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Neuroanatomical markers of familial risk in adolescents with Conduct Disorder and their unaffected relatives

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TITLE PAGE

Neuroanatomical markers of familial risk in adolescents with Conduct Disorder and their unaffected relatives

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Abstract

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Methods: 41 adolescents with CD, 24 unaffected relatives (URs) of CD probands, and 38 healthy controls (aged 12-18), underwent structural magnetic resonance imaging. We performed surface-based morphometry analyses, testing for group differences in cortical volume, thickness, surface area, and folding. We also assessed the volume of key subcortical structures.

Results: The CD and UR groups both displayed structural alterations (lower surface area and folding) in left inferior parietal cortex compared with controls. In contrast, CD participants showed lower insula and pars opercularis volume than controls, and lower surface area and folding in these regions than controls and URs. The URs showed greater folding in rostral anterior cingulate and inferior temporal cortex than controls and greater medial orbitofrontal folding than CD participants. The surface area and volume differences were not significant when controlling for attention-deficit/hyperactivity disorder comorbidity. There were no group differences in subcortical volumes.

Conclusions: These findings suggest that alterations in inferior parietal cortical structure partly mediate the effects of familial risk for CD. These structural changes merit investigation as candidate endophenotypes for CD. Neuroanatomical changes in medial orbitofrontal and anterior cingulate cortex differentiated between URs and the other groups, potentially reflecting neural mechanisms of resilience to CD.

Key words: Conduct Disorder; antisocial behavior; brain structure; surface-based morphometry; endophenotype; family-based designs.

Introduction

Conduct Disorder (CD) is characterized by a repetitive pattern of aggressive and antisocial behavior (American Psychiatric Association, 2000). It results in substantial personal and financial costs for the affected individuals, their families, and society in general (Erskine *et al.,* 2014; Rivenbark *et al.,* 2018), and is the most common reason for referral to Child and Adolescent Mental Health Services in the UK (National Institute for Health and Care Excellence, 2013). CD is also linked to negative adult outcomes, such as mental and physical health problems (Copeland, Wolke, Shanahan, & Costello, 2015; Odgers *et al.*, 2007) and personality disorders (Burt, Donnellan, Iacono, & McGue, 2011; Robins, 1978). It is therefore important to understand its etiology, which may help in developing effective treatments and prevention programs.

There is increasing evidence that CD may have a neurobiological basis, with many studies reporting differences in brain function or structure in children or adolescents with CD or conduct problems compared with typically-developing controls (Alegria, Radua, & Rubia, 2016; Fairchild *et al.*, 2019; Rogers & De Brito, 2016). This research has been extremely valuable in identifying the neuroanatomical and functional *correlates* of CD and callous-unemotional (CU) traits. However, the cross-sectional nature of these studies means it is unclear whether structural or functional abnormalities in regions such as the orbitofrontal cortex or insula precede the disorder or reflect a secondary effect of having CD or lifestyle factors associated with the condition (e.g., substance abuse or sustaining head injuries when fighting). It is possible that these brain abnormalities are caused by the same etiological factors (genetic or environmental risk factors) that led the individual to develop the disorder (Bidwell, Willcutt, Defries, & Pennington, 2007). Relevant to this point, twin studies have shown that brain volume, and particularly volume of the frontal lobe, is highly heritable (Jansen, Mous, White, Posthuma, & Polderman, 2015). It has also been shown that CD and related phenotypes such as criminal behavior and antisocial personality disorder cluster within families. For example, Blazei *et al.* (2008) found a strong resemblance between biological fathers and sons in terms of antisocial behavior – particularly if the father resided in the home. Similarly, Christiansen *et al.* (2008) found that the siblings of those with attention-deficit/hyperactivity disorder (ADHD) *and* conduct problems were 5 times more likely to develop ADHD and conduct problems and 3 times more likely to develop conduct problems than the

siblings of children with ADHD alone. Similar patterns of familial aggregation have been reported in criminological studies – for example, the Cambridge Study in Delinquent Development found that just 6% of the families accounted for 50% of all criminal convictions, and conviction rates were two times higher in the sons of fathers with a history of criminal behavior than the sons of fathers without such a history (Farrington, Barnes & Lambert, 1996).

Although these studies have provided compelling evidence that antisocial behavior clusters within families, far less is known about the brain mechanisms which explain this family resemblance, even though this is an important issue with implications for the development of intervention and prevention programs. One strategy that can be adopted to study the brain mechanisms that may mediate genetic or environmental risk for CD is to study the first-degree relatives of affected probands who do not show the disorder themselves, but may still carry markers of familial risk. For example, Ersche *et al.* (2012) employed this strategy to investigate whether brain abnormalities are associated with familial risk for substance dependence or reflect the neurotoxic effects of prolonged drug use. Substance dependent individuals and their unaffected siblings were found to display common neuroanatomical abnormalities in brain regions involved in inhibitory control, suggesting that they are a risk factor for substance dependence, rather than reflecting the secondary consequences of drug use (i.e., drug-induced damage). A similar study investigating familial risk markers for autism found common reductions in activation in brain regions involved in biological motion perception and social cognition (e.g., the superior temporal sulcus) in children with autism and their unaffected relatives (URs), relative to controls (Kaiser *et al.*, 2010).

Applying this logic to CD, if similar alterations in brain structure are observed in adolescents with CD *and* their URs, this would indicate that neuroanatomical abnormalities and CD co-segregate within families (are inherited together) and that such structural changes may mediate the effects of genetic risk for CD. Studying unaffected first-degree relatives, as well as affected probands with CD, would therefore help us to address the question of whether neuroanatomical changes increase risk for developing CD or reflect the secondary consequences of having CD and associated lifestyle factors. A further advantage of studying URs, who 'beat the odds' by remaining free from severe antisocial behavior despite being at increased risk, is that protective or compensatory brain changes might be

observed in this group which counteract the effects of familial risk. Relevant to this point, Kaiser *et al.* (2010) identified potential compensatory effects in the URs of ASD probands. They showed greater ventromedial prefrontal cortex activity than the autistic or typically-developing control groups when viewing point-light displays of biological motion.

Most existing studies investigating brain structure in CD have employed voxel-based morphometry (VBM) methods which test for differences in gray matter volume across the whole brain. However, using this composite, intensity-based measure is problematic because volume in a given region is a function of its cortical thickness and surface area, as well as cortical folding, which show distinct genetic etiologies (Panizzon *et al.*, 2009), developmental trajectories (Raznahan *et al.*, 2011), and underlying cellular mechanisms (Rakic, 2009).

Accordingly, we compared adolescents with CD and their URs using surface-based morphometry (SBM), to examine whether these groups show common or distinct abnormalities in cortical structure compared with typically-developing adolescents. On the basis of previous SBM findings (Fairchild *et al.*, 2015; Hyatt, Haney-Caron, & Stevens, 2012; Smaragdi *et al.*, 2017; Wallace *et al.*, 2014), we predicted that adolescents with CD would show structural alterations in the insula, orbitofrontal cortex, superior temporal gyrus, and inferior parietal cortex compared with typically-developing adolescents. We also hypothesized that the URs of CD probands would show similar structural abnormalities, albeit possibly at an intermediate level, as their loading of genetic or environmental risk may be lower than the probands. Consistent with this, a recent study found that ADHD probands and their unaffected siblings both showed lower orbitofrontal cortex volume (Bralten *et al.*, 2016). Given prior evidence that ADHD comorbidity may be important in determining the extent of structural changes observed in CD (Fairchild *et al.*, 2015; Smaragdi *et al.*, 2017), we controlled for ADHD symptoms in a supplementary analysis. Although exploratory in nature, we also tested for potential 'compensatory' or 'protective' structural changes in the URs. However, due to the lack of previous studies, we had no *a priori* predictions regarding the loci and direction of such effects. Lastly, based on evidence of subcortical alterations in youths with CD (Rogers & De Brito, 2016; Wallace *et al.*, 2014), we tested for group effects on subcortical volumes. We predicted that the CD group would

show lower amygdala volume compared to the controls, and similar reductions might be observed in URs.

Methods

Participants

Healthy control participants (n=41) were recruited from mainstream schools and colleges, whereas participants with CD (n=43) were mainly recruited from specialist schools, pupil referral units and Youth Offending Services in the Hampshire area. The URs (n=24) were recruited directly from the families of the CD participants, as well as the aforementioned recruitment sources. Participants were aged between 12-18 years. All parents/carers completed a Family History Screen, consisting of three questions assessing current and lifetime psychopathology, behavioral problems, and criminal convictions, in the participants' first-degree relatives. This screen was designed to identify siblings of adolescents with CD who did not meet the diagnostic criteria for CD themselves. In addition, it enabled us to identify the unaffected offspring of parents who had previously displayed CD and ensure that the controls had no family history of CD.

Diagnostic and questionnaire measures

The Kiddie-Schedule of Affective Disorders and Schizophrenia-Present and Lifetime version (K-SADS-PL; Kaufman *et al.*, 1997), a semi-structured diagnostic interview based on DSM-IV-TR criteria (APA, 2000), was used to screen for CD and other common psychiatric disorders. The participants and their parents/carers were interviewed separately to ensure confidentiality, and the information from each interview was combined such that a symptom was considered present if it was endorsed by either informant (Kaufman *et al.*, 1997). Even if the initial screen items for CD and ADHD were not endorsed, the full supplements for these disorders were always completed to obtain dimensional information on these disorders for all participants.

The self-report version of the Inventory of Callous-Unemotional traits (ICU; Essau, Sasagawa & Frick, 2006) was used to assess callous-unemotional traits. It contains 24 items scored on a 0-3 scale, from 'not at all true' to 'definitely true' (Cronbach's Alpha in present sample=0.82). Factor

analysis has revealed that the ICU captures three distinct dimensions of behavior termed callousness, uncaring, and unemotional (Essau *et al.*, 2006), therefore scores for these subscales are also reported. The self-report Youth Psychopathic traits Inventory (Andershed, Kerr, Stattin, & Levander, 2002) was used to measure psychopathic personality traits. It contains 50 items, each scored on a 1-4 point scale, from 'does not apply at all' to 'applies very well' (Cronbach's Alpha in present sample=0.93), and as well as total scores, it can be divided into Grandiose-Manipulative, Callous-Unemotional, and Impulsive-Irresponsible subscales corresponding to the three-facet model of psychopathy (Andershed *et al.*, 2002). Further information about these questionnaires' psychometric properties and their factor structures can be found in Supplementary Materials. The Wechsler Abbreviated Scale of Intelligence was used to estimate full-scale IQ (Wechsler, 1999). Lastly, the Edinburgh Handedness Inventory (Oldfield, 1971) was used to assess handedness.

Ethical Approval

The study was approved by the University of Southampton Ethics Committee, the University Hospital Southampton NHS Trust, Southampton City Council Children's Services and the Hampshire County Council Research and Evaluation Unit. Participants aged ≥16 provided written informed consent, whereas parents provided informed consent and participants provided assent if below age 16.

Procedure

Once they had been screened for psychiatric disorders and standard MRI exclusion criteria, such as claustrophobia, participants were invited to the Southampton General Hospital for a magnetic resonance imaging (MRI) scan lasting 35-40 minutes. The structural (T1-weighted) scan was the first sequence performed during the scanning session, and was repeated as needed until usable data, uncontaminated by movement, had been collected. This was determined by a trained radiographer.

Data Acquisition

Structural MRI data were acquired using a 1.5-Tesla Siemens Avanto scanner (Siemens Medical Solutions, Erlangen, Germany). We acquired T1-weighted three-dimensional MPRAGE images

(voxel size=1.2x1.2x1.2 mm, repetition time=2400ms, echo time=3.62ms, flip angle=8°, 160 slices). Total scanning time was 7 minutes, 41 seconds. After scanning, the structural images were reviewed by a consultant neuroradiologist to screen for neurological abnormalities. We initially included 108 participants, but five participants (3 controls; 2 CD) were excluded due to having cysts or tumors, leaving 103 participants with usable MRI data.

Surface-based morphometry analyses: cortical volume, thickness, surface area (SA) and local gyrification index (*l*GI) and subcortical volumes

MRI-based quantification of cortical volume, thickness, SA and folding (quantified using *l*GI) was performed using FreeSurfer 5.3.0 (http://surfer.nmr.mgh.harvard.edu). This method has been described in detail (Fischl, 2012). Briefly, the procedure involves segmentation of white matter, tessellation of the gray-white matter junction to construct representations of the gray/white matter boundary and cortical surface. Each participant's cortex was then visually inspected and, if necessary, manually edited by one of the authors (N.T.), blind to group status. This involved: (i) realignment of each subject's image to the Montreal Neurological Institute template; (ii) setting intensity normalization control points where brain matter was erroneously skull-stripped; and (iii) adjustment of the watershed parameters of the skull strip. From this reconstruction, vertex-wise estimates of both cortical thickness and cortical area were obtained. lGI, which measures the amount of cortical folding within versus outside the sulcus, was calculated using the method outlined by Schaer *et al.* (2012). In order to map the participants' brains to a common space, reconstructed surfaces were registered to an average cortical surface atlas using a nonlinear procedure that optimally aligns sulcal and gyral features across individuals (Fischl *et al.*, 1999a, b). Finally, we estimated amygdala, hippocampus, caudate, pallidum, putamen, thalamus, and nucleus accumbens volumes using FreeSurfer's automatic segmentation pipeline (Fischl *et al.*, 2002).

Statistical Analyses

We tested for group differences in demographic and clinical variables using analyses of variance, with independent *t*-tests used to follow up significant *F* tests; Chi-Square tests were used for group comparisons of nominal variables (e.g., sex).

For each hemisphere, group differences in cortical volume, thickness, surface area and lGI at each vertex were tested using a general linear model (GLM) with age, sex, IQ and total intracranial volume (TIV) orthogonalized to sex included as covariates of no interest. We also repeated the analyses including lifetime ADHD symptoms as a further covariate (these results are reported in **Supplementary Table 1**, available on-line). Given previous evidence suggesting that childhood-onset (CO-CD) and adolescence-onset (AO-CD) variants of CD may differ quantitatively in brain structure or function (Fairchild *et al.*, 2013), we initially ran analyses comparing these subgroups (i.e., CO-CD>AO-CD, AO-CD>CO-CD). As there were no significant differences between subgroups, they were treated as a combined group in the comparisons with URs and HCs.

After applying a vertex-wise/cluster-forming threshold of p=0.05, the level of statistical significance was subject to a further cluster-wise P (CWP) value correction procedure for multiple comparisons based on a Monte Carlo z-field simulation (Hagler *et al.*, 2006). Clusters are only reported if they met a whole-brain corrected threshold of CWP≤0.05.

Lastly, we tested for group differences in subcortical volumes using one-way analyses of covariance with age, sex, IQ and TIV orthogonalized to sex included as covariates of no interest, whilst applying a False-Discovery-Rate (FDR) correction for multiple comparisons at *q*=.05. Significant group effects were followed up with pairwise *t*-tests. These analyses were repeated including lifetime ADHD symptoms as a covariate.

Results

Sample characteristics

Demographic and clinical characteristics of the sample are reported in Table 1. The groups differed in age (p=0.01), with the URs being around a year younger than the other groups. The groups also

differed in estimated IQ ($p<0.001$), with the CD group having the lowest average IQ and the control group having the highest. However, the groups were matched in sex and handedness. As expected, the CD group reported higher rates of CD symptoms, ADHD symptoms, CU traits, and psychopathic traits than the other groups (*ps*<0.001). Critically, the HCs and URs did not differ on any clinical or personality variable, confirming the 'unaffected' nature of the latter group.

[INSERT TABLE 1 HERE]

Surface-based morphometry results: Potential markers of familial risk for antisocial behavior

Relative to controls, the CD group showed lower left inferior parietal cortex surface area, whereas the URs showed lower cortical folding in this region (Figure 1; Table 2). When controlling for comorbid ADHD symptoms, both the CD and UR groups showed lower left inferior parietal cortical folding (Supplementary Table 1).

[INSERT FIGURE 1 HERE]

Effects related to CD but not observed in unaffected relatives – non-familial risk

In contrast, the CD group showed lower volume in left insula and right pars triangularis extending to right insula compared with controls, and lower surface area in left insula and right pars triangularis/insula compared with both the controls and URs (Figure 2; Table 2).

Further structural differences between the CD and control groups that were not observed in the URs included lower bilateral pericalcarine, left pars opercularis and right precentral gyrus surface area in the CD group (Table 2). In addition, the CD group showed greater cortical thickness in left superior frontal gyrus and superior temporal cortex and right frontal pole compared with the URs (Table 2).

[INSERT TABLE 2 AND FIGURE 2 HERE]

Potential compensatory effects in the unaffected relatives

There were also several effects *unique* to the URs – they showed increased folding in rostral anterior cingulate cortex compared with controls and increased medial orbitofrontal cortex folding compared

with the CD group (Figure 3; Table 2). The URs also showed greater folding in lingual gyrus and inferior temporal cortex compared with controls, and greater folding in bilateral pars triangularis/insula and left superior frontal gyrus compared with the CD group.

[INSERT FIGURE 3 HERE]

Impact of adjusting for comorbid ADHD

When including ADHD symptoms as a covariate, the similarities between the CD and UR groups were amplified – as mentioned above, both groups showed lower left inferior parietal folding compared with controls (Supplementary Table 1). There were also differences in cortical thickness which appeared specific to the CD group – they showed greater medial orbitofrontal cortical thickness compared to controls and greater superior frontal cortical thickness compared to URs (Supplementary Table 1). However, some of the CD-control differences were rendered non-significant, such as differences in left insula and right pars opercularis volume (HC>CD). In addition, the group differences in surface area (e.g., lower insula and pars triangularis surface area in CD) were not significant when adjusting for ADHD symptoms. As might be expected given the low level of ADHD symptoms in URs, differences between this group and the controls remained significant when adjusting for ADHD – including increased rostral anterior cingulate folding.

Subcortical volumes

There were no group differences in amygdala, hippocampus, thalamus, caudate, pallidum, putamen or nucleus accumbens volumes when controlling for multiple comparisons. There was, however, a trend towards a group effect in left thalamus (p=.088, uncorrected), which became nominally significant when IQ was not included as a covariate. Post-hoc tests revealed lower left thalamus volume in CD versus HC participants (p=.028, Hedges's g=-0.50), but no other pairwise differences. See Supplementary Materials for more information.

Discussion

In the present study, we used surface-based morphometry and subcortical segmentation methods to investigate whether probands with Conduct Disorder (CD) and their unaffected relatives (URs) show similar or distinct changes in brain structure compared with typically-developing adolescents, and identify potential protective or compensatory alterations in the URs. The observation of common structural alterations in CD probands and URs would indicate that CD and related neuroanatomical changes co-segregate within families, suggesting the latter may partly mediate the effects of genetic or environmental risk for CD. We also assessed four different measures of cortical structure – cortical volume, thickness, surface area, and folding – and subcortical volumes to provide greater specificity regarding the structural changes associated with CD and its familial risk.

Our first key finding was that similar alterations in left inferior parietal cortical structure were observed in individuals with CD and their URs. The CD participants showed lower surface area in left inferior parietal cortex, whereas the URs showed lower folding in this region, compared with controls. When controlling for comorbid ADHD, both groups showed lower left inferior parietal folding compared with controls. These findings provide the first available evidence that reductions in inferior parietal surface area and folding might constitute a neuroanatomical endophenotype for CD which is present in CD probands *and* their URs. This suggests that alterations in inferior parietal cortical structure may partly mediate the effects of familial risk for CD. Previous SBM studies have reported structural abnormalities in the inferior parietal cortex, or adjacent areas such as supramarginal gyrus, in CD (Fahim *et al.*, 2011; Jiang *et al.*, 2015; Smaragdi *et al.*, 2017; Wallace *et al.*, 2014). The left inferior parietal cortex is implicated in language comprehension, theory of mind, action observation (Molenberghs, Cunnington, & Mattingley, 2012), and perhaps most intriguingly, facial emotion recognition (Zhang, Song, Liu, & Liu, 2016). The latter function appears relevant to our earlier finding that CD probands and their URs show similar deficits in facial emotion recognition (Sully, Sonuga-Barke, & Fairchild, 2015).

Our second key finding was that the volume of the insula and surrounding frontal lobe structures such as the pars opercularis was lower in CD compared with control participants.

Critically, these changes in volume appeared to be driven by reductions in insula and pars opercularis surface area, rather than cortical thickness, in the CD group. The CD group also showed lower insula and pars triangularis surface area compared to URs. To our knowledge, although several VBM studies have reported lower insula gray matter volume in CD (Fairchild et al., 2013; Fairchild et al., 2011; Sterzer, Stadler, Poustka, & Kleinschmidt, 2007) and the insula was identified in a recent meta-analysis of VBM studies (Rogers & De Brito, 2016), this is the first study to examine the underlying basis of such volumetric changes. The finding that reductions in surface area, rather than cortical thickness, drive reductions in insula volume observed in CD is consistent with data from normative studies showing that surface area is more strongly related to volume than cortical thickness (Im et al., 2008). The anterior insula is considered to play a critical role in processing emotional (especially negative) stimuli (Calder, Keane, Manes, Antoun, & Young, 2000), empathy (Singer et al., 2004), and awareness of one's own physiological and emotional states (Craig, 2009). Consequently, structural deficits in the insula might explain why adolescents with CD show deficits in empathy (Martin-Key, Brown, & Fairchild, 2017) and learning from punishment (Kohls et al., 2020), and reduced sensitivity to losses when making decisions (Fairchild et al., 2009). On the other hand, the fact that reductions in insula volume and surface area were not observed in the URs challenges the idea that they mediate the effects of familial risk for CD. Of interest, we found that adolescents with CD, but not their URs, showed heightened risk-taking in a gambling task (Sully, Sonuga-Barke, Savage, & Fairchild, 2016). It should also be noted that these insula volume and surface area differences were rendered non-significant when adjusting for comorbid ADHD symptoms, suggesting that they are not related to CD specifically or are more pronounced in participants with comorbid CD+ADHD.

We also found greater medial orbitofrontal cortical thickness in the CD group compared with controls, although only when adjusting for comorbid ADHD, whereas cortical thickness in the superior frontal gyrus and frontal pole was increased in the CD group compared with the URs. The medial orbitofrontal cortex is involved in representing the reward value of stimuli (Liu, Hairston, Schrier, & Fan, 2011) and social cognitive processes (Molenberghs, Johnson, Henry, & Mattingley, 2016). The frontal pole is implicated in executive functions – especially tasks in which multiple cognitive processes must be monitored simultaneously (Mansouri, Koechlin, Rosa, & Buckley, 2017).

Previous studies have reported lower cortical thickness (Fahim *et al.*, 2011; Jiang *et al.*, 2015; Smaragdi *et al.*, 2017), lower surface area (Fairchild *et al.*, 2015; Sarkar *et al.*, 2015), and atypical folding in the orbitofrontal cortex in CD (Hyatt *et al.*, 2012; Wallace *et al.*, 2014). fMRI studies have also observed atypical medial orbitofrontal cortex activation in adolescents with CD during facial emotion processing (Fairchild *et al.*, 2014; Passamonti *et al.*, 2010).

Although we did not observe reduced superior temporal gyrus cortical thickness in the CD group, contrary to the findings of our previous study (Fairchild *et al.*, 2015) and earlier results obtained in younger children or individuals with non-comorbid CD (Fahim *et al.*, 2011; Hyatt *et al.*, 2012; Wallace *et al.*, 2014), left superior temporal gyrus folding was reduced in CD participants compared with controls. This is in line with previous VBM studies reporting lower superior temporal gray matter volume in adolescents with CD (Rogers & De Brito, 2016), and adults with antisocial personality disorder and psychopathy (de Oliveira-Souza *et al.*, 2008; Muller *et al.*, 2008). As with the insula results, these findings further implicate the superior temporal gyrus in the pathophysiology of CD but challenge the idea that structural changes in this region fall on the causal pathway between familial (genetic or environmental) risk and CD. The superior temporal gyrus is implicated in social cognition, including facial emotion processing, as well as auditory and vocal perception and language comprehension (Redcay, 2008). Of note, a range of social cognitive deficits have been reported in CD, such as impairments in facial and vocal emotion recognition (Blair, Budhani, Colledge, & Scott, 2005; Fairchild, Van Goozen, Calder, Stollery, & Goodyer, 2009), empathic accuracy and affective empathy (Martin-Key *et al.*, 2017, 2020; Schwenck *et al.*, 2012), and social competence in real-life situations (Oliver, Barker, Mandy, Skuse, & Maughan, 2011).

Strengths and limitations

To our knowledge, this is the first neuroimaging study to investigate familial risk markers for antisocial behavior by assessing cortical structure in CD probands and their URs. The use of SBM methods enabled us to disaggregate the cortical properties that give rise to volume and demonstrate that reductions in insula and pars opercularis/triangularis volume were driven by changes in *surface area*. We also assessed the volume of key subcortical structures such as the amygdala. Another

strength of the study was that the URs were truly free of CD – most had no CD symptoms – rather than being elevated in CD symptoms, but not quite meeting the diagnostic criteria. The URs were also relatively free of psychopathology in general and did not differ from controls in personality traits linked to CD, such as callous-unemotional traits. Lastly, our sample was well-characterized from a psychiatric perspective, with data collected from multiple informants (in most cases, participants *and* parents/carers). The participants were also screened carefully for comorbid disorders such as ADHD, and we systematically examined the impact of ADHD comorbidity.

In terms of limitations, it was not optimal that the groups contained a mixture of males and females (although males were over-represented), as the relationship between CD and brain structure may partly differ by sex (Fairchild *et al.*, 2013; Smaragdi *et al.*, 2017). This was almost unavoidable, given the scale of the study and the fact that URs were more likely to be female, although the groups did not differ in sex. Critically, we controlled for sex, age, IQ and total intracranial volume, in our analyses. However, future studies should recruit enough males and females in each group to examine whether similar findings are obtained when analysing data from males and females separately. Stronger familial effects may be observed in the unaffected siblings of female, versus male, probands, as girls might require a higher loading of genetic risk to develop CD (Meier, Slutske, Heath, & Martin, 2011). It should be noted that some of the group differences, particularly those obtained for volume and surface area, were rendered non-significant when controlling for ADHD (e.g., insula), whereas others were only significant when adjusting for ADHD (e.g., differences in inferior parietal folding in CD). This is consistent with previous SBM studies which found that controlling for ADHD symptoms attenuated some of the group differences in cortical structure – particularly for surface area (Smaragdi *et al.*, 2017) – whereas other CD-related effects were only present *when* adjusting for ADHD symptoms (Fairchild *et al.*, 2015). Nevertheless, we note that our unadjusted findings are probably more representative of clinical reality, given that ADHD comorbidity is common in CD (Angold, Costello & Erkanli, 1999) and there is significant genetic overlap between ADHD and CD/ODD (Tuvblad *et al.*, 2009), so controlling for ADHD symptoms might be 'overcorrecting'. Finally, the UR group included both the unaffected siblings of the CD probands who were included in the study and the siblings of CD probands who were unwilling or ineligible to participate in the study

(e.g., due to being too old or incarcerated). It has been argued that investigating URs who are related to the included CD participants and those that are not offers advantages in terms of identifying markers that are specifically related to psychiatric disorders (Kaiser *et al.*, 2010), rather than simply identifying heritable aspects of brain structure, but this also restricted the analyses that we could perform. One benefit of recruiting a large number of CD proband-unaffected sibling pairs would be to investigate whether abnormalities in cortical structure (e.g., in inferior parietal cortex) are shared by both family members. Future studies could also examine whether neuroanatomical abnormalities are transmitted inter-generationally from parents to children and whether this predicts risk for CD (Thissen *et al.*, 2014), consistent with the hypothesis that brain structure abnormalities mediate familial risk for CD.

Conclusions

In the first study to investigate whether neuroanatomical abnormalities associated with CD co-segregate within families, we found evidence that reductions in inferior parietal cortex surface area and folding may be familial risk markers for CD, as these were present in both affected probands and their unaffected relatives. These alterations in inferior parietal cortical structure merit further investigation as candidate endophenotypes for CD. Conversely, we identified neuroanatomical abnormalities that were specific to the CD group, such as lower insula and pars opercularis/ triangularis volume and surface area. Although this suggests that alterations in insula structure play an important role in the development of CD, such that they distinguish between affected and unaffected members of the same families, they also challenge the idea that such structural alterations mediate the effects of familial risk for CD. We also observed increased folding in the rostral anterior cingulate, medial orbitofrontal and inferior temporal cortices in the unaffected relatives compared with the CD and control groups, which may reflect compensatory or protective effects.

Figure Legends:

Figure 1. Neuroanatomical markers of familial risk for Conduct Disorder that were observed in both the CD probands and the unaffected relatives compared with controls. Panel A. Left inferior parietal cortical surface area was lower in participants with Conduct Disorder (CD) compared with healthy controls (HC). B. Left inferior parietal cortical folding was lower in unaffected relatives (UR) than healthy controls. C. Left inferior parietal cortical folding was reduced in participants with CD compared with healthy controls when adjusting for comorbid ADHD symptoms. D. Left inferior parietal cortical folding was lower in unaffected relatives than healthy controls when adjusting for comorbid ADHD symptoms.

Figure 2. Cortical structure alterations observed in the Conduct Disorder group compared to the healthy controls and unaffected relatives, reflecting non-familial risk. Panel A. Right pars triangularis surface area (extending to insula) was lower in participants with Conduct Disorder (CD) compared with healthy controls (HC). B. Right pars triangularis surface area (extending to insula) was lower in participants with CD compared with the unaffected relatives (UR). C. Left pars triangularis cortical folding (extending to insula) was lower in participants with CD compared with the unaffected relatives. D. Right pars triangularis cortical folding (extending to insula) was lower in participants with CD compared with the unaffected relatives.

Figure 3: Potential protective or compensatory structural changes observed in the unaffected relatives compared with the healthy control and Conduct Disorder groups. Panel A. Medial orbitofrontal cortical folding was higher in the unaffected relatives (UR) compared with the Conduct Disorder (CD) group. B. Rostral anterior cingulate cortical folding was higher in the unaffected relatives compared with the healthy controls (HC).

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Figure 1: Effects observed in probands with CD and unaffected relatives – reflecting putative *familial risk markers*

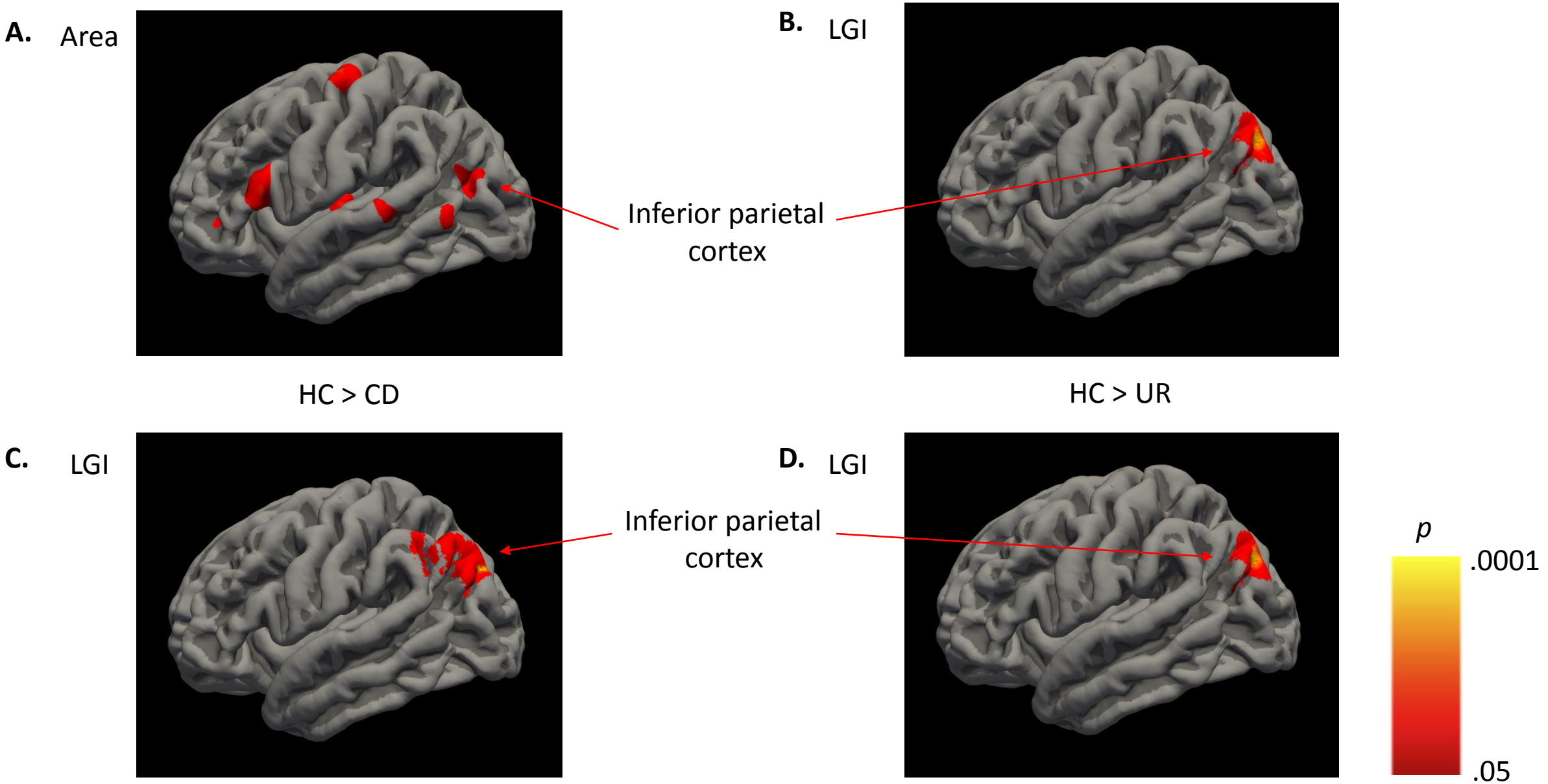


Figure 2: Effects observed in the CD group only – reflecting *non-familial risk*

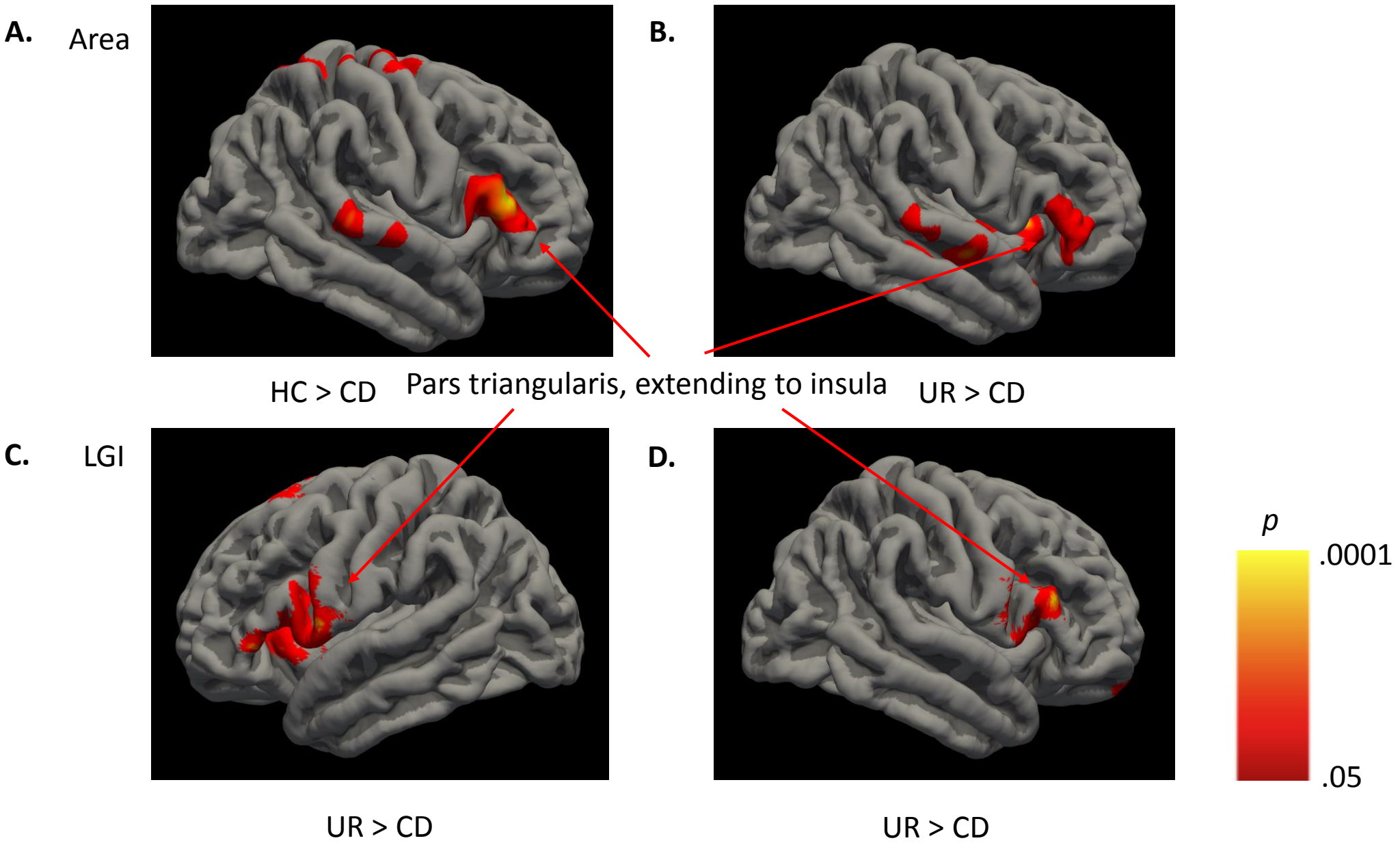


Figure 3: Protective or compensatory effects observed in the unaffected relatives in the prefrontal cortex

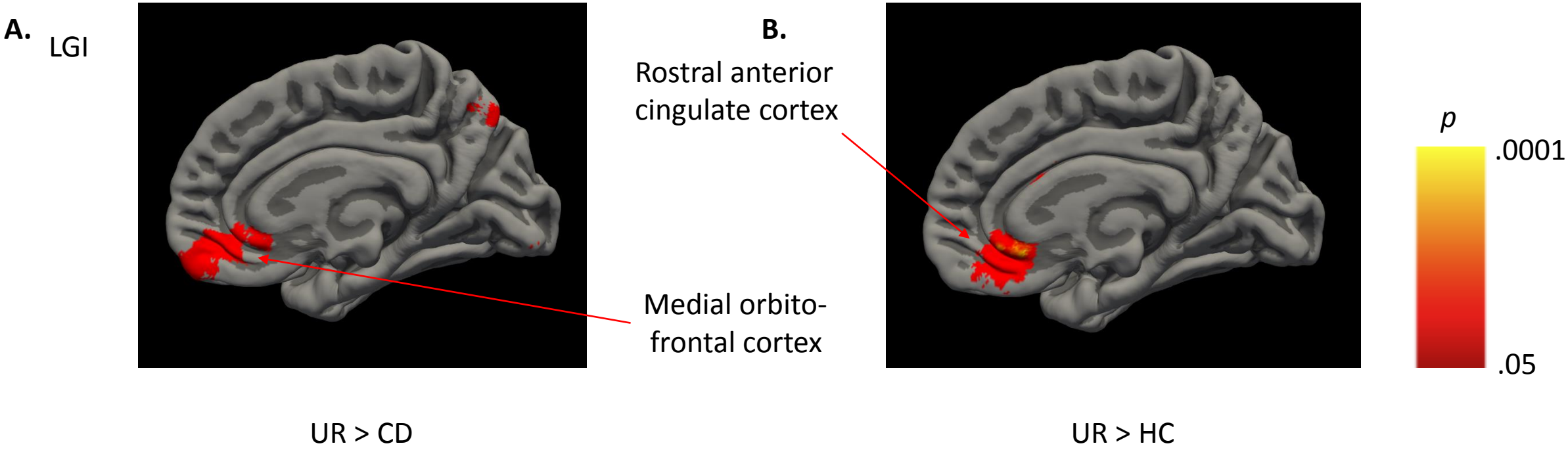


Table 1. *Demographic and clinical characteristics of the sample*

	HC (*n* = 38)	UR (*n* = 24)	CD (*n* = 41)	*p* value	post-hoc
Age (years)	16.51 (1.31)	15.64 (1.49)	16.66 (1.37)	.014	UR < CD
Gender (M:F)	32:6	17:7	37:4	.125	
Handedness (R:L:A)[a]	29:5:3	17:1:6	31:5:5	.336	
IQ	103.79 (9.76)	98.54 (8.78)	93.27 (19.90)	< .001	CD < HC
CD Symptoms	0.21 (0.53)	0.38 (0.58)	8.27 (2.35)	< .001	HC, UR < CD
ADHD Symptoms	0.71 (1.47)	1.46 (2.02)	7.17 (4.31)	< .001	HC, UR < CD
ICU Total[b]	22.46 (7.89)	22.33 (7.66)	31.80 (8.02)	< .001	HC, UR < CD
ICU Unemotional[b]	7.68 (2.60)	7.25 (2.59)	9.17 (2.95)	.012	UR < CD
ICU Callous[b]	6.30 (3.45)	6.96 (3.74)	11.80 (5.12)	<.001	HC, UR < CD
ICU Uncaring[b]	8.49 (4.12)	8.12 (3.78)	11.07 (4.25)	.005	HC, UR < CD
YPI Total[c]	100.8 (16.3)	99.9 (17.6)	121.0 (21.4)	< .001	HC, UR < CD
YPI Callous-Unemotional[d]	29.9 (6.3)	31.8 (6.6)	36.3 (7.3)	<.001	HC, UR < CD
YPI Grandiose-Manipulative[d]	37.4 (7.9)	33.7 (9.8)	40.8 (11.1)	.021	UR < CD
YPI Impulsive-Irresponsible[d]	33.5 (7.0)	34.3 (5.6)	44.7 (7.4)	<.001	HC, UR < CD
Childhood-onset (*n*)			23		
Adolescence-onset (*n*)			18		

Note. Means are presented with standard deviations in parentheses. Group differences were assessed with one-way ANOVA *F*-tests and pairwise Bonferroni-adjusted *t*-tests (continuous variables) and Chi-Squared tests (categorical variables). Key: HC = healthy controls; UR = unaffected relatives; CD = conduct disorder; M = male; F = female; R = right-handed; L = left-handed; A = ambidextrous; IQ = intelligence quotient; ADHD = attention-deficit/hyperactivity disorder; ICU = Inventory of Callous-Unemotional traits (self-report version); YPI = Youth Psychopathic traits Inventory. Participants who developed symptoms of CD before age 10 were classified as having 'childhood-onset' CD, whereas those who only displayed symptoms of CD after age 10 were classified as having 'adolescence-onset' CD.
[a] $n_{missing}$ = 1 (CD group)
[b] $n_{missing}$ = 1 (HC group)
[c] $n_{missing}$ = 2 (HC group)
[d] $n_{missing}$ = 3 (2 HCs, 1 CD)

Table 2. Cortical volume, thickness, surface area and gyrification differences between the Conduct Disorder, unaffected relative and healthy control groups, when *not* including lifetime ADHD symptoms as a covariate.

Group comparison	Brain region	Hemisphere	NVtxs	Size (mm^2)	X	Y	Z	Max	CWP
Cortical Volume									
HC > CD	Insula	L	1927	1216.2	-34	6	10	4.2	<0.001
	Pars opercularis	R	1305	631.6	38	11	8	2.5	0.046
Cortical Thickness									
CD > UR	Superior frontal cortex	L	781	614.1	-14	1	72	4.1	0.021
	Superior temporal gyrus	L	1060	579.1	-49	-26	3	3.5	0.029
	Frontal pole	R	516	640.4	16	62	-18	2.9	0.018
Cortical Surface Area									
HC > CD	Pars opercularis	L	1212	1039.7	-53	20	8	3.1	0.010
	Inferior parietal cortex	L	1305	849.9	-45	-71	12	2.7	0.037
	Pericalcarine	L	915	950.0	-14	-98	-16	1.8	0.018
	Pars triangularis	R	3215	2268.4	51	30	12	3.5	<0.001
	Precentral gyrus	R	2493	1143.4	30	-12	56	3.1	0.014
	Pericalcarine	R	1683	1737.8	16	-95	-3	2.3	0.001
UR > CD	Insula	L	2483	1155.5	-35	5	8	3.6	0.005
	Pars triangularis	R	1340	1213.1	43	42	0	2.2	0.009
Local Gyrification Index									
HC > CD	Superior temporal gyrus	L	1352	863.5	-45	-1	-17	3.0	0.015
	Lateral occipital cortex	L	1840	1390.9	-19	-93	17	2.3	<0.001
	Pars opercularis	L	2039	1133.1	-51	8	-3	2.1	0.002
	Postcentral gyrus	R	2022	954.1	19	-32	75	3.7	0.010
CD > HC	Fusiform gyrus	L	1527	940.2	-41	-38	-26	2.1	0.009
	Pars orbitalis	R	1006	927.5	47	42	-9	2.9	0.011
	Parahippocampal gyrus	R	1710	1064.6	19	-30	-16	1.8	0.005
HC > UR	Inferior parietal cortex	L	1459	1152.0	-39	-79	33	2.2	0.002
UR > HC	Inferior temporal cortex	L	1206	962.2	-43	-16	-39	2.5	0.008
	Lingual gyrus	L	694	719.7	-5	-81	-5	2.4	0.047
	Rostral anterior cingulate	R	1906	1369.1	2	25	-9	3.0	<0.001
UR > CD	Pars triangularis	L	4154	2361.9	-46	26	3	2.4	<0.001

	Superior frontal cortex	L	1219	946.4	-5	8	69	2.0	0.009
	Pars triangularis	R	3336	2139.1	56	27	17	3.2	<0.001
	Medial orbitofrontal cortex	R	2338	2033.0	6	49	-7	2.2	<0.001

Key: ADHD, attention-deficit/hyperactivity disorder; CD, Conduct Disorder; CWP, cluster-wise-P value; HC, healthy control; L, left; NVtxs, number of vertices; Max, maximum -log10(p value) in the cluster; R, right; UR, unaffected relatives. Note: Only significant pairwise comparisons between the groups are reported.

Supplementary Materials to Fairchild et al. ***Neuroanatomical markers of familial risk in adolescents with Conduct Disorder and their unaffected relatives***

Supplementary Table 1. Cortical structure differences between the Conduct Disorder, unaffected relative and healthy control groups, *when including lifetime ADHD symptoms as a covariate of no interest*.

Group comparison	Brain region	Hemisphere	NVtxs	Size (mm^2)	X	Y	Z	Max	CWP
Cortical Volume									
No significant differences between groups									
Cortical Thickness									
CD > HC	Medial orbitofrontal cortex	L	644	553.2	-2	27	-26	2.1	0.037
CD > UR	Superior frontal gyrus	L	852	682.0	-13	1	72	3.3	0.029
Cortical Surface Area									
No significant differences between groups									
Local Gyrification Index									
HC > CD	Inferior parietal cortex	L	2190	1586.5	-39	-80	32	2.1	<0.001
CD > HC	Entorhinal cortex	L	2120	1330.1	-21	-7	-27	1.8	<0.001
	Parahippocampal gyrus	R	4135	2981.6	19	-20	-24	2.9	<0.001
HC > UR	Inferior parietal cortex	L	1497	1184.8	-39	-79	33	2.3	0.001
UR > HC	Inferior temporal cortex	L	1161	962.2	-43	-16	-39	2.4	0.010
	Rostral anterior cingulate	R	2107	1552.9	2	25	-9	3.0	<0.001
UR > CD	Superior frontal cortex	L	1563	1227.7	-13	28	55	2.5	0.001
	Inferior parietal cortex	L	1276	807.4	-46	-59	44	1.7	0.023
CD > UR	Entorhinal cortex	R	2149	1538.3	24	-18	-21	2.0	<0.001

Key: ADHD, attention-deficit/hyperactivity disorder; CD, Conduct Disorder; CWP, cluster-wise-P value; HC, healthy control; L, left; NVtxs, number of vertices; Max, maximum -log10(p value) in the cluster; R, right; UR, unaffected relatives. Note: Only significant pairwise comparisons between the groups are reported.

Factor structure and psychometric properties of the Inventory of Callous-Unemotional traits (ICU) and Youth Psychopathic traits Inventory (YPI)

Factor structure of the ICU:

The self-report version of the Inventory of Callous-Unemotional traits (ICU; Essau, Sasagawa & Frick, 2006) contains 24 items scored on a 0-3 scale. The factor structure of the ICU remains debated with some studies reporting problems identifying factor models with adequate fit without modifications (e.g., item exclusions; Houghton , Hunter, & Crow, 2013). The majority of studies support a three-factor structure comprising the factors callousness, uncaring and unemotional (Essau *et al.*, 2006; Pechorro, Ray, Barroso, Maroco, & Gonçalves, 2016), with some indication that these load onto a first-order general callous-unemotional factor (Essau *et al.*, 2006; Kimonis *et al.*, 2008).

Reliability of the ICU:

Cronbach's alphas for the ICU total score and individual subscales were acceptable to good (.78-.82; Peterson, 1994), except for the ICU unemotional subscale which showed questionable internal consistency (α = .63; see Supplementary Table 2). These reliabilities are broadly in line with previous studies (e.g., Colins, Andershed, Hawes, Bijtterbier, & Pardini, 2016; Pechorro *et al.*, 2016), including a recent meta-analysis (Cardinale & Marsh, 2020). Mean inter-item correlations can be considered good if they fall between 0.15 and 0.50 (Clark & Watson, 1995), which was the case for the total ICU score as well as all three subscales.

Factor structure of the YPI:

The Youth Psychopathic traits Inventory (YPI; Andershed, Kerr, Stattin, & Levander, 2002) contains 50 items scored on a 1-4 point scale, which form 10 subscales (each consisting of five items). Exploratory and confirmatory factor analyses in community, clinical and forensic samples from a range of different countries (Andershed *et al.*, 2002; Colins, Bijttebier, Broekaert, & Andershed, 2014; Declercq, Markey, Vandist, & Verhaeghe, 2009; Hillege, Das, & de Ruiter, 2010; Pechorro, Ribeiro da Silva, *et al.*, 2016; but see Muñoz, Abate, Sharp, & Venta, 2019) suggest that these subscales form a three-factor structure comprising a Grandiose-Manipulative dimension, a Callous-Unemotional dimension, and an Impulsive-Irresponsible dimension, reflecting interpersonal, affective and behavioral

aspects of psychopathy, respectively. However, growing evidence also supports improved fit for a new bifactor model that includes a general psychopathy factor reflecting the shared variance between all items (Pihet, Suter, Meylan, & Schmid, 2014; Wang *et al.*, 2017; Yang *et al.*, 2019).

Reliability of the YPI:

Cronbach's alphas for the YPI total score and the Grandiose-Manipulative, Callous-Unemotional, and Impulsive-Irresponsible subscales were good to excellent (.80-.93; see Supplementary Table 2), in line with values reported in previous studies (e.g., Colins *et al.*, 2014; Neumann & Pardini, 2012). All mean inter-item correlations can be considered good (all between 0.15 and 0.50; Clark & Watson, 1995).

Supplementary Table 2. Callous-unemotional and psychopathic traits by group, as assessed using the self-report Inventory of Callous-Unemotional traits (ICU) and the self-report Youth Psychopathic traits Inventory (YPI)

	HC (*n* = 38)	UR (*n* = 24)	CD (*n* = 41)	*p*-value	post-hoc	α	MIC
ICU							
Missing	1	0	0			6	6
Total	22.46 (7.89)	22.33 (7.66)	31.80 (8.02)	**<.001**	HC, UR < CD	.82	.17
Unemotional	7.68 (2.60)	7.25 (2.59)	9.17 (2.95)	**.012**	UR < CD	.63	.26
Callous	6.30 (3.45)	6.96 (3.74)	11.80 (5.12)	**<.001**	HC, UR < CD	.79	.26
Uncaring	8.49 (4.12)	8.12 (3.78)	11.07 (4.25)	**.005**	HC, UR < CD	.78	.31
YPI							
Missing	2	0	1*			8	8
Total	100.83 (16.26)	99.88 (17.63)	120.96 (21.41)	**<.001**	HC, UR < CD	.93	.20
Callous-Unemotional	29.92 (6.34)	31.83 (6.64)	36.25 (7.28)	**<.001**	HC, UR < CD	.81	.22
Grandiose-Manipulative	37.39 (7.91)	33.71 (9.79)	40.78 (11.12)	**.021**	UR < CD	.90	.33
Impulsive-Irresponsible	33.53 (6.97)	34.33 (5.58)	44.70 (7.40)	**<.001**	HC, UR < CD	.87	.29

p-values are based on one-way ANOVAs and post-hoc pairwise comparisons are based on Bonferroni corrected *t*-tests (equal variances not assumed). HC, healthy controls; UR, unaffected relatives; CD, conduct disorder; α, Cronbach's alpha; MIC, mean inter-item correlation.
*None missing for YPI total.

Detailed results of the subcortical volume analyses

Supplementary Table 3 presents the main effects of Group and the effect sizes for all pairwise group comparisons on subcortical volumes, adjusted for sex, age, total intracranial volume (orthogonalized to sex) and IQ. The displayed *p*-values are not corrected for multiple comparisons. Following the equations provided by Nakagawa and Cuthill (2007), we calculated Hedge's *g* and its 95% confidence interval (CI) as the effect size for each comparison. Hedge's *g* is preferable to Cohen's *d* when group sizes are small (Hedges & Olkin, 1985) but can be interpreted in a similar manner with 0.2, 0.5, and 0.8 indicating a small, medium and large effect, respectively (Cohen, 1992).

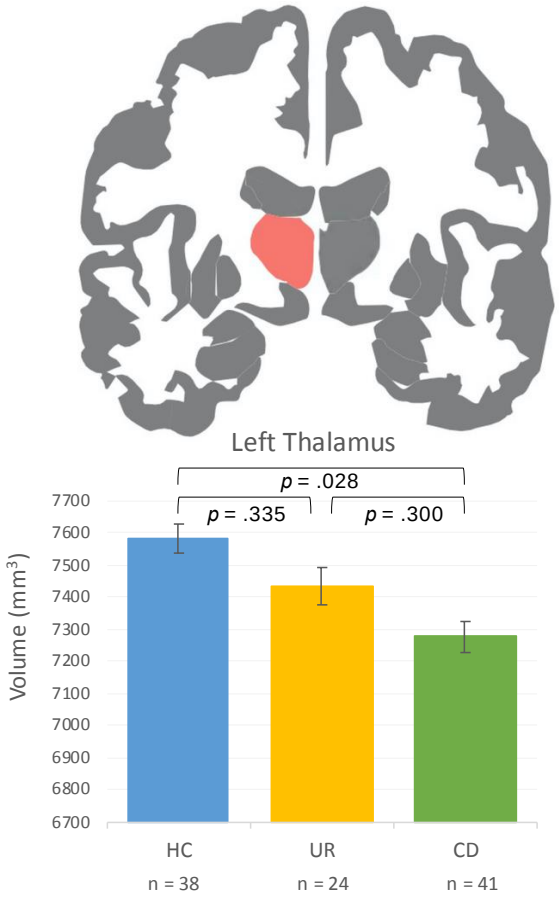
While there were no significant group effects on volumes of the amygdala, hippocampus, caudate, pallidum, putamen, thalamus, or nucleus accumbens, there was a trend towards a group effect in the left thalamus, $F(2,96) = 2.50$, $p = .088$, $\eta_p^2 = 0.05$. Post-hoc tests revealed significantly lower volume in the CD relative to the HC group ($p = .028$, Hedges's $g = -0.50$), whereas the UR group had thalamus volumes that were intermediate between CD and control participants with no significant differences in comparison to either group (Hedges $g = 0.26$ and -0.25, respectively; see Supplementary Figure 1). The group effect in the left thalamus reached significance at the nominal significance level when IQ was not included as a covariate, $F(2,98) = 4.04$, $p = .021$, $\eta_p^2 = 0.08$, but did not survive False Discovery Rate correction for multiple comparisons or adjustment for lifetime ADHD symptoms.

Supplementary Table 3: Main effects of group on subcortical volumes and effect sizes for the post-hoc group comparisons, adjusted for sex, age, IQ, and total intracranial volume (orthogonalized to sex)

Subcortical Region	Group effect		*g*	95% CI_l	95% CI_u	*p*
Left thalamus	$F(2,96)=2.50$, $p=.088$	**HC > CD**	**-0.50**	**-0.95**	**-0.05**	**.028**
		HC > UR	-0.25	-0.76	0.26	.335
		UR > CD	-0.26	-0.77	0.24	.300
Right thalamus	$F(2,96)=0.52$, $p=.594$	HC > CD	-0.23	-0.67	0.22	.310
		HC > UR	-0.14	-0.65	0.37	.593
		UR > CD	-0.10	-0.60	0.41	.704
Left caudate	$F(2,96)=0.77$, $p=.468$	HC < CD	0.22	-0.23	0.66	.333
		HC > UR	-0.06	-0.57	0.45	.810
		UR < CD	0.28	-0.22	0.79	.266
Right caudate	$F(2,96)=0.56$, $p=.572$	HC < CD	0.16	-0.28	0.61	.466
		HC > UR	-0.09	-0.60	0.42	.725
		UR < CD	0.26	-0.25	0.76	.313
Left putamen	$F(2,96)=0.22$, $p=.803$	HC > CD	-0.11	-0.55	0.34	.638
		HC < UR	0.05	-0.46	0.56	.841
		UR > CD	-0.16	-0.66	0.35	.532
Right putamen	$F(2,96)=0.62$, $p=.539$	HC < CD	0.02	-0.42	0.46	.936
		HC < UR	0.26	-0.25	0.78	.306
		UR > CD	-0.24	-0.75	0.26	.340
Left pallidum	$F(2,96)=0.09$, $p=.917$	HC < CD	0.09	-0.35	0.53	.679
		HC < UR	0.04	-0.47	0.55	.870
		UR < CD	0.05	-0.45	0.56	.835
Right pallidum	$F(2,96)=0.12$, $p=.884$	HC > CD	-0.10	-0.54	0.34	.645
		HC > UR	-0.01	-0.52	0.50	.962
		UR > CD	-0.09	-0.60	0.41	.713

Left hippocampus	$F(2,96)=0.67$, $p=.517$	HC < CD	0.25	-0.19	0.69	.264
		HC < UR	0.07	-0.44	0.58	.785
		UR < CD	0.19	-0.32	0.69	.463
Right hippocampus	$F(2,96)=0.34$, $p=.716$	HC < CD	0.03	-0.41	0.48	.878
		HC > UR	-0.17	-0.68	0.35	.519
		UR < CD	0.20	-0.30	0.71	.433
Left amygdala	$F(2,96)=1.51$, $p=.227$	HC < CD	0.07	-0.37	0.51	.763
		HC < UR	0.43	-0.09	0.94	.100
		UR > CD	-0.35	-0.86	0.15	.166
Right amygdala	$F(2,96)=0.67$, $p=.516$	HC < CD	0.07	-0.37	0.51	.743
		HC < UR	0.29	-0.22	0.81	.259
		UR > CD	-0.21	-0.72	0.29	.401
Left accumbens	$F(2,96)=0.26$, $p=.774$	HC > CD	-0.16	-0.60	0.28	.477
		HC > UR	-0.10	-0.61	0.41	.698
		UR > CD	-0.06	-0.57	0.44	.801
Right accumbens	$F(2,96)=0.11$, $p=.896$	HC > CD	-0.08	-0.52	0.37	.736
		HC < UR	0.04	-0.48	0.55	.892
		UR > CD	-0.11	-0.62	0.39	.660

Note. The greater than/less than symbol and the sign of the effect size indicate the direction of the effect. A positive effect size reflects that the respective region is larger in the second group (e.g. CD in the case of HC – CD) whereas a negative effect size indicates that the region is smaller in the second group. Significant differences are marked in bold. *p*-values are not corrected for multiple comparisons. Key: CD = Conduct Disorder; HC = healthy controls; UR = unaffected relatives; *g* = Hedge's *g*; 95% CI_l/CI_u = lower and upper 95% confidence intervals of *g*.



Supplementary Figure 1. Post-hoc group comparisons for left thalamus volume (shown in red in the top panel which displays a coronal view of the brain). Error bars represent standard errors and *p*-values are uncorrected. HC = healthy controls; UR = unaffected relatives; CD = Conduct Disorder.

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TITLE PAGE

Neuroanatomical markers of familial risk in adolescents with Conduct Disorder and their unaffected relatives

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Abstract

Background: Previous studies have reported brain structure abnormalities in Conduct Disorder (CD), but it is unclear whether these neuroanatomical alterations mediate the effects of familial (genetic and environmental) risk for CD. We investigated brain structure in adolescents with CD and their unaffected relatives to identify neuroanatomical markers of familial risk for CD.

Methods: 41 adolescents with CD, 24 unaffected relatives (URs) of CD probands, and 38 healthy controls (aged 12-18), underwent structural magnetic resonance imaging. We performed surface-based morphometry analyses, testing for group differences in cortical volume, thickness, surface area, and folding. We also assessed the volume of key subcortical structures.

Results: The CD and UR groups both displayed structural alterations (lower surface area and folding) in left inferior parietal cortex compared with controls. In contrast, CD participants showed lower insula and pars opercularis volume than controls, and lower surface area and folding in these regions than controls and URs. The URs showed greater folding in rostral anterior cingulate and inferior temporal cortex than controls and greater medial orbitofrontal folding than CD participants. The surface area and volume differences were not significant when controlling for attention-deficit/hyperactivity disorder comorbidity. There were no group differences in subcortical volumes.

Conclusions: These findings suggest that alterations in inferior parietal cortical structure partly mediate the effects of familial risk for CD. These structural changes merit investigation as candidate endophenotypes for CD. Neuroanatomical changes in medial orbitofrontal and anterior cingulate cortex differentiated between URs and the other groups, potentially reflecting neural mechanisms of resilience to CD.

Key words: Conduct Disorder; antisocial behavior; brain structure; surface-based morphometry; endophenotype; family-based designs.

Introduction

Conduct Disorder (CD) is characterized by a repetitive pattern of aggressive and antisocial behavior (American Psychiatric Association, 2000). It results in substantial personal and financial costs for the affected individuals, their families, and society in general (Erskine *et al.*, 2014; Rivenbark *et al.*, 2018), and is the most common reason for referral to Child and Adolescent Mental Health Services in the UK (National Institute for Health and Care Excellence, 2013). CD is also linked to negative adult outcomes, such as mental and physical health problems (Copeland, Wolke, Shanahan, & Costello, 2015; Odgers *et al.*, 2007) and personality disorders (Burt, Donnellan, Iacono, & McGue, 2011; Robins, 1978). It is therefore important to understand its etiology, which may help in developing effective treatments and prevention programs.

There is increasing evidence that CD may have a neurobiological basis, with many studies reporting differences in brain function or structure in children or adolescents with CD or conduct problems compared with typically-developing controls (Alegria, Radua, & Rubia, 2016; Fairchild *et al.*, 2019; Rogers & De Brito, 2016). This research has been extremely valuable in identifying the neuroanatomical and functional *correlates* of CD and callous-unemotional (CU) traits. However, the cross-sectional nature of these studies means it is unclear whether structural or functional abnormalities in regions such as the orbitofrontal cortex or insula precede the disorder or reflect a secondary effect of having CD or lifestyle factors associated with the condition (e.g., substance abuse or sustaining head injuries when fighting). It is possible that these brain abnormalities are caused by the same etiological factors (genetic or environmental risk factors) that led the individual to develop the disorder (Bidwell, Willcutt, Defries, & Pennington, 2007). Relevant to this point, twin studies have shown that brain volume, and particularly volume of the frontal lobe, is highly heritable (Jansen, Mous, White, Posthuma, & Polderman, 2015). It has also been shown that CD and related phenotypes such as criminal behavior and antisocial personality disorder cluster within families. For example, Blazei *et al.* (2008) found a strong resemblance between biological fathers and sons in terms of antisocial behavior – particularly if the father resided in the home. Similarly, Christiansen *et al.* (2008) found that the siblings of those with attention-deficit/hyperactivity disorder (ADHD) *and* conduct problems were 5 times more likely to develop ADHD and conduct problems and 3 times more likely to develop conduct problems than the

siblings of children with ADHD alone. Similar patterns of familial aggregation have been reported in criminological studies – for example, the Cambridge Study in Delinquent Development found that just 6% of the families accounted for 50% of all criminal convictions, and conviction rates were two times higher in the sons of fathers with a history of criminal behavior than the sons of fathers without such a history (Farrington, Barnes & Lambert, 1996).

Although these studies have provided compelling evidence that antisocial behavior clusters within families, far less is known about the brain mechanisms which explain this family resemblance, even though this is an important issue with implications for the development of intervention and prevention programs. One strategy that can be adopted to study the brain mechanisms that may mediate genetic or environmental risk for CD is to study the first-degree relatives of affected probands who do not show the disorder themselves, but may still carry markers of familial risk. For example, Ersche *et al.* (2012) employed this strategy to investigate whether brain abnormalities are associated with familial risk for substance dependence or reflect the neurotoxic effects of prolonged drug use. Substance dependent individuals and their unaffected siblings were found to display common neuroanatomical abnormalities in brain regions involved in inhibitory control, suggesting that they are a risk factor for substance dependence, rather than reflecting the secondary consequences of drug use (i.e., drug-induced damage). A similar study investigating familial risk markers for autism found common reductions in activation in brain regions involved in biological motion perception and social cognition (e.g., the superior temporal sulcus) in children with autism and their unaffected relatives (URs), relative to controls (Kaiser *et al.*, 2010).

Applying this logic to CD, if similar alterations in brain structure are observed in adolescents with CD *and* their URs, this would indicate that neuroanatomical abnormalities and CD co-segregate within families (are inherited together) and that such structural changes may mediate the effects of genetic risk for CD. Studying unaffected first-degree relatives, as well as affected probands with CD, would therefore help us to address the question of whether neuroanatomical changes increase risk for developing CD or reflect the secondary consequences of having CD and associated lifestyle factors. A further advantage of studying URs, who 'beat the odds' by remaining free from severe antisocial behavior despite being at increased risk, is that protective or compensatory brain changes might be

observed in this group which counteract the effects of familial risk. Relevant to this point, Kaiser *et al.* (2010) identified potential compensatory effects in the URs of ASD probands. They showed greater ventromedial prefrontal cortex activity than the autistic or typically-developing control groups when viewing point-light displays of biological motion.

Most existing studies investigating brain structure in CD have employed voxel-based morphometry (VBM) methods which test for differences in gray matter volume across the whole brain. However, using this composite, intensity-based measure is problematic because volume in a given region is a function of its cortical thickness and surface area, as well as cortical folding, which show distinct genetic etiologies (Panizzon *et al.*, 2009), developmental trajectories (Raznahan *et al.*, 2011), and underlying cellular mechanisms (Rakic, 2009).

Accordingly, we compared adolescents with CD and their URs using surface-based morphometry (SBM), to examine whether these groups show common or distinct abnormalities in cortical structure compared with typically-developing adolescents. On the basis of previous SBM findings (Fairchild *et al.*, 2015; Hyatt, Haney-Caron, & Stevens, 2012; Smaragdi *et al.*, 2017; Wallace *et al.*, 2014), we predicted that adolescents with CD would show structural alterations in the insula, orbitofrontal cortex, superior temporal gyrus, and inferior parietal cortex compared with typically-developing adolescents. We also hypothesized that the URs of CD probands would show similar structural abnormalities, albeit possibly at an intermediate level, as their loading of genetic or environmental risk may be lower than the probands. Consistent with this, a recent study found that ADHD probands and their unaffected siblings both showed lower orbitofrontal cortex volume (Bralten *et al.*, 2016). Given prior evidence that ADHD comorbidity may be important in determining the extent of structural changes observed in CD (Fairchild *et al.*, 2015; Smaragdi *et al.*, 2017), we controlled for ADHD symptoms in a supplementary analysis. Although exploratory in nature, we also tested for potential 'compensatory' or 'protective' structural changes in the URs. However, due to the lack of previous studies, we had no *a priori* predictions regarding the loci and direction of such effects. Lastly, based on evidence of subcortical alterations in youths with CD (Rogers & De Brito, 2016; Wallace *et al.*, 2014), we tested for group effects on subcortical volumes. We predicted that the CD group would

show lower amygdala volume compared to the controls, and similar reductions might be observed in URs.

Methods

Participants

Healthy control participants (n=41) were recruited from mainstream schools and colleges, whereas participants with CD (n=43) were mainly recruited from specialist schools, pupil referral units and Youth Offending Services in the Hampshire area. The URs (n=24) were recruited directly from the families of the CD participants, as well as the aforementioned recruitment sources. Participants were aged between 12-18 years. All parents/carers completed a Family History Screen, consisting of three questions assessing current and lifetime psychopathology, behavioral problems, and criminal convictions, in the participants' first-degree relatives. This screen was designed to identify siblings of adolescents with CD who did not meet the diagnostic criteria for CD themselves. In addition, it enabled us to identify the unaffected offspring of parents who had previously displayed CD and ensure that the controls had no family history of CD.

Diagnostic and questionnaire measures

The Kiddie-Schedule of Affective Disorders and Schizophrenia-Present and Lifetime version (K-SADS-PL; Kaufman *et al.*, 1997), a semi-structured diagnostic interview based on DSM-IV-TR criteria (APA, 2000), was used to screen for CD and other common psychiatric disorders. The participants and their parents/carers were interviewed separately to ensure confidentiality, and the information from each interview was combined such that a symptom was considered present if it was endorsed by either informant (Kaufman *et al.*, 1997). Even if the initial screen items for CD and ADHD were not endorsed, the full supplements for these disorders were always completed to obtain dimensional information on these disorders for all participants.

The self-report version of the Inventory of Callous-Unemotional traits (ICU; Essau, Sasagawa & Frick, 2006) was used to assess callous-unemotional traits. It contains 24 items scored on a 0-3 scale, from 'not at all true' to 'definitely true' (Cronbach's Alpha in present sample=0.82). Factor

analysis has revealed that the ICU captures three distinct dimensions of behavior termed callousness, uncaring, and unemotional (Essau *et al.*, 2006), therefore scores for these subscales are also reported. The self-report Youth Psychopathic traits Inventory (Andershed, Kerr, Stattin, & Levander, 2002) was used to measure psychopathic personality traits. It contains 50 items, each scored on a 1-4 point scale, from 'does not apply at all' to 'applies very well' (Cronbach's Alpha in present sample=0.93), and as well as total scores, it can be divided into Grandiose-Manipulative, Callous-Unemotional, and Impulsive-Irresponsible subscales corresponding to the three-facet model of psychopathy (Andershed *et al.*, 2002). Further information about these questionnaires' psychometric properties and their factor structures can be found in Supplementary Materials. The Wechsler Abbreviated Scale of Intelligence was used to estimate full-scale IQ (Wechsler, 1999). Lastly, the Edinburgh Handedness Inventory (Oldfield, 1971) was used to assess handedness.

Ethical Approval

The study was approved by the University of Southampton Ethics Committee, the University Hospital Southampton NHS Trust, Southampton City Council Children's Services and the Hampshire County Council Research and Evaluation Unit. Participants aged ≥16 provided written informed consent, whereas parents provided informed consent and participants provided assent if below age 16.

Procedure

Once they had been screened for psychiatric disorders and standard MRI exclusion criteria, such as claustrophobia, participants were invited to the Southampton General Hospital for a magnetic resonance imaging (MRI) scan lasting 35-40 minutes. The structural (T1-weighted) scan was the first sequence performed during the scanning session, and was repeated as needed until usable data, uncontaminated by movement, had been collected. This was determined by a trained radiographer.

Data Acquisition

Structural MRI data were acquired using a 1.5-Tesla Siemens Avanto scanner (Siemens Medical Solutions, Erlangen, Germany). We acquired T1-weighted three-dimensional MPRAGE images

(voxel size=1.2x1.2x1.2 mm, repetition time=2400ms, echo time=3.62ms, flip angle=8°, 160 slices). Total scanning time was 7 minutes, 41 seconds. After scanning, the structural images were reviewed by a consultant neuroradiologist to screen for neurological abnormalities. We initially included 108 participants, but five participants (3 controls; 2 CD) were excluded due to having cysts or tumors, leaving 103 participants with usable MRI data.

Surface-based morphometry analyses: cortical volume, thickness, surface area (SA) and local gyrification index (*l*GI) and subcortical volumes

MRI-based quantification of cortical volume, thickness, SA and folding (quantified using *l*GI) was performed using FreeSurfer 5.3.0 (http://surfer.nmr.mgh.harvard.edu). This method has been described in detail (Fischl, 2012). Briefly, the procedure involves segmentation of white matter, tessellation of the gray-white matter junction to construct representations of the gray/white matter boundary and cortical surface. Each participant's cortex was then visually inspected and, if necessary, manually edited by one of the authors (N.T.), blind to group status. This involved: (i) realignment of each subject's image to the Montreal Neurological Institute template; (ii) setting intensity normalization control points where brain matter was erroneously skull-stripped; and (iii) adjustment of the watershed parameters of the skull strip. From this reconstruction, vertex-wise estimates of both cortical thickness and cortical area were obtained. lGI, which measures the amount of cortical folding within versus outside the sulcus, was calculated using the method outlined by Schaer *et al.* (2012). In order to map the participants' brains to a common space, reconstructed surfaces were registered to an average cortical surface atlas using a nonlinear procedure that optimally aligns sulcal and gyral features across individuals (Fischl *et al.*, 1999a, b). Finally, we estimated amygdala, hippocampus, caudate, pallidum, putamen, thalamus, and nucleus accumbens volumes using FreeSurfer's automatic segmentation pipeline (Fischl *et al.*, 2002).

Statistical Analyses

We tested for group differences in demographic and clinical variables using analyses of variance, with independent *t*-tests used to follow up significant *F* tests; Chi-Square tests were used for group comparisons of nominal variables (e.g., sex).

For each hemisphere, group differences in cortical volume, thickness, surface area and lGI at each vertex were tested using a general linear model (GLM) with age, sex, IQ and total intracranial volume (TIV) orthogonalized to sex included as covariates of no interest. We also repeated the analyses including lifetime ADHD symptoms as a further covariate (these results are reported in **Supplementary Table 1**, available on-line). Given previous evidence suggesting that childhood-onset (CO-CD) and adolescence-onset (AO-CD) variants of CD may differ quantitatively in brain structure or function (Fairchild *et al.*, 2013), we initially ran analyses comparing these subgroups (i.e., CO-CD>AO-CD, AO-CD>CO-CD). As there were no significant differences between subgroups, they were treated as a combined group in the comparisons with URs and HCs.

After applying a vertex-wise/cluster-forming threshold of p=0.05, the level of statistical significance was subject to a further cluster-wise P (CWP) value correction procedure for multiple comparisons based on a Monte Carlo z-field simulation (Hagler *et al.*, 2006). Clusters are only reported if they met a whole-brain corrected threshold of CWP≤0.05.

Lastly, we tested for group differences in subcortical volumes using one-way analyses of covariance with age, sex, IQ and TIV orthogonalized to sex included as covariates of no interest, whilst applying a False-Discovery-Rate (FDR) correction for multiple comparisons at *q*=.05. Significant group effects were followed up with pairwise *t*-tests. These analyses were repeated including lifetime ADHD symptoms as a covariate.

Results

Sample characteristics

Demographic and clinical characteristics of the sample are reported in Table 1. The groups differed in age (p=0.01), with the URs being around a year younger than the other groups. The groups also

differed in estimated IQ ($p<0.001$), with the CD group having the lowest average IQ and the control group having the highest. However, the groups were matched in sex and handedness. As expected, the CD group reported higher rates of CD symptoms, ADHD symptoms, CU traits, and psychopathic traits than the other groups (*ps*<0.001). Critically, the HCs and URs did not differ on any clinical or personality variable, confirming the 'unaffected' nature of the latter group.

[INSERT TABLE 1 HERE]

Surface-based morphometry results: Potential markers of familial risk for antisocial behavior

Relative to controls, the CD group showed lower left inferior parietal cortex surface area, whereas the URs showed lower cortical folding in this region (Figure 1; Table 2). When controlling for comorbid ADHD symptoms, both the CD and UR groups showed lower left inferior parietal cortical folding (Supplementary Table 1).

[INSERT FIGURE 1 HERE]

Effects related to CD but not observed in unaffected relatives – non-familial risk

In contrast, the CD group showed lower volume in left insula and right pars triangularis extending to right insula compared with controls, and lower surface area in left insula and right pars triangularis/insula compared with both the controls and URs (Figure 2; Table 2).

Further structural differences between the CD and control groups that were not observed in the URs included lower bilateral pericalcarine, left pars opercularis and right precentral gyrus surface area in the CD group (Table 2). In addition, the CD group showed greater cortical thickness in left superior frontal gyrus and superior temporal cortex and right frontal pole compared with the URs (Table 2).

[INSERT TABLE 2 AND FIGURE 2 HERE]

Potential compensatory effects in the unaffected relatives

There were also several effects *unique* to the URs – they showed increased folding in rostral anterior cingulate cortex compared with controls and increased medial orbitofrontal cortex folding compared

with the CD group (Figure 3; Table 2). The URs also showed greater folding in lingual gyrus and inferior temporal cortex compared with controls, and greater folding in bilateral pars triangularis/insula and left superior frontal gyrus compared with the CD group.

[INSERT FIGURE 3 HERE]

Impact of adjusting for comorbid ADHD

When including ADHD symptoms as a covariate, the similarities between the CD and UR groups were amplified – as mentioned above, both groups showed lower left inferior parietal folding compared with controls (Supplementary Table 1). There were also differences in cortical thickness which appeared specific to the CD group – they showed greater medial orbitofrontal cortical thickness compared to controls and greater superior frontal cortical thickness compared to URs (Supplementary Table 1). However, some of the CD-control differences were rendered non-significant, such as differences in left insula and right pars opercularis volume (HC>CD). In addition, the group differences in surface area (e.g., lower insula and pars triangularis surface area in CD) were not significant when adjusting for ADHD symptoms. As might be expected given the low level of ADHD symptoms in URs, differences between this group and the controls remained significant when adjusting for ADHD – including increased rostral anterior cingulate folding.

Subcortical volumes

There were no group differences in amygdala, hippocampus, thalamus, caudate, pallidum, putamen or nucleus accumbens volumes when controlling for multiple comparisons. There was, however, a trend towards a group effect in left thalamus (p=.088, uncorrected), which became nominally significant when IQ was not included as a covariate. Post-hoc tests revealed lower left thalamus volume in CD versus HC participants (p=.028, Hedges's g=-0.50), but no other pairwise differences. See Supplementary Materials for more information.

Discussion

In the present study, we used surface-based morphometry and subcortical segmentation methods to investigate whether probands with Conduct Disorder (CD) and their unaffected relatives (URs) show similar or distinct changes in brain structure compared with typically-developing adolescents, and identify potential protective or compensatory alterations in the URs. The observation of common structural alterations in CD probands and URs would indicate that CD and related neuroanatomical changes co-segregate within families, suggesting the latter may partly mediate the effects of genetic or environmental risk for CD. We also assessed four different measures of cortical structure – cortical volume, thickness, surface area, and folding – and subcortical volumes to provide greater specificity regarding the structural changes associated with CD and its familial risk.

Our first key finding was that similar alterations in left inferior parietal cortical structure were observed in individuals with CD and their URs. The CD participants showed lower surface area in left inferior parietal cortex, whereas the URs showed lower folding in this region, compared with controls. When controlling for comorbid ADHD, both groups showed lower left inferior parietal folding compared with controls. These findings provide the first available evidence that reductions in inferior parietal surface area and folding might constitute a neuroanatomical endophenotype for CD which is present in CD probands *and* their URs. This suggests that alterations in inferior parietal cortical structure may partly mediate the effects of familial risk for CD. Previous SBM studies have reported structural abnormalities in the inferior parietal cortex, or adjacent areas such as supramarginal gyrus, in CD (Fahim *et al.*, 2011; Jiang *et al.*, 2015; Smaragdi *et al.*, 2017; Wallace *et al.*, 2014). The left inferior parietal cortex is implicated in language comprehension, theory of mind, action observation (Molenberghs, Cunnington, & Mattingley, 2012), and perhaps most intriguingly, facial emotion recognition (Zhang, Song, Liu, & Liu, 2016). The latter function appears relevant to our earlier finding that CD probands and their URs show similar deficits in facial emotion recognition (Sully, Sonuga-Barke, & Fairchild, 2015).

Our second key finding was that the volume of the insula and surrounding frontal lobe structures such as the pars opercularis was lower in CD compared with control participants.

Critically, these changes in volume appeared to be driven by reductions in insula and pars opercularis surface area, rather than cortical thickness, in the CD group. The CD group also showed lower insula and pars triangularis surface area compared to URs. To our knowledge, although several VBM studies have reported lower insula gray matter volume in CD (Fairchild et al., 2013; Fairchild et al., 2011; Sterzer, Stadler, Poustka, & Kleinschmidt, 2007) and the insula was identified in a recent meta-analysis of VBM studies (Rogers & De Brito, 2016), this is the first study to examine the underlying basis of such volumetric changes. The finding that reductions in surface area, rather than cortical thickness, drive reductions in insula volume observed in CD is consistent with data from normative studies showing that surface area is more strongly related to volume than cortical thickness (Im et al., 2008). The anterior insula is considered to play a critical role in processing emotional (especially negative) stimuli (Calder, Keane, Manes, Antoun, & Young, 2000), empathy (Singer et al., 2004), and awareness of one's own physiological and emotional states (Craig, 2009). Consequently, structural deficits in the insula might explain why adolescents with CD show deficits in empathy (Martin-Key, Brown, & Fairchild, 2017) and learning from punishment (Kohls et al., 2020), and reduced sensitivity to losses when making decisions (Fairchild et al., 2009). On the other hand, the fact that reductions in insula volume and surface area were not observed in the URs challenges the idea that they mediate the effects of familial risk for CD. Of interest, we found that adolescents with CD, but not their URs, showed heightened risk-taking in a gambling task (Sully, Sonuga-Barke, Savage, & Fairchild, 2016). It should also be noted that these insula volume and surface area differences were rendered non-significant when adjusting for comorbid ADHD symptoms, suggesting that they are not related to CD specifically or are more pronounced in participants with comorbid CD+ADHD.

We also found greater medial orbitofrontal cortical thickness in the CD group compared with controls, although only when adjusting for comorbid ADHD, whereas cortical thickness in the superior frontal gyrus and frontal pole was increased in the CD group compared with the URs. The medial orbitofrontal cortex is involved in representing the reward value of stimuli (Liu, Hairston, Schrier, & Fan, 2011) and social cognitive processes (Molenberghs, Johnson, Henry, & Mattingley, 2016). The frontal pole is implicated in executive functions – especially tasks in which multiple cognitive processes must be monitored simultaneously (Mansouri, Koechlin, Rosa, & Buckley, 2017).

Previous studies have reported lower cortical thickness (Fahim *et al.*, 2011; Jiang *et al.*, 2015; Smaragdi *et al.*, 2017), lower surface area (Fairchild *et al.*, 2015; Sarkar *et al.*, 2015), and atypical folding in the orbitofrontal cortex in CD (Hyatt *et al.*, 2012; Wallace *et al.*, 2014). fMRI studies have also observed atypical medial orbitofrontal cortex activation in adolescents with CD during facial emotion processing (Fairchild *et al.*, 2014; Passamonti *et al.*, 2010).

Although we did not observe reduced superior temporal gyrus cortical thickness in the CD group, contrary to the findings of our previous study (Fairchild *et al.*, 2015) and earlier results obtained in younger children or individuals with non-comorbid CD (Fahim *et al.*, 2011; Hyatt *et al.*, 2012; Wallace *et al.*, 2014), left superior temporal gyrus folding was reduced in CD participants compared with controls. This is in line with previous VBM studies reporting lower superior temporal gray matter volume in adolescents with CD (Rogers & De Brito, 2016), and adults with antisocial personality disorder and psychopathy (de Oliveira-Souza *et al.*, 2008; Muller *et al.*, 2008). As with the insula results, these findings further implicate the superior temporal gyrus in the pathophysiology of CD but challenge the idea that structural changes in this region fall on the causal pathway between familial (genetic or environmental) risk and CD. The superior temporal gyrus is implicated in social cognition, including facial emotion processing, as well as auditory and vocal perception and language comprehension (Redcay, 2008). Of note, a range of social cognitive deficits have been reported in CD, such as impairments in facial and vocal emotion recognition (Blair, Budhani, Colledge, & Scott, 2005; Fairchild, Van Goozen, Calder, Stollery, & Goodyer, 2009), empathic accuracy and affective empathy (Martin-Key *et al.*, 2017, 2020; Schwenck *et al.*, 2012), and social competence in real-life situations (Oliver, Barker, Mandy, Skuse, & Maughan, 2011).

Strengths and limitations

To our knowledge, this is the first neuroimaging study to investigate familial risk markers for antisocial behavior by assessing cortical structure in CD probands and their URs. The use of SBM methods enabled us to disaggregate the cortical properties that give rise to volume and demonstrate that reductions in insula and pars opercularis/triangularis volume were driven by changes in *surface area*. We also assessed the volume of key subcortical structures such as the amygdala. Another

strength of the study was that the URs were truly free of CD – most had no CD symptoms – rather than being elevated in CD symptoms, but not quite meeting the diagnostic criteria. The URs were also relatively free of psychopathology in general and did not differ from controls in personality traits linked to CD, such as callous-unemotional traits. Lastly, our sample was well-characterized from a psychiatric perspective, with data collected from multiple informants (in most cases, participants *and* parents/carers). The participants were also screened carefully for comorbid disorders such as ADHD, and we systematically examined the impact of ADHD comorbidity.

In terms of limitations, it was not optimal that the groups contained a mixture of males and females (although males were over-represented), as the relationship between CD and brain structure may partly differ by sex (Fairchild *et al.*, 2013; Smaragdi *et al.*, 2017). This was almost unavoidable, given the scale of the study and the fact that URs were more likely to be female, although the groups did not differ in sex. Critically, we controlled for sex, age, IQ and total intracranial volume, in our analyses. However, future studies should recruit enough males and females in each group to examine whether similar findings are obtained when analysing data from males and females separately. Stronger familial effects may be observed in the unaffected siblings of female, versus male, probands, as girls might require a higher loading of genetic risk to develop CD (Meier, Slutske, Heath, & Martin, 2011). It should be noted that some of the group differences, particularly those obtained for volume and surface area, were rendered non-significant when controlling for ADHD (e.g., insula), whereas others were only significant when adjusting for ADHD (e.g., differences in inferior parietal folding in CD). This is consistent with previous SBM studies which found that controlling for ADHD symptoms attenuated some of the group differences in cortical structure – particularly for surface area (Smaragdi *et al.*, 2017) – whereas other CD-related effects were only present *when* adjusting for ADHD symptoms (Fairchild *et al.*, 2015). Nevertheless, we note that our unadjusted findings are probably more representative of clinical reality, given that ADHD comorbidity is common in CD (Angold, Costello & Erkanli, 1999) and there is significant genetic overlap between ADHD and CD/ODD (Tuvblad *et al.*, 2009), so controlling for ADHD symptoms might be 'overcorrecting'. Finally, the UR group included both the unaffected siblings of the CD probands who were included in the study and the siblings of CD probands who were unwilling or ineligible to participate in the study

(e.g., due to being too old or incarcerated). It has been argued that investigating URs who are related to the included CD participants and those that are not offers advantages in terms of identifying markers that are specifically related to psychiatric disorders (Kaiser *et al.*, 2010), rather than simply identifying heritable aspects of brain structure, but this also restricted the analyses that we could perform. One benefit of recruiting a large number of CD proband-unaffected sibling pairs would be to investigate whether abnormalities in cortical structure (e.g., in inferior parietal cortex) are shared by both family members. Future studies could also examine whether neuroanatomical abnormalities are transmitted inter-generationally from parents to children and whether this predicts risk for CD (Thissen *et al.*, 2014), consistent with the hypothesis that brain structure abnormalities mediate familial risk for CD.

Conclusions

In the first study to investigate whether neuroanatomical abnormalities associated with CD co-segregate within families, we found evidence that reductions in inferior parietal cortex surface area and folding may be familial risk markers for CD, as these were present in both affected probands and their unaffected relatives. These alterations in inferior parietal cortical structure merit further investigation as candidate endophenotypes for CD. Conversely, we identified neuroanatomical abnormalities that were specific to the CD group, such as lower insula and pars opercularis/ triangularis volume and surface area. Although this suggests that alterations in insula structure play an important role in the development of CD, such that they distinguish between affected and unaffected members of the same families, they also challenge the idea that such structural alterations mediate the effects of familial risk for CD. We also observed increased folding in the rostral anterior cingulate, medial orbitofrontal and inferior temporal cortices in the unaffected relatives compared with the CD and control groups, which may reflect compensatory or protective effects.

Figure Legends:

Figure 1. Neuroanatomical markers of familial risk for Conduct Disorder that were observed in both the CD probands and the unaffected relatives compared with controls. Panel A. Left inferior parietal cortical surface area was lower in participants with Conduct Disorder (CD) compared with healthy controls (HC). B. Left inferior parietal cortical folding was lower in unaffected relatives (UR) than healthy controls. C. Left inferior parietal cortical folding was reduced in participants with CD compared with healthy controls when adjusting for comorbid ADHD symptoms. D. Left inferior parietal cortical folding was lower in unaffected relatives than healthy controls when adjusting for comorbid ADHD symptoms.

Figure 2. Cortical structure alterations observed in the Conduct Disorder group compared to the healthy controls and unaffected relatives, reflecting non-familial risk. Panel A. Right pars triangularis surface area (extending to insula) was lower in participants with Conduct Disorder (CD) compared with healthy controls (HC). B. Right pars triangularis surface area (extending to insula) was lower in participants with CD compared with the unaffected relatives (UR). C. Left pars triangularis cortical folding (extending to insula) was lower in participants with CD compared with the unaffected relatives. D. Right pars triangularis cortical folding (extending to insula) was lower in participants with CD compared with the unaffected relatives.

Figure 3: Potential protective or compensatory structural changes observed in the unaffected relatives compared with the healthy control and Conduct Disorder groups. Panel A. Medial orbitofrontal cortical folding was higher in the unaffected relatives (UR) compared with the Conduct Disorder (CD) group. B. Rostral anterior cingulate cortical folding was higher in the unaffected relatives compared with the healthy controls (HC).

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Table 1. *Demographic and clinical characteristics of the sample*

	HC (*n* = 38)	UR (*n* = 24)	CD (*n* = 41)	*p* value	post-hoc
Age (years)	16.51 (1.31)	15.64 (1.49)	16.66 (1.37)	.014	UR < CD
Gender (M:F)	32:6	17:7	37:4	.125	
Handedness (R:L:A)[a]	29:5:3	17:1:6	31:5:5	.336	
IQ	103.79 (9.76)	98.54 (8.78)	93.27 (19.90)	< .001	CD < HC
CD Symptoms	0.21 (0.53)	0.38 (0.58)	8.27 (2.35)	< .001	HC, UR < CD
ADHD Symptoms	0.71 (1.47)	1.46 (2.02)	7.17 (4.31)	< .001	HC, UR < CD
ICU Total[b]	22.46 (7.89)	22.33 (7.66)	31.80 (8.02)	< .001	HC, UR < CD
ICU Unemotional[b]	7.68 (2.60)	7.25 (2.59)	9.17 (2.95)	.012	UR < CD
ICU Callous[b]	6.30 (3.45)	6.96 (3.74)	11.80 (5.12)	<.001	HC, UR < CD
ICU Uncaring[b]	8.49 (4.12)	8.12 (3.78)	11.07 (4.25)	.005	HC, UR < CD
YPI Total[c]	100.8 (16.3)	99.9 (17.6)	121.0 (21.4)	< .001	HC, UR < CD
YPI Callous-Unemotional[d]	29.9 (6.3)	31.8 (6.6)	36.3 (7.3)	<.001	HC, UR < CD
YPI Grandiose-Manipulative[d]	37.4 (7.9)	33.7 (9.8)	40.8 (11.1)	.021	UR < CD
YPI Impulsive-Irresponsible[d]	33.5 (7.0)	34.3 (5.6)	44.7 (7.4)	<.001	HC, UR < CD
Childhood-onset (*n*)			23		
Adolescence-onset (*n*)			18		

Note. Means are presented with standard deviations in parentheses. Group differences were assessed with one-way ANOVA *F*-tests and pairwise Bonferroni-adjusted *t*-tests (continuous variables) and Chi-Squared tests (categorical variables). Key: HC = healthy controls; UR = unaffected relatives; CD = conduct disorder; M = male; F = female; R = right-handed; L = left-handed; A = ambidextrous; IQ = intelligence quotient; ADHD = attention-deficit/hyperactivity disorder; ICU = Inventory of Callous-Unemotional traits (self-report version); YPI = Youth Psychopathic traits Inventory. Participants who developed symptoms of CD before age 10 were classified as having 'childhood-onset' CD, whereas those who only displayed symptoms of CD after age 10 were classified as having 'adolescence-onset' CD.
[a] $n_{missing}$ = 1 (CD group)
[b] $n_{missing}$ = 1 (HC group)
[c] $n_{missing}$ = 2 (HC group)
[d] $n_{missing}$ = 3 (2 HCs, 1 CD)

Reviewers' comments:

Reviewer #1:

My only request is that the authors provide some more information and details regarding the scales that were employed to assess the antisocial/CD symptoms - namely, please describe the factor structure of these scales and adjust the sample characteristics table (and attendant statistics) to report scores from these factors (e.g., a brief literature search revealed that both the YPI and the ICU have three distinct subfactors—please report these in addition to the total scores). This in important to report/consider in any case, and may have consequences for how familial risk vs. proband-specific risk is interpreted in the present cortical morphometry analyses.

Response: We thank the reviewer for their positive comments on the manuscript. We agree that, although psychopathic and CU traits were not a major focus of the study, it would be valuable to include more information about the subscales of the YPI and ICU. The scores for each of the individual subscales are now reported in Table 1 and reinforce the point that the unaffected relatives were lower than the CD group (and comparable to the control group) in all measures related to antisocial behaviour/personality traits. We also provide information about the factor structure of these scales and related references in the Methods section of the paper (see p. 7, first para) and expand on this and the psychometric properties of these measures in the Supplementary Materials.

Reviewer #2:
Major comments
I only had one major concern regarding this paper, which is the way in which ADHD is dealt with. It's apparent from the results section that including ADHD in the analyses leads to some quite notable changes in the findings, however this is not discussed in detail in the discussion (it is only mentioned briefly on occasion).

* If possible, it would be helpful to repeat Table 2 as a supplementary table, but this time have the results if ADHD is controlled. At the moment it is difficult to compare the findings with and without ADHD directly.

Response: Thank you for this very helpful suggestion. We have now created a single table in the Supplementary Materials showing the results obtained when ADHD symptoms are controlled, and agree it is easier to compare across the two sets of results when presented in this format.

* If I have understood correctly, the "first key finding" described in the discussion was only present when ADHD was not controlled for. However, ADHD was not discussed in the paragraph addressing this finding. I would like to know the authors' interpretation of what is happening with ADHD, and whether some of their findings might plausibly be attributed to ADHD rather than conduct disorder. This could be an important avenue for future research.

Response: The reviewer is correct and we have now modified the Discussion to highlight this point, as well as giving a message of caution regarding controlling for ADHD symptoms. We note that the adjusted findings reflect those specifically related to CD, whereas the unadjusted findings probably reflect clinical reality given that ADHD comorbidity is so common in CD and there is an argument that, due to the overlapping genetic etiology of CD and ADHD, controlling for ADHD symptoms may be 'overcorrecting' in a sense. We have added the following text to page 15 to address this:

'It should be noted that some of the group differences, particularly those obtained for volume and surface area, were rendered non-significant when controlling for ADHD (e.g., insula), whereas others were only significant when adjusting for ADHD (e.g., differences in inferior parietal folding in CD).

This is consistent with previous SBM studies which found that controlling for ADHD symptoms attenuated some of the group differences in cortical structure – particularly for surface area (Smaragdi et al., 2017) – whereas other CD-related effects were only present when adjusting for ADHD symptoms (Fairchild et al., 2015). Nevertheless, we note that our unadjusted findings are probably more representative of clinical reality, given that ADHD comorbidity is common in CD (Angold, Costello & Erkanli, 1999) and there is significant genetic overlap between ADHD and CD/ODD (Tuvblad et al., 2009), so controlling for ADHD symptoms might be 'overcorrecting'.'

* ADHD should probably be mentioned in the abstract where it influences findings - at the moment a reader would not be aware that some of the findings mentioned do not occur when ADHD is controlled for.

Response: We have amended the Results section of the Abstract to address this point, noting that the volume and surface area findings were rendered non-significant when controlling for ADHD.

* Relatedly, please could the authors explain the rationale for controlling for ADHD symptoms, in either the introduction or methods.

Response: We have added a rationale for controlling for ADHD symptoms to the Introduction (p. 5)

It is up to the authors how they approach this issue of ADHD - they may want to restructure their interpretation/discussion. Alternatively, I think it would be ok to keep a similar structure but make it clear that the findings are at the group level (CD/UR/HC), and that some of these findings change when ADHD is included. As a reader I am interested in the authors' interpretation of the effects of ADHD, and would welcome more discussion of this.

Response: We agree that the fact that ADHD comorbidity modulated the findings should have been emphasised more in the manuscript. We have now revised the Abstract, the Introduction, the Method, the Discussion and the Supplementary Tables to address this important comment.

Minor comments

The introduction states that conduct disorders are the most common reason for referral to Child and Adolescent Mental Health Services, but the reference (NICE, 2013) is not in the reference list so I could not verify it. Although conducted in Italy, other research has conduct disorder as quite low on the list of reasons for referral (Pedrini et al., 2015, Child Adol Psychiatry Mental Health). Please could the researchers add further supporting evidence for their statement, or qualify it if necessary (e.g. "one of the most common reasons").

Response: We thank the reviewer for pointing out this omission. According to the NICE guidelines for Conduct Disorder, published in 2013 and updated in 2017:

'Conduct disorders are the most common reason for referral of young children to child and adolescent mental health services (CAMHS). Children with conduct disorders also comprise a considerable proportion of the work of the health and social care system. For example, 30% of a typical GP's child consultations are for behavioural problems, 45% of community child health referrals are for behaviour disturbances and psychiatric disorders are a factor in 28% of all paediatric outpatient referrals. In addition, social care services have significant involvement with children and young people with conduct disorders, with more vulnerable or disturbed children often being placed with a foster family or, less commonly, in residential care. The demands on the educational system are also considerable and include the provision of special-needs education. The criminal justice system also has significant involvement with older children with conduct disorders.'

We now give the full reference for the NICE guidelines for the assessment and treatment of conduct disorder on p. 3 and in the reference list (p. 20, bottom line), and note in the manuscript that this figure applies to the UK.

I have some minor suggestions regarding the language:

* "Co-segregate" - is this the best/most appropriate term? From what I understand "co-segregate" refers to genetics, whereas in this paper it is being used to refer to genetics and environment. Therefore "co-occur" may be better?

Response: We have sought advice from geneticist colleagues on this point and have been informed that it is appropriate to use this term in a broader sense – to refer to anything that segregates (i.e., is inherited) together. Thus, genes and associated brain alterations could co-segregate within families – not just different genes on the same chromosome. We would prefer to continue using this term because 'co-occur' minimises the nature of the association.

* I would change "comparison subjects" to "comparison individuals"

Response: This has now been amended to 'typically-developing controls' on p. 3 (second para).

* The phrase "delinquent fathers" is out-of-date (and potentially offensive!) - so I would put it in inverted commas or use a different term.

Response: We agree that this term is potentially offensive and have switched to an alternative term: 'fathers with a history of criminal behavior' (see p. 4, first para)

Please could the authors clarify why they used different corrections for multiple comparisons for different analyses? (According to the last two paragraphs of Statistical Analyses in the Methods).

Response: Thank for the opportunity to clarify our analysis approach. The surface-based morphometry analysis approach (main analysis) consists of mass-univariate testing of approximately 160,000 vertices per hemisphere. Since the neuroimaging data are smoothed and effects in neighbouring vertices are spatially correlated, it is standard in neuroimaging data analysis to look for significant differences in clusters (i.e., groups of adjacent vertices) rather than in single vertices, which would yield a high rate of artefactual results with low/unclear neurobiological meaning. In order to achieve this, the Monte Carlo methods (which in these contexts simulate a high number of permutations under the null hypothesis and check how often the value of a statistic from the 'true' analysis is exceeded) is especially useful with surface-based morphometry data as traditional random field theory would require a more complex implementation with no theoretical advantages. This need does not arise, with tabular data (like the subcortical data, where a single value for each structure, for each individual, is generated by FreeSurfer) where, instead, parametric approaches are readily available while the implementation of a Monte Carlo method would yield no practical advantage. In short, we adopted the standard approach for correcting for multiple comparisons in each analysis (but this is more nuanced and arguably more powerful in the vertex-wise analysis of the cortical structure data).

The order of findings in the Results (lower left inferior parietal cortex first) is different to the order of findings in the Discussion (insula first). I would switch one of these around so the flow is the same.

Response: We agree with the reviewer's comment about the order of the findings in the Results and Discussion sections and have switched the order of the respective paragraphs in the Discussion to improve flow (see p. 12-13).

Reviewer #3: This study examines neuroanatomical markers of CD as mediators of genetic and environment risk for conduct disorder in a sample of affected adolescents, unaffected relatives, and

healthy controls. Findings in terms of structural differences between groups reveal provide remarkable insight into those neural markers that may be indicative of different levels of risk (and resilience) for CD. Overall, this manuscript is well written, and uses an appropriate methodology to shine light on an understudied area of knowledge related to CD - the role of neural markers. Remarkably, I have very few criticisms of this manuscript, well done! The introduction and discussion are logically organized, clearly and concisely justify the current study, and provide great supporting evidence. The authors use a well-validated approach (FreeSurfer/SBM) to quantify neural features. The results are easily parsable and appropriately presented. Moreover, the discussion excellently supports the results in the context of the current literature, while acknowledging the limitations and suggesting areas of novel research. I applaud the authors for a well-crafted study and well-polished manuscript.

Response: We would like to thank the reviewer for their positive feedback on our study and manuscript.

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May 18, 2021

Dear Professors Kendler and Murray,

Fairchild et al. 'Neuroanatomical markers of familial risk for antisocial behaviour in adolescents with Conduct Disorder and their unaffected relatives - R1'

Thank you for giving us the opportunity to revise our paper for consideration for publication in *Psychological Medicine*. We sincerely the anonymous reviewers for providing constructive comments on the manuscript, and hope that we have addressed these to your satisfaction (and theirs).

In particular, we have fully addressed Reviewer #2's major comment about the impact of controlling for ADHD comorbidity on the group differences in cortical structure. We have extensively revised the Abstract, Introduction, Methods, Discussion and Supplementary Materials to emphasise that some of the findings were no longer significant when controlling for ADHD symptoms, and provide interpretation of these changes – while noting that the unadjusted findings are probably more reflective of clinical reality. We have also provided a stronger justification for our analytic approach and have restructured the Discussion section to increase consistency with the Results section (i.e., the findings are reported, and then discussed, in the same order). As requested by Reviewer #1, we now report data for each of the ICU and YPI subscales in Table 1, in addition to the total scores, and give further information about the factor structure and psychometric properties of these questionnaires in Supplementary Materials. These data strengthen our argument that the unaffected relatives are indeed 'unaffected' because they score lower than the Conduct Disorder group (and similarly to the healthy controls) on the individual facets of psychopathy and three dimensions of callous-unemotional traits. We provide a detailed rebuttal of each of the points made in the Decision Letter in the Response to Reviewers.

We believe that addressing the Reviewers' comments has substantially improved the paper and very much hope that the revised manuscript is now considered acceptable for publication in *Psychological Medicine*. We look forward to hearing from you in due course.

Yours sincerely,

Graeme Fairchild, Ph.D., signed on behalf of all co-authors