_2(\beta2)_3$ and $(\alpha4)_3(\beta2)_2$ stoichiometry, of which the former is more sensitive to nicotine and is found in greater amounts in individuals exposed to nicotine for long periods of time[57,58]. Through GWAS, an association has been reported between nAChR SNPs and nicotine dependence or other phenotypes related to cigarette smoking, and significant associations are found in the group of genes on chromosome 15 consisting of *CHRNA5-CHRNA3-CHRNB4* (Table 2). Stevens, et al. studied a Caucasian population comprised of 1,295 light smokers (≤ 5 cigarettes per day) and 1,452 heavy smokers (≥ 30 cigarettes per day), and they report 13 SNPs in the set of genes on chromosome 15 that are significantly associated with the group of heavy smokers[59]. Saccone, et al. published a study associating nicotine dependence in Caucasian and African American populations. In their study, the control group consisted of individuals with a score of 0-1 in the Fagerström questionnaire, and the nicotine-dependent group consisted of individuals scoring ≥ 4. For the Caucasian population, Saccone, et al. included 1,063 cases and 999 controls, and for African Americans, they included 461 cases and 249 controls. These researchers found five SNPs with significant association in the *CHRNA5* gene, 11 SNPs in the *CHRNA3* gene, and one associated in *CHRNB4*[60]. Li, et al. reported two SNPs within the same genetic region associated with the number of cigarettes consumed in a Korean population[61]. When studying the European population, Berrettini, et al. found haplotypes formed by the *CHRNA5/CHRNA3* genes that predispose to nicotine dependence[62]. A variant reproduced in different populations associated to nicotine dependence is rs16969968 in the *CHRNA5* gene. The presence of this polymorphism causes a change in the amino acid sequence from aspartic acid (allele G) to asparagine (allele A) at the 398 position of the protein, thus leading to a change in the charge of the second intracellular domain of the $\alpha5$ subunits[63]. In 2008, Bierut, et al. found that the presence of the minor allele A of rs16969968 alters function of the nicotinic cholinergic receptor because cellular assays indicated a change in intracellular calcium concentration when this polymorphism is present[64]. This research group concluded that a decrease in the function of the nicotinic cholinergic receptor is associated with significant risk of nicotine dependence because subjects with the allele A in *CHRNA5* require more nicotine to activate the dopaminergic pathway. In the European population with the homozygous AA variant for this SNP, Lips, et al. reported that this variant is significantly associated ($p < 0.001$) with the group of smokers who consume ≥ 20 cigarettes per day (OR: 1.81; 95% CI: 1.30-2.13)[65]. In 2010, Hong, et al. showed that this change significantly affects brain

Table 2. Single nucleotide polymorphisms in *CHRNA5*, *CHRNA3*, *CHRNB4,* and *CHRNA7* associated to nicotine dependence

Gene	SNP	Region	Pop	p	OR (95% CI)	Ref
CHRNA5	rs684513	Intron	Ceu	1.13E-07	0.70 (0.61-0.80)	58
	rs667282	Intron		1.68E-04	0.75 (0.64-0.87)	59
	rs17486278	Intron	Ceu	7.00E-08	1.37 (1.22-1.54)	58
			AA	4.43E-02	1.28 (1.01-1.63)	59
			Ceu	5.74E-07	1.40 (1.23-1.59)	
	rs569207	Intron	Ceu	1.40E-08	0.69 (0.61-0.79)	58
	rs637137	Intron	Ceu	1.30E-08	0.69 (0.61-0.79)	
				1.03E-04	0.74 (0.64-0.86)	59
				0.00184	0.74 (0.62-0.90)	68
	rs951266	Intron	AA	4.51E-02	1.45 (1.01-2.06)	59
			Ceu	9.83E-07	1.39 (1.22-1.57)	
			Kor	0.008	1.48 (1.11-1.98)	60
	rs16969968	No-syn, N398D	Ceu	6.30E-08	1.37 (1.22-1.54)	58
				4.04E-02	1.90 (1.40-2.60)	62
				0.00044	1.34 (1.14-1.58)	68
				1.298E-04	1.31 (1.14-1.50)	69
	rs578776	3´UTR	AA	1.47E-02	2.04 (1.15-3.62)	59
			Ceu	4.14E-07	1.40 (1.23-1.59)	
				1.37E-06	0.75 (0.66-0.87)	58
				0.00038	0.73 (0.61-0.87)	68
	rs1051730	Syn, Y215Y	Ceu	9.30E-08	1.37 (1.22-1.53)	58
				2.007E-04	1.3 (1.13-1.49)	69
CHRNA3	rs3743078	Intron	AA	6.76E-02	1.38 (0.98-1.95)	59
			Ceu	5.00E-09	0.69 (0.60-0.78)	58
	rs1317286	Intron	AA	6.76E-02	1.38 (0.98-1.95)	59
			Ceu	4.44E-06	1.36 (1.19-1.54)	
	rs938682	Intron	Ceu	2.87E-04	0.76 (0.65-0.87)	
	rs11637630	Intron	Ceu	5.00E-09	0.69 (0.60-0.78)	58
	rs7177514	Intron	Ceu	2.10E-04	0.75 (0.65-0.87)	59
			Ceu	3.10E-04	0.76 (0.65-0.88)	
	rs6495308	Intron	Ceu	2.64E-04	0.76 (0.65-0.87)	
	rs8042059	Intron	Ceu	3.23E-04	0.76 (0.65-0.88)	
	rs8042374	Intron	Ceu	5.21E-04	0.77 (0.66-0.89)	
	rs4887069	Intron	Ceu	2.40E-04	0.76 (0.65-0.87)	
	rs17487223	Intron	Ceu	8.07E-07	1.33 (1.18-1.48)	58
	rs12440014	Intron	AA	2.07E-02	1.59 (1.07-2.36)	58
			Ceu	3.45E-07	0.72 (0.64-0.82)	
CHRNB4	rs11636605	Intron	Ceu	1.18E-06	0.72 (0.63-0.82)	69
				6.562E-03	1.27 (1.07-1.50)	
	rs11072768	Intron	Kor	0.028	1.17 (1.02-1.34)	60
	rs12441998	Intron	Ceu	3.807E-03	1.28 (1.08-1.52)	69
	rs1316971	Intron	Ceu	3.18E-06	0.73 (0.64-0.83)	58
				3.910E-03	1.29 (1.08-1.53)	69
CHRNA7	rs1913456	Intron	AA	3.29E-02	1.28 (1.02-1.60)	70
	rs6494212	Intron		2.70E-03	1.44 (1.14-1.83)	
	rs904951	Intron		3.14E-02	1.30 (1.02-1.65)	

SNP: Single nucleotide polymorphism; Pop: population; OR: odds ratio; 95% CI: 95% confidence interval; Ceu: Caucasian; AA: African American; Kor: Korean.

Table 3. Single nucleotide polymorphisms in subunit of cholinergic nicotinic receptors encode outside chromosome 15 and associated to nicotine dependence

Gene/SNP	Region	Pop	p	OR (95% CI)	Ref
CHRNB1					
– rs7215056	Intron	Ceu	2.013E-02	1.21 (1.03-1.43)	69
– rs7210231	Intron		5.24E-03	0.80 (0.69-0.94)	70
			1.372E-02	1.23 (1.04-1.45)	69
– rs2302761	Intron		3.44E-03	0.79 (0.68-0.93)	70
			9.904E-03	1.24 (1.05-1.46)	69
CHRNA4					
– rs2236196	3´UTR	Ceu	4.36E-04	1.30 (1.12-1.50)	70
			0.00093	1.35 (1.13-1.61)	68
– rs3787138	Intron		1.02E-03	1.38 (1.14-1.67)	70
			3.875E-02	1.24 (1.01-1.52)	69
– rs2229959	Syn, P403P		4.28E-03	1.33 (1.09-1.62)	70
– rs2273504	Intron		2.266E-03	1.31 (1.1-1.55)	69
CHRNB3					
– rs4952	Syn, N205N	Ceu	1.08E-02	0.65 (0.47-0.91)	70
– rs4950	5´UTR		4.128E-03	1.64 (1.14-2.31)	69
		AA	1.43E-03	0.78 (0.67-0.91)	70
– rs7838246	Intrón	Ceu	1.001E-04	1.38 (1.17-1.62)	
			7.582E-03	1.59 (1.13-2.23)	
CHRNA6					
– rs2304297	3´UTR	Ceu	4.777E-03	1.26 (1.07-1.47)	69
– rs892413	Intron		3.254E-02	1.20 (1.02-1.42)	
– rs10087172	Intron		3.702E-02	1.20 (1.01-1.41)	
– rs1072003	Intron		8.959E-03	1.26 (1.06-1.5)	

SNP: Single nucleotide polymorphism; Pop: population; OR: odds ratio; 95% CI: 95% confidence interval; Ceu: Caucasian; AA: African American.

signaling in subjects consuming tobacco by observing changes in regions of the ventral striatum, amygdala, and hippocampus[66]. In 2011, Janes, et al. suggested that smokers who have the A variant of rs16969968 are more likely to have memories associated with cigarette smoking and states that the allele A has an important role in memory[67]. In 2010, the Consortium of Tobacco and Genetics of the University of Carolina in the USA conducted a meta-analysis of 16 studies of nicotine dependence with some phenotypes associated with tobacco consumption. Among their findings, *CHRNA5* polymorphisms are highlighted, which are associated with cigarettes smoked per day, including rs16969968 (p = 5.57E-72), and also the A allele of rs1051730, which corresponds to an increase by one in the amount of cigarettes smoked (p = 2.75E-73)[54]. In 2011, Grucza, et al. correlated nicotine dependence to interaction with multiple genes in 811 nondependent Caucasians and 797 dependent smokers (four points or more in the Fagerström nicotine dependence scale)[68]. In terms of nAChR, they found a significant association with three *CHRNA5* SNPs of which rs16969968 and rs578776 were already reported in at least two different populations. In a European population, Saccone, et al. found evidence that SNPs that form haplotypes within the group of genes *CHRNA5-CHRNA3* and *CHRNB3-CHRNA6* are associated with risk of nicotine dependence[69]. In 2010, the same group published data on SNPs associated with nicotine dependence in genes of other nicotinic cholinergic receptors, most notably *CHRNA7*, *CHRNA4*, *CHRNB1*, and *CHRNB3* (Table 3). In addition, this study reproduced the finding made in other populations indicating that the likelihood of quitting smoking

is higher in the African American population compared to Caucasians and that African Americans have a greater risk of nicotine dependence, with fewer cigarettes smoked per day compared to cigarette smoking in Caucasians[70].

GENETIC VARIANTS IN THE *NRXN1* GENE ASSOCIATED WITH NICOTINE DEPENDENCE

In a 2008 study conducted in African American and Caucasian populations, Nussbaum, et al. found three SNPs in an intron[71]. The authors proposed that its intronic location contributes to alternative splicing, which generates isoforms that influence formation of neural circuits. Another member of the family of neurexins associated with nicotine dependence is the *NRXN3* gene, which has also been associated with alcohol dependence. Kelai, et al. reported that this family of genes is involved in drug addiction because results obtained in mouse models reveal that drug exposure induces a change in the expression of these genes[72].

NICOTINIC CHOLINERGIC RECEPTORS AND NEUREXIN 1 IN NICOTINE ADDICTION

To conduct synaptic transmission, the set of ion channels, receptors, and neurons needs to be regulated efficiently and operate in harmony. It has been discovered that the short isoform of neurexin 1 interacts with nAChR $\alpha4\beta2$, concentrating it on presynaptic terminals of neurons[73]. Little is known about the mechanism underlying how short and long forms of neurexin are regulated to generate hundreds of isoforms, but it is possible that certain SNPs can regulate this process and consequently affect the expression of neurexin. These changes in expression of the protein could alter functions mediated by nAChR $\alpha4\beta2$, and, in turn, both can collectively participate in nicotine addiction.

DISCUSSION

Nicotine addiction is a complex process influenced by multiple factors, including sociocultural, family and the individual's own biology. Various studies both on molecular genetics and the environment support the role of genes in tobacco consumption. This proposed genetic risk does not appear to be conferred by one or two main genes that show large effects, but rather different genes that present particular discrete effects. In general, genetic factors only explain half the total variability in nicotine addiction, and the remaining variations are influenced by environmental factors. Knowledge, use, and accurate analysis with existing genomic medicine and bioinformatics tools are essential for clarifying the intricate network of neurobiological mechanisms that are largely mediated by genetic factors of nicotine addiction. Furthermore, in the future, knowledge of genetic risk factors may offer individualized therapeutic measures based on information derived from genetic association studies, as previously reviewed. The multifactorial nature of nicotine addiction makes coordinated research from multiple disciplines necessary. Therefore, it is important to investigate factors associated with genetic risk and their interaction with environmental elements.

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