Design of the Custom TaqMan Gene Expression Array Cards

We hypothesized that maternal weight trajectories could alter the expression of epigenetic machinery genes or genes implicated in metabolism or in development. The originality of this study was that different families of epigenetic modifiers were examined. Based on literature and our previous study [1], 60 epigenetic genes were selected because of their implication in metabolic processes, fetal growth, obesity, type-2 diabetes, developmental conditioning of offspring phenotype or the response to maternal nutrition. The second aim of this expression study was to assess the expression of known target genes of developmental conditioning by maternal nutrition (32 genes):

1) DNA methylation

DNA methyltransferases are implicated in developmental programming, fetal growth restriction or the response to maternal HFD [1–6]. We also studied the genes coding for **DNA hydroxymethylation** enzymes (TETs) to see if maternal metabolism could have an impact of their expression. **Methyl-binding domain-containing proteins** play an important role in brain development and in mental disorders, which associate with alterations in feeding behaviour, and also in glucose homeostasis [2,4,7–14].

2) Histone methylation

Some **lysine methyltransferases** are involved in obesity, diabetes, adipogenesis and the response to maternal HFD [1,15–20]. **Lysine demethylases** are also involved in obesity [1,21–23]. **Arginine transferases** are known regulators of glucose metabolism and adipogenesis [24–28].

3) Histone acetylation

Genes related to histone acetylation were particularly suitable to our selection criterion; thus, 29 members of these families were studied out of 60 epigenetic machinery genes. **Histone deacetylases** are involved in adipogenesis, fetal growth restriction and developmental programming by maternal nutrition [5,6,29–32]. **Sirtuins** are largely implicated in lipid and glucose metabolism, and are important sensors of caloric restriction [33–39]. Some sirtuins are associated with an obese phenotype [40–45]. Many members of the **lysine acetyltransferase** family play a role in metabolic diseases like obesity, diabetes and hepatic steatosis [46–55]. **Bromodomain proteins** 2 and 4 are involved in obesity and in adipogenesis [56–58].

4) Glucose and lipid metabolism: *Pparα, Pparγ, Pparδ, Pepck, Pgc-1α, C/Ebp-α, C/Ebp-β, Gck, Lpl, Rev-erbα, Nocturnin, OxtR*

5) Serotonin signaling: *Tph1, Slc6a4, MaoA, 5-HT-r2a, 5-HT-r2c*

6) Glucocorticoid signaling and metabolism: *11βHsd-1, 11βHsd-2, Gr*

7) Appetite regulation: *leptin, leptin receptor, Pomc, Npy, Bdnf,*

8) Feto-placental growth: *Gcm1, GcGr, Igf2, Igf2r, Slc16a10, Irs-1, InsR*

For the first three categories, we chose all members of the epigenetic machinery for which we found information related to our selection criterion. Genes for which we did not find relevant information or that have never been studied in the context of obesity were not taken into

account: *Kdm1a*, *Mbd4*, *Brd4*, *Prmt3*, *Prmt4*, *Prmt6*, *Phf8*, *Kdm4b*, *Kdm4c*, *Kdm5b*, *Jarid2*, *Kmt2a*, *Kmt2b*, *Kmt2c*, *Kmt2d*, *Kmt3b*, *Kmt5a*, *Kmt6*.
For a subset of genes in categories 4 to 8, epigenetic alterations are documented in the context of developmental conditioning. For example, methylation and histone modifications in the promoter of the ***glucocorticoid receptor*** are altered in response to maternal nutrition or behaviour [4,59–63]. Methylation of the ***leptin*** promoter and the anorexygenic hypothalamic neuropeptide ***Pomc*** promoter is modified by maternal high-fat or low-protein diet [64–67]. The maternal nutritional environment alters epigenetic marks at the level of transcriptional factors involved in the development of metabolic tissues [60,68–73].
All genes assessed in this study are listed in Additional file 2 with their assay ID numbers (Table S1).

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