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A glimpse into the future – Personalized medicine for smoking cessation

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Abstract

The devastating consequences of tobacco smoking for individuals and societies motivate studies to identify and understand the biological pathways that drive smoking behaviors, so that more effective preventions and treatments can be developed. Cigarette smokers respond to nicotine in different ways, with a small number of smokers remaining lifelong low-level smokers who never exhibit any symptoms of dependence, and a larger group becoming nicotine dependent. Whether or not a smoker transitions to nicotine dependence has clear genetic contributions, and variants in the genes encoding the $\alpha 5$ - $\alpha 3$ - $\beta 4$ nicotinic receptor subunits most strongly contribute to differences in the risk for developing nicotine dependence among smokers. More recent work reveals a differential response to pharmacologic treatment for smoking cessation based on these same genetic variants in the $\alpha 5$ - $\alpha 3$ - $\beta 4$ nicotinic receptor gene cluster. We anticipate a continuing acceleration of the translation of genetic discoveries into more successful treatment for smoking cessation. Given that over 400,000 people in the United States and over 5 million people worldwide die each year from smoking related illnesses, an improved understanding of the mechanisms underlying smoking behavior and smoking cessation must be a high public health priority so we can best intervene at both the public health level and the individual level.

Keywords

Nicotine dependence; Smoking cessation; Genetics; Nicotinic receptor genes; Nicotine metabolizing genes

1. Nicotine dependence – a major public health problem

Smoking is the greatest modifiable contributor to premature death in the U.S. and the world (Mokdad et al., 2004; World Health Organization, 2011; Centers for Disease Control, 2012a), and cigarette use will kill one in two long-term smokers (Centers for Disease Control, 2010). Each year over 400,000 people in the United States die of smoking related illnesses (Centers for Disease Control, 2008), and because of increasing cigarette use in developing nations, it is predicted that the worldwide death toll from smoking will increase

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Conflict of interest

Laura J. Bierut is listed as an inventor on Issued U.S. Patent 8,080,371, 'Markers for Addiction,' covering the use of certain SNPs in determining the diagnosis, prognosis, and treatment of addiction. Nancy L. Saccone is the spouse of Scott Saccone, who is also listed as an inventor on the above patent.

from the current 5.4 million persons per year to more than 8 million persons per year by 2030 (World Health Organization, 2011). The economic burden of smoking is correspondingly high. In the United States alone, annual costs are estimated at \$96 billion in direct medical expenses and \$97 billion in lost productivity (Centers for Disease Control, 2008). These important public health issues and associated economic costs motivate studies to identify and understand biological pathways that drive smoking behaviors so that more effective prevention and cessation treatments can be developed.

A revolution of genetic technologies is underway, and these new scientific tools can be brought to bear on the study of smoking behaviors and nicotine dependence. Millions of genetic variants (or single nucleotide polymorphisms; SNPs) can be assessed in the human genomes of tens of thousands of individuals, and the genetic examination of smoking behaviors can be undertaken on a scale not possible 10 years ago. These new technologies have facilitated investigations of many complex diseases, and thousands of new genetic findings have been made in the past decade (Hindorff et al., 2009, 2013). Modern genetic studies have significantly impacted our understanding of illnesses such as obesity, diabetes, and heart disease, and also nicotine dependence and smoking related illnesses. Specifically relevant here, recent genetic findings show promise to improve clinical care for smoking cessation.

2. Development of nicotine dependence – a multi-step process

Nicotine is the compound in tobacco that is primarily responsible for the maintenance of smoking behaviors and the development of dependence (Stolerman and Jarvis, 1995), and many people with severe smoking related illnesses remain unable to stop smoking due to the addictive nature of nicotine (Centers for Disease Control, 2013). To develop nicotine dependence, a person passes through a series of behavioral steps, and both environmental and genetic factors influence each transition. The first step in the development of nicotine dependence is the initiation of smoking, which occurs with the use of a first cigarette. The next step in this process is taken when an individual passes the threshold of smoking 100 cigarettes over a lifetime, and becomes a “smoker,” a definition that has been employed in large-scale epidemiological studies (Bondy et al., 2009). These early steps of smoking behavior typically occur in adolescence and are strongly influenced by environmental factors such as peer smoking, cigarette access, and cigarette cost (Kobus, 2003; Hoffman et al., 2006; Centers for Disease Control, 2012b). As cigarette use continues, smoking behaviors become more established, and a range of different smoking patterns is seen. At one end of behavior, some smokers remain lifelong low level cigarette users, or “chippers”, who never develop any symptoms of dependence. At the opposite extreme of behavior, some people increase their use of cigarettes, smoke cigarettes more intensively, and become addicted, heavy smokers. The addicted smokers also have the least success with smoking cessation. Twin studies convincingly demonstrate that a substantial contribution of genetic factors determines whether one becomes a lifelong light smoker or a nicotine dependent, heavy smoker (Li, 2006).

Fig. 1 shows data from the Collaborative Genetic Study of Nicotine Dependence (COGEN) where we tracked the number of people who transition through each step in this model of the development of nicotine dependence. We assessed individuals in the Detroit and St. Louis communities, 25–44 years of age, and we asked about their smoking history. Slightly over half of those queried reported smoking at least one cigarette in their lifetime; of those who smoked one cigarette, 58% continued using cigarettes and smoked 100 cigarettes in their lifetime. Among these smokers, 45% went on to develop nicotine dependence, defined by a score of 4 or more on the Fagerström Test for Nicotine Dependence (FTND) (Heatherton et al., 1991), and an additional third of smokers had some

symptoms of nicotine dependence. A minority of smokers, 20%, reported low levels of smoking that never escalated to dependence. This differential response to smoking, with a small number of smokers remaining lifelong low level smokers who never exhibit any symptoms of dependence, and another group of smokers becoming nicotine dependent, heavier smokers, identifies two extremes of smoking behaviors. These extremes mark people who are differentially susceptible to the addictive component of cigarettes (nicotine) and have very different rates of smoking cessation. This step of whether a smoker transitions to nicotine dependence has clear genetic contributions. In fact, within just the past few years, several specific genetic variants differentiating these groups of light smokers versus heavy smokers have been successfully identified.

3. Nicotinic acetylcholine receptor subunit genes – targets for genetic studies

Genetic variation in the genes encoding the nicotinic acetylcholine receptor subunits strongly contributes to differences in the risk of developing nicotine dependence among smokers. The most compelling genetic association with nicotine dependence is in the chromosomal region 15q25 that encompasses the $\alpha 5$ - $\alpha 3$ - $\beta 4$ nicotinic acetylcholine receptor subunit gene cluster (*CHRNA5-CHRNA3-CHRNA4*) (Saccone et al., 2007). Many independent studies have validated this association with nicotine dependence and with other smoking behavioral phenotypes such as cigarettes smoked per day (Berrettini et al., 2008; Thorgeirsson et al., 2008). Large-scale studies of over 73,000 European-ancestry individuals in genome-wide association (GWA) meta-analyses unequivocally identify this region as associated with heavy smoking ($p = 5.57 \times 10^{-72}$) (Liu et al., 2010; Thorgeirsson et al., 2010; Tobacco and Genetics Consortium, 2010).

One method to dissect and understand these findings is to compare genetic variant associations across diverse human populations. Genetic risk factors that are consistent across European, Asian, and African populations point to variants that are more likely to cause biologic changes, which in turn lead to disease association. The chromosome 15 region containing the *CHRNA5-CHRNA3-CHRNA4* gene cluster has very different genetic architecture across these three populations, and these differences have been leveraged to narrow down potential functional association signals. In a large, international, collaborative meta-analysis, the association between variation in the *CHRNA5-CHRNA3-CHRNA4* genes and smoking quantity was examined in over 22,000 smokers of European, Asian, and African descent. Despite the diverse genetic backgrounds across these populations and the widely varying frequencies of the risk variation, from 5% to over 35%, the variant rs16969968 is clearly associated with heavy smoking behavior across all populations. This consistent association across populations of various ancestries provides evidence that rs16969968 is most likely a causative functional variant that alters the susceptibility to nicotine dependence (Chen et al., 2012a).

4. $\alpha 5$ nicotinic acetylcholine receptor subunit gene – biologic function

Taking these consistent genetic association findings to the next step, laboratory studies point to potential biological mechanisms that alter the risk of developing nicotine dependence. The variant rs16969968, which is consistently associated across multiple populations, changes an amino acid from aspartic acid to asparagine in the $\alpha 5$ nicotinic acetylcholine receptor subunit protein in a region of the protein that is highly conserved across different species (Bierut et al., 2008). The nicotinic acetylcholine receptor is a pentameric structure, and an *in vitro* functional study finds that $(\alpha 4\beta 2)_2\alpha 5$ receptors that contain the $\alpha 5$ subunit protein with the high genetic risk asparagine amino acid substitution exhibit a reduced response to a nicotine agonist compared with receptors containing the low genetic risk

aspartic acid change (Bierut et al., 2008). Further studies show that $(\alpha 4\beta 2)_2\alpha 5$ nicotinic acetylcholine receptors that contain an $\alpha 5$ subunit protein with the amino acid substitution (asparagine) have lower calcium permeability and increased short-term desensitization compared to receptors that contain the $\alpha 5$ protein with aspartic acid at that position (Kuryatov et al., 2011). More recent biologic experiments have shown similar effects of the $\alpha 5$ amino acid change on the function of other nicotinic acetylcholine receptors that contain the $\alpha 5$ subunit, such as $(\alpha 3\beta 4)_2\alpha 5$ (Tammimaki et al., 2012). These studies demonstrate that the genetic variant, rs16969968, in the $\alpha 5$ nicotinic receptor subunit that increases risk of developing nicotine dependence decreases functional response of the nicotinic acetylcholine receptor.

Mouse model work has been highly informative about the role of the $\alpha 5$ nicotinic acetylcholine receptor subunit in the development of addictive behavior. Addictive behavior reflects a balance between reinforcing and aversive properties of a drug. At lower doses, addictive drug use stimulates reinforcing properties, which leads to increased drug intake (Lynch and Carroll, 2001). At higher levels of drug use, aversive effects become more prominent and begin to limit further intake (Henningfield and Goldberg, 1983; Lynch and Carroll, 1999). Studies of *CHRNA5* knock-out mice show a disassociation between these reinforcing and aversive properties of nicotine intake (Fowler et al., 2011). Both wild type and $\alpha 5$ subunit knockout mice have similar intake of nicotine at lower doses, which reflects the stimulatory effects of nicotine on brain reinforcement systems. At higher doses of nicotine, differences in nicotine intake are seen between wild type and $\alpha 5$ knockout mice. Wild type mice decrease intake at higher doses of nicotine, consistent with stimulation of aversive pathways related to nicotine. However, $\alpha 5$ subunit knockout mice consume greater amounts of nicotine at high doses, which suggests an attenuation of the aversive effects of nicotine. These differences in nicotine intake suggest that the $\alpha 5$ nicotinic acetylcholine receptor subunit plays an important role in the aversive components of nicotine intake and does not have a significant role in reinforcing effects.

Further experiments of mice with the $\alpha 5$ nicotinic acetylcholine receptor subunit knocked out highlight the role of the medial habenula in the control of nicotine intake. Expression of $\alpha 5$ nicotinic acetylcholine receptor subunit is concentrated in the habenulo-interpeduncular pathway (Fowler et al., 2011). The medial habenula projects almost exclusively to the interpeduncular pathway, and high dose nicotine activates this pathway (Herkenham and Nauta, 1979; London et al., 1988). Reintroduction of $\alpha 5$ nicotinic acetylcholine receptor subunits into the medial habenula of $\alpha 5$ knockout mice restores the nicotine intake patterns of wild type mice (Fowler et al., 2011). These findings demonstrate that the $\alpha 5$ nicotinic acetylcholine receptor subunit is a key component in the habenulo-interpeduncular neurocircuit controlling nicotine intake.

In sum, human genetic, functional, and animal studies link differences in smoking behavior to specific genes and genetic variants, and those variants are linked to basic cellular mechanisms and to brain regions. These studies converge on a conceptual framework of a balance between reinforcing and aversive effects of nicotine use. Individuals who smoke more heavily and become nicotine dependent are more likely to have risk variants in the $\alpha 5$ nicotinic acetylcholine receptor subunit. The risk allele, rs16969968, associated with nicotine dependence results in an amino acid change in the $\alpha 5$ nicotinic acetylcholine receptor subunit. The incorporation of this high genetic risk $\alpha 5$ subunit into nicotinic acetylcholine receptors decreases receptor function compared to receptors containing the low genetic risk $\alpha 5$ subunit. Similarly, mice with the $\alpha 5$ subunit gene knocked out show increased nicotine intake, consistent with a loss of the aversive effects related to nicotine. Reintroduction of $\alpha 5$ subunit gene into the medial habenula of knockout mice restores wild type patterns of nicotine consumption. These convergent findings support a model of the

habenulo-interpeduncular pathway as a key neurocircuit that acts as an inhibitory pathway which limits nicotine intake. Within this circuit the $\alpha 5$ subunit plays an important role in nicotinic acetylcholine receptor function, and reduced receptor function decreases the inhibitory signaling that in turn limits nicotine intake. This model is consistent with the finding that individuals who carry risk alleles for nicotine dependence are less sensitive to the aversive effects of nicotine and can smoke more cigarettes.

5. Multiple variants in the $\alpha 5$ nicotinic acetylcholine receptor subunit gene influence risk

Though we have focused on one variant associated with nicotine dependence, rs16969968, further studies demonstrate that multiple variants in this chromosome 15q25 region encompassing the $\alpha 5$ - $\alpha 3$ - $\beta 4$ nicotinic receptor subunit genes independently contribute to the risk of becoming nicotine dependent (Stevens et al., 2008; Saccone et al., 2009, 2010; Liu et al., 2010). A second independent genetic association with nicotine dependence is marked by rs880395 (and other highly correlated SNPs in European-ancestry populations, such as rs588765), and this group of SNPs is strongly associated with *CHRNA5* mRNA expression levels in the brain and lung (Falvella et al., 2009; Wang et al., 2009a,b; Smith et al., 2011). Low level *CHRNA5* mRNA expression is associated with a reduction of the risk of developing nicotine dependence. Fine mapping studies using allele specific gene expression in tissue from European and African American brain narrow down the most likely region containing the functional alleles for expression regulation to a 10 kb region upstream of the transcriptional startsite of *CHRNA5* (Smith et al., 2011). Another signal upstream of *CHRNA5* has recently been identified in a meta-analysis of heaviness of smoking among African Americans (Hamidovic et al., 2011; David et al., 2012) and may represent an additional, distinct associated risk factor for nicotine dependence. These studies imply that complex regulation of nicotinic acetylcholine receptor subunits contribute to a person's overall risk of becoming nicotine dependent.

6. Translation

6.1. Interplay of genes and environments

Once genetic factors contributing to disease susceptibility have been identified, a next goal is to find environmental factors that can alter this genetic vulnerability. One important environmental risk factor for the development of heavy smoking and nicotine dependence is the initiation of smoking at a young age. In a collaborative study of 34 research groups, we investigated the impact of early onset smoking on the association between genetic variation in *CHRNA5* and heavy smoking. This meta-analysis included over 90,000 subjects, and results indicate that the genetic risk for heavy smoking tagged by the variant rs16969968 is greater in early onset smokers (onset at 16 years or younger), as compared to later onset smokers (Hartz et al., 2012). See Fig. 2. In other words, the genetic risk for developing heavy smoking and nicotine dependence is augmented if one starts smoking in adolescence. These findings are supported by animal studies, which demonstrate that the adolescent brain is particularly vulnerable to the addictive effects of nicotine compared to adult animals (Wiling et al., 2012). It is noteworthy that other gene-environment interactions have also been observed with the rs16969968 polymorphism for nicotine dependence. Level of parent monitoring during adolescence (Chen et al., 2009) and number of adolescent peers who smoke (Johnson et al., 2010) both alter the influence of this genetic variation in the $\alpha 5$ nicotinic acetylcholine receptor subunit gene on the risk of developing nicotine dependence. These studies of the interplay of genetic variation and modifiable environments strengthen public health messages to reduce adolescent smoking and may thus decrease genetic risk of developing nicotine dependence.

6.2. Smoking cessation success

The most important public health significance of understanding the genetic underpinnings of nicotine dependence comes from translating that knowledge into improved treatment for smoking cessation. Nicotine dependence is one of the strongest predictors of ongoing smoking. For instance, in our assessment of smoking behavior in the Detroit and St. Louis communities, only 33% of nicotine dependent smokers aged 25–44 years of age quit smoking at the time of the interview, whereas 67% of light smokers had stopped smoking (see Fig. 1). Thus, we anticipate that genetic variants that increase the risk of developing nicotine dependence will also play a role in successful smoking cessation. Though the evidence of association of the chromosome 15q25 region with nicotine dependence and heavy smoking is unequivocal, genetic studies of smoking cessation with the region have shown more conflicting results. Several of the initial studies of smoking cessation have found little to no evidence of genetic associations with the chromosome 15q25 region containing *CHRNA5* and current versus former smoking status (Breitling et al., 2009; Tobacco and Genetics Consortium, 2010).

Smoking cessation is a complex behavior clearly influenced by many environmental factors and biologic predispositions. Growing evidence shows that the more genetically influenced smoking cessation phenotype is captured early in the course of a quit attempt and can be conceptualized as relapse to smoking. The first study to clearly show an association of the chromosome 15q25 region with quitting smoking was an examination of women who smoked during pregnancy (Freathy et al., 2009). Pregnancy can be thought of as a time when environmental and social forces maximally encourage smoking cessation. Because of concerns about the health of the mother and baby, physicians and other health care providers recommend smoking cessation, and socially, smoking during pregnancy is discouraged. Freathy et al. (2009) studied a large cohort of pregnant women, and identified those who smoked during the first trimester of pregnancy. Continued smoking throughout pregnancy (or in other words failed smoking cessation) was predicted by the same genetic variants in *CHRNA5* that increased the risk of developing nicotine dependence. Those who quit smoking during pregnancy were more likely to have the low-risk genetic variants in *CHRNA5*. These results have been confirmed in a second, independent sample of pregnant women who smoked (Thorgeirsson and Stefansson, 2010). In both of these studies, smoking cessation was undertaken in a naturalistic setting and most likely was without pharmacologic intervention. Both studies confirmed the strong genetic influence of variation in *CHRNA5* on smoking cessation during this time limited period when environmental and social influences are maximally encouraging smoking cessation.

As with the general population studies of smoking cessation, many smoking cessation trials that examined the genetic association of *CHRNA5* and successful smoking cessation have also had mixed results. Some of the smoking cessation treatment studies have failed to find an association with these genetic variants in the chromosome 15q25 region and successful quitting (Conti et al., 2008; Bousman et al., 2012), whereas others have seen a modest effect (Munafo et al., 2011). We believe that these conflicting results are due to the complex interplay between pharmacologic treatment and genetic risk factors.

Our recent study demonstrates that the risk of failed smoking cessation is associated with the same genetic risk variants that predict a greater likelihood of nicotine dependence, and this genetic risk can be modified by pharmacologic treatment. See Fig. 3. In this placebo controlled clinical trial of smoking cessation, all subjects underwent rigorous counseling. In the placebo arm of the smoking cessation trial, subjects with the low-risk genetic variants in *CHRNA5*, marked by haplotype 1, have the highest rate of abstinence at the end of treatment. In contrast, subjects with the high-risk genetic variants in *CHRNA5* which increase the risk of developing nicotine dependence and are marked by haplotype 3, show

the lowest rate of abstinence at the end of treatment. A different pattern of abstinence at end of treatment is seen in the pharmacologic treatment arm of the study. With pharmacologic treatment using nicotine replacement therapy, bupropion, or both medications, subjects in the high-risk haplotype 3 group respond vigorously to treatment, with a 2–3 fold increase in abstinence compared to subjects with the haplotype 3 who received placebo medication. In contrast, subjects in the low-risk genetic risk group, haplotype 1, have similar abstinence rates at end of treatment with or without pharmacologic intervention. These results reveal a statistically significant interaction between the genetic variants in the $\alpha 5$ - $\alpha 3$ - $\beta 4$ nicotinic acetylcholine receptor subunit gene cluster and smoking cessation pharmacotherapy on the outcome of abstinence at end of treatment (Chen et al., 2012b). This finding, which is now supported by a combined analysis of multiple clinical trials (Bergen et al., 2013), is complementary to the naturalistic association findings of ongoing smoking during pregnancy. In this smoking cessation trial, difficulty with smoking cessation related to genetic risk in the $\alpha 5$ nicotinic acetylcholine receptor subunit is ameliorated by aggressive pharmacological treatment.

The clinical impact of this genetic and treatment interaction is exemplified by the number needed to treat (NNT). The NNT is the number of people who must receive the treatment in order for one person to benefit. For example, the optimal NNT is one, which means each person who receives treatment benefits from it. On the other hand, a large NNT implies that numerous people receive treatment and most do not benefit from it. In the genetic study of smoking cessation by Chen et al. (2012b), the NNT varies widely depending on haplotype: NNT is 4 for smokers with the high-risk haplotype (H3), and thus the high-risk haplotype group (H3) receives a strong benefit from pharmacologic treatment. On the other hand, the NNT is greater than 1000 for smokers with the low-risk haplotype (H1), indicating that the low-risk haplotype group receives essentially no added benefit from pharmacologic treatment for smoking cessation. This impressive difference in NNT between haplotypes and treatment represents the first investigation in which genetic variant by treatment interaction is identified for smoking cessation in the chromosome 15q25 region. This work is an advance towards achieving our goal of personalized medicine where genetic information can distinguish between patients who are likely to respond strongly to pharmacologic treatment and those who receive no benefit for smoking cessation.

7. Future of genetic studies of nicotine dependence and smoking cessation

This review highlights results from the strongest region associated with nicotine dependence, the genomic area encompassing the $\alpha 5$ - $\alpha 3$ - $\beta 4$ nicotinic receptor subunit gene cluster. As we continue to identify genetic factors that alter risk for developing nicotine dependence, an improved understanding of the underlying biological mechanisms of addiction will follow. Some of these studies must be done in animal models, such as a “knock-in” mouse, where experimental conditions can be more controlled. A knock-in mouse has now been engineered to possess the human rs16969968 variant (*Chrna5* D398N) and thus the amino acid change in the $\alpha 5$ nicotinic acetylcholine subunit protein. This mouse is one of only a very few mice that have been engineered to possess a human polymorphism associated with substance dependence, and it is the first for a human polymorphism associated with nicotine dependence. This novel resource will permit an exploration of important molecular, neurobiological and behavioral mechanisms through which this genetic variant alters risk for nicotine dependence and smoking related illnesses. This approach will provide unique insights into the molecular and neurobiological mechanisms through which this risk polymorphism affects brain function.

It is also becoming clear that both common and less frequent genetic variants in the chromosome 15 region containing the $\alpha 5$ - $\alpha 3$ - $\beta 5$ nicotinic receptor gene cluster contribute to

the development of nicotine dependence. For example, less common (frequency 1%–5%) and rare (frequency <1%) variation in the $\alpha 3$ and $\beta 4$ nicotinic receptor subunit genes is associated with an altered risk for nicotine dependence and cigarette consumption (Haller et al., 2012). These less common variants also alter the protein structure of the $\alpha 3$ and $\beta 4$ nicotinic receptor subunits, and expression of these variants in human cells results in altered dose response curves for nicotine, but not the natural ligand acetylcholine. Many of these variant proteins also show reduced cell surface expression, suggesting that the variants may affect protein folding and trafficking. These findings demonstrate that we must delve more deeply into the regions associated with nicotine dependence to understand the multiple variants that alter the complex biological mechanisms underlying the risk of developing nicotine dependence.

Other genetic regions also contribute to the development of nicotine dependence. Genetic variation in the $\alpha 6$ and $\beta 3$ nicotinic receptor subunit gene cluster is correlated with nicotine dependence (Bierut et al., 2007; Thorgeirsson et al., 2010). There is growing evidence that variants in *CHRNA4* and *CHRNA5* also contribute to the risk of nicotine dependence (Wessel et al., 2010; Xie et al., 2011; Haller et al., 2012). Genetic differences in nicotine metabolism also play an important role in the development of nicotine dependence and heaviness of smoking (Thorgeirsson et al., 2010; Tobacco and Genetics Consortium, 2010). The primary gene that metabolizes nicotine, *CYP2A6*, accounts for the majority of the initial conversion of nicotine to cotinine (Yamazaki et al., 1999). Though a large part of the genetic variance related to the development of nicotine dependence remains unexplained, these initial genetic discoveries begin to uncover genetic underpinnings of smoking behaviors.

Further genetic work on phenotypes and diseases related to nicotine dependence will also be important. The *CHRNA5-CHRNA3-CHRNA4* region is associated not only with nicotine dependence, but also with diseases for which smoking is the major risk factor, such as lung cancer (Amos et al., 2008; Hung et al., 2008; Thorgeirsson et al., 2008) and chronic obstructive pulmonary disease (Young et al., 2008; Pillai et al., 2009). While there is agreement that *CHRNA5-CHRNA3-CHRNA4* variants have a clear effect on smoking behavior, the full degree to which these effects on smoking explain the associations with consequent medical disorders remains under debate. Key biomarkers such as cotinine (Keskitalo et al., 2009; Munafo et al., 2012), other nicotine metabolites, and carcinogen metabolites will provide important information about smoking exposure to help answer these questions. Other smoking behaviors beyond nicotine dependence are of public health importance. For example, smoking initiation, while necessary to the development of nicotine dependence, involves genetic risk factors that differ from those already known for nicotine dependence (Belsky et al., 2013).

8. Conclusion

In summary, though smoking has been greatly reduced in the U.S. over the last two decades, nearly one in five adults remain current smokers (Centers for Disease Control, 2012a). Teenagers continue to initiate cigarette use, with many 12th graders reporting ever smoking (39.5%), smoking in the past 30 days (17.1%), and daily smoking (9.3%) (Johnston et al., 2012). Once smoking behaviors are established and a person has developed nicotine dependence, cessation is difficult. The important contribution of *CHRNA5* to the development of heavy smoking and nicotine dependence has been confirmed in a prospective longitudinal study of smoking behaviors starting in adolescence. Individuals at high genetic risk are more likely to convert to daily smoking as teenagers, progress more rapidly to heavy smoking, develop nicotine dependence more frequently, and are more likely to fail in smoking cessation attempts (Belsky et al., 2013).

Problems with smoking cessation are further illustrated by the high proportion of people with smoking related illnesses who continue to smoke, including those with bronchitis (41.1%), coronary heart disease (29.3%), emphysema (49.1%), and smoking related cancers (other than lung) (38.8%) (Centers for Disease Control, 2007). Smoking cessation is difficult, and individuals continue to smoke even though a majority of current smokers (68%) report wanting to quit (Centers for Disease Control, 2011). Our goal is to use modern genetic approaches to push the boundaries of our understanding of the fundamental neurobiological underpinnings of who develops nicotine dependence and to then translate these findings into clinical interventions to disrupt the development of heavy smoking and aid those who wish to quit smoking. We anticipate an acceleration of this translation of genetic findings into more successful treatments for smoking cessation. Given that over 400,000 people in the U.S. and over 5 million people world-wide die each year from smoking related illnesses, an improved understanding of the genetic and environmental mechanisms underlying smoking behavior and smoking cessation must be a high public health priority so we can best intervene at both the individual and community levels (Institute of Medicine, 2007; U.S. Department of Health and Human Services, 2010; World Health Organization, 2011; Koh and Sebelius, 2012).

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Fig. 1.
Smoking behaviors in the COGEND screening sample.

Fig. 2.

Meta-analysis of the association between the rs16969968 genotype (where A is the risk allele) and heavy smoking (cigarettes per day >20) vs. light smoking (cigarettes per day 10), stratified by early-onset smoking (age at onset ≤16 years) and late-onset smoking (onset >16 years). Odds ratios (ORs) are given relative to late-onset smokers with the GG genotype. Effect of the interaction between the rs16969968 A allele and early-onset smoking on risk of heavy smoking: OR = 1.16, $n = 36,936$, $P = 0.01$. Figure from Hartz et al., 2012.

Fig. 3.

Response to smoking cessation pharmacotherapy varies by *CHRNA5* haplotypes. ^a*CHRNA5* haplotypes are associated with failed smoking cessation in the placebo group (Wald $\chi^2 = 7.02$, $df = 2$, $p = 0.03$). *CHRNA5* haplotypes are not associated with smoking cessation outcomes in the treatment group (Wald $\chi^2 = 1.45$, $df = 2$, $p = 0.48$). The interaction of haplotypes and treatment on abstinence is significant (Wald $\chi^2 = 8.97$, $df = 2$, $p = 0.01$). [^a*CHRNA5* haplotypes based on rs16969968 and rs680244 ($N = 1073$); H1 = G-C (21%); H2 = G-T; (44%) H3 = A-C (35%).] Figure from Chen et al., 2012b.