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Research Report

# Affected functional networks associated with sentence production in classic galactosemia

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ABSTRACT

Patients with the inherited metabolic disorder classic galactosemia have language production impairments in several planning stages. Here, we assessed potential deviations in recruitment and connectivity across brain areas responsible for language production that may explain these deficits. We used functional magnetic resonance imaging (fMRI) to study neural activity and connectivity while participants carried out a language production task. This study included 13 adolescent patients and 13 age- and gender-matched healthy controls. Participants passively watched or actively described an animated visual scene using two conditions, varying in syntactic complexity (single words versus a sentence). Results showed that patients recruited additional and more extensive brain regions during sentence production. Both groups showed modulations with syntactic complexity in left inferior frontal gyrus (IFG), a region associated with syntactic planning, and in right insula. In addition, patients showed a modulation with syntax in left superior temporal gyrus (STG), whereas the controls did not. Further, patients showed increased activity in right STG and right supplementary motor area (SMA). The functional connectivity data showed similar patterns, with more extensive connectivity with frontal and motor regions, and restricted and weaker connectivity with superior temporal regions. Patients also showed higher baseline cerebral blood flow (CBF) in right IFG and trends towards higher CBF in bilateral STG, SMA and the insula. Taken together, the data demonstrate that language

Abbreviations: (a)CBF, (absolute) cerebral blood flow; ASL, arterial spin labeling; BOLD signal, blood oxygenation level dependent signal; CAS, childhood apraxia of speech; EEG, electroencephalography; ERP, event-related potential; (f)MRI, (functional) magnetic resonance imaging; GALT, galactose-1-phosphate uridyl transferase; GLM, general linear model; IFG, inferior frontal gyrus; MTG, middle temporal gyrus; PG, precentral gyrus; POI, patch of interest; PT, planum temporale; SMA, supplementary motor area; STG, superior temporal gyrus; TE, echo time; TR, repetition time

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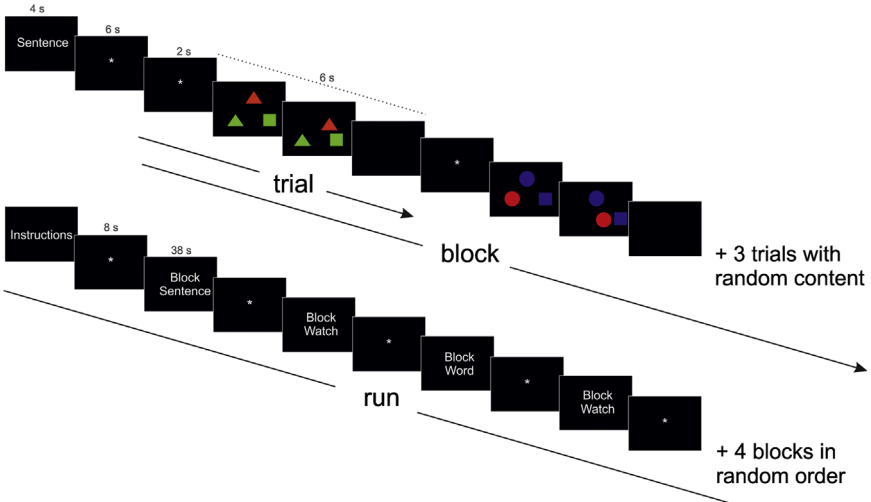
abnormalities in classic galactosemia are associated with specific changes within the language network. These changes point towards impairments related to both syntactic planning and speech motor planning in these patients.



## 1. Introduction

There is neuroscientific evidence for language production impairments in several planning stages in patients with classic galactosemia, an inborn error of galactose metabolism (Timmers et al., 2012). In the current study, we used functional magnetic resonance imaging (fMRI) to investigate potential deviations in functional neural networks involved in language production.

Classic galactosemia is a potentially lethal disorder that results from a profound deficiency of galactose-1-phosphate uridyl transferase (GALT) enzyme activity (Holton et al., 2001). After exposure to breast milk or a milk-based formula, infants suffer a rapid and devastating demise. Early dietary restriction of galactose prevents or resolves the acute manifestations of the disease. However, significant complications appear later in childhood involving the ovaries and the brain (Panis et al., 2004; Rubio-Gozalbo et al., 2010; Waggoner et al., 1990; Waisbren et al., 2012). Cognitive impairments include lower intelligence, memory impairments, slower information processing, as well as voice, motor (speech), and language impairments (Antshel et al., 2004; Doyle et al., 2010; Rubio-Agusti et al., 2013; Timmers et al., 2011; Widhalm et al., 2002). Childhood apraxia of speech (CAS) or verbal dyspraxia has traditionally been reported as an explanation for the speech and language impairments in galactosemia (Nelson et al., 1991; Robertson et al., 2000; Waggoner et al., 1990), although recent estimations indicate that only about 20–25% of the patients with galactosemia meet the criteria (Potter, 2011; Shriberg et al., 2011). In addition, patients with galactosemia have impairments in language planning (Potter et al., 2013). Vocabulary, grammar and word retrieval problems have been described (Schweitzer et al., 1993; Waggoner et al., 1990; Waisbren et al., 1983), as well as cases with clinically significant delays on pre-linguistic skills (at age 13 month) (Lewis et al., 2013), and failures to meet age-appropriate phonological awareness (aged 7–9) (Lewis et al., 2012). The majority of patients with galactosemia and history of speech sound disorders also have language disorders, which cannot be explained by lower cognitive abilities (Potter et al., 2008).




**Description of the task**

| | | |
|---|---|---|
| **Language production** | Sentence 'S' | *'The red triangle flies towards the green square.'* |
| | Word 'W' | *'triangle, red, square, green, to fly towards'* |
| **Passive watching** | Animated 'PA' | watch animated scene |
| | Static 'PS' | watch static scene (figures stay in start position) |

**Fig. 1 – Schematic overview of the language production task. Top: Contents of one block, starting with an instruction of the specific block condition (presented for 4 s), followed by a baseline period (6 s). Trials started with a baseline period (2 s), followed by the onset of the scene (animated plus freeze period; total duration of 4.5 s) and a blank screen (1.5 s). From the onset of the scene on, participants had 6 s to give their response. Total trial duration was 8 s, and total block duration (5 trials of same condition) was 60 s. Bottom: Contents of one run, starting with general instructions of the task and eight blocks – two per condition – preceded by a baseline period (10 s). One run had a duration of approximately 8 min. Further, the conditions and examples of responses are described.**

Previous brain studies in classic galactosemia have shown structural abnormalities, such as diffuse white matter abnormalities and cortical atrophy (Crome, 1962; Haberland et al., 1971; Hughes et al., 2009; Krabbi et al., 2011; Nelson et al., 1992; Ng et al., 1989; Timmers et al., 2015; Wang et al., 2001). In a functional study (Dubroff et al., 2008), widespread baseline decreases in cortical glucose metabolism were found (e.g., in the superior temporal gyrus and cerebellum) in addition to some increases in glucose metabolism. Recently, our group directly examined the relation between brain abnormalities and cognitive impairments, in particular sentence production impairments. Using high temporal resolution electroencephalography/event related potential (EEG/ERP) during an active language production task, we demonstrated that patients with galactosemia showed impairments in several planning stages, from conceptualisation continuing into lexical and syntactic planning (Timmers et al., 2012).

Sentence production requires multiple planning stages over time, including conceptual, lexical, syntactic and phonologic planning (Bock and Levelt, 1994; Levelt et al., 1999; Vosse and Kempen, 2000). Several brain regions are recruited, including areas in the superior temporal lobe, the temporal–parietal junction (or planum temporale, PT) and in the inferior frontal lobe (Hickok, 2009; Indefrey, 2011; Price, 2010, 2012). For syntactic planning, the left inferior frontal gyrus (IFG) has been depicted as (one of) the most important regions (Cappa, 2012; Indefrey et al., 2001, 2004), in addition to the medial frontal gyrus, superior parietal lobule, right insula, left posterior middle temporal gyrus (MTG), and bilateral supplementary motor areas (SMA) (Haller et al., 2005; Menenti et al., 2011; Sahin et al., 2006; Segaert et al., 2011).

The current study will characterize functional networks associated with active language production in patients with galactosemia as compared to healthy controls. We acquired fMRI data to examine activity and connectivity patterns within relevant brain areas. An important issue in patient studies is a possible confounding role of abnormal cerebral blood flow (CBF) that can contribute to an abnormal fMRI signal (the blood oxygenation level dependent [BOLD] signal) (Uludag et al., 2006). The BOLD signal is directly affected by the blood oxy genation and volume, and the blood oxygenation in turn changes with CBF, cerebral blood oxygen consumption (CM RO2), and cerebral blood volume (CBV). Baseline differences in CBF can have effects on the sensitivity of the BOLD signal, which could confound observed BOLD changes, which is why CBF is a relevant control for group comparisons in fMRI. CBF was measured in this study using arterial spin labelling (ASL). In order to associate the high temporal resolution data to high spatial resolution data of the current study, we used a similar paradigm to the one used in the previous EEG study. Participants were instructed to either passively watch the presented visually animated scenes (control condition), or overtly describe it using one of two responses that vary in syntactic complexity (i.e., using sentences or separate words (Fig. 1); see Indefrey et al., 2001; Timmers et al., 2012). We will examine whether there are any differences across groups as to which brain areas are recruited during language production, and more specifically syntactic planning, and as to which brain areas are functionally connected during this task.

## 2. Results

The T1-weighted data of all patients were evaluated by a neuro-radiologist (*PH*). All were evaluated as normal, except one showing evidence of (sub)cortical atrophy (note that this participant was no outlier in any of the outcomes and did not influence the effects reported hereafter).

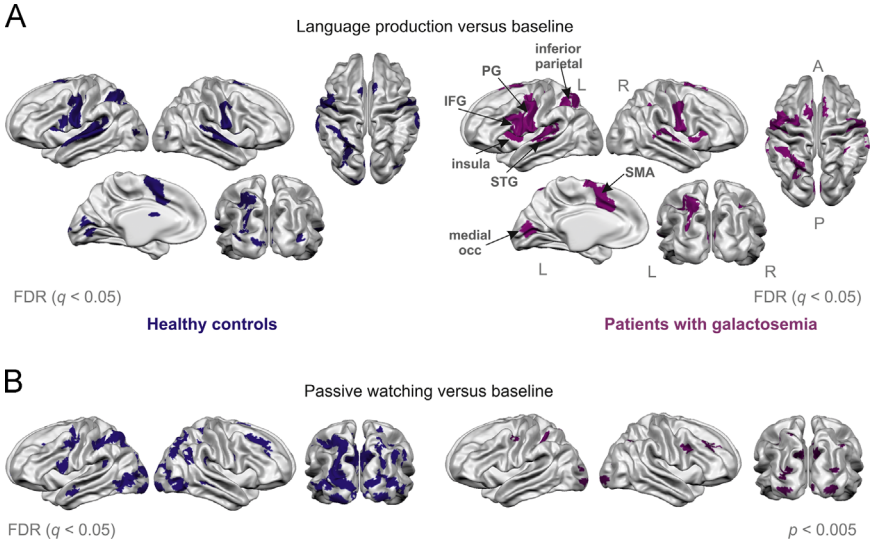



**Fig. 2 – Statistical maps (FDR correction threshold of $q < .05$), separate per group (controls displayed on the left in dark blue; patients with galactosemia on the right side in purple). (A) The contrast language production versus baseline ['S'+'W' > baseline] is shown. (B) The contrast passive watching versus baseline ['PA'+'PS' > baseline]. For illustration purposes, the statistical threshold for this latter contrast was artificially lowered in the patients, to make it more comparable to the other condition and other group ($p < .005$ instead of FDR ($q < 0.05$)). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)**

### 2.1. *Language production sensitive areas*

Statistical maps of both groups displaying the regions that are sensitive to language production in comparison to baseline are shown in Fig. 2A. A generally similar network of regions was observed in both groups, including the bilateral precentral gyrus (PG), bilateral superior temporal gyrus (STG)/PT, bilateral pre-SMA, left IFG, left insula, regions in the left superior parietal and in bilateral medial occipital lobe. Maps in Fig. 2B show the contrast of passive watching versus baseline, revealing that controls recruit several areas during passive watching that are also recruited during active language production, which is not/less present in patients. To avoid confounding by differences in passive watching, all subsequent analyses are restricted to the contrast language production versus baseline.

Based on visual inspection of the maps, several group differences were observed. The patient group showed a more restricted involvement (i.e., less BOLD signal change) of the STG (bilateral) and the PT, and a more extensive recruitment in the left IFG, left PG and bilateral pre-SMA. In addition, the right insula is recruited in the patients, whereas it is not statistically significant in the controls.

The statistical analysis corroborated that there are several regions showing a group difference (see Fig. 3). A lower BOLD signal change in the patient group as compared to the controls was found in left STG, left PT and bilateral occipital regions, while a higher signal change in the patients was observed in left middle and superior frontal regions, bilateral PG and right posterior insula.

### 2.2. *Patch of interest analysis*

A group effect was observed in the right STG and pre-SMA, where the patients showed higher BOLD signal change than controls (Table 2). Further, there was an interaction effect between group and syntactic complexity in left STG: only patients showed increased signal change during the 'S' condition compared to 'W'. In left IFG and right insula, there was a main effect of syntactic complexity: 'S' elicited higher signal change compared to 'W'.

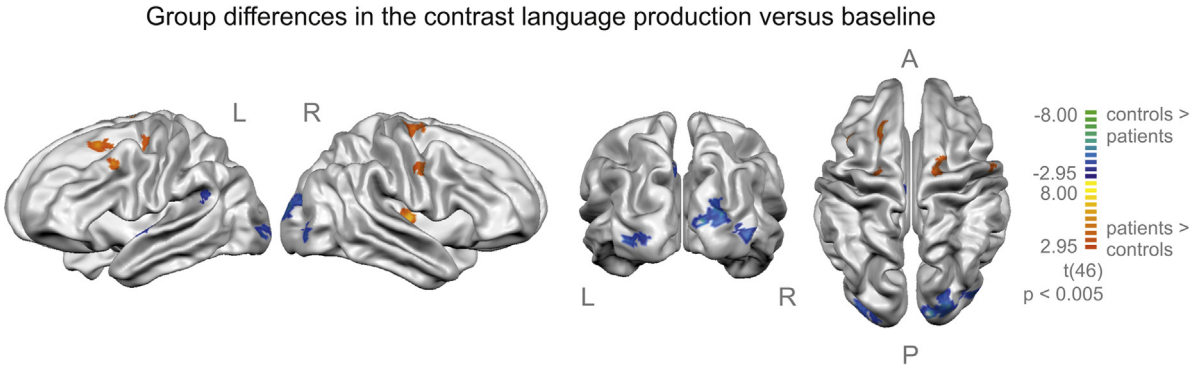



**Fig. 3 – Group differences across the cortical surface in the contrast language production versus baseline ['S'+W' > baseline]. In red, areas are shown in which patients showed higher BOLD signal change compared to controls; in blue regions, the patients showed lower signal change compared to controls. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)**

**Table 1 – Participant characteristics.**

| | Patients | | Controls | |
|---|---|---|---|---|
| | Value | Range | Value | Range |
| Group size | 12 | | 12 | |
| Males/females | 3/9 | | 3/9 | |
| Age (in years)[1] | 17.4 | 14.6–21.1 | 17.1 | 14.0–20.0 |
| Age at initiation of diet (in days) | 11.0 | 0–35 | | |
| GALT activity (in % of reference value)[2] | 0.55% | 0–1.52% | | |
| GALT mutation | 5 (45%) | Q188R/Q188R | | |
| | 1 (9%) | Q188R/L195P | | |
| | 3 (27%) | L195P/K229N | | |
| | 2 (18%) | 400Tdel/unknown | | |
| Special education[3] | 75% | | | |
| Speech therapy[3] | 92% | | | |
| Motor therapy[3] | 42% | | | |

[1] Age did not differ significantly between the groups [$F_{1,22}=0.12$, $p=0.73$].
[2] GALT activity was measured at diagnosis.
[3] At some point in life.

**Table 2 – Patch of interest analyses.**

| Area of interest | F ($p$) values of effects | | | Description of results | | | Peak coordinates | | |
|---|---|---|---|---|---|---|---|---|---|
| | Condition | Interaction | Group | Condition | Group | Interaction | Tal x | Tal y | Tal z |
| Left | | | | | | | | | |
| Superior temporal gyrus | 1.85 (0.187) | 4.87 (0.038)* | 0.45 (0.834) | | | C: 'W'='S' P: 'S'>'W' | −56 | −13 | 6 |
| Supplementary motor area | 0.80 (0.381) | 1.28 (0.271) | 1.64 (0.241) | | | | −6 | −2 | 62 |
| Insula | 2.29 (0.145) | 0.28 (0.602) | 0.30 (0.864) | | | | −27 | 14 | 16 |
| Precentral gyrus | 1.68 (0.209) | 0.31 (0.582) | 1.77 (0.197) | | | | −49 | −9 | 47 |
| Inferior frontal gyrus | 6.61 (0.017)* | 1.44 (0.243) | 0.00 (0.990) | 'S'>'W' | | | −49 | 13 | 22 |
| Medial occipital area | 0.40 (0.534) | 0.07 (0.793) | 0.15 (0.703) | | | | −6 | −77 | 7 |
| Superior parietal area | 1.72 (0.397) | 0.39 (0.589) | 1.86 (0.732) | | | | −15 | −67 | 53 |
| Right | | | | | | | | | |
| Superior temporal gyrus | 0.03 (0.863) | 2.98 (0.098) | 5.11 (0.034)* | | P>C | | 60 | −8 | 4 |
| Supplementary motor area | 2.11 (0.161) | 0.02 (0.892) | 5.10 (0.034)* | | P>C | | 7 | 12 | 57 |
| Insula | 9.89 (0.005)** | 1.22 (0.281) | 0.10 (0.758) | 'S'>'W' | | | 32 | 14 | 6 |
| Precentral gyrus | 0.32 (0.580) | 0.06 (0.810) | 3.63 (0.070) | | | | 52 | −7 | 41 |
| Inferior frontal gyrus | 0.39 (0.540) | 2.38 (0.137) | 0.11 (0.742) | | | | 50 | 1 | 10 |
| Medial occipital area | 0.75 (0.397) | 0.30 (0.589) | 0.12 (0.732) | | | | 15 | −67 | 9 |

'S'=sentence condition; 'W'=words condition; P=patients with classic galactosemia; C=healthy control.
* $p<0.05$.
** $p<0.001$.

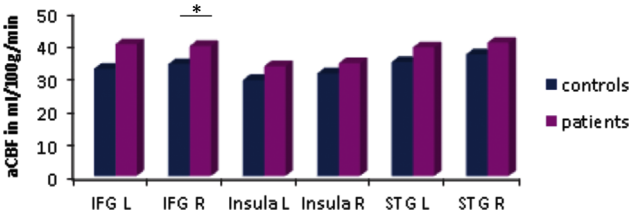



**Fig. 4 – Overview of the baseline cerebral blood flow values, per group and per POI (all POIs consisted of 61 vertices). Although a trend was present in all POIs, the difference was significant only in right IFG. *$p<0.05$.**

### 2.3. *Cerebral blood flow differences during baseline*

The following POIs were covered by the ASL sequence: bilateral IFG, STG and insula. Only the right IFG showed a significant difference across groups [$F_{1,16}=4.84$, $p=0.043$], with patients having a higher aCBF compared to controls (Fig. 4).

### 2.4. *Functional connectivity analysis*

Regions showing a modulation with syntactic complexity – left IFG and right insula – were used as seeds in the correlation analysis. In addition, a seed was placed in left pre-SMA as this region was sensitive to syntactic modulations in healthy adults during the execution of the identical task (forthcoming data) (Fig. 5).

In controls, activity in the left IFG seed region correlated with other regions in the left inferior and middle frontal gyrus (MFG), left insula, left PT/supramarginal gyrus (SMG), left posterior superior and middle temporal gyrus (MTG), and right IFG. In the patients, generally a similar network appeared. However, visual inspection revealed that additional regions showed a correlation with the left IFG, including precentral gyrus and sulcus, more anterior parts of left STG and left MTG, and a larger region in right anterior IFG. The statistical comparison corroborated group differences: stronger connectivity in the patient group was observed with bilateral superior temporal sulcus (STS)/MTG, and with left posterior insula. Weaker connectivity was observed with right PT, and bilateral superior parietal regions.

The activity of the right anterior insula seed correlated with activity in right IFG and MFG, left anterior insula, and bilateral anterior cingulate cortex (ACC) in controls. In patients, visual inspections showed a more extensive network, involving the right precentral and postcentral areas, and right STG. Statistical comparisons revealed stronger connectivity in the patient group with left PG and posterior MFG; and group differences in bilateral parietal areas. In addition, weaker connectivity was found with bilateral central sulcus.

Finally, connectivity maps showed that the left pre-SMA seed was functionally connected with bilateral (pre)SMA, bilateral PG, bilateral STG, left PT, and left superior parietal regions. In patients, visual inspection showed that the seed region correlated more extensively with bilateral (pre)SMA, and PG, and less with bilateral STG and left PT. Statistical

tests confirmed stronger connectivity in the patient group with bilateral (pre)SMA, left MFG, right PT and insula, and weaker connectivity with left posterior STG and MTG, and with bilateral parieto-occipital regions.

## 3. Discussion

This study clearly showed that although the patients recruited a generally similar network during language production – compared to matched healthy controls –, specific group differences in neural activity and functional connectivity patterns were present. More extensive, and additional areas were recruited (in left frontal cortex, PG and SMA; and right insula, respectively), while other areas were less involved (left STG, PT). A similar pattern was observed in functional connectivity maps of the patients, showing stronger connectivity with regions in frontal and motor cortex, and weaker connectivity with posterior superior temporal regions, compared to controls.

The overall pattern suggests abnormalities associated with syntactic planning, as well as with (sensory-)motor planning of speech production. Related to syntactic planning, the data indicates that the left IFG is similarly activated, as both groups showed a modulation with syntactic complexity in left IFG

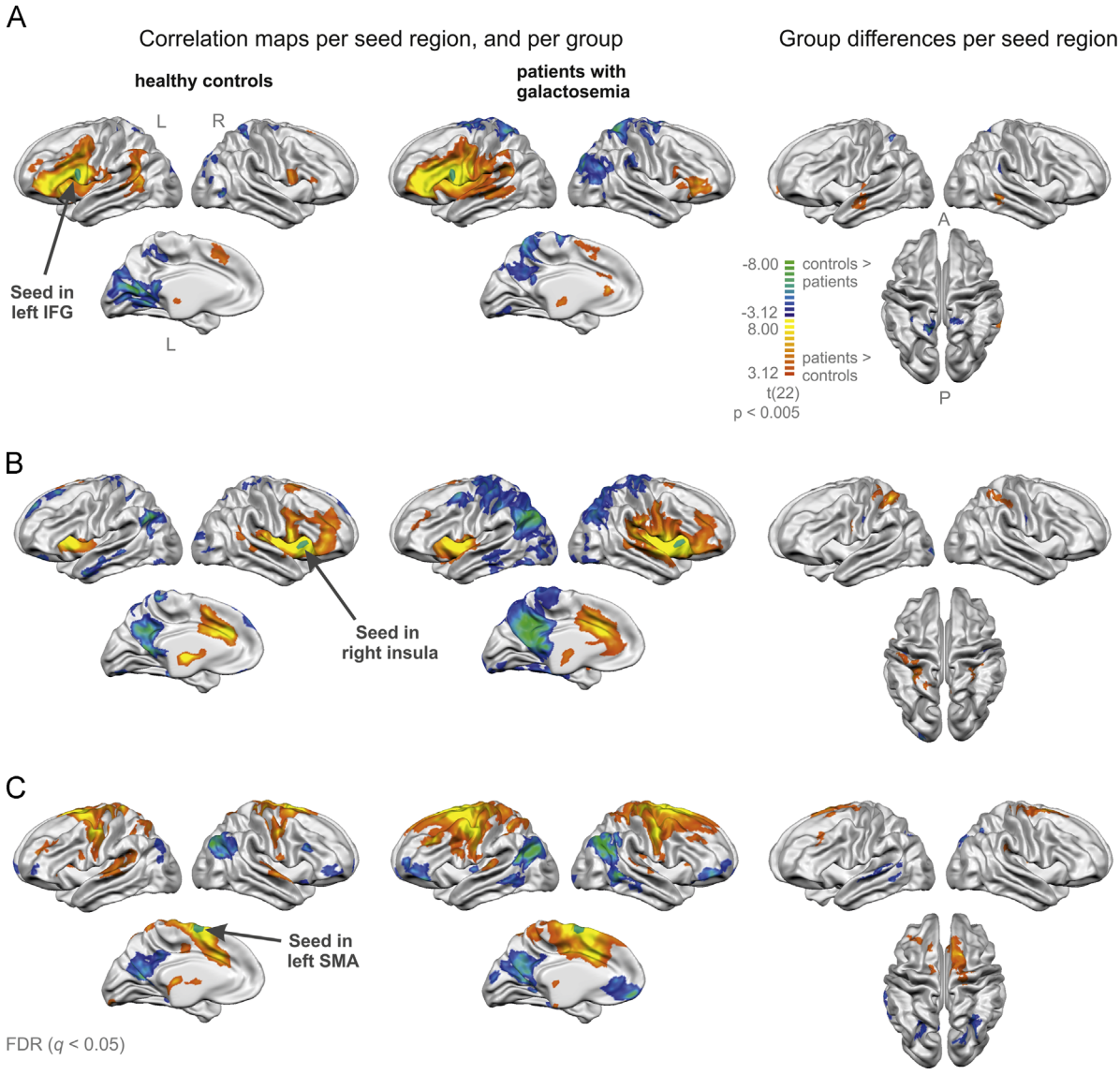



**Fig. 5 – Functional connectivity maps. Left and middle column: Statistical maps resulting from the seed-based functional connectivity analysis, separate per group (red indicates positive correlation, blue indicates negative correlation). Seed regions were defined based on observed syntactic complexity modulation: the left IFG, right insula (see Table 2). In addition, the left (pre)SMA was included as a seed region, as it has been reported sensitive to syntactic modulations in this task in healthy adults (Timmers et al., forthcoming). The seeds are overlaid in green. Maps are thresholded at FDR $q<0.05$. Right column: Group differences in seed-based connectivity maps across the cortical surface, per seed region. In red, areas are shown in which patients showed stronger connectivity compared to controls; in blue regions, patients showed weaker connectivity compared to controls. Maps are thresholded at $p<0.005$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)**

(higher activity for sentences 'S' compared to words 'W'; as expected by previous studies with healthy controls; Indefrey et al., 2001; Segaert et al., 2011), but a group difference is lacking. However, functional connectivity patterns with the left IFG seed region showed stronger connectivity with bilateral STS/MTG in the patient group. This could reflect increased effort during lexical–syntactic processes, probably related to verbal working memory load (in analogy to interactions between left IFG and left MTG in comprehension studies; Snijders et al., 2009). Moreover, patients showed an additional syntactic modulation effect in left STG, a region relevant for understanding syntactically complex sentences, and integrating semantic and syntactic information (Grodzinsky and Friederici, 2006). Recruiting additional areas for syntactic planning indicates the need for additional neural recourses, and as language comprehension in the patients is rather intact, STG might be recruited in patients for production purpose too. Alternative, it might reflect increased monitoring in the complex syntactic (sentence) condition (Christoffels et al., 2007, 2011; Indefrey and Levelt, 2004).

Related to (sensory-)motor planning, less signal change was observed in the PT, pointing towards aberrant sensory-motor integration (Hickok and Poeppel, 2007; Hickok, 2009). This finding would be in line with previously reported motor (speech) disorders in galactosemia (Potter, 2011; Potter et al., 2013; Rubio-Agusti et al., 2013; Shriberg et al., 2011). Hypoactivity of the posterior STG and PT has been observed in patients with specific language impairment (SLI) (Badcock et al., 2012) and CAS as well (Liegeois et al., 2003). Weaker connectivity in patients between IFG and the right PT, and bilateral superior parietal regions supports this claim, suggesting suboptimal communication between frontal areas associated with overall language planning (IFG) and temporal and parietal sensory-motor integration (PT, superior parietal region). Furthermore, patients showed stronger connectivity between the right insula and regions in the left PG and posterior MFG, and bilateral parietal areas, suggesting alterations in neural activity patterns related to motor speech planning and/or sensory-motor integration (Brown et al., 2009; Cauda et al., 2011; Price, 2010, 2012; Sahin et al., 2006). The question is whether this insula network has evolved in patients over time to compensate for lack of connectivity between IFG and PT/parietal areas. Damage to the right insula region is associated to CAS (Ogar et al., 2005). Altered motor planning in these patients is also supported by stronger connectivity with bilateral SMA, MFG, right PT and insula — all implicated in the (sensory-)motor system.

Moreover, in the right IFG a significant baseline CBF difference was found, and trends towards a difference in all other covered regions (the slab covered bilateral inferior frontal, insular, and superior temporal regions). The CBF was higher in the patient group compared to the controls. A higher baseline level of CBF results in lower sensitivity of the BOLD signal, hence a smaller BOLD signal change for the same amount of neuronal activity change (Brown et al., 2003). Thus, the observed increases in the BOLD signal in the patients are not explainable by the observed baseline CBF differences. An explanation for the observed CBF findings (higher in patients compared to controls) is still lacking. In a PET study in patients with galactosemia, the authors also observed increases in baseline glucose metabolism in the cingulate and temporal regions (Dubroff et al., 2008). This would be in line with a tight linkage between baseline CBF and baseline glucose metabolism. However, more prominent in the PET study were widespread decreases in glucose metabolism, including in the STG, medial occipital lobe and superior frontal cortex, which contrast the current findings of increased CBF. However, as the PET study differed in many respects (spatial resolution, age of the patients, sample size), it is difficult to draw direct comparisons.

The current findings are in line with our previous EEG study, in which we used ERPs to study the time course of syntactic encoding using the same paradigm. The ERP wave morphology was similar for controls and patients, but specific differences – including increased ERP amplitudes during time windows associated with lexical and syntactic planning – were identified (Timmers et al., 2012). The described white matter abnormalities in this disease (Nelson et al., 1992; Timmers et al., 2015), may be related to the observed functional network patterns, as it was found that white matter abnormalities showed specific regional profiles and furthermore correlations with outcome (Timmers et al., 2015). However, further studies are warranted to link the electrophysiological and functional imaging data specifically to data on structural abnormalities in the brain.

Limitations of the current study include the relatively small sample size and the specific focus on syntactic planning. Future studies should aim to include other stages of language production and other cognitive domains, to create a more complete picture of affected functional networks. With resting state fMRI (Damoiseaux et al., 2006; Rosazza and Minati, 2011), for instance, an overview of potential deviations in intrinsic functional networks could be gathered. Also, the relation between specific language abnormalities and general cognitive functioning should be explored, as cognitive functioning might be a confounding factor in this study. Another point future analyses and studies should address is potential abnormalities in activation and connectivity patterns in cerebellar and subcortical regions. The current study focused on the cortical surface and hence, potential deviations in cerebellar and subcortical regions were not examined. Finally, although the observed effects can be linked to speech and language functioning quite specifically, we cannot exclude any potential confounding effects of the observed differences in the passive watching condition (e.g., the differences in the occipital regions might be attributable to visual processing differences). Abnormalities in visual and visuo-spatial processing have been described before (e.g., Doyle et al., 2010; Schweitzer et al., 1993), making this a very interesting domain to focus on in future endeavours.

## 4. Conclusion

Altered neural activity and connectivity were observed during active language performance in classic galactosemia revealing impairments in both the cognitive counterpart of language production (including syntactic planning), as well as the motor speech (planning) part. More extensive and additional areas were recruited in left frontal cortex, PG, and SMA; and right insula, respectively, while other areas (left STG, PT) were less involved. A similar pattern was observed in functional connectivity, which was stronger with regions in frontal and motor cortex, and weaker with posterior superior

temporal regions. We showed that these differences are not related to baseline differences between groups in CBF. In addition to higher cognitive load, working memory, and potential visual processing abnormalities, these observations might reflect compensation mechanisms for disease-based functional alterations within the language network, or adaption mechanisms of the patients to cope with cognitive difficulties. Full compensation, however, seems to fail in the context of language production.

This study bridges the language impairments observed in these patients to abnormalities in the neuronal networks required for language planning. Current recommendations in classic galactosemia already include speech evaluation, but should also specifically mandate language planning evaluation to ensure tailored treatment approaches.

## 5. Experimental procedures

### 5.1. Participants

Thirteen adolescent patients with galactosemia and 13 age- and gender-matched healthy controls participated in this study. Recruitment of the patients was based on a previous study of our group (Timmers et al., 2012), in which all patients aged 12–18 years known to the Dutch Galactosemia Patient Organization were invited to participate. For the current study, a subset was invited again (aged 14 years and older) to reach a sample of 12 per group (based on power calculation for a medium effect of 0.4 with a desired power of 0.90; G*power 3.0.10). Classic galactosemia was diagnosed by GALT enzyme activity assay and/or GALT-gene mutational analysis. Two participants were excluded: one patient because of extensive motion during the scanning and one control because of a current health condition. Characteristics of the groups can be found in Table 1. Participants had no other relevant health conditions. All had normal or corrected to normal vision, were native Dutch speakers, were screened for MRI compatibility, and signed informed consent (in case of minors, both parents/caregivers also gave written informed consent). The Medical Ethical Committee of Maastricht University Hospital/Maastricht University (azM/UM) gave ethical clearance for this study.

### 5.2. Language paradigm

Visually animated scenes were presented, consisting of three coloured geometric figures, of which one moved towards another (either 'to fly towards' or 'to bump into'). Participants were instructed to either passively watch the scene, or to describe it overtly using a complete sentence or separate words (i.e., varying syntactic complexity from high to low), resulting in four conditions: passive watching of static scenes ['PS'], passive watching of animated scenes ['PA'], sentence-level syntactic planning ('S'), and word-level planning ('W'). See Fig. 1 and Timmers et al. (2012, 2013) for more details.

### 5.3. Procedure

To prevent excessive motion during scanning, participants received explicit instructions and practiced lying and speaking in a dummy scanner (for approximately 5 min). Participants received instructions and practiced the language paradigm, after which they were placed comfortably in the MRI scanner. Prior to the language task, the arterial spin labelling (ASL) sequence was acquired. The language task consisted of 4 runs in a blocked design (8 blocks per run; Fig. 1). Conditions were randomized per set of 4 blocks (each condition occurring once). Participants were instructed to describe the scenes as fast and accurate as possible. From the onset of the scene on, participants had 6 s to give their response. Stimulus presentation was synchronized with MR data acquisition by triggering the stimulus program (Presentation Software, Neurobehavioral Systems Inc) with the beginning of each trial. During the session, more data was acquired that will be described elsewhere. Total duration of the experiment was approximately 90 min.

### 5.4. Data acquisition

Data were acquired on a 3-T Siemens Magnetom Allegra head scanner using a 8-channel head coil, and a 3-T Siemens Trio whole body scanner (Siemens Medical System, Erlangen, Germany), using a 32-channel head coil (acquisition on two different scanners was necessary because of irresolvable technical problems; scanner parameters were identical unless otherwise specified; of each group four participants were scanned on the Allegra scanner).

*Functional MRI:* 32 axial slices (3.5 mm iso-voxel) covering the entire cortical volume were collected using a standard echo-planar imaging (EPI) sequence (repetition time [TR]=2000 ms, echo time [TE]=30 ms).
In order to be able to post hoc correct the EPI data for magnetic field inhomogeneities, a field map was acquired (TR=704 ms; TE[1]=5.11 ms; TE[2]=7.57 ms).
*Anatomical images:* 1 mm iso-voxel resolution T1-weighted ADNI MPRAGE sequence (TR=2250 ms; TE=2.6 ms). 192 slices were collected covering the whole brain.
*Arterial spin labelling*: we used the PICORE–QUIPSS II ASL sequence with the following parameters: TI1=700 ms, TIs=900 ms and TI2=1400 ms. 100 volumes were collected with 8 slices, positioned to cover the inferior frontal, superior temporal and inferior parietal regions (TR=2000 ms; TE=20 ms [Trio: TR=17 ms]). In the calibration sequence, 10 volumes were acquired with identical slice positioning (TR=10.000 ms; TE=20 ms [Trio: TR=17 ms]).

### 5.5. Data analyses

Data were analysed using BrainVoyager QX 2.6 (Brain Innovation, Maastricht, the Netherlands). Functional data were corrected for geometrical EPI distortions (Breman et al., 2009); pre-processing included correction of slice scan time differences and 3D head motion, linear trend removal, temporal high pass filter (3 cycles/run) and 3D spatial smoothing (Gaussian filter FWHM of 4 mm). The functional runs were co-registered with the anatomical data and normalized in Talairach space. White matter reconstructions of both hemispheres of each participant were created and cortical meshes

were aligned to a dynamic group average using a cortex-based alignment algorithm (Goebel et al., 2006).

Per participant and per run, a design matrix was created with the 4 conditions, adjusted for the hemodynamic response delay. The 6 parameters describing the 3D head motion and the extracted mean signal from the cerebral spinal fluid (CSF) and white matter (WM) (as an estimate of physiological noise) were normalized and added as variables of no interest. On the cortical surface, a univariate random-effects (RFX) analysis was performed per group. From the resulting maps, patches of interest (POIs) were extracted per condition and per participant. Beta values were extracted and fed into a repeated measures General Linear Model (GLM) having age as a covariate in order to evaluate group and syntactic complexity effects. In addition, group differences across the maps were examined.

Further, POIs were selected as seeds for a seed-based functional connectivity analysis in which the entire time course of a specific POI (i.e., seed) is extracted, normalized and regressed with all other vertices on the cortical surface using an RFX analysis with the time course as a predictor. The rationale is that regions showing temporal correlations (or synchronisation) with the seed region are interpreted as forming a functional network with the seed region (Huettel et al., 2004; Li et al., 2009). Statistical contrasts were inspected at the level of the whole cortex, if not otherwise specified: FDR corrected at $q=.05$.

The perfusion-based ASL data were corrected for 3D head motion and co-registered to the anatomical data. Absolute (quantitative) CBF (aCBF) maps were created for each participant (Çavuşoğlu et al., 2009). These maps were projected onto the cortical surface of each participant. From the individual POIs, the aCBF data were extracted and data quality and efficiency of the tagging were evaluated (in comparison to a reference value of around 50 ml/100 g/min for grey matter in visual cortex (Chen et al., 2008); participants with absolute CBF values less than 20 ml/100 g/min – to incorporate safety margin – were discarded from the analysis; two patients and one control were excluded from this analysis). A GLM was performed per POI in order to evaluate group differences in aCBF.

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