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Research Paper

Influence of Gestational Diabetes and Pregestational Maternal BMI on the Brain of Six-Year-Old Offspring

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ABSTRACT

Background: Gestational diabetes (GD) and maternal excess weight are common pregnancy conditions that increase the risk of future complications for both the mother and her offspring. Their consequences on neurodevelopment are widely described in the literature, but less is known concerning the potential transgenerational influence on the brain structure.
Methods: We used a combination of support vectors machine and hierarchical clustering to investigate the potential presence of anatomical brain differences in a sample of 109 children aged six years, born to mothers with overweight or obesity, or to mothers diagnosed with GD during pregnancy.
Results: Significant effects are visible in the brain of children born to mothers with GD associated with pregestational excess weight, especially overweight instead of obesity. No differences in children's brain were observed when considering those born to normal-weight mothers.
Conclusions: Our study highlights the need for clinical attention of pregnant women at risk to develop GD, and especially those with pregestational excess weight, since this status was found to be associated with detectable transgenerational brain changes. These effects may be due to the absence of specific and individualized intervention in these mothers during pregnancy.

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Patient consent statement: Written informed consent forms were obtained at the beginning of the study and before the MRI magnetic resonance session, from all parents or guardians of the children involved in the PREOBE Follow-up study.

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Introduction

Gestational diabetes (GD) is a serious pregnancy complication affecting 16.5% of pregnancies worldwide,[1,2] with long-term health consequences. The most relevant are type 2 diabetes and cardiovascular diseases, in both mother and offspring, and macrosomia, future obesity, and/or GD in the child.[2-4] Prepregnancy excess weight has been also linked to several health consequences and long-term increased risk in the offspring, including child obesity, diabetes, and cardiovascular diseases.[5-7]

The effects of GD and maternal excess weight on the offspring neurodevelopment are widely investigated and well known.[8-13] However, the potential transgenerational effects of those conditions on brain structure and function have been so far rarely investigated.[14-20] Although clinical and sociologic studies pointed out the relevance of environmental factors and domestic habits for the emergence of excess weight and even type 2 diabetes,[21-24] the identification of further associations between maternal health conditions and brain structural characteristics in their offspring could help to consolidate a still developing research topic.

To this aim, we investigated if maternal prepregnancy body mass index (p-BMI) and the presence or absence of GD can be related to anatomical differences between their offspring's brains at age six years. To do so, we adopted combined use of supervised and unsupervised machine learning methodologies. As pointed out by Koul et al.,[25] this twofold approach allows to address two different kinds of research questions. By means of supervised learning, and in the present case support vector machine (SVM), it is possible to determine whether data have "discriminatory information." In other words, SVM can answer the question: are offspring's brains different enough to correctly assign each of them to the group it belongs to? (e.g., child born to mother with GD or without GD). Unsupervised learning, and in the present case hierarchical clustering (HC), allows to analyze the variability of data.[25] This method can answer the questions: can the subjects be organized into groups based on the characteristics of their brain? How many groups must be created? Who enters each of the groups?

In the present work, these questions were answered based on the analyses of the structural properties of the children's brains at six years. Potential differences on these properties were not put into relationship with nonbrain characteristics of the children themselves, but with maternal p-BMI and/or the presence or absence of GD. To evaluate whether results came from the whole brain characteristics or if they were due to specific tissues, analyses had been repeated on the whole brain, and using gray matter (GM) or white matter (WM) only.

Based on scientific literature, we hypothesized that SVM and HC methods can detect differences in the anatomical offspring's brain characteristics associated with transgenerational effects.

Materials and Methods

Study design and selection of subjects

The present work is based on the PREOBE[26] (www.ClinicalTrials.gov, Identifier: NCT01634464) study, a prospective observational cohort study, designed to explore pre- and postnatal influence of maternal overweight, obesity, and GD on their offspring. Mothers were recruited between 2008 and 2012 in the Clinical University Hospital San Cecilio and the Mother-Infant University Hospital of Granada (Granada, Spain) and their peripheral health centers. Briefly, the database consists of medical and sociocultural information concerning 331 pregnant women aged between 18 and 45 years and their full-term and healthy offspring. Full general inclusion and exclusion criteria can be found in Berglund et al.[26] In the present work we focused on the structural magnetic resonance imaging (MRI) acquired up until October 2017 for 155 healthy children aged six years. This age was chosen as younger children are rarely collaborative enough to allow the collection of reliable MRI data. Moreover, six years has been described as the first end point of early brain development and maturation.[27,28] Twelve subjects were excluded because their mothers were already affected by diabetes before becoming pregnant. The remaining 143 subjects were then divided into six groups, on the bases of their calculated maternal p-BMI at the recruiting visit (between weeks 12 and 20 of pregnancy) and the diagnosis of GD at 34 weeks of gestation. Cutoff points for p-BMI were taken as follows: $18.5 \leq$ p-BMI ≤ 25 = normal weight group (NW); $25 \leq$ p-BMI ≤ 30 = overweight group (OW); p-BMI ≥ 30 = obese group (OB) (see Table 1 for group details).

The study was approved by the Human Research Ethics Committee of the University of Granada and conducted in accordance with the Helsinki Declaration for human research studies. Written informed consent forms were obtained at the beginning of the study and before the MRI session, from all parents or guardians of the children involved in the PREOBE Follow-up study.

MRI acquisition and preprocessing

Before the real MRI acquisition, the children participated in a practice session. The children were familiarized with the MRI environment, and they were introduced in a mock scanner and listened to the real scanner's noise. In addition, to reassure and keep them from movement and falling asleep, the children watched a cartoon film during the real acquisition. Furthermore, a foam system was located around the participant's head.

T1 images were acquired for each participant, using a 3T Magnetom Trio scanner (Siemens Medical System, ERLANGEN, Germany), located at Mind, Brain and Behavior Research Centre at the University of Granada. A high-resolution T1-weighted 3D magnetization-prepared rapid gradient-echo (MPRAGE) sequence was acquired with the following parameters: repetition time (TR) = 2.3 ms, echo time (TE) = 3.1 ms, flip angle = 9°, field of view (FOV) = 256 × 256 mm, matrix size = 320 × 320, and number of slices = 208, resulting in an isotropic resolution of 0.8 × 0.8 × 0.8 mm. Acquisition time was 6 minutes 35 seconds. Of note, this scanner provides images on which field inhomogeneity correction has already been performed. These images were independently checked by two expert researchers, to detect artifacts due to motion or other causes. Thirty-four subjects were excluded at this point, after convergent judgment (see Table 1 for details on the group subdivision).

T1 images were preprocessed and segmented into GM and WM using DARTEL[29] as implemented in DBAPI 2.3.[30] To obtain additional tissue segmentation maps with intensity values in it (instead of probability values as provided by DARTEL), the whole brain T1 of each subject was multiplied for either the binarized GM or binarized WM DARTEL maps of the same subject.

Preliminary confounding interaction assessment

To exclude potential confounding effects, we performed linear regression between relevant couples of variables. These analyses were performed on GD groups and non-GD groups separately. In detail, we contrasted maternal pre-pregnancy BMI (p-BMI) versus gestational weight gain (GWG); GWG versus children's BMI at the moment of the MRI evaluation; p-BMI versus children's birth weight; and p-BMI versus children's BMI at the moment of the MRI evaluation. Moreover, we performed a one-way ANOVA on children's BMI at the moment of the MRI evaluation.

TABLE 1.
Characteristics of the Study Population

	Non-Gestational Diabetes (N = 78)			Gestational Diabetes (N = 31)		
	Normal Weight (NW; n = 44)	Overweight (OW; n = 19)	Obesity (OB; n = 15)	Normal Weight (NWGD; n = 12)	Overweight (OWGD; n = 10)	Obesity (OBGD; n = 9)
Maternal p-BMI (kg/m^2)	22.50 (1.65)	27.00 (1.25)	31.96 (1.28)	22.06 (2.01)	27.53 (1.15)	35.96 (4.86)
GWG (kg)	12.93 (5.73)	10.10 (6.67)	9.34 (6.52)	10.10 (7.63)	7.14 (4.25)	1.74 (10.15)
Birth weight (g)	3246.36 (399.92)	3468.95 (574.30)	3382.67 (514.94)	3459.17 (514.94)	3056.00 (347.63)	3454.44 (418.81)
Children's BMI at MRI (kg/m^2)	16.00 (1.50)	17.09 (2.29)	17.28 (2.51)	16.82 (2.60)	17.72 (2.85)	17.00 (2.30)
Maternal age (years)	31.48 (3.75)	32.63 (4.34)	29.00 (4.14)	33.50 (5.20)	33.80 (2.97)	34.44(4.72)
Child sex (M/F)	24/20	8/11	7/8	8/4	4/6	5/4
Gestational age (weeks)	39.57 (1.13)	39.56 (1.89)	39.60 (1.64)	39.58 (1.44)	39.10 (1.52)	39.44 (1.51)
Children's age at MRI (days)	2386.59 (116.10)	2373.84 (115.51)	2293.40 (105.90)	2317.83 (101.15)	2256.10 (75.69)	2256.44 (45.72)
Subjects removed	17	3	3	4	5	2

Abbreviations:
F = Female
GWG = Gestational weight gain
M = Male
MRI = Magnetic resonance imaging
NW = Normal weight
NWGD = Normal weight and gestational diabetes
OB = Obese
OBGD = Obesity and gestational diabetes
OW = Overweight
OWGD = Overweight and gestational diabetes
p-BMI = Prepregnancy body mass index
Data are expressed in mean (S.D.) and refers to the population analyzed after subjects removal.

Neuroimaging analyses

Support vector machine analyses

To investigate whether brain data contained discriminatory information, we performed classification task between the experimental groups, using PRONTO tool.[31] We used a binary support vector machine (SVM), with leave one subject out as cross-validation method, and no hyperparameter optimization. SVM is one of the most widely used classification algorithm in the field of neuroimaging.[32] In this class of algorithms, a hyperplane is searched that optimally separates the items into two, or more, classes.[33] Therefore, the best solution is based only on those items in the proximity of the hyperplane, rather than on the whole sample.[34] Several comparisons were realized, to separately analyze the effect of GD and the effect of p-BMI (see Table 2 for an overview of the comparisons). The results were evaluated considering total accuracy (TA), balanced accuracy (BA), and area under the curve (AUC). Chance level cutoffs for SVM accuracy were set in accordance with the work of Combrisson and Jerbi[35] considering $P < 0.05$ and two classes. This cutoff, which is always specified in the results section, depends on the number of subjects included in each analysis. For those comparisons showing statistically significant accuracy, weight maps were inspected to identify possible brain regions particularly relevant for the classification procedure, which means showing a more marked difference between the compared groups. SVM was applied to the whole brain T1, and to GM and WM separately.

Hierarchical clustering analyses

To further verify the statistically significant SVM results, we performed HC analysis, using Orange 2.7.[36] Clustering algorithms are abundantly used in MRI research,[37] and, generally speaking, they organize items into a set of nested partitions.[34] HC does not require the definition of a predetermined number of clusters, as it is instead necessary for the widely-used k-means clustering.[34] In the present case, a N×M matrix was created, in which each row represented a different subject, and each column, a same voxel through all the subjects. Nonbrain voxels were excluded from the matrix by means of a group mask. Distances between rows (i.e., between subjects) were calculated using Euclidean metric, and Ward linkage was used to build the dendrogram. Ward linkage tries to minimize the variability inside each cluster.[34,38] HC was performed separately for GM and WM, due to computational constraints. Results were evaluated as a ratio between the majority class and the total number of items in each cluster. In other words, we observed if the majority of subjects in every given cluster belonged to the same study group.

Results

Confounding interaction analyses

Overall, none of the linear regression analyses between maternal variables and children's variables highlighted marked confounding effects (Figs. S1, S3, S4, and S6-S9). The only relevant interaction was found between maternal p-BMI and GWG for mothers with GD ($R^2 = 0.4$) (Figs. S1 and S2). The higher the p-BMI was, the lesser the kilograms gained during the pregnancy; however, GWG does not seem to influence children's BMI at the moment of the MRI evaluation ($R^2 = 0.07$) (Figs. S3 and S4). The one-way ANOVA performed on the children's BMI at the moment of the evaluation did not show statistically significant differences (see Fig. S5).

Gestational diabetes effect

GD groups versus non-GD groups

SVM analyses. To assess the effect of GD, independently from maternal p-BMI, children born to mothers with GD (N = 31) or without GD (N = 78) were collapsed into two macrogroups. The SVM analysis at this macrogroups level (N = 109), on whole brain data, showed TA = 73.39%. However, due to the marked unbalance between the two classes, BA = 58.09% (critical cutoff of

TABLE 2.
Results of the SVM Analyses Based on Whole Brain Images

Group Comparison	Total N	Critical Cutoff	Total Accuracy	Balanced Accuracy	AUC
GD effect					
NW vs NWGD	56	60%	76.79%	48.86%	0.66
OW vs OWGD	29	70%	**89.66%**	**87.37%**	0.88
OB vs OBGD	24	70%	54.17%	47.78%	0.50
EW vs EWGD	53	60%	**75.47%**	**70.43%**	0.76
Non-GD vs GD	109	58%	**73.39%**	**58.09%**	0.79
p-BMI effect (GD groups)					
NWGD vs OWGD	22	70%	**72.73%**	**73.33%**	0.69
OWGD vs OBGD	19	70%	63.16%	62.22%	0.78
NWGD vs OBGD	21	70%	52.38%	47.22%	0.42
NWGD vs EWGD	31	62.50%	64.52%	58.77%	0.64
p-BMI effect (non-GD groups)					
NW vs OW	63	60%	68.25%	48.86%	0.30
OW vs OB	34	62.50%	29.41%	26.32%	0.10
NW vs OB	59	60%	74.58%	50%	0.35
NW vs EW	78	58.70%	44.87%	41.11%	0.46

Abbreviations:
AUC = Area under the curve
BMI = Body mass index
EW = Excess weight
EWGD = Excess weight (Overweight and obesity groups) with GD
GD = Gestational diabetes
NW = Normal weight
NWGD = Normal weight and gestational diabetes
OB = Obese
OBGD = Obesity and gestational diabetes
OW = Overweight
OWGD = Overweight and gestational diabetes
p-BMI = Prepregnancy BMI
SVM = Support vector machine
Critical cutoff is based on Combrisson and Jerbi,[33] considering $P < 0.05$ and two classes. Significant accuracy results are in bold.

reference = 58%) must be considered as a more reliable indicator (Fig 1). The AUC was equal to 0.79. Inspection of the weight map showed a global contribution rather than main involvement of specific regions. Our result could be interpreted as a moderate evidence of discriminability between the two groups (i.e., brain imaging from children born to mothers with GD versus without GD). At the same time, it suggests the need of a set of more accurate analyses with a subtler subdivision of the groups. The SVM analysis of both WM and GM alone did not produce statistically significant results (see Tables S1 and S2).

Hierarchical clustering analyses. Coherently with the around-threshold SVM result, the dendrograms obtained on both GM and WM data did not show a net separation between the GD groups and the non-GD groups.

In other words, the brain structural properties did not allow to clearly discriminate between children born to mothers with GD and children born to mothers without GD.

Excess-weight_GD groups versus excess-weight_non-GD groups

SVM analyses. The around-threshold results obtained in previous analyses on the whole GD groups could have been at least in part due to the underlying effect of maternal p-BMI. To test this hypothesis, we proceeded focusing on excess weight groups only. We hence collapsed overweight and obesity groups with GD (EWGD; N = 19) or without GD (EW; N = 34), and let apart normal weight groups. The SVM analysis at this level (N = 53), on whole brain data, showed TA = 75.47%. Again, due to the marked unbalance between the two classes, BA = 70.43% (critical cutoff of reference = 60%) must be considered as a more reliable indicator (Fig 2). The AUC was equal to 0.76. Inspection of the weight map showed a global contribution rather than main involvement of specific regions. Hence, our result can be interpreted as a quite strong evidence of discriminability between the two groups. The SVM analysis of both WM and GM alone produced statistically significant and comparable results as well (see Tables S1 and S2).

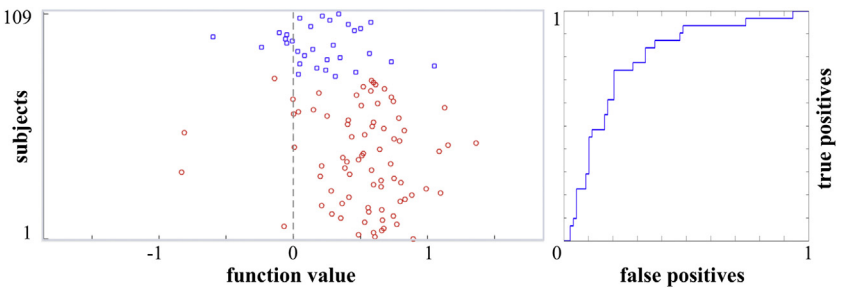

FIGURE 1. Results of the comparison between gestational diabetes (GD) groups and non-GD groups. Left: details of subjects' discrimination based on whole brain images. The farther the mark from the central dashed line, the more reliable the classification of the related subject. Blue squares = GD subjects, red circles = non-GD subjects. Right: Area under the curve. The color version of this figure is available in the online edition.

Hierarchical clustering analyses. The dendrogram obtained on GM data shows the presence of two main clusters, one including 39 subjects and the other including 14 subjects. Thirty-one of 39 subjects (79.5%) in the first cluster belong to the EW group, whereas 11 of 14 subjects (78.6%) in the second cluster belong to the EWGD group (Fig 2). Coherently with the SVM results, the clustering analysis showed a net separation between the two groups. On the contrary, the dendrogram obtained on WM data did not show a net separation between the groups. These results suggest that when focusing on children born to mothers with excess weight only, the separation between children born to mothers with GD and children born to mothers without GD became clearer.

Group by group comparisons

SVM analyses. To further refine the focus of the analyses, we then moved to the comparison between couples of groups with same p-BMI. The only condition to produce statistically significant results was overweight group without GD (OW; N = 19) versus overweight group with GD (OWGD; N = 10). The SVM analysis at this level (N = 29), on whole brain data, showed TA = 89.66% and BA = 87.37% (critical cutoff of reference = 70%) (Fig 3). The AUC was equal to 0.88. Inspection of the weight map showed a global contribution rather than main involvement of specific regions. Our results can be interpreted as a marked evidence of discriminability between the two groups. The SVM analysis of both WM and GM alone produced statistically significant and comparable results as well (see Tables S1 and S2).

Of note, the SVM comparison for normal weight groups with or without GD did not produce significant results (TA = 76.79%, BA = 48.86%, AUC = 0.66). This finding suggests that the barely significant results obtained when comparing all the groups without GD against all the groups with GD could be masked by the similarity among normal weight groups. After removing them, limiting the groups to excess weight condition, results became significant. However, whereas the comparison between overweight groups (OW versus OWGD) resulted in statistically significant results, the comparison for obesity groups (OB versus OBGD [obesity and gestational diabetes]) did not produce significant results (TA = 54.17%, BA = 47.78%, AUC = 0.50). Therefore, results for excess weight groups were probably driven by the discriminability between overweight groups.

Hierarchical clustering analyses. The dendrogram obtained on GM data for the overweight groups (OW versus OWGD) shows the presence of two main clusters, one including 21 subjects and the second one including eight subjects. Eighteen of 21 subjects (85.7%) in the first cluster belong to the OW group, whereas seven of eight subjects (87.5%) in the second cluster belong to the OWGD group. The dendrogram obtained on WM data shows the presence of two main clusters, one including 15 subjects and the second one including 13 subjects. One subject from the OW group remained outside from the two clusters, appearing as an outlier. Thirteen of 15 subjects (86.7%) in the first cluster belong to the OW group, whereas eight of 13 subjects (61.5%) in the second cluster belong to the OWGD group. Coherently with the SVM results, the clustering analysis showed a net separation between these two groups.

Maternal prepregnancy BMI effect

SVM analyses

To explore the effect of maternal p-BMI the same approach used for GD was followed, but in this case p-BMI groups without GD and p-BMI groups with GD were tested separately. In this context, the only comparison to produce statistically significant results was the one testing normal weight group with GD (NWGD [normal weight and gestational diabetes]; N = 12) versus obesity group with GD (OWGD; N = 10). The SVM analysis at this level (N = 22), on whole brain data, showed TA = 72.73% and BA = 73.33% (critical cutoff of reference = 70%) (Fig 4). The AUC was equal to 0.69. Inspection of the weight map showed a global contribution rather than main

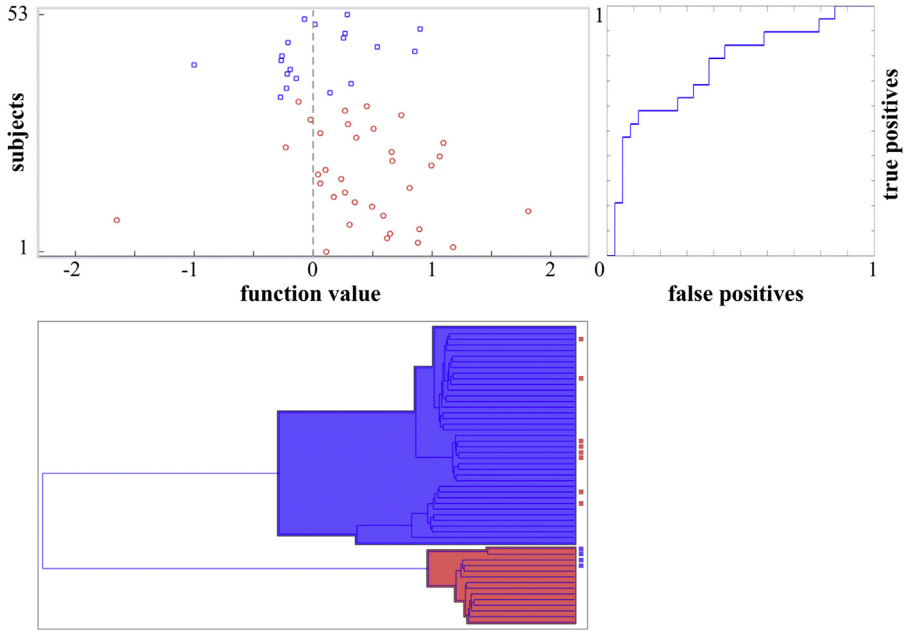

FIGURE 2. Results of the comparison between EWGD (excess weight overweight and obesity groups with gestational diabetes [GD]) group and EW group (overweight and obesity groups without GD). Top left: details of subjects' discrimination based on whole brain images. The farther the mark from the central dashed line, the more reliable the classification of the related subject. Blue squares = GD subjects, red circles = non-GD subjects. Top right: Area under the curve. Bottom: hierarchical clustering results for gray matter (GM). The blue cluster mainly includes EW subjects, whereas the red cluster mainly includes EWGD subjects. The squares on the right side of the dendrogram mark subjects not belonging to the majority group in that cluster. The color version of this figure is available in the online edition.

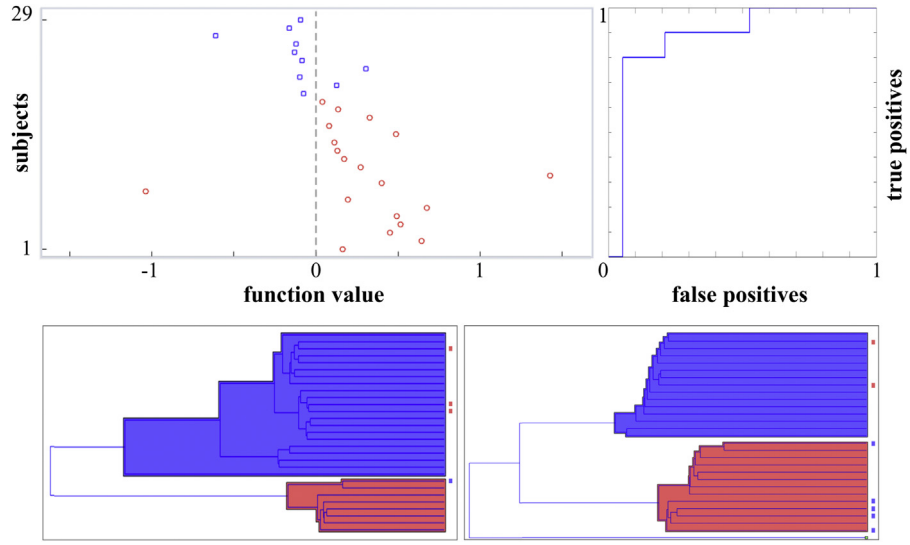

FIGURE 3. Results of the comparison between OWGD group (overweight group with gestational diabetes) and OW (overweight group without gestational diabetes) group. Top left: details of subjects' discrimination based on whole brain images. The farther the mark from the central dashed line, the more reliable the classification of the related subject. Blue squares = GD subjects, red circles = non-GD subjects. Top right: area under the curve. Bottom left: hierarchical clustering results for gray matter (GM). The blue cluster mainly includes OW subjects, whereas the red cluster mainly includes OWGD subjects. The squares on the right side of the dendrogram mark subjects not belonging to the majority group in that cluster. Bottom right: hierarchical clustering results for white matter (WM). Color scheme was used as described for GM. The color version of this figure is available in the online edition.

involvement of specific regions. Our result could be interpreted as a moderate evidence of discriminability between the two groups. The SVM analysis of both WM and GM alone produced statistically significant and comparable results as well (see Tables S1 and S2).

All the other comparisons focusing on maternal p-BMI effect did not produce statistically significant results (see Tables 2 and S1 for an overview).

Hierarchical clustering analyses

Despite the statistically significant SVM result, the dendrograms obtained on both GM and WM data did not showed a net separation neither between p-BMI groups associating with GD nor between p-BMI groups without GD.

Additional SVM comparisons focusing on OWGD, evaluating the interaction between p-BMI and GD, are described in the supplementary material (see also Table S3).

Discussion

GD and maternal excess weight are common pregnancy conditions that can have a negative impact for both the mother and her offspring. In the present study, we tried to understand if these clinical variables are associated with the structural properties of the offspring's brains, using a combination of supervised and unsupervised learning methods. We found overall moderate evidence of differences on the brain of children born to mothers with and without GD. This effect is stronger especially in children born to mothers with overweight and GD, and it was not found in children born to normal weight mothers. In terms of brain involvement, what allowed to differentiate between the compared groups seems to be a global property, rather than marked differences in specific cortical or subcortical regions.

Based on the results, the effect of GD at increasing maternal p-BMI seems to follow a bell-shaped distribution. In fact, GD seems to play a relevant role especially in the case of children born to mothers with overweight. On the contrary, there was no evidence of significant differences between the offspring of mothers with normal weight without GD and those born to mothers with normal weight and GD. Similarly, no significant differences were detected between the offspring born to mothers with obesity and no GD and those born to mothers with obesity and GD.

To the best of our knowledge there is only one previous study that explored the additive association of GD and maternal p-BMI on children brains.[18] The results showed a linear correlation between maternal BMI and hypothalamic dysfunction, but the statistical significance of this effect disappeared when adjusting for GD

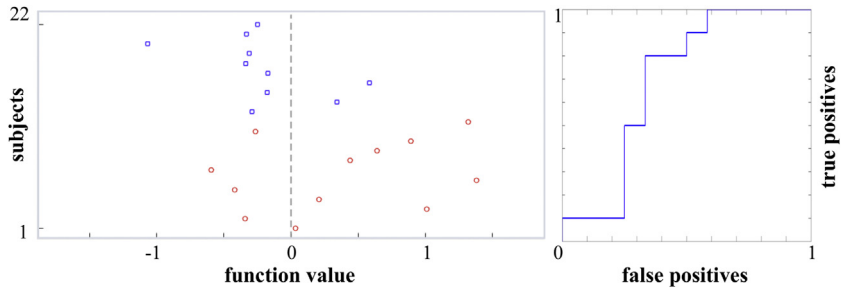

FIGURE 4. Results of the comparison between normal weight and gestational diabetes (NWGD) group and overweight and gestational diabetes (OWGD) group. Left: details of subjects' discrimination based on whole brain images. The farther the mark from the central dashed line, the more reliable the classification of the related subject. Blue squares = overweight subjects, red circles = normal weight subjects. Right: Area under the curve. The color version of this figure is available in the online edition.

exposition. The authors suggest that GD mediates the association between maternal BMI and brain function. Although these results could appear as conflicting with ours, some elements should be noted. First, the authors investigated a functional dysregulation limited to the hypothalamus, whereas the present study focused on whole brain structural properties. Second, the PREOBE children were scanned at the age of six years, whereas the sample analyzed in Page et al. (2019) ranged between 7 and 11 years. Last, our findings suggest a nonlinear interaction, whereas Page and colleagues only tested a linear relationship. Other previous studies that found significant relationships between GD and offspring's neurodevelopment[12,39] did not explore the additive impact of excessive maternal weight before pregnancy.

Our findings are in line with those of a previous study conducted on this same cohort that reported an additive effect of GD and maternal BMI on latencies of visual evoked potentials at age 18 months.[40] A similar bell-shaped relation was in fact found between maternal BMI and latencies for the GD group. The authors claimed that poorer myelination of the auditory system may explain the results. However, previous analyses,[14] including one conducted on the PREOBE cohort,[16] failed to find a significant association of WM of children aged six years with maternal p-BMI. Coherently, in the present study HC results gave better separation when applied to GM rather than WM, possibly suggesting that GD and maternal BMI could influence mainly GM at this stage of development. Nevertheless, the SVM analyses suggested that GM and WM are substantially equally informative to discriminate between groups. Further research at different development phases will help to elucidate the possible different role of maternal p-BMI and GD on these tissues in their offspring brain.

Of note, the children born to mothers who were overweight and had GD were not only found to differ from those born to mothers with comparable BMI but without GD. The children were also different from the offspring of normal weight mothers, with or without GD, as well as from the offspring born to mothers with obesity and no GD. Hence, the co-occurrence of maternal overweight and GD is likely to be the more effective condition determining long-term consequences in their offspring brain. This aspect deserves further clinical consideration in light of the documented increased risk of GD with increasing BMI.[41] On the contrary, maternal p-BMI alone was not found to be associated with differences in offspring of mothers without GD. At present, overweight pregnant women are considered at risk to develop gestational diabetes. Although there are new clinical protocols to identify the presence of GD during the first trimester of pregnancy, there are no specific recommendations or interventions for these pregnant women until they are diagnosed of GD.

The observed bell-shaped effect could also be influenced by different maternal conduct depending on group membership. If, on the one hand, prepregnancy normal weight could act as a protecting factor, on the other hand mothers with obesity could be more aware of the risks related to their BMI. In virtue of this, they could adopt special preventive measures. Moreover, obstetricians could recommend avoiding an excessive gestational weight gain, and supervise them frequently. Conversely, mothers with overweight are still on the way to potentially develop obesity; as a consequence, they could still have those negative habits that were already abandoned (or at least mitigated) by mothers with obesity. Moreover, if the latter are likely to be followed by a specialist, the former could even not be aware of the risks they are exposed to. This hypothesis is supported by a qualitative survey realized on a sample of Latinas women, showing that mothers who were overweight rarely gave importance to body weight, and underestimated the role of diet, compared with both healthy weight women and those with obesity.[42] More generally, Shub et al.[43] found that the majority of the interviewed mothers with prepregnancy excess weight had limited knowledge of the risk associated with maternal obesity. In light of this, it is not possible to exclude that mothers enrolled in PREOBE could have adopted more responsible behaviors for the fact of being part of a research project (a kind of Hawthorne effect). This fact could be reflected in the negative linear relation between p-BMI and GWG, especially marked for mothers with GD. Consequently, the influence between GD/BMI and offspring's brain structural properties in the real population could be more evident.

Finally, it is fundamental to note that at the moment of the MRI evaluation there were no statistically significant differences for the BMI of the children. Coherently, no relationship was found between the maternal p-BMI and the BMI of their children at age six years. Therefore, the results are unlikely attributable to differences in build or development of the subjects.

One potential limitation of the present work is the sample composition. Although quite consistent as a whole size, the decomposition into six subgroups, and the data quality assessment, generated reduced cardinalities. Moreover, some of the comparisons involved unbalanced groups. However, this aspect, which reflects at least in part the rate of incidence of GD,[44,45] was taken into consideration when evaluating the results. Head movement is a second relevant problem, common to the MRI research field, and particularly marked when working with children. To try to limit the influence on data, specific procedures were followed, as explained in the methods section. In addition to this precaution, data were subjected to manual screening and discarded when corrupted. Although many control variables were collected, it is not possible to exclude confounding effects due to uninvestigated parameters. Last, HC had been performed for GM and WM separately, but it was not possible to jointly analyze the two for computational constraints. Interestingly, future studies could benefit from the addition of information concerning the fathers. Since the present study suggests the existence of structural brain differences emerging as a global property, the analysis of cortical parameters, such as cortical thickness and gyrification, could help to clarify the contribution of specific regions instead. At the same time, the absence of specific brain regions sticking out from the global pattern does not facilitate the speculation about possible behavioral implications. To this aim, future investigation of the functional MRI counterpart would be meaningful. Finally, a longitudinal approach would allow to follow the temporal evolution of the transgenerational influences. In fact, although at the time of the analyses the existing structural differences were not related with any sign of impairment, these could possibly evolve into elements of concerns during development, or on the contrary to become no longer detectable.

In the present article we analyzed the potential transgenerational signs of maternal GD and excess weight before pregnancy detectable on the offspring's brains at age six years. Results showed that the relationship with GD is visible for the children born to mothers with excess weight, and in particular those with overweight. On the contrary, no detectable differences emerged when considering mothers with normal weight. Relationship with p-BMI was only found for GD-positive groups, suggesting that maternal p-BMI alone is not associated with transgenerational signs. From the clinical point of view, our study highlights the need for specific care of mothers with excess weight diagnosed with GD, since the combination of these two factors seems to have the capability to induce transgenerational brain signs.

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Supplementary data

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References

1. International Diabetes Federation. IDF Diabetes Atlas. 9th ed. Brussels, Belgium: International Diabetes Federation; 2019.
2. Benhalima K, Devlieger R, Van Assche A. Screening and management of gestational diabetes. Best Pract Res Clin Obstet Gynaecol. 2015;29:339–349.
3. Plows JF, Stanley JL, Baker PN, Reynolds CM, Vickers MH. The pathophysiology of gestational diabetes mellitus. Int J Mol Sci. 2018;19:3342.
4. Pintaudi B, Fresa R, Dalfrà M, et al. The risk stratification of adverse neonatal outcomes in women with gestational diabetes (STRONG) study. Acta Diabetol. 2018;55:1261–1273.
5. Whitaker RC. Predicting preschooler obesity at birth: the role of maternal obesity in early pregnancy. Pediatrics. 2004;114:e29–e36.
6. Chu SY, Callaghan WM, Kim SY, et al. Maternal obesity and risk of gestational diabetes mellitus. Diabetes Care. 2007;30:2070–2076.
7. Reynolds RM, Allan KM, Raja EA, et al. Maternal obesity during pregnancy and premature mortality from cardiovascular event in adult offspring: follow-up of 1 323 275 person years. BMJ. 2013;347:f4539.
8. Adane AA, Mishra GD, Tooth LR. Maternal pre-pregnancy obesity and childhood physical and cognitive development of children: a systematic review. Int J Obes. 2016;40:1608–1618.
9. Yeung EH, Sundaram R, Ghassabian A, Xie Y, Buck Louis G. Parental obesity and early childhood development. Pediatrics. 2017;139, e20161459.
10. Deardorff J, Smith LH, Petito L, Kim H, Abrams BF. Maternal prepregnancy weight and children's behavioral and emotional outcomes. Am J Prev Med. 2017;53:432–440.
11. Sousa RAL, Torres YS, Figueiredo CP, Passos GF, Clarke JR. Consequences of gestational diabetes to the brain and behavior of the offspring. An Acad Bras Cienc. 2018;90:2279–2291.
12. Torres-Espínola FJ, Berglund SK, García-Valdés LM, et al. Maternal obesity, overweight and gestational diabetes affect the offspring neurodevelopment at 6 and 18 months of age–a follow up from the PREOBE cohort. PLoS One. 2015;10, e0133010.
13. Brinciotti M, Napoli A, Mittica A, Bitterman O, Matricardi M. Cortical evoked potentials in children of diabetic mothers. Exp Diabetes Res. 2011;2011:640535.
14. Ou X, Thakali KM, Shankar K, Andres A, Badger TM. Maternal adiposity negatively influences infant brain white matter development. Obesity (Silver Spring, MD). 2015;23:1047–1054.
15. Li X, Andres A, Shankar K, et al. Differences in brain functional connectivity at resting state in neonates born to healthy obese or normal-weight mothers. Int J Obes. 2016;40:1931–1934.
16. Verdejo-Román J, Björnholm L, Muetzel RL, et al. Maternal prepregnancy body mass index and offspring white matter microstructure: results from three birth cohorts. Int J Obes. 2019;43:1995–2006.
17. Salzwedel AP, Gao W, Andres A, et al. Maternal adiposity influences neonatal brain functional connectivity. Front Hum Neurosci. 2018;12:514.
18. Page KA, Luo S, Wang X, et al. Children exposed to maternal obesity or gestational diabetes mellitus during early fetal development have hypothalamic alterations that predict future weight gain. Diabetes care. 2019;42:1473–1480.
19. Lynch KM, Alves JM, Chow T, et al. Selective morphological and volumetric alterations in the hippocampus of children exposed in utero to gestational diabetes mellitus. Hum Brain Mapp. 2022;42:2583–2592.
20. Fernandes DJ, Spring S, Roy AR, et al. Exposure to maternal high-fat diet induces extensive changes in the brain of adult offspring. Transl Psychiatry. 2021;11:149.
21. Bellou V, Belbasis L, Tzoulaki I, Evangelou E. Risk factors for type 2 diabetes mellitus: an exposure-wide umbrella review of meta-analyses. PLoS One. 2018;13, e0194127.
22. Solmi M, Köhler CA, Stubbs B, et al. Environmental risk factors and nonpharmacological and nonsurgical interventions for obesity: an umbrella review of meta-analyses of cohort studies and randomized controlled trials. Eur J Clin Invest. 2018;48, e12982.
23. Haycraft E, Karasouli E, Meyer C. Maternal feeding practices and children's eating behaviours: a comparison of mothers with healthy weight versus overweight/obesity. Appetite. 2017;116:395–400.
24. Patel C, Karasouli E, Shuttlewood E, Meyer C. Food parenting practices among parents with overweight and obesity: a systematic review. Nutrients. 2018;10:1966.
25. Koul A, Becchio C, Cavallo A. PredPsych: a toolbox for predictive machine learning-based approach in experimental psychology research. Behav Res Methods. 2018;50:1657–1672.
26. Berglund SK, García-Valdés L, Torres-Espinola FJ, et al. Maternal, fetal and perinatal alterations associated with obesity, overweight and gestational diabetes: an observational cohort study (PREOBE). BMC Public Health. 2016;16:207.
27. Woodburn M, Bricken CL, Wu Z, et al. The maturation and cognitive relevance of structural brain network organization from early infancy to childhood. Neuroimage. 2021;238:118232.
28. Cao M, Huang H, He Y. Developmental connectomics from infancy through early childhood. Trends Neurosci. 2017;40:494–506.
29. Ashburner J. A fast diffeomorphic image registration algorithm. Neuroimage. 2007;38:95–113.
30. Yan CG, Wang XD, Zuo XN, Zang YF. DPABI: data processing & analysis for (Resting-State) brain imaging. Neuroinformatics. 2016;14:339–351.
31. Schrouff J, Rosa MJ, Rondina JM, et al. PRoNTo: pattern recognition for neuroimaging toolbox. Neuroinformatics. 2013;11:319–337.
32. Orrù G, Pettersson-Yeo W, Marquand AF, Sartori G, Mechelli A. Using support vector machine to identify imaging biomarkers of neurological and psychiatric disease: a critical review. Neurosci Biobehav Rev. 2012;36:1140–1152.
33. Cortes C, Vapnik V. Support-vector networks. Machine Learn. 1995;20:273–297.
34. Khosla M, Jamison K, Ngo GH, Kuceyeski A, Sabuncu MR. Machine learning in resting-state fMRI analysis. Magn Reson Imaging. 2019;64:101–121.
35. Combrisson E, Jerbi K. Exceeding chance level by chance: the caveat of theoretical chance levels in brain signal classification and statistical assessment of decoding accuracy. J Neurosci Methods. 2015;250:126–136.
36. Demsar J, Curk T, Erjavec A, et al. Orange: data mining toolbox in Python. J Machine Learn Res. 2013;14:2349–2353.
37. Mirzaei G, Adeli H. Segmentation and clustering in brain MRI imaging. Rev Neurosci. 2019;30:31.
38. Ward JH. Hierarchical grouping to optimize an objective function. J Am Stat Assoc. 1963;58:236–244.
39. Linder K, Schleger F, Kiefer-Schmidt I, et al. Gestational diabetes impairs human fetal postprandial brain activity. J Clin Endocrinol Metab. 2015;100:4029–4036.
40. Torres-Espínola FJ, Berglund SK, García S, et al. Visual evoked potentials in offspring born to mothers with overweight, obesity and gestational diabetes. PLoS One. 2018;13, e0203754.
41. Kim SY, England L, Sappenfield W, et al. Racial/ethnic differences in the percentage of gestational diabetes mellitus cases attributable to overweight and obesity, Florida, 2004-2007. Prev Chronic Dis. 2012;9:E88.
42. Wang ML, Arroyo J, Druker S, Sankey HZ, Rosal MC. Knowledge, attitudes and provider advice by pre-pregnancy weight status: a qualitative study of pregnant Latinas with excessive gestational weight gain. Women Health. 2015;55:805–828.
43. Shub A, Huning EY, Campbell KJ, McCarthy EA. Pregnant women's knowledge of weight, weight gain, complications of obesity and weight management strategies in pregnancy. BMC Res Notes. 2013;6:278.
44. Mack LR, Tomich PG. Gestational diabetes: diagnosis, classification, and clinical care. Obstet Gynecol Clin North Am. 2017;44:207–217.
45. Rojo-Martínez G, Valdés S, Soriguer F, et al. Incidence of diabetes mellitus in Spain as results of the nation-wide cohort di@bet.es study. Sci Rep. 2020;10:2765.