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Neurologic Deficits in Patients With Newly Diagnosed Celiac Disease are Frequent and Linked With Autoimmunity to TG6

Short Title: neurological deficits in patients with CD

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Abbreviations: CD coeliac disease, GFD gluten-free diet

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Abstract

Background & Aims: Celiac disease is an autoimmune disorder induced by ingestion of gluten that affects 1% of the population and is characterized by gastrointestinal symptoms, weight loss, and anemia. We evaluated the presence of neurologic deficits and investigated whether the presence of antibodies to TG6 increases the risk of neurologic defects in patients with a new diagnosis of celiac disease.

Methods: We performed a prospective cohort study at a secondary-care gastroenterology center of 100 consecutive patients who received a new diagnosis of celiac disease based on gastroscopy and duodenal biopsy. We collected data on neurologic history, and patients were evaluated in a clinical examination along with magnetic resonance imaging (MRI) of the brain, MR spectroscopy of the cerebellum, and measurements of antibodies against TG6 in serum samples. The first 52 patients recruited underwent repeat MR spectroscopy at 1 year after a gluten-free diet (GFD). The primary aim was to establish if detection of antibodies against TG6 can be used to identify patients with celiac disease and neurologic dysfunction.

Results: Gait instability was reported in 24% of the patients, persisting sensory symptoms in 12%, and frequent headaches in 42%. Gait ataxia was found in 29% of patients, nystagmus in 11%, and distal sensory loss in 10%. Sixty percent of patients had abnormal results from the MRI, 47% had abnormal results from MR spectroscopy of the cerebellum, and 25% had brain

white matter lesions beyond that expected for their age group. Antibodies against TG6 were detected in serum samples from 40% of patients–these patients had significant atrophy of subcortical brain regions compared to patients without TG6 autoantibodies. In patients with abnormal results from MR spectroscopy of the cerebellum, those on the GFD had improvements detected in the repeat MR spectroscopy 1 year later.

Conclusions: In a prospective cohort study of patients with a new diagnosis of celiac disease at a gastroenterology clinic, neurological deficits were common and 40% had circulating antibodies against TG6. We observed a significant reduction in volume of specific brain regions in patients with TG6 autoantibodies, providing evidence for a link between autoimmunity to TG6 and brain atrophy in patients with celiac disease. There is a need for early diagnosis, increased awareness of the neurological manifestations amongst clinicians and reinforcement of adherence to a strict GFD by patients in order to avoid permanent neurological disability.

Keywords: wheat, headaches, ataxia, peripheral neuropathy, brain atrophy

What You Need to Know

Background: We evaluated the presence of neurologic deficits and investigated whether the presence of antibodies to TG6 increases the risk of neurologic defects in patients with a new diagnosis of celiac disease presenting to gastroenterologists.

Findings: In a prospective cohort study of patients with a new diagnosis of celiac disease at a gastroenterology clinic, neurological deficits were common and 40% had circulating antibodies against TG6. We observed a significant reduction in volume of specific brain regions in patients with TG6 autoantibodies. MR spectroscopy of the cerebellum improved after 1 year on a gluten-free diet.

Implications for Patient Care: Our findings further emphasize the need for early diagnosis of celiac disease and strict adherence to a gluten-free diet in order to prevent permanent neurological disability.

Introduction

Celiac Disease (CD) is an autoimmune disease triggered by the ingestion of gluten. It affects 1% of the population.[1] CD belongs to the spectrum of gluten-related disorders that encompass diverse manifestations including Dermatitis Herpetiformis (DH), and neurologic dysfunction, the commonest of which is gluten ataxia (GA).[2]

It remains unclear as to why in some patients the initial presentation is with gastrointestinal symptoms whilst in others it is primarily or exclusively with an itchy vesicular skin rash (DH) or with neurologic manifestations.[3,4]

One possible explanation may lie in the primary immunological response targeting different potential auto-antigens (transglutaminases) and thus driving the respective primary clinical manifestation. This would be in keeping with the discovery of TG2 being the autoantigen in CD, TG3 the autoantigen in DH and TG6 the autoantigen in neurologic manifestations.[5,6,7,8] The degree of overlap between CD and extraintestinal manifestations remains unknown, as does the potential for future development of such manifestations with ongoing exposure to gluten.

It is assumed that neurologic symptoms and dysfunction in patients with newly diagnosed CD presenting to gastroenterologists are rare. To our knowledge, no study has utilized both detailed neurologic examination and brain imaging with MR spectroscopy to prospectively evaluate large numbers of such patients at the time of diagnosis.

The primary aim of this study was therefore to establish if TG6 autoimmunity in the context of newly diagnosed CD patients is a marker of current neurological dysfunction. Secondly to clarify the true prevalence of neurologic

involvement as identified by detailed brain imaging and neurologic clinical evaluation at the time of diagnosis of CD, in patients presenting to gastroenterology clinics. Thirdly to prospectively study any changes on neuroimaging 12 months after the diagnosis of CD and the introduction of gluten-free diet (GFD).

Methods

Patient selection and clinical assessments

This was a 3-year prospective study based at the Department of Gastroenterology, Academic Department of Neurosciences and Academic Department of Neuroradiology, Sheffield Teaching Hospitals NHS Trust, Sheffield, UK. The study was approved by the South Yorkshire Research Ethics Committee and informed consent was obtained from all participants. Patients were referred by their General Practitioners for investigation to a gastroenterology clinic run by one of the authors (DSS). All patients were from the catchment area of the Sheffield Teaching Hospitals NHS Trust. Consecutive patients with newly diagnosed CD were recruited during their gastroenterology appointment immediately after the biopsy results confirming CD were available. A history of previous neurologic diagnosis was an exclusion criterion, although none of the patients that agreed to participate had a prior history of neurologic consultation or diagnosis.

Neurologic history and clinical examination

All patients were clinically assessed by Consultant Neurologists. The Neurologists were blinded to the neuro-radiological findings and to the

serological results for TG6 autoantibodies. Patients were specifically asked if they had any balance problems (gait instability, tendency to fall, clumsiness), persisting sensory symptoms (tingling, numbness, peripheral pain in feet and hands) or frequent (weekly) headaches. If there was any clinical evidence of a peripheral neuropathy on clinical examination (distal sensory disturbance and/or areflexia) patients underwent neurophysiological assessments done by 2 of the authors to verify the presence of neuropathy. The neurologic examination included detailed assessment of gait, including ability to tandem walk and stand on each leg in turn.

Brain imaging

Patients underwent MR imaging of the brain, including volumetric T1 weighted and volumetric FLAIR, and MR spectroscopy of the cerebellum (vermis and hemisphere). The methodology has been described previously.[9] Any white matter disease on MRI was analyzed by 2 of the authors, neuroradiologists with over 10 years' experience at specialist neuroradiology consultant level. Both neuroradiologists were blinded to the TG6 serological status at the time of reporting. Scans were graded using the Fazekas scoring system and the number of white matter hyperintensities recorded from the volumetric FLAIR images with discrepancies resolved by consensus.[10] Imaging data from 525 healthy volunteers was used to correct for age related changes in morphometric assessments.

Volumetry of key grey matter (GM) brain regions was based on processing of T1 images to generate normalized brain volume (NBV) data sets. Significant findings were further interrogated by age-controlled volumetry of sub-regions,

and “vertex” shape analysis via “FIRST” where appropriate. Detailed methodology for image processing used is provided in the supplementary material.

Serology

Serological testing for TG2 IgA, antigliadin IgG and IgA (Aesku), endomysium antibodies and HLA typing was undertaken in all patients prior to the biopsy. None of the CD patients had IgA deficiency. All sera collected at baseline were tested for circulating anti-TG6 antibodies as part of the study, using previously described methodology.[7,8]

Gluten-free diet follow-up

The first 52 patients recruited were re-assessed after 1 year on GFD, including repeat brain imaging, telephone interview and serological status. Due to limited funding it was not possible to re-assess the entire cohort.

Statistical analysis

A database was developed using the Statistical Package for Social Science (version 23.0 for Mac; SPSS). Analyses sought to investigate differences in key MR variables relative to TG6 status, characterize the significance of different measures at presentation and examine the progression in relation to MR variables in the 1-year follow-up group. Frequencies and descriptive statistics were examined for each variable. Comparisons between groups were made using the Student’s t-test for the normally distributed continuous variables and the Mann-Whitney’s U test for the non-normally distributed

continuous variables. Categorical variables were compared using the chi square test. Level of significance was set at the 95% confidence level. Statistical models employed to test significance of observed changes on imaging data are given in the Supplemental Materials section.

Results

A total of 100 consecutive newly diagnosed CD patients (60F and 40M) were recruited over 18 months. Reasons for referral included: one or more gastrointestinal symptoms (abdominal bloating, abdominal pain, weight loss, diarrhea, pale stools) in 54% of patients, anaemia in 16%, family screening in 14%, persistent fatigue in 6%, irritable bowel syndrome diagnosed in primary care in 5%, osteoporosis in 4%, presence of other autoimmune diseases in 4% and abnormal liver function tests in 3%. The mean age at the time of diagnosis of CD was 43±15 years (range 19 to 77). In line with a CD diagnosis, 97% of patients had circulating IgA TG2 autoantibodies, 90% had endomysium antibodies and 82% had circulating antibodies to gliadins (88% IgG, 75% IgA and 63% both). All patients carried a CD susceptibility class II MHC, either HLA DQ2 (96%) or HLA DQ8 (4%).

Clinical evaluation

Detailed neurologic history revealed complaints of gait instability in 24% of patients, persisting sensory symptoms in 12%, and 42% with frequent (weekly) headaches, whereby 21% had more than one of these 3 neurological symptoms. On clinical examination, gait instability was found in 29% of patients. Sustained gaze-evoked horizontal nystagmus was observed in 11%

(all apart from 1 of these 11 patients also had gait instability), and distal sensory loss and/or areflexia suggestive of peripheral neuropathy in 10. A neurophysiological assessment indicated 1/10 with evidence of large fiber neuropathy and another with small fiber neuropathy. In total 67/100 patients had symptoms and/or signs of neurological dysfunction (Table 1). Excluding headache, which as a sole neurological symptom was present in 17% of patients, 50% of patients had symptoms and/or signs of neurological dysfunction. We did not identify any other contributory factors (e.g. vitamin deficiencies, presence of Diabetes Mellitus) that could potentially explain the above findings amongst those patients with neurologic deficits.

Brain imaging

MRI and MR spectroscopy was performed in 96 patients. Four patients did not have imaging, 2 due to claustrophobia, 1 was pregnant and one had a recent coronary stent insertion. In one patient the spectroscopy was unsuccessful. MR spectroscopy of the cerebellum was abnormal in 45/95 (47%) patients, in 36 the abnormality was in the vermis (NAA/Cr ratio ≤0.95), in 25 in the hemisphere (NAA/Cr ratio ≤0.99) and in 16 in both. The prevalence of abnormal MR spectroscopy was not significantly different in patients with clinically overt balance problems and those without (53.1% vs 42.2%; p=0.311). The number of patients with white matter lesions over and above what is expected from age was 24/96 (25%). This compared to 18/525 (3%) in healthy volunteers (p<0.001). There was no difference in the vascular risk factors or age between the group of patients with white matter changes and those without.

Stratification for TG6 autoantibodies

Serologic testing for TG6 antibodies was done in 98 patients and were present in 39 (40%) patients (TG6 IgA : 28, TG6 IgG: 22, and both:11). This compared to 4% in the healthy population.[8]

Combined MR imaging and TG6 serology was available in 95 participants of which 37 (40%) had circulating autoantibodies to TG6. Patients with TG6 antibodies (TG6+) were significantly older than those without (TG6-): 46.7±14.8 vs 40.3±14.8 (*t*-test, p=0.043). Abnormal imaging alone was not significantly linked to the presence of TG6 antibodies (26/38 [68.4%] in TG6+ vs 29/57 [50.9%] in TG6-, p=0.09). However, 28/38 (73.7%) of TG6+ patients had abnormal MRS of the cerebellum and/or balance problems compared to 26/57 (45.6%) of TG6- patients (chi square, p=0.007). Table 2 summarizes these findings.

To investigate more specific imaging changes between the 2 groups we carried out a normalized brain volume (NBV) analysis for key regions. This revealed TG6+ patients to have significantly reduced cerebellar gray matter (GM) (NBV_{TG6+} / NBV_{TG6-} = 7.554±0.808 / 7.869±0.718; independent *t*-test p=0.050), and subcortical GM (NBV_{TG6+} / NBV_{TG6-} = 3.403±0.193 / 3.503±0.192; p=0.016), but not cortical GM (NBV_{TG6+} / NBV_{TG6-} = 27.286±1.823 / 27.793±1.632; p=0.168) (Fig. 1). Further analysis controlling for age and including sub-regions was undertaken. This showed that, for the TG6+ group, NBV remained significantly reduced in the primary subcortical GM region, and was significantly reduced in the thalamus, but not in any other comparison (Table 3). There was a trend for abnormal atrophy in the TG6+

group in three further cerebellar regions of interest (VIIb, VIIIa and Crus II, $p<0.07$). FIRST "vertex" analysis was used to investigate the significant finding in the thalamus. Models that did not control for age were first used to visualise the overall spatial pattern of atrophy between the two groups (Fig. 2a), showing a broad pattern of GM loss, with relative sparing of the anterior and pulvinar nuclei. With age correction applied (Fig. 2b), a significant locus of atrophy around the lateral / ventral posterior nuclei became evident.

Follow-up after 1 year of GFD

At one year, during telephone consultation with the first 52 patients recruited 20/24 (83.3%) indicated that their headaches had improved (in 15) or completely resolved (in 5) following the introduction of GFD.

All 52 patients underwent repeat brain imaging at one year (11.6 ± 1 month). Of the 23/52 patients who had abnormal NAA/Cr baseline vermian spectroscopy values, 12 (52.2%) improved to within the normal range. In 15 patients (29%) there was an increase in the number of white matter lesions on the second scan. All apart from one of these patients already had white matter abnormalities at baseline. At the time of their second scan, 20 (47%) out of 43 patients that had repeat serological testing still had positive serology (all had reduced levels compared to baseline; anti-TG6 antibody analysis was not performed at one year), suggesting inadequate compliance with GFD. We found no correlation between increased white matter lesions or change in MR spectroscopy and serological status at one year. The numbers however were very small.

Discussion

To our knowledge this is the largest prospective study to perform detailed neurologic evaluation in patients with newly diagnosed CD presenting to gastroenterologists, using both clinical assessments and brain imaging including MR spectroscopy. The study suggests that 67% of patients with CD already have neurological symptoms and/or signs of neurological dysfunction at the time of diagnosis of CD: gait instability in 24% of patients, persisting sensory symptoms in 12%, and 42% with frequent headaches. Particularly notable was the presence of gait ataxia in 29% of CD patients.

In addition 46% of patients had abnormal MR spectroscopy of the cerebellum and 25% had excessive white matter lesions over and above what is expected from age (3% in healthy population). Such neurological involvement often fails to be identified as it is unreported by patients and not questioned by gastroenterologists. As patients were from the local catchment area and referred by their local General Practitioners we believe that these findings are representative of adult patients with CD presenting to gastroenterologists.

TG6 autoantibodies have been proposed as an early marker for neurological involvement in gluten-related diseases.[8] In addition to TG6 autoimmunity, mutations of the TGM6 gene have been linked to ataxia.[11,12] Our data provide further support for the utility of this marker in newly-diagnosed CD patients as a surrogate for brain involvement, as TG6 antibody positive patients displayed significant atrophy of subcortical brain regions, particularly the thalamus. TG6 is expressed in a subset of neurons in the cerebellar cortex (Purkinje cells) and cerebellar nuclei but also in the thalamus.[13,14] The thalamus is involved in motor control in terms of acting as a relay centre between the cerebellum and

the motor cortex.[15] The observed significant thalamic atrophy and tendency towards cerebellar atrophy are therefore in line with loss or impairment of TG6+ neurons, potentially affecting GABA-ergic inhibitory pathways. This is in keeping with recent publications demonstrating brain hyper-excitability in patients with CD.[16]

We have previously demonstrated that the prevalence of circulating TG6 antibodies in CD patients presenting with ataxia is much higher than CD patients presenting to gastroenterologists (73% vs 40%), whereas the prevalence of TG6 antibodies in paediatric CD patients presenting to gastroenterologists was found to be 25%.[17] The TG6 antibody prevalence in these 3 groups is analogous to what is observed in patients with DH where circulating TG3 antibodies (DH-specific epidermal autoantibodies) are found in up to 71% of patients with DH but in only 50% and 11% of adult and pediatric CD patients, respectively.[18] It is noteworthy that in patients with DH, not all patients have circulating TG3 antibodies, yet 100% have IgA-TG3 deposits in the papillary dermis, the site of the primary manifestation.[19] Therefore, while the presence of these antibodies (TG2, TG3 and TG6) in the serum is diagnostically helpful, their absence in the serum does not preclude a localized response at the level of the target tissue (gut, skin and brain). This observation may explain why some of the patients from this study with neurological symptoms and signs and/or abnormal imaging were negative for serum TG6 antibodies. Such patients may still be positive for TG6-specific plasma cells, may have TG6 antibodies in the cerebrospinal fluid and also have deposition of TG6 antibodies in brain tissue which can only be assessed

retrospectively, post-mortem. Indeed, the presence of such TG6 antibody deposits was demonstrated in the brain of gluten ataxia patients.[7]

Data on the serological prevalence of TG6 in different gluten sensitivity-related populations are sparse and have been performed by mainly using customized in-house TG6 antibody assays. One such study found the prevalence to be just 10% amongst a cohort of a mixture of neurology patients with CD or AGA positivity without enteropathy.[20] Until such time as the widespread availability of a reliable and readily available standardized commercial TG6 assay in everyday clinical practice becomes a reality, it is difficult to draw any major conclusions on the differences in prevalence between published studies.

The prevalence of peripheral neuropathy in the current cohort was low at 2%. Another study demonstrated evidence of peripheral neuropathy in 23% of patients with established CD.[21] A population based epidemiological study on 28,232 patients with CD showed a 2.5-fold increased risk for peripheral neuropathy.[22] The low prevalence of peripheral neuropathy in our cohort suggests that gluten neuropathy may be a late manifestation of CD (the mean age at diagnosis of neuropathy in the cross-sectional study above was 51 compared to 43.8 in the cohort studied here). This is consistent with our observation that for patients with CD presenting with neuropathy the average age at diagnosis of CD was 67.

Twenty five percent of patients had significantly increased number of white matter abnormalities on MR imaging corrected for age, when compared to

healthy controls. Headaches, which subside with adherence to a GFD, and white matter abnormalities on MRI in CD patients have been previously reported using the term gluten encephalopathy.[23] The largest population study on CD patients showed a significantly increased risk of headache-related visits.[24] The majority of patients with CD and headache studied here indicated that their headache had improved or subsided (80%) after a year on a strict GFD. Similar percentage improvement (75%) has been reported in a recent systematic review of headache in CD.[25]

The observation of high prevalence of white matter abnormalities in patients with CD has also been reported in a smaller study of 17 patients who were on GFD.[26] The concern over the white matter abnormalities on MR imaging is their potential contribution to cognitive decline. A population study has suggested an increased risk of vascular dementia in patients with CD.[27] The aetiology of the headache in CD patients remains unclear but an interesting study demonstrated regional cerebral blood flow hypoperfusion in patients with CD not on GFD when compared to healthy controls and patients with CD on GFD.[28]

There are also some limitations to our study. We did not include a healthy control population alongside the CD group as the primary aim was to investigate differences between CD patients positive or negative for TG6 antibodies. It is therefore difficult to know how the prevalence of the clinical findings would compare to a healthy population examined in the same setting. However, the literature provides some guidance. A smaller cross-sectional study in patients with established CD (mean age 51 years), demonstrated stance and gait instability in 33% of patients, a figure similar to what we

found.[29] A study assessing the prevalence of gait instability in 115 healthy volunteers (same mean age as our group) found that none had gait instability.[30] Another study of untreated patients with CD showed the presence of neurological symptoms to be 15% compared to 0% in healthy controls.[31] Only half of the cohort of patients neurologically assessed at baseline underwent repeat imaging at 1 year. This was due to limited funding. However the beneficial effect of GFD in patients with GA, using MR spectroscopy as the outcome measure has already been reported in a much larger cohort of 117 patients.[9]

In conclusion, neurological dysfunction in patients with newly diagnosed CD presenting to the gastroenterologist is common but overlooked. TG6 antibodies are prevalent amongst patients with newly diagnosed CD and are associated with regional brain atrophy. Given the early presence of neurological dysfunction including brain atrophy there is a need for early diagnosis, increased awareness of the neurological manifestations amongst clinicians and reinforcement of adherence to a strict GFD by patients in order to avoid permanent neurological disability.

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Figure legends

Fig. 1 Brain atrophy in patients with TG6 autoantibodies.

Box plots visualizing normalized brain volumes (NBVs) for cerebral cortical (top left), subcortical (top right), and cerebellar cortical (bottom) grey matter for TG6 antibody positive or negative CD patients (asterisk: $p<0.05$, independent *t*-test).

Fig. 2 Visualisation of TG6 antibody-related atrophy in the thalamus.

TFCE-corrected output from FIRST "vertex" analysis of the thalamus before (A) and after (B) correction for age-related changes.

A. Red locations highlight areas which have significantly ($p\leq0.05$) atrophied in the TG6+ group compared to the TG6- group.

B. Red areas as above while blue areas identify locations where ($p\leq0.1$) for reference (top & middle); differences only reached significance in the left thalamus (middle). However, when reducing the threshold for red areas to $p\leq0.06$ in the right thalamus, a very similar pattern to the left is revealed (bottom).

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Table 1: Neurological evaluation of newly diagnosed CD patients

Number of patients with CD presenting to gastroenterologists recruited	100	
Mean age at diagnosis	43±15 years (range 19-77)	
Total number with symptoms and/or signs of neurological dysfunction	67%	
Symptoms	gait instability loss of balance	24%
	persisting sensory disturbance	12%
	frequent headaches	42%
Signs	gait ataxia	29%
	nystagmus	11%
	sensory loss and/or areflexia	10%
Excluding headache, total number with symptoms and signs of neurological dysfunction	50%	
Abnormal MR spectroscopy of the cerebellum	45/95 (47%)	
White matter abnormalities on MR brain imaging (excessive for age)	25% (3% in healthy controls)	
Number of patients undergoing MR imaging at one year	52/100	
Abnormal cerebellar spectroscopy at baseline	23/52 (44.2%)	
Normalization of MR spectroscopy at one year	12/23 (52.2%)	
Number TG6 positive CD patients	40%	

Table 2: Differences between patients with CD positive for TG6 antibodies compared to those negative for TG6 antibodies. * significant

	TG6 +ve	TG6-ve	p value
age	46.7	40.3	0.043*
abnormal brain imaging	68.4%	50.9%	0.09
abnormal MR spectroscopy of the cerebellum and gait ataxia	73.7%	45.6%	0.007*

Table 3: Volumetric analysis of individual brain regions following stratification for TG6 autoantibody positivity revealed significant differences in age-controlled ANOVAs.

	ROI	37 patients TG6+ Mean(SD) NBV	58 patients TG6- Mean(SD) NBV	TG6 positivity *p* value	Age correct. *p* value
Freesurfer Volumes	Subcortical GM	3.403(0.193)	3.503(0.192)	***.045***	***.050***
	Thalamus	0.928(0.066)	0.973(0.085)	***.035***	***.002***
	Caudate	0.397(0.041)	0.400(0.048)	*.406*	***.015***
	Putamen	0.581(0.072)	0.604(0.054)	*.221*	***.005***
	Globus Pallidus	0.177(0.022)	0.179(0.016)	*.823*	***.025***
	Hippocampus	0.519(0.051)	0.523(0.040)	*.750*	*.697*
	Amygdala	0.175(0.022)	0.182(0.017)	*.096*	*.752*
SUIT Volumes	Cerebellum GM	7.554(0.808)	7.869(0.718)	*.110*	*.097*
	I-IV	0.449(0.053)	0.470(0.048)	*.121*	***.027***
	V	0.588(0.061)	0.608(0.054)	*.154*	*.266*
	VI	1.224(0.126)	1.266(0.114)	*.163*	*.218*
	VIIb	0.619(0.063)	0.645(0.057)	*.065*	*.385*
	VIIIa	0.589(0.061)	0.615(0.055)	*.055*	*.407*
	VIIIb	0.478(0.052)	0.497(0.045)	*.118*	*.161*
	IX	0.417(0.051)	0.432(0.043)	*.327*	***.004***
	X	0.060(0.011)	0.064(0.010)	*.209*	***<.001***
	Vermis	0.351(0.041)	0.364(0.037)	*.259*	***.024***
	Crus I	1.625(0.180)	1.700(0.163)	*.103*	***.027***
	Crus II	1.155(0.120)	1.207(0.110)	*.064*	*.222*