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CYP2A6 genetic variation alters striatal-cingulate circuits, network hubs and executive processing in smokers

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Abstract

Background—Variation in the *CYP2A6* gene alters the rate of nicotine metabolic inactivation and is associated with smoking behaviors and cessation success rates. The underlying neurobiological mechanisms of this genetic influence remain unknown.

Methods—Intrinsic functional connectivity strength (FCS), a whole brain, data driven, graph theory based method, was applied to resting state fMRI data in 66 smokers and 92 nonsmokers. A subset of subjects (n=23/20; smokers/nonsmokers) performed the Monetary Incentive Delay (MID) task, probing reward anticipation and a Go/NoGo task, probing response inhibition, on two occasions, in the presence and absence of a nicotine patch.

Results—A significant *CYP2A6* genotype × Smoking effect was found in the dorsal anterior cingulate cortex (dACC) and ventral striatum (VS), such that the normal (vs. slow) genotype individuals showed greater FCS in smokers but not nonsmokers. FCS was negatively associated with nicotine dependence severity in slow metabolizers. Both hubs were biased by inputs from the insula identified from seed-based connectivity. Similar Gene × Environment interactions were seen in VS during smoking abstinence when subjects performed the MID task and in dACC when they performed the Go/NoGo task; both reductions were “normalized” in smokers (and increased in nonsmokers) following acute nicotine administration.

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Conclusions—Since the *CYP2A6* effect was seen only in smokers, these data suggest that the rate of nicotine metabolism, and thus the concentration of nicotine presented to brain over the course of nicotine addiction, shapes brain circuits that, among other functions, compute reward and impulsivity processes.

Keywords

CYP2A6 genotype; smoking; nicotine; functional connectivity strength; striatal-cingulate-insula circuits; neuroadaptations

Introduction

Nicotine is the principal addictive component in tobacco that maintains smoking behavior. Nicotine (and other drug) dependence induces neuroplastic alterations in brain circuits (1), which in turn contribute to the altered behavioral and cognitive processes seen in dependent individuals that impact their ability to stop smoking (2). However, identifying the neuroanatomical loci and then mechanistically linking these dependence-induced neuroadaptations and genetic influences with their behavioral consequences remains very poorly understood. With the consistently poor outcomes following treatment for nicotine dependence (3), empirically based neurobiological mechanisms have the potential to improve treatment efficacy via individualized treatment optimization along with the discovery of novel drug targets.

The hepatic enzyme cytochrome P450 (*CYP2A6*) is the main metabolic pathway responsible for nicotine inactivation and clearance (4); *CYP2A6* is expressed at very low levels in the human brain, or not at all (5, 6). Variation in the *CYP2A6* gene alters the rate of nicotine metabolism, with the slow genotype reducing nicotine metabolism more than 50% (7). Smokers with the reduced (slow and intermediate) *CYP2A6* genotype start smoking earlier in life (8–10), become dependent quicker (11, 12), but smoke fewer cigarettes (10, 13) and have a higher likelihood of quitting smoking (14, 15). Slow metabolizers have higher quit success rates when using either placebo or transdermal nicotine replacement therapy (NRT, i.e. nicotine patch) vs. varenicline (Chantix®) usage, but have greater adverse effects from varenicline, while normal genotype individuals respond better to varenicline than to NRT (16–18). Thus these *CYP2A6* genetic effects on cessation persist months after being nicotine free, suggesting that genetic variations in this metabolic pathway likely created long-lasting brain changes that result in these differential smoking phenotypes and treatment response differences.

The underlying neurobiological mechanisms of the impact of this genetically variable pharmacokinetic (PK) enzyme on smoking-related behaviors and neuroplasticity are largely unknown. Only a single study has been published, which reported that slow metabolizing smokers have reduced limbic responses to smoking cues (19). However, both human and preclinical data indicate the importance of PK influences on drug addiction – both the rate of drug onset (primarily influenced by route of delivery) and offset (primarily influenced by biotransformation and distribution) (20). Smoking (relative to nicotine patch) delivers nicotine to the brain in seconds, while genetically variable rates of nicotine inactivation by

CYP2A6 leads to variable offset time, both of which may result in rapid and long lasting neuroplastic effects and differential abuse potential. Indeed, the rapid onset of action for both nicotine and cocaine preferentially engage mesocorticolimbic (MCL) circuits to promote neurobehavioral plasticity (21). In contrast, variation in the ‘off-time’ can alter the abuse and dependence of drugs from a number of classes, including cocaine, benzodiazepines and nicotine (22). For example, while both cocaine and methylphenidate have high affinity for the dopamine transporter and reach the brain rapidly after intravenous administration, cocaine’s shorter duration of action, due to rapid removal from brain, both promotes and enables its frequent use, while methylphenidate has a much longer duration of action due to its slower brain clearance and has a much lower abuse profile (23).

Since smokers with the slow metabolism *CYP2A6* genotype have relatively constant nicotine plasma levels throughout the day, while those with faster metabolism likely experience a greater surge in plasma nicotine with each cigarette (24), and as changes in PK can change fundamental neurobiological mechanisms, we reasoned that *CYP2A6*-induced modifications in brain nicotine concentration differentially alters nicotinic receptor function and in turn, modifies brain circuits that contribute to the development and/or maintenance of nicotine addiction and its plasticity-related behavioral and cognitive profile. We also reasoned that this would occur only among smokers, while those not exposed to nicotine would have no genotype-induced neuroplastic differences, leading to significant and meaningful Gene $\times$ Environment (*CYP2A6*-smoking) interactions.

However, as smokers present with a spectrum of altered behaviors and neuronal circuits (25), we could not *a priori* predict those circuits that might be most sensitive to variable nicotine exposure. We thus interrogated the effects of *CYP2A6* genotype on whole brain network topological organization using resting state functional connectivity (rsFC) (26) and a data driven analytical graph theory metric, functional connectivity strength (FCS). High FCS of a region is thought to reflect high nodal activity, thought to reflect regions of high neuronal integration (27–29).

To complement this data driven network approach, we also considered smoking status (smoker vs. nonsmoker), current level of smoking (cigarettes per day, CPD) and nicotine dependence severity (Fagerstrom Test for Nicotine Dependence, FTND (30)). We hypothesized that the *CYP2A6* genotype would, among smokers but not among nonsmokers, 1) influence brain FCS; 2) alter FCS within nodes of the MCL dopamine system; and 3) modulate neuronal computations and cognitive processes, specifically reward processing and inhibitory control.

To test these hypotheses, we genotyped smokers and nonsmokers for the *CYP2A6* polymorphism and subsequently classified them as normal, intermediate or slow nicotine metabolizers (16, 19, 31). In addition to acquiring resting state BOLD data, a subset of smokers and nonsmokers also performed the MID reward task and a Go/NoGo response inhibition task on two sessions—once while wearing a nicotine patch and once after 12 hours of smoking abstinence.

Methods and Materials

Participants

Resting state BOLD fMRI data were acquired from non-treatment seeking smokers (n=66) and nonsmokers (n=92), matched by age, sex, IQ and race (Table 1). Subjects were selected from a large cohort of individuals who gave written informed consent under protocols approved by the NIDA-IRP IRB. IRB approval for *CYP2A6* genotyping was granted at the University of Toronto. A subset of smokers (n=23; 15 normal and 8 reduced metabolizers) and nonsmokers (n=20; 9 normal and 11 reduced) also performed two fMRI tasks following resting BOLD data acquisition: the Monetary Incentive Delay (MID) task, which assesses neuronal activity during anticipation of and outcome for monetary rewards (32), and a Go/NoGo task assessing response inhibition (33). See SI for details of fMRI data acquisition and preprocessing and voxel-based morphometry (VBM) data analyses and results.

Genotyping

Genomic DNA was extracted from blood samples and genotyped for *CYP2A6* as described (16, 19, 31). Based on the impact of enzymatic activity (7, 31), subjects were grouped into normal, intermediate and slow metabolizers (16, 19, 31). Due to the more limited cohort size available for task data, we combined the slow and intermediate genotype groups into a single reduced genotype group (19) for task analysis.

FCS analysis

FCS, defined as the sum of functional connectivity between any one voxel and all other brain voxels (27, 28), was computed for each subject from 6 min of BOLD fMRI acquired with subjects' eyes open. Functional connectivity between voxels was measured using Pearson correlation in MATLAB (The MathWorks Inc, Natick, Massachusetts). FCS was computed after thresholding the correlation map at $r > 0.25$ (28). A voxel-wise threshold of $P < 0.01$ and a spatial-extent of 30 contiguous voxels were determined via Monte Carlo simulation to provide a corrected threshold of $P < 0.05$.

MID and Go/NoGo tasks

Subjects performed two tasks on two occasions, separated by 1–7 days. A nicotine or placebo patch was placed, in random order, 2–9 hours prior to scanning. Trials in the MID task included four sequential stimuli: PRIME-1 (gain, loss or neutral), PRIME-2 (win/loss magnitude), TARGET, and FEEDBACK; a button press was required to the TARGET. For the Go/NoGo task, subjects viewed a serial stream of alternating 'X' and 'Y' letters and were to respond to each target while the stimulus was present on the screen. NoGo stimuli, in which the target stimuli did not alternate (e.g. X, X), required response inhibition (34–36).

Since we had strong *a priori* interest in the ventral striatum (VS) for the MID task and dorsal anterior cingulate cortex (dACC) for the Go/NoGo task, we did region of interest analysis and small volume correction ($P_{\text{corrected}} < 0.05$) using respectively, a striatal atlas (<http://fsl.fmrib.ox.ac.uk/fsl>) and dACC mask (37).

Statistical analysis

A 3 (Genotype) $\times$ 2 (Smokers, Nonsmokers) ANCOVA was performed on whole brain FCS maps, using age, gender, IQ, race and years of education as covariates. Post-hoc one-way ANOVA and two-tailed independent *t*-tests identified differences between groups. MID and Go/NoGo tasks were analyzed using GLM (35). Associations between FCS in regions showing significant GENOTYPE $\times$ SMOKING interactions and FTND/CPD were tested using Pearson's correlation analyses. The mean FCS in these regions was regressed against age, IQ and years of education. One and two way ANOVAs followed by Tukey's *t*-test for multiple comparisons and frequency analyses (Pearson's χ^2 -test) were conducted (SPSS 20, IBM, Armonk, New York) to compare demographic and behavioral characteristics among groups.

Results

Demographics, genotypes and behavioral characteristics

For the entire sample, there was no significant difference in the frequency distribution of the three *CYP2A6* groups between smokers and nonsmokers ($P = 0.36$) and the *CYP2A6* genotype frequency distributions were in Hardy-Weinberg equilibrium for both groups. The samples were well matched, although nonsmokers had higher verbal IQ ($P = 0.002$) and years of education ($P = 0.004$) than smokers, which did not differ between the three genotype groups in either cohort. As expected (9–11, 13), there was a significant difference in CPD ($P = 0.04$) between the three genotype smoker groups, such that slow (vs. normal) genotype smokers smoked fewer cigarettes ($P = 0.003$) (See Table 1 for details). Groups were generally well matched in the task subgroup as well except that smokers and reduced genotype individuals both had lower IQ and fewer years of education than nonsmokers (see Table 2). Additionally, there was no difference in the frequency distribution of the four groups ($P = 0.18$) with the *CYP2A6* genotype frequency distributions were in Hardy-Weinberg equilibrium.

FCS and correlation with smoking behaviors

FCS was utilized as a metric of regional connectivity strength (27, 28). A 3 $\times$ 2 ANCOVA on whole brain FCS revealed a significant *CYP2A6* GENOTYPE $\times$ SMOKING status interaction in only two brain regions, the dACC and left VS ($P_{\text{corrected}} < 0.05$) (Fig. 1A and Supplementary Table 1). Based on their anatomical overlap with the FCS analysis, post-hoc analyses (whole brain, voxel-wise, one-way ANCOVA, followed by two sample *t*-test) suggest that in both brain regions smokers with the normal metabolizer genotype had higher FCS than a) all nonsmoker groups and b) smokers with the slow metabolizing genotype (Fig. 1 B–C). Importantly, there was no effect of the *CYP2A6* genotype on FCS among nonsmokers.

Exploratory correlation analysis between FCS and dependence severity (FTND) revealed a negative correlation in both hub regions in the slow but not in the normal or intermediate genotype groups, i.e. as smoking dependence increased, FCS decreased in VS ($r = -0.51$, $P = 0.01$) and dACC ($r = -0.44$, $P = 0.04$) (Fig. 1 D–E). These data recapitulate negative correlations in these addiction-related regions previously identified using different analytical

strategies and samples (37, 38). Finally, there was no relationship between FCS and CPD across the three genotype groups.

To determine those brain circuits contributing to the above GENOTYPE $\times$ SMOKING interaction on FCS, we next used the identified dACC and VS regions as seeds in a functional connectivity analysis. We identified a functional circuit between the dACC and bilateral insula and posterior cingulate cortex that contributed to the dACC FCS hub finding (Fig. 2A), while a functional circuit between the VS and bilateral insula and dACC contributed to the VS interaction (Fig. 2B) ($P_{\text{corrected}} < 0.05$)

Task-induced striatal and dACC activity

To address the functional consequences of the above GENOTYPE $\times$ SMOKING alterations relevant to nicotine dependence, a subset of smokers and nonsmokers ($n=43$) performed the MID reward task and the Go/NoGo response inhibition task on two separate occasions, in the presence and absence of an acute nicotine patch. A standard general linear model (GLM) approach was used in the analysis.

MID—Consistent with previous reports in both healthy individuals (32) and smokers (34, 36), subjects showed greater VS activation in the anticipatory gain compared with the neutral reward expectation condition (there was no effect in the [loss-neutral] condition). Consistent with the pattern seen in the FCS analysis (Fig. 1), there was a significant GENOTYPE $\times$ SMOKING interaction in VS activation in the placebo patch (abstinent) condition such that there was less activation in smokers with the reduced (slow and intermediate) versus normal metabolizing genotype, which was, once again, not seen in nonsmokers (Fig. 3A). In contrast, NRT administration appeared to ‘reverse’ the reduced VS response in smokers such that there was now no longer a genotype specific signal difference when anticipating gains.

Go/NoGo—Consistent with that seen in the MID task, smokers with the reduced genotype showed reduced dACC activity during unsuccessful inhibitions (Fig. 3B) when they were in the abstinent state than normal metabolizers. Acute NRT again appeared to enhance activation in this group up to that of the normal genotype group. Consistent with the metabolic role of the CYP enzyme, task activity was enhanced by NRT in nonsmokers, but only in the reduced metabolizers.

Finally, there was a main effect of *CYP2A6* GENOTYPE in bilateral precentral gyrus and bilateral anterior insula, such that the normal genotype group had higher FCS than those with the slow and intermediate genotypes, regardless of smoking status (Fig. 4 A, C). There was also a main effect of SMOKING in the bilateral superior temporal gyrus, with smokers showing decreased FCS across the three genotypes (Fig. 4 B, D and Supplemental Table 1).

Discussion

Alterations in the environment can modulate the influence of genetics upon behavioral outcomes, including nicotine addiction (39). Individuals with the slow *CYP2A6* genotype, which leads to higher levels and/or longer durations of plasma and brain nicotine after

smoking (7, 15, 24), display distinct smoking behaviors compared to those with the normal genotype, including levels of dependence and quit rates (9, 10, 40). To understand the neurobiological mechanisms underlying this altered smoking phenotype and in service of potential clinical implications, we examined the effect of *CYP2A6*, smoking status and their interaction on brain intrinsic functional connectivity and task-induced activity. We found that in smokers *CYP2A6* modified neuronal circuits involved in computations during the processing of both reward and response inhibition, and critically, does so consistent with the underlying resting network strength, supporting a behaviorally relevant and phenotype specific consequence of this GENOTYPE $\times$ SMOKING network interaction.

Using FCS, a data driven, graph theory network metric coupled with a whole brain search, we found significant GENOTYPE $\times$ SMOKING interactions localized within two key striatal-cingulate circuit components, the VS and dACC, such that FCS in both regions was highest in the normal metabolizing smokers. Further, FCS in both regions was negatively correlated with dependence severity, but only in the slow metabolizing group. Both of these findings are remarkably coincident with those reported previously by our group. Using independent data sets and, critically, independent analysis strategies, we previously identified a resting state functional circuit between the dACC and VS that was negatively correlated to FTND and was driven, in part, by the nicotinic alpha 5 subunit polymorphism (37, 38).

The current VS and dACC neuroanatomical findings, and their consistent identification across studies (41–43) implicate their prominent role in drug dependence. Notably, the VS receives dopaminergic input from the mesencephalic VTA, while also getting descending glutamatergic afferents from multiple frontal cortical regions, including the cingulate (44). The dopaminergic afferents impart a learning signal that identifies a mismatch between an expected and actual reward (45), while top-down cortical modulation is thought to provide context to these teaching signals (45, 46).

Since the VS is activated during reward processing (32, 47, 48) and is differentially activated in smokers vs. nonsmokers during reward (34, 36) or to the presentation of smoking-related cues (49, 50), we next addressed the functional significance of the resting state G $\times$ E FCS VS findings. We identified an identical G $\times$ E pattern of neuronal activity when anticipating a gain during the MID reward task, i.e. genotype had no effect in nonsmokers, while abstinent smokers with the reduced (vs. normal) *CYP2A6* genotype demonstrated less VS activation (Fig 1B & Fig 3A). These data suggest that enhanced brain nicotine concentration as a function of genotype modulated striatal circuit plasticity that is manifest both at rest and during dopamine related neuronal computations of reward anticipation. Since FCS in the VS is negatively correlated with FTND in slow metabolizers (Fig 1D), these data suggest that such smokers not only have a smaller reward attribution signal during nicotine absence, perhaps speaking to their more rapid development of nicotine dependence, but also have relatively greater success when in treatment (9, 15, 16, 18, 40). Notably, our VBM analysis did not demonstrate any G $\times$ E differences in GMV, suggesting that the functional alterations seen here were not influenced by underlying gray matter anatomy differences as previously reported in smokers (51). The coherence of both the resting and task data is consistent with

the well-known linkage between resting connectivity strength, task activity and behavioral performance (52, 53).

Reduced inhibitory control with diminished action monitoring, which is most often localized to the ACC (54, 55), and diminished responsivity to one's errors, represent an executive function profile of addiction (56) that may, at least in part, serve to speed the initiation and perhaps prolong the maintenance of dependence. Reduced metabolizing smokers, but critically not nonsmokers, demonstrated smaller dACC activation when making errors during a response inhibition task, similar to that previously reported in cocaine users (35). That this profile was only seen in reduced metabolizers suggests that such dysregulated neuronal processing does not predate the development of nicotine addiction but may rather be the consequence of chronic nicotine use. That acute nicotine administration (in our case, NRT) appears to reverse or 'normalize' this reduction in processing in both the dACC and the VS suggests a mechanism wherein acute nicotine intake serves to reduce cognitive deficits and potentiate the addiction. That said, since slow metabolizers also have higher quit rates when in treatment, our data encourage aggressive treatment intervention.

The differences in nicotine PK between slow and normal metabolizers may be directly responsible for differences between genotype smoking patterns (CPD and depth of inhalation) (10, 57), and presumably result in differential receptor signaling and the neuroplasticity observed herein. In contrast, during the initiation of smoking, the metabolically induced altered PK may have resulted in differential receptor signaling and neuroplasticity, which in turn altered smoking behaviors. Though not easy to disambiguate, these alternatives from the current design, extant data support the latter model. There is a 3.2 hazard ratio increased risk for conversion to tobacco dependence in adolescent slow relative to normal metabolizers, and as there is no evidence of titration of cigarette consumption *before* dependence (9), this suggests a change in receptor activation among nicotine smokers, resulting in differential response and neurotransmitter release leading to elevated risk for converting to dependence. As the rates of nicotine inactivation are the same among adolescence and adult smokers (58), the very low levels of smoking in slow smokers (4.2 versus 1.8 CPD) (9) are not easily compatible with models of nicotine titration based on PK differences alone. Examination in those smokers who are not yet dependent might tease apart the PK causal relationship between neurochemical changes and smoking behavioral differences among *CYP2A6* genotypes.

The FCS method identifies voxels in the brain that have the greatest connectivity to other brain regions, which can be conceptualized as forming neurocircuit hubs that integrate diverse informational sources (27). Aberrant connections to and from or within such hubs may indicate dysregulation across multiple cognitive domains that underlie neuropsychiatric diseases. Using the FCS identified hubs as seeds in independent functional connectivity analyzes, we identified circuit linkages between bilateral insula, dACC, and posterior cingulate cortex that underlie the dACC alteration, while insula and dACC constituted circuit linkage alterations between the VS hub. Together the dACC, insula and VS form the key components of the salience network (SN) (59), which is thought to link the current homeostatic state of the organism with other brain networks to facilitate appropriate, adaptive responding. Sutherland et al (1) proposed a model linking the SN, default-mode

network (DMN) and executive control network (ECN) for such adaptive responding in smokers such that the strength of the internetwork linkage varied as a function of smoking status, with acute abstinence strengthening the SN – DMN linkage, to the detriment of the SN-ECN. Recent empirical evidence supports this model, with SN - DMN strength correlated with enhanced craving and reduced working memory performance in abstinence vs. sated smoking (60). Consistently, NRT administration in the current study enhanced task induced VS and dACC activation in both genotype groups of smokers.

The current findings have clear implications for smoking cessation. Since the nicotine half-life in slow metabolizers is about 50% greater than in normal metabolizers (169 vs 113 minutes) (7), within a day or so of cessation, all of the nicotine in both genotype groups is cleared from the body. If there is no nicotine present for the *CYP2A6* to metabolize, how is variation in *CYP2A6* mediating these long-term differences in smoking cessation?

Our data for the first time suggests that smoking-induced and genetically modulated nicotine PK differences can alter specific brain circuits and network hubs critically related to nicotine abstinence and recidivism. Slow metabolizers show reduced neural reactivity to smoking related cues (19). Reactivity to smoking cues predicts smoking cessation success (61, 62), while greater insula-dACC coupling at rest predicts enhanced smoking cue-reactivity (50). That smokers with stroke lesions limited to the insula spontaneously quit smoking because their bodies ‘forgot they were smokers’ (63), suggests that the insula (and presumably its regional interconnectivity) process the body sensations and/or perceptions of nicotine withdrawal (64, 65). Thus, the current finding of reduced insula connectivity strength between the VS and dACC (key SN hubs) in the slow metabolizers mechanistically support both the reported reduction in cue responding in this *CYP2A6* genotype and helps explain the epidemiological and experimental observations of enhanced cessation success in slow metabolizers.

There is growing evidence that the *CYP2A6* genotype differentially contributes to smoking cessation treatments. Slow metabolizers respond better to NRT when it is administered for an extended period of time, while the additional treatment time provides no incremental benefit to normal metabolizers (16, 18), even after controlling for genotype differences in baseline CPD, dependence level, and patch-derived plasma nicotine levels (18). These observations suggest that the PK of NRT may more closely resemble the smoking-induced nicotine PK in slow metabolizers that originally shaped the neural circuits, replacing downstream nicotine signaling from cigarettes more effectively than among normal metabolizers. In contrast, normal metabolizers respond more favorably to varenicline than NRT, report fewer drug related side effects and greater cognitive improvement (17). This metabolism group-specific responding is supported in part by the knowledge that varenicline is not a substrate for *CYP2A6* (66); thus one might have anticipated identical responses to varenicline among slow and normal metabolizers if not for genotype differences in neurobiological adaptation as reported herein that must have occurred before cessation treatment.

Taken together, this genetic imaging study demonstrated a strong Gene $\times$ Environment interaction in cingulate and ventral striatal network hubs supported by circuits that

prominently implicate the insula. The ability of these network hubs to process reward signals and stimulus response inhibition are also compromised (although due to the limited sized cohort that performed the MID and Go/NoGo tasks, replication with larger samples is encouraged). Nevertheless, the genetic influence on both task activation and network hubs was seen only in smokers, suggesting that variable brain nicotine levels established during smoking can significantly and differentially modify brain circuits linked to, and perhaps enhance, subsequent nicotine dependence. Our data further suggest that these circuit alterations are a result of, rather than predisposing to, nicotine dependence. This conjecture is further supported by the enhanced BOLD signal seen following acute nicotine administration in nonsmokers. If indeed the identified striatal and cingulate hubs and circuits are altered by chronic nicotine use, they may serve as reversible biomarkers and novel targets for the development of medications to differentially treat nicotine addiction based upon the *CYP2A6* genotype.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Interaction between *CYP2A6* genotype and smoking status

A) FCS network analysis illustrating the interaction between the *CYP2A6* genotype and smoking status in the dorsal anterior cingulate (dACC) and left ventral striatum (VS). B–C) Mean FCS values in the dACC and VS in each of the six groups. In both brain regions smokers with the normal metabolizing genotype had higher FCS than all nonsmoker groups and smokers with the slow metabolizing genotype. There was no effect of the *CYP2A6* genotype on FCS among nonsmokers. D–E) There was a negative correlation in both hubs in the slow but not in the normal or intermediate genotype groups, i.e. as smoking dependence

increased, FCS decreased in both VS ($r = -0.51$, $P = 0.01$) and dACC ($r = -0.44$, $P = 0.04$).
* $P < 0.05$.

Fig. 2. Seed-based resting state functional connectivity

A) The dACC seed based on the *CYP2A6* × smoking interaction from Fig. 1 (green) was significantly connected to (1) dACC (2, 3) bilateral insula and (4) posterior cingulate cortex.

B) The VS seed based on the *CYP2A6* × smoking interaction from Fig. 1 (green) was significantly connected with (1) dACC and (2, 3) bilateral insula.

Fig. 3. *CYP2A6* genotype alters neural activation during the MID-R and Go/NoGo task
 A) Coronal section illustrating the task map from all subjects when performing the MID task with significant activation in bilateral VS when anticipating a gain (vs. neutral) condition. Smokers (but not nonsmokers) with the reduced (slow and intermediate) genotype showed less BOLD signal change compared with those with the normal genotype under the placebo patch condition (i.e. acute abstinence). Acute nicotine patch administration normalized the genotype effect in smokers and appeared to also enhance activity in nonsmokers with the reduced genotype. B) Similarly, dACC activation was smaller in reduced metabolizing

smokers (but not nonsmokers) following unsuccessful inhibitions in the Go/NoGo task under the placebo patch condition. Once again, nicotine patch administration appeared to normalize this effect in reduced metabolizing smokers, while also increasing dACC activity in reduced metabolizing nonsmokers.

Fig. 4. ANCOVA results demonstrating main effect of *CYP2A6* genotype and smoking status
A–B) Main effect of *CYP2A6* genotype, demonstrating that both the slow and intermediate genotype groups showed significantly smaller FCS in bilateral precentral gyrus and bilateral insula than the normal genotype group, regardless of smoking status. C–D) Main effect of smoking status, demonstrating that smokers showed significantly less FCS in the bilateral superior temporal gyrus than nonsmokers, regardless of genotype. L, left; R, right; PCG, Precentral gyrus; STG, superior temporal gyrus; *, $P_{\text{corrected}} < 0.05$.

Table 1

Demographic characteristics (entire cohort)

CYP2A6	NONSMOKERS (n=92)				SMOKERS (n=66)			Statistical Analysis	P
	Normal	Intermediate	Slow		Normal	Intermediate	Slow		
Number of subjects	28	21	43		25	18	23		0.36
Age (years)	34.2±9.6	29.8±7.9	32.4±8.5		36.5±11.0	30.7±10.4	34.5±8.4	all	$P > 0.09$
Gender (M:F)	13:15	12:9	24:19		11:14	10:8	15:8	all	$P > 0.29$
Race									
Black/African American	13	10	35		7	4	17		
Caucasian	12	8	5		18	14	5		
Others	3	3	3		--	--	1		
Years of education ^a	14.1±1.8	14.5±1.2	14.0±2.1		13.2±2.3	13.1±1.6	13.1±1.7	all	$P > 0.88$
IQ (Verbal) ^b	59.8±8.2	59.9±9.0	58.6±10.0		57.6±8.6	53.9±7.5	54.0±6.8	all	$P > 0.36$
FTND					5.4±2.1	5.3±2.0	4.6±1.9		0.33
CPD ^c					20.7±7.4	17.6±8.5	14.5±3.7		0.04
CO					22.4±14.6	14.6±9.2	17.4±8.7		0.12
Interval (hours)					5.3±5.0	3.6±1.2	5.0±4.8		0.62

Pearson's χ^2 -test was used to compare the frequency/gender distribution of the three CYP2A6 genotype groups between smokers and nonsmokers. Two-way ANOVAs were conducted to compare the six groups in age, years of education, and IQ. One-way ANOVA was used to compare differences in FTND, CPD, CO, and interval among the three genotype smokers. Tukey's *t*-test was used for multiple comparisons. Data represents mean ± SD. FTND, Fagerström Test for Nicotine Dependence; CPD, cigarettes per day; M, male; F, female; CO, exhaled carbon monoxide level (ppm); Interval, time from last cigarette smoked to data acquisition beginning.

^aSignificant difference in education years between smokers and nonsmokers ($P = 0.004$).

^bSignificant difference in IQ score between smokers and nonsmokers ($P = 0.002$).

^cSignificant difference in CPD between normal and slow metabolizers ($P = 0.003$).

Table 2

Demographic characteristics (MID task and Go/NoGo cohort)

	NONSMOKERS		SMOKERS		Statistical Analysis
	<i>(n=20)</i>		<i>(n=23)</i>		
	Normal	Reduced	Normal	Reduced	
<i>CYP2A6</i>					<i>P</i>
Number of subjects	9	11	15	8	
Age	30.7±7.8	30.2±6.9	35.5±10.5	35.6±9.4	all <i>P</i> s > 0.08
Race					
Black or African American	1	5	2	4	
Caucasian	7	3	10	3	
Others	1	3	3	1	
Gender (M:F)	5:4	5:6	6:9	5:3	0.23
IQ (Verbal) ^a	64.9±8.9	58.6±3.3	58.3±10.8	51.4±5.9	all <i>P</i> s > 0.01
Years of education ^b	15.6±0.9	14.4±1.6	13.8±1.8	13.2±2.0	all <i>P</i> s > 0.007
FTND			4.8±1.9	5.0±2.2	0.82
CPD			16.8±7.0	13.1±9.0	0.94
CO			6.8±2.8	7.5±4.9	0.68

Pearson's χ^2 -test was used to compare the frequency/gender distribution of the two *CYP2A6* genotype groups between smokers and nonsmokers. Two-way ANOVAs were conducted to compare the four groups in age, years of education, and IQ. Tukey's *t*-tests were used to compare differences in FTND, CPD, and CO between the two genotype smokers, and also were used for multiple comparisons. Reduced: slow and intermediate. Data represents mean $\pm$ SD; FTND, Fagerström Test for Nicotine Dependence; CPD, cigarettes per day; M, male; F, female; CO, exhaled carbon monoxide level (ppm).

^aSmokers had lower IQ than nonsmokers (*P* = 0.011), and reduced genotype individuals had lower IQ score than those with normal genotype (*P* = 0.015).

^bSmokers had fewer years of education than nonsmokers (*P* = 0.007).