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Quantitative MRI brain in congenital adrenal hyperplasia: in vivo assessment of the cognitive and structural impact of steroid hormones

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Quantitative MRI brain in congenital adrenal hyperplasia: \textit{in vivo} assessment of the cognitive and structural impact of steroid hormones

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Context: Brain white matter hyper-intensities are seen on routine clinical imaging in 46\% of adults with congenital adrenal hyperplasia (CAH). The extent and functional relevance of these abnormalities have not been studied using quantitative MRI analysis.

Objective: To examine white matter microstructure, neural volumes and CNS metabolites in CAH due to 21-hydroxylase deficiency (21OHD) and to determine whether identified abnormalities are associated with cognition, glucocorticoid and androgen exposure.

Design, setting and participants: A cross-sectional study at a tertiary hospital including 19 females (18-50 years) with 21OHD and 19 age-matched healthy females.

Main outcome measure: Recruits underwent cognitive assessment and brain imaging including; diffusion weighted imaging of white matter, T1-weighted volumetry and magnetic resonance spectroscopy for neural metabolites. We evaluated white matter microstructure using tract-based spatial statistics. We compared cognitive scores, neural volumes and metabolites between groups and relationships between glucocorticoid exposure, MRI and neurologic outcomes.

Results: Patients with 21OHD had widespread reductions in white matter structural integrity, reduced volumes of right hippocampus, bilateral thalami, cerebellum and brainstem, and reduced mesial temporal lobe total choline content. Working memory, processing speed, and digit span and matrix reasoning scores were reduced in patients with 21OHD, despite similar education and
intelligence to controls. 21OHD individuals exposed to higher glucocorticoid doses had greater abnormalities in white matter microstructure and cognitive performance. 

**Conclusion:** For the first time we demonstrate that 21OHD and current glucocorticoid replacement regimens have a profound impact on brain morphology and function. If reversible, these CNS markers represent a potential target for treatment.

Using quantitative neuroimaging and cognitive assessment, Webb et al demonstrate that 21OHD and current glucocorticoid replacement regimens have a profound impact on brain morphology and function.

**Introduction**

Steroid hormones regulate CNS development, exerting trophic effects on cell survival, differentiation, maturation and synaptogenesis (1,2). The most common variant of the inborn steroidogenic disorder congenital adrenal hyperplasia (CAH), 21-hydroxylase deficiency disrupts adrenal steroidogenesis at critical branch points of glucocorticoid (cortisol) and mineralocorticoid (aldosterone) synthesis. Reduced glucocorticoid feedback to the hypothalamic-pituitary axis results in increased central stimulation of adrenal steroidogenesis. This leads to; increased adrenal androgen production and subsequent systemic androgen excess, glucocorticoid deficiency and, in two-thirds of patients, clinically apparent mineralocorticoid deficiency (3). Medical treatment aims to replace the deficient hormones and to limit exposure to androgen excess. However, in clinical practice, patients are frequently exposed to significant degrees of both glucocorticoid and androgen excess (3). CAH therefore provides a pathophysiological model suited to study the impact of fluctuations in glucocorticoid and androgen exposure on human brain structure and function.

Qualitative evaluation of structural T2-weighted MRI studies in patients with CAH provides evidence white matter hyper-intensities (leukoaraiosis) in up to 46% of patients (4,5) predominantly located in the temporal lobe, amygdala, hippocampus, periventricular and corpus callosum. Leukoaraiosis is an uncommon incidental finding in healthy adults aged <45 years (6). The pathophysiology of leukoaraiosis remains poorly defined (7); however, the presence of local white matter abnormalities appears to be indicative of a global dysregulation of white matter structure, with changes in white matter microstructure in affected individuals not only localised to those areas of abnormality identified on visual inspection (8). Diffusion weighted imaging a non-invasive MRI technique, provides quantitative indices of brain development, enabling the in vivo examination of white matter microstructure and characterization of white matter anatomy including the degree of connectivity between different regions of the brain (9,10). In pathologic conditions, diffusion anisotropy of water molecules is reduced because of altered diffusivity and disorganization of the white-matter fibres. These measurements may become abnormal even before the lesion is morphologically apparent on conventional MRIs, helping both with early detection and with defining the extent of lesions. We hypothesized that diffusion tensor imaging (DTI) would identify significant reductions in quantitative indices of white matter microstructure in patients with CAH, including mean diffusivity, which reflects the degree of water mobility, and fractional anisotropy, which is affected by axonal caliber, fibre density and degree of myelination (11).

Measurements of in-vivo human brain volumes can provide novel insights into the pathophysiology of patients’ cognitive abnormalities with neuroimaging increasingly used in clinical trials as a biomarker to study the impact of treatment modifications. The extent of volumetric abnormalities present in patients with CAH has not previously been investigated using high resolution MRI in conjunction with automated quantitative data analysis. In view of the high
concentrations of androgen, mineralocorticoid and glucocorticoid receptors in the hippocampus, amygdala, thalamus, brainstem and cerebellum (2,12-14), we hypothesized that patients with CAH would have localized volume loss in these regions.

Magnetic resonance spectroscopy (MRS), which enables the in vivo measurement of cerebral metabolites, provides a sensitive tool to measure the impact of changes in steroid hormone exposure on the brain. It has previously been used to investigate how excess glucocorticoid exposure affects cerebral metabolite concentrations in patients with Cushing’s disease (15). Patients with Cushing’s syndrome have reduced total choline, with normalization of the brain metabolite ratios following resolution of Cushing’s disease (15). MRS has not previously been performed in patients with CAH in whom we hypothesized that total choline would be reduced.

Glucocorticoids exert an inverted U-shape influence on human cognition, in particular on acquiring and consolidating memory (2) whereas androgens have an overall beneficial effect on cognitive control, verbal memory and spatial cognition in humans (1,13,16). Whilst previous studies in patients with CAH have consistently identified impairments in short-term and working memory performance, thought to relate to excess glucocorticoid exposure, the relationship between these cognitive abnormalities and changes in brain structure have not previously been investigated (17). We hypothesized that neural abnormalities would correlate with cognitive performance, and that increased glucocorticoid exposure would be associated with poorer performance on neuropsychometric tests, reductions in neural volumes, fractional anisotropy and total choline.

Subjects and methods

Patient selection
Adult women with CAH due to 21-hydroxylase deficiency diagnosed by hormonal and genetic testing were recruited from the endocrine clinic at University Hospital Birmingham and via the patient support group CLIMB, Living with CAH. Local advertisements were placed within University Hospital Birmingham NHS Foundation Trust to recruit control subjects. Exclusion criteria for both groups were pregnancy, hypothyroidism, a medical condition known to affect cerebral anatomy or metal in the body. Additional exclusion criteria for the control group were diagnosed psychiatric disorder, glucocorticoid use (ever) or diagnosed learning disabilities. The study was approved by the national research ethics committee West Midlands and all participants gave written informed consent prior to participation.

Ethnicity, age at diagnosis, handedness, highest educational level, employment category and current glucocorticoid replacement dose were recorded. The genotype of patients with 21OHD was classified into genotype groups null, A, B, and C (18). In patients taking prednisolone instead of hydrocortisone, the equivalent dose was calculated by multiplying total prednisolone dose by four (19). Educational status was graded 1-6 based on the participant’s highest level of educational achievement (1. Completed primary school, 2. Completed middle school to age 16 years, 3. Completed high school to age 18 years, 4. Higher studies beyond school, 5. Degree, 6. Post-graduate degree). All participants with CAH had fasting 9:00-11.00h blood samples taken on the day of MRI acquisition prior to any medication for measurements of plasma renin concentration, serum 17-hydroxyprogesterone (17-OHP), DHEAS, androstenedione and testosterone liquid chromatography and tandem mass spectrometry. Standing height was measured with a stadiometer.

Psychometric assessment
Psychometric testing comprising Wechsler Adult Intelligence Scale (WAIS) IV and Wechsler Memory Scale (WMS) IV assessments was administered by a trained psychology research assistant (20,21).

**Health-related quality of life and mood questionnaires**
All study participants completed the World Health Organization Quality of Life, (WHOQOL) and Hospital Anxiety and Depression Scale (HADS) questionnaires (22,23). WHOQOL is an instrument to assess general well-being and comprises 26 items to broadly measure 4 domains; physical health, psychological health, social relationships and environment. The HADS questionnaire is a 14-scale questionnaire that is used to ascertain the levels of depression and anxiety that a subject is experiencing.

**MRI acquisition**
MR imaging was performed on a 3 T Philips Achieva scanner using a 32-channel head coil. One neuroradiologist (VS) blinded to the clinical data reviewed all images. DTI was performed using a monopolar Stejskal-Tanner sequence over 61 directions, with a single b=0 image and b-factor of 1500 s/mm$^2$. Data was collected in the axial plane, with an imaging matrix of 112x112 over 72 slices at an isotropic resolution of 2 mm. An echo time of 78 ms was used with a SENSE parallel imaging factor of 2. High-resolution 3D T1-weighted images were acquired (TR = 8.40 msec, TE = 3.8 msec, flip angle = 8°, FOV = 288 mm, 175 slices, 2 mm isotropic voxels). MRS data was acquired from an 18x18x18 mm voxel in the left mesial temporal lobe and a 20x20x20 mm voxel in the right parietal lobe. Point-resolved spectroscopy was performed at 3 T, with an echo time of 35 ms, a repetition time of 2000 ms and 128 repetitions. A water unsuppressed MRS was acquired from the same voxel for water referencing.

**Image Analysis**
Diffusion-weighted images were initially processed using the Functional Magnetic Resonance Imaging of the Brain Software Library (FSL; [http://www.fmrib.ox.ac.uk/fsl](http://www.fmrib.ox.ac.uk/fsl)). Data were inspected for movement artifacts and then corrected for eddy current induced distortions. Brain extraction and calculation of diffusion tensor fractional anisotropy and mean diffusivity maps were carried out using FSL tools. Fractional anisotropy and mean diffusivity images were processed using tract-based spatial statistics (TBSS) and automated, observer-independent, voxel-by-voxel whole-brain between-group analysis performed (corrected for age) (24). Initially, every fractional anisotropy image was aligned to the FMRIB58 standard space fractional anisotropy map. Secondly the mean fractional anisotropy image across subjects was created. The mean image was thinned and thresholded at a fractional anisotropy value of 0.2 to create a white matter tract skeleton representing the center of the tracts common to all subjects. Fractional anisotropy data projected onto these skeletons was used in voxel-wise statistical comparisons using the Threshold-Free Cluster Enhancement option (corrected for multiple comparisons across space).

Amygdala, hippocampus, thalamus, cerebellum, brainstem, CSF and total brain volume were determined from the T1-weighted MRI using Freesurfer, an automated segmentation tool (25). We selected the structures in the brain with the highest concentration of androgen, mineralocorticoid and glucocorticoid receptors (2,12-14). No other neural volumes were extracted from the Freesurfer analysis to ensure all analyses performed were hypothesis-driven.

Spectroscopy data were analyzed using the TARQUIN algorithm version 4.3.7 to obtain concentrations for Glutamate, total NAA (N-acetylaspartate and N-acetylaspartylglutamate), total choline (glycerophosphocholine and phosphocholine), total creatine (Creatinine and phosphocreatine) and Glx (glutamine plus glutamate) (26). Since voxels tended to contain grey
matter, white matter and CSF in varying proportions, each voxel was segmented and the metabolite concentrations corrected for the differing water content (27).

**Statistical analysis**

The current study was powered to detect a 20% difference (SD 0.36) in amygdala volume (power=0.85, \( \alpha = 0.05 \)) between patients and controls (28). As the previous published neuroimaging study included only females with CAH (28), and there is a well described sexually dimorphic pattern of brain development (29), all study recruits to the current study were female.

Baseline characteristics of the two groups were compared using unpaired Student’s \( t \) test. Behavioral, quality of life and cognitive assessment scores were compared between patients with CAH and controls using ANCOVA corrected for education level. Correlations were performed to assess the relationships of markers of androgen and glucocorticoid exposure with psychometric assessment scores in patients with CAH. Data was analyzed using SPSS version 22.

Total brain, CSF volume and cerebral metabolite concentrations were compared between patients with CAH and controls using ANCOVA, with age as a covariate. For all other neural volumes, total brain volume was an additional covariate, \( p \)-values were adjusted to control for false discovery rate (FDR) (30).

Partial correlations were used to assess the relationship between neural volumes and metabolite concentrations, when initial results indicated that there was a significant difference in neural volume/metabolite concentration between the two groups, and neuropsychometric scores in patients with CAH, \( p \)-values adjusted for FDR (30). For neural volumes, correlations were also controlled for total brain volume. Correlations were used to assess the relationship between markers of androgen and glucocorticoid exposure, neural volumes, fractional anisotropy and mean diffusivity and metabolite concentrations, (where there was a significant difference in neural volume/metabolite concentration between the two groups) in just patients with CAH, adjusted for FDR (30); Variables examined were those that showed a significant difference between CAH patients and controls in group analyses.

**Results**

**Study cohort characteristics**

Nineteen adult women (mean 30.6 years, range 18-49) with 21-hydroxylase deficiency receiving glucocorticoids (median hydrocortisone dose 11.1mg/\( m^2 \)/day; range 10-13.8) and nineteen healthy women (mean 32.8 years, range 21-50) were recruited between July 2015-September 2016 (Table 1). All participants were right-handed, had no abnormal neurological findings and had completed mainstream schooling. All patients had 21-hydroxylase deficiency confirmed by molecular genetic analysis. Genotype groups null, A, B, and C contained 74\%, 10.5\%, 10.5\%, and 5\% of the patients, respectively (18), which means that the large majority of patients had a classic salt-wasting phenotype. One patient had non-classic CAH. Median age at diagnosis with 21-hydroxylase deficiency was 2 weeks (range birth-17 years). Sixteen out of 19 patients with CAH also had mineralocorticoid deficiency and were on fludrocortisone replacement (median dose 150mcg) at the time of assessment. Eight patients were taking prednisolone and 11 were taking hydrocortisone, the largest dose was taken in the morning in all patients. Fifteen of 19 patients with CAH had been on the same glucocorticoid dose for \( \geq \)3 years. No patients with CAH were taking the oral contraceptive pill.

One individual with CAH was unable to complete the WAIS due to time constraints. One individual with CAH and one control did not tolerate the MRI scan but completed all other
study components. MRI data quality was adequate in all subjects (VS). Temporal voxel MRS spectra were deemed to be suitable for analysis in 17 patients with CAH and 16 controls and parietal voxel spectra were deemed to be suitable for analysis in 18 patients with CAH and 17 controls, as judged by visual inspection by two independent observers (AP, DC).

**Psychometric assessment**

The physical component of QOL was significantly reduced in patients with CAH compared with controls (p<0.001) (Table 2). Patients with CAH scored higher on the depression component of the HADS questionnaire, however no-one scored in the clinical range (p=0.02) (Table 2).

There was no significant difference in full-scale IQ between patients with CAH and controls. When compared to controls patients with CAH had significantly reduced: working memory index (p=0.04), processing speed (p=0.03), digit span (p=0.04) and matrix reasoning scores (p=0.03) (Table 3).

**CNS abnormalities in patients with CAH**

Four of 19 CAH patients (21%) had a type 1 Chiari anomaly; brain MRI was otherwise normal on visual inspection in all subject. In none of the study participants a pituitary gland abnormality was reported; however, specific pituitary views were not acquired.

Widespread reductions in fractional anisotropy were present in the superior longitudinal fasciculus, inferior fronto-occipital fasciculus, corticospinal tract, uncinated fasciculus, cingulate gyrus, the hippocampus and the corpus callosum in patients with CAH (p<0.05) (Fig. 1). Mean diffusivity was increased bilaterally in the superior and inferior longitudinal fasciculus, inferior fronto-occipital fasciculus, anterior thalamic radiation, the corticospinal tract, the uncinate fasciculus, the cingulate gyrus and the hippocampus in patients with CAH (p<0.05).

Although CSF volume was significantly increased in patients with CAH (p=0.003), total brain volume was not significantly different between patients with CAH and controls. After correction for multiple comparisons localised reductions in neural volumes were present in patients with CAH in the right hippocampus, left and right thalami, the cerebellum and the brainstem (p=0.028, 0.007, 0.008, 0.014, 0.03 respectively) (Table 4). There were no significant differences in metabolite concentrations between patients with CAH and controls in the parietal voxel. In the mesial temporal voxel, total choline and total creatine were significantly lower in patients with CAH than controls (p=0.001 and p=0.04 respectively) (Table 4). Only total choline remained significantly reduced after correction for multiple comparisons.

**Correlations between MRI findings and cognitive assessment scores in patients with CAH**

There were significant correlations between cerebellar volume and matrix reasoning scores (r=0.7, p=0.002, Fig. 2A), and between brainstem volume, working memory performance and digit span scores (r=0.6, p=0.009, r=0.59, p=0.01 respectively, Fig. 2B and C). Spectroscopy-acquired left mesial temporal lobe total choline correlated significantly with working memory index (r=0.62, p=0.01, Fig. 2D). There were no significant relationships between fractional anisotropy and mean diffusivity and cognitive assessment scores.

**Correlations between markers of glucocorticoid and androgen exposure, MRI findings and cognitive assessment scores in patients with CAH**

Increased glucocorticoid equivalent dose (mg) correlated significantly with reduced working memory (r= -0.52, p=0.03) and digit span scores (r= -0.51, p=0.03) (Fig. 2E and 2F). There were no significant relationships between markers of androgen exposure and psychometric assessment scores.
Higher hydrocortisone equivalent dose (mg) correlated significantly with reduced mesial temporal lobe total choline (r: -0.72, p=0.001, Fig. 2G) and mean diffusivity (r: -0.5, p=0.03, Fig. 2H), suggesting that patients exposed chronically to higher glucocorticoid doses have greater reductions in mesial temporal lobe total choline and white matter microstructure, reflected by total choline concentration and mean diffusivity. There were no significant differences in any of the cognitive or MRI abnormalities identified between those individuals taking prednisolone and those prescribed hydrocortisone. There were no significant associations between markers of androgen exposure, MRI and cognitive findings.

Discussion

Using multiple quantitative imaging modalities in conjunction with neuropsychological assessment enabled us to identify functionally significant biomarkers of the disease process (CAH) and treatment effects (steroid exposure) in patients with CAH. Patients had global abnormalities of cerebral white matter with localized reductions in neural volumes in regions of the brain that have previously been documented to contain high concentrations of androgen, mineralocorticoid and glucocorticoid receptors (2,12-14). The mesial temporal lobe was affected bilaterally with significant reductions in white matter microstructure, right hippocampal volume and left mesial temporal lobe choline. Interestingly, whilst markers of androgen exposure did not relate to the identified CNS abnormalities, exposure to higher glucocorticoid doses was associated with significantly worse cognitive performance and abnormal mesial temporal lobe total choline and white matter mean diffusivity.

Concordant with finding of previous studies (17,31), we found reduced working memory and digit span scores and impaired quality of life. Whilst we had a priori hypotheses that working memory, processing speed and digit span would be reduced in patients with CAH based on previous studies these differences did not remain after controlling for multiple comparisons (17,32). Cognitive abnormalities in patients with CAH may relate to the supra-physiological glucocorticoid doses used to suppress adrenal androgen production (17). In support of this hypothesis working memory, episodic and declarative memory are adversely affected in patients treated with exogenous glucocorticoids, in adults with Cushing’s syndrome producing excess endogenous glucocorticoids and in healthy human volunteers given glucocorticoids (33,34). Our data provide further evidence that cognitive abnormalities present in patients with CAH relate to the degree of glucocorticoid exposure, with those patients on higher current glucocorticoid doses having significantly worse performance on working memory and digit span tests.

We identified an increased prevalence of type 1 Chiari anomalies in patients with CAH, similar to a previous study of adults with CAH in which 8 of the 39 patients studied had type 1 Chiari anomalies (4). Chiari anomalies are a complex developmental disorder characterized by primary axial skeletal defects and secondary neurological anomalies involving the cranio cervical region (35). We found the cerebellum and hindbrain to be smaller in patients with CAH suggesting that the downwards displacement of the cerebellar tonsils does not relate to brain structural abnormalities. It is possible that in patients with CAH exposure to steroid hormone abnormalities early in embryonic development may affect occipital bone formation antenatally (35). The relevance of this increased prevalence of type 1 Chiari anomalies to the clinical course and ongoing management in patients with CAH remains to be elucidated.

Patients with CAH are exposed to multiple potentially pathological hormone abnormalities and the neural changes we describe are likely to be multifactorial. Hypoglycemia and salt loss at initial presentation and subsequently at the time of adrenal crisis are likely to affect brain
structure. We were unable to examine the difference in CNS changes between patients with and without mineralocorticoid deficiency due to the small number of patients without salt loss recruited to the study. However, we identified no reduction in full scale IQ in our patients with CAH, in contrast to previous studies that report reduced full scale IQ in patients with a history of salt wasting crises requiring inpatient hospital management (36). Future studies should aim to dissect the impact of salt wasting on the CNS by including patients with conditions such as isolated aldosterone synthase deficiency.

Gross structural brain white matter abnormalities have been described previously in patients with CAH, in conjunction with thinning of the corpus callosum and small volume hippocampi (4,37). However, for the first time we have identified significant widespread abnormalities in white matter microstructure in patients with CAH. As longitudinal DTI studies report corpus callosum fractional anisotropy to be higher in men than in women, our findings with regard to white matter microstructure are unlikely to be due to excess androgen exposure (38). Animal and human disease models provide some evidence to suggest that the abnormalities we describe relate to glucocorticoid treatment in patients with CAH. In animal models prolonged exposure to raised glucocorticoid concentrations leads to the inhibition of oligodendrocyte precursor proliferation, negatively impacting on myelin production (39). Patients with Cushing’s disease have significant reductions in fractional anisotropy and increases in mean diffusivity throughout the brain (40). Importantly; partial resolution of neuritic degenerative changes in rodents, significant improvements in cognitive function and reversal of cerebral atrophy in humans occur following withdrawal of glucocorticoids, suggesting that modification of treatment regimens in patients with CAH may positively impact on myelination and cognitive function (41).

In contrast to the expected finding of increased amygdala volume in girls with CAH exposed to excess androgens antenatally Merke et al (28) identified smaller amygdala volumes using a manual tracing method with volumetric MRI acquisition on a 1.5 T. This was interpreted as an effect of postnatal excess glucocorticoid exposure in patients with CAH. Whilst we did not replicate this result, our findings of localized volume loss in the right hippocampus, the left and right thalami, the cerebellum and the brainstem also differs from the pattern of larger limbic, insula and occipital lobe volumes, and smaller parietal lobe and opercular left inferior frontal gyrus volumes seen in association with increased testosterone exposure (1,13,16,36,42).

Mineralocorticoid receptors, which have a similar affinity for cortisol and aldosterone, are located in limbic structures, in particular in the hippocampus, while being almost absent elsewhere in the brain (14). Neurons in the hippocampus do not express 11β-hydroxysteroid dehydrogenase type 2, which elsewhere in the body converts corticosterone and cortisol into inactive metabolites. Therefore, in the hippocampus, the mineralocorticoid receptor is mainly considered to be a glucocorticoid-activated receptor; making the hippocampus selectively vulnerable to non-physiological fluctuations in glucocorticoid concentrations (14). Glucocorticoids increase excitatory amino acids and serotonin concentrations leading to neural damage (33) and may also increase the vulnerability of neurons to other insults such as ischemia, via reduced neuronal glucose uptake (43). Administration of oral hydrocortisone in humans leads to reduced hippocampal metabolism, (44) and patients with Cushing’s syndrome, exposed chronically to high glucocorticoid concentrations, have localized volume loss in the hippocampus and temporal lobes (41). The cerebellum, which we found to be significantly smaller in patients with CAH, has the highest concentration of CNS glucocorticoid receptors (12) with exogenous glucocorticoids impacting negatively on cerebellum growth in mice (45). Whilst the current study design does not enable us to determine the exact etiology of the structural
Impairments identified, the reductions in neural volumes in regions of the brain with high concentrations of glucocorticoid receptors, in areas shown to be affected negatively by increased glucocorticoid exposure in \textit{in vivo} and murine studies suggests that the brain abnormalities we describe in patients with CAH relate to chronic excess glucocorticoid exposure. These findings require replication in larger, independent samples.

We identified a reduction in total choline, a marker of CNS myelination, inflammation and membrane synthesis and repair in CAH patients, which did not extend to the right parietal lobe. Exposure to higher glucocorticoid doses was significantly associated with mesial temporal lobe total choline. This suggests that reduction in total choline in the hippocampal voxel relates to raised glucocorticoid concentrations in patients with CAH as the mesial temporal lobe contains high concentrations of glucocorticoid receptors (2). Khiat \textit{et al} identified a reduction in choline resonance in 13 patients with Cushing’s syndrome in frontal and thalamic areas (15), hypothesized to reflect an inhibition of phospholipase A2 activity by glucocorticoids; phospholipase A2 hydrolyzes specific ester bonds in membrane phospholipids (46). Importantly following normalization of cortisol concentrations, improvements in memory and the brain metabolite profile were documented (15).

**Conclusion**

CAH has a profound impact on normal brain and cognitive development with the effects we describe most marked in the mesial temporal lobe. We also describe a significant association between current glucocorticoid replacement regimens, cognitive and central nervous system abnormalities. Whilst we are not able to control for previous episodes of hypoglycemia and salt loss at initial presentation and subsequently during adrenal crisis incidents, the recent development of more physiological glucocorticoid replacement regimens and other modalities which offer improved control of altered steroidogenesis in CAH (3) may provide opportunities to use the biomarkers identified in the current study (mean diffusivity, mesial temporal lobe total choline) to assess the impact of novel treatments on the CNS in patients with CAH. These findings are also relevant to the wider population of whom 1% are on long-term glucocorticoid therapy (47).

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Figure 1 The association between congenital adrenal hyperplasia and fractional anisotropy (tract based spatial statistics analysis comparing patients with congenital adrenal hyperplasia to healthy controls). The association between congenital adrenal hyperplasia and white matter skeleton fractional anisotropy (FA). Mean FA skeleton overlaid on the mean FA map. Regions of the mean FA skeleton in green represent areas where there were no significant differences in FA values in the patients with congenital adrenal hyperplasia patients compared to healthy controls. Areas in red/yellow are regions where the FA was significantly lower in the congenital adrenal hyperplasia group, and can be observed bilaterally in the superior longitudinal fasciculus, inferior fronto-occipital fasciculus, corticospinal tract, uncinate fasciculus, cingulate gyrus, the hippocampus and the corpus callosum (p<0.05). FA is a dimensionless index.

Figure 2 The association between cognitive function scores and neural volumes, hydrocortisone equivalent dose; cognitive function scores, mesial temporal lobe total choline and mean diffusivity in adults with congenital adrenal hyperplasia. Partial correlations were used to assess the relationship between neural volumes and metabolite concentrations and neuropsychometric test results for WAIS and WMS assessments in patients with congenital adrenal hyperplasia. For neural volumes, correlations were also controlled for total brain volume. Correlations were used to assess the relationship between markers of androgen and glucocorticoid exposure, neural volumes, fractional anisotropy and mean diffusivity and metabolite concentrations, in patients with congenital adrenal hyperplasia. All p-values were adjusted to control for false discovery rate (30).

Table 1: Age, auxology, educational level, glucocorticoid dose and fasting steroid hormone concentrations

|                              | CAH     | Controls | p-value |
|------------------------------|---------|----------|---------|
| Number                       | 19      | 19       |         |
| Mean Age (years) (SD, SE)    | 30.6 (8.9, 2) | 32.8 (8.5, 2) | 0.4    |
| Mean Height (cm) (SD, SE)    | 158.3 (8.3, 1.9) | 164.9 (5.9, 1.4) | 0.008* |
| Mean Weight (kg) (SD, SE)    | 77.1 (16.1, 3.7) | 73.9 (17.8, 4.2) | 0.57   |
Table 2: Hospital Anxiety and Depression Scale and Quality of Life scores in the CAH patients and the controls matched for age, sex and educational status.

| World Health Organization Quality of Life (QOL) questionnaire | CAH | Controls | p-value |
|-------------------------------------------------------------|-----|----------|---------|
| Number of participants                                     | 19  | 19       |         |
| QOL physical                                                | 92 (80-108) | 128 (116-132) | **0.000001** * |
| QOL psychological                                           | 72 (60-96)  | 92 (88-100)  | 0.049   |
| QOL social relationships                                    | 44 (36-52)  | 48 (40-52)   | 0.56    |
| QOL Environment                                             | 116 (108-136)| 124 (108-136) | 0.37    |
| QOL Total                                                   | 90 (77-101) | 108 (96-114) | **0.001** * |

| Hospital Anxiety and Depression Scale (HADS) questionnaire   | CAH | Controls | p-value |
|---------------------------------------------------------------|-----|----------|---------|
| HADS Anxiety                                                 | 7 (5-11) | 6 (4-8)  | 0.4     |
| HADS Depression                                              | 5 (3-7)  | 2 (1-3)  | **0.02** * |

*p-values remain significant after controlling for false discovery rate (30)

Table 3: Cognitive function assessments scores in the CAH patients and the controls matched for age, sex and educational status.

| Wechsler Adult Intelligence Scale–Fourth Edition*            | CAH  | Controls | p-value |
|--------------------------------------------------------------|------|----------|---------|
| Number of participants                                       | 18   | 19       |         |
### Wechsler Memory Scales*

| Measure                     | CAH                  | Controls             | p-value |
|-----------------------------|----------------------|----------------------|---------|
| Number of participants      | 19                   | 19                   |         |
| Design 1 Scaled             | 9 (7-11)             | 11 (9-14)            | 0.02**  |
| Design 2 Scaled             | 11 (9-12)            | 11 (10-16)           | 0.06    |
| Visual Repetition Scaled 1  | 11 (8-13)            | 10 (8-12)            | 0.61    |
| Visual Repetition Scaled 2  | 12 (9-15)            | 12 (10-14)           | 0.97    |
| Content scaled 1            | 10 (9-12)            | 12 (7-13)            | 0.4     |
| Content scaled 2            | 10 (8-12)            | 12 (10-15)           | 0.07    |
| Spatial scaled 1            | 10 (8-12)            | 12 (10-13)           | 0.13    |
| Spatial scaled 2            | 10 (9-13)            | 12 (9-15)            | 0.24    |
| Auditory memory index       | 100 (93-113)         | 109 (94-118)         | 0.27    |
| Visual memory Index         | 105 (95-112)         | 110 (100-117)        | 0.22    |
| Immediate memory index      | 102 (87-111)         | 109 (96-115)         | 0.16    |
| Delayed memory index        | 104 (98-114)         | 115 (104-123)        | 0.14    |

* Results presented as median (Q1, Q3) corrected for educational level  ** Whilst we had a priori hypotheses that working memory, processing speed and digit span would be reduced in patients with CAH based on previous studies these results did not remain significant after correction for false discovery rate (17,32,34).

### Table 4: Neural volumes determined from T1-weighted MRI using Freesurfer, Results corrected for age at scan and total brain volume (25) and Spectroscopy acquired CNS metabolite concentrations; data analysed using TARQUIN (26)

| Measure                     | CAH                  | Controls             | p-value |
|-----------------------------|----------------------|----------------------|---------|
| Number of participants      | 18                   | 18                   |         |
| Total brain volume (IQR)    | 1044732 (115246)     | 1069078 (86972)      | 0.47    |
| CSF                         | 1013 (914-1302)      | 839 (750-918)        | 0.003*  |
| Left hippocampus            | 3792 (3611-4054)     | 4014 (3644-4271)     | 0.16    |
| Right hippocampus           | 3744 (3639-3969)     | 3981 (3682-4272)     | 0.028*  |
| Region                        | N1 (SD, SE)                 | N2 (SD, SE)                 | p-value |
|-------------------------------|-----------------------------|-----------------------------|---------|
| Left amygdala                 | 1509 (1406-1685)            | 1658 (1492-1848)            | 0.055   |
| Right amygdala                | 1680 (1524-1785)            | 1718 (1488-1958)            | 0.6     |
| Left thalamus                 | 6318 (5894-6874)            | 6906 (6262-7578)            | 0.007*  |
| Right thalamus                | 6240 (5689-6730)            | 6622 (6333-7079)            | 0.008*  |
| Cerebellum (SD, SE)           | 113878 (106656-125254)      | 127499 (11484-134100)       | 0.014*  |
| Brainstem                     | 19145 (17569-20910)         | 20527 (19863-21794)         | 0.03*   |

**Mesial temporal voxel metabolites**

| Metabolite        | N1 (SD, SE) | N2 (SD, SE) | p-value |
|-------------------|-------------|-------------|---------|
| Glutamate         | 7.9 (6.2-10.3) | 8 (4.9-10.8) | 0.6     |
| Total NAA         | 5.6 (4.6-6.3) | 6.1 (4.9-7.2) | 0.1     |
| Total choline     | 1.9 (1.5-2.1) | 2.3 (2-2.5)  | 0.001*  |
| Total creatine    | 6.8 (5.8-7.9) | 8 (7.6-8.9)  | 0.04    |
| Glx               | 14.6 (11.2-18.3) | 15 (11-16.9) | 0.7     |

**Parietal voxel metabolites**

| Metabolite        | N1 (SD, SE) | N2 (SD, SE) | p-value |
|-------------------|-------------|-------------|---------|
| Glutamate         | 3.7 (2.9-4.4) | 3.6 (2.4-4.5) | 0.8     |
| Total NAA         | 7.7 (7.2-8.8) | 7.6 (7.3-8.6) | 0.6     |
| Total choline     | 2 (1.6-2.2)  | 1.9 (1.7-2)  | 0.5     |
| Total creatine    | 6.4 (5.8-6.7) | 6.1 (5.8-6.6) | 0.6     |
| Glx               | 4.5 (3.1-7.2) | 6.1 (3.8-4.4) | 0.7     |

*p-value remains significant after controlling for false discovery rate (30)
