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DRD2 methylation and regional grey matter volumes in young adult offspring from families at ultra-high risk for alcohol dependence

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ABSTRACT

Dopaminergic alteration is a prominent feature in those with AD and may influence brain development in those with a family history of AD. MRI scans (3T) from 43 HR offspring (27.4 ± 3.6 years) and 45 controls (24.5 ± 4.1 years) provided whole brain (WB) and region of interest (ROI) analyses. The VBM8 toolbox was used for WB analysis (threshold $p < 0.005$; cluster = 100 voxels); the MarsBaR ROI toolbox provided region of interest data. Pyrosequencing of CpG sites within the DRD2 gene was performed. DRD2 methylation was significantly increased in association with familial high-risk status. Significant familial risk group differences were seen with HR individuals showing reduced volume of the Left Inferior Temporal, Left Fusiform and Left Insula regions relative to LR controls. These regions have previously been linked to social cognition. DRD2 methylation was negatively related to grey matter volumes in these regions. Because these regions, have been previously linked to facial affect perception and social cognition, lesser grey matter volumes in individuals at high-risk for developing AD suggests that neural underpinnings of social cognitive impairment may be a premorbid risk factors for AD.

1. Introduction

There are now numerous reports showing morphological differences between offspring from families with alcohol dependence and those from control families (see Hill, 2010, 2018 for reviews). These morphological differences may be related to differences in neurotransmitter functioning occurring in childhood that influence regional brain development that could potentially persist into adulthood. Previously, gene expression levels have been related to volumetric difference across brain regions (Negi and Guda, 2017). Dopamine mediated signaling has been shown to be critically important in brain neurodevelopment with the potential to alter structure, function and developmental trajectories (See Money and Stanwood, 2013 for review).

The dopamine DRD2 receptor has been studied extensively with respect to addiction with multiple studies pointing to a relationship between differences in receptor number and their level of activation in persons varying by family history of alcohol dependence (Volkow et al., 2006). Because of the significant role that DRD2 receptors play in a variety of addictive disorders, it was of interest to determine if familial risk status influenced DRD2 methylation. Greater familial adversity can be expected to be present in those with family history of alcohol dependence. Previous studies have shown that childhood adversity influences DNA methylation (Essex et al., 2013; Matosin et al., 2017). The effect of the childhood environment on methylation/expression of DRD2 may have implications for childhood brain development that persists into young adulthood.

Because gene expression is influenced by DNA methylation, an experiment was planned to investigate DRD2 methylation and its potential relationship to volumetric differences in brain regions showing familial risk group differences.

2. Methods

2.1. Participants

The present report is based on structural MRI (sMRI) scans of 88 third generation offspring who are part of an ongoing family study that selected families through their parents' generation. Offspring from 65 families were included: a single offspring from 48, 2 siblings from 12 families, 3 siblings from 4 families and one family with four siblings. A sub-sample of the 88 participants had banked DNA ($N = 71$). The goal of the larger longitudinal study was to contrast offspring from high and

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low-risk for alcohol dependence families on the basis of neurobiological and clinical status. Accordingly, offspring were followed through childhood at approximately annual intervals and through young adulthood, biennially. Extensive assessment of alcohol and drug use information was obtained at each follow-up wave. The goal of the present study was to perform a whole brain analysis of differences between offspring with and without a family background of alcohol dependence to provide a discovery analysis of overall differences. A secondary goal was to provide a confirmation analysis of uncovered regions using a region of interest approach. Confirmation included analysis of the role of methylation of D2 in any differences revealed by these analyses.

All participants provided consent with each visit. Children provided assent with parental consent. The study has ongoing approval from the University of Pittsburgh Institutional Review Board.

2.1.1. High-risk families

The high-risk families were identified through a proband pair of alcohol dependent sisters as previously described (Hill et al., 2011). Both members of the proband pair were screened using an in-person structured interview (Diagnostic Interview Schedule) (DIS; Robins et al., 1981) to determine the presence of alcohol dependence and other Axis I psychopathology.

2.1.2. Low-risk control families

Selection of control pedigrees was based on availability of a nuclear family with children between the ages of 8–18 and through parents who were screened for absence of alcohol and drug dependence using the DIS.

2.2. Procedures

2.2.1. Longitudinal clinical assessment of children, adolescents and young adults

All available offspring from high and low-risk families who were 8–18 years old at the initiation of the follow up were eligible for inclusion. Each child/adolescent and his/her parent were separately administered the Schedule for Affective Disorders and Schizophrenia (K-SADS) (Chambers et al., 1985) by trained, Masters' level clinical interviewers and an advanced resident in child psychiatry at each annual evaluation. A reliable best-estimate diagnosis was obtained for all major DSM-III diagnoses at approximately yearly intervals (Hill et al., 2011). Quantity and frequency of use of commonly used substances (e.g., alcohol, cannabis, benzodiazepines, opioids) was also obtained. Young adult assessments included the Composite International Diagnostic Interview (CIDI) (Janca et al., 1992) and CIDI-Substance Abuse Module (CIDI-SAM) (Cottler et al., 1989), providing diagnoses for all DSM-IV diagnoses.

For the present analyses, information concerning lifetime use of substances prior to the MRI was derived from the K-SADS, CIDI, and CIDI-SAM interview data. Exposure was calculated based on reported use across the lifespan starting from the first visit until the last visit prior to the scan. A median split for users was determined and a three group contrast constructed for each substance (0 for Non-Users, 1 for Below Median Users and 2 for those in the Above the Median group).

2.2.2. Life stressors and social resources inventory (LISRES)

The subjects had been part of a longitudinal follow up that included administration of the Life Stressors and Social Resources Inventory (LISRES). The instrument assesses stressors and social support from eight sources: physical health, home and neighborhood, parents, sibling, extended family, school and boyfriend/girlfriend. A total score is calculated for stressors, the Negative Life Events scale, and a total score determined for the same eight sources from which positive support was reported resulting in the Positive Life Events scale. These scores were included in the overall analysis due to the potential for familial alcohol dependence to be associated with the environmental milieu that might alter brain volumes based on a now large literature suggesting that early environment can have effects on brain structure (Geritsen et al., 2015; Hill et al., 2013; McEwen et al., 2015).

2.2.3. Assessment of prenatal use of substances

At the time the offspring were entered into the follow-up study, the first of several follow-up visits to our laboratory, the mother was administered a structured interview (Drinking and Drug Use During Pregnancy) concerning her alcohol, cigarette, and other drug use during each of her pregnancies so that the quantity and frequency of these substances could be determined and used as control variables.

2.2.4. Imaging parameters

The MRI scans were performed at the University of Pittsburgh Medical Center (UPMC) Magnetic Resonance Research Center (MRRC) using a 3T head-only Siemens Trio scanner (Siemens Medical Systems, Ehrlangen, Germany) equipped with a fast gradient system for echo-planar imaging. A standard radiofrequency head coil was used with foam padding to restrict head motion. A 7-min 3D T1-weighted Magnetization Prepared Rapid Gradient Echo Imaging (MPRAGE) sequence (TR = 2300 ms, TE = 2.98 ms, FA = 9°, field of view FOV = 240 mm, acquisition matrix = 240 × 256, in-plane resolution 1.0 × 1.0 mm^2, yielding 160 transversal slices with a thickness of 1.2 mm) was used to acquire a high-resolution anatomical scan for VBM analysis.

2.2.5. Preprocessing

Data preprocessing was performed using Statistical Parametric Mapping software SPM8 (http://www.fil.ion.ucl.ac.uk/spm/) and the VBM8 toolbox (http://dbm.neuro.uni-jena.de/vbm/). The structural images were bias corrected, segmented into grey, white, and cerebrospinal fluid, and affine registration, normalization, and modulation performed. VBM8 uses a maximum posteriori method to segment tissue types, with the segmented images normalized to Montreal Neurological Institute (MNI) space using nonlinear DARTEL normalization. The voxel size was resliced to 1 × 1 × 1 mm^3 for all images.

Manual quality checks of the VBM8 modulated grey matter (GM) images were performed to determine if any structural abnormalities were present followed by a check of all of the images for homogeneity and absence of outliers. Grey matter images with the poorest covariance were removed from the analysis. These quality controlled modulated images were then smoothed using a 12-mm full-width at half-maximum (FWHM) isotropic Gaussian kernel.

SPM statistical analyses of the grey matter images were performed using a two sample *t*-test with contrasts defined as low-risk controls greater than high-risk offspring. The resulting maps were thresholded at $p < 0.005$ with a cluster size of 50 voxels. Family-wise error (F_{we}) rates were calculated to adjust for multiple testing. Additionally, regional volumes were calculated using the MarsBaR ROI toolbox (http://marsbar.sourceforge.net) to compare the GM volume of the specified regions of interests (ROIs) which were further compared in SPSS (version 20). These ROIs calculated for each participant enabled us to perform analyses in SPSS to follow up on areas revealed in the whole brain analyses using multiple covariates of interest.

2.2.6. DNA isolation and genotyping

Genomic DNA was extracted from whole blood or from Epstein Barr Virus (EBV) transformed cells and amplified using PCR. Pyrosequencing of CpG sites within the DRD2 gene were completed on a Biotage PSQ 96MA Pyrosequencer (Biotage AB, Uppsala, Sweden).

3. Results

The participants were compared on demographic and clinical characteristics (Table 1). There were no statistically significant

Table 1
Demographic and clinical characteristics of offspring of alcohol dependent parents and F1 comparison controls with methylation data ($N = 71$).

	No parent with AD $N = 37$	1 parent with AD $N = 19$	2 parents with AD $N = 15$	Group comparison F(df) p, or X^2 (df) p
Male	21	8	3	$N = 32$
Female	16	11	12	$N = 39$
Scan age	24.81 ± 4.26	26.47 ± 3.10	26.20 ± 3.41	$F_{2,68} = 1.47$, ns $F_{2,68} = 4.70$, $p = 0.01$
Education	15.35 ± 1.89	14.42 ± 1.64	13.73 ± 1.83	$F_{2,68} = 0.23$, $p =$ ns (Covary SES)
ICV	1374.77 ± 137.17	1379.50 ± 160.02	1363.19 ± 118.01	$F_{2,68} = 0.06$, ns
Age at DNA collection	13.81 ± 4.59	13.29 ± 4.53	11.54 ± 2.67	$F_{2,68} = 1.53$, ns
Childhood negative life events (Before DNA collection)	47.00 ± 6.30 ($N = 16$)	48.25 ± 11.39 ($N = 8$)	66.00 ± 11.66 ($N = 4$)	$F_{2,27} = 7.80$, $p = 0.002$ $N = 27$
Childhood positive life events (Before DNA collection)	52.87 ± 13.87	60.88 ± 11.96	62.00 ± 13.14	$F_{2,24} = 1.34$, ns $N = 27$

differences in age at MRI scan or DNA collection by parental risk group. The gender representation did not differ by group. The socioeconomic status (SES) did differ between those having no parents with alcohol dependence (AD), those with one and those with two parents meeting criteria for AD. Education varied between groups though this difference was not statistically significant when SES was added as a covariate. Intracranial volumes (ICV) did not show a statistically significant difference by the number of parents with alcohol dependence.

3.1. Brain volumes and SPM

Whole brain analyses were performed in SPM that revealed significant differences in three regions: Left Fusiform, Left Insula, and Left Inferior Temporal regions between offspring from high-risk versus low-risk families. The analyses performed in SPM were adjusted for intracranial volume, personal history of commonly used substances (cigarettes, alcohol and drugs), and prenatal exposure to these substances (Table 2).

3.2. Masks of the regions of interest

Masks of the regions showing whole brain significance in SPM (Left Fusiform, Left Insula, and Left Inferior Temporal regions) were applied to the scan data (Figs. 1–2 and 3) to obtain volumes for further analyses using SPSS (version 20). First, the data were analyzed using a mixed model analysis of variance with family ID included as a random effect to evaluate any potential effect of including multiple siblings from the same family. None of the analyses showed evidence of multiple siblings from the same family contributing to alteration of the results. Therefore, regression analyses were performed that included familial risk, ICV, age at scan, whether or not the subject had met criteria for a substance use disorder that preceded the MRI scan, total days on which the mother reported prenatal use of any drugs, total prenatal use of alcohol (number of drinks), and total cigarette use (number of cigarettes) during pregnancy.

3.3. Analyses of regions of interest

Analyses for each of the three regions showing significant differences in the SPM analyses were further assessed using a mixed model analysis (SPSS 20) to examine all potential covariates with the effect of multiple siblings from the same family used as a random effect. In order to evaluate the effect of familial risk in the context of relevant covariates, the statistical approach involved conducting the analysis with all potentially relevant covariates, removing the non-significant covariates, and re-performing with a reduced model. Because we expected considerable collinearity between familial risk and methylation status, the impact of methylation on volume, in the context of variables found to be significant in the mixed model analyses, was undertaken using linear regression. Also, because collinearity between familial risk and mothers' prenatal use of alcohol, drug, and cigarette**s** was expected based on our previous findings (O'Brien and Hill, 2015) from a larger sample, analyses were performed within the high-risk group using levels of use of these substances.

3.3.1. Left fusiform

For the left fusiform, a mixed model was tested in which family ID was used as a random effect and fixed effects included familial risk (two groups), ICV, age at scan, and a binary value for having or not having SUD before the scan, total drug, alcohol and cigarettes used by the mothers prenatally, sex and SES. Only familial risk ($F = 7.49$, df = 1, 85, $p = 0.008$) and sex ($F = 7.23$, df = 1, 85, $p = 0.009$) had a significant impact on volume of the left fusiform with the high-risk offspring showing reduction in volume.

Next, a regression analysis was performed to assess the combined effects of familial risk, sex and D2 methylation. A significant effect of methylation on left fusiform volume was seen $\beta = -0.254$, SE = 0.033, $p = 0.028$ when evaluated along with familial risk $\beta = 0.209$, SE = 0.013, $p = 0.088$, and sex $\beta = -0.254$, SE = 0.012, $p = 0.028$. The estimated R for this model was 0.507($F = 7.71$. df = 3,67, $p < 0.000$).

Table 2
Low Risk > High Risk with ICV, personal history of SUD, and prenatal exposure to alcohol, cigarettes and drugs as covariates.

Region	MNI coordinates (mm) X	Y	Z	SPM analysis p_{uncorr}	Peak T	Cluster extent	p_{corr} (FWE)	Mixed model analysis (SPSS) p value
Left Fusiform	−23	−52	−14	<0.0001	5.09	3501	<0.0001	0.008 [a]
Left Insula	−38	−12	3	<0.0001	4.48	820	0.001	0.034 [a]
Left Inferior Temporal	−65	−30	−21	<0.0001	4.28	441	0.025	0.016 [b]

[a] A total of 88 subjects were available for analysis; 75 subjects had complete information for all covariates.
[b] A significant difference for the entire sample was not seen but significant results were seen for male subjects in the SPM analysis. A total of 39 male subjects were available for analysis; 34 had complete information for all covariates. SPSS analysis using all participants was significant.

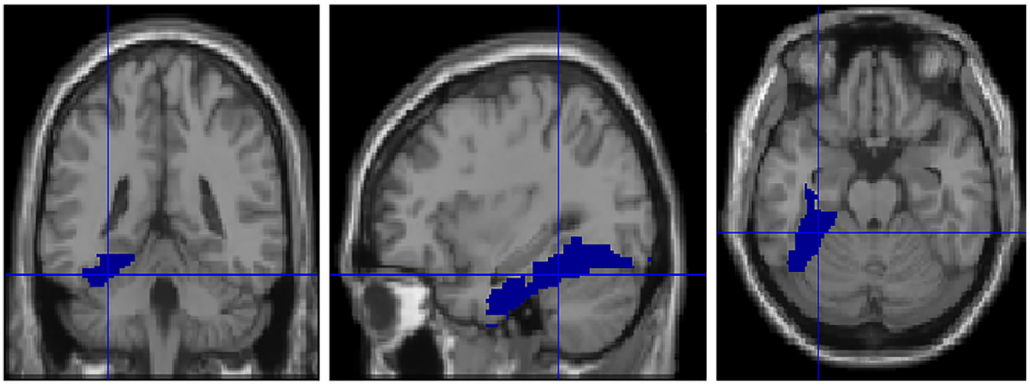

Fig. 1. A whole-brain SPM8 analysis was performed to evaluate the effects of familial/genetic risk group differences for high and low-risk offspring. Analyses were adjusted for intracranial volume, personal history of commonly used substances (cigarettes, alcohol and drugs) and prenatal exposure to these substances. Results for the left fusiform cortex show reduced volume in the high-risk offspring relative to the low-risk controls.

3.3.2. Left insula

A mixed model analysis using the same set of variables used in the model in which the left insula was tested showed that only familial risk had a statistically significant effect ($F = 4.64$, df = 1, 86, $p = 0.034$) on left insula volume.

A regression analysis that included D2 methylation in the analysis did not find familial risk to be statistically significant. The D2 methylation effect was significant $\beta = -0.299$, SE = 0.036, $p = 0.015$ even when familial risk was in the model. The estimated R for this model was 0.392 ($F = 6.16$. df = 2,68, $p = 0.003$).

3.3.3. Left inferior temporal

A mixed model analysis using the same set of variables as those used to test the left fusiform and the left insula was performed for the left inferior temporal region. This analysis revealed a significant age effect ($F = 4.64$, df = 1, 79.51, $p = 0.034$), and a marginal risk effect ($F = 2.93$, df = 1, 60.79, $p = 0.092$).

A regression analysis that included the familial risk effect and D2 methylation status revealed marginally significant effects for both when entered conjointly ($\beta = 0.221$, SE = 0.015, $p = 0.073$ for risk and $\beta = -0.210$, SE = 0.040, $p = 0.089$ for the methylation effect). The estimated R for this model was 0.356 ($F = 4.93$, df = 2, 68, $p = 0.01$).

3.4. Prenatal effects evaluated within the high-risk group

A mixed model analysis was performed within the high-risk group to evaluate potential effects of substance use that may have been incorrectly attributed to familial risk group effects. Because the available number of subjects within this group was reduced to 43, the definition of use was defined first as none (0), less than the median of users (1), and greater than median of users (2). In order to increase power to detect differences by enlarging each contrasted group, a binary variable was tested: drank during pregnancy (yes/no), used any drugs during pregnancy (yes/no) and smoked during pregnancy (yes/no). Analyses were performed for the fusiform (left), insula (left) and the inferior temporal (left). Results of these analyses showed no significant effects for alcohol exposure using the 0, 1, 2 split or the drinker ($N = 18$) versus non-drinker ($N = 23$) groups for the three regions tested. The 0, 1, 2 split for number of drug use days did not reveal significant effects on volume for any of the three structures. Using the binary variable of any drug use during pregnancy versus any use, revealed a marginally significant effect for the left fusiform only ($F = 3.81$, df = 1, 39, $p = 0.058$) with the volume in users being slightly larger. Significant differences were not seen using the three group split for smoking. However, using the binary variable of smoker ($N = 15$) versus non-smoker ($N = 21$) significant effects were seen for the left fusiform ($F = 5.46$, df = 1, 34, $p = 0.025$) and left inferior temporal regions ($F = 9.81$, df = 1,30, $p = 0.004$) with age also showing significant effects ($F = 10.10$, df = 1, 30.0, $p = 0.003$). These differences represent an increase in volume of 4.4% for the left fusiform and 7.6% for the left inferior temporal regions.

3.5. DRD2 methylation and regional volumes

DRD2 methylation was negatively related to grey matter volume of the fusiform cortex (left) ($r = -0.370$, $p = 0.001$), insula (left) ($r = -0.323$, $p = 0.006$), and temporal inferior (left) ($r = -0.316$, $p = 0.007$). Scatterplots depicting the relationship between DRD2 methylation and grey matter volume (Figs. 4, 5 and 6) illustrate that greater methylation is associated with reduced volume in all cases.

3.6. Familial risk status and DRD2 methylation

An analysis was performed to determine if familial risk status was associated with DRD2 methylation status. In this analysis, the 37 offspring from low-risk control families were contrasted with parents with

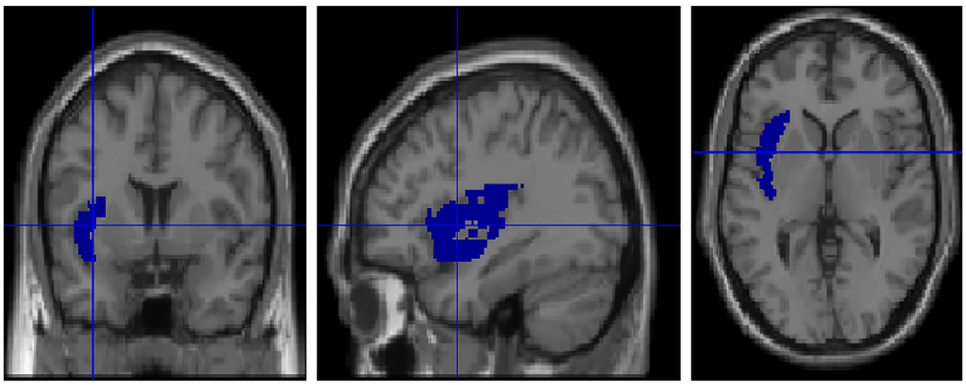

Fig. 2. The whole-brain SPM8 analysis performed to evaluate the effects of familial/genetic risk group differences for high and low-risk offspring show reduced volume in the high-risk offspring relative to the low-risk controls for the left insula.

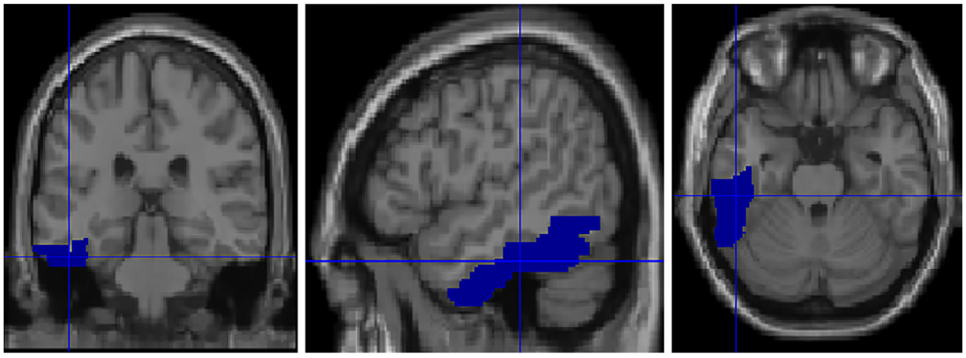

Fig. 3. The whole-brain SPM8 analysis performed to evaluate the effects of familial/genetic risk group differences for high and low-risk offspring show reduced volume in the high-risk offspring relative to the low-risk controls for the left inferior temporal region.

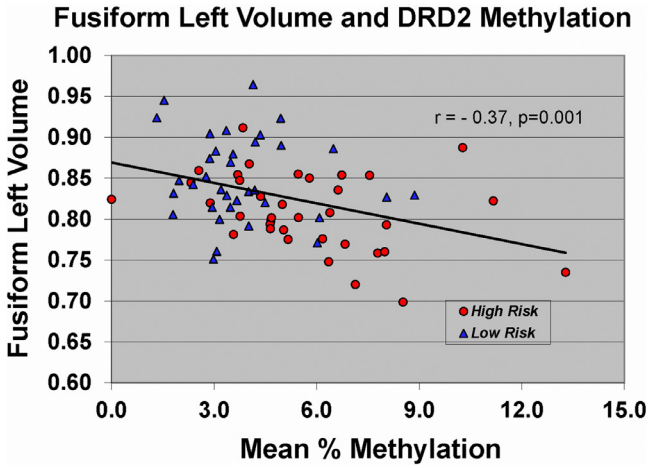

Fig. 4. The relationship between volume of the left fusiform region and DRD2 methylation is illustrated in this scatterplot. Increased methylation was significantly associated with decreased volume.

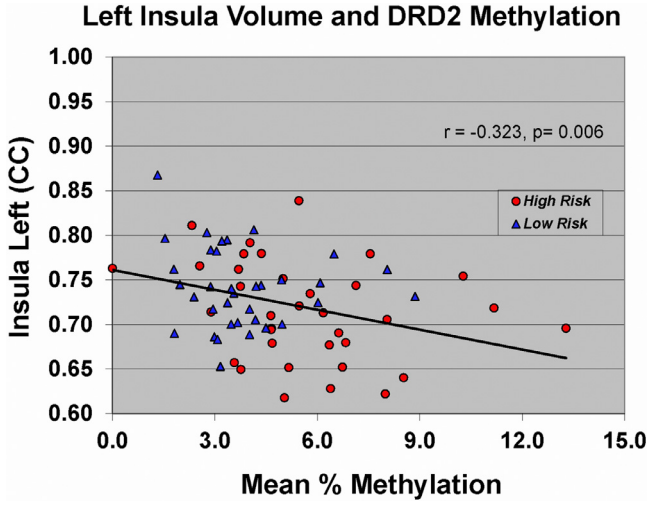

Fig. 5. The relationship between volume of the left insula and DRD2 methylation is illustrated in this scatterplot. Increased methylation was significantly associated with decreased volume.

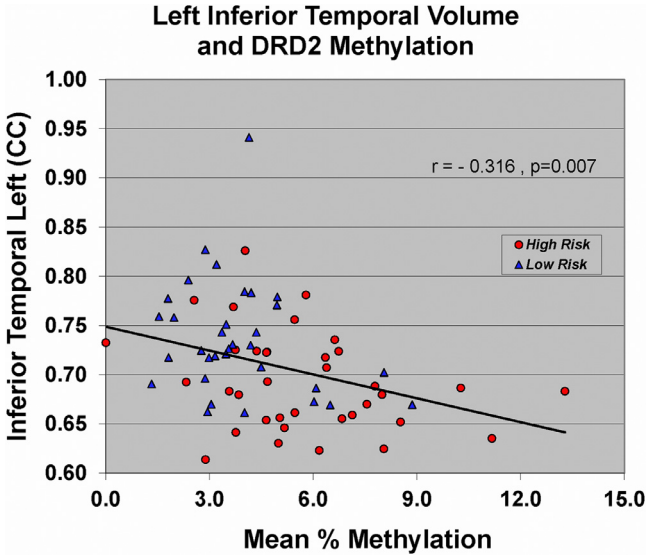

Fig. 6. The relationship between volume of the left inferior temporal region and DRD2 methylation is illustrated in this scatterplot. Increased methylation was significantly associated with decreased volume.

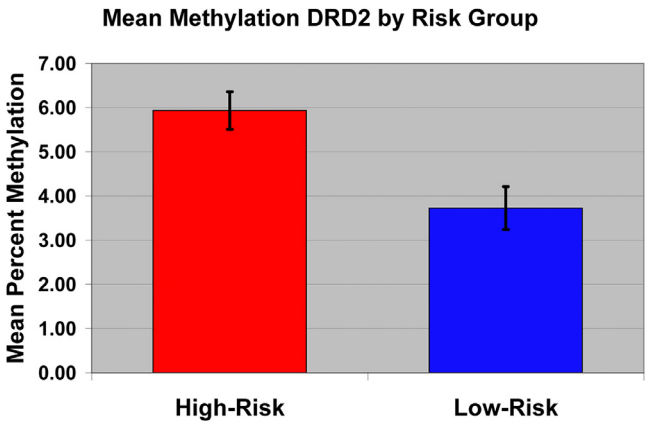

Fig. 7. The relationship between the mean methylation of CpG islands tested and familial risk group is illustrated showing the significant increase in methylation that is associated with membership in a high-risk family.

one or both parents with AD (high-risk). Methylation of CpG sites within the DRD2 gene were significantly increased in high-risk offspring ($p = 0.003$) adjusting for prenatal exposures indicating that those with high-risk familial status had greater DRD2 methylation (Fig. 7). Analysis adjusting for the source of DNA (transformed or not) continued to show a significant familial effect.

3.7. Childhood life events and DRD2 methylation

Longitudinal data collection provided an assessment of total positive life events (PLE) and negative life events (NLE) and data for the Physical Health scales from the LISRES. Data was chosen to be closest in

time to the date of blood collection used for the DNA methylation analysis. The mean age at blood draw for the 69 participants with methylation data and LISRES scores was 12.92 years ± 4.03 years (median = 12 years). The age of the nearest LISRES evaluation was 14.15 ± 3.68 years (median = 13 years). Regression analysis was performed to determine if these measures of environmental conditions were associated with DRD2 methylation in models that included familial risk. The PLE scores were unrelated to the DRD2 methylation values though familial risk status was significant ($\beta = -0.14$ SE = 0.043, $p = 0.002$). NLE scale scores were significantly related to the DRD2 methylation values ($\beta = -0.005$, SE = 0.002, $p = 0.035$). Familial risk was also significant ($\beta = -0.17$, SE = 0.044, $p < 0.001$).

4. Discussion

Significant familial risk group differences were seen with high risk offspring having reduced grey matter volume in the fusiform, insula and inferior temporal regions. Individuals with alcohol use disorders often have interpersonal problems that lead to difficulties in maintaining personal relationships. These difficulties have been linked to deficits in social cognition (Uekermann and Daum, 2008; Valmas et al., 2014). Diminished capacity to recognize emotions in the facial expression of others has been reported in alcohol dependent individuals (Kornreich et al., 2001) along with deficits in face matching (Valmus et al., 2014). Also, youth at high risk for developing substance use disorders show patterns of neural activation that differ from low-risk control offspring when viewing emotional faces and attempting to judge their emotional state (Hill et al., 2007; Hulvershorn et al., 2013) and even when masked faces paradigms are used (Peraza et al., 2015). Preschool age high-risk offspring of alcohol dependent individuals display inhibited behavior in a peer play situation (Hill et al., 1999) that may reflect poorer social cognition and have an underlying neurobiological basis (Hill et al., 2010). Other longitudinal studies have noted that children of alcoholic parents show lesser social competence particularly as young children (Hussong et al., 2005).

Deficits in social cognition seen in alcohol dependent (AD) individuals may be associated with structural variation in brain regions involved in facial recognition and emotional processing. We hypothesized that a decrease in grey matter volume would be seen in high-risk offspring relative to LR controls. Our whole brain analysis revealed differences in regions reported to be involved in facial recognition and social cognition, particularly the prefrontal cortex, insula, fusiform cortices, and the anterior cingulate. Uncovering genetic variation associated with structural variation could provide clues regarding why regional differences in brain volumes may differ between individuals with varying familial risk for alcohol use disorders (Volkow et al., 2006).

Studies addressing the mechanisms involved in these observed differences have largely focused on allelic variation in candidate genes that may have a role in brain morphology. Genes associated with neuroproliferation such as the brain derived neurotrophic factor (BDNF) has achieved a prominent role among these reports (Teh et al., 2012; Hill et al., 2009). Additionally, genome-wide association studies (GWAS) have found statistically significant single nucleotide (SNP) variation associated with brain volume changes in general population samples (Stein et al., 2012). However, the GWAS identified variants often do not overlap with those identified in studies of individuals with psychiatric disorders (Franke et al., 2016), and replicated associations account for no more than 1% of variance (Strike et al., 2015). It has been suggested that GWAS findings for brain morphology in adolescents and adults do not map to those seen in infants because of developmental processes that change the relationship between genes and the brain (Xia et al., 2017). This observation also suggests that epigenetic changes may have a role in the changing relationship between genes and brain morphology. Epigenetic changes due to alteration in histone modification or DNA methylation confer changes in gene expression that can be expected to change the relationship between the gene and brain morphology.

In the present study, we find that hypermethylation of the DRD2 gene appears to contribute to reduction of grey matter in three regions. These regions are of interest with respect to susceptibility to development of addiction because they are specialized for facial affect perception, emotion regulation and social cognition. This reduction in volume may have functional consequences that contribute to deficits in these traits which may, in turn, contribute to the development of SUD. Although only relatively smaller numbers of DRD2 receptors are seen in areas associated with variation in social cognition (insula, fusiform and cingulate) relative to the caudate and putamen (Berneimer et al., 1973), nevertheless, measureable quantities are seen (Hall et al., 1994).

Understanding the factors that are associated with familial risk for alcohol dependence requires a developmental perspective because childhood adversity which has been shown to be associated with brain morphological changes (Tyborowska et al., 2018) may contribute to familial risk group differences. The present results demonstrate that negative life events measured in childhood are associated with changes in D2 methylation in DNA samples obtained in childhood which we find to be associated with volume changes in the fusiform, insula and temporal regions measured in young adulthood.

4.1. Strengths and limitations

One of the principal strengths of present study was the longitudinal nature of the data collection for assessing life stressors and its relationship to DNA methylation. Both of these measures were obtained before the MRI data was obtained so that potential effects of DRD2 methylation on brain volumes could be seen. The young age at which DNA was collected served to insure that any changes seen in methylation were unlikely the result of substance use on part of the individuals who were scanned.

An additional strength is the collection of prenatal exposure data from the mothers of the scanned individuals so that the separate effects of familial/genetic effects could be separated from those associated with prenatal exposures to alcohol, cigarettes and other drugs, an important consideration revealed in an earlier analysis of a larger data set (Sharma and Hill, 2017). Previous work with this sample has shown that women with a familial background of alcohol dependence are significantly more likely to use any substance (alcohol, cigarettes, and or drugs) during their pregnancies (O'Brien and Hill, 2015). Having prenatal information available for analysis allowed for controlling the effect of prenatal use when evaluating the strength of the familial/genetic risk effects.

One limitation of the conclusions that can be drawn from our analyses is that brain volumes were not measured in childhood and related to childhood DRD2 methylation and childhood adversity. Therefore, it is an inference that the volume changes measured in young adulthood reflect a direct effect of volumetric changes occurring in childhood and persisting into young adulthood. However, our results provide the first step in establishing a link between familial risk, childhood adversity, DRD2 methylation and young adult brain volumes. An additional limitation of the data analyzed is the high degree of collinearity between substance use during pregnancy and familial risk status. Although the effects of prenatal use did not reveal significant effects when analyzed as a continuous variable and entered as a covariate in our analyses of familial risk effects, tabulation of the frequencies of use of alcohol, cigarettes and other drugs showed a non-random relationship. An attempt was made to overcome this limitation by analyzing the effect of each exposure (alcohol, cigarettes, and other drugs) within the high risk group. The smaller sample size available to perform this inquiry may have limited our ability to totally separate familial risk from prenatal exposure. These analyses did reveal effects of cigarette smoking on volume of the left fusiform within the high-risk subjects. The analysis showed a significant increase in volume in association with smoking.

Similarly, any drug use during pregnancy within the high risk group was associated with increased volume of the left fusiform. A plausible mechanism for these increases is not readily apparent.

Measurement of DNA methylation in lymphocytes may not reflect changes in brain. Although this may be viewed as a limitation of our study, a report by Davies et al. (2012) has suggested that although methylation varies across tissues, the concordance within individuals is sufficient to recommend the use of peripheral tissues for assessing DNA methylation. Specifically, using post-mortem brain tissue and blood samples obtained pre-mortem, Davies et al. (2012) report correlations of 0.76 and 0.66 (both significant at < 0.001 for cerebellum and cortex, respectively with blood samples. Similarly, using independent samples from buccal scrapings, blood samples and saliva, Cecil et al. (2018) found significant concordance in methylation across the varying tissues, though the same individuals were not assessed.Another potential source of variation in the use of peripheral tissues is variation in methylation status of lymphocytes obtained from fresh versus B lymphocyte transformed cells (Sun et al., 2010). Comparison of peripheral blood cells and transformed B lymphocytes taken from the same 34 individuals assessed at over 27,000 genome-wide methylation sites revealed mean correlations of 0.91 for male and female Caucasian and African-American participants. Because of this potential influence on results, the source of cells (transformed versus non-transformed) was entered into our analyses of familial risk effects on DRD2 methylation as a covariate and found to not detract from the significant effect of familial risk status.

In summary, the present results suggest that a familial diathesis for alcohol dependence confers changes in methylation of the DRD2 gene that have implications for reduction of volumes of the fusiform, insula, and temporal regions in the left hemisphere, structures that are involved in social cognition. Although there is a strong collinearity between prenatal use of substances and familial risk for alcohol dependence, it is unlikely that these would have been responsible for the reduction in volume seen in these regions.

Conflict of interest

The authors declare no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.pscychresns.2019.03.006.

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