Brain regions associated with polygenic risk scores for cigarette smoking in typical development

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ABSTRACT

Tobacco use disorder is highly heritable (Beirut et al., 2015; Xu et al., 2020; Ray et al., 2009), but the impact of cigarette smoking's genetic risk on brain development has not been investigated yet. Investigating which brain regions are associated with a higher genetic risk of tobacco use disorder, prior to the start of cigarette use, could shed light on the physiological mechanisms involved in tobacco use disorder. We used polygenic risk scores for cigarette smoking (PRS-SMK) to determine the influence of genetic risk on brain morphometry in childhood. More precisely, we analyzed the relationships between cortical thickness (CT), surface area (SA), and subcortical brain volumes and the genetic risk for four cigarette smoking phenotypes: smoking initiation (PRS-SI), age of start (PRS-AI), cigarettes per day (PRS-CPD), and smoking cessation (PRS-SC). T1-weighted MRI and whole genome genotyping from 393 participants aged 3 to 21 years old from the Pediatric Imaging Neurocognition and Genetics (PING) dataset and 4627 subjects aged 9 to 12 years old from the Adolescent Brain Cognitive Development (ABCD) dataset (Jernigan et al., 2016; Casey et al., 2018) were used. CIVET was used for brain morphometric measurements (CT, SA) while Freesurfer and MAGeT-Brain pipeline were used to segment subcortical volumes (thalamus, caudate nucleus, putamen, and globus pallidus). For the PING and ABCD datasets, we used the Desikan-Killiany-Tourville (DKT) and Desikan-Killiany (DK) atlases for surface area and cortical thickness, respectively (Klein and Tourville, 2012; Desikan et al., 2006). The genetic risk variants for cigarette smoking were obtained from the Genome Wide Association Study (GWAS) called GWAS and the Sequencing Consortium of Alcohol and Nicotine Use (GSCAN) (Xu et al., 2020), and PRSice was used to calculate the participants' Polygenic Risk Scores (PRS). Linear regressions with brain morphometric data as dependent variable, PRS-SMK as independent variable and sex, age, age squared, intracranial volume, scan, socioeconomic status and ancestry

principal components scores as covariates were computed using matlab2020b. To account for multiple comparisons, we used FDR correction. In the PING dataset, we found that higher PRS-CPD was associated with increased cortical thickness in clusters in right(one) and left temporal(one) lobe while higher PRS-SC was associated with increased cortical thickness in three clusters across the cortex (see Figure 1). Those results were obtained for subjects from 3-21 years old as well as for those from 3-6 years old specifically. For parcellated data and the other morphometric measures, no significant results were obtained (i.e., SA and subcortical brain volumes). In the ABCD cohort, we found that increased cortical thickness in different regions of the frontal and temporal lobes (see Figure 2) was related to higher PRS-SI and PRS-CPD while PRS-SC was associated with reduced cortical thickness in the middle temporal gyrus. Other morphometric measures yielded no significant results (i.e., SA and subcortical brain volumes). Our findings suggest that the genetic risk for cigarette smoking has an impact on early brain development in early and late childhood and might reflect a change in the rate of cortical maturation in frontal and temporal regions previously shown to be associated with tobacco use disorder such as the orbitofrontal and anterior cingulate cortex, and middle temporal gyrus (Kühn et al., 2010; Yangding et al., 2015). Further investigations with another dataset including a larger number of subjects should be conducted to generalize our results.

RÉSUMÉ

Le trouble lié au tabagisme est fortement héréditaire, mais l'impact du risque génétique du tabagisme sur le développement du cerveau n'a pas été étudié. L'étude des régions cérébrales associées à un risque génétique plus élevé de dépendance au tabac, avant le début de la consommation de cigarettes, pourrait éclairer sur les mécanismes physiologiques impliqués dans la dépendance à la cigarette. Nous avons utilisé des scores de risque polygénique pour le tabagisme afin de déterminer l'influence du risque génétique sur la morphométrie cérébrale pendant l'enfance. Plus précisément, nous avons calculé des régressions linéaires entre l'épaisseur corticale (CT), l'aire de surface (SA) et les volumes sous-corticaux et la prédisposition génétique à quatre phénotypes de tabagisme : l'initiation au tabagisme (PRS-SI), l'âge d'initiation (PRS-AI), le nombre de cigarettes par jour (PRS-CPD) et le sevrage tabagique (PRS-SC). L'IRM structurelle (T1w) et le génotypage du génome entier de 393 participants âgés de 3 à 21 ans de l'ensemble de données du Pediatric Imaging Neurocognition and Genetics (PING) et 4 627 sujets âgés de 9 à 12 ans de la cohorte Adolescent Brain Cognitive Development (ABCD) ont été utilisés. CIVET a été utilisée pour les mesures morphométriques cérébrales (CT, SA) tandis que les logiciels Freesurfer et MAGeT-Brain ont été utilisés pour segmenter les volumes sous-corticaux (thalamus, noyau caudé, putamen et globus pallidus). Pour calculer l'aire de surface et l'épaisseur corticale nous avons utilisé les atlas Desikan-Killiany-Tourville (DKT) et Desikan-Killiany (DK) pour les banques de données PING et ABCD, respectivement (Klein et Tourville, 2012 ; Desikan et al., 2006). Les variantes de risque génétique pour le tabagisme ont été obtenues auprès du GWAS et du Consortium de séquençage de l'alcool et de la nicotine (GSCAN) (Xu et al., 2020), et PRSice a été utilisé pour calculer les scores de risque polygénique des participants. Les régressions linéaires avec les données morphométriques cérébrales comme variable dépendante, les scores de risque

polygénique pour le tabagisme comme variable indépendante et le sexe, l'âge, l'âge au carré, le volume intracrânien, le scanner, le statut socio-économique et les scores des composantes principales de généalogie comme covariables ont été calculés à l'aide de matlab2020b. Pour tenir compte des comparaisons multiples, nous avons utilisé la correction FDR. Dans l'ensemble de données PING, nous avons constaté qu'une PRS-CPD plus élevée était associée à une épaisseur corticale accrue dans des sections du lobe temporal droit(une) et gauche(une), tandis qu'une PRS-SC plus élevée était associée à une épaisseur corticale accrue dans plusieurs régions(trois) du cortex (voir la figure 1). Ces résultats ont été obtenus pour les sujets de 3 à 21 ans ainsi que pour ceux de 3 à 6 ans spécifiquement. En ce qui concerne les données parcellisées et les autres mesures morphométriques, aucun résultat significatif n'a été obtenu (c'est-à-dire, pour la SA et les volumes cérébraux sous-corticaux). Dans la cohorte ABCD, nous avons constaté que l'augmentation de l'épaisseur corticale dans différentes régions des lobes frontaux et temporaux (voir Figure 2) était liée à une PRS-SI et une PRS-CPD plus élevées, tandis que la PRS-SC était associée à une réduction de l'épaisseur corticale du gyrus temporal moyen. Les autres mesures morphométriques n'ont donné aucun résultat significatif (c.-à-d., SA et volumes sous-corticaux). Nos résultats suggèrent que le risque génétique associé à la cigarette a un impact sur le développement précoce du cerveau au début et à la fin de l'enfance et pourrait refléter un changement du taux de maturation corticale dans des régions frontales et temporales précédemment associées à la dépendance au tabac telles que les cortex orbitofrontal et cingulaire antérieur et le gyrus temporal moyen (Kühn et al., 2010 ; Yangding et al., 2015). D'autres études incluant un plus grand nombre de sujets devraient être menées pour généraliser nos résultats.

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CONTRIBUTION OF CO-AUTHORS

I declare that I completed the majority of the work in this manuscript. I wrote most of the scripts, used the MaGet-Brain pipeline to pre-process the subcortical volumes, performed all statistical analyses and wrote this entire manuscript. The following highlights the contribution of my co-authors.

Budhachandra Khundrakpam, PhD and *Noor Al-Sharif, PhD candidate* were involved in data pre-processing.

Uku Vainik, PhD and Eric Yu, PhD candidate were involved in PRS calculation.

Christina Tremblay, PhD was involved in conceptualization, methodology, reviewing manuscript and supervision.

Matthias Kirschner, MD PhD, Filip Morys PhD and Andrew Vo, PhD were involved in methodology.

Ziv Gan-Or, PhD was involved in PRS calculation.

Alain Dagher, MD PhD was involved in conceptualization, reviewing manuscript, funding, and supervision.

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CHAPTER 1

1 INTRODUCTION

1.1 General Context

Effective pharmacological treatment coupled with behavioral therapy, economic sanctions and public health warnings have contributed to a remarkable global decrease in cigarette smoking prevalence since 1980 (Freeman et al., 2014). However, the rise of consumption of e-cigarettes and other alternative smoking products leading to cigarette smoking and the complex biological and environmental aspects of nicotine addiction contribute to cigarette smoking being the leading cause of premature death worldwide (Freeman et al., 2014; Benowitz et al., 2010; Casetta et al., 2017). It is associated with high risk for lung cancer, pulmonary diseases, infections, strokes, cardiovascular diseases and neurodegenerative diseases (Benowitz et al., 2010). Cigarettes contains nicotine which is an agonist of the neurotransmitter acetylcholine (Ach) (McKay et al., 2007). Many functions involving acetylcholine and its receptors include muscle action, respiration, heart rate, attention, learning and memory (Gotti et al., 2006). They also trigger the release of other neurotransmitters, which have an impact on mood (Feduccia et al., 2012). When nicotine enters the brain, it binds to acetylcholine nicotinic receptors (nAChRs) and duplicates acetylcholine's effects (Dani et al., 2001). Nicotine also stimulates parts of the brain involved in the production of pleasure, reward, working memory and attention via promoting dopamine (DA) projections to the nucleus accumbens and the prefrontal cortex (Feduccia et al., 2012). Although populations with high levels of stress, mood disorders or cognitive deficits are more vulnerable to nicotine addiction, this addiction remains highly heritable (Bierut, L., & Cesarini, D., 2015). Indeed, nicotine

addiction heritability is estimated to be 0.42–0.57 (Sharp, B. M., & Chen, H., 2019). Furthermore, GWAS studies on cigarette smoking have identified a gene, (CHRNA5) commonly called "Mr Big", that alters the responsiveness of nicotinic receptors to nicotine (Bierut, L., & Cesarini, D., 2015). Another research group found that *CYP2A6* gene variation influences nicotine metabolizing and increases the likelihood of nicotine addiction by increasing the physiological effect of cigarette smoking (Tang et al., 2012). Moreover, GWAS studies have identified genes associated with different smoking behaviors. Smoking initiation, age of initiation, age of cessation and number of cigarettes smoked per day have shown to be associated with different set of genes (Bierut, L., & Cesarini, D., 2015). Yet, the brain regions associated with those genes have not been studied.

Additionally, research for nicotine dependence treatment suggests that dopaminergic processes would have a role in regulating the reinforcing effects of nicotine. The administration of dopamine blockers has been found to reduce cigarette smoking in some individuals, but to increase smoking in others due to compensatory behaviours (Tomkins, D. M., & Sellers, E. M., 2001). Nicotine agonists have been suggested to decrease tobacco use disorder in other studies. However, nicotine agonists such as varenicline and nicotine patches have been proven to cause nausea, headaches, insomnia, and abnormal dreams, lowering adherence to treatment (Drovani et al, 2016), or to be ineffective for smoking cessation in women (Perkins et al., 2008). A medication that targets the receptors linked to anxiety and depression provides an alternative. Bupropion, a molecule without nicotine that was originally created as an antidepressant, has now been licenced as a smoking cessation aid. Its main method of action is to prevent noradrenaline and dopamine from being reabsorbed (Stahl et al., 2004). Bupropion has also been shown to antagonise some nicotinic acetylcholine receptors. The action of this drug results in a reduction of depressive behaviour and

nicotine withdrawal (Warner, C., & Shoaib, M., 2005). Bupropion has been shown in multiple clinical trials to be an effective assistance in promoting smoking cessation (Tomkins, D. M., & Sellers, E. M., 2001). Nevertheless, patients taking Bupropion for smoking cessation have observed side effects similar to those found with nicotine agonists, and they have been reported more frequently (Wilkes et al., 2008).

The financial impact on health care systems, the lack of adherence to treatment and current treatments' side effects emphasize the relevance to uncover the brain regions underlying the genetic risk for cigarette smoking, which might bring further clues about the neurophysiological mechanisms to target.

1.2 Objectives

The primary aim of this study is to determine the influence of genetic risk for cigarette smoking on brain morphometry in childhood. We analyzed the relationships between cortical thickness (CT), surface area (SA), and subcortical brain volumes and four smoking phenotypes (smoking initiation (PRS-SI), age of start (PRS-AI), cigarettes per day (PRS-CPD), and smoking cessation (PRS-SC)). Since higher scores for smoking initiation, number of cigarettes per day and smoking cessation and lower scores in age of initiation are associated with negative outcomes (Liu et al., 2019) and tobacco used disorder has been associated with reduced cortical thickness in regions of the frontal and temporal lobes and increased caudate volume (Li et al., 2015; Zorlu et al, 2017), we hypothesize that subjects with a higher polygenic risk score for smoking initiation, cigarettes per day, and smoking cessation and lower scores in age of initiation, would have thinner cortical thickness and smaller surface area, mostly in prefrontal cortex regions, and increased caudate

nucleus as compared to subjects with a lower polygenic risk score for smoking initiation, cigarettes per day, and smoking cessation and higher PRS-AI.

The secondary objective of the current work is to investigate the relationship between the genetic risk for cigarette smoking and the seven resting state networks. We hypothesize that greater PRS for SI, SC, and CPD, as well as lower PRS-AI, will be associated to resting state networks involved in tobacco use disorder, such as the Default Mode Network (DMN) and the Centre Executive Network (CEN). Several studies have identified the DMN and CEN as key networks in nicotine addiction (Sutherland et al., 2012) due to their roles in cognitive control and emotional regulation.

The third and last aim of this study is to explore the association between the four PRS-SMK and socioeconomic status (SES) as well as impulsivity. Since tobacco use disorder has been associated with low SES and high impulsivity traits (Hiscock et al., 2012; Debry et al.,2018), we hypothesize that greater PRS for SI, SC, and CPD, as well as lower PRS-AI, will be associated with lower SES and higher impulsivity score. Furthermore, even if there is no association between SES and cigarette smoking PRSs, SES may have an independent effect on cigarette smoking, interacting with cigarette smoking PRSs to induce tobacco use disorder.

CHAPTER 2

2 LITERATURE REVIEW

2.1 Tobacco use Disorder, Definition and Prevalence

Tobacco use disorder (TUD) is defined by the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5), as having a minimum of two out of 11 criteria summarized by

heaviness of smoking, craving, tolerance, withdrawal and social impairment for at least 12 months (see Supplementary Table 1 for specific criteria) (Paik et al., 2019). In 2017, more than 1.1 billion individuals smoked tobacco worldwide (McRobbie, H., & Kwan, B., 2021), with TUD affecting 11 percent of Canadians (approximately 3,4 million people) and 12 percent of Americans (39,5 million people) (Zawertailo et al., 2020). TUD is more common in people who have serious mental illnesses including major depressive disorder, schizophrenia, or bipolar disorder (Fornaro et al., 2022). TUD causes chronic obstructive pulmonary disease (COPD), lung cancer, and other fatal diseases, making it the world's leading cause of premature death (Shmulewitz et al., 2022).

2.2 Neurobiology of Tobacco Use Disorder

Nicotine is the major psychoactive component in tobacco, and it is responsible for its addictive behavioural and physiological effects. Nicotine affects the brain via binding to nicotinic receptors, which are specific receptors for the neurotransmitter acetylcholine (ACh). Therefore, those receptors are commonly called nicotinic acetylcholine receptors (nAChR). Particular subtypes of nicotinic receptors, β2 and α4 are found on the cell bodies of dopamine neurons in the ventral tegmental area (VTA). Nicotine increases the activity of these dopamine neurons by binding to these receptors, resulting in an increase in dopamine release in the nucleus accubens (NAcc), which is known to mediate reward (Tomkins, D. M., & Sellers, E. M., 2001; Picciotto, M. R., & Mineur, Y. S. 2014). Increased dopamine projections from the VTA to the NAcc following nicotine administration are also thought to induce impulsive behaviours, which are characteristic of addiction (Feduccia et al., 2012). Moreover, animal studies have highlighted the key role of β2 receptors in nicotine addiction by showing that the absence of this receptor in the VTA is associated with the absence of withdrawal or nicotine seeking behaviour (Picciotto et al., 1998).

Nicotinic receptors significantly enable longitudinal structural changes in the dopaminergic system, but also in the hippocampus and in the amygdala (Feduccia et al., 2012). The β2 receptors coupled with another important subtype of nicotinic receptor, α7, and are also present in hippocampus and in the amygdala. ACh via its projections from the basal forebrain to the hippocampus activates the hippocampus, implicated in memory (Picciotto et al., 2012). Similarly, via Ach projections, the amygdala collaborates with the prefrontal cortex to assess the environment and take appropriate action (Picciotto et al., 2012). Nicotinic receptors in the amygdala and hippocampus play a role in nicotine's well-known cognitive, memory, and learning benefits (Feduccia et al., 2012). Furthermore, data showing that microinfusion of nAChR agonists can enhance memory-related abilities while antagonists decrease them (Feduccia et al., 2012) indicate the role of the hippocampus and amygdala in mediating part of the cognitive-enhancing effects of nicotine. The hippocampus and amygdala may also play a role in nicotine addiction by improving context-drug connections and increasing memories associated with drug use, all of which have been found to predict drug-seeking and relapse in human and animal models (Fuchs et al., 2008).

Nicotinic acetylcholine receptors (nAChRs) are important molecular predictors of nicotine addiction because of their effect on dopamine release, reward, impulsivity, and memory after nicotine administration (Feduccia et al., 2012).

2.3 Polygenic Risk Scores for Cigarette Smoking

Polygenic risk scores (PRS) are estimates of an individual's genetic vulnerability to a characteristic or disease based on their genotype profile and relevant genome-wide association study (GWAS). Because TUD has a strong genetic component, we employed four distinct PRS for smoking (PRS-SMK) estimated based on four different smoking behaviours in our study. Indeed, TUD heritability is expected to be approximately 50%, while single nucleotide polymorphism (SNP) heredity, as calculated by PRSs, is estimated to be around 9%. Because certain rare variations are overlooked during the imputation process of GWASs, SNP heritability is often lower than heritability obtained through twin studies (Deak, J. D., & Johnson, E. C., 2021). GWAS studies on cigarette smoking have found loci and genes related with distinct smoking behaviours in millions of smokers, making PRSs calculations to approximate genetic vulnerability conceivable. The polygenic risk scores of each smoking behaviour in each subject are calculated using SNPs obtained from those loci and genes and detected in the genomes of people from selected datasets. In addition to the CHRNA5 variant that alters the responsiveness of nicotinic receptors (Bierut, L., & Cesarini, D., 2015) and the CYP2A6 variant that induces a fast metabolism of nicotine (Ray et al., 2016), GWAS studies on cigarette smoking have identified four other variants associated with different smoking behaviours: CHRNA5-A3-B4, CHRNA4, DNMT3B and DBH. These six variants are the main variants used by GWASs on smoking behaviour to date to identify genetic risk for cigarette smoking (Deak, J. D., & Johnson, E. C., 2021).

Using GWAS studies on smoking behaviours, Bray and colleagues discovered that cigarette smoking-related PRSs were linked to smoking-related behaviours in European heritage populations. Moreover, they found that PRS generated from smoking behaviours can accurately predict smoking cessation in clinical trials. PRSs were related to successful smoking cessation in

two randomized placebo-controlled trials. The PRS of a later age of initiation predicts a higher rate of successful smoking cessation (Bray et al., 2022). Despite their ability to predict smoking behaviours, PRSs fail to consider the age-dependency of genetic effects (Deutsch, A. R., & Selya, A. S., 2020). Investigating the influence of genetic risk for cigarette smoking on brain development in typically developing cohorts could bring more insights about the age effect.

In the present study we used four smoking phenotypes (smoking initiation (PRS-SI), age of start (PRS-AI), cigarettes per day (PRS-CPD), and smoking cessation (PRS-SC)). PRS-SI denotes the genetic risk of beginning to smoke, PRS-CPD denotes the genetic risk of smoking a higher number of cigarettes per day if people begin to smoke, PRS-SC denotes the genetic risk of having trouble quitting smoking, and PRS-AI denotes the age of the first cigarette. Higher PRS-SI, PRS-CPD, and PRS-SC scores, as well as lower PRS-AI levels, are linked to bad health outcomes such as heavy smoking, difficulty quitting and tobacco use disorder (Liu et al., 2019; Shmulewitz et al., 2022).

2.4 Brain Morphometry

Genes have an impact on the morphometry of the brain. Surface area and cortical thickness are highly heritable (0.89 and 0.81, respectively), but they are not genetically related (genetic correlation = 0.08) (Panizzon et al., 2009). Cortical thickness, surface area, and subcortical volumes are all influenced by genes as they mature, resulting in long-term effects on brain regions and their associated functions (Fjell et al., 2015; Brouwer et al., 2022).

Cortical thickness represents the average of the distance between each cortical white surface vertex and the closest point on the gray matter surface (Fischl & Dale, 2000). Although there is no consensus yet, cortical thinning has been found that cortical thickness increases until the age of three and then follows a linear pattern of thinning until early adulthood in typical development (Wierenga et al., 2014; Fjell et al., 2015; Lyall et al., 2015; Tamnes et al., 2017). While the relationship between synaptic processes and cortical thickness has not been empirically proved, it may be due to mechanisms such as synaptic pruning and intracortical myelination (Fjell et al., 2015). Surface area is generally computed at the interface between the gray and white matter (i.e., at the white surface) or using the surface at the mid-distance between the white and gray cortical matter surface (Winkler et al., 2012). Furthermore, surface area has distinctive pattern of maturation in comparison to cortical thickness. It increases slowly from birth to late childhood when it slowly starts to decrease until early adulthood (Wierenga et al., 2014).

The developmental trajectories of subcortical volumes differ amongst subcortical areas (Goddings et al., 2014). Subcortical brain regions related with decision-making and reward-seeking behaviours such as the striatum (i.e., the putamen, caudate nucleus, thalamus, and globus pallidus) play crucial roles in tobacco use disorder (Goddings et al., 2014; Noel et al., 2013). From birth to adolescence, the volume of those brain regions increases then decreases from adolescence to adulthood (stby et al., 2009; Goddings et al., 2014;).

Environmental factors such as tobacco use disorder can alter the typical maturation of brain features.

2.4.1 Cigarette Smoking and Structural Brain Changes

Tobacco use disorder in adults has been associated with a diffused decrease in cortical thickness, particularly in frontal regions (Karama et al., 2015; Kühn et al., 2010). Zorlu et al. derived similar conclusions from comparing cortical thickness (CT) in smokers and non-smokers with major depressive disorder (MDD) (Zorlu et al, 2017). Indeed, non-smokers with major depression have increased cortical thickness in the left temporal cortex, the right insular cortex and left pre- and postcentral gyrus, but there is no difference in CT in those regions between smokers with MDD and healthy controls. Another research group found reduced frontal cortical thickness (left caudal anterior cingulate cortex (ACC), right lateral orbitofrontal cortex (OFC)), left insula, left middle temporal gyrus, right inferior parietal lobule, and right parahippocampus and increased caudate volume in young adult smokers compared to non-smokers. Moreover, they found a relationship in this study between cortical thickness reduction in the OFC and tobacco use disorder (Li et al., 2015). Despite studies showing a change in cortical thickness associated with cigarette smoking, studies investigating the association between cortical thickness and the genetic risk for cigarette smoking have not been done.

Additionally, most of the regions found to be associated with structural brain changes and tobacco use disorder are part of the Default mode network (DMN) and the Central executive control network (CEN).

2.5 Cigarette Smoking and the Seven Resting State Networks

The implication of the seven resting state networks (Yeo et al., 2011) in cigarette smoking has been previously investigated (Sutherland et al., 2012, Cho et al., 2010). Several groups have identified the default-mode and central executive control networks (CEN) as key networks in

nicotine addiction (Sutherland et al., 2012). Another study discovered that smokers have greater activity in the prefrontal and limbic networks than non-smokers (Janes et al., 2012). These findings support the associations found between regions in the prefrontal cortex and tobacco use disorder. Furthermore, a key region of the CEN, the dorsolateral prefrontal cortex, has been associated with inhibition of impulsive behaviours (Cho et al., 2010). This suggests a role of impulsivity in tobacco use disorder.

2.6 Cigarette Smoking and Impulsivity

Impulsivity is a personality trait representing a wide spectrum of behaviors that include inability to withhold a response, intolerance to delay gratification and perseverance on a nonrewarded response (Kale et al., 2018). Impulsivity is highly associated with different smoking behaviours such as smoking initiation and smoking persistence and is most found amongst smokers in comparison to non-smokers (VanderVeen et al., 2008; Debry et al., 2008). More particularly, smokers generally have higher scores on lack of planning and positive urgency. UPPS-P is a commonly used scale that measures impulsivity and comprised items measuring the lack of planning and positive urgency of a subject (Kale et al., 2018). Smoking behaviours such as smoking initiation and smoking cessation have been, respectively, positively and negatively associated with impulsivity. Moreover, positive urgency, defined as impulsive behaviours when feeling intense positive emotions, is associated with nicotine dependence. Cigarette smoking status is also associated with positive urgency and lack of premeditation (aversion to planning). Finally,

severity of nicotine dependence seems to be mostly associated with positive urgency (Kale et al., 2018).

2.7 Demographic factor Influencing Cigarette Smoking

Different smoking behaviours are related to socioeconomic status (SES). SES has a negative relationship with heavy smoking, but a positive relationship with quitting (Hiscock et al., 2012). Furthermore, lower SES has been linked to a particular trajectory of structural brain development, leading to cognitive deficits and emotional dysregulation (Brito et al., 2014). As a result, this variable could have an impact on the relationship between the PRSs of cigarette smoking and brain morphology.

CHAPTER 3

3. MANUSCRIPT

Introduction

Cigarette smoking is known to be the number one cause of excess mortality. People continue to smoke because of nicotine's addictive properties (Benowitz, N. L., 2010, Casetta et al., 2017). The propensity to nicotine addiction is heritable. In fact, genome-wide association studies (GWASs) on cigarette smoking have identified numerous loci associated with various smoking behaviours such as smoking initiation, cigarettes per day, or smoking cessation (Sharp, B. M., & Chen, H., 2019). Therefore, smoking initiation and persistence could potentially have a neurodevelopmental origin. The genes previously associated with cigarette smoking could influence the development of brain regions involved in nicotine addiction or increase these regions vulnerability to environmental factors that might encourage tobacco use disorder. Indeed,

psychiatric disorders like tobacco use disorder have been more closely related to alterations in developmental trajectories of brain areas than cross-sectional differences in brain regions structures (Wierenga et al., 2014).

In childhood and adolescence, the brain undergoes complex and evolving structural changes driven by genes and the environment (Brouwer et al., 2022). Longitudinal neuroimaging studies consistently show a linear pattern of cortical thinning from childhood to early adulthood (Ball et al., 2019; Wierenga et al., 2014). Cortical surface area follows a different developmental trajectory, having a cubic and quadratic association (Wierenga et al., 2014; Ducharme et al., 2015) with age with a peak around 9 years followed by a slow decrease until early adulthood. Simultaneously, brain development varies across brain regions. Accelerated cortical thinning is observed in frontoparietal regions and in the visual cortex, whereas thinning of the primary motor and sensory regions is slower (Ball et al., 2019). Other groups have found regions from the association cortex to mature later than those from primary and sensory cortex. Longer periods of plasticity in brain areas associated with higher order functions has been associated with vulnerability to psychiatric disorders (Sydnor et al., 2009). However, further investigations on the maturation of higher order functions such as impulse control (involved in smoking cessation) and of sensory areas (involved in perception of smoking cues) is needed.

Studies on cigarette smoking and associated structural changes have observed reduced cortical thickness in frontal regions. Since these studies are mostly cross-sectional, the reduction in cortical thickness could be explained by alterations in the cortical maturation during the development (Brouwer et al., 2022). Investigating the relationship of brain anatomy with genetic risks enables us to study the neuroanatomical correlates in a large population-based cohort of children and

adolescents without confounding factors associated with cigarette smoking, including direct effects of nicotine and other compounds in cigarette smoke, associated cerebrovascular disease or medication use, and co-morbidities like other drug use. Recent longitudinal studies show a high association between polygenic risk scores for smoking (PRS-SMK) and onset of smoking behaviours in adulthood (Belsky et al., 2013). However, studies on PRS-SMK have exclusively investigated cigarette smoking to determine the validity of PRS-SMK on predicting smoking behaviours. Determining the effect of genetic risk for cigarette smoking on the development of brain structures requires investigation of younger non-smoker cohorts and could shed light on the underlying mechanisms involved in smoking initiation, persistence, and heaviness.

Here we address the relationship between PRS-SMK and cortical thickness, surface area and subcortical brain volumes in two large population-based cohort of typically developing children (3 – 21 years) derived from the Pediatric Imaging, Neurocognition and Genetics (PING) study (Jernigan et al., 2016) and the Adolescent Cognitive Brain Development (ACBD) study (9-12 years) (Casey et al., 2018). We hypothesised that higher Polygenic Risk Score for Smoking Initiation (PRS-SI), Polygenic Risk Score for Smoking Cessation (PRS-SC) and Polygenic Risk Score for Cigarettes Per Day (PRS-CPD) and lower Polygenic Risk Score for Age of Initiation (PRS-AI) would be associated with differences in morphometry (cortical thickness, surface area and subcortical volume). We also examined age-group specific variations of PRS-SMK on cortical morphometry across different neurodevelopmental stages. Furthermore, to better understand the functional consequences of any neuroanatomical effect, we looked at the association between PRS-SMK and the average cortical thickness in the 7 resting state networks (Yeo et al., 2011). Finally, we examined the correlations between PRS-SMK and socioeconomic status (SES) and impulsivity traits.

Methods

Participants

Data from participants in two different datasets: the PING (Jernigan et al., 2016) and the ABCD (Karcher, N. R., & Barch, D. M., 2021) were used. In the PING dataset, T1-weighted MRI scans, demographic, and genetic data were derived from 393 typically developing children and adolescents aged from 3 to 21 years old from European ancestry (Jernigan et al., 2016). This is a comprehensive, publicly shared dataset including cross-sectional data from 1493 healthy subjects from ten sites across the United States. Parental questionnaires (https://pingstudy.ucsd.edu) were used to collect demographic data on the individuals (age, gender, socioeconomic status, and impulsivity scores). A total of 393 non-smoker subjects having demographic and genetic data were included in the study. In the ABCD dataset including 11 875 participants, a total of 4627 subjects were included in the analysis after proper quality control of the neuroimaging data and removing participants with missing data. The analysis only included participants who had completed T1-weighted MRI scans, demographic (age, sex, socioeconomic status, impulsivity scores), and genetic data at baseline. The ABCD study is the largest brain development study in the United States and comprises 21 locations (Karcher, N. R., & Barch, D. M., 2021). The participants in this study were normally developing children aged from 9 to 12 years old from European ancestry.

Genomic data

PING cohort

For the PING cohort, genomic data processing and calculation of PRS followed the method in a recent publication from Khundrakpam and colleagues (Khundrakpam et al., 2020). More specifically, 539,865 single nucleotide polymorphisms (SNPs) were genotyped. Data were

prepared for imputation using the "imputePrepSanger" pipeline (https://hub.docker.com/r/eauforest/imputeprepsanger/), implemented on CBRAIN (Sherif et al., 2014) and the Human660W-Quad_v1_A-b37-strand chip as reference. Using Plink genotype files, the pipeline adjusted the strand, position and reference alleles to match the HRC panel. Quality control steps were also performed. These steps ensured that enough data were available for subjects and SNPs (--mind 0.1, --geno 0.1) and kept only common SNPs (--maf 0.05) that have passed the Hardy-Weinberg equilibrium test (-- hwe 5e-8). Indeed, palindromic SNPs, SNPs with differing alleles, SNPs with no match to the reference panel, SNPs with > 0.2 allele frequency difference to the reference and duplicates were removed from the pipeline. The Sanger Imputation Service (McCarthy et al., 2016) was used for data imputation, applying default settings and the Haplotype Reference Consortium, HRC (http://www.haplotype-reference-consortium.org/) as the reference panel. The imputed SNPs were further filtered with Plink v1.90b6.17 and v2.00a3LM (Chang et al., 2015). All imputed SNPs had INFO scores R^2>0.9 and were filtered again using a more stringent filtration criterion (–mind 0.05, --geno 0.05, -- maf 0.05, --hwe 1e6, --maf 0.05, --max-alleles 2) and heterozygosity rates (--het) three standard deviation (SD) units from the mean. After these processing steps, 11,737,360 variants were available to calculate PRSs. Participants were filtered to have 0.95 loadings to the European genetic ancestry factor (coded as "GAF_europe" in the PING dataset), resulting in 526 participants. To model population structure, the same participants were used to calculate the 10 principal components across the variants, excluding areas in high linkage disequilibrium (LD)with each other (--indep-pairwise 50 5 0.2) with Plink 2.

The PRS-SMK were calculated from the GWAS and Sequencing Consortium on Alcohol and Nicotine use (GSCAN) (Liu et al., 2019) comprising 1.2 million individuals. PRS-SMK were

calculated with PRSice-2 (Choi, S. W., & O'Reilly, P. F., 2019). We clumped the data as per PRSice default settings (clumping distance = 250kb, threshold $r^2 = 0.1$), using the GWAS hits ($p< 5\times10^{-08}$) cut-off criterion. After matching with available variants in the data, the PRS-SI was based on 20,542 variants, PRS-AI on 17,406 variant, PRS-CPD on 17,934 variants and PRS-SC on 16,932 variants. PRS-SMK with various p-values were obtained, but we used the PRS-SMK with a p-value of 0.05 (see Supplementary Figure 1-4 for PRS-SMK distributions in PING).

ABCD cohort

In the ABCD cohort, we used the same SNPs associated with four smoking phenotypes: age of initiation, smoking initiation, number of cigarettes smoked per day and smoking cessation identified by GSCAN (Liu et al., 2019). More specifically, 517,724 SNPs were genotyped. Our quality control procedures were executed using the toolset PLINK (Chang et al., 2015). SNPs and individuals with very high level of missingness were filtered out (--mind 0.05, --geno 0.05). Subjects with sex mismatch were removed. Only SNPs with a minor allele frequency (MAF) threshold of 0.05 and that have passed the Hardy-Weinberg equilibrium test (-- hwe 1e-4) were kept. We excluded heterozygosity outliers (15%). We also removed relatedness closer than first cousin. We removed missingness by case control (p-value < 1e-4) and by haplotype (p-value < 1e-4). Finally, we removed non-European subjects, which resulted in 4627 subjects. Imputation was done using the Haplotype Reference Consortium (HRC r1.1 2016 (hg19) phasing Eagle v2.4). All imputed SNPs had INFO scores $R^2>0.8$. Linkage disequilibrium was used to prune variants in the GWAS summary statistic. After imputation, 12,632,435 variants were available for calculation of polygenic scores. Polygenic scores were calculated with PRSice-2 (Choi, S. W., & O'Reilly, P. F., 2019). We clumped the data as per PRSice default settings (clumping distance = 250kb, threshold

r^2 = 0.1), using the GWAS hits ($p< 5\times10^{-08}$) cut-off criterion. After matching with available variants in the data, the PRS-SI was based on 20,304 variants, PRS-AI on 17,544 variants, PRS-CPD on 17,679 variants and PRS-SC on 16,857 variants. PRS-SMK with various p-values were obtained, but we used the PRS-SMK with a p-value of 0.05. All PRS-SMK were normalised for downstream analysis so that all means of PRS, which could otherwise range from positive to negative values, could be compared. To model population structure, the same participants were used to calculate the 10 principal components across the variants, excluding areas in high linkage disequilibrium (LD) with each other (--indep-pairwise 50 5 0.2) with Plink 2 (see Supplementary Figure 5-8 for PRS-SMK distributions in ABCD).

Image acquisition, pre-processing and brain parcellations

PING Cohort

For cortical and subcortical segmentation, each location used a standardised structural MRI protocol that included a 3D T1-weighted inversion prepared RF-spoiled gradient echo scan with prospective motion correction (PROMO). The CIVET processing pipeline (Ab-Dab'bagh et al., 2006) was used to compute cortical thickness, surface area, and brain volume measurements (including total intracranial volume) for 81,924 vertices encompassing the whole cortex. Image acquisition and pre-processing are covered in more details in Jernigan et al., (2016). Two independent reviewers performed quality control (QC) on these data. Data having motion artefacts, a low signal-to-noise ratio (less than 800), artefacts caused by blood vessel hyperintensities, surface-surface junctions or improper positioning of the grey or white matter (GM and WM) surface were rejected (Khundrakpam et al., 2020).

For the brain parcellations analysis, the Desikan-Killiany-Tourville (DKT) atlas (64 cortical regions) was used. The cerebral cortex was divided into 32 regions per hemisphere based on the gyral and sulcal structure, and images were registered based on individual cortical folding patterns to match cortical geometry across subjects (Klein, A., & Tourville, J., 2012).

The automated volumetric measurements approach from FreeSurfer was used to compute subcortical volumes (Fischl et al., 2002). The subcortical volumes of the putamen, caudate nucleus, globus pallidus, and thalamus were used in our analysis.

ABCD cohort

Since it has been shown that at group level cortical thickness-estimates strongly correlate between pipelines (CIVET, FreeSurfer) (Kharabian et al., 2020), FreeSurfer version 5.3.0 was used to accomplish all structural neuroimaging pre-processing in the ABCD cohort (Casey et al., 2018). Subjects that failed FreeSurfer Quality Control were removed from the study. After QC, in total, 4277 participants were included in our study. The ABCD Data Acquisition and Integration Core used the FreeSurfer image analysis software (http://surfer.nmr.mgh.harvard.edu/) to perform cortical reconstruction and volumetric segmentation. These approaches are explained in detail in previous publications (Dale et al., 1999; Fischl et al., 1999).

In a nutshell, this procedure involves removing non-brain tissue using a hybrid watershed/surface deformation procedure (Segonne et al., 2004), automated Talairach transformation, segmentation of the subcortical white matter and deep grey matter volumetric structures (including the globus pallidus, caudate, nucleus, putamen, and thalamus) (Fischl et al., 2002), intensity normalisation,

tessellation of the gray/white matter boundary (Fischl and Dale, 2000) and automated topology correction (Fischl et al., 2001). Finally, it entails surface deformation in response to intensity gradients to properly position the gray/white and gray/cerebrospinal fluid borders where the largest intensity shift identifies the transition to the other tissue class (Fischl and Dale, 2000).

For brain parcellations, the Desikan atlas (DK atlas, 68 cortical regions) was used, images were registered based on individual cortical folding patterns to match cortical geometry across subjects and the cerebral cortex was parcellated into 34 regions per hemisphere based on the gyral and sulcal structure (Desikan et al., 2006).

Statistical analyses

PING cohort

Association between PRS-SMK, cortical thickness and surface area

To determine the association between each of the four PRS-SMK and cortical thickness and surface area, general linear models (GLM) were applied vertex-wise using the SurfStat toolbox (Worsley et al., 2009). This statistical model was used for cortical thickness and surface area:

$$\mathbf{T_i = intercept + \beta_1 PRS\text{-}SMK + \beta_2 Age + \beta_3 Age^2 + \beta_4 PRS\text{-}SMK*Age + \beta_5 PRS\text{-}SMK*Age^2 + \beta_6 Sex + \beta_7 PC10 + \beta_8 Scanner + \varepsilon_I} \quad \textbf{(Equation 1.)}$$

where *i* is a vertex, PRS-SMK is one of the four smoking polygenic risk score (PRS-SI, PRS-AI, PRS-SC or PRS-CPD), *Age* is age in years at the time of scan, PC10 are the first 10 principal components of genomic data to account for population stratification and ε is the residual error. Quadratic *Age* was also used because it had been shown to fit the data better than models with only lower degree age (Kirschner et al., 2021). For each cortical feature, vertex-wise *t*-statistics of the

association with PRS-SMK (β_1PRS-SMK) were mapped onto a standard surface. To assess the significance of PRS-SMK effects on cortical thickness or surface area, whole brain correction for multiple comparisons using Random field theory (RFT) at cluster-level $p \leq .01$ was applied (Hayasaka et al., 2004; Worsley et al.,2004). The subsequent analysis in the PING cohort was restricted to cortical thickness since surface area did not show any significant association with the PRS-SMK.

Age-centered effects of PRS-SMK on cortical thickness in the PING cohort

To verify if the significant results were associated with a specific age, age-specific associations between the four smoking PRS and cortical thickness were assessed. We used six different age range between 3 and 21 years (3 years interval) in the statistical model (Eq. 1). We chose a 3-year interval since it was the shortest interval with enough subjects to have sufficient statistical power. Each t-map was corrected for multiple comparison using RFT at $p \leq .01$ to identify the age bins with a significant relationship between SMK-PRS effects and cortical thickness. There was no significant correlation between age and PRS-SC (r=0.0173, p = 0.7320) or age and PRS-CPD (r = 0,0300, p=0.5535), which could have affected the age-centered analyses (Figure 2).

Parcel-wise and subcortical volume analyses

All the analyses were computed using matlab2019b and followed a general linear regression model (see equation 2) with sex, age, age squared, total brain volume, scanner and the 10 ancestry principal components scores as covariates:

$$T_i = \text{intercept} + \beta_1\text{PRS-SMK} + \beta_2\,\text{Age} + \beta_3\text{Age}^2 + \beta_4\text{PRS-SMK*Age} + \beta_5\text{PRS-SMK*Age}^2 + \beta_6\text{Sex} + \beta_7\text{PC10} + \beta_8\text{Scanner} + \varepsilon_I \quad \textbf{(Equation 2.)}$$

where T_i stands for the mean cortical thickness, surface area or subcortical volume per region and "PC10" stand for each of the 10 principal components. FDR correction was used to account for multiple comparisons.

DKT atlas

In addition to the voxel-wise analysis, we also used the DKT atlas to investigate if the mean cortical thickness or surface area in larger brain regions could be associated with each of the four PRS-SMK. Since we expected those brain morphometry measures to have a linear relationship with our PRS-SMK, we integrated our variables in a linear regression model. We used our four PRS-SMK as the independent variables and the 64 regions of cortical thickness or surface area as the dependent variables.

CAMMOUN atlas

To verify if a higher resolution parcellation will yield similar results to the analysis with DKT atlas, the CAMMOUN atlas with 1000 cortical regions were used (Cammoun et al., 2012). We used our four PRS-SMK as the independent variables and the mean cortical thickness per region as a dependent variable.

PRS-SMK and cortical thickness in the seven resting state networks

Since specific resting state networks such as the DMN and the CEN have been shown to be implicated in cigarette smoking (Sutherland et al., 2012), we evaluated the effect of the genetic risk for cigarette smoking on cortical thickness in the 7 Yeo resting state networks (Yeo et al., 2011). We used linear regression models with our four PRS-SMK and the mean cortical thickness in each of the seven resting state networks as dependent and independent variables, respectively.

Subcortical brain volumes

Since certain subcortical regions are implicated in dopamine production, projections and reward processes underlying tobacco use disorder (Li et al., 2015), we investigated if specific subcortical regions' volumes might be affected by a higher genetic risk for cigarette smoking. Two software packages are mainly used to compute subcortical volumes (i.e., FreeSurfer and MAGeT-Brain), but which one is the more accurate remains a matter of debate (Makowski et al., 2018). To better understand the effect of the software choice in our subcortical brain volumes analysis, we compared the results obtained by both toolboxes.

Subcortical brain volumes using the FreeSurfer software

Freesurfer was first used to segment brain subcortical regions (Fischl, B., 2012). Then, linear regression models with four subcortical brain regions (thalamus, caudate nucleus, putamen, and globus pallidus) and the four smoking phenotypes were computed. The four PRS-SMK were used as the independent variables and the volume of the four subcortical brain regions were used as independent variables.

Subcortical brain volumes using the MAGeT-Brain pipeline

We used MaGeT-Brain, a pipeline developed by a research group at the Douglas Mental Health University Institute (Chakravarty et al., 2013), to segment brain subcortical regions with a high accuracy. T1-weighted MRIs were uploaded into the MaGet-Brain pipeline and the brain volumes were obtained following the documentation from the cobra-lab (Tullo et al., 2018). Linear regression models were computed with three subcortical brain regions in both hemispheres (thalamus, striatum and globus pallidus) and the four smoking phenotypes. The four PRS-SMK

were used as the independent variables and the volume of the three subcortical brain regions (per hemisphere) were used as independent variables.

ABCD cohort

Since the T1-MRI were pre-processed and quality control was done only with Freesurfer in the ABCD cohort, the analysis was performed only with this software for the subcortical volumes and were computed only parcel-wise for the cortical thickness and surface area measures (DK atlas parcellation).

Association between PRS-SMK, cortical thickness and surface area

Linear regressions with brain morphometric data as dependent variable, polygenic risk scores for cigarette smoking as independent variable and sex, age, age squared, intracranial volume, scanner and ancestry principal components scores as covariates were computed using matlab2019b (see equation 2). To account for multiple comparisons, we used FDR correction.

Subcortical brain volumes

Freesurfer was used to segment subcortical brain regions to investigate if specific subcortical regions' development (thalamus, caudate nucleus, putamen, and globus pallidus) was affected by a higher genetic risk for cigarette smoking. Therefore, we did a linear regression model with these four subcortical brain regions and the four smoking phenotypes. Our model followed Eq. 2 and the same statistical analysis for the subcortical volumes than in the PING cohort. The four PRS-SMK were used as the independent variables and the four subcortical brain regions were used as independent variables.

Socioeconomical status

Since SES has been related to cigarette smoking (Hiscock et al., 2012) and could have an impact on brain morphometry (Piccolo et al., 2016), we controlled the influence of this variable on our results. We calculated a compositive score for SES by summing two scores from the ABCD questionnaires: the highest education level achieved between the parents and the child's household income. Before adding SES to our model, we verified the correlation between our composite score and the four different PRS-SMK to avoid collinearity between PRS-SMK and SES, but no correlation was found (see Table 4). We added this compositive score to our initial model (equation 2).

Impulsivity traits

Since cigarette smoking has been related with higher impulsivity (Debry et al., 2008), we examined the correlation between the four PRS-SMK and the combined score of positive urgency and lack of planning (two measures of impulsivity) for the subjects in the ABCD dataset. Expecting a linear relationship between the four PRS-SMK and the impulsivity scores, we used Pearson correlations. Out of the 4627 subjects with Polygenic risk scores, 3449 subjects had a score for both positive urgency and lack of planning (from the UPPS-P children short version questionnaire) and were used for the correlations.

Results

PING cohort

Vertex-wise analysis

When using the whole PING cohort (aged from 3-21 years old), we found that higher cortical thickness in regions situated in both the right and left temporal lobes were related to higher PRS-

CPD while higher PRS-SC were significantly associated with clusters in the left frontal, temporal and parietal lobes (see Figure 1A and 1C, respectively). More specifically, in participants aged 3 to 6, age-dependent analyses demonstrated increased cortical thickness in the same locations for higher PRS-CPD and in clusters in the left temporal and parietal lobes for higher PRS-SC (see Figure 1B and 1D).

Parcel-wise and subcortical volume analysis

However, the analyses with the PING dataset using two atlases with different resolutions (DKT and Cammoun atlas) yielded no significant results for cortical thickness and surface area after correcting for multiple comparisons ($p\text{-value}_{FDR}>0.05$) (see Table 2). Moreover, no significant effect between each of the four PRS-SMK and the mean cortical thickness in each of the seven resting state networks was observed ($p\text{-value}_{FDR}>0.05$) (see Table 2). No significant results were found between the four PRS-SMK and the subcortical volumes using Freesurfer and the MaGeT-Brain pipeline ($p\text{-value}_{FDR}>0.05$) (see Table 2).

ABCD cohort

Parcel-wise analysis and subcortical volume analysis

We showed that larger cortical thickness in two separate regions of the right frontal lobe was related to higher PRS-SI using the DK atlas in the ABCD dataset (Casey et al., 2018) (See Figure 2A). Increased cortical thickness was linked to PRS-CPD in three regions of the left frontal and temporal lobes and two regions of the right temporal lobe (See Figure 2B). The PRS-SC was associated with lower cortical thickness in the right middle temporal gyrus (see Figure 2C) (p-

value$_{FDR}$<0.05). However, no significant results were found between each of the four PRS-SMK and the subcortical volumes using Freesurfer (p-value$_{FDR}$>0.05) (see Table 3).

Relationships with clinical variable and trait

Relationships between the four PRS-SMK and SES and impulsivity were also investigated. However, no significant correlation was found with SES or any of the impulsivity traits (see Table 4). Moreover, adding SES as covariate in the statistical models in the ABCD cohort yielded the same significant regions than the ones previously observed.

Discussion

In this study, we used neuroimaging and genetics data to investigate whether difference in PRS-SMK reflected differences in the neuroanatomy of brain regions in children and adolescents. We characterized the relation between four PRS-SMK and cortical morphometry. We found that cortical thickness was associated with PRS-SMK, but surprisingly, we found a general pattern of increased cortical thickness. Indeed, higher PRS-CPD and PRS-SC were associated with increased cortical thickness across the cortex in typically developing children from 3-21 years old. In children from 9-12 years old, higher scores of PRS-CPD and PRS-SC were associated with increased cortical thickness in areas of the frontal lobes and decreased cortical thickness in the middle temporal gyrus, respectively. Additionally, children from 9-12 years with higher scores of PRS-SI showed reduced cortical thickness in regions of the right frontal lobe. Furthermore, age group models suggest a potential trajectory from PRS-SMK associated cortical thickness increase in early childhood, more specifically from age 3-6 years old.

Surface area and subcortical volumes showed no association with any of the four PRS-SMK, suggesting a differentiation in the mechanisms of maturation in brain cortical thickness, surface area and subcortical volume.

Finally, we also investigated the relationship between the genetic risk for smoking and the seven resting state networks (Yeo et al., 2011), as well as socioeconomic status and impulsivity. We hypothesized that greater PRS for SI, SC, and CPD, as well as lower PRS-AI, would be associated to the resting state networks involved in tobacco use disorder, such as the DMN and CEN (Sutherland et al., 2012), as well as lower SES (Hiscock et al., 2012), and higher impulsivity scores (Debry et al., 2008). No association was found between PRS-SMK and any of the seven resting state networks. This suggests that PRS-SMK have an influence on specific brain regions, potentially affecting different brain functions, but does not affect entire networks. Lastly, no correlation was found between any of the four PRS-SMK and SES or impulsivity traits. This confirms that, while PRS-SMK are associated to cigarette smoking in adults (Deak, J. D., & Johnson, E. C., 2021), cigarette smoking is a result of a much more complex interplay between genes and the environment.

Cigarette smoking in adults has been both related to faster cortical thinning and reduced thickness of cortical regions in longitudinal and cross-sectional studies (Kühn et al., 2010; Li et al., 2015). It's worth noting that the areas implicated in these studies overlap with the areas in the current study that show greater cortical thickening in early and late childhood (i.e., the orbitofrontal cortex, anterior cingulate cortex, and middle temporal gyrus).

The cause of increased cortical thickness in our sample needs to be investigated. A post-mortem study on cigarette smokers has identified a change in gene-expression in the postsynaptic density in the hippocampus (Mexal et al., 2005). It is possible that postsynaptic density is related to cortical thickness and that reduced cortical thickness in adult smokers results from postsynaptic density loss.

The relationship between the PRS-SMK and increased cortical thickness in early and late childhood raises the question of how the inherited risk of cigarette smoking contributes to a different developmental trajectory. Our findings raise the possibility of a delay in cortical thinning in childhood since the orbitofrontal cortex showed an increased cortical thickness correlating with higher PRS in contradiction to the neurotypical development trajectory of cortical thinning in childhood (Wierenga et al., 2014). Moreover, our findings in ages 3-6 and 9-12 are plausible with the observed the reduction cortical thickness in the orbitofrontal cortex of young adult smokers around 19 years old (Li et al., 2015). This is attributable to the fact that in polygenic disorders, several different trajectories might emerge simultaneously or sequentially (Kirschner et al., 2021) due to the complicated and heterochronic nature of cortical maturation during childhood and adolescence (Wierenga et al., 2014; Fjell et al., 2015; Tamnes et al., 2017). A delayed trajectory in the morphometry of areas in the association cortex may represent a general pattern of cortical development in children with higher PRS-SI, PRS-CPD, PRS-SC, and lower PRS-AI. Nonetheless, the PRS-SMK incorporates several genetic factors, each of which may have a different impact on cortical development.

Since the study's cross-sectional design limits the ability to trace cortical thickness trajectories, the findings should be interpreted accordingly. Moreover, the overlap between the new findings and

neuroanatomical studies on smokers point to a link between polygenic risk and smoking clinical phenotype, but given the limited heritability of cigarette smoking ($h^2 = 0.5$) (Bierut, L., & Cesarini, D., 2015) further investigations are necessary. Future research could benefit from larger datasets, longitudinal designs, and longer follow-up to determine which people with higher PRS-SMK will become smokers. A longitudinal study using the ABCD dataset, for example, may look at the children from the current study and their future smoking habits. In addition, the increased cortical thickness associated with the PRS-SMK in early and late childhood (ages 3 to 6 and 9-12) highlights the need for larger datasets with children to target a more specific age range. Finally, the PRS-SMK values used in this investigation were derived using a demographic of European ancestry. Given that it is not clear if our findings can also apply to other populations, more GWAS on cigarette smoking should be computed in other communities, and tools to translate SNPs from GWAS to SNPs from different dataset with different population should be developed.

Conclusion

The findings of this study about the relationship between the genetic risk for cigarette smoking and differences in cortical thickness in children could help create new cigarette smoking neurodevelopmental model. Furthermore, this neuroimaging and genetics-based approach contributes to a better understanding of the complicated relationships between hereditary risk for cigarette smoking and environmental factors. Overall, the current study uncovers brain regions related to tobacco use disorder that could also potentially be implicated in other addictions or to genetically related mental illnesses.

CHAPTER 4

4. GENERAL DISCUSSION

Our study's primary objective was to test whether differences in PRS-SMK would be related with differences in brain morphometry in specific brain regions in children and adolescents, prior to smoking. As hypothesized, differences in cortical thickness were observed in frontal and temporal regions in early and late childhood, but contrary to what we expected, there was a pattern of increased cortical thickness in most of the regions. These findings could be explained by a delay in maturation in areas where cortical thickness has increased.

Our second aim was to examine whether PRS-SMK were associated with the seven resting state networks. Contrary to our hypothesis, the PRS-SMK showed no association with each of the seven resting state networks.

The last aim of the study was to evaluate whether PRS-SMK had similar associations as cigarette smoking has with low socioeconomic status and high impulsivity traits. Contrary to what was previously observed in studies on cigarette smoking (Hoostock et al., 2012; Debry et al.,2018), there was no association between the PRS-SMK and SES or impulsivity traits.

4.1 Further analyses

For further analyses, computing vertex-wise analysis and subcortical volumes with the MAGeT-brain pipeline in the ABCD dataset could be interesting to verify if it yields similar results than the ones obtained with the PING cohort. Furthermore, since the ABCD dataset has longitudinal data available, performing a longitudinal analysis with the participants in the present study could help verify if the PRS-SMK would be related to tobacco use disorder in the future and to longitudinal morphological changes in the same brain regions identified in this study. Additionally, because correlations between SES and impulsivity scores were not conducted in the PING dataset due to missing data, linear mixed-effects models might be used to account for these missing data and compare the results to the ABCD dataset. Moreover, correlating family history of addiction with structural brain measures could be helpful to verify the overlap with the brain regions identified in the present study. Because the CHRNA5 gene causes increased nicotinic receptor responsiveness (Sharp B. M. & Chen H., 2019), which contributes to tobacco use disorder, calculating PRS without this gene and investigating its association with different structural brain measures could shed more light on other genetic mechanisms implicated in tobacco use disorder, such as the lack of impulse control suggested in this study. The current analyses were performed with linear regressions, but using a different statistical method such as partial least square regressions (PLS) could help verify the replicability of our results. In addition, other groups have used genomic structural equation modelling (SEM) when calculating Polygenic Risk Scores (Grotzinger et al., 2019). Combining the different PRS-SMK into one score could help target more specific regions implicated with cigarette smoking. Lastly, genetic risk for tobacco use disorder is highly correlated to alcoholism, cannabis addiction, schizophrenia and other mental illnesses sharing overlapping genes (Clarke et al., 2017; Brouwer et al., 2022; Pasman et al., 2018). Using the PRS-SMK calculated in the present study in addition to structural brain measures from patients

with these different disorders could help understand if the regions related to the PRS-SMK are also affected in other psychiatric disorders.

4.2 Strengths

This study is the first research project investigating the association between the PRS-SMK and brain morphometry in neurodevelopmental cohorts. Indeed, other groups have studied PRS-SMK in neurodevelopmental datasets, but they only investigated the relationship with smoking behaviors. Also, two different software (MaGeT-brain and Freesurfer) were used to compute the subcortical volumes and compared the findings in the PING dataset. Lastly, the two datasets used had large sample sizes (393 subjects and 4627 subjects for PING and ABCD, respectively) and the PING cohort had children with a wide range of ages.

4.3 Limitations

Since the two datasets had different sample size and age range (3-21 years old and 9-12 years old for PING and ABCD, respectively), it did not enable us to compare directly the results obtained with both datasets. Moreover, the Info score used to calculate the PRS-SMK was not matched between the PING (>0.8) and the ABCD cohort (>0.9), which could have affected the values of the SNPs included in the analyses. Furthermore, the PRS-SMK used in this study were calculated using SNPs from populations with European ancestries as research on the application of GWAS from European ancestries to other population has shown that SNPs don't overlap between populations (Carlson et al., 2013). Therefore, other investigations must be performed to verify if the findings from this study can be generalized to different populations. Additionally, because this

study was performed on neurodevelopmental cohorts of non-smokers, other studies will be needed to verify if similar findings are also associated with cigarette smoking. Lastly, although this study used neurodevelopmental cohorts of non-smokers subjects in PING (n=19) and ABCD (n=579) were exposed to prenatal cigarette smoking which could have had an impact on brain morphometry (El Marroun et al., 2014). Further analyses comparing subjects' brain morphometry with and without prenatal cigarette smoking exposure would be useful to control the confounding effect of prenatal exposure on the association between PRS-SMK and brain morphometry.

4.4 Conclusion

In conclusion, our findings suggest that the genetic risk for cigarette smoking has an impact on early brain development around 9 to 12 years of age. This might reflect a change in the rate of cortical maturation in specific frontal and temporal regions previously shown to be associated with tobacco use disorder (Kühn et al., 2010; Li et al.,2015) such as the orbitofrontal and anterior cingulate cortex, and the middle temporal gyrus. Furthermore, PRS-SMK impact brain development in early and late childhood in areas (orbitofrontal cortex, anterior cingulate cortex) involved in reward, motivation and impulse control potentially contributing to smoking initiation and persistence. Further investigations with another dataset including a larger number of subjects should be conducted to generalize our results. These findings could be used to focus public health efforts on children aged 9 to 12, who are more prone to a lack of impulse control in the prevention of tobacco use disorder or other substance use disorders, if they are generalized.

CHAPTER 5

5. Figures & Table

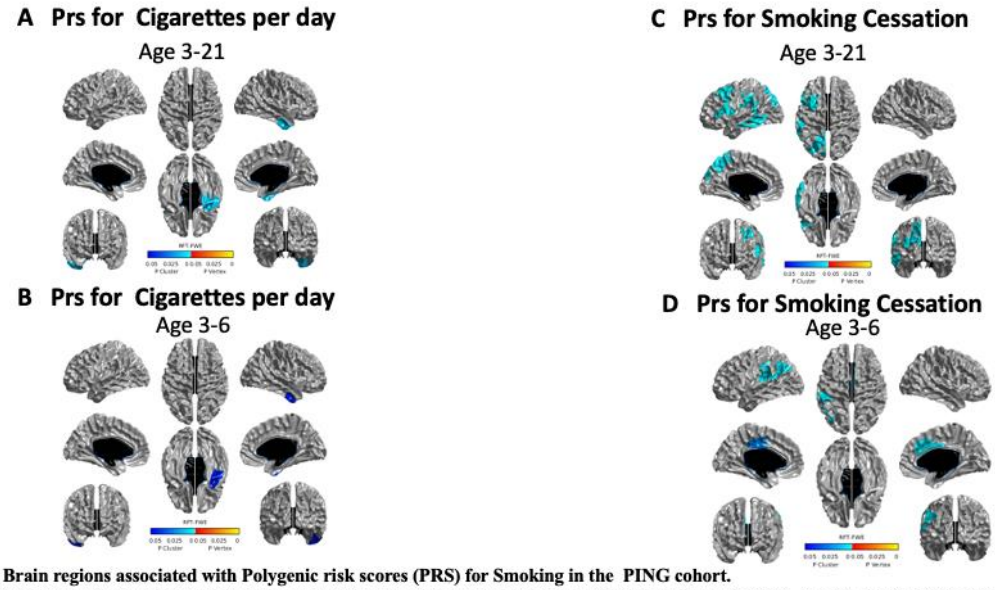


Figure 1. Brain regions associated with Polygenic risk scores (PRS) for Smoking in the PING cohort. (A,B) PRS for Cigarettes per day significantly associated with clusters in the right and left temporal lobes ($p<0.05$, $\beta > 0$). (C,D) PRS for Smoking Cessation significantly associated with clusters across the cortex ($p<0.05$, $\beta > 0$). (A) PRS for Cigarettes per day significantly associated with clusters in the right and left temporal lobes for the whole cohort ($p<0.05$, $\beta > 0$). (B) PRS for Cigarettes per day significantly associated with clusters in the right and left temporal lobes for the subjects aged from 3-6 years old ($p<0.05$, $\beta > 0$). (C) PRS for Smoking Cessation significantly associated with clusters across the cortex for the whole cohort ($p<0.05$, $\beta > 0$). (D) PRS for Smoking Cessation significantly associated with clusters across the cortex for the subjects aged from 3-6 years old ($p<0.05$, $\beta > 0$).

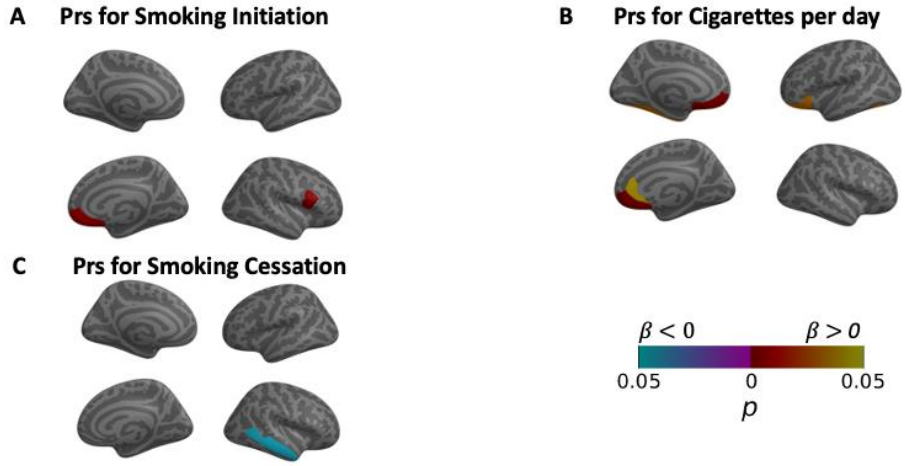


Figure 2. Brain regions associated with Polygenic risk scores (PRS) for Smoking using the DK atlas in the ABCD cohort. (A) PRS for Smoking Initiation significantly associated with right mOFC and Pars opercularis ($p<0.05$, $\beta > 0$). (B) PRS for Cigarettes per day significantly associated with left Fusiform gyrus, mOFC and lOFC and right rACC and mOFC ($p<0.05$, $\beta > 0$). (C) PRS for Smoking Cessation significantly associated with the right Middle temporal gyrus ($p<0.05$, $\beta < 0$). mOFC = Medial Orbitofrontal Cortex; lOFC = Lateral Orbitofrontal Cortex; rACC = rostral anterior cingulate cortex.

Table 1: PRS-SMK and Vertex-wise analysis in the PING cohort

		BRAIN MEASURES	
PRS	**Ages**	**Cortical Thickness**	**Surface Area**
Smoking Initiation	3-21	p>0.05	p>0.05
	3-6	p>0.05	p>0.05
Age of Initiation	3-21	p>0.05	p>0.05
	3-6	p>0.05	p>0.05
Cigarettes per day	**3-21**	**1 cluster in the left and right temporal lobes p<0.05 b>0**	p>0.05
	3-6	**1 cluster in the left and right temporal lobes p<0.05 b>0**	p>0.05
Smoking cessation	**3-21**	**clusters in the left and right frontal temporal, parietal lobes p<0.05 b>0**	p>0.05
	3-6	**clusters in the left and right temporal and parietal lobes p<0.05 b>0**	p>0.05

Table 2: PRS-SMK and Parcel-wise analyses and subcortical volume analyses in the PING cohort

	BRAIN MEASURES						
	DKT ATLAS		CAMMOUN ATLAS				
PRS	**Cortical Thickness**	**Surface Area**	**Cortical thickness**	**Surface area**	**7 resting state networks**	**Subcortical volumes with FreeSurfer**	**Subcortical volumes with MAGeT-Brain pipeline**
Smoking Initiation	p>0.05 For all parcel-lations	p>0.05 For all parcel-lations	p>0.05 For all parcel-lations	p>0.05 For all parcel-lations	p>0.05 For the 7 net-works	p>0.05 For all sub-cortical regions	p>0.05 For all sub-cortical regions
Age of Initiation	p>0.05 For all parcel-lations	p>0.05 For all parcel-lations	p>0.05 For all parcel-lations	p>0.05 For all parcel-lations	p>0.05 For the 7 net-works	p>0.05 For all sub-cortical regions	p>0.05 For all sub-cortical regions
Cigarettes per day	p>0.05 For all parcel-lations	p>0.05 For all parcel-lations	p>0.05 For all parcel-lations	p>0.05 For all parcel-lations	p>0.05 For the 7 net-works	p>0.05 For all sub-cortical regions	p>0.05 For all sub-cortical regions
Smoking cessation	p>0.05 For all parcel-lations	p>0.05 For all parcel-lations	p>0.05 For all parcel-lations	p>0.05 For all parcel-lations	p>0.05 For the 7 net-works	p>0.05 For all sub-cortical regions	p>0.05 For all sub-cortical regions

Table 3: PRS-SMK and Parcel-wise analysis and subcortical volume analysis in the ABCD

	BRAIN MEASURES		
	DK ATLAS		
PRS	**Cortical Thickness**	**Surface Area**	**Subcortical volumes with FreeSurfer**
Smoking Initiation	**Right Medial Orbito-frontal Cortex and Pars opercularis p<0.05 b>0**	p>0.05	p>0.05
Age of Initiation	p>0.05	p>0.05	p>0.05
Cigarettes per day	**Left and Right Medial Orbito-frontal Cortex Left Fusiform gyrus and left lateral Orbito-frontal Cortex Right Rostral Anterior Cingulate Cortex p<0.05 b>0**	p>0.05	p>0.05
Smoking cessation	**Right Middle Temporal Gyrus p<0.05 b<0**	p>0.05	p>0.05

Table 4: PRS-SMK and relation with socioeconomic status and impulsivity trait

		Impulsivity Traits Associated with Smoking	
PRS	**SES**	**Positive Urgency**	**Lack of planning**
Smoking Initiation	r=-0.0208 p=0.1581	r=0.0015 p=0.9277	r=-0.0028 p=0.8675
Age of Initiation	r=0.0056 p= 0.7028	r=-0.0137 p=0.4227	r=-0.0114 p=0.5021
Cigarettes per day	r= 0.0039 p= 0.791	r=-0.0076 p=0.6571	r =0.0028 p=0.8681
Smoking cessation	r=-0.0075 p=0.6083	r = 0.0024 p=0.886	r = 0.0182 p=0.2863

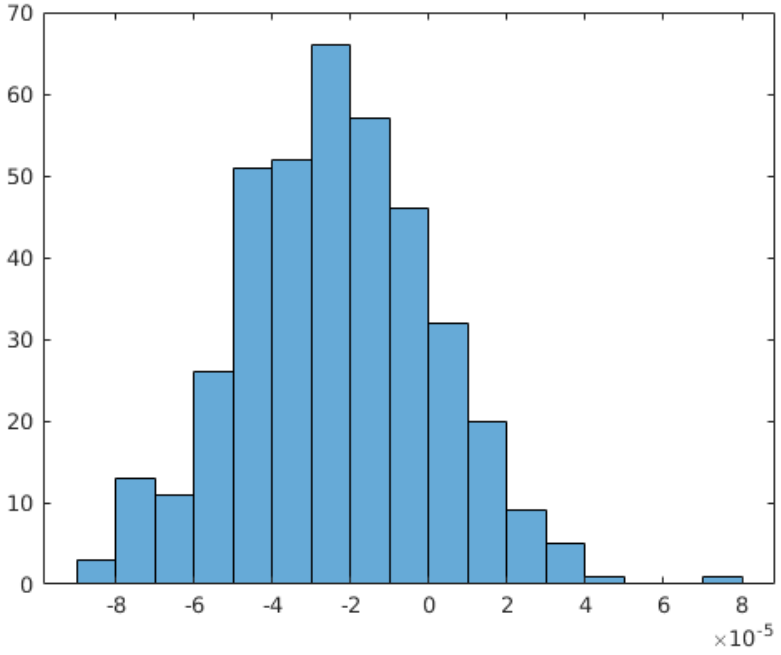
Supplementary Table 1. DSM-5 TUD criteria

1. Tobacco often taken in larger amounts or over longer periods than intended.
2. A persistent desire or unsuccessful efforts to cut down or control tobacco use.
3. A great deal of time spent on activities necessary to obtain or use tobacco.
4. Craving or strong desire or urge to use tobacco
5. Recurrent tobacco use resulting in a failure to fulfill major role obligations at work, school, or home.

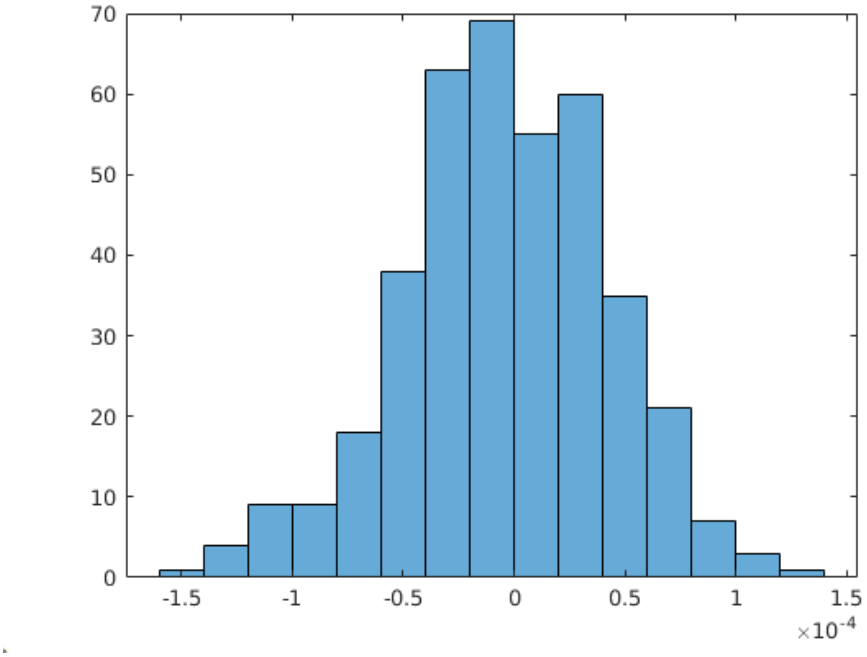
6. Continued tobacco use despite having persistent or recurrent social or interpersonal problems caused or exacerbated by the effects of tobacco.	
7. Giving up or reducing important social, occupational, or recreational activities because of tobacco use.	
8. Recurrent tobacco use in situations in which it is physically hazardous.	
9. Continued tobacco use despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to have been caused or exacerbated by tobacco.	
10. Tolerance, as defined by either the need for markedly increased amounts of tobacco to achieve the desired effect or a markedly diminished effect with continued use of the same amount of tobacco.	
11. Withdrawal, as manifested by either the characteristic withdrawal syndrome or the use of tobacco to relieve or avoid withdrawal symptoms	
Diagnostic threshold	≥ 2 items

(Paik et al., 2019)

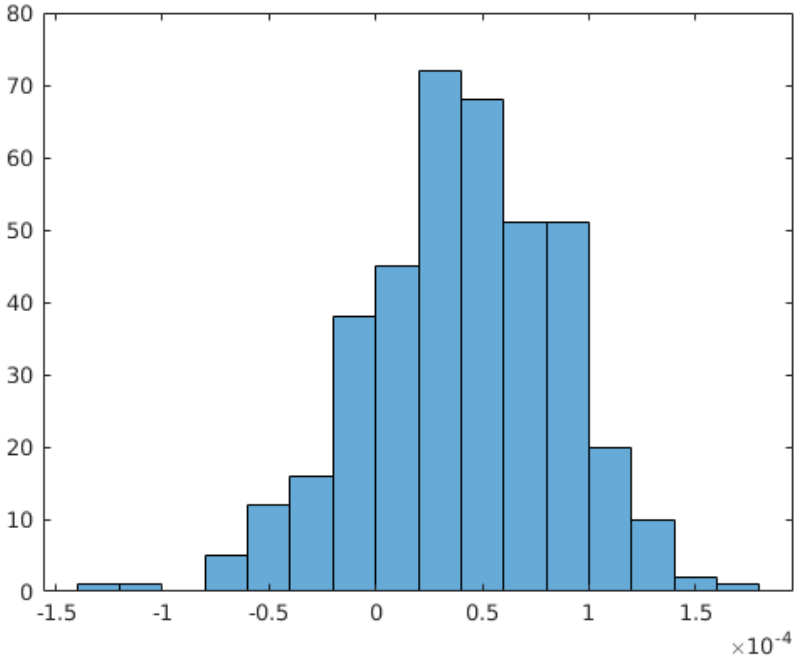
Supplementary Figure 1: Distribution of PRS-AI in the PING cohort



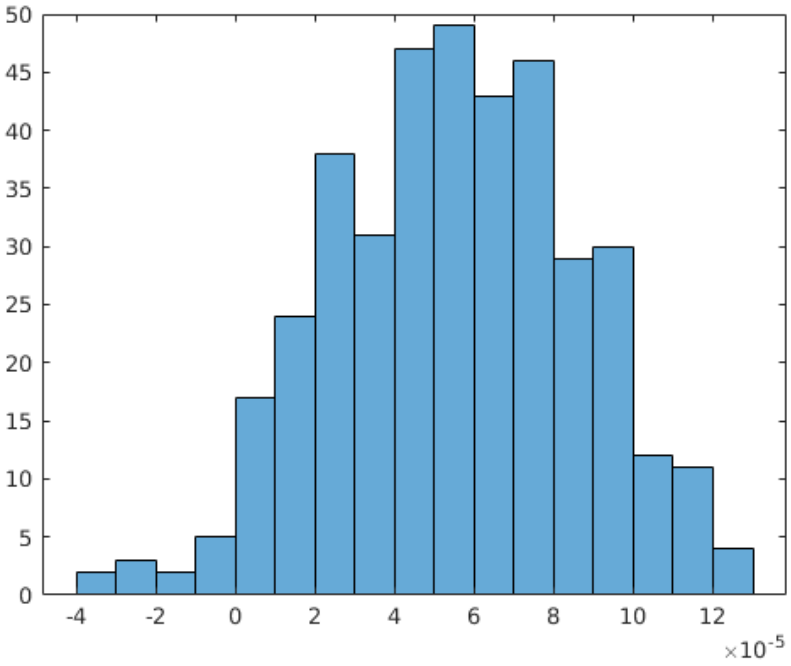
Supplementary Figure 2: Distribution of PRS-CPD in the PING cohort



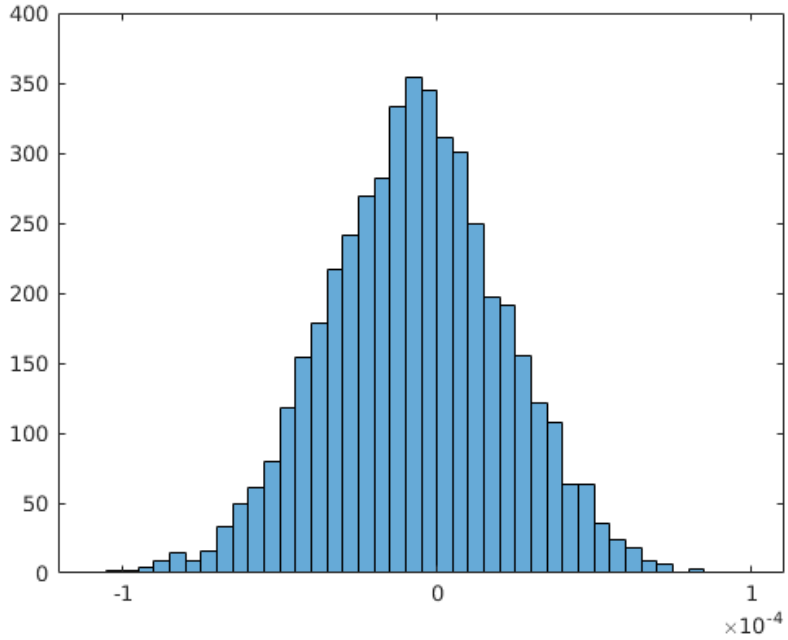
Supplementary Figure 3: Distribution of PRS-SC in the PING cohort



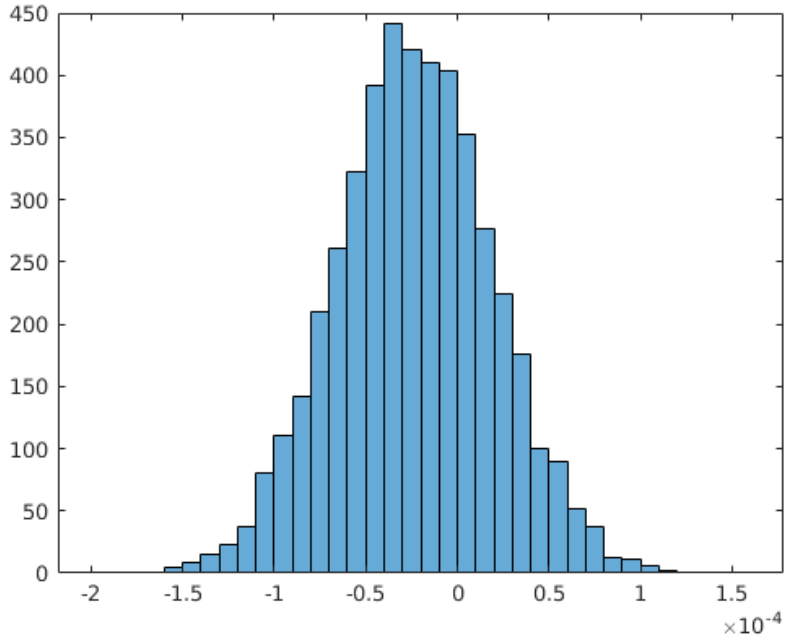
Supplementary Figure 4: Distribution of PRS-SI in the PING cohort



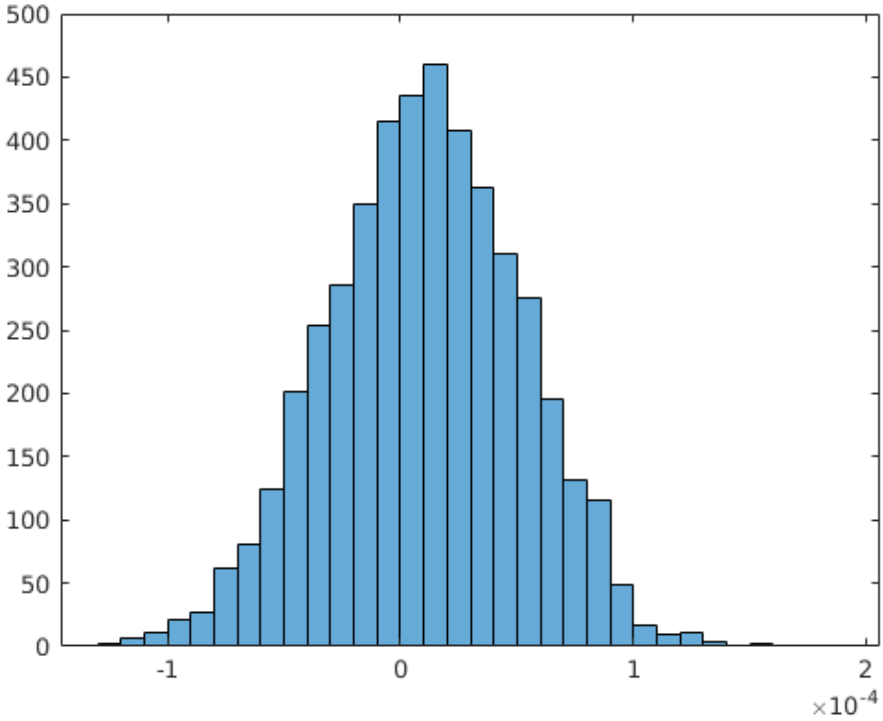
Supplementary Figure 5: Distribution of PRS-AI in the ABCD cohort



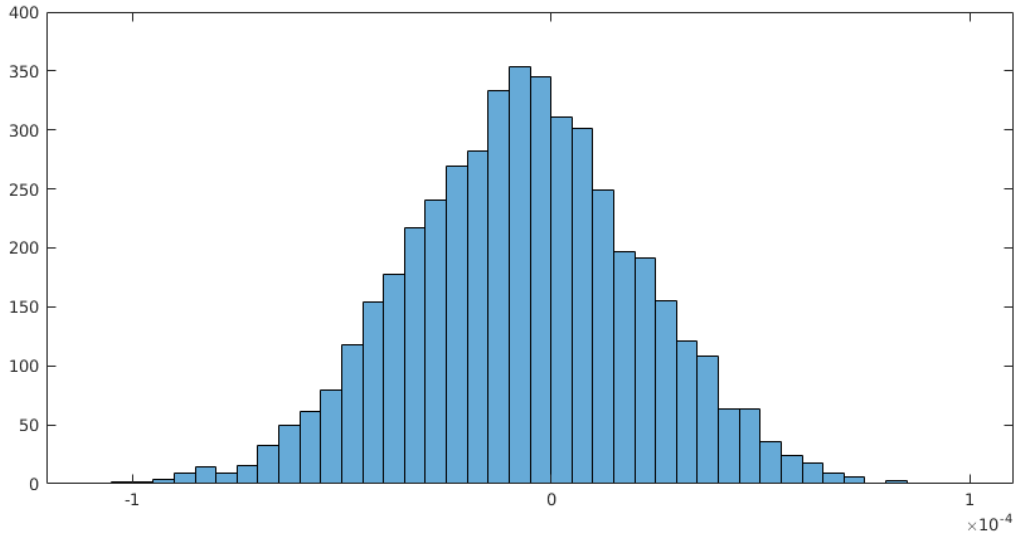
Supplementary Figure 6: Distribution of PRS-CPD in the ABCD cohort



Supplementary Figure 7: Distribution of PRS-SC in the ABCD cohort



Supplementary Figure 8: Distribution of PRS-SI in the ABCD cohort



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