Hypothalamic Inflammation in Human Obesity Is Mediated by Environmental and Genetic Factors

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Obesity is associated with hypothalamic inflammation (HI) in animal models. In the current study, we examined the mediobasal hypothalamus (MBH) of 57 obese human subjects and 54 age- and sex- matched nonobese control subjects by MRI and analyzed the T2 hyperintensity as a measure of HI. Obese subjects exhibited T2 hyperintensity in the left but not the right MBH, which was strongly associated with systemic low-grade inflammation. MRS revealed the number of neurons in the left hypothalamic region to be similar in obese versus control subjects, suggesting functional but not structural impairment due to the inflammatory process. To gain mechanistic insights, we performed nutritional analysis and 16S rDNA microbiome sequencing, which showed that high-fat diet induces reduction of *Parasutterella sp.* in the gut, which is significantly correlated with MBH T2 hyperintensity. In addition to these environmental factors, we found subjects carrying common polymorphisms in the *JNK* or the *MC4R* gene to be more susceptible to HI. Finally, in a subgroup analysis, bariatric surgery had no effect on MBH T2 hyperintensity despite inducing significant weight loss and improvement of peripheral insulin sensitivity. In conclusion, obesity in humans is associated with HI and disturbances in the gut-brain axis, which are influenced by both environmental and genetic factors.

Obesity is now widely viewed as a chronic neuroendocrine disease, with the core abnormality lying in central appetite regulation. The mediobasal hypothalamus (MBH) integrates peripheral anorexigenic and orexigenic signals to determine a net sense of satiety or hunger (1). In recent decades, convincing evidence was provided that leptin, released from adipose tissue, is one of the major anorexigenic signals from the periphery into the brain and that leptin resistance in the MBH is a common pathology in rodent and human obesity (2). While leptin resistance is strongly associated with common single nucleotide polymorphisms (SNPs), e.g., in the melanocortin 4 receptor (MC4R) (3), it is generally accepted that genetic factors can only explain part of the obesity epidemic.

Low-grade inflammation related to obesity was initially described in adipose tissue (4–6). In 2005, the first study was published suggesting that inflammation might also occur in the hypothalamus of obese animals (7). In this index study, long-term feeding of rats with a high-fat diet induced MBH inflammation, and this induced hypothalamic leptin resistance associated with the development of obesity (7). Histologically, hypothalamic inflammation (HI) is characterized by gliosis, which involves infiltration of microglia and proliferation of astrocytes, resulting in increased cell density (8). In animal models, MBH inflammation and gliosis is detectable even before body weight gain, suggesting

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that HI might indeed be causative in the pathophysiology of obesity (9).

While the findings of HI and gliosis were confirmed by several independent groups in rodent and nonhuman primate animal models (8–10), human data on HI are still rare. In 2012 a retrospective analysis of brain MRI from 34 human subjects with a BMI ranging from 17.7 to 44.1 kg/m^2 revealed a hyperintensity in T2-weighted MRI of the MBH in obese humans, which was significantly related to BMI (9). In addition, a prospective study was published in the past year that also used MRI in 67 participants (11). A longer T2 relaxation time was found to be associated with a higher BMI and was more prominent in the left MBH, with the right MBH exhibiting longer T2 relaxation times only in subjects with the highest signal intensity of the left. Importantly, in this recent study Ellen Schur and her coworkers also performed MRI scans on postmortem brain slices and related their MRI data to histological staining for glial fibrillary acidic protein. Using this approach, they convincingly confirmed that MRI was indeed detecting HI and gliosis, validating that this technique is a reliable method for detecting surrogate markers of MBH inflammation in human studies.

In the current study we examined nonobese and obese subjects by combining brain MRI, detailed nutritional analysis, 16S rDNA microbiome sequencing, and array-based genotyping to gain further insights into HI in human obesity.

RESEARCH DESIGN AND METHODS

MRI and Spectroscopy

The MRI examinations were performed on a 3T MRI scanner (Achieva; Phillips Medical Systems, Eindhoven, the Netherlands) with a 32-channel head coil. Spectroscopy data were analyzed using the Philips SpectroView package. For the analysis of metabolites, N-acetylaspartate (NAA) was compared with creatine (Cr) (12,13).

Bacterial 16S rRNA gene sequencing, quality control, and bioinformatics analysis were performed as previously described (14).

Array-Based Genotyping

The FoCus (Food Chain Plus) samples were typed on the OmniExpress Exome array. The genotyping data underwent stringent quality control (15), leaving 1,751 samples with 964,193 common and rare SNPs.

Tissue Collection

Hypothalami of two subjects were dissected at autopsy, fixed by immersion in neutrally buffered 10% formalin, dehydrated, and embedded in paraffin. Serial coronal brain sections were cut from the level of the optic chiasm to the caudal border of the mammillary bodies. Consecutive 4-μm sections of hypothalamus (preoptic, supraoptic, tuberal, and mammillary hypothalamic areas) were deparaffinized and rehydrated in graded alcohols.

Immunohistochemical Stainings and Photomicrography

The human postmortem hypothalamic tissue sections were subjected to a standard citrate-based heat antigen retrieval procedure, washed three times in PBS, and blocked with 0.75% BSA in PBS for 45 min. Sections were subsequently incubated with the primary antibodies (anti–glial fibrillary acidic protein [αGFAP], rabbit monoclonal, or anti–IBA1, rabbit monoclonal [Abcam]) at a 1:500/1:250 dilution in 0.75% BSA at 4°C overnight, respectively. After appropriate wash steps in PBS, tissue bound antibody was detected using biotinylated goat anti-rabbit IgG antibodies (Vector Laboratories) followed by horseradish peroxidase–conjugated avidin, both diluted at 1:200 in PBS. With the primary antibodies omitted, only secondary antibodies and/or horseradish peroxidase–conjugated avidin were used as controls. Standard chromogenic techniques (Vector Laboratories) were used for signal detection.

Statistical Analysis

Statistical analyses and graphics were carried out using R (16). For verification of normal distribution of the data, the Shapiro-Wilk test and visualization methods were used. For group comparisons, a two-tailed t test or a Wilcoxon rank sum test were used. Outliers were identified visually. Unless otherwise stated, P values <0.05 were considered significant or nominally significant. Multiple testing corrections were performed using Bonferroni correction.

Analysis of Inflammatory and Metabolic Markers

Univariate correlation analyses of interleukin (IL)-6, C-reactive protein (CRP), HOMA of insulin resistance (HOMA-IR), and triglycerides with MBH/amygdala signal ratio (L) were performed. Since this analysis might be confounded by BMI, secondly a linear model was used including obesity as a stratified variable (0 = nonobese group, and 1 = obese group) and MBH/amygdala signal ratio (L) as the dependent variable.

Candidate Gene Approach

The genome analysis toolset PLINK (17) was used to extract SNP information. SNPs of four selected candidate genes (c-Jun N-terminal kinase [JNK], MC4R, NF-κB, and FTO) were extracted from the available OmniExpress Exome array data. Setting a minor allele frequency >5%, we obtained for MC4R n = 1 SNP, for JNK n = 6 SNPs, for NF-κB n = 13 SNPs, and for FTO n = 137 SNPs.

Microbiome Analysis

Each genus (count data) of the core measurable microbiome of n = 26 genera was individually correlated with the MBH/amygdala signal ratio of the obese human group (n = 48), whereas P values were computed using algorithm AS_89 (18). All correlations were performed using the Spearman rank correlation test owing to nonparametric data distribution.

MRS Data Analysis

We considered different possible forms of nonlinear relationships between NAA/Cr (L) and MBH/amygdala signal ratio (L) (in both the complete sample and separately for obese and nonobese subjects), in particular threshold

Table 1—Summary of subject characteristics

	Nonobese	Obese
n	54	57
Women	90.74	91.23
Right-handed	88.46	88.89
Age (years)	46 (40.25–48.75)	43 (33–54)
Height (m)	1.72 (1.66–1.76)	1.67 (1.63–1.72)
Weight (kg)	65.2 (59.0–73.5)	130.6 (115.7–140.5)
BMI (kg/m^2)	22.62 (21.62–24.05)	43.73 (40.75–47.05)
Glucose (mg/dL)	89.0 (85.0–96.0)	101 (93–117)
Insulin (μU/mL)	7.30 (5.25–9.65)	24.35 (16.18–37.35)
HOMA-IR	1.56 (1.15–2.17)	5.88 (4.07–11.43)
Triglycerides (mg/dL)	71.5 (63.5–99.0)	146 (107–195)
IL-6 (pg/mL)	2.5 (2.25–3.3)	5 (3.3–6.3)

Data are % or median (interquartile range). The FoCus cohort comprises 1,837 human individuals and was built up to study the effect of nutrition on human health (27,34). All subjects who underwent MRI examination (n = 111) in the FoCus cohort were divided into two groups (nonobese, with BMI <30 kg/m^2 [n = 54], and obese, with BMI ≥30 kg/m^2 [n = 57]), age and sex frequency matched. A subgroup of 10 severely obese humans underwent bariatric surgery. BMI was measured in weight in kilograms divided by the square of height in meters. HOMA-IR = insulin (μU/mL) × glucose (mg/dL)/405.

effects and exponential and U- or S-shaped relationships. After confirming by visual inspection of scatterplots that none such relationships were present, we used rank transformed values of NAA/Cr (L) as the dependent variable in a linear model, which would detect any monotone (also nonlinear) relationship between NAA/Cr (L) and MBH/amygdala signal ratio (L).

Ethics

The study was approved by the local ethics committee in Kiel, Germany (A156/03-4/13), and each proband gave informed consent before inclusion in the study.

RESULTS

HI and Gliosis Are Present in Human Obesity and Are Restricted to the Left Hypothalamus

In the current study, we examined the MBH of 57 human subjects with obesity and 54 control subjects, frequency matched by age and sex, by T2-weighted MR imaging. Their characteristics are given in Table 1. As suggested by Joshua Thaler and coworkers (9), in the present MRI study we related the MBH T2 intensity to the intensity of the corpora amygdala in order to normalize for unspecific neuronal effects (Fig. 1). As shown in Fig. 2*A*, obese human subjects exhibit a significant increase in MBH T2 intensity, indicating inflammation and gliosis, an effect only detectable in the left, not the right, hypothalamus. Additionally, a sensitivity analysis was performed by excluding six overweight subjects from the comparison of the MRT T2 intensity (R) between nonobese and obese subjects, resulting in no significant change of the statistical outcome (P = 0.54). Besides these findings in obesity, it is worth mentioning that in nonobese control subjects we found the MBH T2 intensity to be physiologically lower in the left compared with the

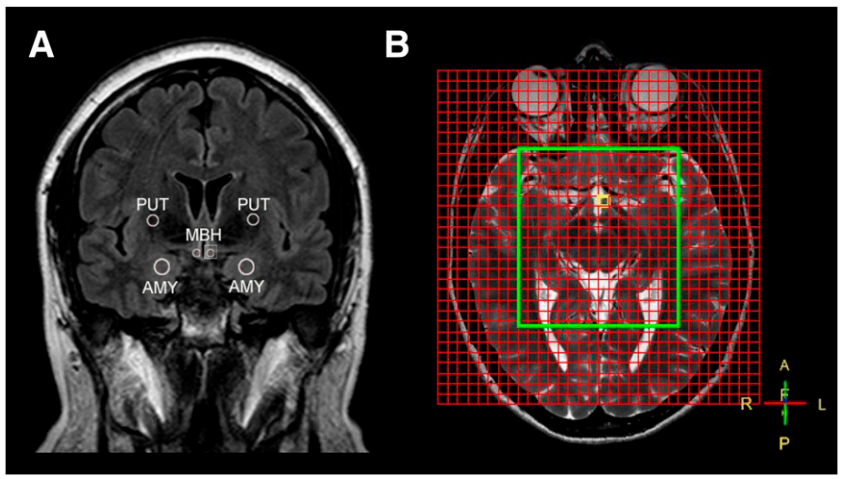

Figure 1—ROIs in T2-weighted fluid-attenuated inversion recovery (FLAIR) images and spectroscopy. *A*: ROI placement in the MBH, the putamen (PUT), and the amygdala (AMY) in coronal T2-weighted FLAIR images. The box marks the region from which the biopsies were taken. For placement of the ROI in the MBH, anatomic landmarks such as the third ventricle and the optic tract were used. Coronal T2-weighted FLAIR sequence was used with a voxel size of 0.9 × 1.13 × 3 mm^3 (echo time [TE] = 160 ms; repetition time [TR] = 12,000 ms). The volumes of the ROIs used for MBH T2 signal ratios were drawn manually and cover an area of circa 2–4 mm^2, a circumference of circa 6–7 mm, a diameter of circa 3 mm, and a thickness of 3 mm. *B*: MRS grid through the bottom of the third ventricle. A multivoxel proton spectroscopy (MRS) using multiply optimized insensitive suppression train (MOIST) water suppression with a voxel size of 10 × 10 × 10 mm^3 (chemical shift imaging point resolved spectroscopy, echo time = 35 ms, repetition time = 2,000 ms) and additional sagittal and axial T2-weighted sequences for the MRS planning was performed. The voxels were placed unilaterally to obtain separated results. For each side, the voxel that includes most parts of the MBH was chosen for spectrum analysis. A, anterior; F, frontal; L, left, P, posterior; R, right.

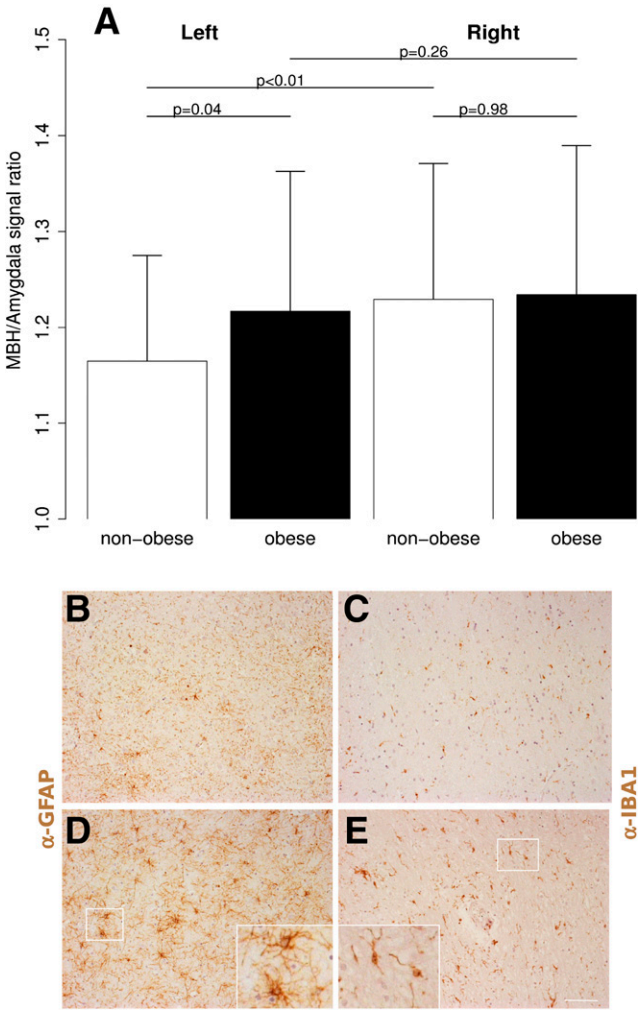

Figure 2—Hypothalamic gliosis in human obesity. *A*: Increased MBH T2 hyperintensity in obese humans in the left MBH. Ratios of mean ROI signal intensity of the MBH and amygdala were calculated for each side to rule out global interindividual signal intensities. Additionally, ratios of mean ROI signal intensity of the ipsilateral putamen and amygdala were calculated as a control. For each hemisphere, MBH T2 intensity (measured as MBH/amygdala signal ratio) was compared between nonobese (n = 54) and obese (n = 57) humans using a Wilcoxon rank sum test revealing significant results only in the left MBH. For each weight group, MBH T2 intensity was compared between the left and right hemisphere using a two-sided t test or a Wilcoxon rank sum test, respectively, showing significantly higher T2 intensity in the right hemisphere of nonobese humans. Data are displayed as form mean (SD). *B–E*: Immunohistochemical stainings of postmortem brain sections from one normal-weight and one overweight human donor. GFAP staining as an astrocyte marker (*B* and *D*) and IBA1-stained sections (activated microglia) (*C* and *E*). All photomicrographs are taken in a similar area (medial zone of the anterior area tuberalis). *B* and *C*: Derived from a 66-year-old male individual who died of cardiac tamponade after myocardial infarction, with BMI 23 kg/m^2. *D* and *E*: Derived from a 64-year-old male individual who died of myocardial infarction, with BMI 28 kg/m^2. Note the increased gliosis and microglia density in the individual with higher BMI. Bar denotes 50 μm.

right MBH ($P < 0.01$). In that respect, a comparison of the MBH/amygdala signal ratio between left-handed (n = 8) and right-handed (n = 93) individuals did not yield a significant difference (P = 0.81) (excluded: n = 9 who were ambidextrous). As an additional control, the ratio of putamen and amygdala was calculated. Neither in the right nor the left hemisphere was a significant difference observable between the two groups regarding the putamen/amygdala ratio (right: P = 0.68; left: P = 0.67 [Wilcoxon rank sum test]). In addition to MRI, we were able to perform immunohistochemical stainings of postmortem hypothalamic biopsies of a lean and an overweight human individual. As shown in Fig. 2*B–E*, these analyses support the MRI findings on inflammation (microglia, α-IBA1) and gliosis (astrocytes, αGFAP) in the left MBH in relation to human obesity.

HI and Gliosis in Human Obesity Are Associated With Systemic Low-Grade Inflammation but Not Peripheral Insulin Sensitivity or Hypertriglyceridemia

We next aimed to address whether HI and gliosis are associated with systemic low-grade inflammation or with peripheral metabolic disturbances, e.g., insulin resistance and/or hypertriglyceridemia. Therefore we measured serum IL-6 levels and CRP levels as systemic inflammatory markers and glucose and insulin (calculation of HOMA-IR) as well as triglyceride levels as metabolic parameters. Correlation analyses revealed a positive correlation of IL-6 and CRP with the MBH/amygdala signal ratio (L), while metabolic factors showed no association (IL-6: $P < 0.01$, ρ = 0.29, n = 90; CRP: P = 0.02, ρ = 0.25, n = 88; HOMA-IR: P = 0.42, ρ = 0.08, n = 110; triglycerides: P = 0.75, ρ = 0.03, n = 111). Additionally, a linear model was applied to determine the impact of each factor with adjustment for obesity, suggesting that HI and gliosis are associated with IL-6 but not metabolic factors (Table 2). It is worth mentioning that the association of IL-6 and MBH T2 hyperintensity (L) was also seen when obese subjects only were examined (P = 0.01, ρ = 0.35, n = 54).

HI and Gliosis in Human Obesity Are Not Associated With a Reduced Neuronal Cell Count

Having found that HI and gliosis are present in the left MBH of obese humans, we next aimed to examine whether the inflammatory reaction is associated with a destruction of neurons, which would suggest irreversibility of the process. Using MRS, we estimated the neuronal cell number by calculating the NAA/Cr ratio in the left hemisphere. A comparison of the NAA/Cr ratio of nonobese versus obese human subjects showed no significant difference, suggesting no reduction of the neuronal cell count in the left hypothalamic region (Fig. 3). Also, no significant difference was found in the NAA/Cr ratio when individuals with and without T2 hyperintensity were examined (P = 0.54 by Wilcoxon test). In addition, a linear model was applied to adjust for obesity, again suggesting the absence of neuronal destruction in the left hypothalamic region.

HI and Gliosis in Obesity Are Associated With Disturbances in the Gut Microbiome

We next examined whether MBH inflammation and gliosis are associated with specific gut microorganisms. Fecal samples of the obese human subjects were subjected to a

Table 2—Linear model of MBH/amygdala signal ratio (L)

	β	*P*
IL-6	0.02	0.005*
CRP	<0.01	0.29
HOMA-IR	−0.01	0.057
Triglycerides	<0.01	0.800

MBH/amygdala signal ratio (L) was tested as a dependent variable on BMI, IL-6, HOMA-IR, triglycerides, and CRP, whereas only IL-6 revealed significant results. *P* value: significance of effect of specified variable on MBH/amygdala (L), β: Regression coefficient. Twenty-three observations were deleted owing to missingness. Two outliers in the triglyceride data and one outlier in the IL-6 data were excluded from the analyses. Obesity was included in the model as a dichotomous variable at a cutoff at BMI >30 kg/m^2. *Significant *P* value ($P < 0.05$).

16*S* rDNA sequencing, and the core measurable microbiome, including *n* = 26 genera, was analyzed. Correlation analyses within the obese humans group identified two bacteria, showing a nominally significant, negative correlation with the MBH/amygdala signal ratio (L): *Parasutterella sp.* and *Marinilabiliaceae* (genus unclassified) (Table 3). Further correlation analysis of the core measurable microbiome species within the genus *Parasutterella sp.* followed by a nucleotide sequence blast (19) identified *P. excrementihominis* to be negatively associated with MBH/amygdala signal ratio (L) in obese humans. None of the examined gut bacteria showed a significant positive association with the MBH/amygdala signal ratio (L).

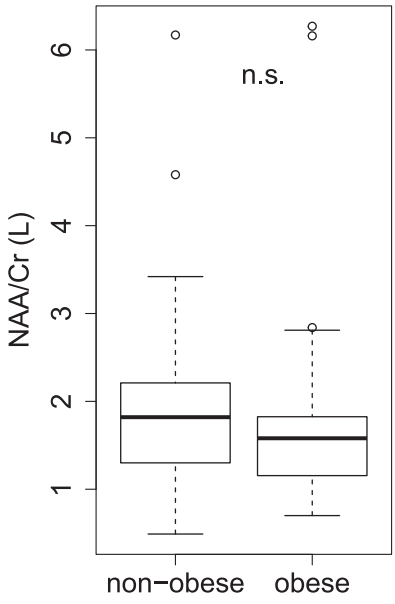

Figure 3—Similar neuronal cell count among the two weight groups. NAA (marker of neuronal and axonal viability and intensity) was compared with Cr (internal reference). Nonobese (*n* = 38) and obese (*n* = 43) patients were compared regarding their left MBH NAA/Cr signal ratios (Wilcoxon rank sum test), showing no significant differences in neuronal cell counts (*P* = 0.12). Thirty-one subjects were excluded from the MRS data analysis because the peaks of the metabolites could not be determined properly or because the MRS plane missed the hypothalamus. n.s., not significant.

Table 3—Correlation analyses of two identified bacteria with MBH/amygdala signal ratio (L) and nutritional parameters

	Parasutterella sp.: phylum Proteobacteria		*Marinilabiliaceae* (unclass.): phylum Bacteroidetes	
	ρ	*P*	ρ	*P*
MBH/amygdala (L)	−0.383	0.0073*	−0.299	0.0398*
Fats	−0.298	0.0439*	−0.187	0.2132
Carbohydrates	−0.075	0.6194	−0.132	0.3801
Proteins	−0.283	0.0567	−0.101	0.5047
Total energy	−0.196	0.1908	−0.166	0.2724

Parasutterella sp. correlates negatively with HI and fat intake, while *Marinilabiliaceae* correlates with HI but not with nutritional parameters. Correlation analyses were performed within the obese subject group (MBH/amygdala signal ratio [L]: *n* = 48 subjects, nutrition: *n* = 45 subjects) using the Spearman rank correlation test. In our exploratory approach of the microbiota, we investigated the correlation of 26 bacteria with HI in order to detect the bacteria with the largest effect sizes (at a nominal significance level of 0.05). In that analysis, the findings were not robust to multiple testing by Bonferroni correction when we used the 26 species as independent cases. However, gut bacteria are not independent from each other, and co-occurrence analysis revealed that the core measurable microbiome in relation to HI can be subdivided into 3 major clusters. Within the clusters, *Parasutterella sp.* but not *Marinilabiliaceae* holds against multiple testing. unclass., unclassified. *Significant *P* value ($P < 0.05$).

Gut Microbiota Disturbances Associated With MBH Inflammation and Gliosis Are Related to the Nutritional Fat but Not Carbohydrate, Protein, or Total Energy Intake

Having shown that the two bacteria *Parasutterella sp.* and *Marinilabiliaceae* are associated with alterations in the MBH/amygdala signal ratio (L), we next aimed to examine whether the nutritional intake influences the abundance of these microorganisms in the gut. Therefore, the Food Frequency Questionnaire was used to obtain information on the nutritional behavior over a time period of 12 months according to the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam protocol (20). Afterward, the total intake of fat, carbohydrates, proteins, and total energy was calculated using the EPIC soft database (20). Correlation analysis revealed that *Parasutterella sp.* were significantly negatively associated with dietary fat intake of obese human subjects (Table 3), while no association was found with the total energy, carbohydrate, or protein intake. More specifically, detailed correlation analysis of 41 nutritional fat components revealed that intake of glycerin and lipoids (glycolipids and phospholipids) ($P = 0.03$, $\rho = -0.32$) and intake of long-chain fatty acids ($P = 0.049$, $\rho = -0.29$) were significantly negatively associated with *Parasutterella. sp*. The *Marinilabiliaceae* family did not show a significant association with nutritional components. Of interest, we did not find a direct effect of dietary

components on HI, suggesting that the microbiome is essential in mediating nutritional effects on neuronal inflammation. However, one caveat is that nutritional questionnaires rely on self-reporting of the probands and must therefore be interpreted with care.

Common Polymorphisms in the JNK Gene or in the MC4R Gene Increase the Individual Susceptibility to HI and Gliosis

Thus far, the data obtained suggested that environmental factors such as the gut microbiome are important in mediating HI and gliosis in humans. In the next step we aimed to examine whether genetic factors might also be important, presumably by increasing the individual susceptibility of obese humans to the identified environmental effectors. We performed a candidate gene approach by focusing on four factors previously described to be associated with either HI and gliosis in animal models (JNK, NF-κB) (7) or human obesity (MC4R, FTO) (3,21). As shown in Fig. 4, this analysis revealed an increased MBH/amygdala signal ratio (L) to be associated with a common SNP in the JNK gene (rs1062225) ($P < 0.01$) as well as with a common SNP in the MC4R gene promoter region (rs11872992) ($P = 0.04$) within the obese patients. Additional analyses of these two SNPs regarding the metabolic parameters BMI, HOMA-IR, and triglycerides and the inflammatory parameters IL-6 and CRP revealed no significant difference in comparison of wild-type and mutant subjects within the obese group. No significant association was found for common SNPs in the NF-κB or FTO gene after multiple testing correction (Bonferroni correction).

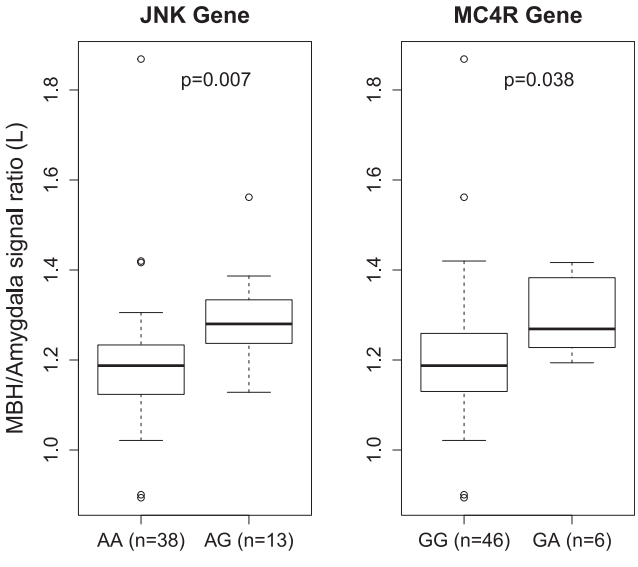

Figure 4—Box plots displaying SNPs within candidate genes as significantly associated with MBH T2 hyperintensity in obese patients. Carrying the heterozygous SNP of the JNK gene (SNP rs1062225) or the MC4R gene (SNP rs11872992) is associated with increased levels of HI within the obese patient group (Wilcoxon rank sum test). One proband with a homozygous mutation for MC4R was excluded from the MC4R analysis. For the SNP in the JNK gene, no probands with homozygous mutations existed.

Weight Loss and Improvement of Peripheral Insulin Sensitivity Due to Bariatric Surgery Do Not Affect HI or Gliosis

In a subgroup ($n = 10$) of the obese individuals of the current study, we also examined the MBH inflammation and gliosis in response to bariatric surgery. As shown in Fig. 5*A* and *B*, in a time period of ~10 months this surgical procedure resulted in a profound weight loss and a dramatic improvement of insulin sensitivity as indicated by a significant reduction of the mean HOMA-IR from 7.4 to 1.7 ($P = 0.02$). In contrast, IL-6 serum levels did not differ before or after the study period, indicating that in this 10-month time period, bariatric surgery is able to improve metabolic function but is not able to reduce systemic low-grade inflammation (Fig. 5*C*). In respect to the hypothalamus, MBH T2 hyperintensities (L) were not significantly normalized after the intervention (Fig. 5*D*). Also, no significant difference could be observed in the NAA/Cr (L) quotient, indicating comparable numbers of neurons before and after the observation period ($P = 0.17$).

DISCUSSION

In previous studies in obesity, MBH T2 hyperintensity as a measure for HI was lateralized to the left hemisphere (9,11), a finding that was reproduced in our prospective study (Fig. 2). While we could exclude an association with left- versus right-handedness, it is notable that other hypothalamic functions, especially parasympathetic outflow, are lateralized to the left side of the brain (22). In addition to our previous observations, our results suggest that the left MBH physiologically has lower MRI T2 intensity compared with the right (Fig. 2*A*, white bars). MRI intensity correlates not only with an increased number of glial cells but also with a lower number of neurons (23). It suggests that the left MBH under healthy conditions might contain a larger number of neurons and might therefore dominate the right MBH in the regulation of autonomic functions.

In the current study, we used MRS in addition to T2-weighted imaging in order to estimate the neuronal cell count by determining the NAA/Cr ratio. Of interest, we found no significant difference in the left hypothalamic region in nonobese versus obese individuals, suggesting that the number of neurons is not negatively affected by the immune process and the gliosis. However, it has to be mentioned that in rodents, neurogenesis does occur within the arcuate and is suppressed by a high-fat diet (24). Thus, a comparable number of neurons might be the result of either absent destruction or the generation of novel neurons. While this finding suggests reversibility, within our 10-month intervention period, bariatric surgery did not beneficially affect the left MBH T2 hyperintensity despite a profound weight loss and a marked improvement of insulin sensitivity. However, the MBH/amygdala signal ratios (L) were reduced by 0.06, which is more than the difference in

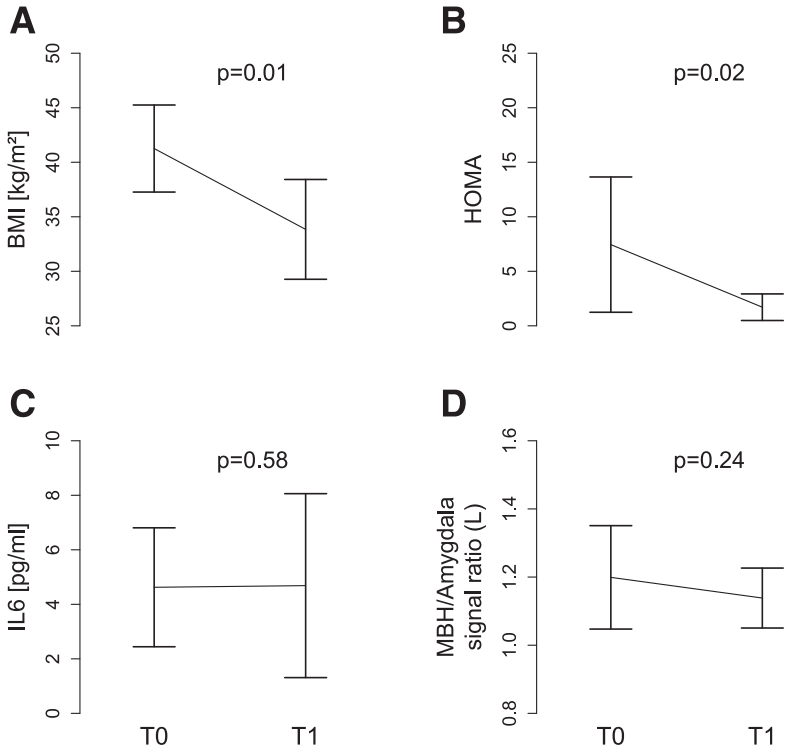

Figure 5—Pre– (T0) and post– (T1) bariatric surgery. Severe obese patients who underwent bariatric surgery (n = 10) showed significant improvement of BMI and HOMA-IR but not in IL-6 or MBH-T2 intensity after a mean follow-up time of 10 months. Two dropouts left the study because of claustrophobia during the MRI measurements, and in some cases phenotypic data were not available *A*: n = 8. *B–D*: n = 7. Paired two-tailed t tests (*A*, *B*, and *D*) and a Wilcoxon signed rank test (*C*) were applied. Data are displayed as mean (SD).

the means between the nonobese and obese group. The postsurgery mean was also below the mean of the nonobese group. These findings might suggest a clinical significance of the difference observed, and the absence of the statistical significance might be due to the small sample size. In addition, since adipose tissue (25) and liver inflammation (26) are also known to reverse much more slowly after weight loss compared with insulin resistance, it can be speculated that after a longer observational period, beneficial changes in HI would become detectable.

The findings of the current study suggest that HI and gliosis are more associated with the overall systemic low-grade inflammation in obesity rather than with metabolic abnormalities, e.g., insulin resistance. This is in contrast to a previous study, in which a significant association of MBH T2 hyperintensity with peripheral insulin resistance was reported (11). However, in that report inflammatory biomarkers were not measured and no weight loss intervention was performed; therefore, it might not have been conclusively possible to ponder inflammatory versus metabolic factors regarding their role in HI and gliosis. In addition, in animal experiments it has been shown that HI proceeds the body weight gain (9), also suggesting that the MBH pathology is not the result of the obesity-associated insulin resistance.

In the current study we recruited our nonobese and obese probands from the FoCus cohort in Kiel, a cohort of n = 1,326 cross-sectional control subjects and n = 511 obese subjects for investigation of the relationship of nutrition, microbiome, and inflammation (15,27,28). The obese subjects in the FoCus cohort were recruited via our outpatient obesity clinics. As is often seen in Europe, these institutions are more frequently visited by female patients compared with male patients, explaining the ~91% female population of our groups. Since the gut-brain axis is generally believed to be important in body weight regulation, we analyzed the microbiome sequencing data with respect to the MBH T2 hyperintensity in the obese patient group. Taking the core measurable microbiome of n = 26 genera into consideration, we were able to identify a significant negative association for two bacterial species with the MBH T2 hyperintensity, while no positive association of any gut bacterium with the hypothalamic abnormality was found. The first, *Parasutterella sp.*, has recently been associated with the intake of anti-inflammatory α-linolenic acid in animals (29), and the second, *Marinilabiliaceae*, is known to be a marine organism (30). Since a diet rich in α-linolenic acid and sea food reflects a more healthy type of nutrition, the negative association of these microorganisms with the MBH/amygdala signal ratio might indicate the importance of nutritional factors in HI and gliosis. But one might argue that obesity itself has a major impact on the human gut microbiome, and therefore the association of the two microorganisms with MBH T2 hyperintensity

might be of an indirect nature. However, in the current study the association of *Parasutterella sp.* and *Marinilabiliaceae* with the MBH/amygdala signal ratio was significant within the obese patients group, suggesting a direct association of the gut microbiome and the inflammatory hypothalamic pathology.

Several animal models indicate that nutritional factors, such as a high-fat diet, induce HI and gliosis (8,9). In addition, nutrition is known to influence the gut microbiome heavily (31,32). Thus, we next examined whether macronutrients are associated with the abundance of the two gut microbes that had been identified to be associated with MBH T2 hyperintensity. Interestingly, for *Parasutterella sp.* we found that a higher fat content in the diet is significantly associated with a lower abundance of the bacterium. Since lower counts of the bacterium are associated with higher MBH/amygdala signal ratio, these findings suggest a pathway whereby nutrition, via the gut microbiome, might affect HI and gliosis. Of importance, the abundance of *Parasutterella sp.* was associated with fat intake but not with total energy intake, again suggesting that it is not caloric intake and/or the obesity phenotype per se that triggers HI but, rather, certain macronutrients and gut microbial species. This suggests that in humans, as in animal models (9), changes in the gut-brain axis are not the result of obesity but, rather, might induce appetite and satiety dysregulation and might therefore be causative for the increase in body weight.

Many chronic diseases are characterized by disturbances in gene-environment interaction. As we had genotyping data available for all of our study subjects, we examined whether common polymorphisms might influence the individual susceptibility for the above-mentioned environmental factors. Since data from animal studies suggested JNK and NF-kB (7) to be important factors in HI and human data suggest MC4R and FTO polymorphism (3,21) to influence satiety, we related common SNPs of these genes to the MRI data in a candidate gene approach. In that analysis we found, for the JNK SNP rs1062225 and the MC4R promoter SNP rs11872992, a significant positive association with MBH inflammation and gliosis in obese humans. This is of particular interest, since in rodent models JNK signaling (7) and MC4R function (33) have been implicated in the modulation of neuronal inflammation. It suggests that human individuals with heterozygous mutations in the corresponding genes are more susceptible to environmental factors triggering HI. Within the obese group, in contrast to MBH T2 intensity, metabolic parameters (HOMA, BMI) did not show a significant association with the MC4R and JNK SNPs tested, again suggesting that factors other than the metabolic phenotype trigger HI, e.g., the individual susceptibility to nutritional components.

Several limitations of our study have to be pointed out. *1*) we are only providing data on associations and correlations, and therefore we cannot prove causality; *2*) the voxels in the hypothalamus (L) that were used for MRS analyses may have variable overlap with the coronal regions of interest (ROIs) placed for T2 ratio; *3*) nutritional data were obtained by self-reported questionnaires and might therefore be biased; and *4*) the number of subjects undergoing bariatric surgery may have been too few for detection of significant effects on HI.

In conclusion, we present a prospective translational study of the role of HI and gliosis in human obesity. Our findings suggest that HI in the future might be considered as an additional dietary and/or pharmacological treatment target to potentiate the effect of bariatric surgery and nonsurgical obesity therapies, especially in the phase of weight maintenance.

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