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Title: Cognitive Deficit and White Matter Changes in Persons with Celiac Disease: a Population-Based Study

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Croall I.D. has no disclosures and contributed to the design, conduct, analysis of the study and writing of the manuscript

Sanders D.S. has no disclosures and contributed to the conduct of the study and writing of the manuscript

Hadjivassiliou M. has no disclosures and contributed to the conduct of the study and writing of the manuscript

Hoggard N. has no disclosures and contributed to the design and conduct of the study, and writing of the manuscript

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Abstract:

Background & Aims: There is debate over the presence and prevalence of brain injury in patients with celiac disease. To validate previous reports, we investigated the prevalence of neuropsychological dysfunction in persons with celiac disease included in the National UK Biobank, which contains experimental medical data from 500,000 adults in the United Kingdom.

Methods: Biobank participants with celiac disease (n=104; mean age, 63; 65% female) were matched with healthy individuals (controls, n=198; mean age, 63 y; 67% female) for age, sex, level of education, body mass index, and diagnosis of hypertension. All subjects were otherwise healthy. We compared scores from 5 cognitive tests, and multiple-choice responses to 6 questions about mental health, between groups using t test and □2 analyses. Groupwise analyses of magnetic resonance imaging brain data included a study of diffusion tensor imaging metrics (mean diffusivity, fractional anisotropy, radial diffusivity, axial diffusivity), voxel-based morphometry, and Mann-Whitney U comparisons of Fazekas grades.

Results: Compared with controls, participants with celiac disease had significant deficits in reaction time (*P*=.004) and significantly higher proportions had indications of anxiety (*P*=.025), depression (*P*=.015), thoughts of self-harm (*P*=.025) and health-related unhappiness (*P*=.010). Tract-based spatial statistics analysis revealed significantly increased axial diffusivity in widespread locations, demonstrating white matter changes in brains of participants with celiac disease. Voxel-based morphometry and Fazekas grade analyses did not differ significantly between groups.

Conclusions: In an analysis of data from the UK Biobank, we found participants with celiac disease to have cognitive deficit, indications of worsened mental health, and white matter changes, based on analyses of brain images. These findings support the concept that celiac disease is associated with neurological and psychological features.

KEY WORDS: TBSS, VBM, nervous system, gut–brain interactions

Introduction

Celiac disease is an autoimmune condition triggered by eating foods containing gluten. Celiac disease affects approximately 1%(1) of the population and its gastrointestinal pathophysiology and phenotype is now well characterised for the clinical setting(2). A strict gluten-free diet (GFD) is the only effective treatment for the condition, and this leads to a complete resolution of intestinal damage and discomfort(3). However, celiac disease also involves a number of non-intestinal functions and those which affect the brain are of particular interest.

In one paper, patients with neurological referrals found brain atrophy in the cerebellum and a number of cerebral areas(4) as well as indications of white matter damage. Another reported approximately half of newly diagnosed patients with celiac disease without any neurological history had symptoms concerning balance and sensation accompanied by abnormal cerebellar MR Spectroscopy readings(5). Cerebellar / thalamic degeneration was also found in those with a brain-expressing, gluten-related antibody (Transglutaminase 6, TG6(6,7)). Cognitive deficit has been reported in a cohort of elderly patients with celiac disease compared to controls(8), although the study had a small sample size (N=18). Finally, other studies have found worsened mental health in celiac disease, particularly where related to depression and anxiety(9).

However, a recent review demonstrates that many papers also find strikingly different prevalence estimates of neurological symptoms in celiac disease, sometimes at 0%(10). As well as being sometimes limited by study power and generalisability, the reports which do show significant findings often come from specialised centres with an interest in the topic, which raises a concern of positive ascertainment and referral bias. Conversely, as patients with celiac disease are primarily cared for via gastroenterology, neurological symptoms may be underestimated and untreated. Validation of previous findings in a well-powered, independent cohort where data collection has been out of the control of the study team is therefore highly desirable.

We used data from the National UK Biobank to compare people with celiac disease against matched controls, investigating for evidence of cognitive deficits, mental health problems and white matter disease. Our hypothesis was that if previous positive reports are due to bias, then we would *not* show differences in cognitive performance, indications of depression and anxiety, or evidence of brain injury from MRI scanning. Our aims were to perform groupwise comparisons between subjects with celiac disease against matched controls in the following scenarios: A) Key cognitive test scores; B) Answers given to questions interrogating mental health; C) Pertinent outcomes from imaging analyses indicating prevalence of white matter lesions, overall white matter health and brain atrophy.

Methods

UK Biobank and Study Participants

The UK Biobank is a "health resource" funded by the Wellcome Trust which has eclectic healthcare data available for 500,000 UK-based adults of any medical background. The initial study recruited these 500,000 adults (aged 40-69), between 2006-2010. The main recruitment method involved postal invitations to join the study, which were sent to people in this age bracket who also lived within travelling distance of an assessment centre. Details of potential participants were gained via NHS patient registers. There were no other inclusion / exclusion criteria. Information about the Biobank can be found at www.ukbiobank.ac.uk.

During this initial study visit data was collected concerning demographic details, medical history information, questionnaires and some physical measures and biological samples. The Biobank study has since expanded its scope in the following years by integrating information such as cancer and death registry data for its participants, as well as inviting participants back for further study-specific assessments. Pertinent to the current experiment, since 2014 participants have been receiving invitations to re-attend a new session which includes MRI brain scanning and cognitive testing. At the time of writing, this is still ongoing and ultimately aims to assess 100,000 of the original subjects by 2022. Access to the UK Biobank data was obtained on the 10th of April 2019 (project code 43043) where data were available for 28,787 of these participants.

Information not available from the Biobank includes date of celiac disease diagnosis/duration of disease and other related medical information such as serological reports and measures of dietary success.

The purpose of the Biobank study is to make this data available to researchers, and Biobank participants were made aware of this and the possibility of invitations for repeat visits when initially agreeing to take part. The ethical agreements for the Biobank study therefore extend to cover experiments which use their data.

Identification of Celiac Subjects and matched Controls

Data which was initially disseminated to the study team comprised a database which included variables for the full Biobank cohort (i.e. all 500,000). This database was reduced to those where brain MRI had been performed. As a subject who only partially completed scanning would still receive this identifier, it was further ensured such subjects also had a minimum of FLAIR, T1 and DTI scans available (the purpose of these is described later).

Participants with celiac disease were then found from this subgroup. Diagnoses are primarily reported in Biobank data by two methods, self-reported (SR) or by Hospital Episodes Statistics (HES). SR diagnoses are given at each assessment visit during a verbal interview and reported conditions are assigned a study code by a Biobank study team member. HES data is initially taken from hospital records (where conditions are coded according to ICD10 criteria) and published by the UK health service after validation procedures. The UK Biobank study acquired this data and implemented further validation

before making it available for its participants. HES data is available for anyone who has been an inpatient since 1997, and studies have indicated it has strong validity with respect to the original diagnoses(11). In order to capture the full population of subjects with celiac disease, any participant reporting the condition by either/or of these methods was identified.

Exclusion criteria were then applied to these participants. These were

- Any history of malignancy or chemotherapy treatment
- Taking any medications which could be considered "psychoactive" at the time of the imaging / cognitive testing (e.g. antidepressants, antipsychotics etc.; these are self-reported during verbal interview after which medication-specific study codes are assigned, similar to the self-reporting of diagnoses)
- Any other significant inter-current diagnoses. This critically included any cardiovascular disease other than a hypertension diagnosis, any neurological or psychiatric condition other than anxiety/depression, rheumatological / inflammatory conditions (such as psoriasis, lupus etc.), any head trauma and any significant gastrointestinal disorder (such as colitis, Crohn's etc.)

Data concerning malignancy was available in Biobank variables dedicated to describing cancer history and other diagnoses were identified by SR and HES data (reporting of a relevant condition in either, at any study point, resulted in exclusion). The final group of subjects with celiac disease therefore had a "minimum" set of imaging data available (T1, FLAIR and DTI), no other notable diagnoses, and were not taking psychoactive medication at the time of the main data collection.

Controls otherwise followed the same exclusion criteria but also did not report celiac disease (SR/HES). Additionally, controls were selected from participants who had the "minimum" set of MRI scans available and also had completed all main cognitive outcome measures (described later) to maximise available data. Controls were matched to the celiac disease group in a 2:1 ratio. Matching was based on age (within 3 years), sex (exact match), age completed education (within 1 year), body-mass index (within 2 BMI points) and if the subject had a diagnosis of hypertension (either SR or HES; exact match). Matching was performed using the case-control matching plug-in "Fuzzy" for SPSS, which was run without sampling replacement, with priority given to exact matches and with the case order randomised.

Initially two matches per case for the whole celiac disease group were sought based on the matching criteria. The diagnoses and medication history of returned suggestions were then inspected "by hand" to identify those which met remaining study criteria and those which must be excluded. This process was repeated until either all subjects with celiac disease had two matches, or no remaining matches were available.

Cognitive Outcomes

At the same time as brain imaging, Biobank participants undergo a number of cognitive assessments. The total cognitive data was reduced to five key outcomes which are representative of different domains, acquired via a computer interface. Outcomes were **reaction time** (the mean response time across 12 rounds of a "snap"-like card game task), **digit span** (from the digit span task), **pairs matching errors** (the total number of errors made in a "pairs matching" task, with 6 possible pairs), **fluid intelligence** (the total correct score from a series of logic questions in the Biobank's "fluid intelligence" test) and **trail making B - A** (the difference between the time to complete the "A" and "B" conditions in the trail making task).

Mental Health Outcomes

During the initial Biobank assessment, subjects were asked a number of questions (multiple-choice answers) which interrogated mental health. These were reduced to 6 for the current study, which in turn focused on anxiety, depression, happiness with own health, suicidal thoughts, thoughts of self-harm and sleep quality. Responses given which did not indicate a clear answer (e.g. "Prefer not to answer") were removed to increase sensitivity in comparing meaningful information. The answers given indicating if subjects ever had suicidal or self-harm type thoughts were also modified to "no" or "yes" responses, from "no", "yes, once" and "yes, more than once"; the two "yes" type answers were combined. This modification was also to increase sensitivity of the analyses in detecting the most pertinent outcome.

MRI Data

MRI data were collected on one of three identical Siemens Skyra 3T scanners based in Biobank assessment centres in Manchester, Newcastle and Reading (UK) which ran matched protocols. The full acquisition included:

T1-Weighted (T1W) images. These are standard "structural" MRI scans that are ideal for showing the brain's anatomy with good image contrast between grey and white matter tissue. T1W scans are commonly used in analyses which investigate for differences in brain volume between groups. The major acquisition parameters for these are: *3D "MPRAGE", 1mm^3 resolution, TR/TE=880/2000ms*

FLAIR images. These are similar to T1W scans in that they are also "structural" but the image contrast in them is most effective at highlighting "white matter lesions", which are a common type of brain pathology reported in conditions such as dementia (and have been previously reported in celiac disease(4)). The major acquisition parameters for these are: *3D "SPACE", 1.05x1x1mm, TI/TR=1800/5000ms*

DTI images. DTI is an "advanced" type of MRI scan. This does not produce a conventional image of the brain, but instead gives raw data which describes the direction and distance which water molecules diffuse in throughout the brain. After processing of this data, DTI

scans can be used to give metrics which summarise this behaviour, and these measures have been shown to be very sensitive at detecting damage to the brain's white matter which cannot be "seen" on more conventional imaging such as T1W and FLAIR scans(12). The major acquisition parameters for these are: *multishell data with 50 directions at each b=1000 and b=2000, 5x b=0, 2mm^3 resolution (5x b=0 images are also available with phase-encoding reversed), TR/TE=92/3600ms*

Image Processing

DTI & Tract-Based Spatial Statistics

The objective of DTI processing was to run a "Tract-Based Spatial Statistics" (TBSS) analysis. In this analysis, the raw DTI data which describes the direction and distance of water diffusion in a 3D space throughout the brain is processed to create new "images". Four different images were created in the current experiment which are commonly used in DTI studies; Fractional Anisotropy (FA), Axial Diffusivity (AD), Radial Diffusivity (RD) and Mean Diffusivity (MD). The voxel values in these images, which describe different mathematical properties of the water diffusion, are used as markers for the health of the white matter tissue which surrounds the water. Changes in these measures are therefore of interest in showing damage to the brain's white matter. A TBSS analysis can be used to perform this sort of investigation by comparing the images from the celiac disease group against the images from the control group, and highlighting areas of white matter which are consistently different between the groups. TBSS analysis of DTI scans is a widely used investigative method throughout neuroscience research(13).

Technical details for this processing are as follows. The raw DTI images were reduced to create a "single shell" volume of the b=1000 and b=0 acquisitions. Processing was conducted using FSL's "FDT" pipeline including TOPUP and EDDY processing to correct for geometric distortion (based on the first b0 of the main and secondary datasets). DTIFIT then calculated maps of FA, AD (i.e. the L1 map), RD (i.e. the average of L2 and L3 maps) and MD.

These four maps were then processed using the TBSS pipeline. This involves nonlinear registration of the FA images to a standard space target FA image, thresholding of FA values at a value of 0.2 and creation of an FA "skeleton" for each subject. The calculated transformations for the FA images are then also applied to AD, RD and MD maps to create equivalent standard-space skeletons of those. These 4D datasets were subject to voxelwise comparison between celiac disease and control groups in an independent *t*-test-type model using the Randomise tool with Threshold-Free Cluster Enhancement (TFCE) for multiple comparisons correction and 10,000 permutations per statistical contrast.

Four celiac disease scans were not included in this analysis as on inspection their DTI data had been collected with a "pilot" MRI scan only used in the early stage of the Biobank imaging study that is not scientifically comparable with the main sequence later implemented. One control scan was also not included due to processing errors resulting from the image having been acquired with incorrect orientation settings.

Volumetry & White Matter Lesions

The objective of T1W scan analysis was to investigate for regions of grey matter volume difference in subjects with celiac disease (i.e. to detect any areas where the brain appears to have atrophied). This was done via a "Voxel-Based Morphometry" (VBM) analysis which, similar to the TBSS approach, conducts a statistical analysis between the celiac disease T1W images and the control T1W images to highlight regions of the brain where values (representing the volume of grey matter at that point in space) are consistently significantly different between groups in the same local area.

The technical details for this processing are as follows: T1 scans were bias-field corrected using "N4"(14) and then processed with the VBM pipeline implemented in FSL(15). The study-specific template for this, which needs to be equally representative of both experimental groups, was based on all participants with celiac disease and the first of each control match (due to subjects with celiac disease who did not return any matches this still left the groups marginally unbalanced; an additional 3 control subjects were selected at random to balance the groups with N=104 each). After registration of GM tissue probability maps to this across the whole cohort (with modulation), smoothing was applied with a sigma value of 2. An independent *t*-test-type comparison of the modulated GM probability values was made between celiac disease and control groups using Randomise with TFCE correction applied (10,000 permutations per contrast).

FLAIR scans were graded using the Fazekas scale (16) by a consultant neuroradiologist (N.H.) who was blinded to study group. This is a clinical rating scale to assess the severity of white matter lesions in a person's brain, and is done by visual inspection of the FLAIR images.

Statistical Analyses

DTI and brain volumetry analyses were implemented within FSL and have been fully described in their image processing sections. Other statistics were conducted in SPSS (Version 25). The normality of all variables were visually inspected before analyses to inform if parametric or non-parametric approaches should be used. In some analyses there were missing cases; all available data were used in comparisons.

Cognitive outcomes were compared by independent samples *t*-test between groups. In the case that significant differences were found in both DTI and cognitive analyses, post-hoc analysis would be conducted to relate the two in a TBSS analysis examining the interaction between the cognitive and DTI variables in question, between experimental groups (TFCE-corrected, 10,000 permutations).

Mental health questions were compared between groups by X^2 comparison.

Fazekas score was compared between groups by Mann-Whitney U analysis.

Post-hoc analyses were conducted to investigate any differences between the HES and SR celiac disease groups which may confound primary findings. Participants with celiac disease who had an HES diagnosis were compared against those with only an SR diagnosis in groupwise testing on all demographic variables, and any variable which was found to be significant in the main analyses.

Results

Participant Overview

104 subjects were included in the celiac disease group; 53 of these were identified by Hospital Episodes Statistics (HES) data and 51 by self-reporting (SR). Two control matches were found for 98 of these; only one match was available for three of the subjects with celiac disease, while no matches were found for the remaining three. This gave a total of 198 controls.

The celiac disease group had a mean age of 63.0 years and was 65.4% female. The control group had a mean age of 62.5 years and was 66.6% female. These, and other key demographic variables were not significantly different between groups. Comparisons between HES and SR celiac disease subgroups were also non-significant. These variables and analyses are summarised in Table 1.

Cognitive & Mental Health Comparisons

Independent *t*-test showed the celiac disease group was significantly slower on the reaction time task (celiac= 621.2±124.0ms, controls= 583.9±95.7ms, p=0.004). Comparisons between digit span (p=0.858), pairs matching errors (p=0.857), fluid intelligence (p=0.196) and trails A-B (p=0.448) were not significant.

X^2 analyses showed subjects with celiac disease were significantly more likely to answer "yes" to the "**anxiety**" question (celiac = 31.5% yes, controls= 18.0% yes, p=0.025), the "**depression**" question (celiac = 58.4% yes, controls= 41.4% yes, p=0.015), the "**self-harm**" question (celiac = 19.5% yes, controls= 9.0% yes, p=0.025), and gave answers indicating less "**general happiness with [their] own health**" (p=0.010). The remaining questions which explored suicidal thoughts and sleep quality were not significantly different, but did give p values which "approached" significance (i.e. p<0.1). See Table 2 for full results.

Imaging Analyses

TBSS comparison of FA, RD and MD values showed no significant differences between groups, however there were significant changes when examining AD. Here, AD was found to be increased in the celiac disease group in locations which included cerebellar, brainstem and thalamic white matter, as well as the forceps major of the corpus callosum and a segment of the superior longitudinal fasciculus (Figure 2). The mean(SD) of AD in across regions found to be significantly different in the TBSS analysis was $1.366^{10\text{^-}3}(0.034^{10\text{^-}3})$ in the control group, and $1.395^{10\text{^-}3}(0.036^{10\text{^-}3})$ in the celiac disease group.

Analysis was conducted to relate the significantly-different cognitive reaction time scores with AD, by re-analysing the TBSS data and examining the interaction between study group and RT/AD correlation. While there were multiple locations which reached p<0.1 (Figure 3), this did not reach significance.

The VBM analysis did not reveal any areas of significant difference. The Mann-Whitney U comparison of Fazekas scores also did not show any significant differences between groups (p=0.203).

Post-hoc analyses comparing HES and SR diagnosed CD participants

HES-diagnosed participants with celiac disease were not significantly different to the SR-diagnosed group in any demographic variable. They were also not significantly different in reaction time or any of the mental health questions. A TBSS analysis found the HES group had significantly higher AD than the SR group in a number of locations. To confirm the influence of this on the main analysis, further TBSS investigation compared only the HES celiac disease group against their matched controls and found extensive areas of increased AD in a similar pattern to the main finding, and generally at higher levels of significance. (Figure 4).

Discussion

Previous literature has indicated celiac disease to involve cognitive deficit and brain injury, although debate remains about the possibility of positive ascertainment and referral bias. We therefore hypothesised that there would be no indications of cognitive or neurological dysfunction in participants with celiac disease (and without any neurological diagnoses) taken from an independent dataset. However, using the UK Biobank our comparisons against matched controls revealed a reaction time deficit, compromised mental health, and extensive white matter tract changes; we therefore reject our hypothesis. This study validates previous findings showing celiac disease to involve neuropsychological harm. It is the first study to use a DTI analysis in this context, and overall highlights the meaningful ways in which patients with celiac disease may be psychologically and neurologically impacted by their condition.

The literature has examined neurological effects in celiac disease for many years now, although there remains debate as to the prevalence of clinically-relevant consequences. While some research finds neurological symptoms in as many as 50% of newly-diagnosed patients(5), other reports find none(10). Variability in these findings is likely due in part to the specialisation of study groups which are usually focused on either gastroenterology or neurology, raising the question of if brain injury in celiac disease is ultimately under-diagnosed or over-diagnosed. This study, which has found a range of neurological findings in an independent cohort of patients with celiac disease, is therefore an important addition to this discussion.

Previous studies reporting brain injury in celiac disease indicate a phenotype that largely resembles ischemia-driven white matter disease. These papers show reactivity between gluten-related antibodies and blood vessels throughout the body(17–19), atherosclerotic changes(20), decreased cerebral perfusion(21), white matter lesions(4,5), cognitive impairment(8) and an increased risk of vascular-type dementia(22). Our findings support and expand on these studies. DTI is regarded as a particularly sensitive technique in detecting white matter changes(23), and after implementing this acquisition in a TBSS analysis we report widespread increases in AD. While “conventional” interpretation of DTI values may expect AD to decrease following injury(24), raised glial cell activity in response to damage has been shown to increase AD(25), while raised AD has also been reported as an outcome in contexts such as mild traumatic brain injury(26) and tract degeneration due to Friedreich's ataxia(27). As the celiac disease group also had impaired reaction time compared to controls, the confidence that the tract changes represent overall worsened health should remain high. While a significant relationship directly between the AD changes and the cognitive deficit was not found, those comparisons were “approaching” significance (i.e. $p<0.1$) so the possibility of this being due to a lack of power should be considered. Taken together these results highlight that physiological white matter impairment is prevalent in celiac disease, and that these occur alongside compromised cognitive ability.

We also report indications of worsened mental health. It is not necessarily surprising that subjects with celiac disease were more overall unhappy with their own health as patients report a long term, high degree of perceived disease “burden”(28). The tendency towards

anxious and depressive thoughts also support previous literature (29,30), which are accompanied in the present experiment by increased thoughts around self-harm. It is unclear what drives these outcomes, and while a component of them is likely an understandable psychological response to living with a chronic condition, other research has found a relationship between depression and presence of gliadin antibodies in people both with and without celiac disease(31). This implies that there may also be a physiological basis to gluten-related mood problems.

Neither increases in white matter lesions or brain atrophy was reported in the current experiment. These have been previously reported(4,5) in celiac disease, although those studies focused on patients with neurological referrals / on a sub-groups of patients who also had antibody positivity against TG6 (in whom cerebellar and thalamic atrophy was found). Direct comparison with the present study of "typical" patients with celiac disease with unknown TG6 status is therefore difficult. It is possible that these effects were present but only in a selection of the participants, or spread across participants but of an effect size which the analyses were not powered to detect. It should be noted that the positive imaging findings do show changes in similar brain regions to these previously-reported with the cerebellum, thalamus, and the tract which connects them being focal points of the TBSS white matter results. DTI changes can be found as a precursor to white matter lesions which later become identifiable on FLAIR scans(32), so it is additionally possible the TBSS results were detecting this.

These results highlight the importance of awareness about neurological involvement in celiac disease, and the ways in which these can meaningfully affect patients. DTI changes such as raised AD have been suggested as clinically relevant in a range of conditions including stroke, Parkinson's, traumatic brain injury etc.(37), while finding significant cognitive changes is arguably an immediate demonstration of such a meaningful impact. Patients with celiac disease were found to have impaired reaction time, which is a measure of the "processing speed" cognitive domain(33). While it should be noted that reaction time also has a motor speed component to it, when a reaction time deficit is also accompanied by evidence of damage to white matter tracts (the physiological basis of processing speed(34)) as it is in the current experiment, it is most likely to be representative of this domain. Processing speed is a focus of study in contexts such as vascular dementia(35) and subjective cognitive impairment (SCI (36)). SCI is perhaps of particular relevance; generally regarded as a pre-dementia state SCI sufferers are significantly more likely to convert to dementia(38), consistently report lowered quality of life(39) and in addition to impaired processing speed also show altered white matter DTI measures (including raised AD(40)). While increased dementia risk is generally not supported in celiac disease (except for vascular dementia(22)), the phenotype of syndromes such as SCI are overall highly comparable to the celiac disease results reported in the current study and elsewhere, both in terms of physiological and cognitive changes. While the impact of these neurological consequences are unlikely to be clinically "morbid", they should therefore neither be considered trivial. Indeed it is likely because of their level of severity being relatively lower that symptoms often go unrecognised when assessed by non-specialist clinicians(10).

Understanding this is vital so that physicians can give properly targeted care to patients with celiac disease.

Research supports that neurological damage in celiac disease is driven by gluten exposures(41,42). While gastrointestinal injury is generally understood to recover on a GFD, the brain is far less capable of this(41) and acquired neurological deficits will accumulate and persist for the rest of the patient's life. This is supported by studies such as one showing cognitive difficulties to persist in a cohort of patients with celiac disease who had been on a GFD for an average of 5.5 years(8). Strict adherence to a GFD is the best measure to limit further progression of such injury, and studies such as the current one may hopefully bolster motivation from both clinicians and patients in delivering this. This also raises the issue of how people with gluten-sensitivity who continue a gluten-containing diet likely suffer much more pronounced outcomes than those studied in experiments such as this one. Undiagnosed celiac disease cases would be a pertinent example of this at-risk population, as well as people who have neurological gluten sensitivity without the warning-signs of enteropathy.

Factors in study design may have limited the ability to detect true-positive findings. It should be highlighted that the celiac disease group investigated in this study underwent extensive exclusion criteria, which included a number of gastroenterological conditions which are known to be associated with CD such as all forms of colitis(43), and other potentially-linked inflammatory problems such as psoriasis(44). While this was important in order to maintain a well-defined experimental group, it also means that the population studied is likely to be an unusually "healthy" one. Further, conducting disease-specific research with eclectic 3rd party resources such as the UK Biobank does have limitations. In this case information such as time since diagnosis, dietary success and serological status etc. are not available, meaning that there will be a relatively high degree of heterogeneity in the celiac disease group. This is relevant as factors such as cognition are known to improve after some time on a GFD(45). Data noise will therefore be overall increased in the study outcomes, and the sensitivity of analyses will be reduced. This means that confidence in the study findings being true positives should remain high, but it should be considered that these may if anything under-represent the scale of neurological problems in celiac disease.

Further limitations that should be considered involve the accuracy of celiac disease "diagnoses", particularly within the self-reported (SR) group. While hospital episodes statistics (HES) data also has limitations it should have comparatively good accuracy in correctly identifying subjects with clinically diagnosed celiac disease (11). Post-hoc analyses seeking to confirm if the main study findings were disproportionately influenced by SR or HES subgroups found that these differed from each other in only the TBSS analysis, where the HES group drove the main study finding. This therefore maintains confidence that the main results are representative of appropriately-defined celiac disease.

In conclusion, this study has used an independent dataset to validate previous reports of cognitive deficit and neurological changes in typical celiac disease. We reported an impairment in reaction time, worsened mental health and white matter changes in UK Biobank participants with celiac disease compared to matched controls. When combined,

these findings show both evidence of physiological brain injury as well as meaningful impacts on patients with regards to their psychological faculties and wellbeing.

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Table / Figure Legends

Table 1. Left before bold line: Overview of demographic information in each study group, with results of statistical tests examining for any differences (none were significant). Right after bold line: overview of the same information compared between celiac disease subgroups based on "diagnosis" by either Hospital Episodes Statistics (HES) and Self-Reported (SR) data. The same analyses are repeated for these (none were significant).

Table 2. Overview of answers given to questions exploring mental health, with results of X^2 (Chi-squared) analyses examining for differences between study groups

Figure 1. Boxplot showing significant differences in reaction time between celiac disease and control groups

Figure 2. TBSS outputs showing areas of white matter tracts where Axial Diffusivity (AD) is significantly higher in the celiac disease group (red / yellow locations; green indicates no significant change). TOP ROW- cerebellar white matter; MIDDLE ROW- ascending white matter including the red nucleus (left), brainstem (middle) and extending into the thalamus (right); BOTTOM ROW- forceps major (left and middle) and superior longitudinal fasciculus (right).

Figure 3. White matter tract areas with p values "approaching" significance (i.e. p=0.05-0.1, blue locations) from an analysis examining the interaction between study group and the relationship between axial diffusivity (AD) and reaction time scores.

Figure 4. TBSS outputs showing results of an analysis comparing AD between the HES-diagnosed participants with celiac disease and their matched controls. AD is raised in the HES group (red/yellow areas) in widespread locations which largely overlap with the main finding. TOP ROW - inferior temporal lobe fibres (left), which extend superiorly and are accompanied by the red nucleus (left-middle), thalamus (right-middle) and superior longitudinal fasciculus (right). BOTTOM ROW - the posterior half of the corpus callosum (left), with a large focus on the forceps major (middle and right)