FAMILY HISTORY OF SUBSTANCE USE PROBLEMS ON CORTICAL MORMPHOMETRY
IN HEALTHY CHILDREN

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ABSTRACT

Background
Common structural brain abnormalities reported in substance use related (SUD) related regions were found in children with family history (FH) of SUD. However, the effect of FH-degree of SUD is not well examined. Therefore, the influence of degree of FH of substance use problems (SUP) on cortical morphometry was investigated.

Methods
921 typically developing children (ages 3-20 years old; 470 boys/451 girls) had structural MRI and executive functions measures. The influence of first and/or second-degree FH (Any-FH) and the separate effects of first-degree (First-FH) and second-degree FH of SUP (Second-FH) on cortical structures were investigated using FreeSurfer. The relationships between FH related cortical alterations and executive functioning were examined. Sex interactions with FH-degree on cortical morphometry were also investigated.

Results
Any-FH children had volume alterations mediated by changes in surface areas. FH children have larger left prefrontal and right superior areas and volumes when compared to No-FH children. First-FH and Second-FH show a variable effect on cortical volumes particularly for regions in the posterior frontal and anterior parietal regions as well as the right fusiform gyrus. Sex differences for cortical morphometry between First-FH and Second-FH children were widespread, but strongest in the left frontal regions. The left supramarginal and right fusiform gyri, regions specifically reduced for First-FH children, were also related to differential executive functioning and this same association was found in First-FH girls. Importantly, the majority of results remained even when controlling for confounders such as ADHD.

Conclusion
The children with FH of SUP have overall differences in prefrontal regions. First-FH and Second-FH children showed different morphometric alterations in parietal and temporal regions, which may contribute to deficits in executive function. Further studies are need to explore the sex-specific influences in cortical morphometry of children with FH of SUP, and the possible consequences on cognition.
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LIST OF ABBREVIATIONS

Any-FH: Children with first and/or second-degree family members with substance use problems

AUD: Alcohol use disorders

First-FH: Children with first-degree family members with substance use problems

FH: Family History

GAF: Genetic Ancestry Factor

No-FH: Children with no first and/or second-degree family members with substance use problems

PING: Pediatric Imaging, Neurocognition, and Genetics

SAMSHA: The Substance Abuse and Mental Health Services

Second-FH: Children with second-degree family members with substance use problems

SNP: Single nucleotide polymorphism

SUD: Substance use disorders

SUP: Substance use problems
CHAPTER 1. INTRODUCTION

Substance use disorders (SUD) are a pervasive problem in our society with approximately 8.2% of the population, ages 12 and over having SUD (SAMSHA, 2013). Family history (FH) of SUD is a strong risk factor for the development of drug and alcohol use disorders (Biederman et al. 2000; Bierut et al. 1998; Merikangas et al. 1998; Nurnberger et al. 2004), making a person eight times more likely to develop SUD across a range of substances (Merikangas et al. 1998). Increased density of family members with SUD has also been associated with early onset of alcohol use (Hill and Yuan 1999) and is a risk factor for poorer long-term outcomes for adolescent-onset alcohol use disorders (AUD) (Khoddam et al. 2015).

Inhibitory control, a skill that keeps us from acting impulsively, is an executive function. SUD individuals have impaired inhibitory control as they have lost their ability to inhibit the desire to pursue immediately pleasurable stimuli such as alcohol and drugs (Kalivas & Volkow, 2005). Deficits in inhibitory control play a critical role in increased risk for SUD in youth (Ivanov et al. 2008, for review). In a longitudinal study, poorer baseline inhibitory control in children with SUD parents was predictive of children’s later adolescent alcohol and drug use (Nigg et al. 2006). Therefore, poorer inhibitory control in children with FH of SUD is a strong predictor of substance use in youth.

Past studies of the influence of FH on cortical morphometry in youth have often investigated the frontal cortices (Benegal et al. 2007; Hill et al. 2009; Squeglia et al. 2015), due to the association of frontal alterations for adolescent SUD (De Bellis et al. 2005; Medina et al. 2008; Churchwell et al. 2010; Fein et al. 2013; Doallo et al. 2014). However, findings are inconsistent and may be due in part to different definitions of FH used in each study. Orbitofrontal cortex volumes did not differ between controls and youth with first- and second-degree relatives with AUD (Squeglia et al. 2015), although an association was found for volume of the right orbitofrontal cortex relative to the left for siblings with two alcohol dependent brothers (Hill et al. 2009). Another frontal region, the superior frontal cortex, was found to be smaller bilaterally in high-risk youth who were alcohol naïve but sons of fathers with early onset AUD (Benegal et al. 2007). Although studies of cortical morphometry for FH of SUD children are limited, findings suggest FH of SUD is associated with abnormal frontal brain regions and smaller frontal volumes is associated with later substance use in longitudinal studies (Cheetham et al. 2012;
Therefore, the underlying alterations in cortical volumes, surface area and thickness, may provide insight in the association between FH of SUD and later substance use.

Overall, past studies in FH youth have separately found underlying cortical (Benegal et al. 2007; Hill et al. 2009) or cognitive (Acheson et al. 2011; Aytaclar et al. 1999; Gierski et al. 2013; Nigg et al. 2006) abnormalities in youth and adults with a FH of SUD. However, only a single study of FH youth to date has evaluated the association between FH and cortical gray matter morphometry measures on cognitive performance and found no association, even when the association was investigated within each sex separately (Silveri et al. 2008).

SUD differs between the sexes as men have a higher prevalence of SUD (SAMSHA 2013), while women tend to develop SUD more rapidly and relapse more (Brady and Randall 1999; Ignjatova and Raleva 2009). Development of SUD may be due in part to the differential normal development of the brain between sexes. Boys overall have larger gray matter volumes and sex differences also exist in the development of cortical structures for boys and girls with girls generally peaking in volume about a year before boys (Giedd et al. 1997; Giedd et al. 1999). However, sex differences have only been found for FH of SUD children for white matter (Silveri et al. 2008) and subcortical regions (Hanson et al. 2010; Cservenka et al. 2015). Sex differences have been found for the cortical structures of adolescent alcohol users in comparison to same sex controls although the sample size was small (Medina et al. 2008). In addition, sex differences for executive functioning have been found for FH of SUD children, with girls performing worse on executive function tasks in comparison to same sex controls with the opposite findings within boys. In healthy children, inhibitory control also differs between sexes in childhood with girls having better inhibitory control (Else-Quest et al. 2006). However, once children reach adulthood the female benefit is inconsistently found (Hosseini-Kamkar and Morton 2014).

This study aims to address several limitations from previous studies of FH children. First, past FH studies of cortical morphometry in youth have focused only on brain volumes (Benegal et al. 2007; Hill et al. 2009; Squeglia et al. 2015), but ignored cortical surface area and thickness. Second, FH studies have often grouped first and second-degree FH together without examining potential differences in degree of FH (Hanson et al. 2010; Herting et al. 2010; Herting et al. 2010).
2011; Silveri et al. 2008; Squeglia et al. 2015) or evaluated only relatives of individuals with either alcohol (Acheson et al. 2011; Aytaclar et al. 1999; Benegal et al. 2007; Gierski et al. 2013; Hill et al. 2009; Nigg et al. 2006; Silveri et al. 2008; Sjoerds et al. 2013; Squeglia et al. 2015) or stimulant use disorders (Ersche et al. 2012; Ersche et al. 2013). However, little is known about the common risk of SUD on relatives. Third, FH studies have sometimes included (or not specifically excluded) children who may have had prenatal exposure to alcohol or drugs (Benegal et al. 2007; Hill et al. 2009; Ersche et al. 2012; Ersche et al. 2013; Sjoerds et al. 2013) although children with prenatal substance exposure have structural abnormalities (Chang et al. 2004; Akyuz et al. 2014; Rajaprakas et al. 2014; Gautam et al. 2015, for example) and may differ from structural alterations associated with FH of SUD.

Therefore, the association between FH and FH-degree and cortical morphometry measures (cortical thickness, surface area and volumes) was investigated. In addition, the association of sex and FH-degree on cortical morphometry was examined. Lastly, the association between FH and cortical morphometry on executive function and the role of sex in these associations was studied.

Specific Aims

Specific Aim 1: Evaluate the association of FH of any alcohol and/or substance problems (SUP) on measures of cortical surface area, thickness, and volume in a wide range (ages 3-20) of typically-developing children.

Hypothesis: FH of SUP children will have smaller frontal volumes based on a past finding of children with FH of SUD having smaller superior frontal cortices (Benegal et al. 2007). Additionally, in longitudinal pediatric studies smaller frontal volumes were associated with later substance use (Cheetham et al. 2012; Squeglia et al. 2014).

Specific Aim 2: To investigate the separate influences of first-degree FH (First-FH) and second-degree FH (Second-FH) on cortical morphometry measures.

Hypothesis: Similar to the previous hypothesis, FH of SUP children will have smaller volumes based on the aforementioned studies (Benegal et al. 2007; Cheetham et al. 2012; Squeglia et al. 2014). However, it is predicted that the cortical morphometry of First-FH children will be the most different from children with no FH of SUP (No-FH) children, and Second-FH children will
be in between the First-FH and No-FH group due to the fact that First-FH children should have a higher genetic loading and environmental influence of FH.

Specific Aim 3: Investigate the association of sex and FH-degree on cortical morphometry measures.

**Hypothesis:** First-FH girls will have smaller prefrontal volumes than Second-FH girls and the opposite pattern will be found for boys. This is based on a past finding that girls with AUD had smaller volumes than healthy control girls and the opposite relationship was found within boys (Medina et al. 2008) as it is expected that First-FH children will be more likely to resemble adolescents with AUD and Second-FH children will be similar to the healthy controls.

Specific Aim 4: Investigate the association of FH and cortical morphometry on inhibitory control.

**Hypothesis:** For children with FH of SUP, lower frontal volumes will be associated with worse inhibitory control. This is based on a previous finding that smaller superior frontal volumes were associated with worse externalizing behavior (Benegal et al. 2007).
CHAPTER 2. METHODS

The cross-sectional Pediatric Imaging, Neurocognition, and Genetics (PING) study created a normative dataset of behaviors, brain functions and structures from 1,493 typically developing children. Data were collected for children ages 3-20 years from ten sites across the United States, and are now publicly accessible at http://ping.chd.ucsd.edu. The dataset includes computerized neuropsychological assessments, genome single nucleotide polymorphism (SNP) data, and high-resolution brain MRI (Jernigan et al., 2015). The institutional review boards at each site approved the study’s experimental design and assent/consent forms.

Participants

Participant recruitment and characteristics have been reported previously (Brown et al., 2012; Fjell et al. 2012; Akshoomoff et al. 2014; Douet et al. 2014; Douet et al. 2015) and are available on the PING website (http://ping.chd.ucsd.edu). Briefly, children recruited were of any sex and ethnicity, and provided consents for participation if they were ≥18 years old or had parental consents in those ages 3-17 years. Additionally assent was needed for children ages 7-17 years old. The children were excluded for any diagnosis of neurological, developmental or psychiatric disorders, preterm birth (<36 weeks), maternal prenatal daily substance use for more than one trimester, history of head trauma with loss of consciousness of >30 minutes, acute claustrophobia, any MRI contraindications, and pregnancy. Since premature birth is associated with abnormal brain structural development (Ajayi-Obe et al. 2000; Ball et al. 2012), 17 participants born premature (<36 weeks) were found in the database and removed from the analysis. Additionally, 61 participants were excluded from analyses because they reported drug use within the last 30 days, to avoid the confounding effects of adolescent substance use (Medina et al. 2008; Jacobus et al. 2012, for example). Due to the possible confounding of prenatal drug exposure on brain morphometry (Chang et al. 2004; Akyuz et al. 2014; Rajaprakas et al. 2014; Gautam et al., 2015) children with prenatal substance exposure (N=106) were also removed from the current study.

FH of substance use problems

Data for FH of substance use problems (SUP) were collected by self-report from at least one parent/guardian for the minor participants or from the participants ≥ 18 years, for a total of 1,293
participants. Self-reported FH of SUP data consisted of answering “yes” or “no” to alcohol and drug problems for each relative. The relatives included parents, siblings, grandparents, and aunts/uncles. The children were considered to have FH of SUP if they had first- and/or second-degree relatives with SUP (Any-FH). First-degree relatives were defined as relatives sharing at least 50% of their genes with the children (First-FH). Second-degree relatives were defined as relatives sharing at least 25% but less than 50% of their genes with children (Second-FH). FH density was calculated by summing various weights for all family members reporting SUP, as follows. Each first-degree relative (i.e., parents and siblings) was given a weight of 0.5 and each second-degree relative (i.e., grandparents, uncles, and aunts) was given a weight of 0.25 (Cservenka et al., 2015). Unlike previous reports, biological siblings were also included in the FH density analyses and were given a weight of 0.5. Both maternal and paternal relatives were included in the FH density measures.

Genetic ancestry factor

Saliva samples were obtained from each participant and used for genomic DNA extraction. Genetic ancestry factors (GAF) were evaluated using multidimensional scaling analysis and estimates of local ancestry in admixed populations (LAMP), as previously described (Akshoomoff et al. 2014; Douet et al. 2014; Douet et al. 2015).

Structural MRI

MRI scan methods and analyses have been reported previously (Brown et al. 2012; Fjell et al. 2012; Walhovd et al. 2012; Douet et al. 2014; Douet et al. 2015) and are accessible on the PING website. Neuroimaging data were collected using 3 Tesla scanners from three manufacturers: General Electric (Milwaukee, WI, USA, 1 scanner), Phillips (Andover, MA, USA, 2 scanners), and Siemens (Erlangen, Germany, 7 scanners). Scans included a 3-D T1-weighted structural scan (MP-RAGE, 1.0 x 1.0 x 1.2 mm$^3$ resolution, 8 minutes acquisition time). MRI scans were sent to the University of California, San Diego, to ensure protocol adherence, and the images were inspected visually for artifacts. Images determined to be unacceptable were either not included in analyses or the scans were repeated to obtain usable images. Morphometric measures included volumes, surfaces areas and thickness for all cortical regions. Image analysis was completed using a modified Freesurfer software (http://surfer.nmr.mgh.harvard.edu/), which parceled each
brain hemisphere into 33 clusters (Brown et al., 2012). A total of 921 participants had usable scan data for cortical measurements (surface area, thickness, and volume) as well as data for age, GAF, scanner device, SES, and sex, and were used for the final analyses.

Cognitive Functioning

The NIH Toolbox® is a computerized battery used to assess six cognitive domains: Attention, Episodic Memory, Executive Function, Language, Processing Speed, and Working Memory (Weintraub et al. 2013; Akshoomoff et al. 2014). This analysis focused on the Flanker Inhibitory Control and Attention Test (Flanker), which measures inhibitory control. Toolbox data were only analyzed for children ages 9 years or older, since many children at younger ages had difficulty completing the task. Of the 627 children who were age 9 or older, 624 had usable Flanker scores.

Statistical Analyses

Demographic analyses were performed in SAS using either a one-way ANOVA or a chi-square test to analyze group differences between FH groups (Any-FH, First-FH, and Second-FH) and controls (No-FH), and group difference between children with First-FH and those with Second-FH.

All other analyses were performed using the online PING Dataportal (https://ping-dataportal.ucsd.edu/) (Bartsch et al. 2014), which uses a data driven general additive model (GAM) as enacted by the R program (http://www.rproject.org). GAM is similar to multiple linear regression, except that covariates or variables are allowed to be smooth functions with smoothness being data-driven and not pre-specified.

To analyze the effect of FH status on brain morphometry, significance maps for cortical volume, surface area and thickness were created in the vertex model using age, SES (indicated by household income and highest parental/guardian education), GAF, sex (Akshoomoff et al., 2014), and scanner device as covariates. The false discovery rate (FDR) was used to threshold the statistical maps and perform multiple comparisons corrections at the 5% level (Benjamini and Hochberg, 1995). When vertex models showed differences in large regions that corresponded to the parceled FreeSurfer regions of interest (ROI), those findings were analyzed using the ROI models plotted across age. Since the underlying vertex findings were already corrected with FDR, ROI group analyses were not additionally corrected for multiple
comparisons. However, ROI interaction values were corrected for multiple corrections using Bonferroni correction when not modeled previously on vertex analyses. Analyses were run using sex, SES, GAF, and scanner device as covariates. Since no group differences or group-by-age interactions were found for the whole hemisphere measures of cortical surface area, thickness or volume as well as intracranial volume in either right, left or both hemispheres (no p-value<0.002 after Bonferroni correction), these measures were not included as covariates in the regional cortical ROI analyses.

Linear regressions were used to further evaluate whether FH density or number of first-degree family members with SUP correlated with morphometric measures of brain regions that showed group differences based on FH status on the vertex maps. Age, sex, scanner device, GAF and SES were included as covariates in these models.

To determine the influence of sex on FH-degree, significance maps of sex-by-FH-degree interactions on cortical measures were generated. Additionally, a two-way ANCOVA using age, scanner device, SES, and GAF as covariates was utilized to validate the sex-by-FH-degree interactions in regions showing group differences on the significance maps. The additive effects of sex and FH-degree were analyzed using a two-way ANCOVA without the interaction term. Age, SES, GAF and scanner device were used as covariates.

Group differences for Flanker scores were examined across age with sex, SES, and GAF as covariates. The interactive effects of FH and ROI on executive function measures was analyzed via a two-way ANCOVA using SES, GAF, age and sex as covariates. To determine if sex influenced the interactive effects of FH and ROI on executive functioning, a three-way ANCOVA using GAF, SES, and age as covariates was also performed.
CHAPTER 3. RESULTS

Participant characteristics (Table 1)

The 921 children included in the analyses were 11.78±0.16 years old (470 boys, 451 girls). Compared to No-FH children, children grouped by either Any-FH, First-FH or Second-FH were similar in age, sex proportion, and handedness. First-FH children were also older and had higher FH density and lower household income than Second-FH children.

Children with FH of SUP showed differences in their genetic ancestry factor (GAF); regardless of grouping, children with FH of SUP had more European GAF. FH of SUP children also had less East Asian and Central Asian GAF compared to children with No-FH, consistent with lower SUD prevalence rates for Asians in comparison to other ethnic/racial groups (SAMSHA, 2013). Only children with either Any-FH or First-FH showed more American Indian GAF than No-FH children. The distributions of African and Oceania GAF were not different between the children in Any-FH and No-FH groups. Additionally, no GAF differences were found between children with First-FH and Second-FH. Compared to children with No-FH, those with FH of SUP had lower SES, as measured by the combination of household income and parental/guardian education, while only children with Second-FH had similar household incomes to No-FH children. Given some of these group differences, SES and GAF as well as sex and scan device type were systematically included as covariates in statistical models.

Since FH of SUP and SUP itself were previously associated with increased symptoms of externalizing behaviors (Benegal et al. 2007; Bava and Tapert 2010; Weiland et al. 2014) and attention-deficit/hyperactivity disorders (ADHD) in youth (Hill et al. 2007; Venkatasubramaniana et al. 2007; Hill et al. 2011), possible group differences in the ADHD diagnosis were also assessed. In this cohort, 4.6% of the children with No-FH had a diagnosis of ADHD, while 9.6% or twice as many of the First-FH sub-cohort had the diagnosis. Therefore, the data were evaluated with and without controlling for the ADHD diagnosis.

Influence of Any-FH on cortical structures

On whole cortical analyses (vertex model), cortical surface areas and volumes, but not thickness, were different between children with Any-FH and No-FH independently of age (Figure 1, left panel). Specifically, compared to those with No-FH, Any-FH children had a smaller right precuneus area, but larger cortical surface areas and volumes for the left frontal
pole, left pars orbitalis, left pars triangularis, left rostral middle frontal, left orbitofrontal and right superior frontal cortices (Figure 1, left panel). ROI analyses validated that Any-FH children had a larger surface area for the left lateral orbitofrontal cortex (Figure 1a; Age-p<0.0001; Any-FH-p=0.02; R²=0.25). Among all the ROIs examined, FH density positively correlated with cortical surface area in the left lateral orbitofrontal cortex (p=0.003; R²=0.26) and left rostral middle frontal cortex (p=0.02; R²=0.26), and volume for the left rostral middle frontal cortex (p=0.05; R²=0.40) (Figure 1b-d). In contrast, higher FH density was associated with smaller surface areas for the left frontal pole (p=0.04; R²=0.12).

**Differential Effects of First-FH and Second-FH influences on cortical structures**

On whole cortical analyses, both First-FH and Second-FH children had different cortical surface areas and volumes compared to No-FH children (Figures 2&3). Specifically, First-FH children had smaller surface areas and volumes (independently of age) in the left caudal middle frontal cortex, left supramarginal gyrus, right fusiform gyrus, right precuneus, and the pre- and post-central cortices bilaterally, as well as larger surface areas and volumes for the left frontal pole, left pars orbitalis, left pars triangularis, left rostral middle frontal cortex, left lingual gyrus, left orbital frontal cortex (Figure 2, left panel). Additionally, First-FH children had larger surface areas for the right superior frontal cortex and the right superior temporal sulcus and smaller surface areas for the right posterior and isthmus cingulate gyri (Figure 2, left panel). On relevant ROI data, First-FH children had smaller surface areas for the left supramarginal gyrus (Age-p<0.0001; First-FH-p=0.04; R²=0.24), left caudal middle frontal cortex (Age-p<0.0001; First-FH-p=0.007; R²=0.20), and right fusiform gyrus (Age-p<0.0001; First-FH-p=0.02; R²=0.23) (Figure 2a-c) as well as smaller volumes in these same regions (Table 2).

On the whole cortical analyses, Second-FH children had larger surface areas and volumes for the right superior frontal cortex, left frontal pole, left pars orbitalis, left pars triangularis, left rostral middle frontal cortex, left orbitofrontal cortex and the left cingulate gyrus but smaller surface area in the right precuneus and the posterior superior temporal gyri bilaterally compared to children with No-FH (Figure 3, left panel). Additionally, Second-FH children showed widespread larger volumes throughout the frontal, parietal, temporal, and occipital lobes compared to children with No-FH (Figure 3, left panel). Due to similarities of Second-FH and Any-FH whole cortical analyses, ROI analyses for Second-FH children focused on the same
regions showing group differences on the Any-FH vertex models (Figure 3, right panel) and confirmed that Second-FH children had a larger surface area for the left lateral orbitofrontal cortex (Age-p<0.0001; Second-FH-p=0.05; R²=0.26) and a larger volume for the right superior frontal cortex (Age-p<0.0001; Second-FH-p=0.04; R²=0.33) than children with No-FH (Figure 3a-b).

Furthermore, whole cortical analyses showed that First-FH children had a larger surface area than Second-FH children only in the right superior temporal sulcus (Figure 4, upper panel, light yellow). However, First-FH children had smaller volumes than Second-FH children in the right fusiform and left supramarginal gyri, as well as smaller surface areas and volumes in the left caudal middle frontal cortex, left pre-central cortex, and bilaterally for the post-central cortices (Figure 4, upper panel, light blue). ROI analyses of these regions showed smaller surface areas in the right post-central cortex (Age-p=0.0006; FH-degree-p=0.05; R²=0.24) and smaller volumes in the left supramarginal gyrus (Age-p=0.006; FH=0.02; R²=0.33), right fusiform gyrus (Age-p=0.44; FH-degree-p=0.03; R²=0.24), and right post-central cortex (Age=0.0001; FH-degree-p=0.03; R²=0.31). Moreover, morphometric measures of brain regions with the greatest group difference between First-FH and Second-FH children showed an association with the number of First-FH relatives. Specifically, a greater number of first-degree family members correlated negatively with volumes of the left caudal middle frontal cortex (p=0.002; R²=0.27), left supramarginal (p=0.02; R²=0.35) and right fusiform (p=0.008; R²=0.24) volumes (Figure 4a-c), and with surface area of the left caudal middle frontal cortex (p=0.006; R²=0.20). To ensure these associations were not driven by the single subject with 3 first-degree family members, analyses were repeated without this subject and all associations remained.

Effects of Sex and FH on cortical morphometry

Independent of age, sex-by-FH-degree interactions were found across the whole cortex on surface areas and volumes, but particularly for medio-frontal, parietal and occipital lobes in both hemispheres (Figure 5, upper panel, bright yellow). ROI analyses of brain regions that showed group differences on the vertex model for Any-FH analyses (Table 3), showed sex-by-FH-degree interactions for volumes of the medial orbitofrontal (FH-degree-p=0.06; Sex-p=0.25; FH-degree x Sex-p=0.009; R²=0.30, Figure 5a), lateral orbitofrontal (FH-degree-p=0.18; Sex-p=0.70; FH-
degree x Sex-p=0.03; $R^2=0.33$, Figure 5b), and rostral middle frontal cortices (FH-degree- p=0.06; Sex-p=0.86; FH-degree x Sex-p=0.02; $R^2=0.38$, Figure 5c) only in the left hemisphere. First-FH boys and girls had similar volumes for the left medial orbitofrontal cortex, while Second-FH boys had larger volumes than Second-FH girls (Figure 5a). Volumes of the lateral orbitofrontal and rostral middle frontal cortices differed between First-FH boys and girls; however, this difference in volume was more pronounced between Second-FH boys and girls (Figure 5b-c). No-FH children were not included in the analyses, but are shown as a reference in the Figures.

FH-degree-by-sex interactions were not found on brain regions that showed group differences for FH-degree; therefore, the main effects of sex and FH-degree were evaluated without the interaction term. Independently of FH-degree, boys consistently showed larger surface areas and volumes of all assessed regions compared to girls (all Sex-p≤0.005). An additive effect of FH-degree and sex on volumes for the left supramarginal gyrus (FH-p=0.02, Sex-p<0.0001) and right postcentral cortex (FH-p=0.04, Sex-p<0.0001) (Figure 5d-f) was also found, with First-FH children having larger volumes than Second-FH children.

**Effects of Family History of SUP on Inhibitory Control**

Flanker scores, reflective of inhibitory control, improved with age independently of the FH status (all p-values<0.0001). However, the scores were not different between FH groups (Any-FH, First-FH, and Second-FH) when compared to No-FH children and did not show interactive effects between FH status by age (all p-values>0.05).

**Cortical volumes and FH status on executive function**

Since only brain volumes showed significant effects on FH status and sex using the ROI model, FH-by-cortical volume interactions on Flanker task scores were examined solely for regions with FH group volume effects (Table 4). In all children with First-FH (both sexes combined), larger volumes of the left supramarginal gyrus (Figure 6a) and the right fusiform gyrus (Figure 6b) were related to better Flanker scores. In contrast, performance on the Flanker task for No-FH children was essentially independent of the volumes in these regions (Figure 6a-b). FH-by-volume interactions were not found on Flanker scores in children with Second-FH in comparison to children with No-FH (all p-values > 0.05).
Due to the sex differences observed for FH-degree on the left supramarginal gyrus and the right fusiform gyral volumes, sex-by-First-FH-by-selected volumes interactions on Flanker scores were further assessed, but neither of the interactions were significant (p >0.05). When each sex was evaluated separately, First-FH girl’s performance was worse with smaller volumes of either the left supramarginal or the right fusiform gyri, while Flanker scores did not vary with the volumes in No-FH girls (First-FH x Volume p<0.05 for both regions; Figure 6c-d).

Conversely, no association was found between these volumes and the Flanker scores for either First-FH or No-FH boys (Figure 6e-f). To eliminate potential outlier effects, since only the No-FH children had the larger volumes, the analyses were repeated excluding subjects with larger volumes in the left supramarginal gyrus (>18k) and the right fusiform gyrus (>12k); the resulting p-values were comparable to those of the initial analyses.

Lastly, all statistical analyses were repeated with ADHD as a covariate to account for the possible ADHD confounder effect, and most findings remained significant. However, two findings narrowly missed significance: left orbitofrontal area (Second-FH versus No-FH children, Second-FH p=0.06) and right post-central area (First-FH versus Second-FH children, FH-degree p=0.06).
CHAPTER 4. DISCUSSION

Overall, Any-FH and Second-FH children had larger frontal regions that were larger for children across all ages, suggesting these differences did not result from developmental delay. Similar to findings in SUD adolescent studies, First-FH children had smaller volumes of the left supramarginal and right fusiform gyri that may reflect an increase of susceptibility for SUP development. Poorer inhibitory control performance was related to smaller volumes of the left supramarginal and right fusiform gyri, primarily in girls; since individual with SUP have poorer inhibitory controls (Nigg et al. 2006), the smaller volumes may render these individuals more vulnerable to SUP later in life (Caldwell et al. 2005; Velanova et al. 2009; Mahmood et al. 2013; Hatchard et al. 2015). Lastly, boys had larger cortical volumes than girls, especially in the left frontal regions, but the volume differences were more pronounced in children with Second-FH than those with First-FH, indicating sex-specific development related to cortical development in FH children.

Stable morphometric alterations in children with FH of SUP

Past findings have shown altered frontal regions for FH youth (Benegal et al. 2007; Hill et al. 2009), although this is the first study to show larger frontal surface areas for FH of SUP youth. Alterations of the frontal regions have consistently been shown both structurally and functionally for adolescents (De Bellis et al. 2005; Churchwell et al. 2010; Chung et al. 2011; Norman et al. 2011; De Bellis et al. 2013; Fein et al. 2013; Heitzeg et al. 2014) and adults (Tanabe et al. 2009; Nestor et al. 2011; Ide et al. 2014, for example) with substance use disorders. Therefore, these regions appear to be susceptible to the influence of substance use starting in adolescence and persisting into adulthood. These findings extended the previous literature in youth with FH of SUP (Benegal et al. 2007; Hill et al. 2009; Squeglia et al. 2015).

Larger frontal regions observed in children with FH of SUP appear to be stable neuroanatomical markers for FH of SUP rather than from developmental delays mediated by FH of SUP (Sjoerds et al. 2013). Compared to the brain areas of children with No-FH, larger cortical areas in children with Any-FH and Second-FH were already present at age 3, and persisted across the age-span. Varying findings in the literature between children and adults with FH may be due to differences in study methods and populations. Findings of smaller bilateral superior frontal cortices were found for young males with early AUD fathers (Benegal et al. 2007), but no
differences were found for male and female adults (mean age of late 30’s) with mood disorders in addition to FH of SUD (Sjoerds et al. 2013). In addition, a developmental delay was found for FH youth with two AUD brothers; however, findings were only for the cerebellum (Hill et al. 2007) suggesting developmental delays exist in other brain regions.

Unique pattern of brain development in children with First-FH SUP

First-FH children have a unique pattern of structural development with smaller gray matter volumes for the posterior frontal and anterior parietal regions bilaterally and for the right fusiform gyrus in comparison to both Second-FH and No-FH children. Both the left supramarginal and right fusiform gyri were associated with individuals with substance use disorders (Park et al. 2007; James et al. 2011; Hicks et al. 2012; Jacobus et al. 2012; Epstein and Kumra 2014). For example, the left supramarginal gyrus of adolescent marijuana users had reduced cerebral blood flow compared to healthy controls (Jacobus et al. 2012). Cerebral blood flow vary with age during normal brain development (Takahashi et al. 1999), thus abnormal blood flow may be related to altered neurodevelopmental processes. Additionally, alterations of brain activation in the fusiform gyrus correlated with level of craving in AUD youth (Park et al. 2007). Lower volume of left supramarginal and the right fusiform gyri was associated with a higher number of first-degree family members. Similarly, adolescent and adult substance users had smaller volumes in these regions (James et al. 2011; Hicks et al. 2012; Epstein and Kumra 2014). Moreover, smaller volumes in adolescents initially naïve to substance use predicted later cannabis and heavy alcohol use in two longitudinal pediatric studies (Cheetham et al. 2012; Squeglia et al. 2014). Therefore, smaller volumes of the left supramarginal and right fusiform gyri in the First-FH children in the current study may be associated with later development of substance use, although longitudinal studies are needed to determine this relationship.

Relationships between unique morphometric pattern in First-FH children and inhibitory control

Although poorer inhibitory control was related to the development of substance use in FH children (Nigg et al. 2006), in this study, FH of SUP across all children did not influence this cognitive domain as measured by the Flanker task. The findings are consistent with previous studies of FH children and healthy controls who performed similarly (Heitzeg et al. 2010; DeVito et al. 2013) or differed only in their reaction times for inhibitory control tasks (Acheson
et al. 2014; Heitzeg et al. 2014). This suggests executive function deficits mediated by FH are fully expressed only for children who are substance users or may be a result of substance use in these children. However, First-FH children who performed worse on Flanker scores had smaller volumes of the left supramarginal and right fusiform gyri, which were unique structural alterations in the First-FH children. The brain activation in these two brain regions were also associated with executive function in healthy children (Velanova et al., 2009) and adolescents (Mahmood et al. 2013) as well as in AUD adolescents (Caldwell et al. 2005; Hatchard et al. 2015). Moreover, girls but not boys with smaller volume for either the left supramarginal or right fusiform gyri performed worse on the Flanker task, suggesting that only girls with these smaller brain volumes had poorer inhibitory control, which in turn may be associated with a higher risk of SUP development. Since girls typically outperform boys on inhibitory control tasks (Else-Quest et al. 2006), the poorer performance in the First-FH girls in the current study may be due to other sex-specific influences from FH of SUP. For example, stressful childhood situations such as child abuse and neglect were associated with later substance use only for females (Wilson et al. 2009), and stress also led to increased activation of the left supramarginal and right fusiform only in women (Wang et al. 2007; Mather et al. 2010). Therefore, these underlying sex differences may account for First-FH girls being more affected on executive function and thus having a higher risk for later SUP than boys.

Alterations of Cortical Areas and not Thickness in Children with FH-SUP

The altered cortical surface areas, but not cortical thickness, contributed to the changes in brain volumes are consistent with a past extended family pedigree study that found better correlations between surface area, rather than thickness, with brain volumes (Winkler et al. 2010). Furthermore, genetic influences were stronger than environmental influences on surface area for the frontal, parietal and temporal poles in a pediatric twin population (Yoon et al. 2012). Therefore, the associations between the FH of SUP in all children and their cortical surface areas may be influenced by their genetic susceptibilities. For instance, MECP2 may be a candidate gene for future studies of FH of SUP in children since it is a well documented determinant of brain development, particularly for changes in surface areas (Joyner et al. 2009).

FH implies a shared environment and genetic load. Similar genetics/environmental factors appear to be influencing prefrontal regions, while influences diverge for regions in the posterior
frontal, parietal and temporal lobes. First-FH children have higher environmental and genetic loading than Second-FH children, which may play a role in the distinctive findings for cortical morphometry. Although environmental influences should be less for Second-FH children, SES may still be similar within families, and SES could potentially be an important environmental factor, as it is known to influence surface area development (Noble et al. 2015). While Second-FH children did have lower parental education, SES was controlled for in all analyses to account for some environmental differences although this likely does not fully capture the full influence of environment. Genetic loading within First-FH children should more drastically impact cortical morphometry than loading for Second-FH children. For instance, variations in genes related to alterations in cortical morphometry for FH of SUD children (Hill et al. 2009) or AUD adolescents (Dalvie et al. 2014), such as the 5-HTT or BDNF genes, may be generally more prevalent in First-FH children and have an additive effect that ultimately leads to the full expression of brain alterations. Additionally, genes associated with normal brain development such as COMT (Raznahan et al. 2011; Knickmeyer et al. 2013), may play a role in differential development. COMT is also associated with sexually dimorphic brain function and may be associated with differences (Tunbridge et al. 2011). However, additional studies are needed to elucidate the genetic influences on cortical morphometry for children at risk of SUP development along with the environmental factors.

**Sex-specific differences on cortical morphometry in relation to FH-degree of SUP**

The findings in the current study are consistent with past studies showing sex differences related to typical brain development (Giedd et al. 1997; Lenroot et al. 2007) and to adolescent substance use development (Hammerslag et al. 2015, for review). Strong sex-by-FH-degree interactions were found on surface area and volumes, especially in the medio-frontal, parietal and occipital lobes. However, the smaller left frontal volumes in girls with Second-FH children compared to those in the First-FH children (who should have a greater genetic influence from FH-degree) is somewhat inconsistent with a prior study of adolescents with AUD, which found smaller than normal prefrontal volumes in the girls and larger than normal prefrontal volumes in the boys (Medina et al. 2008). Past studies of FH have also found sex-by-FH-degree interactions but mainly for subcortical (Hanson et al. 2010; Cservenka et al. 2015) and white matter (Silveri et al. 2008) volumes. However, the frontal cortices in particular are targets for substance use
alterations in adolescents (De Bellis et al. 2005; Medina et al. 2008; Churchwell et al. 2010; Squeglia et al. 2012; Fein et al. 2013; Doallo et al. 2014) and past studies have shown sex differences for the frontal regions between adolescents and adults with SUD compared to controls (Medina et al. 2008; Squeglia et al. 2012). Further studies need to be conducted to elucidate the underlying mechanisms leading to sex differences in FH children.

Strengths and limitations

The current study has several strengths. Compared to previous studies (Benegal et al., 2007; Hill et al. 2009; Squeglia et al. 2015), this study has a large cohort sample size that allows enough power to disentangle the effect of the FH-degree/load on cortical development and cognition. Additionally, past studies of cortical morphometry in youth have focused on relatives of individuals with AUD; however, this study examined children with FH of any alcohol and/or drug use problem. Furthermore, past studies solely examined alterations in cortical volumes in FH youth (Benegal et al. 2007; Hill et al. 2009; Squeglia et al. 2015), while this study provides a more comprehensive analyses of cortical morphometric differences related to FH of SUP by investigating thickness and surface area, and their contributions to volumes. Additionally, children with any reported prenatal exposure were intentionally excluded, which was not explicitly done in past studies (Benegal et al. 2007; Hill et al. 2009). Finally, this study accounted for the influence of a possible confounder, such as ADHD diagnosis.

One of the main limitations is that this was a cross-sectional study. In order to determine risk factors associated with later SUD development, longitudinal studies are needed to follow these children, before they start using drugs, through adulthood. Additionally, the influence of FH of any substance use problems was examined. However, drugs may have variable effects on cortical morphometry (Morales et al. 2012; Durazzo et al. 2014; Mon et al. 2014). The next step would be to examine the influence of FH based on the type (or class) or amount of drug(s) used, and investigate the additive or interactive effects on brain development. Due to the wide age range of children in this study, it is also likely that some adolescents have already begun substance use, but this data was not available for all participants, but any participants who reported substance use were excluded from the analysis. Further, family history was self-reported by parents or adult participants which may be under-reported by parents who did not want to admit to SUP, or lacked the knowledge of family history of SUP. Finally, First-FH and Second-FH children were
examined separately, but some First-FH children additionally reported Second-FH; however, this is not surprising due to the high prevalence of SUP within families. Unfortunately, strict categorization of First-FH and Second-FH would require a third group with both First and Second-FH.

Conclusions

These findings showed that FH-mediated alterations are mainly localized in the frontal regions that were consistently reported to be associated with SUP in adolescence and adulthood. Moreover, those frontal alterations appear as early as childhood (>3 years old) even before the development of SUP and remain consistent throughout childhood. Larger frontal regions may be a stable neuroanatomical marker for SUP development, but longitudinal studies are needed. Furthermore, First-FH and Second-FH children have differential cortical development with specific regions showing unique alterations in First-FH children. Future studies of children with FH of SUP should evaluate First-FH and Second-FH children separately since their brains may be affected differently. Moreover, the regions altered specifically in First-FH children are known to be relevant to executive function, which correlated with inhibitory control performance. Within the First-FH children, only girls had this same association, suggesting an important role of sex within the interactive and additive effects of FH, cortical morphometry and executive function performance. Altogether these findings provide insights into how brain development and cognition are influenced by FH-degree, and the First-FH children may be particularly at risk for later SUP. The sex-specific influence of FH of SUP on brain development also needs to be explored further.
APPENDIX A.
Table 1. Participant and Parent/Guardian Characteristics by Family History Group

All values are in mean ± standard errors. All analyses were done using a one-way ANOVA or a chi-square test. Children with FH of SUP differ from children with No-FH for socioeconomic status (SES, which combined highest education of parents/guardians and household income) and genetic ancestry factor (GAF). ADHD diagnosis was higher only for First-FH children. Additionally, First-FH children were older, had higher FH density and lower SES than children with Second-FH. Due to group differences statistical models included GAF and SES variables for group comparisons.

<table>
<thead>
<tr>
<th>Children Characteristics</th>
<th>No FH of SUP (N=542)</th>
<th>Any FH (N=379)</th>
<th>First FH (N=114)</th>
<th>Second FH (N=265)</th>
<th>One-way ANOVA or X², p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>11.90 ± 0.21</td>
<td>11.61 ± 0.24</td>
<td>12.56 ± 0.41</td>
<td>11.21 ± 0.28</td>
<td>0.38 0.18 0.06 0.008</td>
</tr>
<tr>
<td>Boys /Girls (%)</td>
<td>271/271 (50.0/50.0)</td>
<td>199/180 (52.5/47.5)</td>
<td>58/56 (50.9/49.1)</td>
<td>141/124 (53.2/46.8)</td>
<td>0.45 0.86 0.39 0.68</td>
</tr>
<tr>
<td>Handedness (Left/Right/Mixed/Not yet established)</td>
<td>57/461/20/4</td>
<td>37/321/19/2</td>
<td>12/96/5/1</td>
<td>82/686/34/5</td>
<td>0.75 0.99 0.65 0.89</td>
</tr>
<tr>
<td>GAF Europe</td>
<td>0.57 ± 0.017</td>
<td>0.73 ± 0.017</td>
<td>0.70 ± 0.031</td>
<td>0.74 ± 0.020</td>
<td>&lt;0.0001 0.001 &lt;0.0001 0.25</td>
</tr>
<tr>
<td>GAF Africa</td>
<td>0.14 ± 0.012</td>
<td>0.11 ± 0.013</td>
<td>0.13 ± 0.024</td>
<td>0.11 ± 0.015</td>
<td>0.10 0.61 0.08 0.45</td>
</tr>
<tr>
<td>GAF American Indian</td>
<td>0.04 ± 0.004</td>
<td>0.06 ± 0.006</td>
<td>0.06 ± 0.011</td>
<td>0.05 ± 0.007</td>
<td>0.01 0.02 0.06 0.50</td>
</tr>
<tr>
<td>GAF East Asia</td>
<td>0.20 ± 0.015</td>
<td>0.09 ± 0.011</td>
<td>0.10 ± 0.022</td>
<td>0.09 ± 0.013</td>
<td>&lt;0.0001 0.004 &lt;0.0001 0.79</td>
</tr>
<tr>
<td>GAF Oceania</td>
<td>0.01 ± 0.001</td>
<td>0.006 ± 0.001</td>
<td>0.008 ± 0.003</td>
<td>0.005 ± 0.001</td>
<td>0.20 0.97 0.11 0.21</td>
</tr>
</tbody>
</table>
Table 1. (Continued) Participant and Parent/Guardian Characteristics by Family History Group

<table>
<thead>
<tr>
<th></th>
<th>GAF Central Asia</th>
<th>Diagnosed ADHD (%)*</th>
<th>FH density</th>
<th>Parent/Guardian Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.04 ± 0.007</td>
<td>0.004 ± 0.002</td>
<td>0.004 ± 0.002</td>
<td>0.004 ± 0.002</td>
</tr>
<tr>
<td>Diagnosed ADHD (%)*</td>
<td>25 (4.6%)</td>
<td>27 (7.1%)</td>
<td>11 (9.6%)</td>
<td>16 (6%)</td>
</tr>
<tr>
<td>FH density</td>
<td>0</td>
<td>0.57 ± 0.02</td>
<td>0.94 ± 0.04</td>
<td>0.41 ± 0.02</td>
</tr>
<tr>
<td>Household Income^</td>
<td>7.04 ± 0.11</td>
<td>6.62 ± 0.11</td>
<td>5.72 ± 0.22</td>
<td>7.00 ± 0.13</td>
</tr>
<tr>
<td>Highest Education#</td>
<td>5.94 ± 0.05</td>
<td>5.64 ± 0.06</td>
<td>5.33 ± 0.11</td>
<td>5.77 ± 0.07</td>
</tr>
</tbody>
</table>

vs: versus

*6 participants missing ADHD data (1 First FH, 3 Second FH, 2 No FH of SUP).

^Household Income: (1=<$5K, 6= 40K-50K, 12=≥$300K)

#Highest Education: (7=Professional, 4=High School Graduate, 1=<7 yrs of school)
Table 2. Regions of interest for children with First-FH in comparison to children with No-FH

Regions of interest (ROI) were analyzed across age. GAM analyses were run using sex, SES, GAF, and scanner device as covariates.

<table>
<thead>
<tr>
<th>Region of Interest</th>
<th>Age</th>
<th>First FH</th>
<th>Age x First FH</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Surface Area</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left Caudal Middle Frontal Cortex</td>
<td>&lt;0.0001</td>
<td>0.007</td>
<td>0.08</td>
<td>0.20</td>
</tr>
<tr>
<td>Left Frontal Pole</td>
<td>0.02</td>
<td>0.10</td>
<td>N.S.</td>
<td>0.12</td>
</tr>
<tr>
<td>Left Lateral Orbitofrontal Cortex</td>
<td>0.007</td>
<td>0.07</td>
<td>0.03</td>
<td>0.25</td>
</tr>
<tr>
<td>Left Lingual Gyrus</td>
<td>&lt;0.0001</td>
<td>0.17</td>
<td>N.S.</td>
<td>0.17</td>
</tr>
<tr>
<td>Left Medial Orbitofrontal Cortex</td>
<td>0.18</td>
<td>0.64</td>
<td>N.S.</td>
<td>0.19</td>
</tr>
<tr>
<td>Left Pars Orbitalis</td>
<td>0.43</td>
<td>0.27</td>
<td>N.S.</td>
<td>0.15</td>
</tr>
<tr>
<td>Left Pars Triangularis</td>
<td>0.0005</td>
<td>0.52</td>
<td>N.S.</td>
<td>0.07</td>
</tr>
<tr>
<td>Left Post-central Cortex</td>
<td>0.009</td>
<td>0.30</td>
<td>N.S.</td>
<td>0.23</td>
</tr>
<tr>
<td>Left Pre-central Cortex</td>
<td>&lt;0.0001</td>
<td>0.62</td>
<td>N.S.</td>
<td>0.31</td>
</tr>
<tr>
<td>Left Rostral Middle Frontal Cortex</td>
<td>0.31</td>
<td>0.09</td>
<td>N.S.</td>
<td>0.23</td>
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<tr>
<td>Left Supramarginal Gyrus</td>
<td>&lt;0.0001</td>
<td>0.04</td>
<td>N.S.</td>
<td>0.24</td>
</tr>
<tr>
<td>Right Fusiform Gyrus</td>
<td>&lt;0.0001</td>
<td>0.02</td>
<td>N.S.</td>
<td>0.23</td>
</tr>
<tr>
<td>Right Isthmus Cingulate Gyrus</td>
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<td>0.73</td>
<td>N.S.</td>
<td>0.13</td>
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<tr>
<td>Right Post-central Cortex</td>
<td>0.001</td>
<td>0.09</td>
<td>N.S.</td>
<td>0.19</td>
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<tr>
<td>Right Posterior Cingulate Gyrus</td>
<td>0.37</td>
<td>0.26</td>
<td>N.S.</td>
<td>0.15</td>
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<tr>
<td>Right Pre-central Cortex</td>
<td>&lt;0.0001</td>
<td>0.85</td>
<td>N.S.</td>
<td>0.24</td>
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<tr>
<td>Right Precuneus</td>
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<td>0.18</td>
<td>0.19</td>
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<tr>
<td>Right Superior Frontal Cortex</td>
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<td>0.77</td>
<td>N.S.</td>
<td>0.26</td>
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<td>Right Superior Temporal Sulcus</td>
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<td>0.59</td>
<td>N.S.</td>
<td>0.15</td>
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<tr>
<td><strong>Volume</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left Caudal Middle Frontal Cortex</td>
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<td>0.003</td>
<td>0.06</td>
<td>0.27</td>
</tr>
<tr>
<td>Left Frontal Pole</td>
<td>&lt;0.0001</td>
<td>0.28</td>
<td>N.S.</td>
<td>0.23</td>
</tr>
<tr>
<td>Left Lateral Orbitofrontal Cortex</td>
<td>0.09</td>
<td>0.54</td>
<td>N.S.</td>
<td>0.35</td>
</tr>
<tr>
<td>Left Lingual Gyrus</td>
<td>&lt;0.0001</td>
<td>0.40</td>
<td>N.S.</td>
<td>0.20</td>
</tr>
<tr>
<td>Left Medial Orbitofrontal Cortex</td>
<td>&lt;0.0001</td>
<td>0.41</td>
<td>N.S.</td>
<td>0.32</td>
</tr>
<tr>
<td>Left Pars Orbitalis</td>
<td>0.67</td>
<td>0.47</td>
<td>0.04</td>
<td>0.17</td>
</tr>
<tr>
<td>Left Pars Triangularis</td>
<td>&lt;0.0001</td>
<td>0.95</td>
<td>N.S.</td>
<td>0.12</td>
</tr>
<tr>
<td>Left Post-central Cortex</td>
<td>&lt;0.0001</td>
<td>0.15</td>
<td>N.S.</td>
<td>0.32</td>
</tr>
<tr>
<td>Left Pre-central Cortex</td>
<td>0.50</td>
<td>0.90</td>
<td>N.S.</td>
<td>0.33</td>
</tr>
<tr>
<td>Left Rostral Middle Frontal Cortex</td>
<td>0.002</td>
<td>0.21</td>
<td>0.01</td>
<td>0.41</td>
</tr>
<tr>
<td>Left Supramarginal Gyrus</td>
<td>0.003</td>
<td>0.01</td>
<td>N.S.</td>
<td>0.35</td>
</tr>
<tr>
<td>Right Fusiform Gyrus</td>
<td>0.62</td>
<td>0.02</td>
<td>N.S.</td>
<td>0.24</td>
</tr>
<tr>
<td>Right Post-central Cortex</td>
<td>&lt;0.0001</td>
<td>0.07</td>
<td>N.S.</td>
<td>0.30</td>
</tr>
<tr>
<td>Right Pre-central Cortex</td>
<td>0.99</td>
<td>0.94</td>
<td>N.S.</td>
<td>0.27</td>
</tr>
<tr>
<td>Right Precuneus</td>
<td>&lt;0.0001</td>
<td>0.35</td>
<td>0.02</td>
<td>0.44</td>
</tr>
</tbody>
</table>
Table 3. Interactive effects of sex and FH-degree on cortical surface areas and volumes influenced by Any-FH

Regions of interest (ROI) were analyzed across age. Two-way ANCOVA analyses were run using SES, GAF, and scanner device as covariates.

<table>
<thead>
<tr>
<th>Region of Interest</th>
<th>Sex p-value</th>
<th>FH-Degree p-value</th>
<th>FH-Degree x Sex p-value</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Surface Area</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left Frontal Pole</td>
<td>0.55</td>
<td>0.37</td>
<td>0.63</td>
<td>0.13</td>
</tr>
<tr>
<td>Left Lateral Orbitofrontal Cortex</td>
<td>0.30</td>
<td>0.45</td>
<td>0.51</td>
<td>0.17</td>
</tr>
<tr>
<td>Left Medial Orbitofrontal Cortex</td>
<td>0.55</td>
<td>0.64</td>
<td>0.22</td>
<td>0.20</td>
</tr>
<tr>
<td>Left Pars Orbitalis</td>
<td>0.93</td>
<td>0.21</td>
<td>0.13</td>
<td>0.11</td>
</tr>
<tr>
<td>Left Pars Triangularis</td>
<td>0.05</td>
<td>0.99</td>
<td>0.49</td>
<td>0.56</td>
</tr>
<tr>
<td>Left Rostral Middle Frontal Cortex</td>
<td>0.34</td>
<td>0.28</td>
<td>0.16</td>
<td>0.22</td>
</tr>
<tr>
<td>Right Precuneus</td>
<td>0.23</td>
<td>0.81</td>
<td>0.28</td>
<td>0.22</td>
</tr>
<tr>
<td>Right Superior Frontal Cortex</td>
<td>0.10</td>
<td>0.83</td>
<td>0.70</td>
<td>0.20</td>
</tr>
<tr>
<td><strong>Volume</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left Frontal Pole</td>
<td>0.45</td>
<td>0.85</td>
<td>0.16</td>
<td>0.18</td>
</tr>
<tr>
<td>Left Lateral Orbitofrontal Cortex</td>
<td>0.70</td>
<td>0.18</td>
<td>0.03</td>
<td>0.33</td>
</tr>
<tr>
<td>Left Medial Orbitofrontal Cortex</td>
<td>0.25</td>
<td>0.06</td>
<td>0.009</td>
<td>0.30</td>
</tr>
<tr>
<td>Left Pars Orbitalis</td>
<td>0.58</td>
<td>0.15</td>
<td>0.10</td>
<td>0.15</td>
</tr>
<tr>
<td>Left Pars Triangularis</td>
<td>0.23</td>
<td>0.94</td>
<td>0.86</td>
<td>0.09</td>
</tr>
<tr>
<td>Left Rostral Middle Frontal Cortex</td>
<td>0.86</td>
<td>0.06</td>
<td>0.02</td>
<td>0.38</td>
</tr>
<tr>
<td>Right Superior Frontal Cortex</td>
<td>0.50</td>
<td>0.92</td>
<td>0.28</td>
<td>0.27</td>
</tr>
</tbody>
</table>
Table 4. Effect of cortical volumes and FH status on Flanker performance

We analyzed the interactive effects of FH and ROI on executive function measures via a two-way ANCOVA using SES, GAF, age and sex as covariates.

<table>
<thead>
<tr>
<th>Region of Interest</th>
<th>Volume</th>
<th>Family History</th>
<th>Family History × Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>First-FH</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L Caudal Middle Frontal Cortex</td>
<td>0.69</td>
<td>0.09</td>
<td>0.06</td>
</tr>
<tr>
<td>L Supramarginal Gyrus</td>
<td>0.49</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>R Fusiform Gyrus</td>
<td>0.25</td>
<td>0.03</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>Second-FH</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R Superior Frontal Cortex</td>
<td>0.99</td>
<td>0.66</td>
<td>0.76</td>
</tr>
</tbody>
</table>
APPENDIX B.

Figure 1. Vertex maps and regions of interest for cortical morphometry for Any-FH versus No-FH children

(Left panel) Statistical p-value maps for cortical surface area and volumes generated at each vertex using a general additive model for Any-FH children versus No-FH children. On the whole cortical analysis, independent of age, Any-FH children had a smaller right precuneus area, but showed larger surface areas and volumes for the left rostral middle frontal cortex, left frontal pole, left pars orbitalis, left pars triangularis, left orbitofrontal cortex and right superior frontal cortex than No-FH children. Sex, GAF, SES, Age, and scanner device were included as covariates, and models were corrected for multiple comparisons with the false discovery rate (FDR; 0.05). (a) Using the ROI model (right panel), compared to children with No-FH, Any-FH children had a larger left lateral orbitofrontal cortex. Sex, GAF, SES and scanner device were included as covariates. (b-d) More FH density positively correlated with larger surface areas for the left lateral orbitofrontal (b) and left rostral middle frontal (c) and volume for the left rostral middle frontal (d). GAF, SES, sex, age and scanner device were included as covariates.
(Left panel) Statistical p-value maps for cortical surface area and volumes generated at each vertex using a general additive model for First-FH children versus No-FH children. Sex, GAF, SES, age and scanner device were included as covariates, and models were corrected for multiple comparisons with the false discovery rate (FDR; 0.05). On the whole cortical analysis, independent of age, First-FH children had a smaller surface area and volume for the right precuneus, right fusiform, left caudal middle frontal cortex, left supramarginal gyrus and the pre- and post-central cortices bilaterally (left panel). Larger surface areas and volumes were found for the left rostral middle frontal cortex, orbitofrontal cortex, pars orbitalis, pars triangularis, frontal pole, and lingual gyrus (left panel). First-FH children had larger surface areas for the right lateral superior frontal cortex and superior temporal sulcus and smaller surface areas for the right posterior and isthmus cingulate gyri (left panel). (a–c) Using the ROI model (right panel), compared to No-FH children, First-FH children had a smaller surface area for the left supramarginal gyrus (a), left caudal middle frontal (b), and right fusiform gyrus (c). Sex, GAF, SES and scanner device were included as covariates.
Figure 3. Vertex maps and regions of interest for cortical morphometry for Second-FH versus No-FH children

(Left panel) Statistical p-value maps for surface area and volume generated at each vertex using a general additive model for Second-FH children versus No-FH children. For surface area, Second-FH children had larger left anterior frontal cortex, right superior frontal cortex, left cingulate gyrus and smaller surface areas for the left superior temporal sulcus and right precuneus. For volume, Second-FH children had widespread larger regions throughout the frontal, temporal, parietal and occipital lobes. Sex, GAF, SES, age and scanner device were included as covariates, and models were corrected for multiple comparisons with the false discovery rate (FDR; 0.05). (a-b) On the ROI model (right panel), a larger surface area was found for the left lateral orbitofrontal cortex and larger volume was found for the right superior frontal cortex for Second-FH children in comparison to No-FH children. Sex, GAF, SES and scanner device were included as covariates.
Figure 4. Vertex maps and regions of interest for cortical morphometry for First-FH versus Second-FH children

(Upper panel) Statistical p-value maps for cortical surface area and volumes generated at each vertex using a general additive model for First-FH versus Second-FH children. First-FH children had larger surface areas only in the right superior temporal sulcus. Smaller cortical surface areas and volumes were found for the First-FH children in comparison to Second-FH children for the left caudal middle frontal cortex, left pre-central cortex and the post-central cortices bilaterally. Additionally, volumes for the right fusiform gyrus and left supramarginal gyrus were smaller for First-FH children. Sex, GAF, SES, age and scanner device were included as covariates, and vertex models were corrected for multiple comparisons with the false discovery rate (FDR; 0.05). (a-c) Using the ROI model (lower panel), smaller volumes for the left caudal middle frontal cortex, left supramarginal gyrus, and right fusiform gyrus were all associated with a higher number of first-degree family members. GAF, SES, age, sex and scanner device were included as covariates.
Figure 5. P-value maps of sex-by-FH-degree interaction on brain cortical morphometry

P-value maps show widespread sex-by-FH-degree interactions for cortical surface area and volumes throughout the brain and particularly in the frontal, parietal and occipital lobes (Figure 5, upper panel). (a-c) On the ROI model (Figure 5, lower panel), volumes for the left medial orbito frontal, lateral orbito frontal, and rostral middle frontal cortices also showed sex-by-FH-degree interactions. Boys are shown in blue and girls are shown in pink. We used t-tests to examine sex differences for children with either First-FH or Second-FH showing First-FH boys and girls were not as different as Second-FH children. Analyses were done using a two-way ANCOVA using age, SES, scanner device and GAF as covariates. (d-e) Volumes for the left supramarginal gyrus and right postcentral cortex show an additive effect of sex and FH-degree. Analyses were done with a two-way ANCOVA without the interaction term and using scanner device, age, SES, and GAF as covariates. No-FH children were not included in analyses, but are shown for reference.

p-values: #=0.03; ^=0.007; * < 0.0001
(a-b) For children with First-FH, an interaction between FH status and left supramarginal gyrus and the right fusiform gyrus volumes were found on Flanker score. First-FH children showed an association between smaller volumes and lower Flanker score while no relationship existed for No-FH children. (c-f) We then examined girls and boys separately. (c-d) We found girls followed the same pattern as we found for the overall group. (e-f) For boys, no association existed between either the left supramarginal gyrus or the right fusiform gyrus volumes and FH groups on Flanker scores. Analyses were done with a two-way ANCOVA using SES, GAF, age and sex as covariates. All graphs were plotted using the adjusted values for the Flanker scores.
REFERENCES


