Grey matter density decreases as well as increases in patients with classic galactosemia

Brain Research 1648 (2016) 339–344

Contents lists available at ScienceDirect

Brain Research

journal homepage: www.elsevier.com/locate/brainres

Research report

Grey matter density decreases as well as increases in patients with classic galactosemia: A voxel-based morphometry study

Inge Timmers [a,b,*], Lisanne D. van der Korput [c], Bernadette M. Jansma [a,b], M. Estela Rubio-Gozalbo [c,d]

[a] Department of Cognitive Neuroscience, Maastricht University, PO Box 616, 6200 MD Maastricht, The Netherlands
[b] Maastricht Brain Imaging Center (M-BIC), PO Box 616, 6200 MD Maastricht, The Netherlands
[c] Department of Pediatrics, Maastricht University Medical Center, PO Box 5800, 6202 AZ Maastricht, The Netherlands
[d] Laboratory Genetic Metabolic Diseases, Maastricht University Medical Center, PO Box 5800, 6202 AZ Maastricht, The Netherlands

ARTICLE INFO

Article history:
Received 12 January 2016
Received in revised form
13 July 2016
Accepted 4 August 2016
Available online 5 August 2016

Keywords:
Classic galactosemia
GALT
Neuroimaging
Grey matter
Voxel-based morphometry

ABSTRACT

Brain impairments have been observed in patients with classic galactosemia, an inherited metabolic disorder resulting in a particular neuro-cognitive profile. Neuroimaging studies showed abnormalities such as diffuse white mater (WM) abnormalities and grey matter (GM) atrophy. Our current study analysed grey matter density using voxel-based morphometry (VBM) and compared the brains of eight adolescent patients with classic galactosemia with eight healthy gender- and aged-matched controls. GM density differences were found in several regions. Decreased GM density was found in the patients in the bilateral putamen and bilateral occipital cortex. Increased GM density in the patients, on the other hand, was found in the bilateral inferior frontal and medial prefrontal cortex. The anatomical profile of the abnormalities is in line with the neuro-cognitive profile of patients with classic galactosemia, including motor dysfunction, speech and language difficulties and higher order cognitive problems. Less favourable GM densities in patients (either increased or decreased compared to controls) correlated with younger age, a worse visual working memory performance, and an older age at initiation of the galactose-restricted diet. To conclude, this explorative study is the first to analyse the GM using VBM in this population, and demonstrates a mixed profile of both increased and decreased GM density in these patients.

1. Introduction

Classic galactosemia is a rare autosomal recessive disorder, caused by mutations of the *GALT* gene (Holton et al., 2001). The mutations lead to a severe deficiency of the GALT (galactose-1-phosphate uridylyltransferase) enzyme activity, causing an impaired metabolization of galactose. Galactose is derived from dietary (i.e., milk) and endogenous sources. After neonates start to drink milk, life-threatening symptoms occur including lethargy, poor feeding, jaundice, and hepatomegaly. A galactose restricted diet resolves the acute symptoms quickly. However, despite the diet, most patients experience a variable spectrum of neuro-cognitive impairments with varying severity, typically in the low to low-average range. Patients with classic galactosemia often experience language production impairments (including problems with lexical and syntactic planning), speech (motor) and voice abnormalities (e.g., childhood apraxia of speech and dysarthria), and/or motor dysfunction (e.g., coordination disorders) (Potter et al., 2008; Potter, 2011; Rubio-Agusti et al., 2013; Timmers et al., 2012; Timmers et al., 2015a), Further, studies have reported neurological signs (e.g., tremor or ataxia), slower information processing, memory and executive functioning deficits, mathematical difficulties, visuo-spatial abnormalities, and generally a lower intelligence level going alongside with lower educational attainment (Antshel et al., 2004; Doyle et al., 2010; Schadewaldt et al., 2010; Waggoner et al., 1990; Waisbren et al., 2012). The pathophysiology of these neural complications is not yet understood completely.

Previous studies looking at brain structure have focused mainly on white matter (WM) as the underlying pathogenic mechanism of cognitive abnormalities in this disease. Several groups observed diffuse WM abnormalities on T1-weighted Magnetic Resonance

Abbreviations: GALT, galactose-1-phosphate uridylyltransferase; WM, white matter; MRI, magnetic resonance imaging; GM, grey matter; VBM, voxel-based morphometry

* Corresponding author at: Department of Cognitive Neuroscience, Maastricht University, PO Box 616, 6200 MD, Maastricht, The Netherlands.
E-mail address: inge.timmers@maastrichtuniversity.nl (I. Timmers).

http://dx.doi.org/10.1016/j.brainres.2016.08.005
0006-8993/

Imaging (MRI) (Hughes et al., 2009; Nelson et al., 1992; Otaduy et al., 2006; Rubio-Agusti et al., 2013). We recently analysed WM in more detail, and demonstrated abnormalities in density and orientation dispersion of neurites, two indices characterizing WM (Timmers et al., 2015b). Moreover, not only did the profile of the affected regions correspond with the profile of language, motor and cognitive abnormalities of patients with classic galactosemia, we also identified correlations with behavioural outcome, age, and age at onset of the diet.

In addition to WM abnormalities, brain atrophy has also been observed in patients with classic galactosemia. Nelson et al. (1992) found mild cerebral and cerebellar atrophy. Abnormal MRI findings, in particular a volume loss of the cortex, were furthermore found in patients suffering from motor complications (Rubio-Agusti et al., 2013), and Hughes and colleagues (2009) observed progressive cerebellar atrophy in their MRI study. These atrophy findings suggest grey matter (GM) abnormalities in patients with classic galactosemia. However, these abnormalities have not been studied in a quantitative manner, nor have they been related to the neuro-cognitive profile.

This current MRI study therefore examines the GM in patients with classic galactosemia in more detail using a voxel-based morphometry (VBM) approach. VBM is used to analyse potential differences in GM density between patients and healthy controls, and to examine clinical correlations with anatomical findings. VBM is an unbiased whole brain approach, comparing different brains on a voxel-by-voxel basis using structural (T1-weighted) MR images (Ashburner and Friston, 2000; Mechelli et al., 2005). The aim is to identify differences in the local composition of brain tissue, while discounting large scale differences in gross anatomy and position. The result can be used to detect atrophy and brain tissue expansion (Whitwell, 2009). By using such an explorative approach, we aim to get an overview of the GM abnormalities in these patients.

2. Results

2.1. Differences across groups

The patients showed several clusters of GM density abnormalities, comprising both decreases and increases (see Fig. 1). A decreased GM density (bilaterally) was seen in the putamen, part of the subcortical nuclei of the basal ganglia. Decreased density was also observed in the bilateral medial occipital cortex. An overview and more details of all clusters can be found in Table 1.

Increased GM density was found in both hemispheres, but to a higher extent in the right hemisphere (Fig. 1). The increases were observed in the medial prefrontal and the inferior frontal region (comprising BA 45, and in the right hemisphere also the anterior insular region).

2.2. Correlations with regions of decreased GM density

Significant positive correlations were found between the age of the patients and the GM density of the bilateral occipital regions (right: $r=0.78$ [Fig. 2], $p=0.024$ and left: $r=0.77$, $p=0.026$). Thus, the older the patients, the higher (or less abnormal) the GM

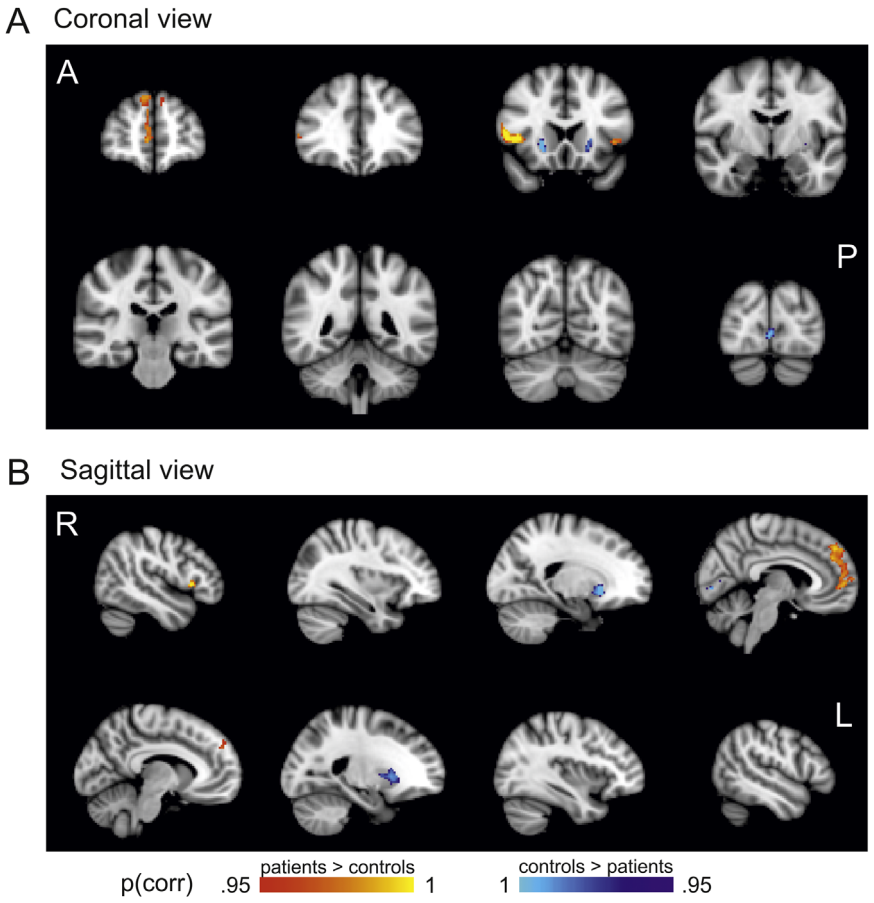

Fig. 1. Overview of GM density decreases and increases. Presented are coronal (A) and sagittal (B) slices of T1-weighted images overlaid with statistical maps of the group differences in GM density, showing both decreases (in blue) and increases (in yellow/orange). Presented results are TFCE-corrected and thresholded at a corrected alpha-level of 0.05. Note that images are in radiological conventrion (left is right).

Table 1
An overview of the regional GM density differences across groups and correlations.

	Mean GM density values		Coordinates of peak difference (MNI)					Correlations		
	Patients	**Controls**	**x-axis**	**y-axis**	**z-axis**	**cluster size**	***p-corr***	**Measure**	***r***	***p-uncorr***
GM increases										
Right medial prefrontal region	0.79	0.53	4	46	38	305	0.007	Visual working memory	−0.77	0.027
								Age at start diet	0.71	0.048
Left medial prefrontal region	0.88	0.61	−8	50	32	30	0.040			
Right inferior frontal region	0.95	0.64	44	16	2	195	0.001			
Left inferior frontal region	0.82	0.57	−42	18	0	21	0.013			
GM decreases										
Right putamen	0.29	0.48	22	18	−2	104	0.008			
Left putamen	0.21	0.37	−18	12	−2	162	0.010			
Right occipital region	0.44	0.67	4	−84	−2	129	0.002	Age	0.78	0.024
Left occipital region	0.47	0.70	0	−84	−2	95	0.002	Age	0.77	0.026

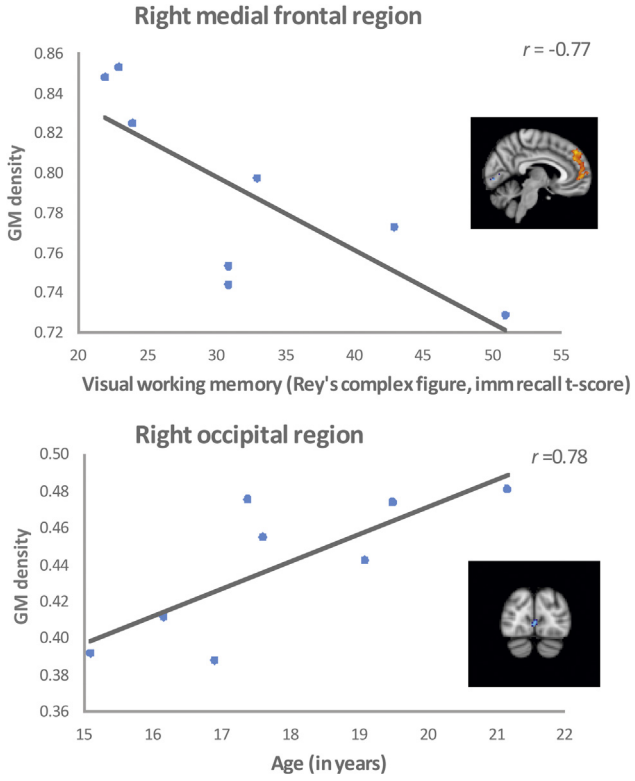

Fig. 2. ROI correlation analyses. A selection of two significant correlations of regions of interests (ROIs) based on group differences in GM density. Added are a linear trend line, the correlation, and an illustration of the location of the ROI.

density was. No other correlations were found within this region, nor with the other regions. For an overview of the correlations, see Table 1.

2.3. Correlations with regions of increased GM density

A significant negative correlation was found between the density of the right medial prefrontal region and visual working memory performance ($r = -0.77$, $p=0.027$; see Fig. 2). Thus, a better visual working memory performance was related to a lower (or less abnormal) GM density. In addition, age at initiation of diet showed a positive correlation with the density of the same region (right medial prefrontal region; $r=0.71$, $p=0.048$). The older the patients were at initiation of the galactose restricted diet, the more abnormal the GM density of this region. No other correlations were found with this region, nor with any of the other regions showing increased GM density.

3. Discussion

This study investigated GM abnormalities in patients with classic galactosemia, for the first time using a quantitative approach. Based on previous literature finding atrophy, we hypothesised that decreases in GM density in the patients would be observed in regions corresponding with the cognitive profile of these patients. Indeed, GM density decreases were found, but unexpectedly also GM density increases were revealed as compared to the healthy controls.

3.1. GM density decreases

Several regions were found to show differences in GM density across the groups. Decreased GM density, which could be interpreted as atrophy or volume loss, was found in bilateral putamen as well as in bilateral occipital regions. The putamen, as part of the basal ganglia, is implicated in motor functions and control. The putamen has connections to the premotor areas and the motor cortex (Draganski et al., 2008), providing a potential link between these findings and the motor (speech) abnormalities in this population (Potter et al., 2013; Rubio-Agusti et al., 2013). In addition, through its connections with other cortical areas (e.g., prefrontal cortex) the putamen is linked to higher order cognitive functions as well (e.g., executive functioning, working memory, sequence learning). Some previous studies have also identified hypodense abnormalities in the putamen in these patients (Böhles et al., 1986; Haberland et al., 1971), while others have not (Crome, 1962; Rubio-Agusti et al., 2013). This might reflect the variability within patients. Using a group-based VBM-approach, we were able to clearly identify GM abnormalities in bilateral basal ganglia. However, we were not able to relate this to any of our behavioural measures. Unfortunately, these measures did not include a pure motor task, but included speech-motor functioning as reflected in voice onset times (i.e., a measure which might not be suited to reflect basal ganglia functioning). In general, the GM findings are in line with our recent WM analysis (Timmers et al., 2015b), showing reduced neurite density and increased orientation dispersion in the internal capsule, a WM tract with projection fibres passing through the basal ganglia. As part of the corticospinal tract, it is of major importance in motor functions, connecting the midbrain and the cortex.

In addition to these subcortical findings, we also observed GM decreases in bilateral occipital cortex, which correspond with the cerebral atrophy described in previous studies (Böhles et al., 1986; Nelson et al., 1992), although these descriptions were rather general (and not specifically point towards the occipital cortex). Visuo-spatial abnormalities have been reported in classic galactosemia before (Doyle et al., 2010), and might be a reflection of these findings. The abnormalities were correlated with age, indicating that the GM density was less abnormal in older patients, but not with any of the other behavioural measures. In contrast, it has previously been suggested that classic galactosemia is progressive in nature, with several studies demonstrating cognitive decline with age (Doyle et al., 2010; Waggoner et al., 1990). Also in the WM, we have found that age was related to a worsening of WM microstructure properties (Timmers et al., 2015a). Others, in contrast, have found no evidence for such a claim (Waisbren et al., 2012; Schadewaldt et al., 2010). Although we should be careful interpreting the current findings, given the sample size and the cross-sectional nature of this study, it does indicate that the question of decline with age is not straightforward and might even be dependent on the outcome.

3.2. GM density increases

Based on previous observations of atrophy, we did not expect any increases in GM density, as compared to healthy controls. The brain regions in which increases were found are the medial and inferior frontal cortex (more specifically BA 45, and on the right also the anterior insula). The inferior frontal gyrus (IFG) plays a role in language production (Hagoort, 2005; Price, 2012), and has traditionally been linked to syntactic processing, although other studies have implicated the region in lexical and phonological processing as well (Sahin et al., 2009). Also in language comprehension, the IFG has been found to be important in retrieving and integrating lexical information (Snijders et al., 2009), and theories have suggested an integrating/unifying role for the IFG (Hagoort, 2005). In a recent functional MRI study of our group, we demonstrated functional abnormalities in this region (Timmers et al., 2015a). We found more extensive recruitment of several regions during active language production, including the inferior frontal gyrus (IFG). This fits with our event-related potential (ERP) study revealing higher amplitudes in several language-related components, reflecting the need for more resources during active language production (Timmers et al., 2012). In addition, we found stronger functional connectivity patterns with the frontal regions, compared to healthy controls. Moreover, the cerebral blood flow (CBF) was found to be higher in right IFG. The more intense recruitment and increased blood flow (in rest) of this region might be related to the more dense GM we found in this study. Lastly, also WM abnormalities were found in tracts connecting the inferior frontal regions to other regions of the brain (i.e., the uncinate fasciculus and the superior longitudinal fasciculus) (Timmers et al., 2015b). From various perspectives, it is clear that the inferior frontal cortex is a region of key importance in classic galactosemia.

In addition to the inferior frontal region, increases were observed in the medial prefrontal cortex. The GM density of the right medial prefrontal region was correlated to visual working memory (Rey Osterrieth Complex Figure Immediate Recall) performance in these patients, which is in line with the importance of this region in learning and storing memories/associations and responses (Euston et al., 2012; Riga et al., 2014). Accordingly, in our WM study, we also found abnormalities in the corona radiate and forceps minor, carrying information into the cortex, and connecting lateral and medial surfaces of the frontal cortex, respectively (Timmers et al., 2015b).

The GM density increases might reflect abnormal GM maturation in these patients. During brain maturation, the GM volume slowly reduces as a result of pruning processes, while the WM volume increases (Giorgio et al., 2010). In normal development, this GM development shows regional differences and can continue in cortical regions up until 20 years of age (Giedd et al., 1999). A different or delayed developmental course of the GM could thus result in an increased GM volume compared to healthy controls (Douaud et al., 2009). In terms of pathophysiological mechanisms, this aberrant maturation might be linked to the accumulation of metabolites due to the deficiency to metabolise galactose (e.g., Gal-1-P and galactitol). In addition to being toxic for tissue and organs, it might also result in (myo)inositol deficiencies, potentially involved in abnormal signalling and development of the brain in classic galactosemia (Berry, 2011).

3.3. Lack of cerebellar abnormalities

Interestingly, our results did not show any abnormalities in the cerebellar GM. Other studies described cerebellar atrophy (Böhles et al., 1986; Nelson et al., 1992; Rubio-Agusti et al., 2013). Our WM study also did not detect any cerebellar abnormalities in this group. A possible explanation of these contrasting findings is the different clinical phenotypes of the participants, as our patients did not suffer from clear neurologic symptoms such as tremor or ataxia (as a rarer subgroup of patients do). The phenotypic variability of patients with classic galactosemia is enormous and only a sub-group of patients show cerebellar signs and symptoms (Waisbren et al., 2012). Alternatively, more in-depth analyses are needed to confirm the lack of any cerebellar abnormalities in our patient group, as our approach might not have picked up any cerebellar abnormalities, due to limited power in this region.

3.4. Limitations

There are several limitations of the current study. First, the sample size was limited. Despite this, however, the group differences in GM density survived stringent statistical testing and corrections for multiple comparisons across space. For the correlations, however, we did not correct for multiple comparisons. The observed correlations – although high – therefore should only be taken as first explorations to link brain abnormalities to neurocognitive functioning. Furthermore, the number and extent of behavioural measures was limited (and only available for the patient group), urging the need for additional studies with elaborate neuropsychological testing to confirm and extend our correlational findings. Finally, the data presented here are cross-sectional, limiting the conclusions we can make on the temporal course of abnormalities. Longitudinal studies should be performed to tackle questions about changes in GM density over time.

4. Conclusion

We demonstrate GM density abnormalities in adolescent patients with classic galactosemia compared to healthy age- and gender-matched controls. A decreased GM density in patients was found in the bilateral putamen and the bilateral occipital cortex, whereas increased GM density in the patients was found in the inferior frontal and medial prefrontal cortex of both hemispheres. The anatomical locations of the affected regions are in line with the neuro-cognitive profile of patients with classic galactosemia, including motor (speech) abnormalities, language production impairments, and memory and executive functioning deficits. In addition, despite the limited sample size, high correlations of the GM regions with visual working memory, age, and age of initiation

of diet were identified. Future studies are warranted to confirm and extend our results. For instance, more detailed analyses of the GM (e.g., cortical thickness, density and orientation dispersion of the GM using NODDI) and longitudinal investigations of the GM will advance our knowledge on the GM characteristics and development in classic galactosemia even more.

5. Methods

5.1. Participants

Eight patients with classic galactosemia (2 males, 6 females) and eight age- and gender-matched controls (2 males, 6 females) participated in this study. The included patients were 15–21 years old and were diagnosed with classic galactosemia using GALT enzyme activity assay and/or *GALT*-gene mutational analysis. All patients were on a galactose restricted diet since diagnosis. Recruitment was based on a previous study (Timmers et al., 2012) in which all patients aged 12–18 years in the Netherlands were invited to participate. For this study, patients older than 14 years old were invited again. Controls were recruited mainly via regional schools. Characteristics of the participants, including behavioural data from an earlier study, can be found in Table 2. Participants with another disease or disorder which could influence the cognitive function independent of classic galactosemia were excluded. All participants were Dutch native speakers. The participants were screened for MRI compatibility and signed informed consent. In case of minors, both parents/caregivers also gave informed consent. The study took place at Maastricht University, and ethical approval for the study was given by the Medical Ethical Committee of Maastricht University Hospital/Maastricht University.

5.2. Data acquisition

Imaging was performed on a 3-T Siemens Trio whole body scanner using a 32-channel head coil (Siemens Healthcare, Erlangen, Germany). The structural MRI data, a T1-weighted ADNI MPRAGE sequence, were obtained with full brain coverage with the following parameters: 1 mm iso-voxel resolution, TR=2250 ms; TE=2.6 ms, 192 slices.

5.3. Data analyses

VBM was performed using the FSL-VBM 5.0 tool, part of the FMRIB Software Library [FSL] version 5.0 (Douaud et al., 2007; Smith et al., 2004). An optimised VBM protocol was used (Good et al., 2001), which includes extra steps to prevent interpretation errors caused by misclassification of non-brain voxels.

Pre-processing included positioning of the T1-weighted MR images in the same orientation as the standard MNI template, brain extraction using the brain extraction tool (*BET*, FSL), and segmentation into cerebrospinal fluid, white and grey matter. For spatial normalisation, the GM images were non-linearly registered to the GM ICBM-152 template using *FNIRT* of FSL (Andersson et al., 2007). The GM images were modulated as a correction of local contraction or enlargement the nonlinear component of the spatial normalisation caused. Finally, the images were smoothed applying an isotropic Gaussian kernel with a sigma of 2 mm.

Table 2
Characteristics of the participants.

	Patients		**Controls**	
	Value/mean (median)	**Range (IQR)**	**Value/mean (median)**	**Range (IQR)**
Group size	8		8	
Males/females	2 / 6		2/6	
Age (in years)[a]	17.9 (17.3)	15.9–21.2 (2.8)	17.2 (17.8)	14.7–20.0 (3.6)
GALT activity (in % of reference value)[b]	0.54% (0%)	0–1.52% (.008%)		
***GALT* gene mutation**	4	Q188R/Q188R		
	2	400Tdel/unknown		
	1	L195P/K229N		
	1	unknown		
Age at initiation of diet (in days)	11.8 (6.5)	0–35 (18.5)		
Educational level	7	Lower/vocational level (3 secondary; 4 tertiary education)	3	Lower/vocational level (tertiary education)
	1	Higher level (secondary education)	3	Pre-university level (secondary education)
			2	Unknown
Special education[c]	63%			
Physical therapy[c]	75%			
Speech therapy[c]	88%			
Visual working memory (*t*-score)[d]	32.3 (31.0)	22–51 (11.8)		
Sustained attention (mean RT in seconds)[e]	13.8 (13.2)	11.3–18.1 (1.3)		
Verbal working memory (scaled score)[f]	3.9 (3.5)	3–7 (2.6)		
Voice onset time sentence production (in seconds)[g]	1.97 (2.01)	1.49–2.20 (0.18)		

[a] No significant difference in age between the groups was found [$F_{1,16}=0.44$, $p=0.519$].
[b] The percentage of GALT activity at diagnosis.
[c] At one point in their lives.
[d] T-score determined by Rey Osterrieth Complex Figure Immediate Recall (Meyers and Meyers, 1995).
[e] Response time determined by mean reaction time in Bourdon-Vos task (Vos, 1988) – as a reference: the mean RT in the control group from Timmers et al., 2012, was 14.4 s (median 13.7 s) with a range of 10.7–21.3 s (IQR: 4.2 s).
[f] Scaled score determined by Digit Span subtest of WISC-R, with an average of 10 (SD 3).
[g] Average voice onset time in a sentence production task – as reference, the average voice onset time from the control group from Timmers et al., 2012, was 1.89 s (median 1.88 s) with a range of 1.25–2.62 s (IQR: 1.09 s) (see Timmers et al., 2012, for more information on these behavioural measures). All neuropsychological testing was performed by author IT.

For the statistical analysis, randomized permutation testing ($n=5000$) was performed, correcting for multiple comparisons across space using the Threshold-Free Cluster Enhancement (TFCE) option (Smith and Nichols, 2009). A design was used having group as a between-subjects factor and age as a covariate. In addition, regions of interest (ROIs) showing significant group differences were extracted for further correlation analyses. Correlations were calculated with the behavioural measures (i.e., visual and verbal working memory, sustained attention, and voice onset times in a language production task) and the patient characteristics age, age of initiation of diet, and GALT activity (here, no correction for multiple testing was applied due to the small sample size, but only correlations $>|0.7|$ are reported). For the significance level, an alpha of 0.05 (corrected for multiple comparisons where applicable) was used.

Acknowledgements

We kindly acknowledge the Dutch Galactosemia Patient Organisation (GVN) for logistic support. We further thank the participants and their parents for their time, effort and cooperation, and the anonymous reviewer for helpful suggestions.

The current study was funded by a Galactosemia Research Fund (GOF) from the Dutch Patient Organisation (GVN), and a Maastricht University Incentive ("Women in higher positions in science").

References

Andersson, J.L.R., Jenkinson, M., Smith, S., 2007. Non-linear Registration, Aka Spatial Normalisation. FMRIB technical report TR07JA2 from www.fmrib.ox.ac.uk/analysis/techrep.

Antshel, K.M., Epstein, I.O., Waisbren, S.E., 2004. Cognitive strengths and weaknesses in children and adolescents homozygous for the galactosemia Q188R mutation: a descriptive study. Neuropsychology 18, 658–664.

Ashburner, J., Friston, K.J., 2000. Voxel-based morphometry – the methods. Neuroimage 11, 805–821.

Berry, G.T., 2011. Is prenatal myo-inositol deficiency a mechanism of CNS injury in galactosemia? J. Inherit. Metab. Dis. 34, 345–355.

Böhles, H., Wenzel, D., Shin, Y.S., 1986. Progressive cerebellar and extrapyramidal motor disturbances in galactosaemic twins. Eur. J. Pediatr. 145, 413–417.

Crome, L., 1962. A case of galactosaemia with the pathological and neuropathological findings. Arch. Dis. Child. 37, 415–421.

Douaud, G., Smith, S., Jenkinson, M., Behrens, T., Johansen-Berg, H., Vickers, J., James, S., Voets, N., Watkins, K.E., Matthews, P.M., James, A.C., 2007. Anatomically related grey and white matter abnormalities in adolescent-onset schizophrenia. Brain 130, 2375–2386.

Douaud, G., Mackay, C., Andersson, J., James, S., Quested, D., Ray, M.K., Connell, J., Roberts, N., Crow, T.J., Matthews, P.M., Smith, S., James, A., 2009. Schizophrenia delays and alters maturation of the brain in adolescence. Brain 132, 2437–2448.

Doyle, C.M., Channon, S., Orlowska, D., Lee, P.J., 2010. The neuropsychological profile of galactosaemia. J. Inherit. Metab. Dis. 33, 603–609.

Draganski, B., Kherif, F., Klöppel, S., Cook, P.A., Alexander, D.C., Parker, G.J., Deichmann, R., Ashburner, J., Frackowiak, R.K., 2008. Evidence for segregated and integrative connectivity patterns in the human Basal Ganglia. J. Neurosci. 28, 7143–7152.

Euston, D.R., Gruber, A.J., McNaughton, B.L., 2012. The role of medial prefrontal cortex in memory and decision making. Neuron 76 (6), 1057–1070.

Giedd, J.N., Blumenthal, J., Jeffries, N.O., Castellanos, F.X., Liu, H., Zijdenbos, A., Paus, T., Evans, A.C., Rapoport, J.L., 1999. Brain development during childhood and adolescence: a longitudinal MRI study. Nat. Neurosci. 2, 861–863.

Giorgio, A., Watkins, K.E., Chadwick, M., James, S., Winmill, L., Douaud, G., De Stefano, N., Matthews, P.M., Smith, S.M., Johansen-Berg, H., James, A.C., 2010. Longitudinal changes in grey and white matter during adolescence. Neuroimage 49, 94–103.

Good, C.D., Johnsrude, I.S., Ashburner, J., Henson, R.N., Friston, K.J., Frackowiak, R.S., 2001. A voxel-based morphometric study of ageing in 465 normal adult human brains. NeuroImage 14, 21–36.

Haberland, C., Perou, M., Brunngraber, E.G., Hof, H., 1971. The neuropathology of galactosemia: a histopathological and biochemical study. J. Neuropathol. Exp. Neurol. 30, 431–447.

Hagoort, P., 2005. On Broca, brain, and binding: a new framework. Trends Cognit. Sci. 9, 416–423.

Holton, J.B., Walter, J.H., Tyfield, L.A., 2001. Galactosemia. In The Metabolic & Molecular Bases of Inherited Disease.In: Scriver, C.R., Childs, B. (Eds.), Vol., McGraw-Hill, New York, pp. 1553–1587.

Hughes, J., Ryan, S., Lambert, D., Geoghegan, O., Clark, A., Rogers, Y., Hendroff, U., Monavari, A., Twomey, E., Treacy, E.P., 2009. Outcomes of Siblings with Classical Galactosemia. J. Pediatr. 154, 721–726.

Mechelli, A., Price, C.J., Friston, K.J., Ashburner, J., 2005. Voxel-based morphometry of the human brain: methods and applications. Curr. Med. Imaging Rev. 1, 105–113.

Meyers, J.E., Meyers, K.R., 1995. Rey Complex Figure Test and Recognition Trial: Professional Manual. Vol. Psychological Assessment Resources, Inc., Odessa.

Nelson, M.D., Wolff, J.A., Cross, C.A., Donnell, G.N., Kaufman, F.R., 1992. Galactosemia: evaluation with MR Imaging. Radiology 184, 255–261.

Otaduy, M.C.G., Leite, C.C., Lacerda, M.T.C., Costa, M.O.R., Arita, F., Prado, E., Rosemberg, S., 2006. Proton MR spectroscopy and imaging of a galactosemic patient before and after dietary treatment. Am. J. Neuroradiol. 27, 204–207.

Potter, N.L., Lazarus, J.A.C., Johnson, J.M., Steiner, R.D., Shriberg, L.D., 2008. Correlates of language impairment in children with galactosaemia. J. Inherit. Metab. Dis. 31, 524–532.

Potter, N.L., 2011. Voice disorders in children with classic galactosemia. J. Inherit. Metab. Dis. 34, 377–385.

Potter, N.L., Nievergelt, Y., Shriberg, L.D., 2013. Motor and Speech Disorders in Classic Galactosemia. JIMD Rep. 11, 31–41.

Price, C.J., 2012. A review and synthesis of the first 20 years of PET and fMRI studies of heard speech, spoken language and reading. NeuroImage 62, 816–847.

Riga, D., Matos, M.R., Glas, A., Smit, A.B., Spijker, S., Van den Oever, M.C., 2014. Optogenetic dissection of medial prefrontal cortex circuitry. Front. Syst. Neurosci., 8.

Rubio-Agusti, I., Carecchio, M., Bhatia, K.P., Kojovic, M., Parees, I., Chandrashekar, H. S., Footitt, E.J., Burke, D., Edwards, M.J., Lachmann, R.H.L., Murphy, E., 2013. Movement disorders in adult patients with classical galactosemia. Mov. Disord. 28, 804–810.

Sahin, N.T., Pinker, S., Cash, S.S., Schomer, D., Halgren, E., 2009. Sequential processing of lexical, grammatical, and phonological information within Broca's area. Science 326, 445–449.

Schadewaldt, P., Hoffmann, B., Hammen, H.W., Kamp, G., Schweitzer-Krantz, S., Wendel, U., 2010. Longitudinal assessment of intellectual achievement in patients with classical galactosemia. Pediatrics 125, 374–381.

Smith, S.M., Jenkinson, M., Woolrich, M.W., Beckmann, C.F., Behrens, T.E.J., Johansen-Berg, H., Bannister, P.R., De Luca, M., Drobnjak, I., Flitney, D.E., Niazy, R., Saunders, J., Vickers, J., Zhang, Y., De Stefano, N., Brady, J.M., Matthews, P.M., 2004. Advances in functional and structural MR image analysis and implementation as FSL. Neuroimage 23, 208–219.

Smith, S.M., Nichols, T.E., 2009. Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. Neuroimage 44, 83–98.

Snijders, T.M., Vosse, T., Kempen, G., Van Berkum, J., Petersson, K.M., Hagoort, P., 2009. Retrieval and unification of syntactic structure in sentence comprehension: an FMRI study using word-category ambiguity. Cereb. Cortex 19, 1493–1503.

Timmers, I., Jansma, B.M., Rubio Gozalbo, M.E., 2012. From mind to mouth: event related potentials of sentence production in classic galactosemia. PLoS One 7, e52826.

Timmers, I., van den Hurk, J., Hofman, P.A., Zimmermann, L.J., Uludağ, K., Jansma, B. M., Rubio-Gozalbo, M.E., 2015a. Affected functional networks associated with sentence production in classic galactosemia. Brain Res. 1616, 166–176.

Timmers, I., Zhang, H., Bastiani, M., Jansma, B.M., Roebroeck, A., Rubio-Gozalbo, M. E., 2015b. White matter microstructure pathology in classic galactosemia revealed by neurite orientation dispersion and density imaging. J. Inherit. Metab. Dis. 38 (2), 295–304.

Vos, P.G., 1988. Bourdon-Vos-Test [Bourdon-Vos Test]. Vol., Swets & Zeitlinger, Lisse.

Waggoner, D.D., Buist, N.R.M., Donnell, G.N., 1990. Long-term prognosis in galactosemia: results of a survey of 350 cases. J. Inherit. Metab. Dis., 802–818.

Waisbren, S.E., Potter, N.L., Gordon, C.M., Green, R.C., Greenstein, P., Gubbels, C.S., Rubio-Gozalbo, M.E., Schomer, D., Welt, C., Anastasoaie, V., D'Anna, K., Gentile, J., Guo, C.Y., Hecht, L., Jackson, R., Jansma, B.M., Li, Y.J., Lip, V., Miller, D.T., Murray, M., Power, L., Quinn, N., Rohr, F., Shen, Y.P., Skinder-Meredith, A., Timmers, I., Tunick, R., Wessel, A., Wu, B.L., Levy, H., Elsas, L., Berry, G.T., 2012. The adult galactosemic phenotype. J. Inherit. Metab. Dis. 35, 279–286.

Whitwell, J.L., 2009. Voxel-based morphometry: an automated technique for assessing structural changes in the brain. J. Neurosci. 29, 9661–9664.