Chapter 2

Family history of alcohol dependence and gray matter abnormalities in non-alcoholic adults

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ABSTRACT

Background. Alcohol-use disorders in adolescents are associated with gray matter (GM) abnormalities suggesting neurotoxicity by alcohol. However, recently similar GM abnormalities were found in non-drinking children with a family history (FH) of alcohol dependence (AD). The question thus rises whether these abnormalities represent a transient delay in brain maturation or a persistent risk factor for developing neuropsychiatric disorders, rather than a (neurotoxic) consequence of AD. This study investigated whether a FH of AD in non-drinking adults is associated with abnormal GM-volumes similar to those observed in drinking and non-drinking adolescents with a FH of AD.

Methods. GM-images were analyzed using Voxel-Based Morphometry in non-alcoholics with (FH+; N = 36) and without (FH–; N = 107) familial AD. Additionally we controlled for possible confounders: diagnosis of depression/anxiety, childhood trauma and familial depression/anxiety.

Results. Smaller GM-volumes were shown in the right parahippocampal gyrus in FH+ compared with FH–. Results were unaffected by confounders.

Conclusions. We demonstrated an effect of familial AD in non-alcoholic adults on GM volume in the parahippocampal gyrus, similar to drinking and non-drinking FH– adolescents. These findings suggest that GM abnormalities in the parahippocampal gyrus represent a persistent biological susceptibility for AD or related psychopathology and not neurotoxicity of alcohol or delayed brain maturation.
INTRODUCTION

Structural brain abnormalities have been repeatedly observed in adult patients with alcohol use disorders (Agartz et al. 1999; Mechtcheriakov et al. 2007) and are generally interpreted as neurotoxic consequences of chronic excessive alcohol use. Moreover, recent studies indicate that gray matter (GM) abnormalities are also present in adolescents with alcohol use disorders (De Bellis et al. 2000; 2005) suggesting neurotoxic damage already at an early age after a relatively brief drinking career, which may be related to toxic vulnerability of the developing brain. Recently, however, similar GM abnormalities were observed in non-drinking children and adolescents with a positive family history (FH+) of alcohol dependence (AD) (Benegal et al. 2007). This latter finding suggests that GM abnormalities in children and adolescents with alcohol use disorders may also result from premorbid non-neurotoxic factors associated with family drinking history. However, since the brain of the adolescent has not reached full maturation yet (Jernigan et al. 1991), the question remains whether these abnormalities represent a familial transmitted transient delay in brain maturation or a persistent (familial) risk factor for the development of AD or other neuropsychiatric disorders.

The risk to develop AD is higher for children of alcohol dependent individuals than for children without such a family history (FH−) (Hasin et al. 1997; Nurnberger et al. 2004). This increased risk within families could be explained by a genetic predisposition (Agrawal and Lynskey 2008; Devor and Cloninger 1989) or by environmental influences such as role models, inadequate psychological support, poor diets, childhood abuse, or maternal alcohol use during pregnancy (Campbell et al. 2009; Gilman et al. 2007; Latendresse et al. 2008), risk factors that have also been shown to increase the vulnerability to develop other psychiatric disorders, such as depression and anxiety disorders later in life (Heim and Nemeroff 2001; Kessler 1997).

Mapping brain characteristics associated with FH of AD could contribute to a better understanding of neurobiological processes predating the development of AD and related psychopathology. Until now, however, direct anatomical evidence for the permanent effects of the alleged genetic and environmental influences mediated by familial alcohol dependence is scarce. So far, a study in adult AD patients has shown smaller intracranial volumes in FH+ patients compared with FH− patients (Gilman et al. 2007), suggesting reduced premorbid brain growth. In addition, De Bellis and colleagues (2000; 2005) found smaller bilateral prefrontal cortex and hippocampus in adolescent alcoholics compared with age- and gender-matched controls, but suggested that these structural differences might have been present prior to the onset of heavy drinking. Moreover, other magnetic resonance imaging studies have shown neuroanatomical deficits in high-risk non-drinking FH+ adolescents and children, like smaller right amygdala and cerebellar volume (Hill et al. 2007b), laterality differences in the orbitofrontal cortex (2009), white matter volumetric abnormalities of the corpus callosum (Venkatasubramanian et al. 2007), and lower structural fronto-cerebellar connectivity (Herting et al. 2011). These studies in young non-alcoholics suggest that, independent of drinking history, family status is of influence on brain structure volumes and connectivity through genetic, epigenetic, or environmental factors.

However, these studies used different techniques to determine the volume of relevant brain areas, most often involving manual tracing of anatomical structures. An alternative approach is voxel-based morphometry (VBM), an automated approach allowing simultaneous, rater independent analysis of different brain regions, that is more time-efficient (Ashburner and Friston 2000; Wright et al. 1995), and has equal sensitivity to manual segmentation (Bergouignan et al. 2009). Recently, Benegal and colleagues (2007) used an optimized VBM approach in non-alcoholic
adolescents with and without first degree AD family members, showing GM volume abnormalities in the superior frontal gyrus, cingulate gyrus, parahippocampal gyrus, amygdala, thalamus and cerebellum in high-risk (FH+) compared with low-risk (FH−) adolescents. These findings suggested that brain abnormalities in adolescents are a cause/vulnerability factor rather than a (neurotoxic) consequence of AD. However, the question remains whether these abnormalities in non-alcoholic FH+ adolescents represent a transient delay in brain maturation or a persistent risk factor for developing neuropsychiatric disorders.

In order to address this issue, we used an optimized VBM approach to assess regional GM volume in a sample of non-alcoholic adults with and without a history of AD in first degree relatives, i.e. in subjects that have fully matured brains and who therefore do not suffer from a transient delay in brain maturation. Additionally, we tested for possible confounders and effects that could explain the main findings apart from familial alcohol dependence status, e.g. maternal alcohol use and the presence of psychopathology. Our sample was drawn from the large Netherlands Study of Depression and Anxiety (NESDA) cohort, containing both healthy controls as well as people with a depression and/or anxiety disorder. Elaborating on previous results by Benegal and colleagues (2007) we hypothesized that non-alcoholic FH+ adults have smaller GM volumes of the parahippocampal gyrus, superior frontal gyrus, medial frontal gyrus, amygdala, and cingulate gyrus compared with non-alcoholic FH− adults. If volume abnormalities in these brain areas are observed in non-alcoholic adults with familial alcoholism, similar to non-alcoholic FH+ adolescents, these brain abnormalities are likely to represent persistent abnormalities associated with familial AD rather than alcohol-related neurotoxicity or a transient neurodevelopmental delay.

METHODS

Sample
Participants included healthy controls and patients with a depression and/or anxiety disorder from the Netherlands Study of Depression and Anxiety (NESDA)-neuroimaging study. NESDA is a large cohort study aimed to examine the long-term course and consequences of depressive and anxiety disorders. Of the 2981 participants with a baseline assessment, 301 took part in the NESDA-neuroimaging study. For a detailed description of the NESDA study design and NESDA-neuroimaging study the reader is referred to (resp. Penninx et al. 2008; Van Tol et al. 2010), respectively. It is important to note that the NESDA-neuroimaging study applied the following exclusion criteria: 1) DSM-IV Axis-I disorders other than major depressive disorder (MDD) or anxiety disorders (social anxiety disorder, panic disorder, generalized anxiety disorder); 2) history of major internal or neurological disorder; 3) hypertension (>180/130 mmHg); 4) general MRI-contraindications. All DSM-IV diagnoses were established by a trained interviewer using the structured Composite International Diagnostic Interview (CIDI, WHO version 2.1).

For this report we selected participants from the NESDA-neuroimaging study using the following exclusion criteria: 1) imaging data of poor image quality; 2) current or lifetime diagnosis of alcohol dependence or alcohol abuse; and 3) use of psychotropic medication including selective serotonin reuptake inhibitors (SSRIs). This latter exclusion criterion was set because chronic use of antidepressants may influence the shape and/or size of the hippocampal formation through enhanced neurogenesis (Czeh et al. 2001), an area of a-priori interest (see VBM analysis section). The remaining subjects were divided in two groups: (1) those with at least one first degree family member (parents, siblings) with a history of AD (FH+ ; N = 36) and (2) those with no family members with a history of AD or other substance use disorder (FH− ; N = 107) In addition to assess-
ments focusing on depression and anxiety, information on lifetime and familial alcohol use was obtained.

The research protocol was approved by the Medical Ethical Review Board of the participating universities and all respondents provided written informed consent.

**Assessment**

Family history of AD was obtained using the family tree method (Fyer and Weissman 1999), an interview assessing the presence of psychiatric disorders among relatives of the participant, with acceptable inter-rater reliability to assess presence of AD in relatives (Andreasen et al. 1977). For the purpose of adequate sample description, and to control for potential confounders, a number of relevant clinical characteristics were measured. Addiction-related variables were assessed using the Alcohol Use Disorder Identification Test (AUDIT) (Babor et al. 1989) for alcohol use and the Fagerström questionnaire (Heatherton et al. 1991) for smoking behavior. Extraversion, a trait associated with the risk to develop AD and possibly related to morphological brain variations, was assessed using the NEO Five-Factor Inventory (NEO-FFI) (Costa and McCrae 1995). At the day of scanning, severity of depression was assessed using Dutch versions of the Inventory of Depressive Symptomatology (IDS; Rush et al. 1996), the Montgomery-Åsberg Depression Rating Scale (MADRS; Montgomery and Asberg 1979), and anxiety using the Beck Anxiety Inventory (BAI; Beck et al. 1988). To take into account negative environmental influences during childhood, information about childhood trauma exposure was assessed with the NEMESIS questionnaire (de Graaf et al. 2002), in which respondents were asked whether they had experienced emotional neglect, psychological abuse, physical abuse or sexual abuse before age 16.

**Image acquisition**

The main outcome variable was regional GM volume differences between participants with and without a FH of AD. Therefore, high resolution sagittal 3D gradient-echo T1-weighted brain images (Repetition time = 9 ms, Echo time = 3.5 ms, matrix size = 256 × 256, voxel size = 1 x 1 x 1 mm, 170 slices, duration: 4.5 min.) were obtained for every participant with the use of 3T Philips MR-systems (Best, The Netherlands) located either at the Academic Medical Center (AMC) Amsterdam, the Leiden University Medical Center (LUMC) or the University Medical Center of Groningen (UMCG). A SENSE-8 (UMCG, LUMC) and a SENSE-6 (AMC) channel head coil were used for radio frequency transmission and reception.

**Image processing**

Images were processed with SPM5 (http://www.fil.ion.ucl.ac.uk/spm/software/) implemented in Matlab 7.3.0 (The Mathworks Inc., Natick, MA). After reorientation along the AC-PC line, the T1 structural images were segmented into gray matter, white matter and cerebrospinal fluid, using the standard unified segmentation option in SPM5. Registration, normalization, and modulation of the T1 images was performed using Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra (DARTEL; Ashburner 2007), implemented in SPM5. The resulting modulated GM images were further normalized to Montreal Neurological Institute (MNI) space and smoothed with a kernel of 8 mm full-width at half-maximum (FWHM).

**Statistical analyses**

**Socio-demographic and clinical data**

Socio-demographic and clinical data were analyzed using SPSS 15.0 and tested for normality using the Shapiro-Wilk test. Differences between patients with and without a positive FH for AD were te-
VBM analysis

Voxel-based analyses of the GM maps were performed within the framework of the general linear model (GLM). To test for the main effect of FH on regional GM volume, a whole-brain independent samples t-test was set up with family status as independent factor and the normalized, modulated and smoothed GM maps as dependent factor. Age, gender, total GM volume, and scan location (by means of two dummy variables) were added as covariates.

In order to control for possible confounding effects by FH related variables, we performed a series of secondary analyses, by either exclusion of participants or additional multiple regression analyses. Because the sample was drawn from a large cohort with the main aim to study affective disorders, we also tested for the possible confounding influence of the presence of a current diagnosis of MDD and/or anxiety disorder. An additional analysis was performed for the presence of intermediate factors that could explain an environmental cause of familial alcoholism, like childhood adversities and the presence of familial depression/anxiety. In order to rule out effects of maternal alcohol use during pregnancy, we performed the same main analysis, excluding FH+ individuals reporting that their mother had AD.

Each voxel-wise comparison was masked with an explicit optimal threshold mask, created using the Masking Toolbox (Ridgway et al. 2009). Based on previous findings by Benegal and colleagues (2007), we focused on the superior frontal gyrus, medial frontal gyrus, parahippocampal gyrus, amygdala, and cingulate gyrus as a priori areas of interest. For these areas we used the Automated Anatomical Labeling (AAL) atlas (Maldjian et al. 2003), implemented in the WFU-PickAtlas toolbox (Wake Forest University School of Medicine [http://fmri.wfubmc.edu/software/PickAtlas]) to protect against type I error. Effects were explored at $P < .001$ whole-brain uncorrected, and had to meet $P < .05$ Family Wise Error (FWE) corrected for the spatial extent of the small volume ($P_{SVC}$) as defined by the AAL templates, to be considered significant. For other areas of no a-priori interest a threshold of $P < .05$ FWE whole-brain corrected was set ($P_{FWE}$).

RESULTS

Demographic and Clinical characteristics

Table 2.1 shows the socio-demographic and clinical characteristics of the sample. Age, years of education, gender, total GM-volume, and scan location did not differ between the FH+ and FH− groups. Both groups had the same distribution of healthy controls and subjects with depression and/or anxiety disorder. However, within the latter group, the frequency of current depression was higher in the FH+ group. Age of onset of depression and anxiety disorder, anxiety symptoms (BAI score), objective depression symptoms (MADRS), drinking behavior (AUDIT score low in both groups), and smoking behavior (Fagerström score) did not differ between the FH+ and the FH− group. Compared with the FH− group, the FH+ reported significantly more depressive symptoms (IDS) at the day of scanning and significantly lower extraversion scores. Prevalence of familial depression/anxiety was significantly higher in the FH+ group than in the FH− group. Finally, the FH+ group reported more psychological and emotional neglect, sexual abuse and parental loss before age 16 than the FH− group.
### Table 2.1 - Sample Characteristics

<table>
<thead>
<tr>
<th></th>
<th>FH– (N = 107)</th>
<th>FH+ (N = 36)</th>
<th>t</th>
<th>U</th>
<th>χ²</th>
<th>df</th>
<th>P</th>
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<tbody>
<tr>
<td>Women, No. (%)</td>
<td>78 (73)</td>
<td>24 (67)</td>
<td>0.511</td>
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<td>.48</td>
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<td>Age, M(SD)</td>
<td>38.1 (10.7)</td>
<td>38.2 (9.8)</td>
<td>1913.0</td>
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<td>.95</td>
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<td>Years of education, M(SD)</td>
<td>13.2 (3.2)</td>
<td>12.7 (3.0)</td>
<td>2774.5</td>
<td>141</td>
<td>.47</td>
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<td>Right handedness, No. (%)</td>
<td>99 (93)</td>
<td>32 (89)</td>
<td>0.463</td>
<td>1</td>
<td>.50</td>
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<td>Scan location, No. (%)</td>
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<td>0.607</td>
<td>2</td>
<td>.74</td>
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<td>AMC</td>
<td>30 (28)</td>
<td>12 (33)</td>
<td></td>
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<td>LUMC</td>
<td>43 (40)</td>
<td>12 (33)</td>
<td></td>
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<tr>
<td>UMCG</td>
<td>34 (32)</td>
<td>12 (33)</td>
<td></td>
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<td>Total GM, M(SD)</td>
<td>728.3 (74.4)</td>
<td>720.4 (98.8)</td>
<td>0.58</td>
<td>141</td>
<td>.56</td>
<td></td>
<td></td>
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<tr>
<td>Depression and/or Anxiety status</td>
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</tr>
<tr>
<td>No history of MDD/Anxiety, No. (%)</td>
<td>41 (38)</td>
<td>8 (22)</td>
<td>3.1</td>
<td>1</td>
<td>.08</td>
<td></td>
<td></td>
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<tr>
<td>Current MDD only, No. (%)</td>
<td>20 (19)</td>
<td>14 (39)</td>
<td>6.063</td>
<td>1</td>
<td>.02*</td>
<td></td>
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<tr>
<td>Current Anxiety only, No. (%)</td>
<td>29 (27)</td>
<td>3 (8)</td>
<td>5.463</td>
<td>1</td>
<td>.02*</td>
<td></td>
<td></td>
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<tr>
<td>Comorbid MDD and Anxiety, No. (%)</td>
<td>17 (16)</td>
<td>11 (31)</td>
<td>3.680</td>
<td>1</td>
<td>.06</td>
<td></td>
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<tr>
<td>Onset age MDD, M(SD)</td>
<td>23.29 (9.9)</td>
<td>20.81 (8.9)</td>
<td>540.0</td>
<td>49</td>
<td>.19</td>
<td></td>
<td></td>
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<tr>
<td>Onset age Anxiety, M(SD)</td>
<td>18.0 (11.9)</td>
<td>15.4 (7.6)</td>
<td>342.5</td>
<td>49</td>
<td>.53</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IDS score, M(SD)</td>
<td>14.4 (13.3)</td>
<td>19.7 (13.7)</td>
<td>1352.0</td>
<td>141</td>
<td>.03*</td>
<td></td>
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<tr>
<td>MADRS score, M(SD)</td>
<td>8.95 (10.0)</td>
<td>12.43 (11.1)</td>
<td>1488.5</td>
<td>141</td>
<td>.07</td>
<td></td>
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<tr>
<td>BAI score, M(SD)</td>
<td>9.9 (10.5)</td>
<td>12.0 (10.8)</td>
<td>1582.0</td>
<td>141</td>
<td>.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FH of Affective Disorders, No. (%)</td>
<td>73 (68)</td>
<td>31 (86)</td>
<td>4.3</td>
<td>1</td>
<td>.04*</td>
<td></td>
<td></td>
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<tr>
<td>Childhood trauma before age 16, No. (%)</td>
<td>46 (43)</td>
<td>25 (69)</td>
<td>7.5</td>
<td>1</td>
<td>.01*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extraversion (NEO-FFI subscale), M(SD)</td>
<td>37.6 (8.5)</td>
<td>33.7 (11.5)</td>
<td>2.18</td>
<td>141</td>
<td>.03*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking (Fagerström Total score), M(SD)</td>
<td>0.9 (1.6)</td>
<td>0.9 (1.7)</td>
<td>1919.5</td>
<td>141</td>
<td>.97</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol use (AUDIT Total score), M(SD)</td>
<td>3.9 (3.9)</td>
<td>3.3 (3.8)</td>
<td>1659.0</td>
<td>141</td>
<td>.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>How often do you take an alcoholic drink?*, Mean(SD)</td>
<td>3.2 (1.4)</td>
<td>2.7 (1.7)</td>
<td>1611.0</td>
<td>141</td>
<td>.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>How many standard drinks per day? Mean(SD)</td>
<td>2.2 (1.9)</td>
<td>1.8 (1.6)</td>
<td>1685.0</td>
<td>141</td>
<td>.14</td>
<td></td>
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</tbody>
</table>

Abbreviations: AMC: Academic Medical Center; AUDIT: Alcohol Use Disorder Identification Test; GM: Gray Matter; BAI: Beck Anxiety Inventory; FH: Family History; IDS: Inventory of Depressive Symptomatology; LUMCG: Leiden University Medical Center; M, Mean; MADRS: Montgomery-Åsberg Depression Rating Scale; MDD: Major Depressive Disorder; NEO-FFI: NEO Five-Factor Inventory; UMCG: University Medical Center Groningen.

*1 = Never; 2 = Monthly or less; 3 = 2-4 times a month; 4 = 2-3 times a week; 5 = 4 or more times a week
**VBM analysis**

The FH+ group showed significantly smaller GM volumes in the right parahippocampal gyrus (PHG) compared with the FH– group ($Z = 4.23; P_{SVC} < .05$) (see figure 2.1). We did not observe significantly smaller volumes of the prefrontal cortices, amygdala and cingulate gyrus in the FH+ as compared with FH– at the set threshold.

Lowering the threshold ($P < .005$ uncorrected) showed smaller GM volumes in the right superior frontal gyrus ($Z = 2.96$) and the right medial frontal gyrus ($Z = 2.87$) in the FH+ group compared with the FH– group.

After adding current MDD/anxiety disorder to the model, the PHG remained significantly smaller in the FH+ group compared with the FH– group ($Z = 4.16; P_{SVC} < .05$). Adding childhood adversity and familial anxiety and/or depression to the model also did not significantly change the main effect of a positive family history of AD on regional brain volume in the PHG ($Z = 3.96; P_{SVC} < .05$; and $Z = 4.21; P_{SVC} < .05$, respectively).

Furthermore, removing participants from the main analysis who reported maternal alcohol use did not change the main results in the PHG ($Z = 3.70; P_{SVC} < .05$).

![Figure 2.1](image)

**Figure 2.1 - Main effect of Family History**

a) Comparison of FH+ with FH– showing smaller gray matter volume of the right parahippocampal gyrus in FH+ (displayed at $P < .005$ uncorrected). Colored bar: $Z$-scores from 0 to 4.23.

b) Plot of group differences in gray matter densities at peak voxel $x=20$ $y=-37$ $z=-11$, contrast estimates at confidence interval 90%.

FH+, positive family history of alcohol dependence; FH–, negative family history of alcohol dependence.
DISCUSSION

The present study aimed to investigate the influence of familial alcohol dependence (AD) on regional gray matter (GM) volumes in a sample of non-alcoholic adults. Findings were consistent with our hypothesis that a positive family history of AD (FH+) in non-alcoholic adults is associated with smaller right parahippocampal gyrus (PHG) volume. This finding partly replicates results of previous studies in non-alcoholic adolescents with an increased familial risk to develop AD (Benegal et al. 2007) and in young alcoholics (De Bellis et al. 2000). However, because of their adolescent and young adult samples, these previous studies could not distinguish between a transient developmental delay in brain maturation caused by FH status, and a persistent familial vulnerability to develop AD or other related neuropsychiatric disorders. In the current sample we were able to rule out a transient developmental delay by only including adults. Additionally, since the FH+ and FH− group in the current study were free of a diagnosis of lifetime alcohol dependence or abuse, and did not differ in daily alcohol use, we were able to rule out neurotoxic effects of alcohol, in contrast to several family history studies that have been performed in AD patients (De Bellis et al. 2000; 2005; Gilman et al. 2007). Also, the possibility that prenatal alcohol exposure is (partly) responsible for our findings through neurotoxicity, could be ruled out, since excluding six of 36 FH+ participants who reported to have a mother with AD from the main analysis, did not influence the results.

Previously, smaller prefrontal volumes have also been reported in FH+ adolescents (Benegal et al. 2007), however results of the current study indicated smaller prefrontal volumes only at sub-threshold level among FH+ adults. This discrepancy in results appears to be at odds with the existence of a specific developmental delay in prefrontal brain maturation in non-alcoholic, high-risk adolescents; the prefrontal cortex is a brain area that is known to develop later than the rest of the brain and reaches maturity only in early adulthood (Gogtay et al. 2004). We did not replicate previous findings of smaller volumes of the amygdala and cingulate gyrus in high-risk individuals, which were possibly also due to a transient developmental delay in the FH+ sample of Benegal (2007).

The hippocampal formation, including the parahippocampal gyrus, is known for its critical role in memory functions (Eichenbaum et al. 1992; Squire et al. 1989). Additionally, the parahippocampal gyrus is considered a key component of a behavioral inhibition mechanism (Gray and McNaughton 2000), modulating functions such as impulse control and emotion regulation. Impulsivity has repeatedly been shown to be associated with an increased risk to develop substance use disorders (e.g., Verdejo-Garcia et al. 2008). Moreover, in the last decade decreased PHG volumes have been found in several anxiety disorders (Liao et al. 2011; Massana et al. 2003), and other neuropsychiatric disorders associated with (excessive) stress (Sapolsky 2000). Reciprocal connections between the medial temporal lobe, the orbitofrontal gyrus and amygdala show its involvement in processing negative information (Iidaka et al. 2001). Together these findings suggest a role for the parahippocampal gyrus in emotion regulation, and indicate that decreased volume in this region related to a positive family history of AD may be a mediating factor in the development of anxiety and depression as well as for the development of addictive behaviors.

By definition, the adults in the current study had not (yet) developed an alcohol use disorder. However, although objective depression measurements (MADRS) did not differ between groups, MDD was more prevalent in the FH+ than in the FH− group. Thus it seems that other psychiatric pathology, often associated with alcohol use disorders, may have increased under the familial load. This increased presence of affective disorders in FH+ individuals is consistent with previous reports (Araujo and Monteiro 1995; Dawson and Grant 1998). Yet, future studies are needed to further...
explore such heterotypic associations between an alcoholic family history and the development or the severity profile of affective disorders (Milne et al. 2009).

Findings of decreased PHG volumes in posttraumatic stress disorder (PTSD) (Nardo et al. 2010; Wignall et al. 2004), correlated with trauma load (Woodward et al. 2009), suggest that negative environmental stress factors associated with familial alcoholism, e.g. during childhood, may affect volumes in the hippocampal area. A decrease in volume could thus be mediated through a mechanism of increased cortisol levels (Lindauer et al. 2006; Pruessner et al. 2007) or stress-related decreased brain derived neurotrophic factor (BDNF), reducing neurogenesis in the hippocampal area (Hajek et al. 2012; Montag et al. 2009). However, in contrast to studies suggesting smaller hippocampal volume as a consequence of stress factors, Gilbertson and colleagues (2002) provided evidence in their PTSD twin study that a smaller hippocampal formation is a pre-existing condition rendering the brain more vulnerable to the development of pathological stress responses. In our sample, we did find significant differences in childhood trauma characteristics between the two groups, indicating that the FH+ group experienced more childhood adversities before the age of 16 years than the FH– group. An additional analysis for childhood adversities did not affect our main findings, indicating that the reduced PHG volume in FH+ subjects is likely not mediated by negative childhood experiences. This was corroborated by another report from the NESDA neuroimaging-study on the influence of childhood adversities on regional brain volumes, where no PHG volume differences were found between those that had and those that had not experienced childhood adversities (van Harmelen et al. 2010).

The observed smaller PHG volume in FH+ adults could be mediated by pre-existing factors, similar to those found by Gilbertson and colleagues (Gilbertson et al. 2002). Impairments in the inhibition mechanism, associated with PHG volume decrease, have been shown to have a genetic rather than environmental origin (King et al. 2009), supporting the hypothesis that the influence of FH on parahippocampal volume also reflects a large genetic predisposition. Additionally, the manipulation of genes by environmental factors such as perinatal stress, or medication use, also known as epigenetics, could mediate regional volume changes in FH+. However, in this study it was not possible to determine the precise factors that could influence epigenetics. Nonetheless, a series of additional analyses showed that some of the possible epigenetic pathways (childhood adversity and stress, and prenatal alcohol exposure) could not explain the main results.

The use of a whole-brain unbiased segmentation approach (VBM-DARTEL) can be seen as an important strength in this study, since we did not limit our analysis to a restricted number of brain structures, but were able to detect volumetric changes across the whole brain in an unbiased manner. The sensitivity of the VBM-DARTEL approach has been shown to correlate with manual segmentation in psychiatric studies (Kroes et al. 2011; Uchida et al. 2008), specifically for detecting hippocampal atrophy in MDD patients (Bergouignan et al. 2009). While we focused solely on GM volumes in this analysis, it is important to mention that white matter (WM) abnormalities have also been associated with FH of AD in young samples (Herting et al. 2011; Venkatasubramanian et al. 2007). It remains unknown, however, how these abnormalities are related to developmental delay or persistent neurobiological abnormalities in adults. Future studies on the role of family history of AD should simultaneously focus on GM volumes, WM volumes and tract analyses like diffusion tensor imaging (DTI) to gain more insight on connections between brain areas and to shed light on the mechanisms that trigger macrostructural and functional brain changes.

As part of the NESDA study, this analysis could be performed in a relatively large sample size (36 FH+ and 107 FH– subjects), and the careful screening of subjects within the framework of the NESDA study allowed us to test for a broad range of possible confounders. Even though NESDA originally aimed at studying the onset and course of depression and anxiety disorders, both healthy
controls and people with depression and/or anxiety were scanned. To capitalize on the sample size we included healthy controls as well as people with an affective disorder. In an additional test we did control for the presence of depression and anxiety, however it should be noted that adjusting for anxiety and/or depression status could result in overcorrection, since alcohol use disorders and affective disorders are highly comorbid, and literature shows that a FH of AD and presence of affective disorders in an individual is common (Araujo and Monteiro 1995; Dawson and Grant 1998), suggesting that the current sample has adequate ecological validity. Nevertheless, in this study the relationship between FH and PHG volume was not affected by depression and/or anxiety status (note that results of VBM comparisons between anxiety and depression groups within NESDA have been reported elsewhere (Van Tol et al. 2010)). Finally, while the family tree method is likely to be inherently subjective, this would however presumably result in false negatives and hence an underestimation of the true effects of familial alcoholism.

In conclusion, the present study provides new insights in the association between a family history of AD and regional GM volume in non-alcoholic adults, allowing us to differentiate between neurotoxic consequences, transient developmental delays in brain maturation and a persistent neurobiological risk for the development of AD and/or AD-related neuropsychiatric disorders. Our main finding that FH+ is associated with a smaller right parahippocampal volume in non-alcoholic adults suggests the presence of a persistent or even permanent brain abnormality in non-alcoholic FH+, possibly mediating the development of alcohol use disorders but also other neuropsychiatric disorders related to AD, as suggested in this study. This has implications for prevention; people with FH+ have a greater chance of PHG abnormalities and thus the development of associated disorders, i.e. AD and AD-related neuropsychiatric disorders, including major depression. The findings of this study also changes our risk perception of the effects of alcohol use in youngsters since PHG abnormalities in alcohol using youngsters are not necessarily the (direct) consequence of their (excessive and thus harmful) alcohol use. Moreover, this knowledge on permanent structural influences of an alcoholic family history could be used as a selection criterion for genetic and imaging research.

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