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Genetics of Smoking Behavior and its Consequences: the Role of Nicotinic Acetylcholine Receptors

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Biological psychiatry seeks to understand brain biology and its role in behavior, and to use the knowledge for treatment of individuals troubled by their own actions or the content of their consciousness. Nicotinic acetylcholine receptors (nAChRs) symbolize both the principle on which the discipline is founded and its mission, binding both acetylcholine, the first neurotransmitter, and nicotine, a substance with particular appeal to those the field seeks to help, with epic consequences on behavior and health. Recent genetic studies have revealed solid evidence that sequence variants within a cluster of nAChR genes on chromosome 15 correlate with smoking behavior and nicotine dependence (ND)(1-3). In addition, there is unequivocal evidence of association of the same region with risk of smoking-related diseases(3-5). How these findings are connected is a subject of some debate. A paper by Gruzca *et al*, appearing in this issue of *Biol Psych*, brings another twist to this story, suggesting that the variant most solidly associated with smoking-related phenotypes, protects against cocaine dependence(6).

The convergent findings came from genome-wide association (GWA) studies revealing that markers located within the CHRNA5/CHRNA3/CHRNA4 cluster on the long arm of chromosome 15 were among the top hits in several independent GWA studies of smoking and smoking-related diseases(1-5) (Table 1). GWA studies compare single nucleotide variants in the sequence of the human genome in cases and controls to search for the ones correlating with disease. Based on data from the first GWA study of ND, a non-synonymous SNP in the CHRNA5 gene, rs16969968, was highlighted as it was among the strongest signals when a set of functional candidate genes was considered(1). Association has now been unequivocally demonstrated with number of cigarettes per day (cpd)(2, 3), ND(1, 3), lung cancer (LC)(3-5) and peripheral arterial disease (PAD)(3). The associated allele is common within populations of European and Middle Eastern origin, with frequencies ranging between approximately 30% and 40% in various European populations, but the variant is less common in Asian and African populations.

The biological connection between psychiatry and other aspects of medicine crystallizes in this sequence variant conferring risk of several diseases affecting both brain and body. The genetic basis of lung and vascular diseases is here to an extent rooted in the biological psychiatry of nAChRs. Direct measurement of the risk of LC and PAD revealed allelic odds ratios of about 1.3(3-5) and 1.2(3), respectively, and given the frequency of the variant it may explain a substantial fraction of cases and the associated death toll(3).

That a sequence variant influencing smoking behavior plays a role in smoking-related diseases is in principle expected. However, it constitutes a surprise that the impact on the

consequences of the behavior is stronger than on the behavior itself. The association with the smoking behavior variables measured cannot fully explain the observed impact on LC(3-5) and PAD(3). The simplest explanation might be that there are aspects of smoking behavior, not measured by the studies and *incompletely measured by ND and cpd*, that are more strongly influenced by the variant and account for the observed difference. The relatively crude measures applied cannot be expected to capture the complexity of smoking behavior. Alternatively, the variant may also render carriers more susceptible to the harmful effects of tobacco smoke. This would require a role in peripheral tissues with an effect on the susceptibility of both the lungs and the vascular system. Finally, there is the possibility that the variant confers risk of LC and PAD independently of smoking behavior. Ironically, arguments along the lines of R.A. Fisher's objections to the early anti-smoking campaign in the wake of the first epidemiological studies correlating smoking with LC, namely that a common gene might influence both smoking and LC without a causal relationship between smoking and LC, are actually weakened by the very discovery of precisely such a gene! This stance now requires postulating different roles for one or more nAChR genes ignoring the common thread, the fact that the gene products act by binding nicotine, a key ingredient of tobacco smoke. To rule out the last possibility, the impact of the variant on LC and PAD among never smokers needs to be measured in a large sample, which probably would require a meta analysis to achieve the necessary sample size. Further genotype-phenotype correlation studies of various smoking behaviors (*eg* total number of cigarettes smoked, depth of inhalation, inhalation time, how far down a cigarette is smoked, *etc*), and queries into the role of nicotine itself in LC and PAD, are among the studies necessary to distinguish between the former two possibilities. It should be emphasized, that while contemplating the relationship between the variant and smoking-related diseases, the focus is strictly on how this variant confers risk. The situation can be quite different for other genes affecting these diseases, and the biology of LC and PAD in general is a different matter as well.

The dissection of the smoking phenotypes has begun, and the variant may affect whether a pleasurable buzz is experienced during early experimentation with smoking(7), and it has been suggested that that the impact on the risk of ND is contingent on early age at onset of regular smoking(8). Further elucidation of the primary phenotype of the variant may even bring into question the usefulness of our current definitions of nicotine addiction from a public health perspective. That is, under the assumption that the impact on smoking-related diseases is entirely through smoking behavior, it follows that our current definitions of ND may have ignored an important and particularly dangerous aspect of smoking, such that smoking-related diseases like LC and PAD turn out to be better surrogates of nicotine addiction than available ND measures.

The impact of this particular variant, with regard to its effect on smoking-related diseases, is rooted in past behavior. Its impact on the future incidence of deadly smoking-related diseases is going to be influenced by its interaction with ongoing smoking prevention efforts, and perhaps most importantly, by how the findings will be put to use in aiding smoking prevention and cessation. Genotype-phenotype studies of smoking cessation success and data from clinical trials of cessation aids may provide key insights. Once it becomes clear which nAChR subunit is the culprit, therapeutic compounds could perhaps be

developed. Genetic findings can quickly provide diagnostics that can be used to influence decision making of individuals, and DNA-based diagnostics will inevitably be included in the psychiatric diagnostic systems of the future.

This region on chromosome 15 is currently the focus of an intense effort, and the paper by Gruzca et al, is one of three recent papers(6, 9, 10) further studying the role of the CHRNA3/CHRNA5/CHRNA4 cluster in addiction from a group of researchers including those who first highlighted the association with ND based on GWA data(1). The studies utilize three samples, the COGA sample, the Family Study of Cocaine Dependence (FSCD), and the NICSNP sample used for the GWA study of ND. In addition to obtaining further support for the association with ND within the COGA sample, and the results on cocaine dependence, the reported findings include an additional SNP within the cluster associating with ND and multiple SNPs associating with alcohol dependence, and the results of *in vitro* studies of the D398N variant of the $\alpha 5$ receptor.

While these new findings on alcohol and cocaine dependence have intriguing implications, the evidence for association is currently weak. Several factors cause weaker inference: sample size, familial relations among subjects, and overlap between the study samples, with the discovery sample in one study used for replication in another. While the reports all concern the same region on chromosome 15, additional markers were studied such that the total number of tests is large enough to impact significance and several phenotypes and models were tested. Further replication studies are needed to demonstrate statistical significance, and obtain reliable effect size estimates.

If the results of Gruzca et al are replicated, the list of associated diseases would expand to include cocaine dependence. It is surprising that the association with cocaine dependence is in the opposite direction of that for ND. Although cocaine may bind strongly enough to nAChRs to mediate an effect, initial attempts at seeking explanations should focus on action mediated by nicotine. As smoking is prevalent and nicotine is probably often the first psychoactive substance used, its effects on the brain of smokers might influence susceptibilities to other substance disorders, for example through an effect on initiation. The authors speculate that the results can be explained by the reduced receptor function of the D398N variant of the $\alpha 5$ -receptor gleaned from their *in-vitro* studies(9) and explain the opposite direction of the association by considering the dual role of nAChRs (in particular the $\alpha 5\beta 2\alpha 4$ isoform) on excitatory dopaminergic and inhibitory GABA-ergic neurons(9).

The nAChR variant provides an exciting model of gene-environment correlations and interactions linking an addiction susceptibility genes to other diseases. The utility and importance of biological psychiatry research is exemplified by the fact that the convergent and only genome-wide significant finding of two large GWA studies of LC constitutes a sequence variant affecting behavior. The new results on cocaine dependence call for additional studies to unequivocally establish the association and to obtain a reliable estimate of effect size. In parallel, other substance disorders and psychiatric disorders need to be examined as well to figure out whether the variant acts in a nicotine-specific manner or has a more general role in addiction.

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Table 1
Associations with smoking behavior and smoking-related diseases

The results listed are from GWA studies(1-5) and direct studies of the variant (8, 9). Thorgeirsson et al performed a GWA study of smoking behavior and subsequently tested the variant in ND, LC, and PAD. Results are reported for the markers rs16969968 (1), rs1051730(3, 4), rs8034191(4, 5), rs6495308 and rs1317286(2). Rs16969968 and rs1051730 are for all practical purposes equivalent in Caucasian populations($R^2=0.99-1.00$). The remaining markers are highly correlated with rs16969968/rs1051730. Almost identical results were obtained for rs1051730 and rs8034191 for LC (4). Weiss et al obtained their result by regression using a haplotype [see (8)]. The risk alleles for rs16969968 and rs1051730 are fixated on this haplotype background.

Study Design	OR	p-value	Cases / Ctrls	References
ND and Smoking Behavior				
FTND (4+) vs FTND(0)	1.4	6×10^{-4}	1050 / 879	Saccone et al.(1)
Cigarettes per day - QTL, sample I		7×10^{-5}	~7500	Berrettini et al.(2)
Cigarettes per day -QTL, sample II		3×10^{-6}	~7500	Berrettini et al.(2)
Cigarettes per day - QTL		6×10^{-20}	13945	Thorgeirsson et al.(3)
DSM or FTND(4+)* vs ctrls (<10 cpd)*	1.40	7×10^{-15}	2394 / 3506	Thorgeirsson et al.(3)
DSM or FTND(4+)* vs pop ctrls	1.17	3.3×10^{-6}	2394 / 28455	Thorgeirsson et al.(3)
FTND (6-10 vs 0-4)**	1.5	1.3×10^{-4}	795 / 256	Weiss et al.(8)
Habitual vs light smoking***		7×10^{-3}	955 / 281	Bierut et al.(9)
Lung Cancer				
LC cases vs population ctrls	1.32	$< 1 \times 10^{-17}$	3878 / 4831	Amos et al.(4)
LC cases vs population ctrls	1.30	5×10^{-20}	4435 / 7272	Hung et al.(5)
LC cases vs population ctrls	1.31	1.5×10^{-8}	1024 / 32244	Thorgeirsson et al. (3)
Peripheral Arterial Disease				
PAD cases vs population ctrls	1.19	1.4×10^{-7}	2738 / 29964	Thorgeirsson et al. (3)

* The subjects included are a subset of the subjects used in the QTL study of cpd.

** Restricted to subjects progressing to regular smoking by age 16.

*** Family-based association study. The study sample (219 families) is from the collaborative study of the genetics of alcoholism (COGA).