Shared genetic influences on ADHD symptoms and very low-frequency EEG activity: a twin study

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Background: Attention deficit hyperactivity disorder (ADHD) is a common and highly heritable neurodevelopmental disorder with a complex aetiology. The identification of candidate intermediate phenotypes that are both heritable and genetically linked to ADHD may facilitate the detection of susceptibility genes and elucidate aetiological pathways. Very low-frequency (VLF; <0.5 Hz) electroencephalographic (EEG) activity represents a promising indicator of risk for ADHD, but it currently remains unclear as to whether it is heritable or genetically linked to the disorder. Methods: Direct-current (DC)-EEG was recorded during a cognitive activation condition in 30 monozygotic and dizygotic adolescent twin pairs concordant or discordant for high ADHD symptom scores, and 37 monozygotic and dizygotic matched-control twin pairs with low ADHD symptom scores. Structural equation modelling was used to quantify the genetic and environmental contributions to the phenotypic covariance between ADHD and VLF activity. Results: Attention deficit hyperactivity disorder was significantly associated with reduced VLF power during cognitive activation, which suggests reduced synchronization of widespread neuronal activity. Very low-frequency power demonstrated modest heritability (0.31), and the genetic correlation (0.80) indicated a substantial degree of overlap in genetic influences on ADHD and VLF activity. Conclusions: Altered VLF activity is a potential candidate intermediate phenotype of ADHD, which warrants further investigation of underlying neurobiological and genetic mechanisms. Keywords: ADHD, EEG, very low-frequency activity, endophenotype, genetics, heritability.

Introduction

Attention deficit hyperactivity disorder (ADHD) is one of the most prevalent neurodevelopmental disorders, and is characterized by impairing levels of inattentive, impulsive and hyperactive symptoms. ADHD persists beyond childhood in around 65% of cases, and is associated with high levels of clinical, psychosocial and economic burden (Faraone, Biederman, & Mick, 2006; Kendall, Taylor, Perez, & Taylor, 2008). Family and twin studies suggest that ADHD is under substantial genetic influence, with an average heritability estimate of 0.76 (Faraone et al., 2005). Candidate gene association studies support the role of genetic factors; however, these studies have shown inconsistency and nonreplication of findings, which suggests a complex genetic inheritance with a small risk conferred by individual genetic variants (Faraone et al., 2005).

One strategy to facilitate the detection of susceptibility genes and elucidate aetiological pathways is the identification of neurobiological processes that underlie the disorder and potentially mediate between genes and behaviour (Gottesman & Gould, 2003). Several candidate endophenotypes have been reported for ADHD (Kuntsi, McLoughlin, & Asherson, 2006; McLoughlin, Kuntsi, Brandeis, & Banaschewski, 2005). Electrophysiological abnormalities as measured using electroencephalography (EEG), in particular, are among the most promising indicators of increased genetic risk for ADHD with consistent associations and moderate-to-high heritability (McLoughlin et al., 2005; Tye, McLoughlin, Kuntsi, & Asherson, 2011).

Investigations of brain function in ADHD have recently extended to include very low-frequency activity (VLF; <.05 Hz) that can be measured using direct-current (DC)-coupled EEG recordings. Spontaneous VLF fluctuations synchronize activity across functionally specific, but widespread distributed neural networks, demonstrating a high degree of coherence within these circuits (Balduzzi, Riedner, & Tononi, 2008; Buszaki & Draguhn, 2004; Fransson, 2005; Vanhatalo et al., 2004). It has been suggested that VLF activity represents a reflection of the brain’s default-mode network (DMN) that is typically characterized by a low-frequency BOLD signal (Fox et al., 2005; Sonuga-Barke & Castellanos, 2007). Specifically, based on findings from fMRI studies, VLF fluctuations are posited to reflect toggling between two anti-correlated brain networks: a task-negative network that is active during wakeful resting states and characterized by these slow oscillations, and the
task-positive network that activates during goal-oriented activity (Fox et al., 2005; Sonuga-Barke & Castellanos, 2007). In addition, EEG studies have reported that VLF activity modulates the activity of higher frequency bands, suggesting regulation of gross cortical excitability (Monto, Palva, Voipio, & Palva, 2008; Vanhatalo et al., 2004). During an active task condition, slow cortical activity can also be generated in response to stimuli, and as such reflect event-related rather than spontaneous activity. These time-locked slow cortical potentials have been posited as an index of conscious perception (He & Raichle, 2009).

Abnormalities in VLF activity are associated with several neuropathological disorders (Broyd et al., 2009). Adults with attention problems demonstrated reduced spontaneous VLF power and reduced rest-to-task VLF attenuation (Helps, Broyd, James, Karl, & Sonuga-Barke, 2009; Helps, James, Debener, Karl, & Sonuga-Barke, 2008), which was replicated in a sample of adolescents with ADHD (Helps et al., 2010). In addition, reduced rest-to-task VLF attenuation has been associated with poor task performance (Helps et al., 2010) and similar slow fluctuations in task performance (Helps et al., 2009; Monto et al., 2008), which may underlie the deficits in task performance exhibited in ADHD (Castellanos et al., 2005). This is supportive of the default-mode interference hypothesis, which proposes that VLF activity, usually exhibited at rest, persists during cognitive activation in ADHD producing periodic lapses of attention (Fox et al., 2005; Sonuga-Barke & Castellanos, 2007). Measurement of VLF activity during a cognitive task allows investigation of this brain-behaviour association and its persistence during activation states that are altered in ADHD (Sergeant, 2000).

Twin research conducted on frequency bands above 1 Hz suggests that EEG is highly heritable (Smit, Posthuma, Boomsma, & Geus, 2005). A preliminary study of affected sibling pairs with ADHD indicated high sibling correlations of 0.53–0.76 during a cognitive activation condition (Loo & Smalley, 2008) although smaller estimates (0.22–0.61) have been reported in a larger study of multiplex families with ADHD (Loo et al., 2010). These studies have used family designs that are unable to discriminate between genetic and environmental influences. The twin design, however, allows separation of these effects by utilizing the different levels of genetic relatedness between monozygotic (MZ; 100%) and dizygotic (DZ; 50%) twin pairs (Neale & Cardon, 1992).

No twin study on VLF activity or its genetic overlap with ADHD has been conducted to date. This study aims to evaluate VLF activity as a potential intermediate phenotype of ADHD during a key developmental period by (a) estimating the heritability of VLF activity, (b) quantifying the strength of the phenotypic relationship of VLF activity with ADHD symptoms, and (c) examining the genetic and environmental overlap with ADHD symptoms. We measured VLF activity during a cognition activation condition [cued continuous performance test (CPT-OX)]; (Doehnert, Brandeis, Straub, Steinhansen, & Drechsler, 2008; McLoughlin et al., 2010; Valko et al., 2010) in adolescent MZ and DZ twin pairs (12–15 years old) concordant and discordant for high and low ADHD symptom scores. Structural equation modelling was applied, enabling separation of the phenotypic covariance between these two parameters (i.e. ADHD and VLF activity) into genetic and environmental components (Toulopoulou et al., 2007). Significant heritability and genetic overlap between ADHD and VLF activity would support this measure as a candidate endophenotype for the disorder, reflecting mediating processes on ADHD (Gottesman & Gould, 2003) or pleiotropic effects of genes (Kovas & Plomin, 2006).

Methods

Sample

The sample was selected from the Twins’ Early Developmental Study (TEDS), a birth cohort study of all twins born in England and Wales between 1994 and 1996 (Trouton, Spinath, & Plomin, 2002). Zygosity was determined using a zygosity questionnaire that has been shown to have 95% accuracy (Price et al., 2000); For cases where zygosity remains unclear from this questionnaire, DNA testing was conducted. The TEDS sample is representative of the general population in terms of parental education, ethnicity and employment status (Kovas, Haworth, Dale, & Plomin, 2007).

The Neurophysiological Study of Activity and Attention in Twins (NEAAT) subset used in this study consisted of 67 male twin pairs in groups of 22 pairs concordant for high levels of ADHD symptoms (MZ: 11; DZ: 11), eight pairs discordant for ADHD symptoms (MZ: 2; DZ: 6) and 37 control pairs discordant for low levels of ADHD symptoms (MZ: 21; DZ: 16). Twin pairs were selected based on an analysis of symptom development over time using the program MPLUS (Data S1). This identified sub-groups of individuals who have been stably high or stably low at ages 8, 12 and 14, using the 18 DSM-IV ADHD items from the Long Version of the Conners’ Parent Rating Scale (Conners, Sitarenios, Parker, & Epstein, 1998a). This ensured that the selected twin pairs were consistently concordant or discordant for high levels of ADHD symptoms (corresponding to a clinical diagnosis) or unaffected controls who had consistently low ADHD symptoms. Demographic characteristics are given in Table 1. Participating families gave their written informed consent, and the study was approved by King’s College London Psychiatry, Nursing and Midwifery Research Ethics Sub-Committee (PNM/08/09-89).

Task and stimuli

The cued-CPT (flanker version; (Doehnert et al., 2008; McLoughlin et al., 2010, 2011; Valko et al., 2010) consists of a black letter array formed of a centre letter flanked on each side by distractor letters, presented in

Table 1: Raw scores and mean comparisons of demographic characteristics adjusted for genetic relatedness.

<table>
<thead>
<tr>
<th></th>
<th>All ADHD versus all controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
</tr>
<tr>
<td>MZ Control (n = 46)</td>
<td>14.45</td>
</tr>
<tr>
<td>DZ Control (n = 35)</td>
<td>14.56</td>
</tr>
<tr>
<td>MZ ADHD (n = 22)</td>
<td>14.01</td>
</tr>
<tr>
<td>DZ ADHD (n = 21)</td>
<td>14.03</td>
</tr>
<tr>
<td>IQ* (n = Raven's matrices, WISC-III-PI multiple choice vocabulary subtests)</td>
<td></td>
</tr>
<tr>
<td>MZ Control (n = 27, 28)</td>
<td>103.28</td>
</tr>
<tr>
<td>DZ Control (n = 21, 23)</td>
<td>103.51</td>
</tr>
<tr>
<td>MZ ADHD (n = 6, 6)</td>
<td>91.88</td>
</tr>
<tr>
<td>DZ ADHD (n = 7, 9)</td>
<td>96.80</td>
</tr>
</tbody>
</table>

ADHD, attention deficit hyperactivity disorder.

*General cognitive ability was assessed at age 14 as part of ongoing TEDS web-based data collection. The twins were tested on the WISC-III-PI vocabulary multiple choice subtests (Wechsler, 1992) and Raven’s standard and advanced progressive matrices (Raven, 1996). Missing scores were imputed from multiple IQ subtest scores across ages 7, 12 and 14 using the ICE command in Stata for each outcome variable. A g score was created with equal weights for the two tests by summing their standardized scores within the NEAAT sample. Further information about g as measured in TEDS can be found elsewhere (e.g., Haworth et al., 2010). Measures of g and IQ correlate highly and both provide an index of general intelligence (Jensen, 1998), and as such, IQ can be calculated from standardized g using the formula IQ = (g × 15) + 100.

**EEG recording and analysis**

Electroencephalography was recorded using 62 channel DC-coupled recording system (extended 10–20 montage). Electrode impedance was kept below 5 kΩ. The reference electrode was positioned at FCz. Vertical and horizontal electro-oculograms (EOGs) were simultaneously recorded from electrodes above and below the left eye and at the outer canthi. The signal was digitized at 500 Hz sampling rate, stored and analysed offline.

Data were analysed in Brain Vision Analyzer (2.0) (Brain Products, Munich, Germany). The signal was re-referenced offline to the average reference, and
downsampled to 256 Hz. We applied 0.02–4 Hz (12dB/Oct) Butterworth filters. Ocular artefacts were removed from the data using biased infomax Independent Component Analysis (ICA (Jung et al., 2000) The extracted independent components were manually inspected, and ocular artefacts were removed by back-projection of all but those components. Continuous EEG was segmented into 50 s epochs. Segments with artefacts exceeding 120 μV peak-to-peak in any channel were rejected. A DC-Detrend command was executed to remove linear drifts from the data. Fast-Fourier Transform analysis was performed on the data from each of the electrodes for each participant. Fifty second Hanning windows were used, and power was calculated. The current study focused on the frequency band 0.02–0.2 Hz that has been implicated previously in ADHD (Helps et al., 2010). To allow comparison with other studies investigating familial effects on higher frequency bands in ADHD (Loo et al., 2010), VLF power was compared across frontal (Fz, F3, F4), central (Cz, C3, C4), parietal (Pz, P3, P4) and, in addition, occipital (Oz, O1, O2) scalp electrode locations.

**Statistical analysis**

Two participants were excluded from analysis due to excessive artefact (DZ control) and extreme commission errors (n = 37) indicative of insufficient task engagement (MZ ADHD).

For the analysis of performance data, the effects of age and general intelligence were regressed out of the data using Stata. All measures had pronounced heterogeneity of variance and skewed distributions, and were log-transformed (optimized minimal skew through the lnskew0 command in Stata).

**Preparation of EEG data prior to model fitting.** The effects of age and general intelligence were regressed out of the data using Stata due to significant associations between both ADHD (Table 1) and VLF activity (age r = −0.12 to −0.19; g r = −0.26 to −0.33). Although general cognitive ability is confounded by the presence of psychiatric disorder, given that age and IQ are highly predictive of cognitive performance, regressing out these effects before twin modelling ensures straightforward interpretation; any reported associations between VLF activity and ADHD are independent of general cognitive ability and neurodevelopmental changes. Simultaneous analyses of dichotomous and continuous data could not be performed in the Mx program, and so both ADHD, which was scored as a dichotomous attribute, and the VLF measure, which was scored as a continuous variable, were modelled as threshold traits. Accordingly, each VLF measure was ordinalized into five equal classes in terms of proportions, which should capture most of the information in the continuous data. To provide a normal distribution for a more successful ordinal cut and to fulfill twin model assumptions, outliers were removed separately for each VLF parameter (±3 SD from the mean; final numbers shown in Table 3) and log-transformed (optimized minimal skew through the lnskew0 command in Stata). The Mx software for structural equation modelling (Neale, Boker, Xie, & Maes, 1999) was then used to estimate polythetic correlations and genetic model parameters using maximum likelihood statistics, while correcting for the selected nature of the sample.

**Comparison of means.** The comparisons of mean values were analysed by means of a regression command in Stata that allows for nonindependent observations (e.g. twin pairs) using a robust cluster command to estimate standard errors. The association between the VLF activity and ADHD symptom scores, and VLF activity and performance measures, was investigated using Pearson’s product moment correlation coefficient on transformed residuals. Performance measures for correlation analysis were selected on the basis of trends towards case-control differences.

**Twin correlations.** Twin correlations between VLF activity and ADHD were estimated by fitting a constrained correlational model to the observed MZ and DZ data to produce (a) one overall within-twin across-trait correlation regardless of zygosity, i.e. between ADHD and VLF, (b) the MZ and (c) the DZ cross-twin within-trait correlation for VLF, and (d) the MZ and (v) DZ cross-twin cross-trait correlation, by comparing one twin’s ADHD score and the co-twin’s VLF score. The MZ and DZ cross-twin correlations were fixed according to the point estimates for ADHD derived from the heritability estimate of a meta-analysis (MZ = 0.76, DZ = 0.38; Faraone et al., 2005), due to the uncertain ascertainment process for twins, concordant and discordant for ADHD symptoms [see below Accounting for Selection; (Toulopoulou et al., 2007)].

**Genetic model fitting.** A more sophisticated approach to the analysis of twin data is using structural equation models that model correlations between variables within individuals and across twins using relationships between observed and latent variables (Rijndijk & Sham 2002). Liability-threshold models were used for both ADHD and VLF variables (Falconer & Mackay, 1996). The liability-threshold models for the dichotomized ADHD phenotype (affected versus nonaffected) assume that risk is normally distributed on a continuum and that the disorder occurs only when a certain threshold is exceeded (Neale & Kendler, 1995). Both affected and unaffacted individuals were assumed to be part of the same distribution of liability to the disorder, with each individual being either below or above the threshold.

Through examination of the differences in correlations between MZ and DZ twin pairs, the variance of VLF activity can be decomposed into additive genetic (A), shared environmental (C) and unique environmental (E) components to estimate (a) the heritability of VLF activity, and (b) genetic and environmental correlations of ADHD with VLF activity. The applied ACE bivariate correlated factors liability-threshold model is illustrated in Figure 1. The parameter estimates from the model can be used to estimate the correlation between the genetic factors for ADHD and VLF activity (rG), which is an index of shared genetic effects between these parameters, and similarly for correlations of unique environmental factors (re). As the rG and re correlations do not take into account the heritability of either trait, it is possible for a large genetic correlation to actually explain a very small portion of the observed covariation between these two traits. Combining the information from the rG and re with the heritabilities of each trait, we
can establish the genetic (ρph-a) and unique environmental (ρph-e) contributions to the total phenotypic correlation (ρph) between ADHD and VLF activity (Toulouropoulou et al., 2007).

Accounting for selection. As the data are from twins selected for high or low ADHD symptoms scores, rather than a random sample, the heritability of ADHD cannot be estimated. Selected samples are more efficient and can be more powerful when studying low prevalence disorders (Neale, Eaves, & Kendler, 1994), but model fitting analyses will usually require an ascertainment correction. As selection is based on ADHD and blind to VLF values, the required ascertainment correction will depend only on the model for ADHD, and therefore the model parameters for ADHD were fixed to constant values supported by a meta-analysis of 20 studies of ADHD (model 1: $h^2 = 0.76$, $c^2 = 0$, $e^2 = 0.24$; (Faraone et al., 2005); univariate twin modelling of DSM-IV based ADHD scores on the Conners’ scores at age 8 in the TEDS sample (model 2: $h^2 = 0.89$, $c^2 = 0$, $e^2 = 0.11$ (Ronald, Simonoff, Kendler, & Plomin, 2008) and at age 12 in the TEDS sample (model 3: $h^2 = 0.73$, $c^2 = 0.13$, $e^2 = 0.14$; C. Greven, unpublished data). In these models, the variance components for VLF activity, as well as their relationship with ADHD, are free parameters to be estimated from the data (Figure 1). The different parameter estimates for ADHD from the different models had no effect on the variance components for VLF activity, and thus, we only report those of the first model. In addition, we fixed the ADHD prevalence rate to a lifetime risk of 5% (Polanczyk, de Lima, Horta, Biederman, & Rohde, 2007).

Results

Results from the regression and correlation analyses

Regression analyses indicated no significant differences between ADHD and control twins for cognitive performance measures (Table 2). VLF power was highest in central regions and was significantly reduced for central and parietal locations in the ADHD group, with the strongest association with central locations (Table 3). Therefore, to reduce the total number of variables and subsequent multiple testing bias VLF power at central regions was used in correlations between symptom scores and performance measures, and in twin modelling analyses to minimize heteroscedasticity. Significant associations between VLF power and symptom scores were found in the control sample only (Table 4), suggesting that increased symptoms of inattention and hyperactivity/impulsivity are associated with increased VLF power in typically developing adolescents. Reduced VLF activity was significantly associated with increased response variability in the ADHD group only (Table 5). All other associations were nonsignificant.

Results from the twin modelling analyses

The MZ cross-twin within-trait correlation for VLF power ($r = 0.37; 95%$ CI, 0.002 to 0.64) was greater...
ADHD, attention deficit hyperactivity disorder; DZ, dizygotic; MZ, monozygotic; VLF, very low-frequency power.

Table 3 Summary statistics and mean comparisons adjusted for genetic relatedness for VLF power based on transformed age- and IQ-regressed scores with extreme outliers removed

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All ADHD versus all controls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLF frontal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MZ Control (n = 45)</td>
<td>4.36</td>
<td>0.36</td>
<td>−1.88</td>
<td>.07</td>
</tr>
<tr>
<td>DZ Control (n = 35)</td>
<td>4.36</td>
<td>0.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MZ ADHD (n = 21)</td>
<td>4.10</td>
<td>0.45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DZ ADHD (n = 27)</td>
<td>4.28</td>
<td>0.48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLF central</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MZ Control (n = 46)</td>
<td>5.29</td>
<td>0.18</td>
<td>−3.03</td>
<td>.003</td>
</tr>
<tr>
<td>DZ Control (n = 35)</td>
<td>5.28</td>
<td>0.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MZ ADHD (n = 20)</td>
<td>5.17</td>
<td>0.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DZ ADHD (n = 26)</td>
<td>5.18</td>
<td>0.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLF parietal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MZ Control (n = 46)</td>
<td>4.92</td>
<td>0.27</td>
<td>−2.25</td>
<td>.03</td>
</tr>
<tr>
<td>DZ Control (n = 35)</td>
<td>4.87</td>
<td>0.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MZ ADHD (n = 21)</td>
<td>4.79</td>
<td>0.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DZ ADHD (n = 27)</td>
<td>4.74</td>
<td>0.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLF occipital</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MZ Control (n = 46)</td>
<td>4.34</td>
<td>0.46</td>
<td>1.99</td>
<td>.05</td>
</tr>
<tr>
<td>DZ Control (n = 36)</td>
<td>4.28</td>
<td>0.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MZ ADHD (n = 21)</td>
<td>4.20</td>
<td>0.51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DZ ADHD (n = 26)</td>
<td>4.10</td>
<td>0.38</td>
<td></td>
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</tr>
</tbody>
</table>

ADHD, attention deficit hyperactivity disorder; DZ, dizygotic; MZ, monozygotic; VLF, very low-frequency power.

Table 4 Correlations between VLF power at central scalp locations and symptoms of ADHD (Pearson’s product moment correlation on transformed age and IQ-regressed scores)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>MZ</th>
<th>DZ</th>
<th>ADHD</th>
<th>MZ</th>
<th>DZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inattention**</td>
<td>0.41**</td>
<td>0.19</td>
<td>0.70**</td>
<td>−0.01</td>
<td>0.04</td>
<td>−0.05</td>
</tr>
<tr>
<td>Hyperactivity/</td>
<td>0.44**</td>
<td>0.34*</td>
<td>0.71**</td>
<td>−0.09</td>
<td>−0.22</td>
<td>0.02</td>
</tr>
<tr>
<td>impulsivity*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p < .05, **p < .01
ADHD, attention deficit hyperactivity disorder; DZ, dizygotic; MZ, monozygotic.

ADHD symptom scores based on the Long Version of the Parent Conners’ Rating Scale (Conners et al., 1998a) collected on the day of testing.

Table 5 Correlations between VLF power at central locations and performance measures (Pearson’s product moment correlation on transformed age and IQ-regressed scores)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>MZ</th>
<th>DZ</th>
<th>ADHD</th>
<th>MZ</th>
<th>DZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD-RT</td>
<td>0.07</td>
<td>0.02</td>
<td>0.11</td>
<td>−0.21</td>
<td>−0.46*</td>
<td>0.20</td>
</tr>
<tr>
<td>CV</td>
<td>0.08</td>
<td>0.06</td>
<td>0.09</td>
<td>−0.28*</td>
<td>−0.49*</td>
<td>0.04</td>
</tr>
</tbody>
</table>

*p < .05
ADHD, attention deficit hyperactivity disorder; DZ, dizygotic; CV, coefficient of variation (SD-RT/MRT); MRT, mean reaction time in milliseconds; MZ, monozygotic; SD-RT, within-subject variability in RTs in milliseconds.

Table 6 Correlation on transformed age and IQ-regressed scores (Pearson’s product moment correlation between ADHD and VLF power)

<table>
<thead>
<tr>
<th></th>
<th>MZ ADHD</th>
<th>DZ ADHD</th>
<th>MZ Control</th>
<th>DZ Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inattention**</td>
<td>0.41**</td>
<td>0.34*</td>
<td>0.46**</td>
<td>0.20</td>
</tr>
<tr>
<td>Hyperactivity/</td>
<td>0.44**</td>
<td>0.34*</td>
<td>0.71**</td>
<td>−0.22</td>
</tr>
<tr>
<td>impulsivity*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p < .05
ADHD, attention deficit hyperactivity disorder; DZ, dizygotic; MZ, monozygotic.

ADHD symptom scores based on the Long Version of the Parent Conners’ Rating Scale (Conners et al., 1998a) collected on the day of testing.

Discussion
This study aimed to evaluate very low-frequency (VLF) neuronal activity as an intermediate phenotype of ADHD in a sample of adolescent monozygotic and dizygotic twin pairs concordant and discordant for ADHD symptoms. Genetic analyses showed that VLF activity demonstrates modest heritability, with no evidence of significant shared environmental effects. Structural equation modelling revealed a significant phenotypic association between high ADHD symptom scores during adolescence and reduced VLF activity during cognitive activation. Genetic factors were the main source of this association, and unique environmental factors were not significant. This is the first study to support VLF activity as an electrophysiological marker of genetic risk in ADHD.
Very low-frequency EEG intermediate phenotypes of ADHD

Heritability of VLF activity during a cognitive activation condition is consistent with reports of high heritability (Smit et al., 2005) and high sibling similarity in an ADHD sample (Loo et al., 2010) in higher frequency bands. This suggests that VLF activity has a genetic basis combined with a moderate contribution from unique environmental effects. The substantial genetic correlation between ADHD and VLF activity indicates that they are substantially influenced by the same genes, supporting VLF activity as a putative intermediate phenotype of the disorder. This finding is an important step in understanding the neurobiological pathways involved in the disorder, and potentially to facilitate in the detection of susceptibility genes. For example, findings suggest that a catecholaminergic deficiency underlies abnormal VLF oscillations, which is also widely reported in ADHD (Castellanos et al., 2005). Indeed dopamine reuptake inhibitors (e.g. methylphenidate), which are used as treatment for ADHD, have been found to modulate slow oscillations in subcortical structures (Ruskin et al., 2001). Causal tests of mediation are necessary to identify if these electrophysiological markers mediate aetiological effects on ADHD, rather than pleiotropic (or epiphenomenal) processes (Walters & Owen, 2008).

The phenotypic association between ADHD and VLF activity is consistent with previous studies reporting reduced VLF power at rest in ADHD (Helps et al., 2008, 2010). In addition, previous studies report an association between increased rest-to-task VLF attenuation and a higher number of inattention symptoms in typical adults (Helps et al., 2009) and a clinical ADHD group (Helps et al., 2010). In the present study, higher VLF activity was associated with increased levels of inattention and hyperactivity/impulsivity in the control group. Such discrepancies between control and ADHD participants may reflect neuropathological differences between groups, and also the robustness of the longitudinal method employed for group selection compared with investigating symptom scores at the single time-point of data collection.

Overall, the findings for both groups support spontaneous VLF activity, typically associated with resting periods as exhibited by slow fluctuations of the BOLD signal, as present during cognitive activation, suggesting that it represents a continuous process that is present during both resting and goal-oriented periods (Fransson, 2006). VLF activity are proposed to be an index of information integration or functional connectivity through synchronization over widely distributed neuronal networks (Biswal, Yetkin, Haughton, & Hyde, 2005; He & Raichle, 2009). The association between ADHD and reduced VLF power in this study may therefore reflect reduced synchronization of these widespread circuits. As VLF activity is proposed to relate to the DMN, one of several widely distributed networks as defined by fMRI (Sonuga-Barke & Castellanos, 2007), these findings suggest that individuals with ADHD demonstrate reduced DMN synchronization during cognitive activation or abnormal toggling between the anti-correlated introspective task-negative and exteroceptive task-positive networks, producing impairment to the functions they each serve (Fox et al., 2005; Fransson, 2005; Sonuga-Barke & Castellanos, 2007).

The measurement of VLF activity during this cognitive task may include both spontaneous VLF activity and event-related VLF slow cortical potentials time-locked to the cue stimulus (the contingent negative variation). Although in this particular study there is limited evidence for a relationship between spontaneous VLF activity and event-related VLF activity (see Data S2), it is still important that future work considers this relationship, particularly because without correction for multiple testing, there is a small yet significant correlation between these measures. Along with the significant correlation between VLF activity and delta activity, such phenotypic overlap may suggest that the genetic contributions reported may be shared across frequency bands. In accordance, VLF phase has been shown to correlate with the magnitude of higher frequency bands suggesting a modulation of gross cortical excitability (Monto et al., 2008; Vanhatalo et al., 2004). Further work investigating phase locking with higher frequency bands as a measure of synchronization will increase our understanding of the mechanisms underlying this overlap, particularly when used in genetically sensitive designs.

Advances in EEG source localization techniques are likely to provide further insight into the relationship between the BOLD signal and EEG; recent work using sLORETA identified differential localization of rest-task VLF attenuation dependent on ADHD status, in the absence of case-control differences in rest-task attenuation itself (Broyd, Helps, & Sonuga-Barke, 2011). This highlights potential topographical and source differences between control and clinical populations. In accordance with this, in the current study, we identified group differences in VLF activity at central and parietal scalp locations only, and genetic effects emerged primarily for central scalp locations. Localization differences may reflect the measure of VLF activity used (i.e. a comparison of VLF activity between rest and task, as opposed to VLF activity during the task itself) and task-specific demands that particular brain processes/regions subserve. Examination of changes over time and space in VLF activity in well-validated, and robust EEG/ERP paradigms is required.

For measures of task performance, phenotypic associations with ADHD were nonsignificant. This is contrary to findings reporting slower and more variable reaction times in ADHD (Klein, Wendling, Huettnner, Ruder, & Peper, 2006; Kuntsi et al., 2010) and increased omission errors (Willcutt, Doyle, Nigg, Faraone, & Pennington, 2005). The lack of a group...
difference in the performance data is likely to be due to the use of the CPT-OX with flankers that was not specifically designed to optimally measure cognitive performance. Another possible reason for our lack of cognitive performance findings is our use of a population sample, rather than a clinical sample, albeit one selected for low and high ADHD symptom scores.

One study reported increased RT variability in a population sample using ADHD symptom scores (similar to the study design here), but the data were collected at a younger age, in a larger sample and using task conditions that maximise RT variability, such as a slow event rate and lack of rewards (Kuntsi, Wood, Van der Meere, & Asherson, 2009). The greater association between ADHD and neurophysiological markers compared with task performance suggests EEG as a more sensitive index of genetic loading for ADHD than the cognitive performance measures, at least as derived from the task used in this study.

According to the default-mode interference hypothesis, a failure to fully attenuate VLF activity in cognitive activation conditions contributes to attention lapses associated with ADHD (Sonuga-Barke & Castellanos, 2007). VLF activity was not associated with task performance measures in this study across the whole sample, but when collapsing the sample by ADHD status and zygotosity, significant associations were found between reduced VLF power and increased response variability in the ADHD group only. This suggests that reduced synchronization during a task is associated with poor performance in ADHD, and is in accordance with previous studies that report associations between rest-task attenuation and increased errors and variability (Helps et al., 2010). Further work incorporating analysis of trial-to-trial changes in the synchronicity between task performance and VLF activity is required (Castellanos et al., 2005; Helps et al., 2009), and linking this to optimal measures of RT variability will help to clarify this association.

Certain limitations must be taken into consideration. First, the sample size is relatively small for a twin study, such that although the estimates are modest to large, the confidence intervals around these estimates are also large introducing an overlap in the confidence intervals for MZ and DZ twins. This might suggest that the contribution from genetics is not significant; nevertheless, combining the significant MZ correlations with the significant mean correlation differences suggests a familial nature that is likely to be of a genetic basis based on the fixed parameters used. This limited statistical power also restricted the estimation of shared environmental (as suggested by cross-twin within-trait correlations for VLF power) and nonadditive genetic influences (as suggested by cross-twin cross-trait correlations between ADHD and VLF power). We acknowledge that such influences may have contributed to the estimates of heritability and genetic overlap between ADHD and VLF activity in our study, although the genetic contribution presented is not overestimated.

The substantial contribution of unique environmental influences on VLF activity may reflect error or instability in the measure. Reliability sets an upper limit on the estimates of heritability; any deviations from perfect reliability will increase measurement error and therefore unique environmental influences (Kuntsi et al., 2006). Studies of test–retest reliability are required to investigate the stability of both phenotypic associations and genetic influences on VLF activity (de Geus, 2002). In addition, the imputation of a relatively substantial proportion of general cognitive ability as a potential confounding influence may introduce further error arising from predicted scores, although the use of a robust multiple-imputation method is likely to have reduced this error variance. As we do not know whether the underlying causes of altered VLF activity are shared or disorderspecific, particularly as abnormalities are reported in several other neuropathological disorders such as autism and schizophrenia (Broyd et al., 2009), it is important that future work directly compares VLF activity profiles across disorders.

In conclusion, applying genetic model fitting for the first time to VLF neuronal activity in ADHD, we have demonstrated modest heritability of VLF activity and substantial genetic overlap between ADHD and VLF activity. This supports the relevance of this novel measure of physiological arousal to our understanding of ADHD, and indicates that lower VLF activity during cognitive activation is a potential intermediate phenotype for ADHD. This provides a basis for identification of specific genes that influence both ADHD and VLF activity, and warrants the investigation of the underlying mechanisms of VLF activity to provide insight into the pathophysiological underpinnings of ADHD.

Supporting information
Additional Supporting Information may be found in the online version of this article:

Data S1. Growth mixture modelling on longitudinal TEDS ADHD data
Data S2. Associations between VLF power and higher frequency bands

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Key points

- The identification of neurobiological processes that mediate between genes and behaviour may elucidate the aetiological pathways underlying complex psychiatric disorders such as ADHD.
- The current study investigated genetic effects on very low-frequency (VLF) neuronal activity in adolescent twin pairs concordant or discordant for high or low ADHD symptom scores.
- Subjects with high ADHD symptom scores demonstrated reduced VLF power that was associated with poor performance.
- VLF activity demonstrated moderate heritability and shared genetic influences with ADHD symptoms, suggestive of an ideal intermediate phenotype of the disorder.
- Investigating these processes provides insight into the pathophysiological underpinnings of ADHD, and may help in understanding their impact on social and academic functioning.

References


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